Antidiabetic Activity of Green Synthesized Zinc Oxide Nanoparticles Using *Quercus infectoria*

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**Abstract:** Diabetes mellitus is the common endocrine disorder that affects more than 10% of people worldwide. Some of the transition metals that are used in nanotechnology are gold, platinum, iron, cadmium, nickel, cobalt etc. Zinc oxide (ZnO) nanoparticles, as one of the important metal oxide nanoparticles, are employed in various fields due to their specific physical and chemical properties. Plant mediated synthesis of nanoparticles has been increasingly gaining popularity due to its improved bioavailability, enhancing aqueous solubility, and increasing resistance time in the body. This study focuses on synthesis of zinc oxide nanoparticles using gallnut extract of *Quercus infectoria* and Characterization by UV-visible (UV-vis) spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), X-Ray diffraction (XR), and SEM analysis. *Quercus infectoria* nutgalls have been widely employed in traditional Asian medicine for several treatments like wound healing, skin disorder, antimicrobial, anti-oxidant and for antidiabetic. The study comprises of antidiabetic activity of aqueous gallnut extract of *Quercus infectoria*.

**Keywords:** Nanoparticles, Zinc, Antidiabetic, *Quercus infectoria*, Gallnut

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**Introduction**

In modern research era of any branch of science, nanotechnology has gained enormous interest. Nanoparticles (NPs) play an essential role as building blocks of nanotechnology. Nowadays, nanoscience as well as nanotechnology is widely applied in different fields mainly in sensor, electronic, antibacterial, water purification, cosmetic, biomedical, pharmaceutical, environmental, catalytic, and material application. The size, crystallinity and morphology of the nanomaterial can greatly influence their catalytic, magnetic, electronic and optical properties (Cui and Lieber, 2001). The main advantages of nanoparticles synthesis at room temperature and from plant extracts partly fulfill the green synthesis (Jones, 2011). Synthesis of nanoparticles has gained great significance during the last few years due to their unique properties and application (Garlapati \textit{et al.}, 2010). Chemical methods are among the most important approaches in metallic nanoparticles synthesis. However, these methods use high cost and toxic
reagents as reducing and stabilizing agents (Prabhu and Poulose, 2012). Currently, there is a growing need to develop inexpensive and environmentally friendly nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol (Philip et al., 2011). Green synthesis of nanoparticles is gaining interest worldwide because of its advantages, such as being eco-friendly, non-toxic, and economic, over chemical and traditional physical methods (Mohanpuria et al., 2008). The recent growth in the field of porous and nanometric materials prepared by non-conventional processes has stimulated the search of new applications of ZnO nanoparticles (Amekura et al., 2008).

Zinc oxide is an interesting semiconductor material due to its application on solar cells, gas sensors, ceramics, catalysts, cosmetics and varistors (Abhulimen, 2005). Recently, ZnO NPs have been used in food packaging materials and various matrices and methods for incorporation of ZnO into those matrices have been reported. ZnO is incorporated into the packaging matrix, free to interact with the food materials offering preservatory effects (Espitia et al., 2012). Presently, ZnO NPs have found application in sunscreens, paints and coatings as they are transparent to visible light and offer high UV absorption and are also being used as an ingredient in antibacterial creams, ointments and lotions, self-cleaning glass, ceramics and deodorants. ZnO nanoparticles have been lately tested for their antimicrobial potential and seem to possess both antibacterial and antifungal potential. They are active against both Gram-positive and Gram-negative bacteria and also show considerable activity against more resistant bacteria spores. It was also observed that doping of ZnO NPs with other metals such as gold, silver, chromium etc. improved the antimicrobial activity of ZnO nanoparticles. Also, inhibitory effects of ZnO nanosuspension are correlated with their size and concentration, with smaller particles offering better inhibitions in higher concentration (Franklin et al., 2007). Zinc oxide nanoparticles have excellent thermal and chemical stability with exceptional optical behavior (Iravani, 2006).

Green synthesis is an alternative to conventional physical and chemical methods. Green synthesis of nanoparticles is gaining importance due to its cost-effectiveness and reduction of toxic chemicals. The use of plants for the synthesis of nanoparticles is a rapid, low-cost, eco-friendly option and is safe for human use (Chung, 2017). Nanoparticle synthesis is mediated by physical, chemical and green methods (Rajakumar et al., 2017). The physical method involves the use of costly equipment, high temperature and pressure, large space area for setting up of machines (Afifi et al., 2015). The chemical method involves the use of toxic chemicals which can be hazardous for the environment and the person handling it. The literature states that some of the toxic chemicals that we use in physical and chemical methods may reside in the NPs formed which may prove hazardous in the medical field (Chandrasekaran et al., 2016). Thus, we needed an environment friendly and cost-effective method for nanoparticle synthesis. Biosynthesis of nanoparticles is an approach of synthesizing nanoparticles using microorganisms and plants having biomedical applications. This approach is an environment-friendly, cost-effective, biocompatible, safe and green approach (Dhandapani et al., 2014). Green synthesis includes synthesis through plants, bacteria, fungi, algae etc. They allow large scale production of ZnO NPs free of additional impurities (Abdul et al., 2014).

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world (Yuvakkumar et al., 2014). Diabetes is one of the leading causes of death in humans and
The presence of diabetes confers increased risk of many devastating complications such as cardiovascular diseases (CVD), peripheral vascular diseases (PVD), stroke, neuropathy, renal failure, retinopathy amputations and blindness (Burke et al., 2003). Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas are available for the treatment of diabetes. However, none of these medications is ideal due to toxic side effects and in some cases diminution of response after prolonged use (Bajaj and Madan, 1993). Medicinal plants and their bioactive constituents are used for the treatment of diabetes throughout the world, especially in countries where access to the conventional anti-diabetic agents is inadequate. Although several medicinal plants have gained importance for the treatment of diabetes, many remain to be scientifically investigated (David, 1996).

Both insulin and glucagon (pancreatic endocrine hormones) are responsible for controlling blood glucose level within the body in an adequate level based on the body needs. Normally, insulin is secreted by the β-cells present in the islets of Langerhans in response to high levels of blood sugar. It potentiates the ability of muscle, red blood cells, and fat cells to absorb sugar out of the blood and consume it in other metabolic processes, which restore the sugar levels to the normal level. On the contrary, glucagon is secreted by α-cells of the pancreas as a result of low blood-glucose level in between meals and during exercise. It stimulates the liver and other cells in muscles to release glucose out of the stored block in our bodies (Dixit and Joshin, 1985). The severity of damage triggered by hyperglycemia on the respective organ systems may be related to how long the disease has been present and how well it has been controlled. Several symptoms such as thirst, polyuria, blurring of vision, and weight loss also accompany diabetes (Punitha et al., 2006). The Indian subcontinent has emerged as the capital of this diabetes population. Indians show a significantly higher prevalence of diabetes when compared with several other populations. Asian Indians display a higher insulin level which is an indicator of peripheral insulin resistance (Gupta and De 2012). The insulin resistance in Indians is thought to be due to their higher body fat percentage. Excess body fat, lack of physical activity and racial predisposition may explain the prevalence of hyperinsulinemia and increased development of type 2 diabetes in Indians (Sperling, 2014).

Diabetes characterized by metabolic deregulation primarily of carbohydrate metabolism, manifested by higher blood glucose level resulting from the defect in insulin secretion, insulin action, or both. Uncontrolled diabetes leads to many complications which are leading to peripheral vascular disease, nephropathy, neuropathy, and retinopathy (Dong et al., 2012). According to the World Health Organization (WHO), up to 80% of the population in developing countries uses plants and its products as a traditional medicine for primary health care needs. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India. There are about 800 plants which have been reported to show antidiabetic potential. Vast collections of plant-derived phytoactive principles representing numerous natural bioactive compounds have established their role for possible use in the treatment of diabetes (WHO, 2002).

It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025. Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Most of the food we eat is broken down into simple sugar called glucose. This glucose is the main source of fuel to get energy for the body. After digestion the glucose reaches our blood stream where it is available for body cells to utilize for energy. But insulin is needed for the glucose to get into the cells. It is the function of the pancreas to secrete the adequate amount of insulin, to transport glucose from
blood into different cells of the body.

The galls of *Quercus infectoria* have also been pharmacologically documented to possess astringent, antidiabetic, antitremorine, local anaesthetic, antiviral, antibacterial, antifungal, larvicidal and anti-inflammatory activities. The main constituents found in the galls of *Quercus infectoria* are tannin (50-70%) and small amount of free gallic acid and ellagic acid. The wide range of pharmacological activities of this plant might support the efficacy of extract preparation of *Quercus infectoria* that are widely used in Malaysia for treating many kinds of health problems since many decades. The nutgalls have been pharmacologically documented for their antiamoebic, anticariogenic and anti-inflammatory activities, to treat skin infections and gastrointestinal disorders. It has been used as dental powder and in the treatment of toothache and gingivitis. Traditionally dried fruit powder used in the treatment of women disorders. Whole plant is useful in the treatment of microbial infection. Hence, this research work is mainly focused on simple process as a green technology using aqueous extract of *Quercus infectoria* galls for the first time for the biosynthesis of zinc nanoparticles without the usage of hazardous and toxic solvent. The process has several advantages with low cost, compatibility, stability and also has proved antidiabetic activity. Keeping the above point in view, we have decided to do synthesis of the zinc oxide (ZnO) nanoparticles by using the aqueous extract of *Quercus infectoria* and also access its antioxidant and anti-diabetic potential.

**Materials and Methods**

*Collection of samples and preparation of aqueous extract:*

The medicinal plants used for the experiment were galls of plant of *Quercus infectoria* (Figs. 1, 2) which were collected from the local medicinal farms.

Fresh galls of *Quercus infectoria* were collected and washed in running tap water followed by double distilled water and coarsely grinded. The aqueous extract of sample was prepared by boiling the freshly coarsely grinded (25 g), with 100 ml of distilled water, at 60 °C for about 20 min, until the colour of the aqueous solution changed from watery to light yellow. Then the extract was cooled to room temperature and filtered using filter paper and used for further experiments.

![Fig. 1: Quercus infectoria plant.](image1.jpg)

![Fig. 2: Galls of Quercus infectoria.](image2.jpg)

**Synthesis of zinc oxide nanoparticles:**

15 ml of leaf extract of *Quercus infectoria* was added to 2.195 g of zinc acetate dehydrate (Fig. 3) dissolved in 35 ml of distilled water (overall concentration 200 mM solution). The reaction mixture was kept on magnetic stirrer for 6 h. After 6 h, 2M NaOH (4 g of NaOH pellet in 50 ml of Milli-Q water) was added to the solution and it was placed in incubator at 60 °C with magnetic stirring for overnight. White mixture was centrifuged at 14,000 rpm for 15 min. The precipitate was subjected to washing with alcohol and distilled...
Fig. 3: Color changing of fruit extract of *Quercus infectoria* from pale yellow to reddish brown after the addition of zinc acetate.

Water three times each. Precipitate was dried in an incubator at 40–50°C and fine powder was prepared with the help of ceramic pestle and mortar. Fine powder was used for characterization with SEM, FTIR, XRD, and UV-Vis.

**Characterization of ZnO nanoparticles:**

**UV-Vis spectroscopy:**

The reduction of zinc ion was monitored by measuring optical density through UV-Vis spectroscopy of the reaction medium after diluting small aliquots of reaction mixture ten times diluted with Milli-Q water and transferred to cuvette, and analysis was done using UV-Vis spectrophotometer.

**X-ray diffraction:**

The formation and quality of compounds were investigated by X-ray diffraction technique. For this purpose, synthesized zinc oxide NPs were centrifuged (1400 rpm) for 15 min, pellet was washed three times with ethanol and finally with sterile Milli-Q water for three cycles. The purified ZnO NP precipitate was dried in an oven at 60°C and powdered with ceramic mortar–pestle. Powdered sample was analyzed using X-ray diffractometer.

**FTIR analysis:**

FTIR was used to identify the possible functional groups involved in the reduction of zinc ion and capping of reduced zinc oxide nanoparticles. FTIR spectrum was recorded using infrared (IR) double beam spectrophotometer. FTIR analysis of dried ZnO nanoparticles (NPs) was carried out through potassium bromide (KBr) pellet method in 1:30 ratios (NPs:KBr) and spectrum was recorded in transmittance mode at a resolution of 4 cm⁻¹. The peaks (stretching) obtained were plotted as transmittance at Y-axis and wave number (cm⁻¹) at X-axis. The spectrum was recorded in the wave number range 500–4500 cm⁻¹ and analyzed subtracting the spectrum of pure KBr.

**SEM analysis:**

Scanning electron microscopy (SEM) analysis was used to obtain the surface image and the size of the synthesized zinc nanoparticles. Thin film of nanoparticle powder sample was prepared on carbon coated tape by adhering small amount of dried fine powder of sample on the grid, excess sample was removed with the help of blotting paper. The film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min. The SEM analysis was used to determine the surface structure of biogenically synthesized ZnO NPs.

**Determination of Antioxidant activity:**

**DPPH radial scavenging assay:**

The free radical scavenging activity of the green synthesized nanoparticle and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl). 100 µl of the tested nanoparticles were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L⁻¹) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated, using the following equation:

\[
\text{DPPH scavenged (\%) = } \left\{ \frac{(A_c - A_t)}{A_c} \right\} \times 100
\]

Where Ac is the absorbance of the control reaction and At is the absorbance in presence of the sample. The nanoparticles of the *Quercus*
*infectoria* value was defined as the concentration in mg of dry material per ml (mg/ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

**ABTS radial scavenging assay:**

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS⁺) scavenging activity was measured according to the method described by (Mensor et al., 2001). ABTS was dissolved in water to a 7 mM concentration. The ABTS radicals were produced by adding 2.45 mM potassium persulphate (final concentration). The completion of radical generation was obtained in the dark at room temperature for 12–16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 ± 0.02. To determine the scavenging activity, 1 ml of diluted ABTS⁺ solution was added to 5, 10, 20, 40, 80, 160, 320 mg/ml of green synthesized nanoparticles, and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

\[
\text{ABTS scavenged (\%)} = \left(\frac{A_c - A_t}{A_c}\right) \times 100
\]

where Ac and At are the absorbencies of the control and of the test sample, respectively. From a plot of concentration against % inhibition, a linear regression analysis was performed to determine the value of the sample.

**Determination of Antidiabetic assay:**

**In Vitro α-amylase inhibitory assay:**

A starch solution (1% w/v) was prepared by stirring 1g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride. The enzyme solution was prepared by mixing 27.5 mg of porcine pancreatic amylase α-amylase (PPA) in 100 ml of 20 mM of phosphate buffer (PBS, pH 6.9) containing 6.7mM of sodium chloride. To 100 μl of green synthesized nanoparticles (10, 20, 40, 80, 160, 320 μg/ml), 200 μl porcine pancreatic amylase was added and the mixture was incubated at 37 C for 20 min. To the reaction mixture 100 μl (1%) starch solution was added and incubated at 37 C for 10 min. The reaction was stopped by adding 200 μl DNSA (1g of 3,5 di nitro salicylic acid, 30 g of sodium potassium tartarate and 20 ml of 2N sodium hydroxide was added and made up to a final volume of 100 ml with distilled water) and kept it in a boiling water bath for 5 min. The reaction mixture was diluted with 2.2 ml of water and absorbance was read at 565 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 200 μl in distilled water. Control, representing 100% enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol.

**Results and Discussion**

UV-visible spectroscopy is usually conducted to confirm the synthesis of zinc oxide nanoparticles. Conducting electrons start oscillating at a certain wavelength range due to the surface plasmon resonance effect which represents the UV-visible spectra of freshly prepared zinc oxide nanoparticles. Peak obtained at 383 nm clearly demonstrates the presence of zinc oxide nanoparticles (Fig. 4).

![UV-Visible of Zinc Oxide Nanoparticles](image)

**X-Ray Diffraction:**

XRD patterns of synthesized zinc oxide nanoparticles reflected that all the diffraction peaks of zinc oxide nanoparticles matched with...
the standard zinc oxide nanoparticles data. Diffraction peaks of XRD were very well matched with the hexagonal wurtzite structure. The sharp and intense peaks indicated that the samples were highly crystalline. From the XRD data, it was found that the peaks are broad; suggesting that the crystallites have sizes in the nanometer range.

**FT-IR Analysis:**

FTIR measurement was carried out to identify the possible biomolecules in *Quercus infectoria* galls extract responsible for capping leading to efficient stabilization of the zinc oxide nanoparticles. The FTIR spectra showed the presence of bonds due to $\sim$OH stretching frequency. The region between 1400 cm$^{-1}$ was assigned for metal-oxygen bond. In addition to the absorption bands of the biomolecules used as reduction and stabilization (capping agents), the absorption peak at 440 cm$^{-1}$ indicated the presence of zinc oxide nanoparticles.

**SEM Analysis:**

After the substantiation of XRD results the sample was further preceded for the SEM study. The size, shape and surface morphology of the zinc oxide nanoparticles was clearly indicated by SEM analysis and structural characterizations demonstrated that the synthesized products were spherical and crystalline in structure.

**Anti-Oxidant Activity:**

**DPPH radical scavenging assay:**

The evaluation of anti-radical scavenging properties of the green synthesized zinc oxide nanoparticles from *Quercus infectoria* and ascorbic acid were executed by DPPH radical scavenging assay (Tables 1, 2; Figs. 5, 6, 7). The inhibition of DPPH radical by the plant extract was determined, a lower value reflected greater antioxidant activity of the sample. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or synthesized nanoparticles. The antioxidant effect is proportional to the disappearance of the purple colour of DPPH in test samples. Thus, antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colourless stable molecule 2,2'-diphenyl1-hydrazine is formed and as a result of which the absorbance (at 517 nm) of the solution is decreased. Hence, the more potent antioxidant, more decrease in absorbance is seen and consequently the value will be minimum.

Oxidative damage to cells is a crucial factor in the pathogenesis of several clinical disorders as well as the normal process of aging. Deficient natural antioxidant defenses are implicated as the etiological or pathological factors of various degenerative disorders (Chambel, *et al*., 2015). The onset and progression of these disorders can be arrested or delayed by antioxidant defense supplementation. Plant-based antioxidants such as resveratrol and quercetin potentiate the body's antioxidant defense and are relatively safe. Thus, a huge body of scientific research focuses on exploring plants with antioxidant potential.

In addition, Elham *et al.* (2021) reported that GA possesses antioxidant effects and protects the oxidation of fat. Additionally, the appropriate concentration of GA should be selected to avoid unwanted effects. Low concentrations of GA ($\leq$ 25 $\mu$gml$^{-1}$) can enhance mitochondrial antioxidant capacity, increase cell proliferation, and repair chemical substances and radiation damage by inhibiting the expression of 17-9p, MIR-21, MIR-421, and other antioxidant target genes of glioblastoma T98G, stimulating cell and tissue regeneration. However, the effect of high concentrations of GA ($>25$ $\mu$gml$^{-1}$) has the opposite effects on the above miRNAs (Elham *et al*., 2021).

**ABTS Radial Scavenging Assay:**

The antioxidant activity of the green synthesized zinc oxide nanoparticles from *Quercus infectoria* and ascorbic acid using ABTS assay were also carried out (Tables 3, 4; Figs. 8, 9). The antioxidant effect is proportional to the disappearance of the colour of ABTS in test samples. Concentration of sample that could scavenge free radical was used
Table 1: DPPH radial scavenging assay of galls of zinc oxide nanoparticles

<table>
<thead>
<tr>
<th>S. No</th>
<th>Zinc Oxide Nanoparticles Concentration (mg/ml)</th>
<th>Percentage Of Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5 mg</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>10 mg</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>20 mg</td>
<td>60</td>
</tr>
<tr>
<td>4.</td>
<td>40 mg</td>
<td>62</td>
</tr>
<tr>
<td>5.</td>
<td>80 mg</td>
<td>65</td>
</tr>
<tr>
<td>6.</td>
<td>160 mg</td>
<td>66</td>
</tr>
<tr>
<td>7.</td>
<td>320 mg</td>
<td>70</td>
</tr>
</tbody>
</table>

Fig.5: Inhibition of DPPH radial by the galls of zinc oxide nanoparticles.

Table 2: DPPH radial scavenging assay of standard drug Ascorbic acid

<table>
<thead>
<tr>
<th>S. No</th>
<th>STANDARD Concentration (mg/ml)</th>
<th>PERCENTAGE OF INHIBITION (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5 mg</td>
<td>7</td>
</tr>
<tr>
<td>2.</td>
<td>10 mg</td>
<td>36</td>
</tr>
<tr>
<td>3.</td>
<td>20 mg</td>
<td>62</td>
</tr>
<tr>
<td>4.</td>
<td>40 mg</td>
<td>75</td>
</tr>
<tr>
<td>5.</td>
<td>80 mg</td>
<td>75</td>
</tr>
<tr>
<td>6.</td>
<td>160 mg</td>
<td>75</td>
</tr>
<tr>
<td>7.</td>
<td>320 mg</td>
<td>75</td>
</tr>
</tbody>
</table>
Fig. 6: Inhibition of DPPH radial by the standard drug Ascorbic acid.

Fig. 7: DPPH Radial Scavenging Assay of Ascorbic Acid and ZnO Nanoparticles.

Table 3: ABTS radial scavenging assay of galls of zinc oxide nanoparticles

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (mg/ml)</th>
<th>Percentage Of Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5 mg</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>10 mg</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>20 mg</td>
<td>6</td>
</tr>
<tr>
<td>4.</td>
<td>40 mg</td>
<td>32</td>
</tr>
<tr>
<td>5.</td>
<td>80 mg</td>
<td>60</td>
</tr>
<tr>
<td>6.</td>
<td>160 mg</td>
<td>74</td>
</tr>
<tr>
<td>7.</td>
<td>320 mg</td>
<td>86</td>
</tr>
</tbody>
</table>
Fig. 8: Inhibition of ABTS radial scavenging assay of galls of zinc oxide nanoparticles.

Table 4: ABTS radial scavenging assay of standard drug ascorbic acid

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (mg/ml)</th>
<th>Percentage of Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5 mg</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>10 mg</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>20 mg</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>40 mg</td>
<td>98</td>
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<tr>
<td>5.</td>
<td>80 mg</td>
<td>98</td>
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<tr>
<td>6.</td>
<td>160 mg</td>
<td>98</td>
</tr>
<tr>
<td>7.</td>
<td>320 mg</td>
<td>98</td>
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</tbody>
</table>

Fig. 9: Inhibition of ABTS radial scavenging assay of standard drug ascorbic acid.
to determine antioxidant capacity of sample compared to standard. Sample that had QI21 < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with QI21 > 150 ppm.

**Anti-diabetic Activity:**

*In Vitro α-amylase inhibitory assay:*

In this study the *in vitro* α- amylase inhibitory activities of the green synthesized zinc oxide nanoparticles from *Quercus infectoria* was investigated (Fig. 10). The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against α-amylase enzyme. The synthesized zinc oxide nanoparticles in different concentrations exhibited potent α-amylase inhibitory activity in a dose-dependent manner. The extract showed inhibitory activity from 0.14 to 1.63 with an QI21. Acarbose is a standard drug for α-amylase inhibitor. Acarbose at various concentrations showed α-amylase inhibitory activity from 0.34 to 1.63. A comparison of α-amylase inhibitory activity between the standard drug and nanoparticles has been depicted in Figure 10. So the nanoparticles might be used as starch blockers since it prevents or slows the absorption of starch into the body mainly by blocking the hydrolysis of 1, 4-glycosidic linkages of starch and other oligosaccharides into maltose, and other simple sugars. In our study, the green synthesized zinc oxide nanoparticles showed maximum α-amylase inhibitory activity.

**Conclusion**

In this work, we have synthesized zinc oxide nanoparticles by a very simple and efficient precipitation method using *Quercus infectoria* galls extract as natural precursor. The main advantage of this green synthesis is simple, cost-effective and eco-friendly. The formation of zinc oxide nanoparticles was confirmed by UV-Vis, FTIR, XRD, and SEM analysis. The synthesized zinc oxide nanoparticles effectively scavenge the free radicals such as DPPH and ABTS comparable to
that of standard in order to exhibit its antioxidant nature. The synthesized zinc oxide nanoparticles were studied for antidiabetic activity by Alpha-amylase enzyme inhibitory potential. On the basis of our current findings, it is possible to conclude that the biosynthesized zinc nanoparticles could be considered as eco-friendly and effective therapeutic agents for controlling diabetes mellitus after several experimental validations.

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