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In vitro antioxidant activity of green-synthesized silver nanoparticles (AgNPs), silver-zinc oxide nanoparticles (Ag-ZnO NPs) using leaf extracts of *Corallocarpus epigaeus*

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ABSTRACT

The present study evaluates the *in vitro* antioxidant capacity of silver nanoparticles (AgNPs), green silver–zinc oxide nanoparticles (Ag–ZnO NPs), and the methanolic leaf extract of *Corallocarpus epigaeus*. The *in vitro* antioxidant activity was determined by the H_2O_2 radical-scavenging assay, reducing power assay, and DPPH radical-scavenging assay. The methanolic leaf extract and standard ascorbic acid were prepared accordingly and used to assess antioxidant potential using the H_2O_2 radical-scavenging assay, reducing power assay, and DPPH radical-scavenging assay. UV-Vis spectrophotometry confirmed the biosynthesis of silver and silver–zinc oxide NPs. UV-Vis spectrophotometry confirmed the green-synthesized AgNPs and Ag-ZnO NPs by surface plasmon resonance at 460 nm and 450 nm, respectively.

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The fabricated Ag-ZnO NPs showed significant antioxidant activity compared with AgNPs and the leaf extract in the H_2O_2 radical-scavenging assay (72.33±0.21%), reducing power assay (0.325±0.12), and DPPH radical-scavenging assay (79.49±0.15%), in a dose-dependent manner. Therefore, in the present investigation, the antioxidant potential of Ag-ZnO NPs can be used as an alternative antioxidant, and antioxidants can mitigate the harmful effects of reactive oxygen species (ROS).

Keywords: *Corallocarpus epigaeus*, Methanolic leaf extract, Silver-nanoparticles, Silver-zinc oxide nanoparticles, Radical scavenging.

1. INTRODUCTION

An imbalance in the synthesis and removal of Reactive Oxygen Species (ROS) may cause cellular damage. The scavenging system has been linked to the pathophysiology of various human illnesses [1]. Oxidative stress is well acknowledged as a major contributing element in the etiology of many chronic and degenerative disorders. These problems include atherosclerosis, ischemic heart disease, aging, diabetes, cancer, immunological suppression, and neurodegenerative illnesses [2, 3, 4]. Antioxidants are essential for effectively eliminating free radicals, which are the major cause of oxidative stress. Plant-derived natural antioxidants have become more popular in recent years. The increased usage of natural antioxidants in the food industry has several uses, including improved stability, inclusion into nutraceuticals, and potential as phytochemicals, all of which have significant implications for human health and disease prevention [5]. The presence of oxygen-free radicals has been accepted for over five decades; nevertheless, their role in disease progression was only understood in the past two decades. As a result, many studies have been investigating the positive effects of antioxidants [6].

The growing field of nanotechnology has had a significant influence on the development of material science research by precisely manipulating material properties at microscopic scales. Traditional nanoparticle synthesis techniques often entail the use of chemical reagents that may be harmful to the environment and human health [7]. The green synthesis strategy, on the other hand, uses natural sources with inherent reducing and stabilizing properties, such as plant extracts, microbes, and biomolecules, to decrease environmental impact and increase biocompatibility [8]. Green synthesis has sparked substantial attention as a solution to these difficulties because of its cheap cost, biocompatibility, and environmental friendliness [9]. Among the many uses of nanotechnology, the synthesis of nanoparticles is a pioneering area that combines scientific research with technical innovation. Within this context, the green synthesis of silver nanoparticles has received a lot of interest for its ability to integrate cutting-edge nanotechnology with ecologically friendly procedures [10]. Zinc oxide (ZnO) is a metal oxide of great economic and industrial relevance owing to its unique properties and wide range of practical applications [11]. Using plant extracts in green synthesis provides a simple and efficient way for large-scale nanoparticle production. Plant extracts, rich in bioactive chemicals, which operate as powerful reducing and stabilizing agents in the green synthesis of nanoparticles, affected their characteristics [12]. The plant parts of *Corallocarpus epigaeus* (leaves, stem, and root) possess a wide range of medicinal properties that have been recognized and exploited in traditional medicine [13]. The current study involves the synthesis of silver nanoparticles (AgNPs) and silver-zinc oxide nanoparticles (Ag-ZnO NPs) by a straightforward co-precipitation procedure. *C. epigaeus* leaf extract is utilized as a capping and stabilizing agent during the synthesis process.

The present study aims to evaluate the antioxidant properties of biofabricated nanoparticles. This was accomplished through the implementation of several assays, including the reducing power assay, H_2O_2 radical scavenging assay, and DPPH radical scavenging assay.

2. MATERIAL AND METHODS

2.1. Collection of Plant Material

The leaves of *C. epigaeus* were collected from the healthy plant (Fig. 1) and the leaves were cleansed, desiccated, and pulverized, resulting in a fine particulate substance.

2.1.1. Preparation of Leaf Methanolic Extract and Standard

The powdered leaf sample (5g) was added with 50 ml methanol and incubated in an orbital shaker at 120 rpm for 48 h, then the resulting mixture was filtered using Whatman no.1 filter paper. The different concentrations of methanolic leaf extract and the standard ascorbic acid were prepared accordingly.

2.1.2. Green Synthesis Of Silver Nanoparticles

The aqueous leaf extract (1:10 w/v) was boiled for 10 min, cooled, and filtered. The filtered extract was centrifuged at 3000 rpm for 15 min (Fig. 1). After centrifuging, 10 ml of the solution was mixed with 90 ml of 1 mM silver nitrate solution (Fig 1) and incubated in the dark for 24-48 hours. The synthesis of AgNPs was shown by the color shift from yellow to reddish-brown (Fig. 1). After synthesis, the silver nanoparticles were washed with deionized water and centrifuged at 10,000 rpm for 15 min. The synthesized AgNPs were confirmed by a UV-Visible spectrophotometer between the wavelength ranges of 200-800 nm.

2.1.3. Synthesis of Ag-Zno Nanoparticles

To synthesize Ag-ZnO nanoparticles, 100 ml of leaf extract was mixed with 50 ml of silver nitrate (0.1 M) and zinc acetate (0.2 M). To which, 2 M sodium hydroxide was added until pH 12.0 was reached. The mixture was then stirred on a magnetic stirrer at 60 °C for 2 h. After heating, the reaction mixture was cooled and filtered using Whattman filter paper. The filtered pellet was washed twice with sterile distilled water and oven-dried at 60 °C. Dry pellet was calcined at 400 °C for 4 h. The calcinated pellet was pulverized using a mortar and pestle and kept in a hermetically sealed glass vial for UV-Vis spectrophotometer analysis. The sample absorbance was measured between the wavelength ranges of 200-800 nm.

2.2. Statistical Analysis

Each assay was performed thrice, and the data collected were analyzed statistically using one-way ANOVA to detect the significant difference between the means of samples at 5% level of significance using SPSS software. The values are presented as mean ± SD.

2.3. Procedures for Antioxidant Assays

2.3.1. H_2O_2 Free Radical Scavenging Assay

The ability to scavenge H_2O_2 free radicals has been quantified through this assay. Various concentrations of leaf methanolic extracts, green-synthesized AgNPs, Ag-ZnO NPs and reference standard (ascorbic acid) were prepared accordingly. After this, 0.6 ml of H_2O_2 (40 mM) solution was added. Spectrophotometer determines the optical density of samples after incubation for 10 min in the dark [14]. Control consists of 40 mM H_2O_2 in phosphate buffer, and blank consists of phosphate buffer. The formula for quantifying free radical scavenging ability of the sample was determined as follows:

2.4. Reducing Power Assay

Reducing power assay was used to evaluate the antioxidant capacity of the tested samples. 2.5 ml of phosphate buffer (0.02%) and potassium ferricyanide (1%) were added to 1ml of test samples (50, 100, 150, 200, 250 and 500 µg/ml) and reference standard. The reaction mixture was incubated at 50ºC for 20 min.

After uniform mixing, 10% trichloroacetic acid solution was added, followed by centrifugation at 3000 rpm for 10-15 min. Thereafter, 2.5 ml of distilled water and 0.5 ml of freshly made ferric chloride were added and centrifuged again. Following an incubation period of 10 min, absorbance was read at 700 nm using a spectrophotometer [14]. In addition, a blank was prepared by mixing all the reagents except the test. The increase in absorbance of the samples was used as the criterion for determining the reducing power of the samples.

2.5. DPPH Assay

The test samples and reference standard were mixed with methanol (1 ml) containing DPPH (0.2 mM). The reaction mixture was properly mixed and incubated in the dark for 30 min. The optical density of the samples was measured at 517nm using a spectrophotometer. The methanol containing 0.2 mM DPPH was used as a control [15]. The inhibition percentage of the free radical scavenging was calculated using the above formula (Eq. 1).

3. RESULTS

The comparative antioxidant potentiality of leaf methanolic extract, green synthesized AgNPs, and Ag-ZnO NPs of *C. epigaeus* was evaluated by using H_2O_2 free radical scavenging assay, reducing power assay, and DPPH radical scavenging assay.

3.1. Confirmation Of Agnps And Ag-Zno Nps Synthesis

The AgNPs produced from aqueous leaf extracts of *C. epigaeus*, exhibited unique absorption spectra at a wavelength of 460 nm by UV-vis spectrophotometer (Fig 2). However, Figure 3 presents the UV-visible absorption spectrum of the synthesized Ag-ZnO NPs. A wide band at 450 nm signifies the existence of Ag nanoparticles and the successful production of ZnO NPs.

3.2. H_2O_2 Free Radical Scavenging Assay

H_2O_2 free radical scavenging activity of the test samples was depicted in Fig. 4. When exposed to a concentration of 250 µg/ml, the ascorbic acid effectively eliminated 84.58±0.27% of the free radicals present. Whereas considerable radical scavenging activity of tested samples was ranked in the hierarchy of Ag-ZnO NPs (72.33±0.21%), followed by green synthesized AgNPs (67.93±0.41%) and leaf methanolic extract (62.75±0.31%).

3.3. Reducing Power Assay

The increase in absorbance indicates the reducing power of the samples. The absorbance of the tested samples showed reducing power in a dose-dependent manner. The greatest absorbance value was observed at 500 µg/ml concentration. The ascorbic acid recorded highest absorbance of 0.396±0.28, followed by the Ag-ZnO NPs, AgNPs and leaf methanolic extract with 0.325±0.24, 0.290±0.30 and 0.250±0.22 absorbance, respectively (Fig 5). Ag-ZnO NPs showed considerably higher antioxidant activity than the other samples tested.

3.4. DPPH Assay

DPPH radical scavenging activity of the samples was analyzed by the reduction in the amount of DPPH. DPPH reduction was found to increase with increase in concentration, and maximum DPPH reduction was observed at 250 µg/ml. The results indicate that ascorbic acid showed higher inhibition rate of 84.39±0.41%, whereas Ag-ZnO NPs, AgNPs, and leaf extract showed an inhibition rate of 79.49±0.25%, 70.39±0.32% and 67.23±0.27% respectively (Fig 6).

4. DISCUSSION

The synthesis of AgNPs was primarily identified by observation of a color transition from light yellow to reddish brown and further confirmed by using a UV-Vis spectrophotometer [16]. Similar surface plasmon resonance of AgNPs at a wavelength of 450nm was also reported earlier in AgNPs synthesized from *Ocimum sanctum [17]* and as a *foetida* resins [18]. Factors like shape, size, and dielectric constant of AgNPs were known to determine the shape and position of the absorption spectrum [19]. The absorption spectra of *Saccharum officinarum* and *Bombax ceiba* also exhibited identical results at wavelengths 480 nm and 470 nm, respectively [20, 21]. Whereas Ag-ZnO NPs exhibited a broad band at 450 nm, a similar wide band of ZnO/Ag NPs was reported earlier by [22].

4.1. H_2O_2 Free Radical Scavenging Assay

Ascorbic acid exhibited maximum percentage inhibition of free hydroxyl radicals in a concentration-dependent manner [23]. When compared to standard ascorbic acid, the highest radical scavenging activity was shown by green-synthesized Ag-ZnO NPs, followed by AgNPs and leaf methanolic extract in a dose-dependent manner. H_2O_2 free radical scavenging of samples was found to be statistically significant at $p=0.05$ (level of significance), similar findings were reported earlier in *Nothapodytes foetida* [24] and *Solanum khasianum* [25]. Leaf extract of *C. epigaeus* showed lower inhibition than all the tested samples; however, a considerable increment in percentage inhibition of free radicals was observed at 250 µg/ml.

Primarily, hydrogen peroxide (H_2O_2) has little reactivity; nonetheless, it possesses the capacity to generate free radicals, hence potentially inducing toxicity inside physiological systems. So, it is crucial to remove H_2O_2 from food systems [26]. According to Kamalanathan, substances that could counteract the harmful effects of free radicals are often referred to as antioxidants [27]. Among the tested samples, green-synthesized Ag-ZnO NPs exhibited more antioxidant activity than AgNPs and leaf methanolic extract.

4.2. Reducing Power Assay

The increase in absorbance with the increase in concentration suggests the presence of antioxidant capacity. Among the samples analyzed, Ag-ZnO NPs exhibited significantly greater reducing power in comparison to AgNPs and leaf methanolic extract (Fig. 4). Whereas, leaf extract of *C. epigaeus* primarily exhibited a very low level of reducing power in comparison to the standard. The reducing agents within the plant samples can donate protons, leading to the reduction of Fe_3^+ to Fe_2^+. This reduction develops the formation of a Prussian blue colored complex and was quantified at a wavelength of 700 nm [28]. Similar findings were reported in all plant extracts of *Carica papaya* [28] synthesized AgNPs using leaf extract of *Holoptelea integrifolia* [29]. *S.khasianum* [30,] and *Muntingia calabura* [31].

4.3. DPPH Assay

The assessment of antioxidant capacity of the samples was performed using the DPPH reduction method, wherein the change in color from violet to yellow was employed as an indicator for quantifying the radical scavenging capacity [30]. DPPH, a nitrogen-centered free radical known for its remarkable stability and often used as an assay for evaluating the free radical scavenging activity [32]. The process of DPPH reduction included the transfer of a proton onto the DPPH molecule, which was observed by the change in color.

Previous reports on the antioxidant activity of tuberous extract of *C. epigaeus* also showed significant antioxidant activity in a concentration-dependent manner [33]. To the best of our knowledge, the current study using leaf extract, AgNPs, and Ag-ZnO NPs has not been reported earlier. Among all the tested samples, Ag-ZnO NPs showed a significantly high amount of DPPH radical scavenging capacity, validating the antioxidant capacity of the Ag-ZnO NPs. Similar findings of the potential antioxidant activity of green-synthesized AgNPs were reported earlier in *Morinda citrifolia* [9]. The antioxidant potential of Ag-ZnO NPs was found to improve in comparison to AgNPs.

5. CONCLUSION

The antioxidant activity of the samples was found to increase in a dose-dependent manner. However, there is no significant difference in the antioxidant activity of silver-zinc oxide nanoparticles of *C. epigaeus* and standard ascorbic acid. The antioxidant capacity of green-synthesized Ag-ZnO NPs was found to be higher than that of AgNPs and leaf methanolic extract. The present study provides crucial information on the potential of green-synthesized nanoparticles over the leaf extract of the *C. epigaeus*.

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Authors' Contributions

All authors contributed equally to the writing of this article. All authors have read and approved the manuscript.

Conflict of Interest Disclosures

Authors declare that they have no competing interest.

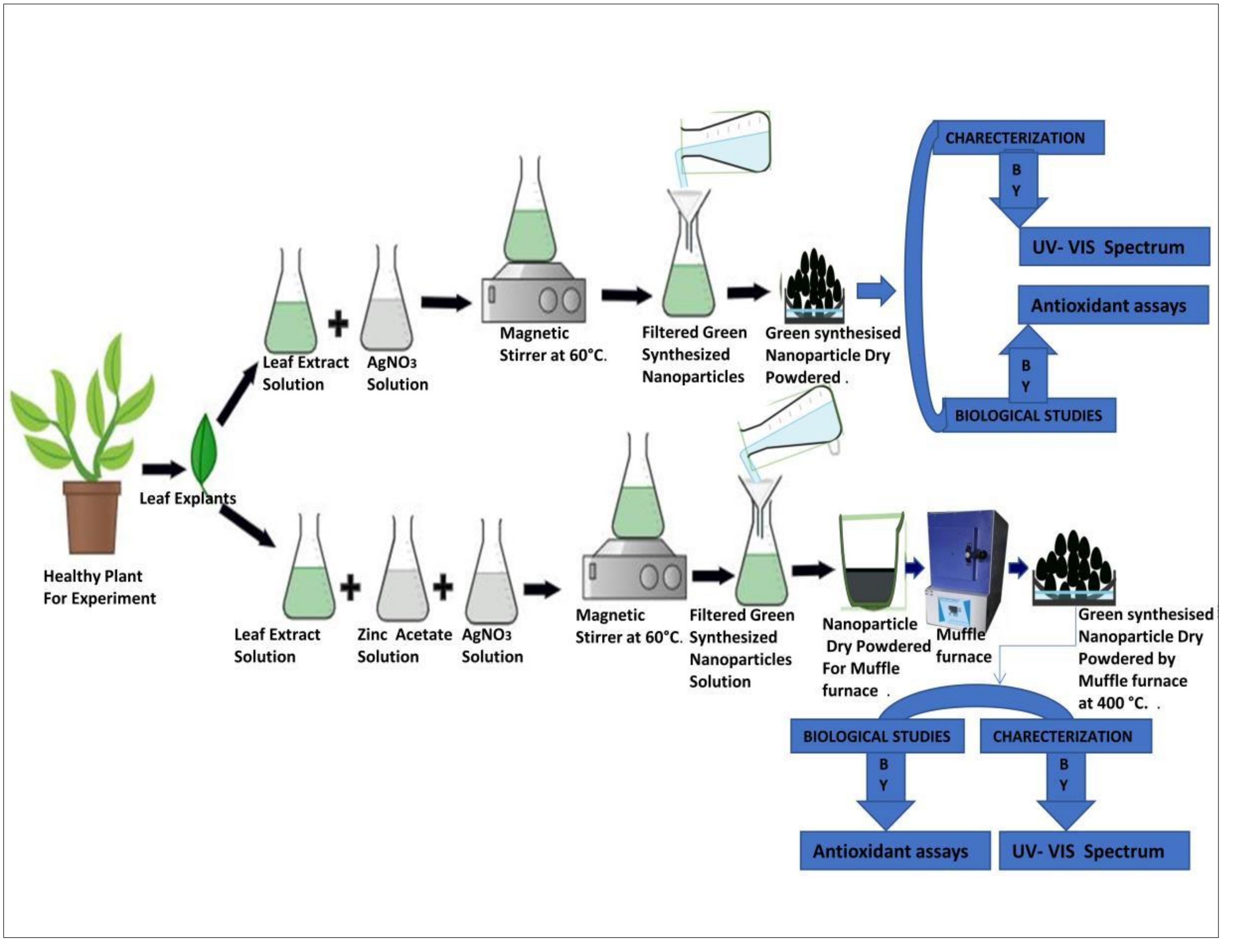

Figure 1. Schematic flow of Ag undoped and Ag-ZnO doped green synthesized nanoparticles from *C. epigaeus* leaf extract.

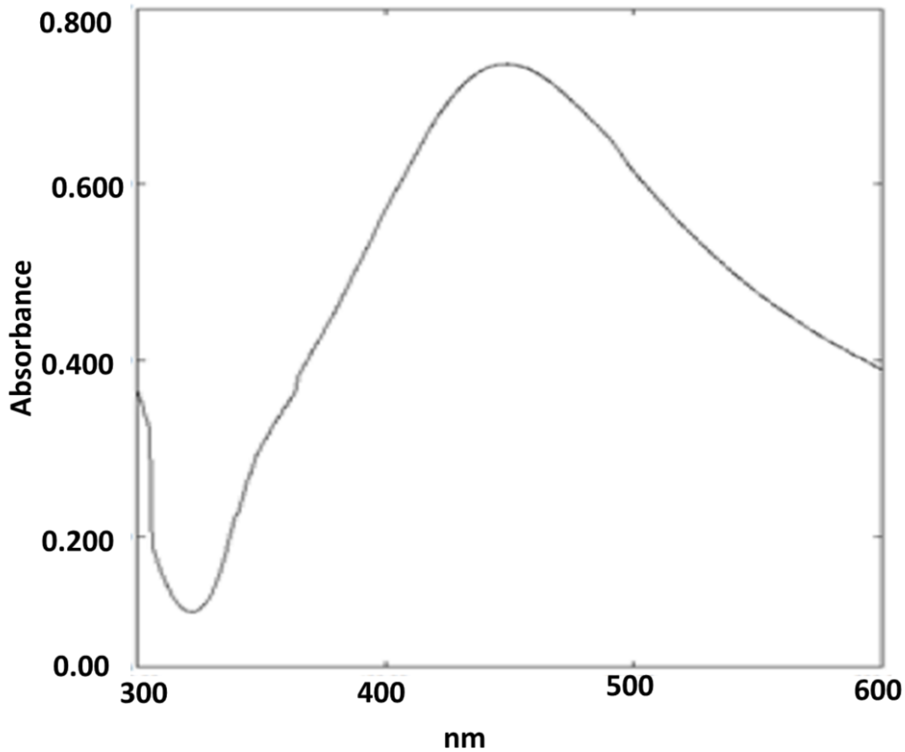

Figure 2. UV- Vis spectrum of silver nanoparticles synthesized from *C. epigaeus* leaf extract.

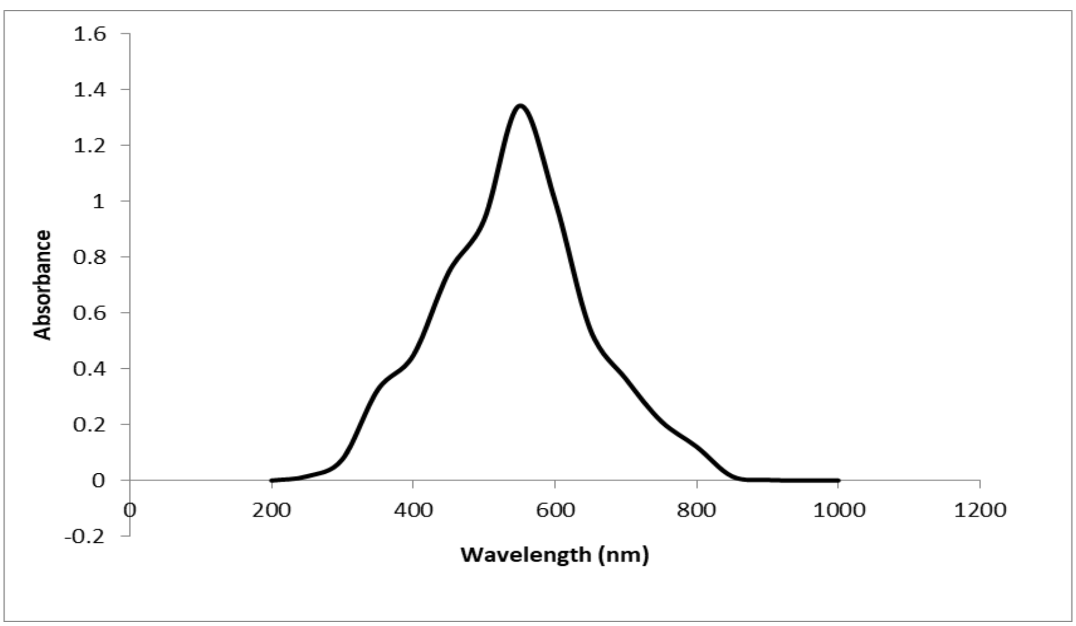

Figure 3. UV- Vis spectrum of silver- zinc oxide doped nanoparticles synthesized from *C. epigaeus* leaf extract.

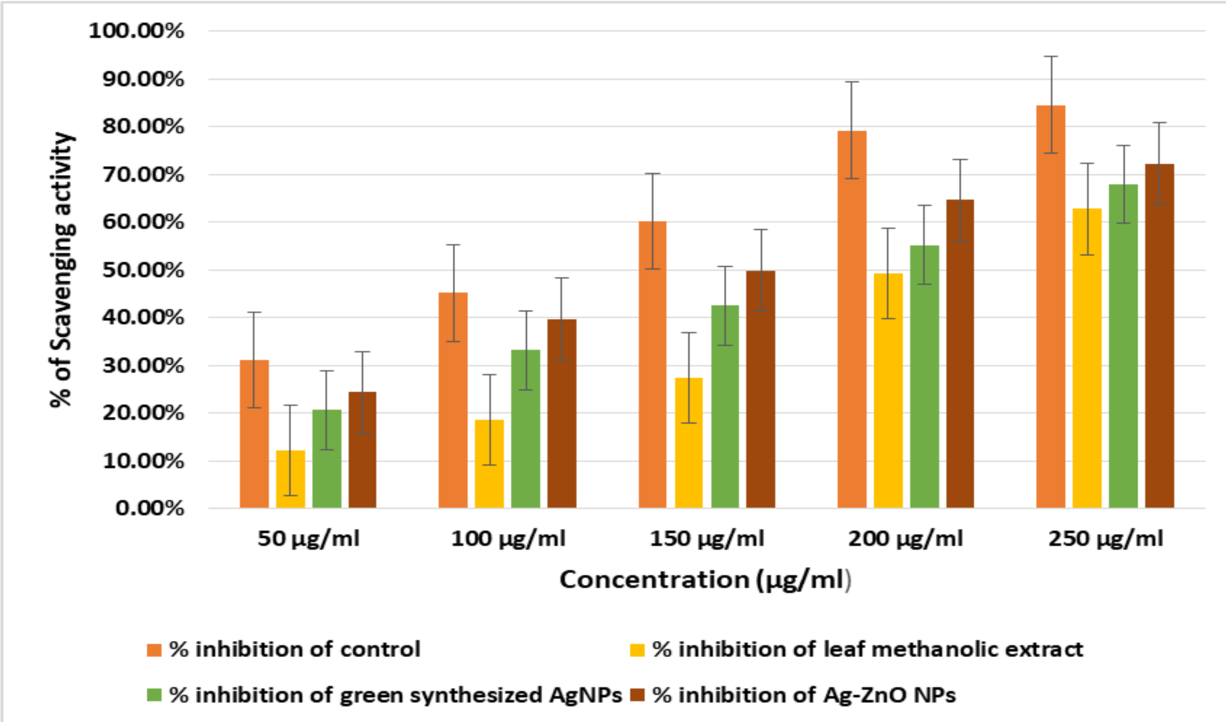

Figure 4. H_2O_2 free radical scavenging assay of the leaf methanolic extract, synthesized AgNPs, and doped Ag-ZnO-NPs of *C. epigaeus*

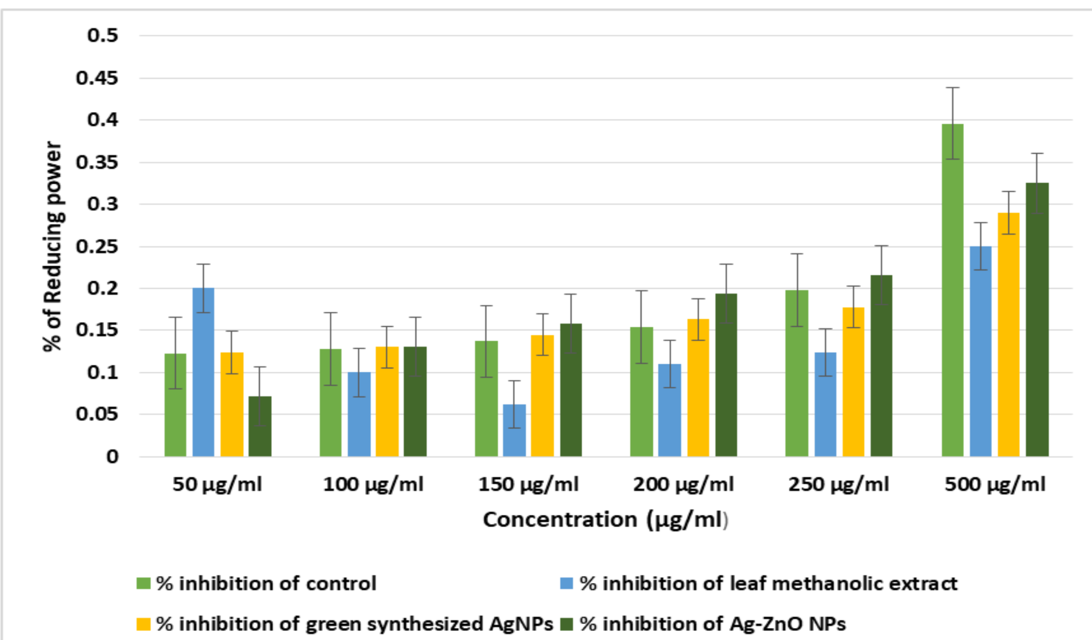

Figure 5. Reducing power assay of the leaf methanolic extract, synthesized AgNPs, and droped Ag-ZnO-NPs of *C. epigaeus*.

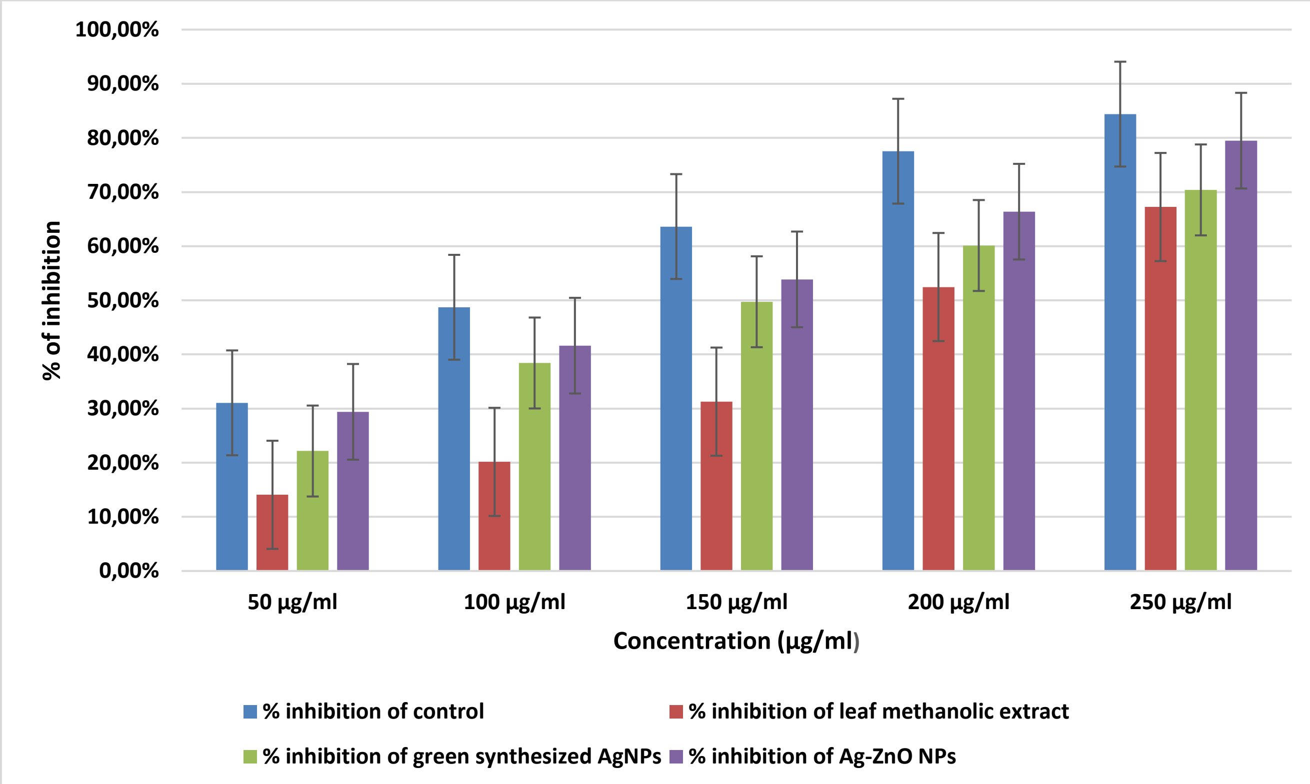

Figure 6. DPPH free radical scavenging assay of the leaf methanolic extract, synthesized AgNPs, and doped Ag-ZnO-NPs of *C. epigaeus*

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