Green synthesis of zinc oxide nanoparticles using Anoectochilus elatus, and their biomedical applications

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**Abstract**

Zinc and its derivatives requirement increased to enhance human immunity against the different pandemics, including covid-19. Green synthesis is an emerging field of research. Zinc oxide (ZnO) nanoparticles have been prepared from Anoectochilus elatus and characterized using absorption, vibrational and electron microscope analysis. They were carried for antibacterial, inflammatory control tendency, and potential antioxidant activities. The brine shrimp lethal assay tested the biologically derived nanomaterial toxicity and the lethal concentration (LC50) is 599.79 mg/ml. The inhibition against the important disease-causing pathogens was measured against four-gram negative, gram-positive bacteria and two fungus pathogens. The nanomaterial exposed inhibition zone for gram-positive bacteria between 17 mm and 25 mm. The inhibition zone against gram-negative bacteria exists between 19 mm and 24 mm. The anti-inflammatory activity was assessed by inhibition of protein denaturation and protease inhibitory activity using nanomaterial. The antioxidant activity was examined using four assays for the therapeutic activities. The average size range of 60–80 nm nanoparticles has prepared and exposed the good biological activity between 50 mg/ml and 100 mg/ml. The comparative results of anti-inflammatory and antioxidant assay results with standards such as Aspirin and vitamin C exposed that two to three times higher concentrations are required for the fifty percent of inhibitions. The prepared low-cost nanoparticle has exhibited excellent biological activity without any side effects and may enhance immunity.

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1. Introduction

Green synthesis has received more attention for various applications because of the low-cost, eco-friendly, safe nanoparticles (NPs) preparation (Govindarajan et al., 2016a,b; Govindarajan and Benelli, 2017; Balalakshmi et al., 2017; Suganya et al., 2017; Balasooriya et al., 2017). Due to the size and shape of the nanomaterials, they have shown various applications in medicinal fields (Narayanan and Sakthivel, 2011). Synthesis of new molecules using the biosynthetic technique is a growing field and the method using bio-extracts has expected more responsiveness than chemical and physical methods for the nanomaterial preparation (Iravani, 2011).
Therefore, there is a growing need for substituent or alternate approaches with various requirements such as eco-safe and cost-effective. Green synthesis is an effective nanoparticle synthesis method, which is inexpensive with risk-free, hypo-allergic within a step synthesis (Rosi and Mirkin, 2005). Nanotechnology is a field of research about less than 100 nm size particles with the distribution, morphology (Senthilkumar et al., 2017). The new method required overcoming the exorbitant, utilizing toxic chemicals imposing environmental and biological risks of the various nanoparticle synthesis methods such as precipitation, electrophoretic, chemical, hydrothermal, sol-gel, and microwave-assisted combustion no chemical techniques (Li et al., 2014).

Based on the reported research of silver NPs by green synthesis and green nanotechnology review, nano-synthesis was carried for the green method’s biological applications (Jagtap and Bapat, 2013; Roy et al., 2013; Nath and Banerjee, 2013). It was reported that several biological organisms such as bacteria, plants and fungus were used for the synthesis of metal oxide NPs by simple methods and had more advantages over conventional physico-chemical methods (Gunalan et al., 2012; Ahmad et al., 2019; Raja et al., 2018). Zinc is an essential trace metal. Recent reports indicate that the NPs, including Zinc oxide nanoparticles (ZnONPs) synthesized by the green technique, was non-toxic, bio-safe, bio-compatible, and had good interaction against various microorganisms compared to chemically derived nanoparticles (Agi et al., 2020; Cheng et al., 2020; Naseer et al., 2020). The ZnONPs by the green technique can be used for drug delivery purposes (Kalpana and Devi Rajeswari, 2018; Selim et al., 2020). The phytoremediation was explained for plants’ green synthesis of NPs (Husen and Siddiqi, 2014; Baharara et al., 2014; Priyanka and Prasanna, 2020). Since ancient times medicinal plants were widely used in the traditional phytotherapy with various herbal formulations despite all the controversy concerning their efficacy and safety, which believed to be an important source of various phytoconstituents such as saponins, alkaloids, terpenes, flavonoids and glycosides due to their potential therapeutic effects (Fugh, 2000; Mathivanan et al., 2010; Govindarajan, 2011; Govindarajan et al., 2013; Govindarajan and Benelli, 2016).

Due to various bioactive metabolites such as flavonoids, alkaloids, saponins, glycosides, terpenoids and steroids present in the plants, which act as significant pharmaceutical potential action as anti-diabetic, anticancer, anti-inflammatory, anti-malarial and anti-dysentery properties and in this present covid-19 pandemic situation, the plants and their extracts act as one of the major sources of drugs in both modern and traditional system of medicine (Yuan et al., 2016; Arumugam et al., 2016). It was reported that the use of non-toxic materials like plant extracts and bacteria for the synthesis of zinc nanoparticles offers various benefits in the field of pharmaceuticals.

Nowadays, metal-based NPs are evolving as new drug carriers that provide a way for site-specific targeting and drug delivery (Ajitadas et al., 2015). Anoectochilus elatus Lindl. (Nagathali) is belongs to the family Orchidaceae. Orchids require nutrients include (Ajithadas et al., 2015).

2. Materials and methods

2.1. Materials

All the chemicals and the microbiological media were obtained from Himedia, India. Ultrapure MilliQ water was used throughout the study. All bacterial and fungus strains were purchased from the American-type culture collection.

2.2. Collection and extraction of A. elatus leaf

A. elatus was collected from the region around Kolli hills, Tamilnadu, India and Botanist authenticated it. Then the air-dried plant leaves ground well. The dried plant powder was extracted in water and concentrated after the charcoal treatment. Fifty grams of whole leaf powder yielded 8.0 g after being dried under a vacuum and stored in a refrigerator.

2.3. Qualitative phytochemical analysis of A. elatus leaf extract

The A. elatus leaf extracts were subjected to qualitative analysis for phenolic groups, glycosides, alkaloids, flavonoids, tannins, terpenoids, saponins steroids by using different solvents,

2.4. Synthesis of Zinc oxide nanoparticles

50 ml of aqueous extract of A. elatus was heated to 60 ºC and then 5 g in 50 ml water solution of zinc nitrate was added slowly to the extract at the same temperature. The reaction mixture was heated until it transformed into a deep yellow suspension. After 4 h, the paste was transferred in a ceramic crucible and the formed ZnONPs of A. elatus (Ae-ZnONPs) was washed by hot water. Then, the sample was heated in an air heated furnace at 100 ºC for 2 h. A light white coloured powder was obtained after heating the powder in a muffle furnace at 400 ºC for 3 h and carried for the characterization (Doan et al., 2020).

2.5. Characterization of Ae-ZnONPs

The adsorption spectrum of Ae-ZnONPs was analyzed in between 200 and 450 nm range in the UV–Visible spectrophotometer. The vibrational behaviour of the nanoparticles was investigated using the Thermo Nicolet FTIR model iS5 Spectrophotometer. The prepared test specimen surface was captured by using Philips XL30 Scanning Electron Microscope.

2.6. Brine shrimp lethal assay

The in vivo brine shrimp lethal assay in seawater was investigated for the prepared Ae-ZnONPs and lethal concentration for fifty percent mortality (LC50) was calculated (Jayaprakash et al., 2016).

2.7. Antimicrobial activity of Ae-ZnONPs

The antimicrobial effects of the Ae-ZnONPs against totally eight bacterial strains and two fungal strains were used throughout the investigation. The bacteria used were Bacillus subtilis, Staphylococcus aureus, E. coli, Pseudomonas aerogenosa, Enterococcus faecalis, Micrococcus luteus, Shigella flexneri, and Serratia marcescens. The fungal strains used were Aspergillus niger and Candida albicans. The bacterial culture inoculation of microorganisms was prepared for the assay as per the reported method (Janaki et al., 2015).

The Ae-ZnONPs (10 mg/ml) solution was prepared in sterile distilled water. Different concentrations, maximum of 100 μl extract, were transferred into wells using sterile Pasteur pipettes. Incuba-
tion completed at 37 °C for 24 h plates outcomes were examined for the effectiveness. Antimicrobial activities were estimated by determining the growth-controlled zone area in diameters. The susceptibility test was carried using standard Ciprofloxacin (100 μg/ml) for the bacterial pathogens and Amphotericin B (100 μg/ml) (Alastruey et al., 2015) for the fungus study as a positive control. The inhibition zone was measured in millimeters and compared.

2.8. Anti-inflammatory activity of Ae-ZnONPs

In vitro anti-inflammatory studies analyzed the suppression of both inflammation and arthritis of Ae-ZnONPs by inhibition of albumin denaturation was performed by the reported methods (Pant et al., 2012) and membrane stabilization test using Alsever mixture (Anosike et al., 2012).

2.9. Antioxidant activity of Ae-ZnONPs

The effect of in vitro antioxidant potential activities of Ae-ZnONPs was studied using free radical scavenging activity such as 1-diphenyl-2-picrylhydrazyl (DPPH) (Chowdhury et al., 2014), reducing potential, 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Kumari et al., 2016), hydrogen peroxide methods (Gülçin et al., 2010). A Nitric oxide (NO) scavenging assay was performed using sodium nitroprusside, which can be determined (Gülçin et al., 2010). A Nitric oxide (NO) scavenging assay was performed using sodium nitroprusside, which can be determined using the Griess-Illosvoy reaction.

3. Results

3.1. Phytochemical studies

The prepared A. elatus leaves extracts using ethanol, benzene, petroleum ether, chloroform, and water were screened for the presence of different phytoconstituents shown in Fig. 1 and Table 1. The result exposed that the polar solvents consist of all phytoconstituents except oil, gums & mucilage, chlorogenic compound and remaining exhibit the minimum chemicals.

3.2. Characterization of Ae-ZnONPs

Optical characterization of the Ae-ZnONPs was carried out using a UV–Visible absorption spectrophotometer in the range of 200–700 nm. The recorded UV spectrum of Ae-ZnONPs has two peaks at 275 nm, 380 nm, respectively (Fig. 2a). Tauc plot values obtained from the UV data also coincide with the reported values for Ae-ZnONPs. 275 nm and 386 nm exposed the bandgap energy of 2.54 eV, 3.12 eV, respectively. Tauc plot was drawn by the online tool (https://www.instanano.com/2020/05/draw-tauc-plot-fro-m-UV-vis-absorbance.html) using UV data and the plotted Tauc plot between Energy and (z(hv))² using UV data in origin which has shown in Fig. 2b. Vibrational spectroscopy has been used to study the ZnO bonding in nanoparticles. FTIR of Ae-ZnONPs is presented in Fig. 2c. From the recorded vibrational spectra, major peaks are observed at 3429 cm⁻¹, 1389 cm⁻¹, 1119 cm⁻¹, 619 cm⁻¹ and 480 cm⁻¹. Strong broadband at 480 cm⁻¹ is denoted for a pure ZnO bond.

The absorption band for O–H stretching vibration of water molecule was detected at 3429 cm⁻¹. The Zn-O stretching for the absorption band is exhibited between 450 cm⁻¹ and 500 cm⁻¹ for ZnO nanoparticles and is almost coincidental with the reported value. The bond nature was confirmed by force constantly. It was calculated by the online tool (https://www.instanano.com/2020/03(force-constant-calculator-from-ftir.html) and the values are presented in Table 2. The scanning electron microscope (SEM) image of synthesized Ae-ZnONPs is displayed in Fig. 2d. The SEM image reveals the powder form of the Ae-ZnONPs and the layers are observed in the morphology. On the surface, macroporous nature was noticed.

3.3. LC₅₀ calculation

The preliminary toxicity limit of the Ae-ZnONPs was studied by BSLA method. For the serially diluted samples, the percentage of mortality was calculated and shown in Table 3. The concentration versus % of mortality graph plotted with regression calculation and lethal concentration for 50% (LC₅₀) was measured. The regression graph is shown in Fig. 3. The Ae-ZnONPs exposed the higher LC₅₀ of 599.79 μg/ml, valuable data for further drug-oriented studies within the limit.

3.4. Antimicrobial activities of Ae-ZnONPs

The antibacterial activity of the Ae-ZnONPs showed a significant reduction in bacterial growth in terms of zone of inhibition. The anti-fungal activity of Ae-ZnONPs against the plant and human disease-causing pathogens such as Candida albicans, Aspergillus niger using serially diluted solutions and compared with usual standard. The inhibition zone observed for both the pathogens is 18 mm, 13 mm, respectively. The results of serially diluted inhibition zones are presented in Table 4. The test plates and comparison chart of the inhibition zones are shown in Fig. 4a and b.
Table 1
Phytochemical constituents of different extracts of *Anoectochilus elatus* leaves.

| S. No. | Test for | Aqueous | Ethanol | Benzene | Petroleum ether | Chloroform |
|-------|----------|---------|---------|---------|----------------|------------|
| 1.    | Alkaloid | ++      | +       | ND      | ND              | ND         |
| 2.    | Flavonoid| ++      | ++      | ++      | +              | ++         |
| 3.    | Triterpenoid| ++   | ++      | +       | ND              | +          |
| 4.    | Phenolic compound| +++ | *     | ++      | *              | +          |
| 5.    | Protein | +       | +       | ND      | ND              | ND         |
| 6.    | Carbohydrates| +++ | +       | ND      | ND              | ND         |
| 7.    | Saponin | +++     | ++      | +       | ND              | ND         |
| 8.    | Steroids| +++     | +       | ND      | +              | ND         |
| 9.    | Glycosides| ++ | +       | ND      | ND              | +          |
| 10.   | Amino acid| +     | +       | ND      | ND              | +          |
| 11.   | Tannin | +++     | ++      | +       | +              | +          |
| 12.   | Oil | ND      | ND      | ND      | ND              | ND         |
| 13.   | Gums & mucilage| ND | ND      | ND      | ND              | ND         |
| 14.   | Chlorogenic compound| ND | ND      | ND      | ND              | ND         |

+++ = high; ++ = moderate; + = low; ND = not detectable.

Table 2
Online calculated Force constant for the IR frequency.

| Frequency | Force Constant in dyne/cm |
|-----------|----------------------------|
|           | Single bond | Double bond |
| 3429      | 8,957,414    | 517,914,829 |
| 1385      | 1461,326.5   | 292,265,2.9  |
| 1119      | 9539,11.0    | 15807,22.1   |
| 863       | 5673,73.8    | 11347,47.6   |
| 619       | 291896.6     | 583793.1     |

Table 3
BSLA result.

| Conc. in µg/ml | Number of Mortality | % of Mortality | LC50 (µg/ml) |
|----------------|---------------------|----------------|--------------|
| 62.5           | 0                   | 0 ± 0.000      | 599.79       |
| 125            | 0                   | 0 ± 0.000      |              |
| 250            | 3                   | 15 ± 0.013     |              |
| 500            | 11                  | 40 ± 0.000     |              |
| 1000           | 16                  | 90 ± 0.942     |              |

Fig. 2. Characterization of Ae-ZnONPs. (a) UV spectra; (b) Tauc plot; (c) FTIR spectra; (d) SEM image.
3.5. In vitro anti-inflammatory effect of Ae-ZnONPs

3.5.1. Inhibition of albumin denaturation

Denaturation of proteins is well-documented due to the cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, the ability of serially diluted Ae-ZnONPs protein denaturation was studied. It was active in impeding the albumin denaturation by induced heat effect. The results for the serially diluted test samples are presented in Table 5.

The Maximum inhibition of 76.02% was observed for Ae-ZnONPs and the standard aspirin drug showed the maximum inhibition of 86% inhibition for 50 µg/ml concentrations. The result obtained from the regression graph (Fig. 5) and the calculated IC50 value of Albumin denaturation is 63 µg/ml.

3.5.2. Membrane stabilization test

Similarly, like albumin denaturation, test samples (10–100 µg/ml) inhibited the heat-induced haemolysis of RBCs to a varying degree, as shown in Table 5. It showed the maximum inhibition of 80.06 ± 0.37% at 100 µg/ml. Using the regression graph equation (Fig. 5), IC50 was observed at 53 µg/ml. Standard aspirin drug showed the maximum inhibition, 89.92% at 50 µg/ml.

3.6. Radical scavenging activity of Ae-ZnONPs

3.6.1. DPPH assay

The DPPH radical scavenging activity was detected and compared with ascorbic acid. The activity of DPPH radical scavenging of Ae-ZnONPs and ascorbic acid was shown in Table 6 and Fig. 6a. The inhibition % for serial concentration like 20, 40, 60, 80 and 100 µg/ml was observed such as 38.11 ± 0.2, 52.39 ± 1.0, 69.03 ± 0.2, 80.15 ± 0.9 and 92.74 ± 0.3. However, for ascorbic acid were found to be 42.27 ± 1.5, 59.08 ± 0.5, 71.56 ± 1.0, 83.40 ± 0.7, and 95.01 ± 0.3 for serial dilution of maximum 50 µg/ml. From the plotted regression graph, IC50 values for DPPH scavenging activity for Ae-ZnONPs and Vit-C were 35 µg/ml, 14 µg/ml, respectively.

3.6.2. Reducing power assay

Potential antioxidant activity can be measured from its reducing tendency of a compound. The Fe3+ to Fe2+ transformation was investigated in the presence of the Ae-ZnONPs to reduce power activity. Similar to the other antioxidant assays, the reducing power of Ae-ZnONPs increased with increasing dosage. Table 7 shows the percentage of inhibition results of the nanoparticles and the standard. A regression graph (Fig. 6b) was plotted and IC50 was calculated for both.

3.6.3. Hydrogen peroxide radical assay

Table 8 and Fig. 6c shows the control percentage of H2O2 with serial concentrations such as 20, 40, 60, 80 and 100 µg/ml were observed in 66.94 ± 1.6, 51.04 ± 0.5, 38.26 ± 0.3, 23.02 ± 0.7, and 17.46 ± 0.2 respectively. Likewise, the inhibition percentage of Vitamin-C for 10, 20, 30, 40 and 50 µg/ml were found to be 24.10 ± 0.3, 40.42 ± 1.2, 52.09 ± 0.7, 64.36 ± 1.5 and 78.62 ± 0.4.

3.6.4. Nitric oxide assay

The percentage of inhibition in the nitric oxide of different concentrations was measured and compared with the same standard Vitamin-C. The outcomes after the colorimetric estimation were tabulated and presented in Table 9. They were using the inhibition percentage regression equation calculated from the graph (Fig. 6d). The IC50 values for nitric oxide scavenging activity for Ae-ZnONPs and Vit-C were 62 µg/ml and 16 µg/ml, respectively. The ratio between the two IC50 values was 3.9 times which showed higher concentration when compared to other ratios.

4. Discussion

The qualitative data of A. elatus reveals the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins and steroids. The spectrum showed a resemblance with the reported UV of ZnO nanoparticles (Talam et al., 2012). The observed narrowing peaks confirmed the reduction of zinc nitrate into ZnONPs. These two peaks exposed the nano nature of ZnO nanoparticles. The observed band gap energy values are almost coincidence with the cubic ZnO and wurtzite ZnONPs was 3.692 keV, 3.691 keV, respectively. In FTIR, the higher values

Table 4
Antimicrobial activity of Ae-ZnONPs.

| S. No. | Microorganisms     | Zone of inhibition (mm) |
|-------|-------------------|-------------------------|
|       |                   | 100 µg/ml | 75 µg/ml | 50 µg/ml | 25 µg/ml | Standard* |
| **Gram-Positive Bacteria** | | | | | | |
| 1.    | Bacillus subtilis  | 25        | 23       | 20       | 15       | 21        |
| 2.    | Staphylococcus aureus | 19       | 17       | 15       | 13       | 24        |
| 3.    | Enterococcus faecalis | 20       | 18       | 16       | 11       | 22        |
| 4.    | Micrococcus luteus | 17        | 16       | 14       | 12       | 25        |
| **Gram-Negative Bacteria** | | | | | | |
| 5.    | E. coli           | 24        | 20       | 17       | 14       | 26        |
| 6.    | Pseudomonas aeruginosa | 19       | 17       | 14       | 13       | 22        |
| 7.    | Serratiamanescens | 21        | 16       | 17       | 13       | 23        |
| 8.    | Shigella flexneri | 22        | 19       | 16       | 12       | 24        |
| **Fungal strains** | | | | | | |
| 9.    | Candida albicans  | 18        | 16       | 14       | 11       | 22        |
| 10.   | Aspergillus niger | 13        | 11       | 10       | 08       | 20        |

* Ciprofloxacin (100 µg/ml) for the bacterial pathogens and Amphotericin B (100 µg/ml) for the fungus study as a positive control.
showed the strong bond nature of ZnONPs. The force of the one atom on the other atom based on the molecular weight was higher in the ZnONPs. The higher elongation property of the nanoparticles may be the reason for the higher penetrating tendency of the nanoparticles into the cell. Due to the higher concentration, the morphology of the $Ae$-ZnONPs is changed to layers. The surface morphology is merely a coincidence with the reported morphology (Bodke et al., 2018). Somewhere, the particles have shown spherical and rod-like structures (Indramahalakshmi, 2017).

The $Ae$-ZnONPs showed dose-dependent activity with an increase in the concentration of nanoparticles. $Ae$-ZnONPs and their ability to bind with the cell membrane generate reactive oxy-
The present study was formulated to understand the phytochemical analysis, biologically synthesis of nanoparticles, phychochemical analysis, characterization, BSLA toxicity, antimicrobial, anti-inflammatory, and potential antioxidant activities of Ae-ZnONPs. The green synthesized Ae-ZnONPs revealed for its characterization studies by UV, FTIR and SEM. The prepared nanoparticle characterization reports are almost similar to the earlier reported results. The nanoparticle behaviour was confirmed through characterization. The synthesized nanoparticles exhibited low toxicity against the brine shrimp. Also, it possesses significant antimicrobial activity and can be alternating existing antimicrobial agents. Due to particle permeability, the particles exposed good antimicrobial inhibition zones against all kinds of selected pathogens and better against the thicker walls containing gram-positive pathogens. The zones are less than 2 to 3 mm compared to standard.
ciprofloxacin and showed 4 mm more inhibition zone against *Bacillus subtilis* when compared with standard. Additionally, the particles possess good anti-inflammatory and antioxidant tendencies of nanoparticle cell interaction behaviour. The inhibition zone concentrations and IC50 are lower than the LC50 except for the reducing power assay IC50. Global hardness is higher for the ZnO molecules and it won’t be an excellent biological inhibitor. However, the prepared *Ae*-ZnONPs showed promising activity in all studies. These results confirmed that the particle size is a tiny and permeable character inside the pathogen’s cell wall.

This work successfully synthesized bioactive nanomaterial from the plant for future drug innovations, and materials that can be a drug were concluded.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Table 7

| Treatment | Concentration (µg/mL) | OD AT 700 nm | IC50 Value (µg/mL) |
|-----------|-----------------------|-------------|--------------------|
| *Ae*-ZnONPs | 100 | 0.9810 | | |
| | 80 | 0.7501 | 56 |
| | 60 | 0.5964 | |
| | 40 | 0.4059 | |
| | 20 | 0.2732 | |
| Ascorbic acid | 50 | 1.3417 | 26 |
| | 40 | 1.0490 | |
| | 30 | 0.9022 | |
| | 20 | 0.7316 | |
| | 10 | 0.5803 | |

#### Table 8

| Treatment | Concentration (µg/mL) | % of inhibition | IC50 Value (µg/mL) |
|-----------|-----------------------|----------------|--------------------|
| *Ae*-ZnONPs | 100 | 66.94 ± 1.6 | 76 |
| | 80 | 51.04 ± 0.5 | |
| | 60 | 38.26 ± 0.2 | |
| | 40 | 23.02 ± 0.7 | |
| | 20 | 17.46 ± 0.2 | |
| Vitamin – C | 50 | 78.62 ± 0.4 | 28 |
| | 40 | 64.36 ± 1.5 | |
| | 30 | 52.09 ± 0.7 | |
| | 20 | 40.42 ± 1.2 | |
| | 10 | 24.10 ± 0.3 | |

#### Table 9

| Treatment | Concentration (µg/mL) | % of inhibition | IC50 Value (µg/mL) |
|-----------|-----------------------|----------------|--------------------|
| *Ae*-ZnONPs | 100 | 79.20 ± 1.0 | 62 |
| | 80 | 62.59 ± 0.8 | |
| | 60 | 49.00 ± 1.5 | |
| | 40 | 30.37 ± 0.6 | |
| | 20 | 19.44 ± 0.1 | |
| Vitamin – C | 50 | 84.73 ± 14 | 16 |
| | 40 | 76.80 ± 1.0 | |
| | 30 | 61.43 ± 0.7 | |
| | 20 | 53.74 ± 1.3 | |
| | 10 | 44.29 ± 0.5 | |

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**Fig. 6.** Radical Scavenging activity of *Ae*-ZnONPs. a) DPPH assay; b) Reducing power assay; c) Hydrogen peroxide assay; d) Nitric Oxide assay.
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Anopheles stephensi

Artemia

artocarpus heterophyllus

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