



## Biofabrication of Zinc Oxide Nanoparticles using the Isolated Flavonoid from *Combretum ovalifolium* and its Anti-oxidative Ability and Catalytic degradation of Methylene blue Dye

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### ABSTRACT

Medicinal plants engrossed great attention for the synthesis of new chemical entities and its biomolecules has fabulous application in many fields such as corrosion, nanotechnology, pharmaceutical companies, and cosmetic industries. The main purpose of the present work is biosynthesis of zinc oxide nanoparticles via simple homogeneous precipitation method using the isolated flavonoid quercetin, which was derived from the medicinal plant *Combretum ovalifolium*. Antioxidant activity and photocatalytic degradation of methylene blue dye were studied on the synthesized ZnONPs. Zinc acetate dihydrate ( $Zn(CH_3COO)_2 \cdot 2H_2O$ ) was utilized as a precursor and quercetin as a reducing agent. Formed Quercetin mediated zinc oxide nanoparticles (Q-ZnONPs) were characterized by UV-Visible, FT-IR, XRD, EDX, and SEM. Fiber shaped Q-ZnONPs were obtained with single crystalline nature, its average diameter was found to be 31.24nm. The results shows that, 168.045 $\mu$ g/ml of Q-ZnONPs scavenged 50% of DPPH on antioxidant activity. The degradation behaviour of the nanomaterial was analysed. The results showed that on the addition of biosynthesized Q-ZnONPs, the degradation of methylene blue increased up to 76.01% at 240 minutes.

**Keywords:** Zinc acetate dihydrate, Quercetin, Biosynthesis of ZnONPs, Characterization, Synthesis Mechanism, Antioxidant activity, Degradation of Methylene blue.

### INTRODUCTION

Quercetin is a flavonoid compound (polyphenol) which can be easily isolated from plant sources. Quercetin being accepted in the phase I clinical trial against the cancer studies, has shown to be effective towards oxidative injury and cytotoxicity. However, due to its poor water solubility, instability and permeability it has limited

applications in pharmaceutical field. Quercetin has limited applications in Alzheimer's diseases owing to its difficulty in crossing BBB adsorption (blood-brain-barrier) and poor oral bioavailability.<sup>1,2</sup> To enhance its solubility and exert its bioactivity, novel preparations (encapsulation) such as liposomes, polymers, micelles and nanoparticles derived quercetin were developed. Since polymer materials (encapsulation) are not stable in long term storage, nanotechnology



methods (complexation with metals) are suggested for preparation of quercetin based drugs especially in the cancer therapy treatments.<sup>3-6</sup>

Nanotechnology is blaze technology. It involves the synthesis and utilization of materials in nanoscale dimension (nanoparticles). It is a bridge between bulk and molecular or atomic structures; with size dependent property being observed. Due to nanomaterials specific optical property, unusual mechanical property, electronic property, high catalytic activity, and low melting point, synthesis of nanomaterials has got the scope in the modern science.<sup>7,8</sup> Different physical and chemical methods exists for the synthesis of a large number of nanomaterials and among all, the most widely used methods are chemical precipitation method, simple solution-based method, sol-gel method, hydrothermal/solvothermal, photochemical and electrochemical reduction.<sup>9</sup> Physical methods possess certain drawbacks such as use of costly equipment, large space for setting up machines, high pressure and temperature. Correspondingly in chemical methods, toxic chemicals are used in the synthesis which exhibits toxic effects on its applications, especially in medicinal fields. In both physical and chemical methods addition of stabilizing agents are needed for the synthesis.<sup>10-12</sup> Alternatively, a green approach is established in a recent decade which has more attention for the synthesis of nanoparticles in an eco-friendly and inexpensive way.

Biosynthesis of nanoparticles is an alternative approach to the synthesis of nanoparticles using plant extracts, fungi, bacteria, yeast, algae, etc.<sup>13,14</sup> Plant extracts mediated nanoparticles approach is most biocompatible, effective, safe and low-cost process than other methods. Furthermore, plant extract also acts as stabilizing and reducing agents in nanomaterials synthesis.<sup>15</sup> Literature states that plant extract are successfully used to synthesize nanoparticles such as copper, silver, gold, cobalt, platinum, palladium, magnetite and zinc.<sup>16</sup> Plant extracts mediated nanomaterials have an effective remedy for cancer, hepatitis, malaria, and other acute diseases, even though, plant extract mediated nanomaterials has excess of additional impurities which are due to numerous phytochemicals present in the plant extracts which can affect the shape, size, and property of the resulted nanomaterials.<sup>17,18</sup>

Zinc oxide nanoparticles have the potential to reduce (or) eliminate the toxic chemicals such as sulfur, arsenic from water as well as it degrades the organic dyes in the polluted wastewater.<sup>19</sup> Although ZnO employed for drug delivery, it still has some limitation in the medicinal field due to its cytotoxicity effect which is not yet to be resolved.<sup>20</sup>

To overcome the above drawback of the existing chemical, physical and green methods, present work focused on the synthesis of zinc oxide nanomaterials using specific bioactive flavonoid which is isolated from medicinal plant. Since bioactive flavonoid is used, it may reduce the toxicity of the resulted zinc oxide nanomaterial. Also it can control the reaction (size & shape) and minimize the impurity of nanomaterials. Using the biosynthesized zinc oxide nanoparticles, antioxidant activity and photocatalytic degradation of methylene blue dye were studied.

## MATERIALS AND METHODS

### Materials

Precursor zinc acetate dihydrate, DPPH, methylene blue, double distilled water and methanol(Merck product)were purchased from Ponmani & Co biochemicals suppliers, Trichy, India. Flavonoid quercetin was previously isolated and characterized from the medicinal plant *Combretum ovalifolium*.<sup>21</sup>

### Biofabrication of quercetin mediated zinc oxide nanoparticles (Q-ZnONPs)

0.01M concentration of quercetin was prepared by dissolving 0.302236 g of quercetin using 100 mL methanol in separate standard flask. Initially, 0.18348 g of zinc acetate dihydrate was taken in a conical flask followed by the addition of 100 mL of double-distilled water and then placed on a magnetic stirrer at 60°C. After the dissolution of zinc acetate, 10 mL of quercetin was added drop wise then allowed to react. After that, every 30 min interval 10 mL of quercetin was added drop wise. After 7 h, the whole solution was taken from the magnetic stirrer and allowed to settle overnight. Formed zinc oxide nanoparticles were collected by centrifugation at 6000rpm and final residue were washed with double distilled water followed by methanol. Collected zinc oxide nano particles were dried in a hot air oven for the complete conversion of Zn(OH)<sub>2</sub>

into quercetin mediated zinc oxide nanoparticles (Q-ZnONPs).<sup>22-24</sup>

### Characterization of Q-ZnONPs

Formed zinc oxide nanoparticles were initially confirmed by UV-Visible spectroscopy and a small portion of ZnONPs were taken for the FT-IR analysis in the range of 500 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> to identify the functional groups involved in its synthesis by comparing it with the spectra of quercetin. ZnONPs were mounted on aluminium stubs using carbon tape further sputtered with gold to study the surface analysis at low and high magnifications with the help of SEM. Obtained ZnONPs were crushed and made it into powder to examine the crystalline phase of the crystals by XRD analysis under Cu-K $\alpha$  with  $\lambda$ -1.54056, Ni filter of Bragg's angles 10 $\circ$ <2 $\theta$ <80 $\circ$  at room temperature. Elemental composition in the ZnONPs was analyzed with the help of EDX.<sup>25-27</sup>

### Antioxidant activity of Q-ZnONPs

0.3 mM concentration of DPPH reagent was prepared by dissolving 11.82 g of DPPH in 100 mL of methanol. Obtained Q-ZnONPs were mixed with 1 mL of methanol. From that, 50 $\mu$ g/mL, 100 $\mu$ g/mL, 150 $\mu$ g/mL and 200 $\mu$ g/mL concentrations were prepared. To the 1 mL of each different concentration 5 mL of DPPH reagent was added separately and kept in a dark room for thirty minutes. Then, the absorbances of the samples were recorded at 517nm and the percentage of radical scavenging activity i.e anti-oxidant activity was calculated by following standard formulae-1. Control reading was recorded by taking 5 mL of DPPH reagent with 1 mL of methanol.<sup>28-30</sup>

$$\text{Percentage of DPPH Scavenged} = \frac{\text{Ab of Control} - \text{Ab of test}}{\text{Ab of Control}} \times 100 \quad (1)$$

**Ab of control** – Control's absorbance,

**Ab of test**- Test solution's absorbance

The IC<sub>50</sub> values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested sample and the ordinate, the average percent of radical scavenging activity.

### Evaluation of the degradation property of biosynthesized Q-ZnONPs on methylene blue dye

The catalytic behaviour of the nanomaterial in the reduction of methylene blue dye was evaluated

after every 30 min interval by taking 3 mL of test sample (methylene blue of 0.1M) treated with 500 $\mu$ g/mL of Q-ZnONP placed on a magnetic stirrer. The reaction progress was noted by recording its absorbance using UV-Visible spectrophotometer. The percentage removal was calculated with the help of the standard calibration curve obtained by measuring the absorbance of untreated methylene blue of varied concentrations(0.1M to 1M) using the formula-2.<sup>31,32</sup>

$$\% \text{ of Methylene Blue Degraded} = \frac{I.C - F.C}{I.C} \times 100 \quad (2)$$

Where,

*I.C*- Initial Concentration of Methylene blue

*F.C*- Final (Sample) Concentration of Methylene blue

## RESULTS AND DISCUSSION

### Characterization of isolated Flavonoid

Flavonoid quercetin was previously isolated from the leaf and stem parts of *Combretum ovalifolium*. Then its structure was characterized with the help of spectroscopic methods.<sup>33</sup>

### Mass spectrum of Quercetin

Mass spectrum of quercetin is shown in Fig. 1 and it clearly explains that the major parent molecular ion (M+1) peak appeared at m/z=302 and it is further fragmented at m/z=285, 257,229, 201, 165, 153, 137 and 30.

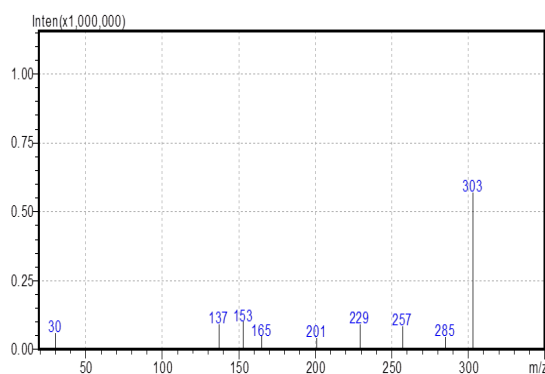


Fig. 1. Mass Spectra of isolated flavonoid quercetin

### NMR spectra of isolated quercetin

<sup>1</sup>H & <sup>13</sup>C-NMR spectra of isolated flavonoid quercetin are shown in Fig. 2 and 3. The following data confirms its structure.

<sup>1</sup>H NMR:  $\delta$  9.335 (OH in C3,s), 12.512 (OH

in C5,s), 6.203-6.198 (1H in C6,dd), 10.810 (OH in C7,s), 6.424-6.419 (1H in C8,d), 7.695-7.690 (1H in C2', dd), 6.909-6.888 (1H in C3',dd), 9.623 (OH in C4',s), 9.393 (OH in C5',s), 7.568-7.542 (1H in C6'.dddd).

$^{13}\text{C}$  NMR:  $\delta$  145.402 (C2), 136.172 (C3), 176.263 (C4), 161.144 (C5), 98.607 (C6), 164.304 (C7), 93.781 (C8), 103.440 (C9), 156.559 (C10), 122.385 (C1'), 120.410 (C2'), 116.034 (C3'), 148.124 (C4'), 147.210 (C5'), 115.476 (C6').

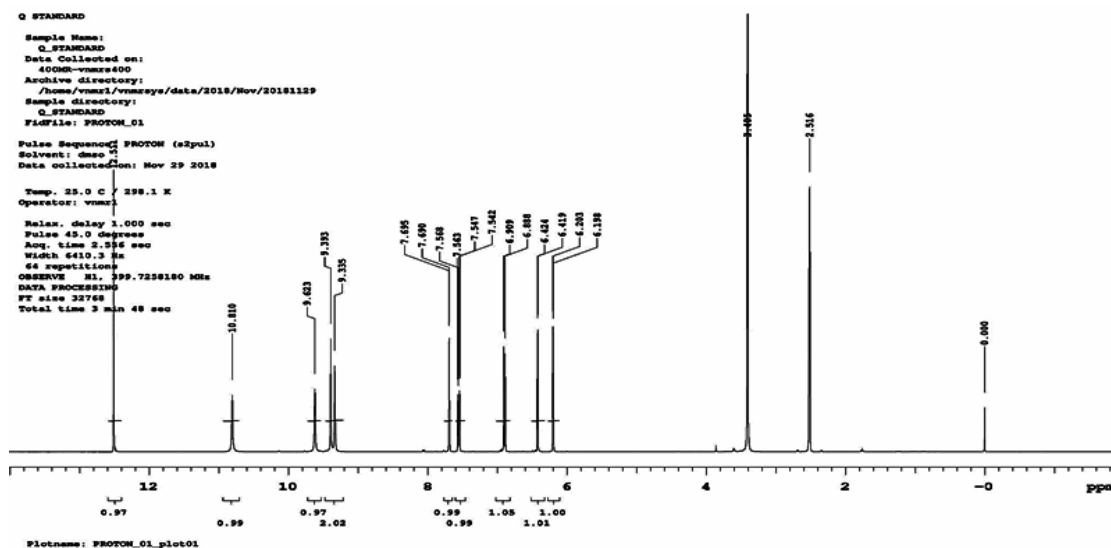


Fig. 2.  $^1\text{H}$ -NMR of spectra of isolated flavonoid quercetin

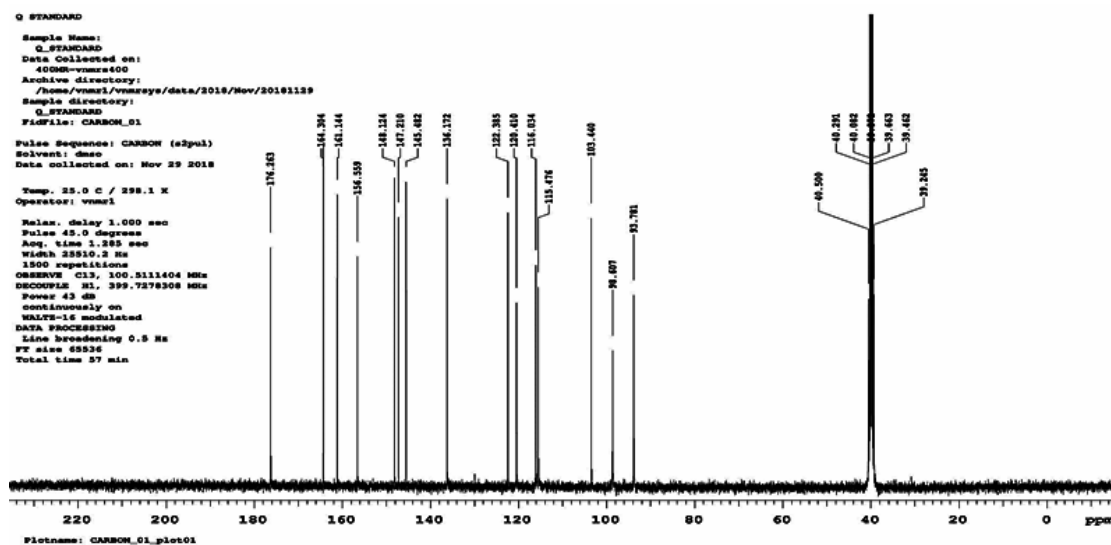


Fig. 3.  $^{13}\text{C}$ -NMR of spectra of isolated flavonoid quercetin

The molecular formula of the isolated compound is  $\text{C}_{15}\text{H}_{10}\text{O}_7$ , accurate molecular weight is found to be 302.236 and name of the isolated flavonoid is flavone-3-rutinoside (quercetin). Numbering and structure of the isolated flavonoid quercetin is shown in the Figure 4.

### Characterization of ZnONPs

Formation of zinc oxide nanoparticles was

preliminarily confirmed by colour changes before and after the addition of reducing agent (quercetin). 34 Initially (in fig-5 before addition), the colour of the isolated quercetin is pale yellow, on adding the zinc acetate solution the colour changes from pale yellow to yellow (stage-1). When time extends the colour of the solution mixer gets darkened and became

colloidal which is shown from stage-2 to stage-4. This colour transformation of quercetin with zinc acetate solution is due to the vibration of surface plasmon resonance of zinc.

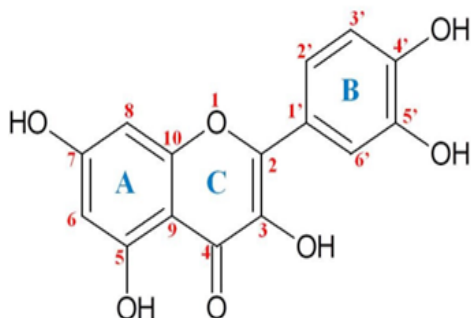


Fig. 4. Numbering and structure of the isolated flavonoid quercetin

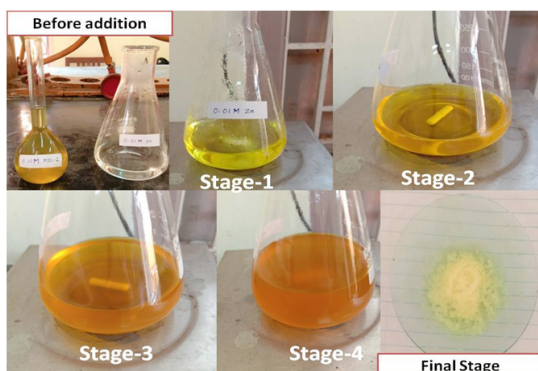


Fig. 5. Various stages of formation of Q-ZnONPs

#### UV-Visible of biosynthesized Q-ZnONPs

Generally, flavonoids show two absorption maxima in UV-Visible spectra, one of which occurs in the range 240-285nm (band-II for ring B cinnamoyl system) and the other in the range 300-400nm (band-I for ring A benzoyl system). Fig. 6 shows the UV-Visible spectra of quercetin before and after synthesis of Q-ZnONPs. Spectral results shows that, before reaction quercetin exhibits  $\lambda_{\max}$  at 298nm, and 354nm which are then shifted to 374nm and 457nm after formation of ZnONPs. It is therefore evidenced that rings A and B of quercetin are involved in the reduction of  $Zn^{2+}$  to  $Zn^0$ .<sup>35,36</sup>

#### FT-IR spectra of biosynthesized Q-ZnONPs

FT-IR spectra of quercetin and quercetin mediated ZnONPs (Q-ZnONPs) is ascertained from the Fig. 7 and the resulted spectral data reveals that quercetin has the functionalities of OH ( $3409\text{ cm}^{-1}$ ), C-H ( $2794\text{--}2712\text{ cm}^{-1}$ ), C=O ( $1806\text{ cm}^{-1}$ ), C=C ( $1611\text{--}1522\text{ cm}^{-1}$ ) and C-H ( $1014\text{ cm}^{-1}$ ) bending vibrations.

In the case of quercetin mediated ZnONPs, OH frequency occurred at  $3427\text{ cm}^{-1}$ , C-H, C=O, C=C, and C-H bending vibrations at  $2987\text{--}2910\text{ cm}^{-1}$ ,  $1600\text{--}1203\text{ cm}^{-1}$ , and  $1064\text{ cm}^{-1}$  correspondingly. Multiple peaks appeared at  $600\text{--}400\text{ cm}^{-1}$  which are responsible for metal-oxygen bonding in quercetin mediated ZnONPs and shift in C=O and suppression of OH frequencies confirm the formation of bonding between zinc and quercetin.<sup>37</sup>

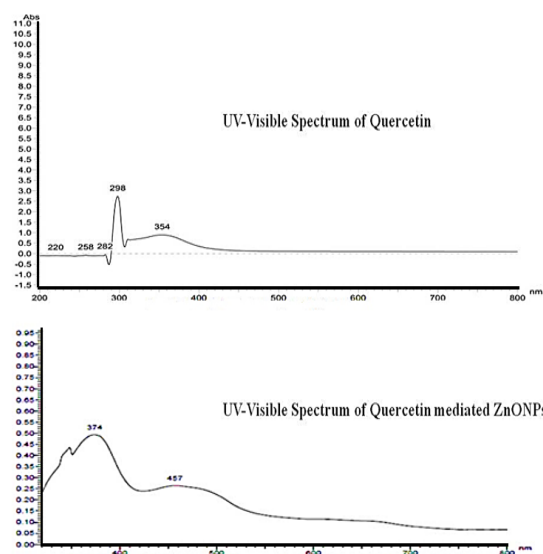


Fig. 6. UV-Visible spectra of quercetin and quercetin mediated zinc oxide nanoparticles Q-ZnONPs

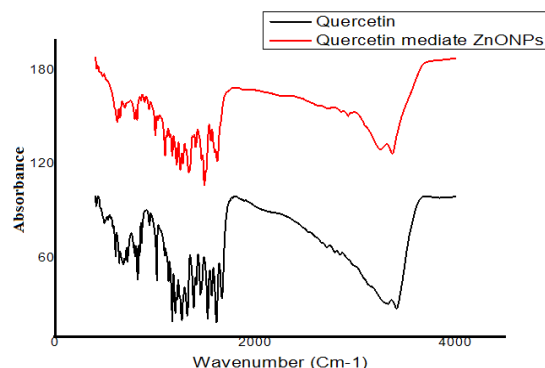


Fig. 7. FT-IR spectra of Quercetin and Quercetin mediated Zinc oxide nanoparticles

#### XRD of Results of Biosynthesized Q-ZnONPs

Figure 8 shows the XRD results of quercetin mediated ZnONPs which gives several Bragg reflections. These are indexed based on the wurtzite structure of zinc oxide. Diffraction peaks of quercetin mediated zinc oxide nanoparticles found at (100), (002) and (101) plane are closer to the reported values of JCPDS standard card no: 36-1451. The

average size of the particles was calculated by Debye-Scherrer's equation; the average size of the quercetin mediated ZnONPs is 31.24nm.<sup>38,39</sup>

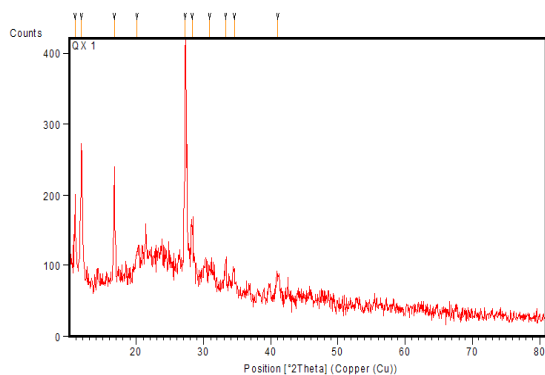


Fig. 8. XRD results of Biosynthesized Q-ZnONPs

#### EDX analysis of Biosynthesized Q-ZnONPs

The EDX of biosynthesized quercetin mediated ZnONPs was done to identify the elemental composition present in it. Fig. 9 reveals zinc and oxygen elements are mostly present in the quercetin mediated zinc oxide nanoparticles along with the presence of little impurities which can be seen.

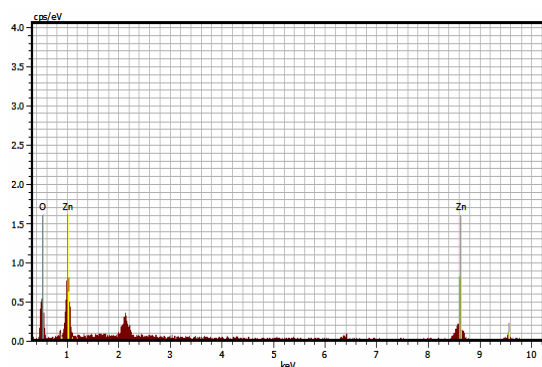


Fig. 9. EDAX of Biosynthesized Q-ZnONPs

#### SEM images of biosynthesized Q-ZnONPs

Morphology of the synthesized quercetin mediated ZnONPs were analyzed with the help of SEM imaging. Quercetin mediated ZnONPs are single crystalline in nature. This is because even, quercetin act as reducing and stabilization agent along with that they act as a capping reagent so that it controls the shape and size of the zinc nanomaterials (uniformly). Quercetin mediated zinc oxide nanomaterials shows zinc nano fiber type morphology which is shown in Figure 10.<sup>40,41</sup>

#### Possible complexation and mechanism of Q-ZnONPs with spectral evidences

Schematic diagram Fig. 11 explains the

common possible intra molecular mechanism of nanoparticles formation. Zinc oxide nuclei initially formed (nucleation formation) subsequently either oriented aggregation or random aggregation developed. After forming aggregation the formed nanoparticles can assemble themselves (oriented aggregation) and forms uniform structure (or) diverse morphology/shapes (random aggregation).

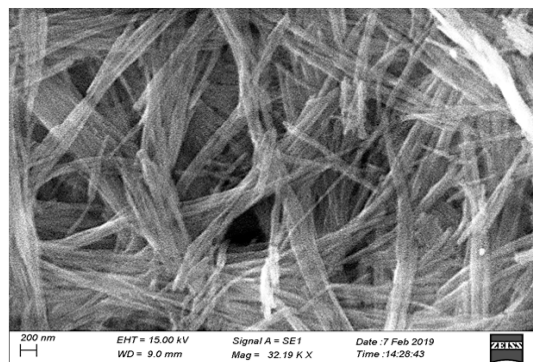


Fig. 10. SEM magnification Image of Biosynthesized Q-ZnONPs

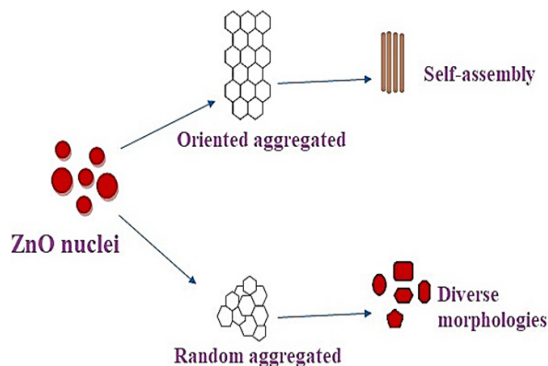


Fig. 11. Schematic diagram represents the common possible intra molecular mechanism of synthesis of nanoparticles

Similarly obtained Q-ZnONPs followed the oriented aggregation mechanism which is given in Fig. 12. In the first step in order to activate the zinc acetate-quercetin reaction mixture was heated at 60°C temperature with constant stirring. Second step zinc element forms complex with quercetin C=O of C-4 carbon and OH of C-5, C-3, C-4' and C-5' (in Fig. 4). From the UV-Visible spectral report ring A and B involves in the bonding which has hydroxyl groups and it can easily form M-O bond with zinc element. In the same way FT-IR spectral results also confirms the functionalities of C=O and -OH are involved in the synthesis which is present in A, B and C rings of quercetin.

Growth aggregation i.e oriented aggregation has occurred in the third step, then Q-ZnONPs assemble itself and form uniform fiber shaped nanomaterials which are formed in the slow process. From the SEM image Fig. 13 the individual fiber in the beam is seen. At fourth step an individual fibers are aggregated and forms the beam like shape which is shown in Fig. 14. Final step (V) is fast step in that all the nanoparticles are accumulated and forms large size particles (in Fig. 15). Accumulated particles can be separated individual fiber by crushing it.

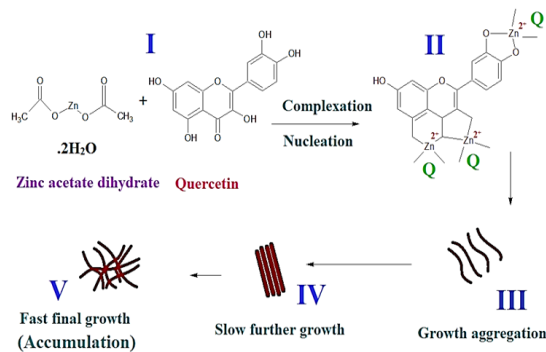


Fig. 12. A Schematic diagram represents probable intra molecular mechanism of Q-ZnONPs

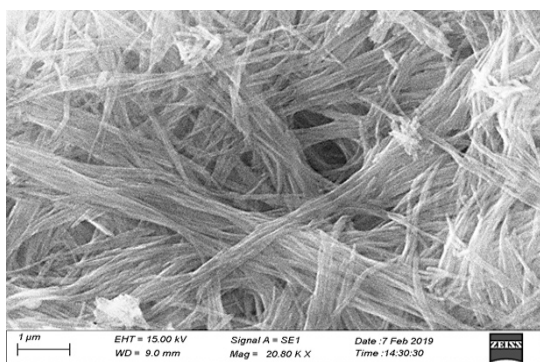


Fig. 13. Step-III image (growth aggregation image) of biosynthesized Q-ZnONPs

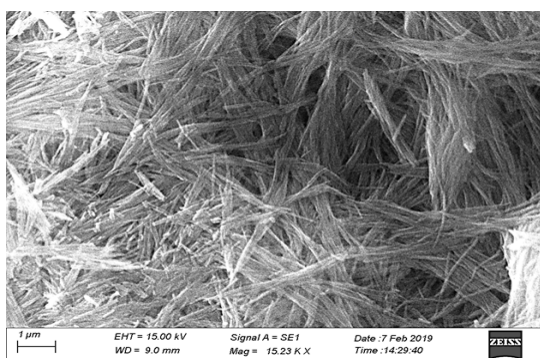


Fig. 14. Step-IV image (slow further growth) of biosynthesized Q-ZnONPs

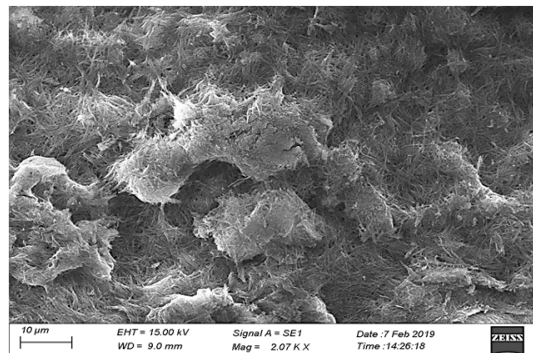


Fig. 15. Step-V image (Fast final growth) of biosynthesized Q-ZnONPs

**Antioxidant activity of Biosynthesized Q-ZnONPs**

Table 1 shows the antioxidant activity of biosynthesized quercetin mediated ZnONPs. Inhibition percentage of DPPH (antioxidant activity) on quercetin mediated ZnONPs increased with increasing concentration of synthesized ZnONPs which is shown in Fig. 16. Moreover, the absorbance values of synthesized ZnONPs get decreased with increasing its concentration. IC<sub>50</sub> value of quercetin mediated ZnONPs is 168.045μg/mL.

Table 1: Antioxidant activity of Biosynthesized Q-ZnONPs

S.No.	Concentration of (Q-ZnONPs) (μg/ml)	Control OD	Sample OD Q-ZnONPs	Inhibition % of Q-ZnONPs
1	50	1.73	1.444	16.53
2	100	1.73	1.193	31.04
3	150	1.73	0.969	43.99
4	200	1.73	0.700	59.55

IC<sub>50</sub> Values      Qu- ZnONPs is = 168.045μg/ml

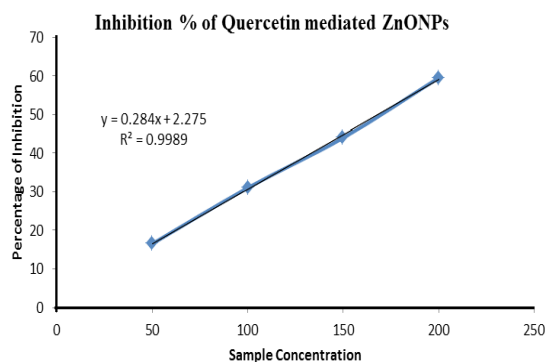


Fig. 16. Anti-oxidant activity of the quercetin mediated ZnONPs

**Photocatalytic degradation of methylene blue dye by using biosynthesized Q-ZnONPs**

Zinc oxide nanomaterials have great

application in the reduction of organic dyes as well as degradation of toxic metals and chemicals. Standard graph of methylene blue is shown in Fig.17. Untreated and Q-ZnONPs treated methylene blue dye UV-Visible spectral results is shown in Fig.18. Spectral results clearly explain that untreated methylene blue shows maximum absorbance, while Q-ZnONPs treating methylene blue exhibits lower absorbances which is shown in Fig. 15. Quercetin mediated zinc oxide nanoparticles (Q-ZnONPs) suppressed or degraded 76.01% of methylene blue at 240 mins (Table 2).

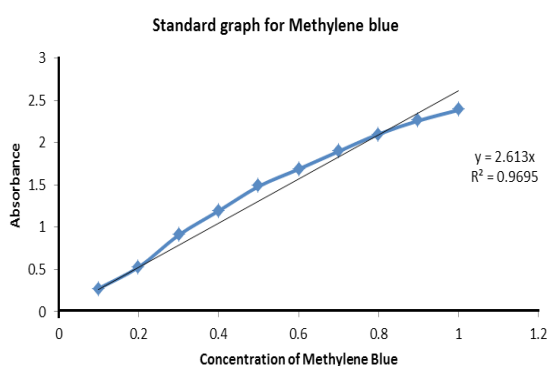


Fig. 17. Standard graph of methylene blue dye

Table 2: Results of percentage of degradation of methylene blue dye by Q-ZnONPs

S.No	Time in Minutes	Percentage of Degradation of Methylene blue by Q-ZnONPs
1	0	0
2	30	11.37
3	60	22.10
4	90	35.53
5	120	47.16
6	150	58.62
7	180	68.13
8	210	70.74
9	240	76.01

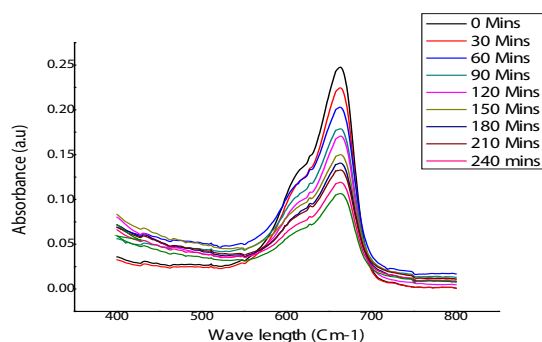


Fig. 18. UV-Spectrum of untreated and Q-ZnONPs treated methylene blue

## CONCLUSION

Above study explore that biomolecule especially flavonoids from plant source can be used in the synthesis of nanomaterials. This bioreductive approach is simple, effective and of ecofriendly nature. Usage of biomolecules in ZnONPs synthesis can be an alternative process for reducing the toxicity of zinc in medicinal applications. Addition of stabilizing and capping reagents might not be needed while using natural flavonoid. Quercetin plays the role of reducing, stabilizing and capping reagents. The results summarized that quercetin mediated ZnONPs were successfully synthesized by simple homogeneous precipitation method and characterized by UV-Visible spectroscopy, FT-IR, XRD, EDX, and SEM analysis. Biosynthesized zinc oxide nanoparticles are single crystalline nature as well as zinc fiber type zinc oxide seeds and its probable mechanism are first time reported in bioreduction method. Because of the free radical scavenging property of Q-ZnONPs, it can be used in free radical diseases like cancer, asthma, diabetes and inflammatory joint diseases. Owing to its catalytic activity of dye degradation, Q-ZnONPs may be applicable in dye industries for wastewater treatment.

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## Conflicts of interest

The authors have declared no conflicts of interest.



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