ANTIOXIDANT ACTIVITY AND PHYSICO-CHEMICAL PROPERTIES OF GREEN SYNTHESIZED ZINC OXIDE NANOPARTICLES USING ERUCA SATIVA LEAF EXTRACT

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ABSTRACT:

The aim of this work is to synthesize zinc oxide (ZnO) nanoparticles from Eurca Sativa water extract which is a medicinal plant cultivated in home gardens in Kurdistan Region-Iraq. The biosynthesis of nanoparticles has been extensively studied due to their numerous applications. Among them, zinc oxide nanoparticles (ZnO NPs) have gained significant attention for wide range of its applications. To investigate the optical, chemical, structural, and morphological properties, different techniques; UV-VIS spectrophotometer, Fourier Transform Infrared Analysis (FT-IR), X-ray diffraction (XRD), Field emission scanning electron microscopy (FESEM), Energy-dispersive X-ray spectroscopy (EDX) were used.

The results revealed that typical ZnO absorption spectra exhibit a well-defined exciton band at 371.6 nm that is near the bulk exciton absorption of ZnO (373 nm) with an energy band gap of 3.029 eV, confirming the production of ZnO nanoparticles. FTIR study demonstrated the existence of bioactive compounds such as flavonoids, polyphenols, tannins, and saponins that can function as reducing and capping agents of ZnO nanoparticles. FESEM picture revealed that ZnO NPs show spherical morphologies with an average diameter of 71.07 nm. The antioxidant activities of biosynthetic ZnO NPs were studied using non-enzymatic methods; 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power assay, and total antioxidant activities. The results showed that the biosynthesized ZnO-NPs nanoparticles had significant antioxidants compared with ascorbic acid as a reference. The obtained results showed that the present method is eco-friendly, less cost-effective, and safe for human health and this method plays a vital role in the industrial and biomedicine fields.

KEYWORDS: ZnO nanoparticles, Erucha Sativa leaves, green synthesis, non-enzymatic antioxidant methods.

1. INTRODUCTION

Anotechnology has a great focus on nano-sized semiconductors in the range of 1–100 nm. It has been an important subject in the fields of basic and applied sciences [1]. These nanoparticles are studied extensively for their specific catalytic, magnetic, electronic, optical and antimicrobial [2, 3], wound healing and anti-inflammatory properties. They have wide range applications due to their size, morphology, excellent chemical stability, and thermal stability [4].

Green nanotechnology involves the biosynthesis of nanomaterials from natural bioactive agents including plant materials, microorganisms, and diverse biowastes like agricultural waste, eggshells, vegetable waste, fruit peels, and others [5]. Moreover, green nanotechnology is considered a key component of clean technologies intended to clean up the environment and turn extra-bioactive compounds into green nanomaterials that are more profitable and ecologically safe. It is a low-cost, biosafe approach for creating nanomaterials for uses in sensors, water desalination, water purification, solar cells, medicine, industrial sectors, and air purification [6, 7].

For the production of NPs, three different methods are available: chemical, physical, and biological [8-10]. The physical methods need very complicated apparatus that can operate under great pressure and heat while chemical techniques have a detrimental influence on using chemical substances as reducing agents, which causes a lot of concerns for both the environment and the workforce [11]. Recent advances in NP synthesis have made the use of biological approaches or “green processes” new, convenient, and affordable [12]. This process, which is thought to be an alternative to traditional physical and chemical procedures, is regarded to be safe and ecologically beneficial for the synthesis of nanomaterials [13].

Researchers now have a novel pathway to use to make metal, metal oxide, and semiconductor NPs in a single pot thanks to this technique. In reality, there are several benefits to adopting green synthesis techniques to create NPs, particularly for biological applications [14].

Plants are the most abundant source of phytochemicals, including flavonoids, polyphenols, glycosides, terpenoids, and proteins, making them one of the most effective green technologies. First, the NPs are reduced, capped, and stabilized by the phytochemicals contained in the plant extractions, which is the main distinction between green synthesis and other methods of creating NPs [15]. Second, plant extracts are a simple way to access these phytochemicals, which are also antioxidants and environmentally safe. Third, some remaining functional groups are attached to the NPs and used to cap the NPs following NP production. As a result, the NPs are more responsive than NPs created using other techniques. The chemical method claims that it is not environmentally friendly. Numerous reducing agents are dangerous, meaning they have negative impacts on human health and the environment and are prohibitively costly [16-20].

Metal oxide NPs have drawn considerable scientific interest due to their unique characteristics and broad application possibilities [21]. Among various nanoparticles, zinc oxide nanoparticles (ZnONPs) are versatile multifunctional inorganic semiconductors that display significant optical, piezoelectric, semiconducting, spintronic, and photonic properties. Wide and straight band gap semiconductors like ZnO NPs (3.37 eV) are essential. High excitonic binding energy (60 meV), which
enables ZnO NPs to perform efficiently in optical systems at or above room temperature, is one of the most remarkable characteristics of this material [22]. A number of approaches are available for the production of ZNPs such as chemical, physical and biological utilize less time for synthesizing large quantities of nanoparticles, they require toxic chemicals as capping agents to maintain stability, thus leading to toxicity in the environment [23]. Due to a wide range of characteristics, zinc oxide is a significant economic and industrial choice. It may be used in a variety of sectors and fields, including the rubber industry, the treatment of metallic surfaces, and the biomedical field [24]. The characteristics of zinc oxide (ZnO) that stand out among its various attributes are its antimicrobial activity, UV absorption capabilities, semi-conductivity, and vulcanization stimulator [25].

Numerous studies and applications of ZnO for photocatalysis[26], as an antibacterial agent [27], in energy cells [28], and in sensors [29] have been documented. The present applications of ZnO NPs in biomedical engineering include tissue engineering, implant coating, bioimaging, wound healing, and the development of anti-cancer medications [30]. Due to their ability to prevent viral entry, reproduction, and organ-wide dissemination, ZnO NPs have direct antiviral efficacy against many viruses. This induces viral death by activating reactive oxygen species, which results in oxidative stress and triggers viral death [31]. As a result, researchers are attempting to create other strategies for enhancing the synthesis of metals and their related oxides using biological materials such as bacteria, fungi, yeast as well as raw materials as plant leaves, fruits and vegetables allow for the large-scale production of ZNPs particles free of impurities [32, 33]. Some plant components, such as roots, leaves, stems, seeds, and fruits, have also been used for zinc oxide nanoparticles synthesis, as plant extracts are rich in phytochemicals, which act as reducing and stabilization agents [34-40]. One of these plants is Eruca Sativa, also known as Rocket plant, belongs to the Brassicaceae family which is regarded as a large family of plants and, an important chemo-preventive plant family [41, 42]. This plant has been grown in Mediterranean area since Roman times and, nowadays is cultivated in different place for its use in salads [43].

Many researchers and groups specifically in our region (Kurdistan-Iraq) synthesized ZnO NPs using plant extracts as green route. Table 1 summarized their work showing ZnO NPs preparation regarding the precursors used, synthesis conditions, properties and application of zinc oxide nanostructure creation.

### Table 1: ZnO NPs synthesis using different plants, precursors,

<table>
<thead>
<tr>
<th>Plant used</th>
<th>Precursor</th>
<th>Synthesis Condition</th>
<th>Properties and Application</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus kotschyanus</td>
<td>(Zn(CH\textsubscript{2}O\textsubscript{2})\textsubscript{2})</td>
<td>Agitated: 60 °C for 30 min. Dip coating process annealing process: 1 h at 450 °C</td>
<td>hexagonal ZnO NPs</td>
<td>[44]</td>
</tr>
<tr>
<td>Euphorbia petiolarata</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}</td>
<td>reflux-condition at 85 °C for 2 hrs. Annealed at 400 °C for 2 hrs.</td>
<td>hexagonal-wurtzite construction with a typical particle size of 55-60 nm</td>
<td>[45]</td>
</tr>
<tr>
<td>Mentha longifolia L.</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}</td>
<td>magnetic stirring at 75 °C for 40 min. Annealing followed out on the Bunsen burner flame at ~ 500 °C for ~ 1 h</td>
<td>spherical shaped nanoparticle with crystalline size of 60–70 nm</td>
<td>[46]</td>
</tr>
<tr>
<td>Punica granatum</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}</td>
<td>stirring at 80 °C for 40 min. Annealing at 500 °C for 1 h</td>
<td>ZnO NPs shows the hexagonal wurtzite arrangement. crystalline dimension of the ZnO NPs is nearly 60 nm. ZnO thin films utilised as a photovoltaic material.</td>
<td>[47]</td>
</tr>
<tr>
<td>Eucalyptus globulus Labill</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}6H\textsubscript{2}O</td>
<td>Stirring at 60 °C for 1 h. annealing at 400 °C for 2 h</td>
<td>ZnO NPs are vastly hexagonal in shape with the average diameter of 35 nm. ZnO NPs possess good stability which is used to absorb dyes, and heavy metal ions from aqueous systems.</td>
<td>[48]</td>
</tr>
<tr>
<td>Thyme</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}6H\textsubscript{2}O</td>
<td>Stirring at RT for 1 h. annealed for 2 h at 150 °C, 250 °C, 350 °C, and 450 °C.</td>
<td>ZnO NPs shows the hexagonal wurtzite arrangement. ZnO NPs have a spherical shape with an average size 39.4–51.86 nm. ZnO NPs synthesized at 450 °C showed a high quality compared to the ZnO NPs synthesized at other calcination temperatures.</td>
<td>[49]</td>
</tr>
<tr>
<td>Pinus Brutia</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}6H\textsubscript{2}O</td>
<td>stirring at 75 °C degrees for 30 minutes. calcination emperatures (200 to 500) °C.</td>
<td>ZnO NPs have hexagonal wurtzite crystal structures. ZnO NPs have particle sizes falling between (10 to 24) nm. The ZnO NPs were calcined at a temperature of 500 °C had superior quality compared to those produced at other calcination temperatures.</td>
<td>[50]</td>
</tr>
<tr>
<td>Pinus Brutia</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}6H\textsubscript{2}O</td>
<td>stirring at 75 °C degrees for 30 minutes. Different pH ranged from 6 - 12</td>
<td>ZnO NPs have hexagonal and wurtzite crystal structure, having particle sizes within the (16.9– 24.15) nm range</td>
<td>[51]</td>
</tr>
<tr>
<td>Allium Calcephalum Wendelbow</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}6H\textsubscript{2}O</td>
<td>stirred at 1 hour at 60 °C. Annealing for 2 hours at 500°C in a furnace</td>
<td>ZnO NPs have hexagonal and wurtzite crystal. A spherical form of ZnO NPs having an (average) size of (21.61-63.12) nm. ZnO NPs synthesized from Zinc Nitrate Hexahydrate showed very high-quality than other zinc salts.</td>
<td>[52]</td>
</tr>
<tr>
<td>Parsley</td>
<td>Zn(NO\textsubscript{3})\textsubscript{3}6H\textsubscript{2}O</td>
<td>stirring at 70°C for 30 min</td>
<td>ZnO NPs had a spherical shape and an anisotropic nature with an average size of 65 nm.</td>
<td>[53]</td>
</tr>
</tbody>
</table>
Eruca Sativa plant is rich with many bioactive compounds which considered as natural antioxidants such as flavonoids, polyphenols, carotenoids and, a good source of vitamin C [54-56]. Therefore, this research work aimed to biosynthesized ZnO-NPs using Eruca Sativa leaves extract. The morphological, structural and, optical properties of biosynthesized ZnO-NPs were investigated. It is also important to evaluate the functional properties of Eruca Sativa leaves in terms of its phytochemical contents to explore the effect of the leaves extract as a capping and reducing agent for ZnO-NPs synthesis. The novelty of this work was the determination of polyphenol contents (total phenol content and total flavonoid content) and to evaluate the antioxidant activities of biosynthesized ZnO NPs using non-enzymatic methods such as DPPH radical scavenging, reducing power assay and total antioxidant methods and compared with ascorbic acid as standard compound.

2. MATERIALS AND METHODS

2.1 Materials Required
Zinc nitrate \((\text{Zn(NO}_3)_2, \text{purity} > 98\%)\) and sodium hydroxide pellets \([\text{NaOH}, \text{purity} \geq 98\%]\) were purchased from Sigma-Aldrich and used as received.

2.2 Preparation of Eruca Sativa Leaf Extract
20 g of Eruca Sativa fresh leaves (Fig. 1a) were purchased from local market in Zakho city, washed several times with tap water and then with distilled water, then cut into small pieces. These leaves were boiled with 200 ml of distilled water at 65°C for 30 minutes [57]. After boiling, colour of the solution changed to light yellow and cooled at room temperature (Fig. 1b). This extract was filtered through Whatman filter paper (No.1) and stored in refrigerator for further studies.

2.3 Phytochemical tests of the plant extract
The preliminary phytochemical qualitative tests were carried out on the aqueous extract of Eruca Sativa leaves to investigate the presence of phenolic compounds, alkaloids, saponins, tannins, flavonoids, proteins, carbohydrates. These phytochemicals were analysed using standard protocols with slight modification [58-62].

2.4 Green Synthesis of ZnO Nanoparticles
For the synthesis of ZnO nanoparticles, 1M of zinc nitrate \([\text{Zn(NO}_3)_2]\) was dissolved in 50 ml of distilled water and kept under stirring for 15 minutes (Fig. 2a). Then 1M of Sodium hydroxide \([\text{NaOH}]\) pellets were dissolved in 20 ml of distilled water and kept under stirring for 15 minutes (Fig. 2b). The two solutions were mixed together (Fig. 2c) and then 25 ml of Eruca Sativa leaves extract was added drop wise to the above mixture and continuously stirred at 65°C for 1 hour (Fig. 2d) until the colloidal solution is obtained. The colour of the resultant solution changes to white colour (Fig. 2e) which confirms the presence of ZnO nanoparticles. The pH of the mixture was set to 12. The precipitate was centrifuged at 10000 rpm and powdered specimen was collected (Fig. 2f). This white-coloured sample was calcinated using a furnace operating at 400 °C for 2 hour and crushed using ceramic mortar and pestle to get fine Zinc Oxide (ZnO) nanoparticles and stored in air-tight bottles for further characterization studies [63].

The interaction of plant extract with metal salts changes the colour of the reacted mixture. This change indicates the starting of reaction with the nucleation process of the ZnO NPs. Therefore, the white colour of the solution indicated the formation of ZnO NPs with specific shape and size as can be seen from (Fig. 2f). Besides, the creation of nanostructure can be characterized by different techniques.

2.5 Characterization of zinc oxide Nanoparticles
For the characterization of zinc oxide nanoparticles, the following techniques were used: UV-Vis, FT-IR, XRD, and SEM.

2.5.1 UV-visible measurements
The use of UV-visible absorption spectroscopy for investigation the optical properties of nanoparticles is important [64]. Therefore, the optical properties of zinc oxide nanoparticles...
were analyzed and scanned using UV-VIS Spectrophotometer (JENWAY 6850 UV/Vis spectrophotometer) in the wavelength 200-1100 nm. One milliliter of the sample was transferred in a quartz cell and analysed at room temperature, using distilled water as a reference solvent.

2.5.2 Fourier Transform Infrared Analysis (FT-IR)

FT-IR spectral analysis was carried out to find the functional groups present in ZnO nanoparticles. Therefore, FTIR spectra were recorded using FTIR Spectrophotometer (IRAffinity-1- SHAMADZU) at the wave number resolution of 1 cm⁻¹ in the range 4000-400 cm⁻¹ on the transmittance mode.

2.5.3 X-ray diffraction analysis (XRD)
The crystal-phase structure and the crystal size of the ZnO NPs were determined using the X-ray diffraction system X-Pert Pro with a scanning range of 2θeta set between 10° and 80° of wavelength λ = 1.5418Å from CuKa operating at 40 kV. 30 mA. The ZnO NP crystal size was calculated using the Scherrer equation [65]:

\[ D = \frac{K\lambda}{β\cos θ} \] …1

Where:
D – the crystal size (λ: 0.15406nm) for CuKa,
λ – the wavelength of the x-ray radiation
K- usually taken as 0.89
β the line width at half- maximum height

2.5.4 Scanning Electron Microscopic Analysis (SEM)
The morphology was investigated by field emission scanning electron microscopy (FE-SEM) (MIRA3 TESCAN). The chemical composition of the prepared nanostructures was measured by energy-dispersive X-ray (EDX).

2.6. Evaluation of antioxidant activity of ZnO nanoparticles

2.6.1. Total phenolic content: Total phenolic content of ZnO nanoparticles had been estimated by Folin-Ciocalteu method with modifications. A standard curve prepared by the same method using serial concentrations of standard tannic acid solution (50-500 mg/ ml) was used for determine the concentration of TPC in ZnO nanoparticles [66].

2.6.2. Total flavonoid content: The aluminum chloride method was used to determine the total flavonoid content (TFC) and with slight modifications. Quercetin was used as a standard in this method and all assessments were made by plotting the calibration curve of quercetin (10- 180 µg/ml) [67].

2.6.3. DPPH free radical-scavenging activity: The 1,1-diphenyl-2-picyrylhydrazyl (DPPH) radical was used to evaluate the water extract of ZnO nanoparticles’s ability to scavenge free radicals using a method described by Sadeq et al. [68] with modification. The percent inhibition of DPPH scavenging of free radicals was determined by using following Eq.1:

% Inhibition of scavenging free radical = [(Ac- As) / Ac] ×100

Where: Ac = absorbance of freshly prepared DPPH and As = absorbance of extracts and standard.

2.6.4. Reducing power activity: The activity of reducing power for water extract of ZnO nanoparticles was measured by a method described by Oyaizu [69].

2.6.5. Total antioxidant capacity, Phosphomolydbate assay: The phosphomolydbenum method was used to determine the antioxidant capacity for water extract of ZnO nanoparticles and ascorbic acid as standard [70].

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis of Eruca Sativa leaf extract

Table 2 displays the findings of a qualitative analysis of the bioactive components (primary and secondary metabolites) of an extract of Eruca sativa leaves. The aqueous leaf extract of Eruca sativa was used for this screening process, and the presence of saponins, tannins, phenolic compounds, and flavonoids was highlighted. This was confirmed by FTIR spectrum, and these compounds may be responsible for the effective capping and chelating agent of nanoparticles, while alkaloids and carbohydrates are not present.

Table 2: Phytochemical analysis of Eruca Sativa

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemicals</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloid tests</td>
<td>Dragendroff’s reagent</td>
<td>Wagner’s reagent</td>
</tr>
<tr>
<td>2.</td>
<td>Saponin test</td>
<td>Aqueous mercury chloride</td>
<td>Foam test</td>
</tr>
<tr>
<td>3.</td>
<td>Tannin test</td>
<td>Lead acetate reagent</td>
<td>Ferric chloride reagent</td>
</tr>
<tr>
<td>4.</td>
<td>Phenolic compound test</td>
<td>Ferric chloride ammonia solution</td>
<td>Millon reagent</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoid test</td>
<td>Alcoholic potassium hydroxide reagent</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Ninhydrin test</td>
<td>α amino acids group test</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Carbohydrate test</td>
<td>Molish reagent</td>
<td>-</td>
</tr>
</tbody>
</table>

Our results are in agreement with results obtained from literature as they confirmed that *Eurica Sativa* leaves are rich of many phytochemicals such as flavonoids, sterols, and terpenoids [71, 72]. The most significant concentration of these phytochemicals are flavonoids which many studies reported that they possess antimicrobial, anti-inflammatory, antioxidant and cytotoxic activities. The majority of all flavonoids are flavanols which are quercetin, kaempferol and isorhamnetin as shown in [Fig. 3] commonly found as O-glycosides [73, 74].

![Fig. 3: Main flavonoids exist in Eruca Sativa.](image)

A possible reaction mechanism for the synthesis of ZnO using *Eurica Sativa* leaves extract is shown in Fig. 4. This reaction occurs between the hydroxyl functional groups the exist in flavanols [Fig. 3]; quercetin, kaempferol and isorhamnetin of the *Eurica Sativa* leaves and the zinc precursor, therefore, these bioactive compounds which present in the leaves extract act as...
ligand agents which consequently form a highly coordinated complex ligands with zinc ions as can monitor this reaction by UV-VIS spectroscopy [47, 75]. These phytochemicals in addition to act as ligand agents, they act as a reducing and stabilizing agent during the process of nucleation, nanoparticles are stabilized and formed with different shapes and dimensions [53, 76]. Then, the complex ligands in which ligation takes place between the functional groups of *Eruca Sativa* and the zinc precursor is decomposed when calcination at 400 °C for 2 hours resulting in the release of ZnO nanoparticles [48].

![Figure 4](image)

**Figure 4:** A possible reaction mechanism for the synthesis of ZnO using *Eruca Sativa* leaves extract.

### 3.2 Fourier Transform Infrared Analysis (FT-IR)

The presence of these bioactive compounds (primary and secondary metabolites) is confirmed by FT-IR technique. FTIR spectral analysis of *Eruca Sativa* leaves extract and ZnO nanoparticles was carried out to find the functional groups present in ZnO nanoparticles (Fig. 5).

In Fig. 5, FTIR spectra of *Eruca Sativa* (black line) was shown and revealed the bands at 3236.65, 2360.87, 2322.29, 1747.5, 1647.20, 1473.31 cm\(^{-1}\) in the region of 4000 cm\(^{-1}\) to 500 cm\(^{-1}\). The band 3236 cm\(^{-1}\) was assigned to the O-H stretching vibration while band at 2360.87 cm\(^{-1}\) is due to C-H stretching vibration. The bands 1747.5 and 1647.20 cm\(^{-1}\) may correspond to C=O anhydride and ester respectively. The stretching vibrational bands observed at 1473.31 cm\(^{-1}\) correspond to N-O functional group.

The FTIR spectral analysis of the biosynthesized ZnO NPs (red line) is shown in Fig. 5. The band 3163.25 cm\(^{-1}\) corresponds to N-H stretching while bands at 2920.22 and 2854.64 cm\(^{-1}\) attributed to C-H stretching mode of these bonds of *Eruca sativa* leaf extract. The peaks 2360.87 and 2322.29 cm\(^{-1}\) indicate the presence of C-H stretching vibration of an aromatic aldehyde. The bands at 1747.5 indicate C=O stretch in polyphenol while band at 1647.20 cm\(^{-1}\) is attributed to the C=C stretch in aromatic ring. The peak at 1546.91 cm\(^{-1}\) corresponds to the C=O of flavonoids while band 1473.61 cm\(^{-1}\) refers to the presence of aromatic ring while the bands 1373.31 and 1311.59 cm\(^{-1}\) refer to the presence of phenols. The bands at 1203.58 and 1153.43 cm\(^{-1}\) indicate the presence of ester. The bands in the region 1080.13 cm\(^{-1}\) indicate the stretch of C-OH whereas the band at 918.11 cm\(^{-1}\) may correspond to O-H bending vibrations of carboxylic acid and C-H bending vibration. The region between 500 – 650 cm\(^{-1}\) correspond to the M-O stretching of ZnO which confirm the formation of ZnO NPs using *Eruca sativa* leaf extract in the presence of these bioactive compounds such as flavonoids and polyphenols as reducing and capping agents.

![Figure 5](image)

**Figure 5:** FTIR spectra obtained for *Eruca Sativa* leaf extract and, biosynthesized ZnO nanoparticles.

### 3.3 XRD Results

X-ray diffraction was used to confirm the crystallinity of ZnO nanoparticles. The XRD patterns of ZnO powder synthesized is shown in Fig. 6.

The XRD peaks were identified as (100), (002), (101), (110), and (103). This shows that the formed nanoparticles are polycrystalline which could be indexed as ZnO hexagonal structure (wurtzite), and show agreement with CPDS data (Card No: 5368) data. The (101) peak with relatively high intensity indicates the preferred orientation of the sample. Besides, peaks belong to Zn (OH): identified as (131), (201), (102), (230), (320), (321), (323) were also appeared and compared CPDS data (Card No:6934). The origin of the impurity could be derived from oxidation processes or from reaction of any preformed zinc hydroxide. The narrow and strong diffraction peaks of ZnO NPs show that the resulting products have a good crystallinity. The crystallite size of the biosynthesized zinc oxide nanoparticles was determined using Eq. (1). It was around 188.89 nm, corresponding to (101) peak. This might be the effect of extract that consequently effect on the parameters and volume of the lattice, leading to increase the grain’s size [77].

![Figure 6](image)
3.4 FESEM Results

The morphology and size of Zinc Oxide (ZnO) nanoparticles examined using Field Effect Scanning Electron Microscopic (FESEM) analysis is shown in Fig. 7a. Fig. 7b shows the particle size distribution histogram of ZnO NPs. It can be seen from figure 7a that the synthesized ZnO NPs exhibit spherical shapes. The average particle size was about 71.07 nm from Fig.7b. The reduction of Zn (II) ions and stabilisation of the resultant nanostructures are the two functions of plant extracts in the synthesis of ZnO NPs [78, 79]. The size, shape, and uniformity of the ZnO NPs that are formed are all influenced by the extract’s concentration [80]. The production of ZnO NPs may be significantly changed by modifying the reducing capabilities of different plant species, as they possess differing quantities of active, reducing molecules. Still, when plant extract concentrations rise, ZnO NPs tend to get smaller [80, 81]. The influence of phytochemical compounds that shape, develop, and stabilise the crystals, along with their slower rate of production, is the cause of the size reduction. This has led scientists to create synthesis techniques that enable more precise control over size and shape in a range of applications [82, 83].

Figure 7: (a)FESEM image of biosynthesized ZnO NPs and (b) particle size distribution histogram of ZnO NPs.

The difference in grain size measured by the XRD and FESEM may be explained by the fact that the XRD measurement is dependent on the size of the defect-free volume, whereas the FESEM measurement visualises the grains directly without accounting for structural defects. This also suggests that the big particles shown in the FESEM are made up of several tiny crystallites, each of which size can be calculated using the Scherrer equation. Moreover, an error rate may arise when estimating grain size from FESEM images due to blurring grain boundaries.

The EDX results of ZnO NPs composition are depicted in Figs. 8a and b.

The EDX analysis of the biosynthesized ZnO nanoparticles using Eruca Sativa leaves extract confirms the presence of ZnO nanostructures. The strong intensity and narrow width of ZnO diffraction peaks indicate that the ZnO were highly crystalline in nature. There are signal for C and K also present in the EDX spectrum. The Zn percentage was 39.5% and 49.33 % for O respectively. The EDAX analysis shows that the optical absorption peaks of ZnO Nanoparticles and these peaks which are due to the surface plasmon resonance effect of zinc oxide Nanoparticles. The origin of these elements lies in the phytocomponents which are existed along with ZnO Nanoparticles [84].

Figure 8: EDX analysis of biosynthesized ZnO nanoparticles, a: EDX spectrum and, b: The composition percentage of Zn and O.

3.5 UV-visible Spectroscopic Results

UV-visible absorption spectroscopy is a powerful widely used technique to characterize the optical properties of nano-sized particles. The absorption spectra of ZnO NPs prepared at temperature of 65°C is shown in Fig.9.

Figure 9: UV-visible spectrum of biosynthesized ZnO nanoparticles at temperature 65°C.

The characteristic absorption spectrum of ZnO shows a well-defined exciton band at 371.6 (Fig. 9) nm which is close to the bulk exciton absorption of ZnO (373 nm). It is known that the ZnO nanoparticles have free electrons due to which Surface Plasmon Resonance (SPR) absorption band. This broad absorption peak indicates the reduction of Zn$^{2+}$ ions in the reaction medium which authenticates the formation of ZnO nanoparticles [85, 86]. Due to the presence of a broad peak in the UV-vis spectra, the grown ZnO NPs showed excellent optical properties [87].

From the UV-visible graph (Fig.9), the energy band gap is calculated using Tauc’s equation as shown below [88, 89]:

\[
(ah\nu)^{1/2} = B(\nu - \nu_0) \quad \text{......... (2)}
\]

Where $a$ is the absorption coefficient that is given by $(\sigma = 2.303 \log (T/d))$, $A$ is the absorption, $h\nu$ is the incident photon energy, $E_g$ is the bandgap energy, $T$ is the transmittance, and $d$ is the film thickness [89, 90].
The calculated band gap was found to be 3.029 eV as shown in Fig.10. Due to the presence of a broad peak in the UV-vis spectra, the grown ZnO NPs showed excellent optical properties.

Figure 10: Band gap (Eg) estimation of the prepared ZnO nanoparticles from Tauc's relation [88].

Numerous biological substances found in plants, such as plant metabolites, aldehydes, alkaloids, amino acids, aromatic amines, flavonoids, phenolic compounds, ketones, polysaccharides, proteins, saponins, steroids, sugars, tannins, and terpenoids, have been linked to the creation of metallic NPs. These substances function as stabilising or reducing agents. The metal salt, temperature, pH, reaction time, quantity of plant extracts, and kinds of biological molecules present may all be changed to provide the ideal circumstances for synthesising NPs with the required size and form [91].

Optimising the reaction parameters is essential for the synthesis of metallic nanoparticles (NPs) from plant extracts. The amounts and ratios of plant extracts to metal salts, as well as pH, reaction time, and temperature, must all be closely monitored and controlled. It is possible to adjust the size and form of the NPs by changing the concentration of plant extract. It has been demonstrated that longer response times result in more NP generation. On the other hand, it has been discovered that increased temperatures reduce NP production and average size. Studies show that pH affects how well metal ions attach to the biomolecules in the extracts. It is possible to create NPs with tetrahedral, hexagonal, spherical, rod-shaped, and irregular forms at various pH values. In general, smaller NPs are produced at higher pH levels [92].

The size, shape, and rate of formation of metallic nanoparticles (NPs) are significantly influenced by the concentration of plant extract employed in their synthesis. Larger amounts of secondary metabolites were produced when the concentration of leaf extract was increased, which led to the production of more stable and smaller NPs. Additionally, it had an impact on the NPs' absorption peak, with an increase in concentration resulting in a sharper peak [93].

Additionally, the main factor influencing the synthesis of nanoparticles change in size, shape, and degree of synthesis is temperature. It is possible to adjust the dimensions of the synthesis of nanoparticles in relation to temperature, as well as the different forms (triangle, octahedral platelets, spherical, and rod). The nucleation centres are forming more strongly as the temperature rises due to the reaction response rate [94].

Phytochemical-containing plant extracts are usually heated for a period of time below 60°C. Extended exposure to elevated temperatures may result in the breakdown of phyto-constituents found in biomass extract. Maintaining the stability of plant metabolites requires operating at room temperature. The generation of NP rises with temperature because it promotes the creation of nucleation centres [93, 95, 96]. The morphology of NPs is greatly influenced by the solvent selection. The absorbance peak increased as the amount of the watery fraction increased [93]. Depending on the organism utilised to synthesise the NPs, different biomolecules are involved in the green production of NPs. According to most research, the average size of NPs decreases as the concentration of precursor increases. More nuclei develop when the precursor quantity is large, and the capping agents stabilise them rapidly. But when the precursor concentration was too high, the number of phytochemicals was no longer enough to stabilise a large number of nuclei [97].

3.6. Antioxidant activities of ZnO nanoparticles

The total phenolic content and total flavonoid content of ZnO nanoparticles synthesised using Eura Sativa leaf extract are shown in Table 3. The total phenolic content of ZnO nanoparticles was determined from the standard curve (y = 0.0006X - 0.0113 with the value of R2 = 0.9854). Total phenolic content equals to 168.3867± 0.1772 which is calculated as milligram of tannic acid equivalents (mg TAE/g) of dry weight of extract. On the other hand, the total flavonoid content of ZnO nanoparticles synthesised using Eura Sativa leaf extract is determined from the standard curve (y = 0.0019X - 0.0027 and R2 = 0.9927) using quercetin as a standard. Total flavonoid content is expressed as milligram of quercetin equivalents (mg QE/g) of dry weight of extract which equals to 15.75 ± 0.0735. From our results, we confirmed that the presence of polyphenols compounds (phenols and flavonoids) is considered as reducing and stabilizing agents in the synthesizing of metal oxide nanoparticles.

Table 3. Total Phenolic and total flavonoid contents of ZnO nanoparticles

<table>
<thead>
<tr>
<th>Extraction type</th>
<th>Total phenolic content (mg TAE/gm)</th>
<th>Total flavonoid content (mg QE/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (ZnO)</td>
<td>168.3867± 0.1772</td>
<td>15.75± 0.0735</td>
</tr>
</tbody>
</table>

The scavenging activity of ZnO nanoparticles water extract conducted a dose dependent increase in DPPH radical scavenging in the concentration range of 25 to 100 μg/ml as well as the scavenging activity of standard ascorbic acid and both are statistically significant p<0.0002 as presented in Table 4. At 25 μg/ml and 50 μg/ml of the ZnO nanoparticles water extract had a stronger DPPH scavenging activity than standard of ascorbic acid.

Table 4: DPPH scavenging activity of ZnO nanoparticles water extract and standard ascorbic acid.

<table>
<thead>
<tr>
<th>Conc</th>
<th>DPPH scavenging activity (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>Ascobic acid (standard)</td>
<td>p &lt; 0.0002</td>
</tr>
<tr>
<td>25</td>
<td>50.95 ± 0.01</td>
<td>40.74 ± 0.01</td>
</tr>
<tr>
<td>50</td>
<td>57.46 ± 0.52</td>
<td>46.43 ± 0.02</td>
</tr>
<tr>
<td>75</td>
<td>62.51 ± 0.11</td>
<td>85.56 ± 0.11</td>
</tr>
<tr>
<td>100</td>
<td>63.82 ± 0.02</td>
<td>97.06 ± 0.02</td>
</tr>
<tr>
<td>IC50</td>
<td>12.77</td>
<td>41.53</td>
</tr>
</tbody>
</table>

Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly p < 0.05
Additionally, the IC50 values of ZnO nanoparticles water extract and standard ascorbic acid are 12.77 and 41.53 respectively. This analysis provided scientific evidence for the high antioxidant activity of this plant. Moreover, in the synthesis of nanoparticles, the plant extract acted as reducing agents. The reducing power of ZnO nanoparticles water extract increased with the increasing concentration of nanoparticles water extract which is considered to be statically significant p < 0.05 as shown in Table 5. The ZnO nanoparticles water extract slightly lower reducing power than ascorbic acid at all concentrations ranged between 25 to 100 μg/ml.

Table 5: Reducing power assay of ZnO nanoparticles water extract and standard ascorbic acid

<table>
<thead>
<tr>
<th>Conc</th>
<th>Reducing power assay (O.D 700 m)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water extract</td>
<td>Ascorbic acid (standard)</td>
</tr>
<tr>
<td>25</td>
<td>1.5153 ± 0.0012</td>
<td>2.32 ± 0.006</td>
</tr>
<tr>
<td>50</td>
<td>1.5207 ± 0.001</td>
<td>2.33 ± 0.003</td>
</tr>
<tr>
<td>75</td>
<td>1.5233 ± 0.002</td>
<td>2.35 ± 0.007</td>
</tr>
<tr>
<td>100</td>
<td>1.5480 ± 0.004</td>
<td>2.37 ± 0.018</td>
</tr>
</tbody>
</table>

Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly p < 0.05.

Total antioxidant capacity of zinc oxide nanoparticles synthesized by of Eruca sativa leaf water extract is showed in Table 6. Antioxidant capacity of zinc oxide nanoparticles and standard ascorbic acid were increased with increasing concentration of zinc oxide nanoparticles and ascorbic acid respectively. Moreover, it is shown that the antioxidant activity of ascorbic acid is higher than the ZnO nanoparticles for all concentration ranged between 25 to 100 μg/ml that is statically significant p < 0.05.

Table 6: Total antioxidant capacity of ZnO nanoparticles water extract and standard ascorbic acid

<table>
<thead>
<tr>
<th>Conc</th>
<th>Total antioxidant capacity (O.D 695 nm)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water extract</td>
<td>Ascorbic acid (standard)</td>
</tr>
<tr>
<td>25</td>
<td>0.0353 ± 0.0003</td>
<td>0.26 ± 0.0002</td>
</tr>
<tr>
<td>50</td>
<td>0.0627 ± 0.0001</td>
<td>0.34 ± 0.0000</td>
</tr>
<tr>
<td>75</td>
<td>0.0753 ± 0.0003</td>
<td>0.48 ± 0.0001</td>
</tr>
<tr>
<td>100</td>
<td>0.0923 ± 0.0003</td>
<td>0.54 ± 0.0002</td>
</tr>
</tbody>
</table>

Note: Results are expressed Mean ± S. Error, n=3. Numbers in same column followed by a similar letter do not differ significantly p < 0.05.

CONCLUSIONS

In this present study, eco-friendly, easy synthesis, low-cost, non-hazardous, organically effective and innovative approach of the biosynthesized ZnO nanoparticles using Eruca Sativa leaf extract have been reported. Firstly, screening of bioactive compounds that exist in Eruca Sativa plat is performed using qualitative methods and confirmed by FT-IR technique as these phytochemicals present in the leaf extract acts as a biological stabilizing and reducing agent for the synthesis of metal oxide nanoparticles. The presence of ZnO nanoparticles was confirmed using UV-visible, FTIR, XRD and FESEM techniques. The absorption band observed at 371.6 nm with an energy band gap of 3.029 eV is confirmed by UV-visible spectroscopic studies. FTIR result confirms the presence of functional groups in ZnO nanoparticles. XRD shows the good crystalline quality of the ZnO product with very well-defined peaks along (002), (100), (101), which are the highest peaks intensities and indexed as a hexagonal structure (wurtzite), therefore to obtain ZnO NPs with high crystallinity, a plenty of hydroxyl groups are required to reduce Zn ions. Different shapes are observed from FESEM: a mixture of rod like and spherical shapes are obtained. Additionally, a formation of ZnO NPs almost equals 71.07 nm in size were conducted in this study.

The novelty of this work was the determination of both total phenol content using Folin-Ciocalteu method with tannic acid as standard reference and total flavonoid content using aluminum chloride method with quercetin as standard for biosynthesized ZnO NPs. These results approved the role of polyphenols compounds as reducing and stabilizing agents in the synthesizing of metal oxide nanoparticles. Furthermore, evaluation the antioxidant activities of biosynthesized ZnO NPs using three non-enzymatic methods: DPPH radical scavenging, reducing power assay and total antioxidant methods and compared with ascorbic acid as standard compound. Our results regarding antioxidant activities of ZnO NPs showed a dose dependent concentration, i.e., increased with increasing concentration of ZnO NPs and ascorbic acid respectively.

Overall, these findings will lead to a better understanding of the plant’s suitability and importance as a plant with a broad range of well-established bioactive metabolites for nanoparticles synthesis.

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