

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

Lina Y. Mohammed

Department of Biomedical Science, College of Medicine, University of Zakho, Kurdistan Region, IRAQ

Received: 13 Jul., 2023 / Accepted: 19 Nov., 2023 / Published: 24 Dec., 2023.

<https://doi.org/10.25271/sjuoz.2023.11.4.1173>**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, Eurca Sativa leaves, green synthesis, non-enzymatic antioxidant methods.

**1. INTRODUCTION**

Nanotechnology 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 (ZNPs) 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

\* Corresponding author

This is an open access under a CC BY-NC-SA 4.0 license (<https://creativecommons.org/licenses/by-nc-sa/4.0/>)

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,

Plant used	Precursor	Synthesis Condition	Properties and Application	Ref
<i>Thymus kotschyanus</i>	Zn (CH <sub>3</sub> CO <sub>2</sub> ) <sub>2</sub>	Agitated: 60 °C for 30 min. Dip coating process annealing process: 1 h at 450 °C	hexagonal ZnO NPs	[44]
<i>Euphorbia petiolata</i>	Zn (NO <sub>3</sub> ) <sub>2</sub>	reflux-condition at 85 °C for 2 hrs. Annealed at 400 °C for 2 hrs.	hexagonal-wurtzite construction with a typical particle size of 55-60 nm	[45]
<i>Mentha longifolia</i> L.	Zn (NO <sub>3</sub> ) <sub>2</sub>	magnetic stirring at 75 °C for 40 min. Annealing followed out on the Bunsen burner flame at ~ 500 °C for ~ 1 h	spherical shaped nanoparticle with crystalline size of 60–70 nm. ZnO NPs showed a scolicidal activity.	[46]
<i>Punica granatum</i>	Zn (NO <sub>3</sub> ) <sub>2</sub>	stirring at 80°C for 40 min. Annealing at 500°C for 1 h,	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.	[47]
<i>Eucalyptus globulus</i> Labill	Zn (NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	Stirring at 60 °C for 1 h. annealing at 400 °C for 2 h	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.	[48]
Thyme	Zn (NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	Stirring at RT for 1 h. annealed for 2 h at 150 °C, 250 °C, 350 °C, and 450 °C.	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.	[49]
<i>Pinus Brutia</i>	Zn (NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	stirring at 75 °C degrees for 30 minutes. calcination emperatures (200 to 500) °C.	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.	[50]
<i>Pinus Brutia</i>	Zn (NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	stirring at 75 °C degrees for 30 minutes. Different pH ranged from 6 - 12	ZnO NPs have hexagonal and wurtzite crystal structure, having particle sizes within the (16.9–24.15) nm range	[51]
<i>Allium Calocephalum</i> Wendelbow	Zn (NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O Zn (CH <sub>3</sub> CO <sub>2</sub> ) <sub>2</sub> ZnCl <sub>2</sub>	stirred at 1 hour at 60 °C. Annealing for 2 hours at 500°C in a furnace	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.	[52]
<i>Parsley</i>	Zn (NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	stirring at 70 °C for 30 min	ZnO NPs had a spherical shape and an anisotropic nature with an average size of 65 nm.	[53]

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 [ $Zn(NO_3)_2$ , purity > 98%] and sodium hydroxide pellets [NaOH, 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.

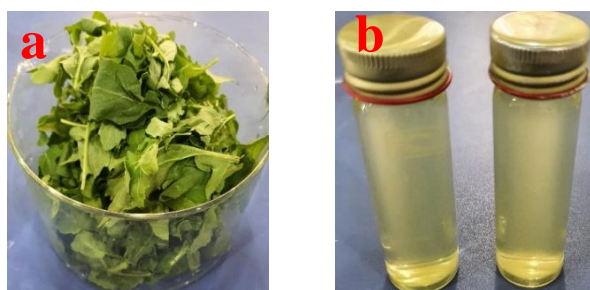


Figure 1:(a) Eruca Sativa Fresh leaves and (b) Eruca Sativa Leaf Extract

### 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 [ $Zn(NO_3)_2$ ] was dissolved in 50 ml of distilled water and kept under stirring for 15minutes (Fig. 2a). Then 1M of Sodium hydroxide [NaOH] pellets were dissolved in 20 ml of distilled water and kept under stirring for 15minutes (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.

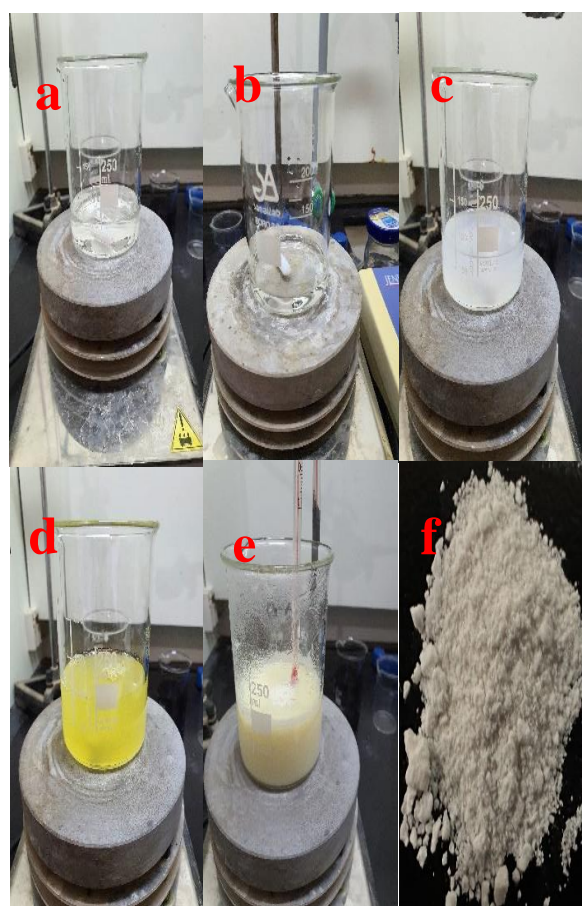


Figure 2: (a)  $Zn(NO_3)_2$  solution, (b) NaOH solution, (c)  $Zn(NO_3)_2$  and NaOH mixture, (d)  $Zn(NO_3)_2$ , NaOH mixture and Eruca Sativa leaves extract, (e) ZnO colloidal solution, and (f) ZnO nanoparticles powders

### 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-SHAMADZO) at the wave number resolution of 1 cm<sup>-1</sup> in the range 4000-400 cm<sup>-1</sup> on the transmittance mode.

**2.5.3 X-ray diffraction analysis (XRD)**

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

$$D = \frac{K\lambda}{(\beta \cos\theta)} \dots 1$$

Where:

D – the crystal size (λ= 0.15406nm) for CuKα,

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-picrylhydrazyl (DPPH) radical was used to evaluate the water extract of ZnO nanoparticle’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:

$$\% \text{ Inhibition of scavenging free radical} = [(A_c - A_s) / A_c] \times 100$$

Where: A<sub>c</sub> = absorbance of freshly prepared DPPH and A<sub>s</sub> = 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, Phosphomolybdate assay:** The phosphomolybdenum 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

S. No.	Phytochemicals	Test	Result
1.	Alkaloid tests	Dragendroff's reagent Wagner's reagent Hager's reagent	-
2.	Saponin test	Aqueous mercury chloride Foam test	+
3.	Tannin test	Lead acetate reagent Ferric chloride reagent	+
4.	Phenolic compound test	Ferric chloride ammonia solution Millon reagent	+
5.	Flavonoid test	Alcoholic potassium hydroxide reagent	+
6.	Ninhydrin test	α amino acids group test	+
7.	Carbohydrate test	Molish reagent	-

Our results are in agreement with results obtained from literature as they confirmed that *Eruca 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].

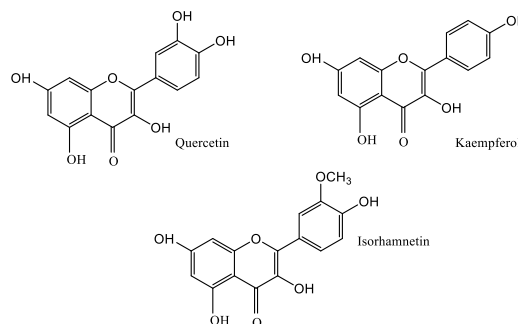


Fig. 3: Main flavonoids exist in Eruca Sativa.

A possible reaction mechanism for the synthesis of ZnO using *Eruca 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 *Eruca 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].

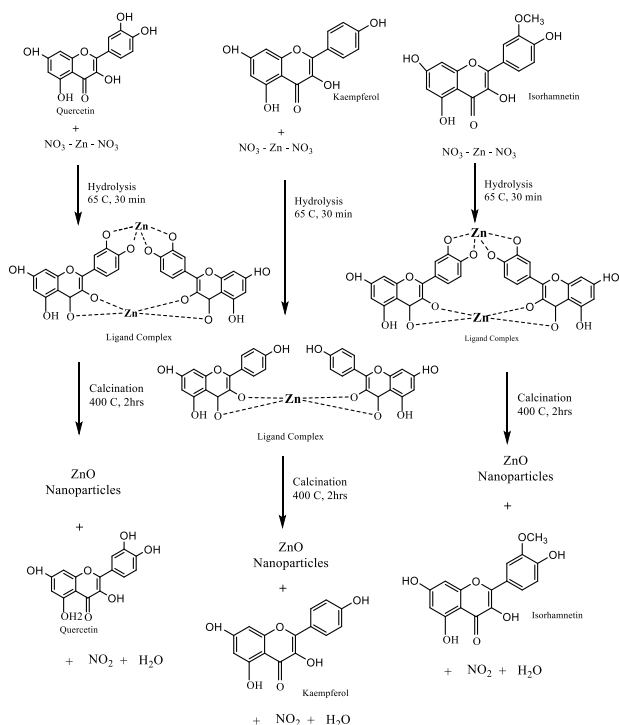


Figure 4: A possible reaction mechanism for the synthesis of ZnO using *Eruca Sativa* leaves extract.

### 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  $\text{cm}^{-1}$  in the region of 4000  $\text{cm}^{-1}$  to 500  $\text{cm}^{-1}$ . The band 3236  $\text{cm}^{-1}$  was assigned to the O-H stretching vibration while band at 2360.87  $\text{cm}^{-1}$  is due to C-H stretching vibration. The bands 1747.5 and 1647.20  $\text{cm}^{-1}$  may correspond to C=O anhydride and ester respectively. The stretching vibrational bands observed at 1473.31  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  corresponds to N-H stretching while bands at 2920.22 and 2854.64  $\text{cm}^{-1}$  attributed to C-H stretching mode of these bonds of *Eruca sativa* leaf extract. The peaks 2360.87 and 2322.29  $\text{cm}^{-1}$  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  $\text{cm}^{-1}$  is attributed to the C=C stretch in aromatic ring. The peak at 1546.91  $\text{cm}^{-1}$  corresponds to the C=O of flavonoids while band 1473.61  $\text{cm}^{-1}$  refers to the presence of aromatic ring while the bands 1373.31 and 1311.59  $\text{cm}^{-1}$  refer to the presence of phenols. The bands at 1203.58 and 1153.43  $\text{cm}^{-1}$  indicate the presence of ester. The bands in the region 1080.13

indicate the stretch of C-OH whereas the band at 918.11  $\text{cm}^{-1}$  may correspond to O-H bending vibrations of carboxylic acid and C-H bending vibration. The region between 500 – 560  $\text{cm}^{-1}$  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.

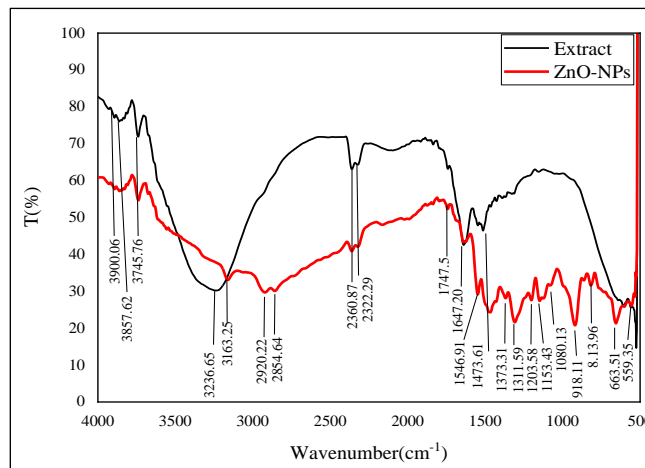


Figure 5: FTIR spectra obtained for *Eruca Sativa* leaf extract and biosynthesized ZnO nanoparticles.

### 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  $\text{Zn}(\text{OH})_2$  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].

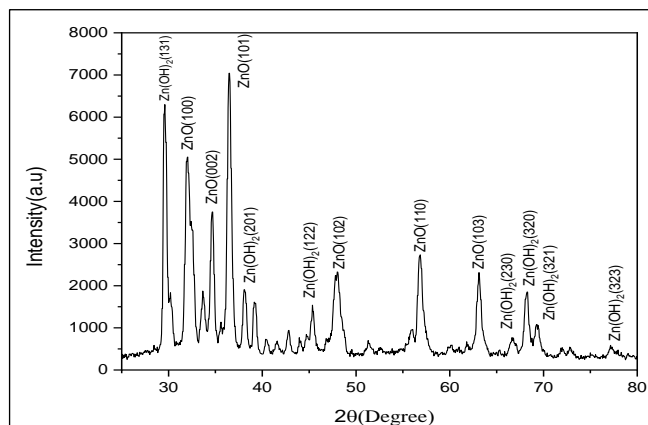


Figure 6: XRD pattern of bio-synthesized ZnO NPs.

### 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 exhibits 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].

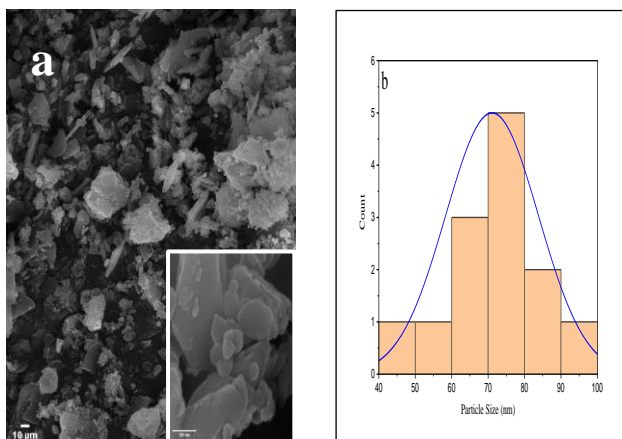


Figure 7: (a)FESEM image of biosynthesized ZnO NPs and (b) particle size distribution histogram of ZnO NPs.

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

phytochemicals which are existed along with ZnO Nanoparticles [84].

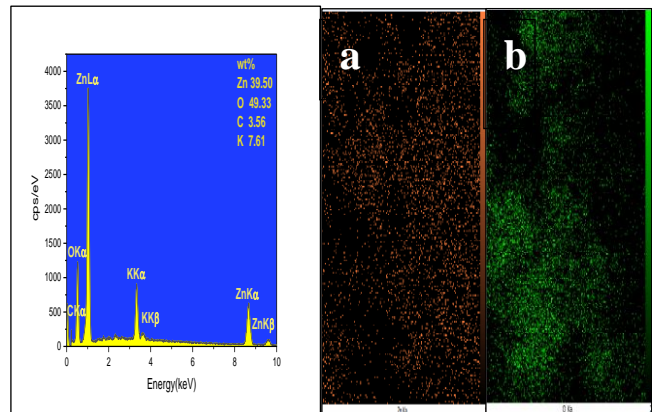
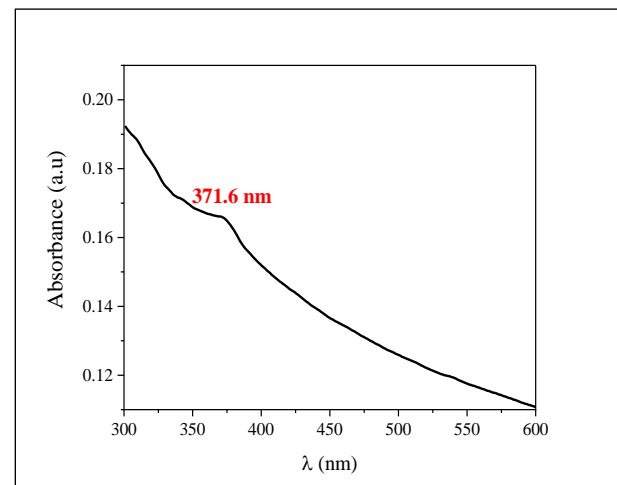


Figure 8: EDX analysis of biosynthesized ZnO nanoparticles, a: EDX spectrum and, b: The composition percentage of Zn and O.

### 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<sup>2+</sup> 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]:

$$(ah\nu)^{1/\gamma} = B(h\nu - E_g) \dots\dots\dots (2)$$

Where  $\alpha$  is the absorption coefficient that is given by ( $\alpha = 2.303 \log (T/d)$ ), 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.

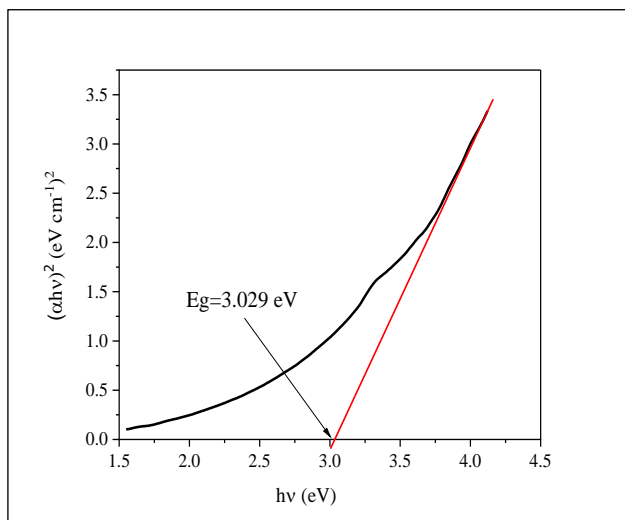


Figure 10: Band gap ( $E_g$ ) estimation of the prepared ZnO nanoparticles from Tauc's relation [88].

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 element influencing how the 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 synthesize 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 synthesized using *Eurca 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  $R^2 = 0.9854$ ). Total phenolic content equals to  $168.3867 \pm 0.1772$  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 synthesized using *Eurca Sativa* leaf extract is determined from the standard curve ( $y = 0.0019X - 0.0027$  and  $R^2 = 0.9927$ ) 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  $15.75 \pm 0.0735$ . 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

Extraction type	Total phenolic content (mg TAE /gm)	Total flavonoid content (mg QE/gm)
Water (ZnO)	$168.3867 \pm 0.1772$	$15.75 \pm 0.0735$

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.

Conc	DPPH scavenging activity (%)		p-value
	Water extract	Ascorbic acid (standard)	
25	$50.95^c \pm 0.01$	$40.74^a \pm 0.01$	$p < 0.0002$
50	$57.46^d \pm 2.52$	$46.43^b \pm 0.02$	
75	$62.51^e \pm 0.01$	$85.56^f \pm 0.11$	
100	$63.82^e \pm 0.02$	$97.06^g \pm 0.02$	
IC50	12.77	41.53	

Note: Results are expressed Mean  $\pm$  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  $\mu\text{g/ml}$ .

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

Conc	Reducing power assay (O.D 700 m)		p-value
	Water extract	Ascorbic acid (standard)	
25	1.5153 <sup>a</sup> ±0.00120	2.32 <sup>c</sup> ± 0.006	p < 0.0006
50	1.5207 <sup>a</sup> ± 0.001	2.33 <sup>cd</sup> ± 0.003	
75	1.5223 <sup>a</sup> ±0.002	2.35 <sup>de</sup> ± 0.007	
100	1.5480 <sup>b</sup> ±0.004	2.37 <sup>e</sup> ± 0.018	

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  $\mu\text{g/ml}$  that is statically significant  $p < 0.05$ .

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

Conc	Total antioxidant capacity (O.D 695 nm)		P-value
	Water extract	Ascorbic acid (standard)	
25	0.0353 <sup>a</sup> ± 0.0003	0.26 <sup>e</sup> ± 0.0002	P < 0.0003
50	0.0627 <sup>b</sup> ±0.001	0.34 <sup>f</sup> ± 0.0000	
75	0.0753 <sup>c</sup> ±0.0003	0.48 <sup>g</sup> ± 0.0001	
100	0.0923 <sup>d</sup> ±0.0003	0.54 <sup>h</sup> ± 0.0002	

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.

### REFERENCES:

Santhoshkumar, J., S.V. Kumar, and S. Rajeshkumar, *Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen*. Resource-Efficient Technologies, 2017. 3(4): p. 459-465.

Mustafa, S.M., et al., *Green synthesis of Ni doped ZnO nanoparticles using dandelion leaf extract and its solar cell applications*. Ceramics International, 2022. 48(19): p. 29257-29266.

Barzinjy, A.A., et al., *Nanostructured device in sensing applications: A review*. Eurasian J. Sci. Eng, 2018. 4(1): p. 82-98.

Fakhari, S., M. Jamzad, and H. Kabiri Fard, *Green synthesis of zinc oxide nanoparticles: a comparison*. Green chemistry letters and reviews, 2019. 12(1): p. 19-24.

Zhang, D., et al., *Green synthesis of metallic nanoparticles and their potential applications to treat cancer*. Frontiers in Chemistry, 2020. 8.

Kumar, J.A., et al., *Kinetics and regression analysis of phenanthrene adsorption on the nanocomposite of CaO and activated carbon: Characterization, regeneration, and mechanistic approach*. Journal of Molecular Liquids, 2021. 334: p. 116080.

Kumar, J.A., et al., *Enhanced PAHs removal using pyrolysis-assisted potassium hydroxide induced palm shell activated carbon: batch and column investigation*. Journal of Molecular Liquids, 2019. 279: p. 77-87.

Iravani, S., et al., *Synthesis of silver nanoparticles: chemical, physical and biological methods*. Research in pharmaceutical sciences, 2014. 9(6): p. 385.

Rajput, N., *Methods of preparation of nanoparticles-a review*. International Journal of Advances in Engineering & Technology, 2015. 7(6): p. 1806.

Hyeon, T., *Chemical synthesis of magnetic nanoparticles*. Chemical communications, 2003(8): p. 927-934.



- Piro, N.S., et al., *Green synthesis magnetite (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles from Rhus coriaria extract: a characteristic comparison with a conventional chemical method*. IEEE Transactions on NanoBioscience, 2022. 22(2): p. 308-317.
- Nadaroglu, H., A.A. GÜNGÖR, and İ. Selvi, *Synthesis of nanoparticles by green synthesis method*. International Journal of Innovative Research and Reviews, 2017. 1(1): p. 6-9.
- Parveen, K., V. Banse, and L. Ledwani. *Green synthesis of nanoparticles: Their advantages and disadvantages*. in *AIP conference proceedings*. 2016. AIP Publishing.
- Chandra, H., et al., *Medicinal plants: Treasure trove for green synthesis of metallic nanoparticles and their biomedical applications*. Biocatalysis and Agricultural Biotechnology, 2020. 24: p. 101518.
- Rahimzadeh, C.Y., et al., *Green synthesis of SiO<sub>2</sub> nanoparticles from Rhus coriaria L. extract: Comparison with chemically synthesized SiO<sub>2</sub> nanoparticles*. PLoS One, 2022. 17(8): p. e0268184.
- Ahmed, A.A., et al., *Neodymium oxide nanoparticles synthesis using phytochemicals of leaf extracts of different plants as reducing and capping agents: Growth mechanism, optical, structural and catalytic properties*. Journal of the Chinese Chemical Society, 2022. 69(3): p. 462-475.
- Mubeen, I. and M.A. Farrukh, *Mechanisms of green synthesis of iron nanoparticles using Trifolium alexandrinum extract and degradation of methylene blue*. Inorganic and Nano-Metal Chemistry, 2023. 53(1): p. 23-32.
- Kumar, H., et al., *Fruit extract mediated green synthesis of metallic nanoparticles: A new avenue in pomology applications*. International journal of molecular sciences, 2020. 21(22): p. 8458.
- Iravani, S., *Green synthesis of metal nanoparticles using plants*. Green Chemistry, 2011. 13(10): p. 2638-2650.
- Gour, A. and N.K. Jain, *Advances in green synthesis of nanoparticles*. Artificial cells, nanomedicine, and biotechnology, 2019. 47(1): p. 844-851.
- Gomaa, E.Z., *Microbial mediated synthesis of zinc oxide nanoparticles, characterization and multifaceted applications*. Journal of Inorganic and Organometallic Polymers and Materials, 2022. 32(11): p. 4114-4132.
- Mohamed, H.E.A., et al., *Structural, morphological and biological features of ZnO nanoparticles using Hyphaene thebaica (L.) Mart. fruits*. Journal of Inorganic and Organometallic Polymers and Materials, 2020. 30: p. 3241-3254.
- Nithya, K. and S. Kalyanasundharam, *Effect of chemically synthesis compared to biosynthesized ZnO nanoparticles using aqueous extract of C. halicacabum and their antibacterial activity*. OpenNano, 2019. 4: p. 100024.
- Gujel, A., et al., *Evaluation of vulcanization nanoactivators with low zinc content: characterization of zinc oxides, cure, physico-mechanical properties, Zn<sup>2+</sup> release in water and cytotoxic effect of EPDM compositions*. Polymer Engineering & Science, 2018. 58(10): p. 1800-1809.
- Ahmoum, H., et al., *Impact of position and concentration of sodium on the photovoltaic properties of zinc oxide solar cells*. Physica B: Condensed Matter, 2019. 560: p. 28-36.
- Roshitha, S.S., et al., *Photocatalytic degradation of methylene blue and safranin dyes using chitosan zinc oxide nano-beads with Musa x paradisiaca L. pseudo stem*. Bioresource Technology Reports, 2019. 5: p. 339-342.
- Rad, S.S., A.M. Sani, and S. Mohseni, *Biosynthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from leaf extract of Mentha pulegium (L.)*. Microbial pathogenesis, 2019. 131: p. 239-245.
- Mahmood, K., et al., *Enhanced efficiency and stability of perovskite solar cells using polymer-coated bilayer zinc oxide nanocrystals as the multifunctional electron-transporting layer*. Journal of colloid and interface science, 2019. 538: p. 426-432.
- Arafat, M., J.Y. Ong, and A. Haseeb, *Selectivity shifting behavior of Pd nanoparticles loaded zinc stannate/zinc oxide (Zn<sub>2</sub>SnO<sub>4</sub>/ZnO) nanowires sensors*. Applied Surface Science, 2018. 435: p. 928-936.
- Iqbal, J., et al., *Plant-extract mediated green approach for the synthesis of ZnONPs: Characterization and evaluation of cytotoxic, antimicrobial and antioxidant potentials*. Journal of Molecular Structure, 2019. 1189: p. 315-327.
- Jha, S., R. Rani, and S. Singh, *Biogenic Zinc Oxide Nanoparticles and Their Biomedical Applications: A Review*. Journal of Inorganic and Organometallic Polymers and Materials, 2023: p. 1-16.
- Zikalala, N., et al., *Biosynthesis protocols for colloidal metal oxide nanoparticles*. Nano-Structures & Nano-Objects, 2018. 16: p. 288-299.
- Yuvakkumar, R., et al., *Novel green synthetic strategy to prepare ZnO nanocrystals using rambutan (Nephelium lappaceum L.) peel extract and its antibacterial applications*. Materials Science and Engineering: C, 2014. 41: p. 17-27.
- Vanaja, M., et al., *Phytosynthesis of silver nanoparticles by Cissus quadrangularis: influence of physicochemical factors*. Journal of Nanostructure in Chemistry, 2013. 3: p. 1-8.
- Barzinjy, A.A. and V.N. Hamadamen, *Investigating Physical Properties and Formation Mechanism of Biosynthesized Zinc Oxide Nanoparticles using Dill (Anethum graveolens) Leaf Extract*. Nanoscience & Nanotechnology-Asia, 2022. 12(6): p. 27-38.
- Nagajyothi, P., et al., *Green route biosynthesis: Characterization and catalytic activity of ZnO nanoparticles*. Materials Letters, 2013. 108: p. 160-163.
- Mustafa, S.M., A.A. Barzinjy, and A.H. Hamad, *An environmentally friendly green synthesis of Co<sup>2+</sup> and Mn<sup>2+</sup> ion doped ZnO nanoparticles to improve solar cell efficiency*. Journal of Environmental Chemical Engineering, 2023. 11(2): p. 109514.
- Xiao, L., et al., *Zinc oxide nanoparticles induce renal toxicity through reactive oxygen species*. Food and Chemical Toxicology, 2016. 90: p. 76-83.
- Ramesh, M., M. Anbuvaran, and G. Viruthagiri, *Green synthesis of ZnO nanoparticles using Solanum nigrum leaf extract and their antibacterial activity*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2015. 136: p. 864-870.
- Azeeza, H.H. and A.A. Barzinjya, *Biosynthesis zinc oxide nanoparticles using Apium graveolens L. leaf extract and its use in removing the organic pollutants in water*. Desalination Water Treat, 2020. 190: p. 179-192.
- Zong, Y., et al., *Synthesis and high photocatalytic activity of Eu-doped ZnO nanoparticles*. Ceramics international, 2014. 40(7): p. 10375-10382.
- Kim, B., Y.E. Choi, and H.S. Kim, *Eruca sativa and its flavonoid components, quercetin and isorhamnetin, improve skin barrier function by activation of peroxisome proliferator-activated receptor (PPAR)-α and suppression of inflammatory cytokines*. Phytotherapy Research, 2014. 28(9): p. 1359-1366.
- Higdon, J.V., et al., *Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis*. Pharmacological research, 2007. 55(3): p. 224-236.
- Aspoukeh, P.K., A.A. Barzinjy, and S.M. Hamad, *A novel approach to the green synthesis of zinc oxide nanorods*

- using *Thymus kotschyanus* plant extract: effect of ammonium hydroxide and precursor concentration. Nano Express, 2023. 4(4): p. 045001.
- A Barzinjy, A., *Characterization of ZnO nanoparticles prepared from green synthesis using Euphorbia petiolata leaves*. Eurasian Journal of Science & Engineering, 2019. 4(3): p. 74-83.
- Shnawa, B.H., et al., *Scolicidal activity of biosynthesized zinc oxide nanoparticles by Mentha longifolia L. leaves against Echinococcus granulosus protoscolices*. Emergent Materials, 2021: p. 1-11.
- Barzinjy, A.A., et al., *Biosynthesis and characterisation of zinc oxide nanoparticles from Punica granatum (pomegranate) juice extract and its application in thin films preparation by spin-coating method*. Micro & Nano Letters, 2020. 15(6): p. 415-420.
- Barzinjy, A.A. and H.H. Azeez, *Green synthesis and characterization of zinc oxide nanoparticles using Eucalyptus globulus Labill. leaf extract and zinc nitrate hexahydrate salt*. SN Applied Sciences, 2020. 2(5): p. 991.
- Karam, S.T. and A.F. Abdulrahman. *Green synthesis and characterization of ZnO nanoparticles by using thyme plant leaf extract*. in *Photonics*. 2022. MDPI.
- Ismail, S.M. and S.M. Ahmed, *THE EFFECT OF CALCINATION TEMPERATURES ON THE PROPERTIES OF ZNO NANOPARTICLES SYNTHESIZED BY USING LEAVES EXTRACTS OF PINUS BRUTIA TREE*. Science Journal of University of Zakho, 2023. 11(2): p. 286-297.
- Ismail, S.M.M., et al., *Characterization of green synthesized ZnO nanoparticles by using pinus brutia leaves extracts*. Journal of Molecular Structure, 2023. 1280: p. 135063.
- Abdulqudos, A.N. and A.F.F. Abdulrahman, *Biosynthesis and Characterization of ZnO Nanoparticles by using Leaf Extraction of Allium Calocephalum Wendelbow Plant*. Passer Journal of Basic and Applied Sciences, 2022. 4(2): p. 113-126.
- Barzinjy, A.A., et al., *Biosynthesis, characterization and mechanism of formation of ZnO nanoparticles using Petroselinum crispum leaf extract*. Current Organic Synthesis, 2020. 17(7): p. 558-566.
- Jin, J., et al., *Analysis of phytochemical composition and chemoprotective capacity of rocket (Eruca sativa and Diplotaxis tenuifolia) leafy salad following cultivation in different environments*. Journal of agricultural and food chemistry, 2009. 57(12): p. 5227-5234.
- Martínez-Sánchez, A., et al., *Controlled atmosphere preserves quality and phytonutrients in wild rocket (Diplotaxis tenuifolia)*. Postharvest Biology and Technology, 2006. 40(1): p. 26-33.
- Martínez-Sánchez, A., et al., *A comparative study of flavonoid compounds, vitamin C, and antioxidant properties of baby leaf Brassicaceae species*. Journal of agricultural and food chemistry, 2008. 56(7): p. 2330-2340.
- Ingle, K.P., et al., *Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts*. Journal of Pharmacognosy and Phytochemistry, 2017. 6(1): p. 32-36.
- Ali, S., et al., *Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder*. BMC complementary and alternative medicine, 2018. 18: p. 1-15.
- Archana, P., et al., *Preliminary phytochemical screening from leaf and seed extracts of Senna alata L. Roxb-an ethno medicinal plant*. Int J Pharm Biol Res, 2012. 3(3): p. 82-89.
- Logeswari, P., S. Silambarasan, and J. Abraham, *Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties*. Scientia Iranica, 2013. 20(3): p. 1049-1054.
- Wansi, J.D., et al., *Alkaloids from the medicinal plants of Africa*, in *Medicinal plant research in africa*. 2013, Elsevier. p. 557-605.
- Mandal, S.C., V. Mandal, and A.K. Das, *Essentials of botanical extraction: Principles and applications*. 2015: Academic press.
- Malabadi, R.B., et al., *Antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of Clitoria ternatea*. Research in Pharmacy, 2015. 2(4).
- Ranjan, S., N. Dasgupta, and E. Lichtfouse, *Nanoscience in food and agriculture 2*. Vol. 20. 2016: Springer.
- Raj, A. and R. Lawrence, *Green synthesis and characterization of zno nanoparticles from leaf extracts of rosa indica and its antibacterial activity*. nutrition, 2018. 11(3): p. 1339-1348.
- Marjoni, M.R., et al., *The Effect of Different Extraction Solvents on Total Phenolic and Flavonoid Total of Snake Plant (Sansevieria trifasciata var. Laurentii)*. Journal of Pharmaceutical Negative Results, 2023. 14(1): p. 38-43.
- Diab, F., et al., *Influence of Simulated In Vitro Gastrointestinal Digestion on the Phenolic Profile, Antioxidant, and Biological Activity of Thymbra spicata L. Extracts*. Antioxidants, 2022. 11(9): p. 1778.
- Sadeq, O., et al., *Phytochemical screening, antioxidant and antibacterial activities of pollen extracts from micromeria fruticosa, achillea fragrantissima, and phoenix dactylifera*. Plants, 2021. 10(4): p. 676.
- Ergün, F., *EFFECTS OF DRYING METHODS ON AMOUNTS OF PHENOLIC AND FLAVONOID COMPOUNDS AND ANTIOXIDANT CAPACITY OF PLANTAGO LANCEOLATA L.* JAPS: Journal of Animal & Plant Sciences, 2023. 33(1).
- El Khomsi, M., et al., *In vitro studies on the antimicrobial and antioxidant activities of total polyphenol content of Cynara humilis from Moulay Yacoub area (Morocco)*. Plants, 2022. 11(9): p. 1200.
- Khan, H. and M.A. Khan, *Antiulcer effect of extract/fractions of Eruca sativa: Attenuation of urease activity*. Journal of Evidence-Based Complementary & Alternative Medicine, 2014. 19(3): p. 176-180.
- Thiab, A., S. Hussein, and M.N. Hamad, *POSSIBLE GASTRIC PROTECTIVE AND THERAPEUTIC EFFECT OF Eruca sativa LEAVES EXTRACT AGAINST GASTRIC ULCER INDUCED BY ETHANOL IN RATS*. Pharmacie Globale, 2016. 7(3): p. 1.
- Garg, G. and V. Sharma, *Eruca sativa (L.): Botanical description, crop improvement, and medicinal properties*. Journal of Herbs, Spices & Medicinal Plants, 2014. 20(2): p. 171-182.
- Jaafar, N.S. and I.S. Jaafar, *Eruca sativa Linn.: Pharmacognostical and pharmacological properties and pharmaceutical preparations*. Asian J Pharm Clin Res, 2019. 12(3): p. 39-45.
- Thi, T.U.D., et al., *Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities*. RSC advances, 2020. 10(40): p. 23899-23907.
- Ovais, M., et al., *Green synthesis of silver nanoparticles via plant extracts: beginning a new era in cancer therapeutics*. Nanomedicine, 2016. 12(23): p. 3157-3177.
- Muthuvel, A., M. Jothibas, and C. Manoharan, *Effect of chemically synthesis compared to biosynthesized ZnO-NPs using Solanum nigrum leaf extract and their photocatalytic, antibacterial and in-vitro antioxidant*

- activity. Journal of Environmental Chemical Engineering, 2020. 8(2): p. 103705.
- Sorbiun, M., et al., *Green synthesis of zinc oxide and copper oxide nanoparticles using aqueous extract of oak fruit hull (jaft) and comparing their photocatalytic degradation of basic violet 3*. International Journal of Environmental Research, 2018. 12: p. 29-37.
- Rathnasamy, R., et al., *Green synthesis of ZnO nanoparticles using Carica papaya leaf extracts for photocatalytic and photovoltaic applications*. Materials Science: Materials in Electronics, 2017. 28: p. 10374-10381.
- Zhu, W., et al., *Green synthesis of zinc oxide nanoparticles using Cinnamomum camphora (L.) Presl leaf extracts and its antifungal activity*. Journal of Environmental Chemical Engineering, 2021. 9(6): p. 106659.
- Ishak, N.M., S. Kamarudin, and S. Timmiati, *Green synthesis of metal and metal oxide nanoparticles via plant extracts: an overview*. Materials Research Express, 2019. 6(11): p. 112004.
- Zeghoud, S., et al., *A review on biogenic green synthesis of ZnO nanoparticles by plant biomass and their applications*. Materials Today Communications, 2022: p. 104747.
- Alharbi, F.N., Z.M. Abaker, and S.Z.A. Makawi, *Phytochemical Substances—Mediated Synthesis of Zinc Oxide Nanoparticles (ZnO NPS)*. Inorganics, 2023. 11(8): p. 328.
- Saravanakkumar, D., et al., *Green synthesis of ZnO nanoparticles using Trachyspermum ammi seed extract for antibacterial investigation*. Der Pharma Chemica, 2016. 8(7): p. 173-180.
- Thirunavukkarasu, C., et al., *Preparation and characterization of ZnO nanoparticles using Moringa oleifera extract by green synthesis method*. Asian Journal of Phytomedicine and Clinical Research, 2016. 4(3): p. 121-132.
- Kavitha, S., et al., *Synthesis and characterisation of zinc oxide nanoparticles using terpenoid fractions of Andrographis paniculata leaves*. International Nano Letters, 2017. 7: p. 141-147.
- Hussain, A., et al., *Biogenesis of ZnO nanoparticles using Pandanus odorifer leaf extract: anticancer and antimicrobial activities*. RSC advances, 2019. 9(27): p. 15357-15369.
- Viezbicke, B.D., et al., *Evaluation of the Tauc method for optical absorption edge determination: ZnO thin films as a model system*. physica status solidi (b), 2015. 252(8): p. 1700-1710.
- Makula, P., M. Pacia, and W. Macyk, *How to correctly determine the band gap energy of modified semiconductor photocatalysts based on UV-Vis spectra*. 2018, ACS Publications. p. 6814-6817.
- Mohammed, K.A., S.M. Ahmed, and R.Y. Mohammed, *Investigation of structure, optical, and electrical properties of CuS thin films by CBD technique*. Crystals, 2020. 10(8): p. 684.
- Alphandéry, E., *Natural metallic nanoparticles for application in nano-oncology*. International Journal of Molecular Sciences, 2020. 21(12): p. 4412.
- Rónavári, A., et al., *Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical applications*. Molecules, 2021. 26(4): p. 844.
- Sulaiman, M., *Factors Influencing the Green Synthesis of Metallic Nanoparticles Using Plant Extracts: A Comprehensive Review*. Pharmaceutical Fronts, 2023.
- Rana, A., K. Yadav, and S. Jagadevan, *A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity*. Journal of Cleaner Production, 2020. 272: p. 122880.
- Roy, A., et al., *Biologically derived gold nanoparticles and their applications*. Bioinorganic Chemistry and Applications, 2022. 2022.
- Vijayaram, S., et al., *Applications of Green Synthesized Metal Nanoparticles—A Review*. Biological Trace Element Research, 2023: p. 1-27.
- Miu, B.A. and A. Dimischiotu, *New Green Approaches in Nanoparticles Synthesis: An Overview*. Molecules, 2022. 27(19): p. 6472.