RESEARCH PAPER

Comparative Studies of Biosynthesized Zinc Oxide Nanoparticles

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ARTICLE INFO

Article History:
Received 21 Aug 2022
Accepted 27 Dec 2022
Published 29 Jan 2023

Keywords:
ZnO nanoparticles
Phytochemical
Aqueous leave extract
EDS
TEM
Percentage yield

ABSTRACT

Zinc oxide nanoparticles are one of the known safe compounds, and their low toxicity makes them suitable for waste water remediation. An environment-friendly approach of synthesizing nanoparticles is the use of plant extracts which are rich sources of phytochemicals as reducing agents. In this research, aqueous extracts of four different plants' leaves were analyzed and found to contain anthocyanins, steroids, phenolics, saponins, flavonoids, and terpenoids. Optimization study prepared the precursors (zinc salt and plant extracts) in varying volume-ratios of 1:1, 1:2 and 2:1; a higher yield was observed for the ratio 1:1 having calculated percentage yields of 50.0 %, 64.1 %, 21.8 %, and 68.0 % for leaves of guava, tropical almond, lemon, and Mexican a, respectively. The TEM characterization of these biosynthesised nanoparticles have sizes in the range 0.22 nm and 5.80 nm, while the EDS spectra presented these nanoparticles as highly pure having major elements of zinc and oxygen. The small sizes and very high purity of the biosynthesised nanoparticles make them fit for adsorption applications such as waste water remediation.

How to cite this article
Oyetola E. O., Comparative Studies of Biosynthesized Zinc Oxide Nanoparticles. Nanochem Res, 2023; 8(1): 31-39
DOI: 10.22036/ncr.2023.01.003

INTRODUCTION

Nanoparticles (NPs) are materials with sizes between 1 – 100 nm in thier atomic, molecular or macro-molecule level [13]. Zinc and Zinc oxide Nanoparticles have been useful in plant development by formulating chemicals that protect plants and crops from insects, fungi, bacteria and pests. They are also useful in water, soil, and air remediation. [2]. ZnO nanoparticles have several advantages which include unique chemical and thermal stability, robustness, and long shelf life over other metal oxides such as TiO_2, WO_3, SiO_2, and Fe_2O_3 [5]. In addition, Food and Drug Administration (FDA) considers zinc oxide as one of the safest metal oxides that can be used in food industries [6].

The synthesis of nanoparticles can be categorized into three main methods i.e. physical, chemical, and biological methods. The biological method, also known as the biosynthetic method, in comparison to the other two methods have been adjudged to be a synthetic route that is, straightforward, cost-effective, dependable, sustainable, and reproducible [11].

According to Verma and Mehata (2015), the ratio of metal salt to plant extract affects the yield and mophology of the biosynthesised nanoparticles. *Azadirachta indica* broth and silver salt were experimented, and it was found that the absorption intensity increases monotonically with increasing the concentration of *Azadirachta indica* broth which shifted the absorption spectrum towards red (from 408 to 421 nm). This indicates an increase in the size of silver nanoparticles [20].

In their study entitled, "Effect of accelerator in green synthesis of silver nanoparticles," Darroudi *et al.* (2010) found that the obtained

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silver nanoparticles absorption peaks, which is the characteristic surface plasmon resonance (SPR) band for silver, shifted from 407 to 424 nm in response to the addition of different NaOH volumes. This blue-shift was related to a decrease in the particle size of Ag-NPs as suggested by Heath (1989) and the results demonstrated good agreement with the results obtained in the transmission electron microscopy (TEM) images of Ag-NPs and their particle size distribution which recorded a decrease in size from 20 nm to 10.7 nm on average [8].

Energy dispersive X-ray spectroscopy (also known as EDS, EDX or EDXA) is a powerful technique that enables scientists to analyze the elemental composition of a desired sample. The major operating principle that allows EDS to function is the capacity of high energy electromagnetic radiation, X-ray to eject 'core' electron (electrons that are not in the outermost shell) from an atom. This principle is known as Moseley's law, which relates a direct correlation between the frequencies of light released and the atomic number of the atom. Removing these electrons from the system will leave behind a hole that a higher energy electron can fill in, and it will release energy as it relaxes. The energy released during this relaxation process is unique to each element on the periodic table, and as such bombarding a sample with X-rays can be used to identify which elements are present and with what proportions [10].

The physical and chemical properties of nanophase materials rely on their crystal and surface structures. Transmission electron microscopy (TEM) is a powerful and unique technique for structure characterization. TEM is unique in identifying and quantifying the chemical and electronic structure of individual nanocrystals [21].

The percentage yield of nanoparticles is highly important in a cost-effective production of nanoparticles. This research seeks to bring to the limelight the effect of varying volumes of reacting mixtures of zinc sulphate heptahydrate and leaves extract in order to ascertain the best volume-mixture ratio. On the other hand, the EDS elemental composition gives knowledge of elements that made up the nanoparticles and shows the proportion of interfering elements (elements that are neither zinc nor oxygen) and thereby indicates the purity level of the biosynthesized nanoparticles.

This work aims to compare elemental compositions, percentage yields, and particle sizes of the zinc oxide nanoparticles synthesised using four different aqueous leave extracts as reducing agents at varying volume ratios of the zinc sulphate precursor.

MATERIALS AND METHODS

Plant sample collection and preparation

Fresh leaves of the plants, *Psidium guajava* (guava fruit), *Terminalia catappa* (tropical almond fruit) *Cymbopogon citratus* (lemon grass), *Tithonia diversifolia* (mexican sunflower) were collected from Osun State Polytechnic, Iree, Nigeria (Geographical coordinates, latitude and longitude – 7.935237, 4.7303397). They were washed and rinsed twice with distilled water, and cut into smaller pieces with knife.

Preparation of plant extracts solutions

A 10 % leaves concentration was prepared by weighing 1000 g each of these leaves and soaked into 10,000 mL of distilled water at room temperature of 28 °C, these were left for 7 days, after which a clean meshed cloth was used to separate out the filtrate, and 1 mm Whatmann filter paper was then use to re-filter the filtrate.

Preparation of Zinc salt and Base solutions

0.01 M $ZnSO_4.7H_2O$ was prepared by dissolving 2.88 g of the salt into 1 L of distilled water using 1000 mL standard flask. While, 0.01 M NaOH was prepared by dissolving 0.4 g of the alkali into 1 L of distilled water using 1000 mL standard flask. In calculating for percentage yield, it is important to note that, 0.52 g, 0.78 g and 1.04 g of zinc salt will be contained inside of 180 mL, 270 mL, and 360 mL of the standard solutions, respectively. All chemicals and reagents used in this work were of analytical grade and used without any purification. Standard solutions were prepared and used where required.

Phytochemical Screening

Standard procedures were employed to test for the presence of phytochemicals:

(i) Saponins: 5 mL of the extract was mixed with 20 mL of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of Saponins [18].

(ii) Phenols: Crude extract was mixed with 2 mL of 2% $FeCl_3$ solution. A blue green or black colour

Table 1: Different Biosynthesis process mixture

Sample Label	Reaction mixture (Metal salt : Leave extract : Stabilizing agent)	Reacting volumes	Ratio
B_1*	Zn salt : Guava extract : NaOH	270 mL : 270 mL : 180 mL	1:1
B_1**	Zn salt : Guava extract : NaOH	180 mL : 360 mL : 180 mL	1:2
B_1***	Zn salt : Guava extract : NaOH	360 mL : 180 mL : 180 mL	2:1
B_2*	Zn salt : tropical almond extract : NaOH	270 mL : 270 mL : 180 mL	1:1
B_2**	Zn salt : tropical almond extract : NaOH	180 mL : 360 mL : 180 mL	1:2
B_2***	Zn salt : tropical almond extract : NaOH	360 mL : 180 mL : 180 mL	2:1
B_3*	Zn salt : Lemon extract : NaOH	270 mL : 270 mL : 180 mL	1:1
B_3**	Zn salt : Lemon extract : NaOH	180 mL : 360 mL : 180 mL	1:2
B_3***	Zn salt : Lemon extract : NaOH	360 mL : 180 mL : 180 mL	2:1
B_4*	Zn salt : Mexican Sunflower extract : NaOH	270 mL : 270 mL : 180 mL	1:1
B_4**	Zn salt : Mexican Sunflower extract : NaOH	180 mL : 360 mL : 180 mL	1:2
B_4***	Zn salt : Mexican Sunflower extract : NaOH	360 mL : 180 mL : 180 mL	2:1

indicated the presence of phenols and tannins [16].

(iii) Flavonoids: Few drops of 20% sodium hydroxide solution was added to 1mL of the extract. A change to yellow color which on addition of acid changed to colourless solution indicated the presence of flavonoids [16].

(iv) Anthraquinones: Few drops of 2 % hydrochloric acid was added to the extract, Appearance of red color indicates the presence of anthraquinone [17].

(v) Anthocyanins: 2 mL of extract was added to 2 mL of 2 M HCl and ammonia. The appearance of pink-red which turned to blue-violet indicated the presence of anthocyanins [16].

(vi) Coumarins: 3 mL of 10 % NaOH was added to 2 mL of extract and the formation of yellow color demonstrated the presence of coumarins [16].

(vii) Emodins: 3 mL of NH_4OH and 5 mL of Benzene were added to 2 mL of the extract. Appearance of red colour indicated the presence of emodins [4].

(viii) Terpenoid: 2 mL of the plant extract was added to 2 mL of acetic anhydride and concentrated H_2SO_4. The formation of blue green rings indicated the presence of terpenoids [16].

(ix) Steroids: 1 mL of the plant extract was dissolved in 10 mL of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids in each of the plant leave aqueous extract [16].

Biosynthesis/optimization process

In order to know the precursor combination with the best yield, reacting mixtures in Table 1 were prepared at room temperature and were throughly mixed by stirring with glass rod for 2 minutes and left for 5 days to bring the biosynthesis reaction into completion at room temperature of 28 °C (during the biosynthesis reaction, +2 oxidation state of zinc in the $ZnSO_4.7H_2O$ standard solution was being reduced to zero i.e. $Zn^{2+} \rightarrow Zn^0$). The supernant was carefully decanted off and the nanoparticles centrifuged at 4000 rpm for 30 minutes; this was done thrice with fresh distilled water to remove unreacted components of the mixture, and then, the oven was dried at 90 °C to obtain the dried nanoparticles (zinc particles get easily oxidized in the presence of oxygen from the atmosphere and the heat drying treatment to form zinc oxide) [15].

TEM particle size characterization

JEM-ARM200F-G TEM was employed for the study under an electron microscope. The sample was made to be semi-transparent to allow the passage of electron beams through it. To achieve this semi-transparent nature, the sample is sectioned into fine sections using the glass attached to a device known as ultra-microtome. The device has a trough that is filled with distilled water, the sections cut were collected in this trough which are then moved to a copper grid to be viewed under the microscope.

Table 2: Phytochemical screening results of the aqueous leaves extract

Test	Guava	Tropical Almond	Lemon	Mexican Sunflower
Anthraquinones	No	No	No	No
Anthocyanins	Yes	Yes	No	Yes
Coumarins	No	No	No	No
Emodins	No	No	No	No
Flavonoids	Yes	Yes	No	Yes
Phenolics	Yes	Yes	No	Yes
Terpenoids	Yes	No	Yes	No
Saponins	Yes	Yes	Yes	Yes
Steroids	Yes	Yes	Yes	Yes

NB: Present (Yes) / Absent (No)

Table 3: Percentage yield of the dried nanoparticles

Sample label	Mass of Zn salt (g)	Weight of dried nanoparticle (g)	Percentage (%)
B_1*	0.78	0.39	50.0
B_1**	0.52	0.25	48.1
B_1***	1.04	0.25	24.1
B_2*	0.78	0.50	64.1
B_2**	0.52	0.29	55.8
B_2***	1.04	0.29	27.9
B_3*	0.78	0.17	21.8
B_3**	0.52	0.06	11.6
B_3***	1.04	0.11	10.6
B_4*	0.78	0.53	68.0
B_4**	0.52	0.33	64.1
B_4***	1.04	0.38	36.6

RESULTS AND DISCUSSION

Phytochemical screening

Nine phytochemicals present were tested on all the aqueous leaves extracts of guava fruit, tropical almond fruit, lemon grass, and Mexican sunflower. Table 2 presents the results.

Saponins and steroids were present in all the extracts, while anthraquinones, coumarins and emodins, which are anthraquionone derivatives, were absent.

Lack of phenol derivatives such as flavonoids and anthocyanin may have a negative impact on the reducing strength of the lemon aqueous extract. This result is in agreement with the phytochemicals result of Umar *et al*, (2016) where phenolics were absent in the lemon leave extract [19].

The absence of terpenoids, a known isoprenoid, suggests that non-aromatic structures may inhibit the reductive ability of guava and lemon plant extract. This probably brought about lower yields as compared to the yields of Mexican sunflower and tropical almond.

Percentage yield calculations

The percentage yield of the nanoparticle was calculated using equation 1 and the calculated values are presented in Table 3.

$$\text{Percentage yield} = \frac{\text{weight of the dried nanoparticle}}{\text{calculated weight of the salt}} \times 100\% \qquad \text{-- Eq 1}$$

The weight of the dried nanoparticles with respect to the mixture-volume ratio shows that the ratio 1:1 of Zn salt to aqueous leave extract gave a better yield compared to the two other mixture ratios i.e. 1:2 and 2:1. B1 has a mean value of 40.7, B2 of 49.3, B3 of 14. 7, and B4 of 56.2, implying that the reducing power trend of the aqueous extract

Table 4: Elemental composition of nanoparticles sample labeled B_1* B_2* B_3* and B_4*

Sample label	Zn (%)	O (%)	C (%)	Al (%)	Fe (%)	Na (%)	Si (%)
B_1*	71.40	5.20	3.10	15.00	3.20	2.10	0.00
B_2*	60.24	20.26	4.50	8.30	4.30	2.40	0.00
B_3*	76.28	20.48	2.36	0.00	0.00	0.00	0.88
B_4*	77.76	20.24	2.00	0.00	0.00	0.00	0.00

Table 5: EDS Percentage Yield of the dried Nanoparticles

Sample	Mass of salt (g)	Wt of nanoparticle	Wt x EDS	Percentage
B_1*	0.78	0.39	0.30	38.4
B_2*	0.78	0.50	0.40	51.3
B_3*	0.78	0.17	0.17	21.8
B_4*	0.78	0.53	0.52	66.7

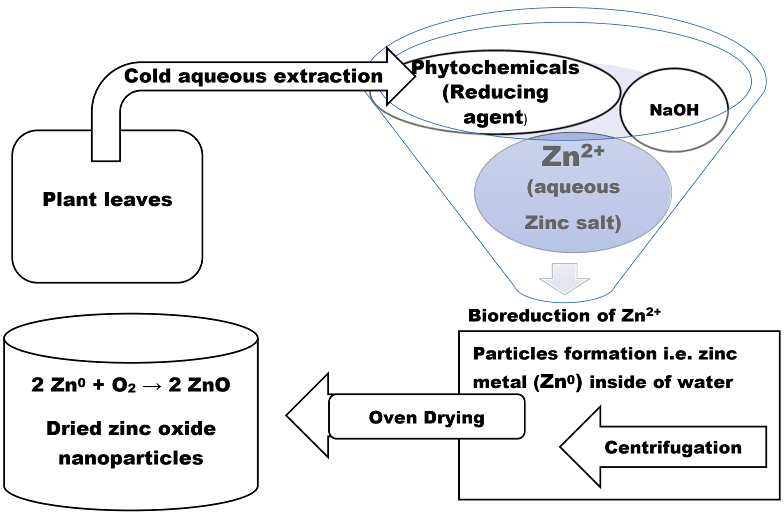

Fig 1: Schematic diagram of the biosynthesis process

against the Zn sulphate salt solution is presented as B_4 > B_2 > B_1 > B_3 i.e. Mexican sunflower > tropical almond > guava > lemon. The phytochemical results, if related to the yield of the nanoparticles, suggest that the number of phytochemicals present is not chiefly the reason for the reducing ability of the extracts but rather the nature of the phytochemicals present.

Elemental composition

The EDS instrument was used to know the purity level of the biosynthesized nanoparticles. Figs. 1 – 4 present the EDS spectra of the 1:1 volume mixture ratio of the nanoparticles produced using the four aqueous extracts as reducing agents, while Table 4 summarizes the spectra peaks with their corresponding percentages.

The EDS result of the selected nanoparticles shows that the biosynthesis process gave a pure Zn metal oxide as the result indicates that over 75 % of the nanoparticles elemental contents are Zn and oxygen. The percentages of Zn with oxygen content in the samples B_1* and B_2* are 76.6 % and 80.5 %, respectively, which incorporated three other metals, namely aluminium, iron, and sodium which makes them of lower purity compared with B_3* and B_4*samples. The sample B_4* which is the biosynthesized nanoparticles using Mexican sunflower as a reductant gave the purest dried nanoparticles which is free of other metallic species

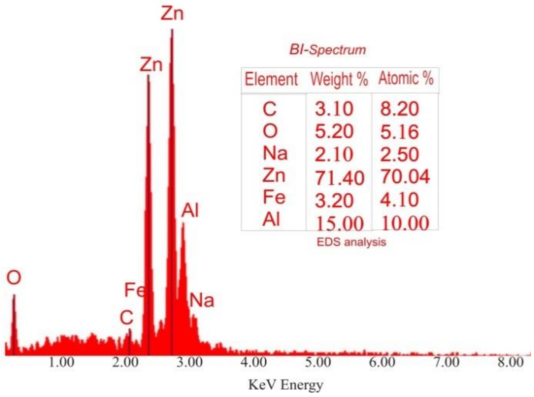

Fig. 1: EDS Spectrum of B1*

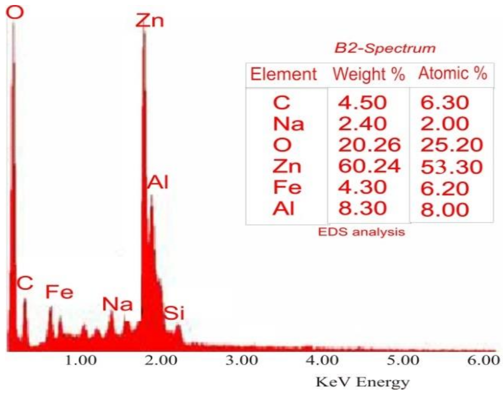

Fig. 2: EDS Spectrum of B2*

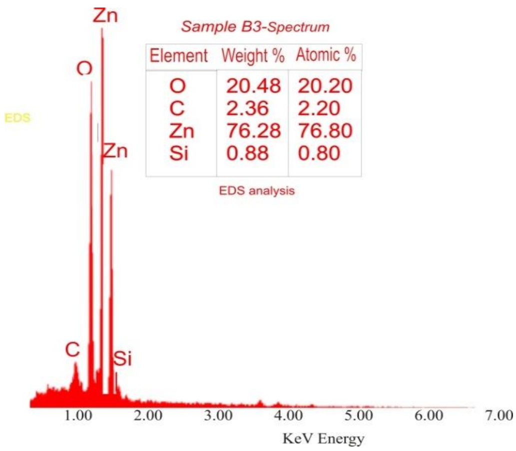

Fig. 3: EDS Spectrum of B3*

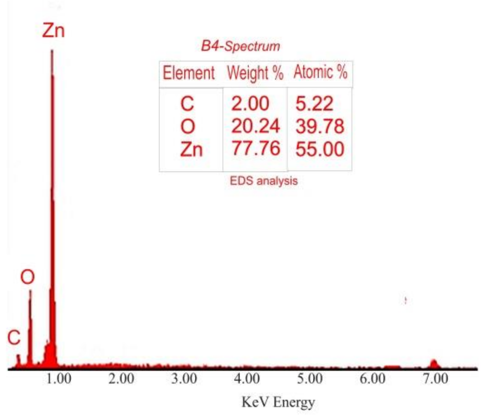

Fig. 4: EDS Spectrum of B4*

and silicon present in sample B_3* i.e. 98.0 % as against 96.8 %. This result compares favourably with Chu *et al*, (2022) where, Zr of 69.29 % and O of 30.71 % in the nano-synthesized ZrO_2 were adjudged to be pure [14].

The yield percentages in Table 3 do not consider the purity data as presented by the EDS weight percentage results. Table 5 presents the calculated percentage yields of the four selected nanoparticles using equation 2.

EDS % yield =

$$\frac{\text{weight of the dried nanoparticle} \times \text{\% weight of Zn and O from the EDS}}{\text{calculated weight of the salt}} \times 100\%$$

--Eq. 2

The calculation of percentage yields which incorporated the percentage weight of EDS elemental results provided a more accurate way of knowing the yield as the level of the purity of dried weighed nanoparticles was considered. The results

Table 6: Deviation percentage yield of the sample using EDS result

Sample	% (Initial)	% (Final)	% (Deviation)
B_1*	50.0	38.4	11.6
B_2*	64.1	51.3	12.8
B_3*	21.8	21.8	0.00
B_4*	68.0	66.7	1.30

maintain the trend as discussed in Table 3 where, $B_4 > B_2 > B_1 > B_3$ i.e. Mexican sunflower > tropical almond > guava > lemon. However, a study of the deviation in the value of these percentage yields confirms that sample B_3* (lemon) is the purest of the biosynthesized nanoparticles having zero deviation while B_2* (tropical almond) is the least in purity, as presented in Table 6 using equation 3.

% Deviation = Percentage yield without EDS – Percentage yield with EDS   Eq. 3

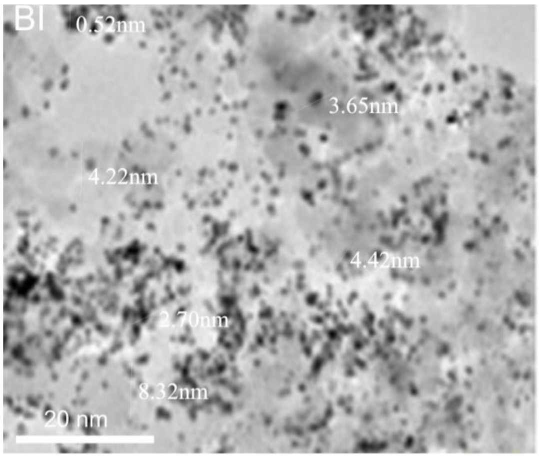

Fig. 5: TEM Micrograph B1*

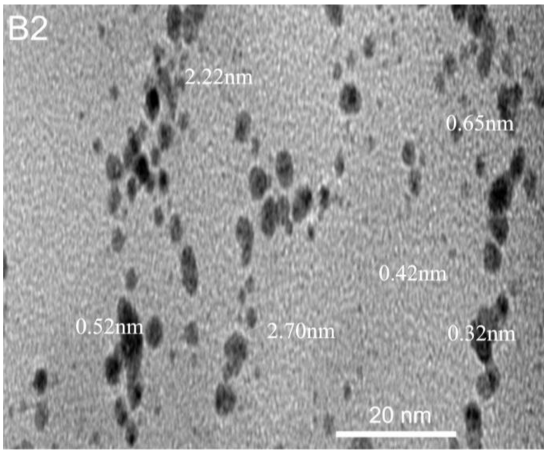

Fig. 6: TEM Micrograph B2*

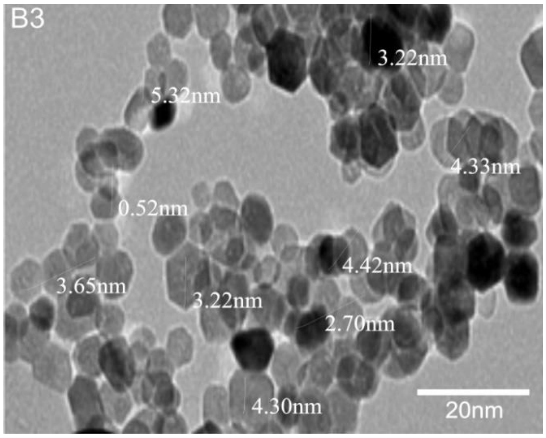

Fig. 7: TEM Micrograph B3*

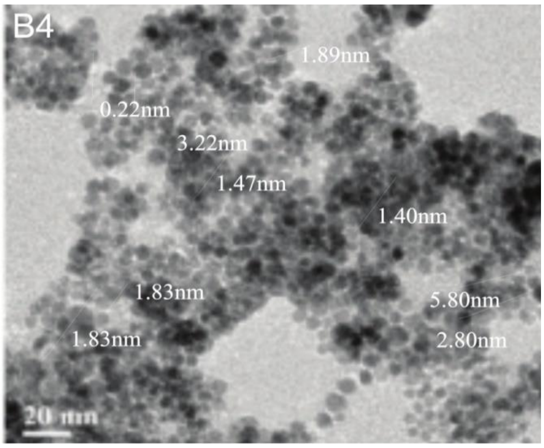

Fig. 8: TEM Micrograph B4*

Table 7: Summary of TEM displayed particle sizes

Nanoparticle	Captured particle sizes (nm)	Mean size (nm)
ZnO guava	0.52, 2.70, 3.65, 4.22 , 4.42 & 8.32	3.97
ZnO tropical almond	0.32, 0.42, 0.52, 0.65, 2.22 & 2.70	1.14
ZnO lemon	0.52, 2.70, 3.22, 3.65, 4.30, 4.33, 4.42 & 5.32	3.56
ZnO Mexican sunflower	0.22, 1.40, 1.47, 1.83, 1.83, 1.89, 2.80, 3.22 & 5.80	2.27

Transmission Electron Micrographs

The micrograph of the selected samples i.e. B_1*, B_2*, B_3* and B_4* are presented in Figs 5 to 8 and the summary of TEM displayed particle sizes is observed in Table 7.

The particle sizes invariably mean large surface area, which make the biosynthesized nanoparticles suitable in adsorption applications in the field of remediation of waste water. The trend in mean particle sizes of the samples are: ZnO tropical almond > ZnO Mexican sunflower > ZnO lemon > ZnO guava with sizes ranging from 0.32 – 2.70 nm, 0.22 – 5.80 nm, 0.52 – 5.32, 0.52 – 8.32, respectively. The TEM images help to confirm the nano-scale sizes of biosynthesized nanoparticles which is a key factor in using such samples as an adsorbent.

Ali (2016) worked on the leaves extract of *Aloe barbadensis* (Aloe Vera) as a reducing agent to produce ZnO NPs in sizes ranging from 8–20 nm using XRD [3]. *Ocimum basilicum L. var.*

purpurascens (Lamiaceae) Red Rubin basil Leaf extract was used as a reducing agent to produce Zn Oxide nanoparticles, the size of which is around 50nm using TEM [1]. *Azadirachta indica* (Meliaceae) Neem Leaf was used as a reducing agent to produce Zn Oxide nanoparticles, the size of which ranges from 9.6–25.5 nm using TEM [7]. Of all the reviewed nanoparticle sizes from plant extract as reducing agent, this research work presented a smaller particle size.

Krishna and Swamy (2012) in their study on the effect of particle size, adsorbent particles of sizes 0.6 mm, 0.8 mm and 1.7 mm particle size were used to adsorb on Cr (IV) from water sample, the quantity of this metal ion adsorbed on the adsorbent were 3.01, 2.66 and 2.34 mg g^{-1}, respectively. It was then concluded that the increase in the particle size diminishes the percentage removal, and at a fixed adsorbent dosage the decrease in particle size increases the metal uptake. The rise in the uptake by smaller particles was said to be due to the greater accessibility to pores and to the greater surface area for bulk adsorption per unit mass of the adsorbent.

In line with the work of Krishna and Swamy (2012), the biosynthensized nanoparticles propose a positive result if applied in waste water remediation

CONCLUSION

The TEM micrographs confirm that nanoparticles were synthesized having sizes between 1 and 100 nm, while the EDS presents a high level of purity i.e., 98 % of the synthesized nanoparticles using Mexican sunflower extract as a reducing agent are Zinc and Oxygen. The best yield was noted in the ratio 1:1 where Zn sulphate salt and Mexican sunflower aqueous extract gave a percentage yield of 68.0 %. However, the biosynthesized ZnO NPs which used tropical almond as its reducing agent is most preferred owing to having the smallest mean particle size of 1.14 nm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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