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Chemical Analysis of Iraqi *Ruta graveolens* .L leaf extract by GC–MS and its application in the green synthesis of zinc oxide nanoparticles

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Abstract

Background: Nanoparticles are used in numerous medical and other fields thanks to their physical and chemical properties. Green synthesis using plant extracts is an environmentally friendly approach for *Ruta graveolens*. L was used to prepare ZnO-NPs because it contains compounds that act as reducing and stabilizing agents.

Aims: This study aimed to chemically characterize the leaf extract of *Ruta graveolens* L using GC-MS technology, and then utilize it in the green synthesis of ZnO-NPs.

Materials and Methods: The plant leaves were collected and extracted using methanol. The extract was then used to prepare nanoparticles, which were characterized using DLS, SEM, and EDS techniques.

Results: GC-MS results revealed ten identified chemical compounds belonging to furanocoumarins, aliphatic ketones, terpene alcohols, and fatty acids. Psoralen, 3-(α, α-dimethylallyl)- was the most abundant compound at 11.773%, followed by 2-Undecanone at 11.370%. DLS analysis showed that the prepared particles fell within the nanometre scale range, averaging a size of 94.3 nm, a Z-average of 64.5 nm, and a dispersion index (PI) of 0.703, indicating relative size heterogeneity. SEM images showed that the particles had a spherical to irregular shape with a clear tendency to clump together, while some direct measurements of their dimensions ranged from 29.86 to 32.89 nm. EDS analysis confirmed that zinc and oxygen were the main elements in the sample, with zinc comprising 77.91% and oxygen 22.09% by weight.

Conclusion: The study concludes that the alcoholic extract of *Ruta graveolens* .L leaves has clear potential for use in the green synthesis of zinc oxide nanoparticles, and that the various characterization results supported the successful preparation of particles within the nanoscale with morphological and elemental properties that confirm the successful formation of ZnO-NPs.

Keyword: *Ruta graveolens .L, DLS, SEM, EDS. ZnO-NPs, green synthesis, nanoparticles*

1. Introduction

Medicinal plants occupy an important place in modern chemical and biological research, not only for traditional uses, but also because of their wide chemical diversity, which makes them an important

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source of natural compounds with pharmaceutical and research value. The plant (*Ruta graveolens* .L) is one of the medicinal plants belonging to the *Rutaceae* family, and many recent studies have shown that it contains multiple types of byproducts from secondary metabolism , including alkaloids, coumarins, flavonoids, volatile oils, and phenolic acids [1]. The chemical importance of *Ruta graveolens*. L is particularly evident when considering its content of volatile and semi-volatile compounds.

Specialized studies of Ruta genus oils have shown that this genus is characterized by containing many volatile compounds, especially 2-undecanone and 2-nonanone, which are considered important compounds for many of the studied samples [2]. An analytical study on *Ruta graveolens*.L showed that the volatile oil isolated from the above-ground sections of the plant was identified through GC-MS technology, with clear major compounds being recorded within the chemical content of the plant [3]. Thus, the chemical characterization of *Ruta graveolens*.L leaf extract using GC–MS represents a key step in identifying the predominant chemical components in the extract before its use in subsequent applications [2], [3]. In the field of nanotechnology, ZnO-NPs have garnered significant research attention, despite the fact that traditional physical and chemical methods for their preparation are often associated with the use of toxic chemicals or harsh preparation conditions.

Therefore, green synthesis has emerged as a more environmentally friendly approach, and specialized studies have confirmed that biological sources, particularly plants, have become key media in this type of preparation [4]. Recent studies have also shown that green synthesis based on plant extracts has become an important trend in the preparation of ZnO-NPs, and that the nature of the plant compounds present in the extract plays an important role in the properties of the particles formed [5]. Green synthesis of ZnO-NPs has shown many advantages, such as simplicity, environmental friendliness, and biosafety [6].

In reference [7], the study concluded that the alcoholic extract of *Ruta graveolens*. L could be used in the biosynthesis of ZnO-NPs, confirming the ability of this plant to enter this biological pathway [7]. One study indicated that combining the chemical characterization of *Ruta graveolens* leaf extract using GC–MS with the use of this extract in green synthesis of ZnO-NPs represents a related research direction, as it combines the study of the chemical profile of the plant extract with its potential in green preparation of nanomaterials within a single scientific framework [1],[7]. Therefore, this study aimed to prepare the alcoholic extract of *Ruta graveolens* leaves, then investigate its active compounds using GC-MS technology, and then employ this extract in the eco-friendly production of ZnO-NPs.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh leaves of *Ruta graveolens* were collected from the College of Agricultural Sciences, University of Baghdad, during January. They were thoroughly washed with tap water and then with distilled water to remove dirt and impurities. The sample went through a process of drying in the shade and at room temperature over the span of several days until all moisture was removed. The samples were then ground using an electric grinder into fine plant powder for storage in clean, dry containers until needed.

DOI: 10.69946/ojms/2026.04.01.09, pp(86-96)

2.2 Sample preparation for extraction

The samples were weighed using a precision balance, 50 g of the dry plant powder inside a Whatman No. 1, then it was sealed, and a Soxhlet extractor was operated with 400 ml of methanol for a duration of 6 hours. The sample was left to cool before being placed in a Rotary Evaporator for 7 minutes to concentrate it and acquire the crude extract.

It was then stored in sterile glass bottles at 4°C until analysis and use in the preparation of nanoparticles as described in [8].

2.3 Chemical Analysis by GC-MS.

Conducted chemical analysis using the extracts from the leaves of *Ruta graveolens* .L. We identified each chemical compound by GC/MS (gas chromatography-mass spectrometry). These analyses were performed on an Agilent Technologies A GC System with a GC-MS 7890 model manufactured in the United States, and operated in the Basra Oil Company's Laboratory. The analysis was started with the use of the thermal program in Table 1 below. Peaks detected by the instruments were identified from the "NIST" database and the "memory" listing for that instrument, as described in [9].

Table 1: Operating conditions of the gas chromatography-mass spectrometer (GC-MS)

Section	Parameter	Value
Mass Spectrometer	Instrument	Agilent 5977 A MSD
	Software	MassHunter software for GC/MS data collection, and MassHunter software for qualitative analysis.
	Ion Source Temperature	230 ℃
	Quadrupole Temperature	150 °C
	Interface Temperature (MSD Transfer Line)	290 °C
	Solvent Cut Time	4.00 min
	Start Time	4.00 min
	End Time	35.00 - 40 min
	Acquisition Mode	Scan
	Scan Speed	1562 (N2)
	Mass Range (m/z)	35 - 650
Gas Chromatography	Instrument	Agilent 7890 B
	Column Oven Temperature Program	Initial 40 C, hold 5 min; rate 10 C/min; final temperature 300 C to end run
	Injection Temperature	290 C
	Injection Mode	Pulsed splitless
	Flow Control Mode	Constant flow
	Pressure	7.0699 psi
	Total Flow	19 mL/min
	Column Flow	1 mL/min
	Purge Flow	3 mL/min
	Injection Volume	1 uL
	Column Type	HP-5MS, 5% phenyl methyl siloxane, 30 m x 0.25 mm x 0.25 um

DOI: 10.69946/ojms/2026.04.01.09, pp(86-96)

2.4 Green Synthesis of ZnO-NPs

A homogeneous solution was prepared in a 500 ml glass beaker by adding 50 grams of $Zn(NO_3)_2$ $6H_2O$ to 100ml of deionized water. Gradually, during the addition of 100ml of previously prepared *Ruta graveolens* .L extract at a temperature of 60 °C. Sodium hydroxide was added dropwise until the pH of the final solution reached 9. To produce a 1M solution of sodium hydroxide, 2 grams of sodium hydroxide were dissolved in 500ml of deionized water. The solution was left to stand for 48 hours to allow a light green precipitate to form. The precipitate was separated using a centrifuge, filtered, and washed with deionized water and ethanol. It was then dried at 300 °C in an oven for 10 hours, leaving a white powder. This powder represents the ZnO-NPs and was stored for testing [10].

3. Results

3.1 Analysis and Separation of Active Ingredients Using (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) analysis of the alcoholic extract of *Ruta graveolens* .L, as shown in Table 2 and Fig. 1, identified the chemical compounds and selected the most prominent ones based on retention time, molecular formula, and % area. Based on GC-MS analysis of *Ruta graveolens*.L The extract was analysed by using GC-MS. The identification of chemical compounds can be observed by the following: Table 2 shows a list of chemical compounds identified during the analysis with their respective retention times, molecular formulas, and area percentages. Fig. 1 shows the graphical representation of the same information.

These compounds were distributed across several chemical groups, including furanocoumarins, aliphatic ketones, terpene alcohols, and fatty acids. The compound Psoralen, 3-(α, α-dimethylallyl)- had the highest occurrence rate at 11.773% with a retention time of 24.894 minutes, followed by 2-Undecanone at 11.370%. Phytol, Chalepin, Bergapten, and α-Linolenic acid also appeared at varying rates, while 2-Nonanone had the lowest occurrence rate at 1.595%.

Table 2: Main active bioactive compounds identified in the alcoholic extract of *Ruta graveolens* .L using GC-MS technology

Area%	Molecular Formula	Detention time (minutes)	Chemical class	The diagnosed compound	
11.773	C16H14O3	24.894	Furanocoumarin	Psoralen, 3-(α,α-dimethylallyl)-	1
11.370	C11H22O	14.861–14.917	Aliphatic ketone	2-Undecanone*	2
4.845	C20H40O	24.076	Terpenoid alcohol	Phytol	3
4.620	C19H22O4	28.752	Furanocoumarin	Chalepin	4
3.371	C12H8O4	23.878	Furanocoumarin	Bergapten (5-methoxypsoralen)**	5
3.312	C18H30O2	24.377	Unsaturated fatty acid	α-Linolenic acid	6
2.316	C16H32O2	22.662	Saturated fatty acid	n-Hexadecanoic acid	7
2.061	C18H34O3	25.997	Oxidized fatty acid	Ricinoleic acid	8
1.913	C11H6O3	21.584	Furanocoumarin	Ficusin	9
1.595	C9H18O	11.769	Aliphatic ketone	2-Nonanone	10

DOI: 10.69946/ojms/2026.04.01.09, pp(86-96)

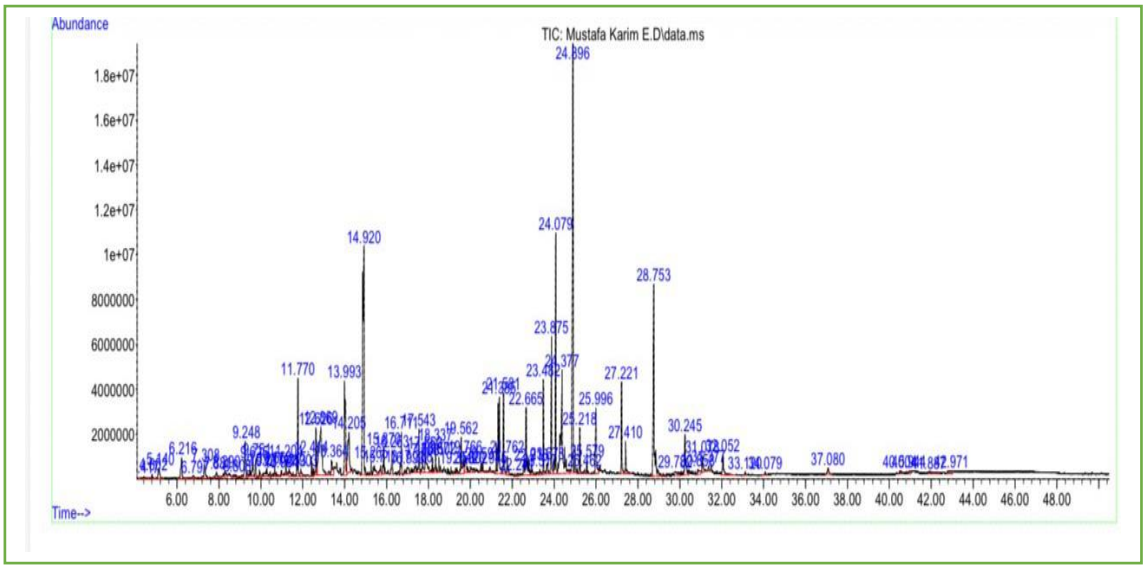

Fig. 1 Chromatograph of GC-MS analysis of the alcoholic extract of *Ruta graveolens*.L

3.2 Green Synthesis of ZnO-NPs

The results showed the successful green synthesis of ZnO-NPs using the alcoholic extract of *Ruta graveolens* L. A color change and precipitate formation were observed, indicating nanoparticle formation. DLS, EDS, and SEM assays supported this formation, confirming the successful preparation of the nanoparticles and elucidating some of their structural and morphological characteristics.

3.2.1 Nanoparticle Size Analysis Using (DLS)

The results of the ZnO-NPs size analysis using a HORIBA SZ-100 instrument, as shown in Table 3 and Fig. 2, indicate that the sample exhibited a monoclinic size distribution, averaging a size of 94.3 nm, a Z-Average of 64.5 nm, and a Mode of 87.3 nm, resulting in a standard deviation of 29.1 nm. The dispersion index (PI) was 0.703, indicating that the prepared particles have a medium-wide size distribution and exhibit some degree of relative heterogeneity, which may be attributed to partial agglomeration or dimensional variations in the suspension medium.

Table 3: Results of measuring the size of ZnO-NPs using DLS technology

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	94.3 nm	29.1 nm	87.3 nm
Total	1.00	94.3 nm	29.1 nm	87.3 nm

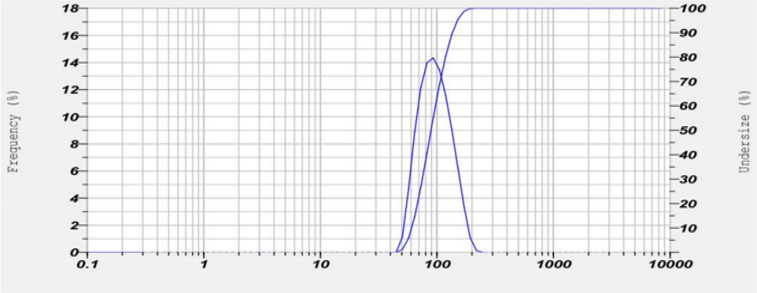

Fig. 2 ZnO-NPs size distribution curve measured by DLS technique.

DOI: 10.69946/ojms/2026.04.01.09, pp(86-96)

3.2.2 Scanning Electron Microscopy (SEM) Imaging of ZnO-NPs

Showed that SEM images in Fig. 3, the prepared ZnO-NPs had a spherical to irregular shape and a clear tendency to aggregate and cluster into nano-clustered structures of varying sizes. The images also revealed that the particles were closely packed and clustered, a characteristic common in nanoparticles prepared using the green method. Direct measurements from the images showed that the dimensions of several particles were in the range of 29.86–32.89 nm, confirming the nanoscale nature of the prepared sample.

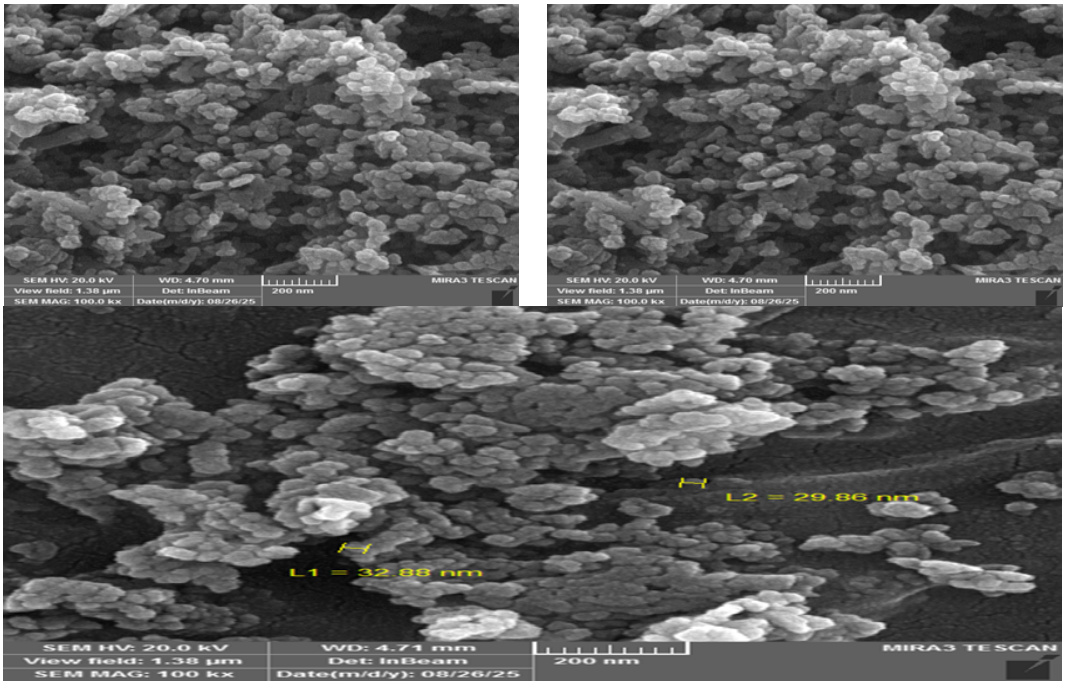

Fig. 3 SEM images of prepared ZnO-NPs at different magnifications, showing the surface shape, nano-assemblies, and some approximate particle size measurements.

3.2.3 Elemental Analysis of ZnO-NPs Using EDS

The EDS analysis, shown in Tables 4 and 5 and Fig. 4, revealed the presence of several elements in the spectrum, including C, O, Na, Zn, Nb, Re, Te, and Cs. However, quantitative analysis showed that only Zn and O were present in significant quantities in the sample. Zn comprised 77.91% by weight and 46.32% by atomic weight, while O comprised 22.09% by weight and 53.68% by atomic weight. These results indicate that Zn and O are the two main and most important elements in the prepared sample, confirming the effective creation of ZnO-NPs.

Table 4: Elements appearing in the EDS spectrum according to the results of automatic identification.

Prob%	keV	Int	Line	Elt
100	0.290	26.4	Ka1	C
100	0.520	76.4	Ka1	O
100	1.050	223.7	Ka1	Na
100	8.660	91.0	Ka1	Zn
100	2.180	42.1	La1	Nb
100	8.650	3.4	La1	Re
100	3.780	4.3	La1	Te
100	4.300	4.3	La1	Cs

DOI: 10.69946/ojms/2026.04.01.09, pp(86-96)

Table 5: Main elements in the sample according to the quantitative results of the EDS analysis

Elt	Line	Int	Error	K	Kr	W%	A%	ZAF	Ox%	Pk/Bg	Class	LConf	HConf	Cat#
O	Ka	73.9	3.6379	0.1317	0.1095	22.09	53.68	0.4954	0.00	116.42	A	20.47	23.72	0.00
Zn	Ka	102.7	1.0509	0.8683	0.7215	77.91	46.32	0.9261	0.00	27.25	A	73.05	82.77	0.00
				1.0000	0.8310	100.00	100.00		0.00					0.00

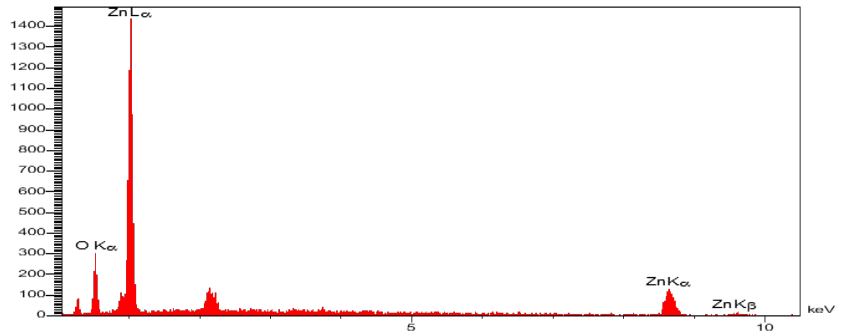

Fig. 4 EDS spectrum showing the elements present in the prepared zinc oxide nanoparticle

4. Discussion

GC–MS analysis of the alcoholic extract of *Ruta graveolens* .L indicates that this extract has a multi-component chemical signature, containing compounds belonging to more than one chemical family, including furanocoumarins, aliphatic ketones, terpene alcohols, and fatty acids. This diversity in the current results is consistent with what has been reported in recent studies on *Ruta graveolens* .L, which have shown that this plant is a rich source of secondary metabolites, particularly furanocoumarins, alkaloids, and volatile compounds, with the final chemical profile of the extract being influenced by the type of plant part used, the extraction method, and the conditions [10, 11].

The appearance of Psoralen, 3-(α,α-dimethylallyl)- alongside Chalepin, Bergapten, and Ficus in indicates a clear presence of the furanocoumarin class in the studied sample, which is one of the chemical classes associated with *Ruta graveolens* .L as mentioned [10]. In contrast, the appearance of 2-Undecanone and 2-Nonanone supports the known chemical pattern of the oils of this plant genus, as specialized studies have shown that *Ruta graveolens* L. oils are predominantly composed of aliphatic ketones, particularly 2-undecanone and 2-nonanone, as the most common and dominant components in this genus[12]. Likewise, the appearance of Phytol, α-Linolenic acid, n-Hexadecanoic acid and Ricinoleic acid indicates that the alcoholic extraction was not limited to the extraction of furanocoumarins only, but also included components of a terpenoid and fatty nature, which is consistent with what was mentioned [11].

Recent studies on the extraction of bioactive compounds from *Ruta graveolens* L have shown that varying extraction conditions and techniques lead to the acquisition of diverse chemical families from a single plant. Based on this, the potential functional and biological significance of the extract can be explained by its chemical components, rather than by any single compound. This explanation is particularly important in light of recent studies on *Ruta graveolens* L extracts demonstrating antioxidant, anti-inflammatory, and antimicrobial activities at the macro-extract level, which

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supports the assertion that the biological effect of this plant is often linked to the cumulative and synergistic effect of its various chemical components [13].

The DLS analysis results showed that the prepared ZnO-NPs fall in the nanoscale range, with a Z-average of 64.5 nm and a Mode of 87.3 nm. The presence of a single main peak in the distribution curve indicates that the sample has a predominance of particles in the suspension medium. However, the dispersion index (PI = 0.703) suggests size variation and relative heterogeneity among the particles. [14]. The DLS technique is one of the established tools for characterizing the physical properties of ZnO-NPs. Synthesis factors, such as temperature, pH, reaction time, and the ratio of plant extract to chemical composition, directly influence the size, shape, and phase of the nanoparticles; this logically explains the size variation observed in the results. Furthermore, the PI values obtained in these results can be explained by partial agglomeration or variations in particle size within the liquid medium. This aligns with the findings of [15].

DLS analysis of ZnO-NPs prepared from *Ruta graveolens* L. extract showed an average hydrodynamic diameter of 337.3 nm with a PDI value of 0.400, which was interpreted as indicating moderate volumetric pluripotency and particle aggregation, a common behavior in biologically synthesized systems. [16] The extract-to-salt ratio and mixing time clearly affected particle size and size variation, with increased mixing time resulting in larger and more variable particles. These findings support the interpretation that size variation does not reflect a failure of synthesis, but rather is related to the nature of the bioreactor and the preparation conditions. The discrepancy between the size recorded using DLS and the dimensions obtained from other microscopic examinations is scientifically expected.

The authors in [17] reported that the hydrodynamic diameter measured by DLS for ZnO-NPs was 335 nm with a PDI value of 0.187, which they considered evidence of a relatively homogeneous colloidal size distribution. This is explained by the fact that DLS measures the hydrodynamic diameter of particles in a suspension medium, a diameter influenced by the hydration layer, the biosphere, and the dispersion or agglomeration state, and not solely by the actual size of the particle nucleus. Therefore, the current results confirm the successful formation of ZnO-NPs within a suitable size range, with acceptable size variation related to the nature of the green synthesis and the biosphere used. Scanning electron microscopy images indicate that the prepared ZnO-NPs possess a quasi-spherical to irregular morphology, with a clear tendency to agglomerate and cluster into nanoclustered forms. This morphological pattern is expected in particles prepared using the green method, and is consistent with the findings of [18], SEM images of bioprepared ZnO-NPs nanoparticles using *Ruta graveolens* L showed a spherical structure with the particles clustering in clustered structures, which is directly consistent with the current results.

The authors in [14] also explained that the morphological pattern may be related to the role of plant chemical compounds as reducing and stabilizing agents, which may be reflected in the surface coverage of nanoparticles and the degree of their aggregation and partial clustering. In terms of size comparison, [19] indicated that SEM analysis of ZnO-NPs prepared using the green synthesis method showed average sizes ranging from 30 to 72 nm, depending on the pH, a range close to the direct measurements recorded in the current images (Figure 2) (29.86–32.89 nm). Therefore, the microscopic images confirm the success of ZnO-NP nanosynthesis, demonstrating that the resulting particles retained a distinct nanoscale character with partial agglomeration and a quasi-spherical morphology features common to many ZnO-NP systems prepared using the green synthesis method.

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The elemental analysis using EDS (as shown in Table 5) revealed that the prepared sample consisted primarily of zinc and oxygen. The weight percentage of zinc was 77.91%, compared to 22.09% for oxygen, while the atomic percentages were 46.32% for zinc and 53.68% for oxygen. These values, as shown in Fig. 4, indicate that zinc and oxygen are the main components of the sample, which supports the successful formation of zinc oxide nanoparticles. This result is consistent with what was stated in reference [7], as the study of ZnO-NPs nanoparticles prepared by green synthesis using the alcoholic extract of Pavonia zeylanica showed that the characterization of the particles included EDS analysis, and that zinc and oxygen were the main elements that appeared in the spectrum, with the possibility of the appearance of signals for other elements related to the biosynthesis medium.

The study [20] also supported the successful biosynthesis of ZnO-NPs nanoparticles mediated by Streptomyces baarnensis filtrate, as the results of HR-TEM, DLS and XRD confirmed the formation of the nanoparticles and their structural properties, which reinforces the explanation for the successful preparation of the particles in the current study. Also, the quantitative analysis in this study was limited to zinc and oxygen, despite the appearance of signals for other elements in the automated identification of the spectrum. This is consistent with what was mentioned by [21], as they explained that EDS confirmed the presence of zinc and oxygen as the primary elements in the sample, with the appearance of secondary amounts of other elements assigned to botanical metabolites or related minerals adsorbed on the surface.

In reference [22], the authors indicated that the EDS analysis of ZnO-NPs prepared from *Retama raetam* extract showed zinc and oxygen as the two primary components comprising a weight percentage of 55.6% and 18.5%, respectively, which supports the dominance of Zn and O in the current results, as the expected pattern of the prepared ZnO-NPs represents green synthesis. The EDS results indicate that zinc and oxygen are the two main elemental components of the prepared nanoparticles, which is consistent with the expected composition of zinc oxide nanoparticles. The presence of carbon in some samples does not necessarily indicate the absence of ZnO-NPs, but rather may be attributed to residues of plant-based organic compounds adsorbed onto the particle surface. This interpretation was supported by [23] when they studied ZnO-NPs prepared using *Leonotis ocymifolia* extract. EDS analysis revealed the presence of carbon in the source plant samples, and the researchers linked this to plant bioactive compounds responsible for coating and stabilizing the nanoparticles. Therefore, the current results can be considered a supporting indicator for the successful biosynthesis of ZnO-NPs with the predicted elemental composition.

5. Conclusions

The results obtained in this study indicate that the alcoholic extract of *Ruta graveolens* leaves possesses clear potential for use in the green synthesis of ZnO-NPs, due to its content of bioactive compounds capable of supporting reduction and stabilization processes. Furthermore, various characterization results demonstrated the successful preparation of particles within the nanoscale, exhibiting morphological and elemental properties that confirm the successful formation of ZnO-NPs. Therefore, the plant extract of *Ruta graveolens* leaves can be considered a promising bio-mediator for the environmentally friendly preparation of nanoparticles, supporting the potential application of this approach in future bio-nanotechnology applications.

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