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PHYTOCHEMISTRY AND BIOLOGICAL USES OF LEONOTIS NEPETAEFOLIA (L.) R.BR. ETHANOLIC EXTRACT ZINC OXIDE NANOPARTICLES

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Abstract

In the current work, a phytochemical analysis of the hydroethanolic extract of *Leonotisnepetaefolia* leaves was performed using the GC-MS analysis method. The extract was discovered to contain several phenolic chemicals. Plant extracts can be used to make metal nanoparticles, which is a quick, dependable, and inexpensive process when compared to other synthesis techniques. The biosynthesis, characterisation, and antibacterial activity of ZnONPs created from an extract of *Leonotisnepetaefolia* were examined in this study. Advanced instrumentation was used to analyze the shape, elemental composition, and other properties of nanoparticles. The average particle size was discovered to be 20 nm. The most visible damage is seen in scanning electron micrographs of the gram-negative bacteria Escherichia coli (E. coli) and the yeast Micrococcus luteus, which show damage to the cell walls. Between 320 and 335 nm, ZnO shows an impressive increase in absorbance in the UV-Vis spectrum. In the FTIR spectra, ZnO tensile vibrations at 426 cm-1 and 540 cm-1 were measured. The SEM investigation revealed that the particle size was between 30 and 40 nm. Particle size and particle load in zeta-size studies were 19 nm and -36 meV, respectively. Studies on the antibacterial activity of the produced nanoparticles revealed that they inhibited the growth of Escherichia coli (E. coli) and Micrococcus luteus. This study showed that ZnONPs may be made inexpensively and could be used as a carrier system for novel drug formulations in clinical therapy.

INTRODUCTION

The goal of nanotechnology is to create materials with dimensions of 1–100 nm, and this field of study is gaining prominence. Nanoparticles have several applications in fields as diverse as medicine, nutrition, aerospace, and the beauty industry. As nanoscale manufacturing has advanced, new applications have emerged for metal oxides like silver oxide (AgO), gold oxide (AuO), and other metallic nanoparticles. Scientists are very interested in zinc oxide (ZnO) nanoparticles because of their distinctive optical and electrical properties [15]. Using nanoparticles in biological systems is not addressed in any one section of the literature. After production, nanoparticles are studied for their activity in a wide range of applications. Zinc oxide nanoparticles (ZnONPs) have been shown to have cytotoxic effects against several different cancer cell lines and to impede the growth of several different bacterial strains [12]. It has been said that ZnONPs are superior drug delivery methods. ZnONPs with a particle size greater than 100 nm are reported to be biocompatible [1], and the US Food and Drug Administration approves of their usage as a drug delivery mechanism.

Keywords:
Leonotisnepetaefolia, Hydroethanolic extract, Zincoxide nanoparticles Phytochemical analysis, Antibacterial activity.

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Because it is affordable, biocompatible, and less poisonous than other metal oxide nanoparticles [14], ZnO offers a wider application potential. The bulk of pharmaceutically active compounds are proven not to interact with ZnONPs [19]. studied how the shape and size of ZnO nanoparticles affect their antimicrobial efficacy. Researchers found that the shape of nanoparticles significantly affected their antibacterial efficacy against Micrococcus luteus and Escherichia coli, with spherical nanoparticles being the most effective, followed by ellipsoidal and prismatic nanoparticles [2–4]. Green synthesis has replaced physical and chemical synthesis since it is cheaper and has fewer negative impacts on the environment. The term "green synthesis" refers to the process of creating nanoparticles without using any toxic chemicals [6]. The combination of medicinal plants and conventional medicines is common practice; depending on the plant and the medicine, this can either increase or decrease the therapeutic effect and so alter the anticipated outcome. The purpose of this study was to characterize and test the antimicrobial activity of ZnONPs synthesized from extracts of Leonotisnepetaefolia leaves grown in Africa, Madagascar, India, Indonesia, Australia, and the Pacific Islands using a straightforward and easy-to-carry-out procedure. GC-MS research looked at the photochemistry of the leaf extract, while UV-Vis, FTIR, SEM, zeta potential, and EDS analyses were used to characterize the produced ZnONPs. Nanoparticles produced in a lab were tested for their antibacterial activities against Micrococcus luteus and Escherichia coli [8–10].

MATERIALSANDMETHODS

All of the chemicals and reagents that were utilized in the research were obtained from Merck, a good chemical company in South Dakota, and ranged in purity from 98 to 99% AR grade. In order to produce the solutions, double-distilled water with a pH of 7.02 was used.

Plantmaterial

The whole Leonotisnepetifolia plant includes a diterpenoid called labdane, which has the chemical formula 8,17:9,13-diepoxylabdane-16,15:19, 6 dilactone. Coumarins include nepetaefolinol, leonotinine, and 4,6,7-trimethoxy-5-methyl chromen-2-one. Labdane diterpene (nepeteafolin, methoxynepetae-folin) is found in the leaves. Morin, apigenin, 3, 6-dihydroxyflavanes, p-coumaric acid, caffeic acid, kaempferol, 3, 7-dihydroxyflavane, galangin, naringenin, 6-hydroxyflavane, o-coumaric acid, and flavone have all been found in Leonotisnepetifolia.The plant is used to cure renal illness, rheumatism, dysmenorrhea, bronchial asthma, fever, and diarrhoea in Madagascar, Brazil, Canada, Kenya, and many African nations. Antibacterial, antirheumatic, anti-inflammatory, analgesic, and anticancer properties have all been attributed to this medication. According to K. Vasuki et al. [18].

Figure1:*Leonotisnepetaefol*

Obtaining leaf extract of *Leonotisnepetaefolia*

500 g of fresh leaves were hand-harvested from a Leonotisnepetaefolia plant in Kannauj District's rural area for use in the extraction testing. The leaves were gathered in the month of November 2022 and then dried for 15 days in the shade. After this period of drying, the samples' humidity was measured using a Dean and Stark instrument [13]. After that, the leaves were homogenized by being crushed in a multiprocessor for 30 seconds before being placed in properly sealed glass vials for storage. Before being used in the extractions, the raw material was kept in a 4°C household refrigerator. Following the methods outlined by Redfern et al. [10], 18 grams of sample (leaf powder) and 100 mL of solvent (80% water/ethanol solution) were placed in an Erlenmeyer flask. Cling film was used to cover the Erlenmeyer flask. Over the course of 24 hours, with intermittent stirring, the extraction was completed. After the extraction procedure was complete, the substance was put through a vacuum filtration system. The solvent was then completely evaporated from the extracts by heating them in an oven. The yield was ultimately determined by weighing the extracted material. The output of the procedure was determined using labor input [11]. Soxhlet extraction yields were between 1.53 and 19.74%. According to some reports, the solvent used has a significant

impact on the substances extracted. The variation in yields can be attributed to the presence of phytoconstituents such as steroids, terpenes, alkaloids, and phenols, which exhibit antibacterial, anti-inflammatory, and antioxidative activity when extracted using non-polar solvents. This will be confirmed via chromatographic studies of the extracted materials.

Characterization by gas chromatography Coupled to Mass Spectrometer (GC-MS)

The QP2010 Ultra GC/MS instrument and a ZB-5HT column (30 m x 0.25 mm x 0.25 m) were used for the analyses. The following were the parameters under which it was conducted: After heating for 1 minute at 60 °C, further 30 minutes are needed at 260 °C. The ion source was operating in scan mode, with an electron energy of 70 eV, an interface temperature of 120 °C, a carrier gas flow rate of 1 ml/min (helium), and no other parameters being changed. Each extract's components were identified after injection of 1L by comparing them to NIST17R libraries and Willey 8. [19]

Synthesis of ZnO nanoparticles

The resultant ethanolic extract of Leonotisnepetaefolia was mixed with 700 mL of 0.5 M zinc acetate dihydrate (Figure 2B). At 80 degrees Celsius, the liquids were swirled constantly for three hours. Precipitates formed after the reaction period was terminated by lowering the pH to about 7.0 with sodium hydroxide (0.5 M). After centrifuging the mixture at 4000 rpm for 20 minutes, we rinsed the sediment off using distilled water and 96% ethanol. After being dried at 100 degrees Celsius for an entire night, the samples were collected and claimed at 300 degrees Celsius. The extract synthesis resulted in a solid particle material, which was stored in hermetic containers until it could be described and used (Figure 2D).

Figure 2: (A)Ethanolic extract of *Leonotisnepetaefolia (*B) Zinc acetate di hydrate solution and (C)ZnONPs solution (D) ZnONPs solidparticulate material

Characterization of nanoparticles

Ultraviolet-visible spectroscopy (UV-Vis):

Band gap analysis required dispersing 50 mg of ZnO nanoparticles in 5 ml of water for each sample to make an aqueous solution, and then ultrasonically treating the solutions for 180 seconds. The samples were run through a 190-to-700-nanometer wavelength range using a Perkin Elmer UV/VIS Lamda 365 spectrophotometer for analysis.

FT-IR analysis: With Nicolet IS10 Thermo Scientific FT-IR equipment and a sweep from 500 cm-1 to 4000 cm-1 using the ATR method (100 scans were performed per reading and three readings were taken), we analyzed about 100 mg of ZnONPs using FT-IR.

Scanning electronmicroscopy (SEM) and Electronic dispersive X-ray (EDS) spectroscopic analysis: The sample was coated in gold using cathodic sputtering (JFC-110 ion sputter, JEOL) to improve the resolution of the final image. A scanning electron microscope (JEOL JSM7600F Tokyo, Japan) was used to take 5 Kev images of the samples. We used ImageJ, a program for processing and analyzing images, to determine the number of grains and crystals present and their average diameter. Histograms showing the distribution of sizes were created with the help of the graphics and data analysis application Origin Pro 8. We were able to determine the average diameter of each particle or crystal by measuring at least 180-200 in each of 5 photos. The chemical make-up of the sample was analyzed using energy dispersive spectroscopy (EDS) throughout an accelerating voltage range of 0-12 keV, again using the same JEOL JSM7600F electron microscope.

X-ray diffraction (XRD): The Bruker D2-Phaser X-ray diffractometer was used for the study of X-ray diffraction. The ZnO particles were crushed in an agate mortar before being loaded onto a support and placed atop a quartz plate for exposure to Cu K-1 radiation at a wavelength of 1.5406. The diffractometer was operated at 30 kV and 10 mA, with a two-range of 10-70°, a step size of 0.02°, and a count time of 1 s/step. Each Jade 6 diffraction pattern was used to calculate an estimated crystallite size using the Scherrer equation (Cullity, 1956).

High-resolution transmission electron microscopy (HRTEM)- ZnO samples synthesized with Leonotisnepetaefolia extracts were analyzed using transmission electron microscopy with lateral resolution of 0.23 nm and longitudinal resolution of 0.14 nm, respectively. The samples were prepared by ultrasonic dispersion of isopropyl alcohol, and a small amount of the suspension was deposited on a carbon film-coated copper grid and allowed to dry at

room temperature. A Gatan CCD camera (model SC200) was used to take the micrographs digitally. ImageJ and Origin Pro 2016 were used to create histograms and quantify particle sizes from TEM images.

DLS and Zeta potential (ζ) analysis: At room temperature (27°C), the hydrodynamic diameter (Dh)was determined by measuring dynamic light scattering (DLS) with a Zetasizer nano-ZS from MalvernInstruments (ZEN3500). This experiment used an ultrasonic bath to mix 0.001 grams of the samplewith 100 ml of water for 15 sec. Keep in mind that this technique provides an estimate of the meansize of the particles in the medium, even if those particles are too small to be detected individually.The zeta potential of nanoparticle solutions was also measured using the same apparatus at variousHCl and NaOH-adjusted pH levels. The effectiveness of 300 ppm solutions of Dh was measured atroomtemperature.

Determination of the minimum inhibitory concentration (MIC) of the treatment with ZnONP against *Micrococcus luteus and Escherichia coli*a liquid culture medium: These tests with a wide range of doses of both treatments with nanoparticles were conducted in sterile, 96-well plates with a flat bottom and a cover. Both the treatment and control wells were supplied with 50 L of a 4X-enriched minimum culture medium. Based on these calculations, the relevant volumes of sterile distilled water and nanoparticle solution were added to reach the targeted treatment concentrations. Microorganism solution at a concentration of 1x108 m.o./mL was added when the capacity in each well reached 200 L. The third, fourth, and fifth wells were used as "controls," or "no treatments" wells. Gentamicin at 5 micrograms per milliliter was used to cultivate Micrococcus luteus and Escherichia coli as a positive control. The plates were incubated at 37 degrees Celsius for 24 hours. There was no evidence of any microbe growth at the concentration. As a result, that was considered the MIC[16].

Determination of MIC of ZnONP against *P. aeruginosa* and *S. aureus* in solid culturemedium.: These experiments utilized an agarose-solidified enriched minimum culture medium. Before the media solidified, the treatments were added at the appropriate concentrations and stirred until uniform. In the absence of treatments, negative controls were created. To ensure sterility, each set of Petri plates was incubated at 37 degrees Celsius for a whole day. Each agar plate contained 200 l of a bacterial solution at a concentration of 1000 CFU/ml, which is equal to 200 CFU. All plates were incubated at 37 degrees Celsius for 24 hours, and then the colony forming units (CFUs) were counted and compared to the controls.

Results

GC-MSanalysis

Through GC/MS analysis, the qualitative and quantitative composition of the leaf extracts of *Leonotisnepetaefolia* was determined using the Soxhlet extraction method results were given (Figure3 and Table1). More than19 significant compounds were identified.

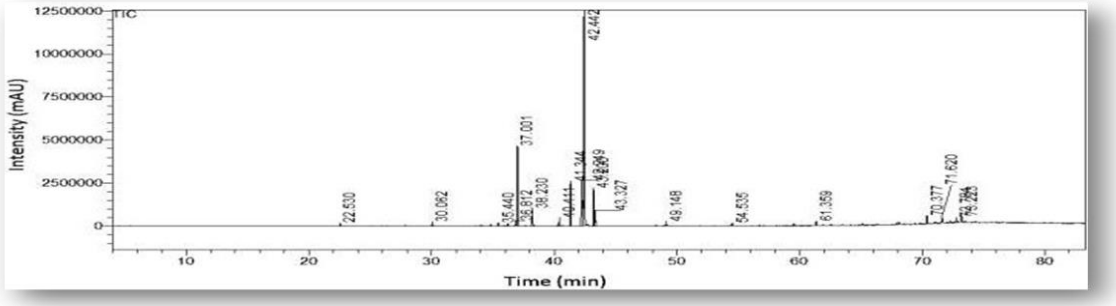


Figure 3: GC-MS of the hydroethanolic extract from the leaves of *Leonotisnepetaefolia obtained* by the hotextractionmethod using Soxhlet.

Table1:Chemical composition of the crude hydroethanolic extract from the leaves of *Leonotisnepetaefolia* obtained by the hot extraction method using Soxhlet

Peak	RT (min)	Compound	(%) GC-MS
1	22.53	Methyl laurate	0.38
2	30.06	Methyl myristate	0.64
3	35.44	Phytol	0.49
4	36.81	NI	0.87
5	37.00	Methyl palmitate	13.67
6	38.23	NI	2.92
7	40.41	γ-Undecanolide	1.32
8	41.34	γ-Decanolide	7.67
9	42.25	9,12-Octadecadienoic acid methyl ester	9.21

10	42.44	Methyl linoleate	46.98
11	43.20	6-Octadecynoic acid methyl ester	6.49
12	43.33	Methyl stearate	2.79
13	49.15	Arachidic acid methyl ester	0.74
14	54.54	Docosanoic acid methyl ester	0.45
15	61.36	Squalene	0.62
16	70.38	Stigmasterol	1.62
17	71.62	Stigmast-5-en-3β-ol	1.11
18	72.78	Stigmast-7-en-3β-ol	0.95
19	73.22	NI	1.07
Total	-	-	**95.13**

Obtainingandcharacterizing$ZnONPs$

Images captured during the synthesis of the ZnONPs are presented in Figure 4. These images show a similar reddish precipitate during the Leonotisnepetaefolia process to those reported in earlier studies [17]. The nature and chemical make-up of the natural extracts would be reflected in the hues of the resulting solutions and precipitate. Similar to what was reported by Al-Shabib et al. (2018), calcination gave the particles a grayish hue. In addition, the pH of the Leonotisnepetaefolia solution was 6.34 0.01 before being adjusted with sodium hydroxide (NaOH).

Figure 4: UV-Vis spectra of Leonotis nepetaefolia ethanolic extract-synthesized ZnO nanoparticles (solution color change). (pH 11, plant extract:metal ion ratio 3:7, 180 min reaction duration).

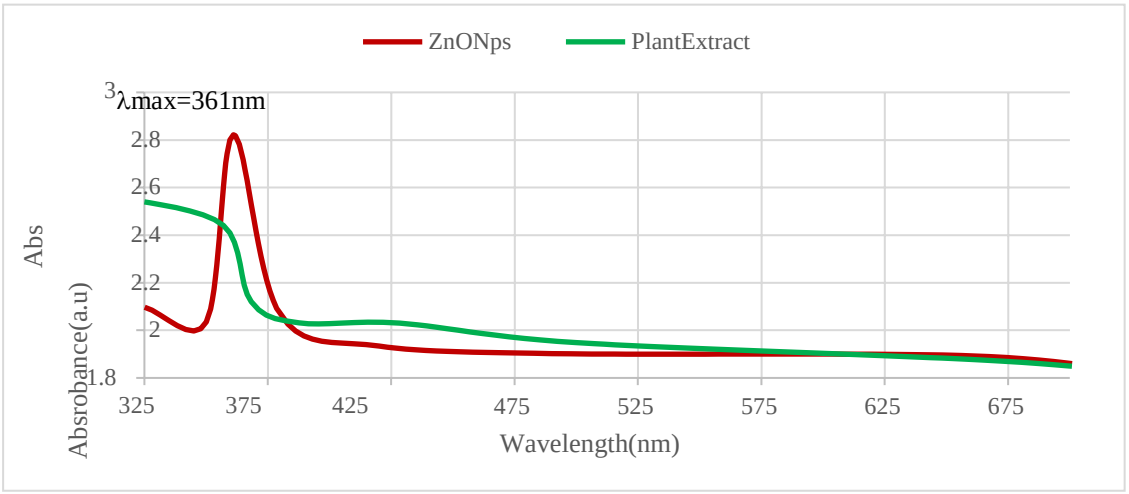
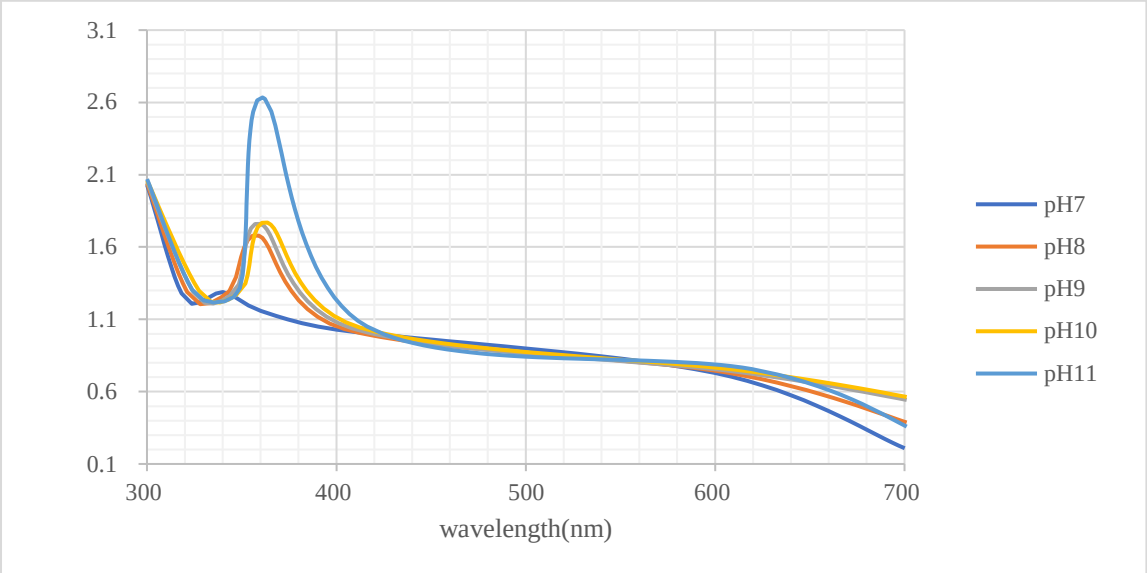


Figure 5. The effect of pH on the green synthesis of ZnONPs.



In Figure 4, the spectrum of the reactants (Leonotisnepetaefolia only) shows no characteristic absorption, unlike illustration nanoparticles, which change in the Uv-Visible spectrum after processing. As shown in Figure 5, the distinctive SPR band of ZnONPs was not visible at pH 7, but it rose with pH and peaked at pH 11.Figure 6 shows that when Leonotisnepetaefolia/metal ratio is at least 3:7, the solution turns light cherry color in 15 min and shows the characteristic surface plasman resonance absorption band (SPR) in the 320-370 nm region .

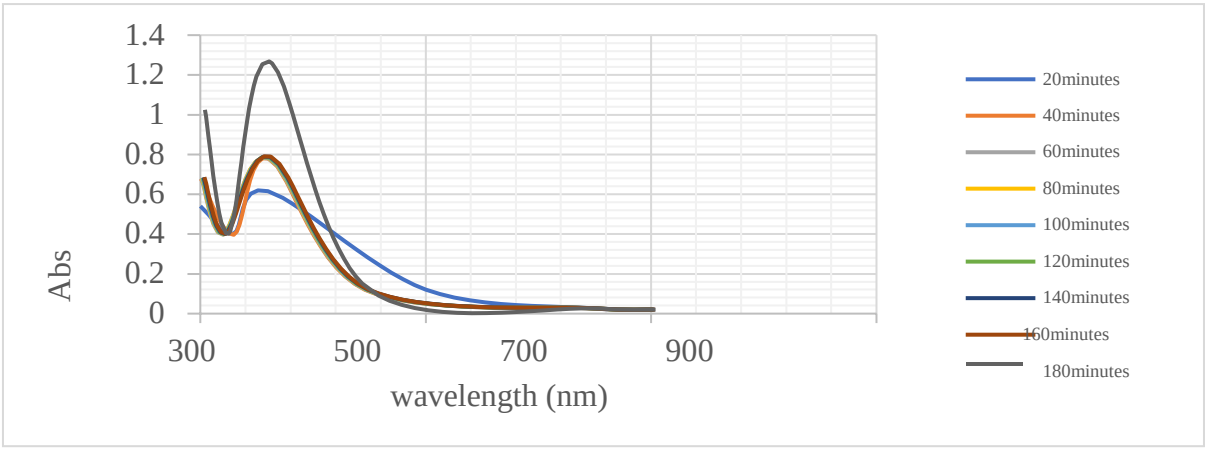


Figure6: The effect of reaction time on the formation of ZnONPs (*Leonotisnepetaefolia*/metal concentration ratio (3:7)(20, 40,60, 80, 100,120,180minfor 1-7 curves, respectively)

X-ray diffraction(XRD)

The results of X-ray diffraction performed on the ZnO sample produced in water are shown in Figure 7. Eleven ZnO-corresponding planes were seen, but their intensities did not stack up in the same order as the reference card's diffraction pattern. The peak (0 0 2) corresponded to the most intense diffracted plane, the peak (1 0 1) to the second most intense, and the peak (1 0 0) to the third most intense (Figure 7). For ZnO, the crystallographic card's intensity readings should go as follows: the plane with the highest intensity (1 0 1), the plane with the second highest reading (1 0 0), and the plane with the third highest reading (0 0 2). These three peaks are the only ones considered because they are in line with ZnO's signature planes [8]. Eleven peaks in the image have been pinpointed at coordinates (in degrees theta) 31.88°, 34.55°, 36.40°, 47.74°, 56.82°, 63.13°, 66.65°, 68.24°, 69.37°, 72.90°, and 77.28°. Additional peaks to ZnO were seen in the ZnO(p) sample, which were associated with the presence of Mg from the chlorophyll of the infusion utilized as a reducer during the ZnO manufacturing process [5].

Figure7:X-ray diffraction spectrum of ZnONPs synthesized from *Leonotisnepetaefolia* extract
SEM-EDS Characterization of nanoparticles

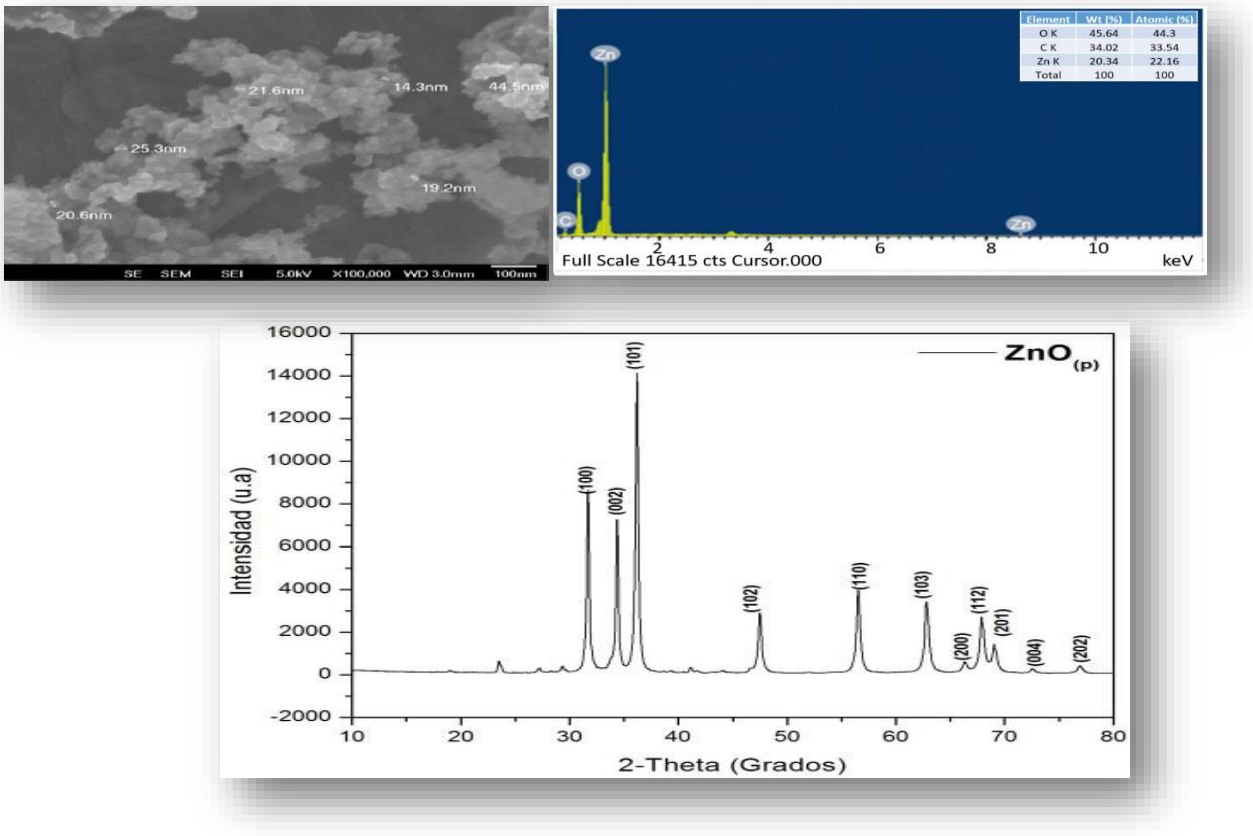


Figure8:(a) Scanning electronmicroscopy, in which zincoxide Nanoparticles, with a size range of 14.3to44.5nm.(b). SEM micrographs of ZnONPs and itsEDSstudy.

A topographic analysis was carried out by scanning electron microscopy to determine the morphologyand size of the ZnO nanoparticles obtained by green synthesis [5].Figures 8show the SEM-EDS studies of ZnONPs. In this case, the composition values of each of the elements present in the form of W%(percentage by weight) were obtained. The three samples show a similar composition; only as light increase in the amount of potassium ions is observed as the concentration of the extract used in the synthesis increases.

Zinc was confirmed by EDS spectra, which showed three peaks between 1 and 10 keV, the strongest of which was at one keV (Figure 8b). Since the stoichiometric ratio of oxygen to zinc is 60:30, thismeans there are no impurity peaks in the sample. As a capping agent for manufactured nanoparticles, stabilizing compounds from the plant extract could explain the spectra's carbon content. Multiple studies have discovered that Zn peaks in the same place on EDS spectra.

Transmission electron microscopy (TEM) and SAEDanalysis

Figure 9 shows the transmission electron microscopy (TEM) characterization results of the sample of the ZnO nanoparticles synthesized using plant extract. For the size of ZnO nanoparticles, more than250 nanoparticles were analyzed using Image J, showing the size distribution found for each sample.In Figure 8, the micrograph of ZnO can be seen, in which irregular shapes with minimal tendency toform semi-circles (ovoid) can be distinguished and with the study of size distribution nanoparticlesrangingfrom20to 40nm.

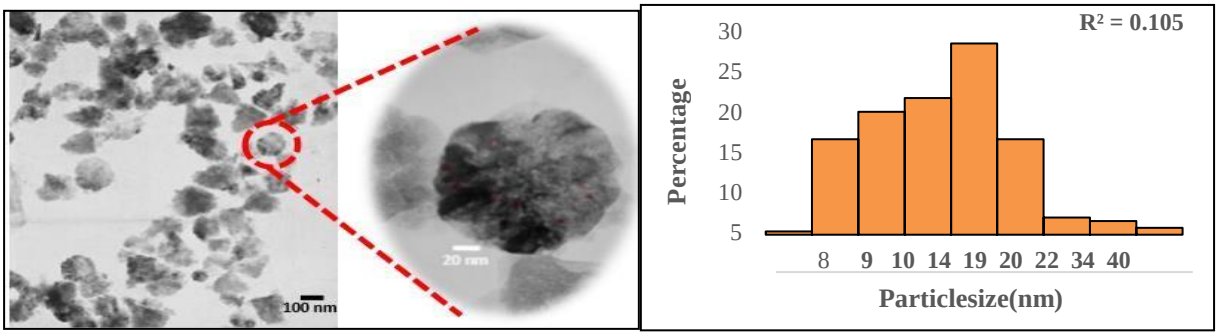
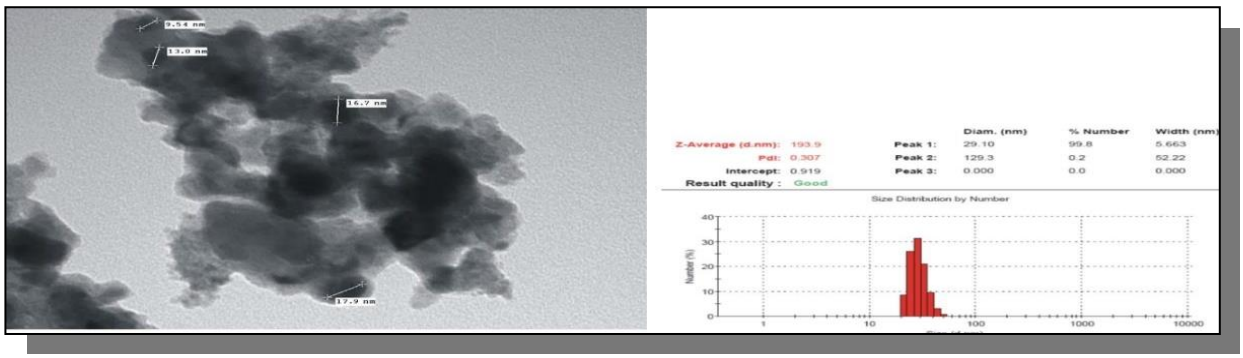


Figure9:TEM image and size distribution of ZnO nanoparticles.

The HRTEM micrographs are shown in Figure 9, where the effect on the morphology and sizes of thenanoparticles produced by the concentration of extract used in the synthesis [7]. Inaddition, the (101) plane is clearly observed for the three samples with an interplanar space of 0.24nm, directly associated with ZnO's hexagonal crystal system, proving that we have ZnO. The SAEDpatterns are shown in the boxes of Figure10, which were indexed according to the hexagonal structure(P63m),JCPDScardnumber36-1451,wherediffractionrings1,2,3and4,correspond ingtoplanes(100),(101),(102)and(101)respectivel y.

Figure 10: Morphology of ZnO nanoparticles through HRTEM. Show the electron diffraction pattern of ZnO.

Dynamic light scattering(DLS)analysis



Previously prepared stocks with a concentration of 1 g/l were used to prepare the samples with ZnOnanoparticles. DLS measurements of the ZnO nanoparticle size distribution are shown in Figure 11.The particles have an average size of 2R = 3 nm with a relatively narrow polydispersity ΔR = 0.6 nm.The size parameters in nm correspond to the hydrodynamic diameters obtained by DLS[4]. Apart from these results, the different types of distributions regarding size in intensity, numberand volume are also obtained using the Zetasizer Nano ZS software; which can provide additionalinformation. Figure 11 shows the size distribution of the ZnO nanoparticles obtained with the DLSnanosized, where it can be seen that 99.9% of the particles have an average diameter of 9 to 18nm and 0.1% have an average diameter of more than 20nm without showing that there are large agglomerations of nanoparticles and having a narrow size distribution.

Figure11:TEM analysis and DSL measurement of ZnO nanoparticles

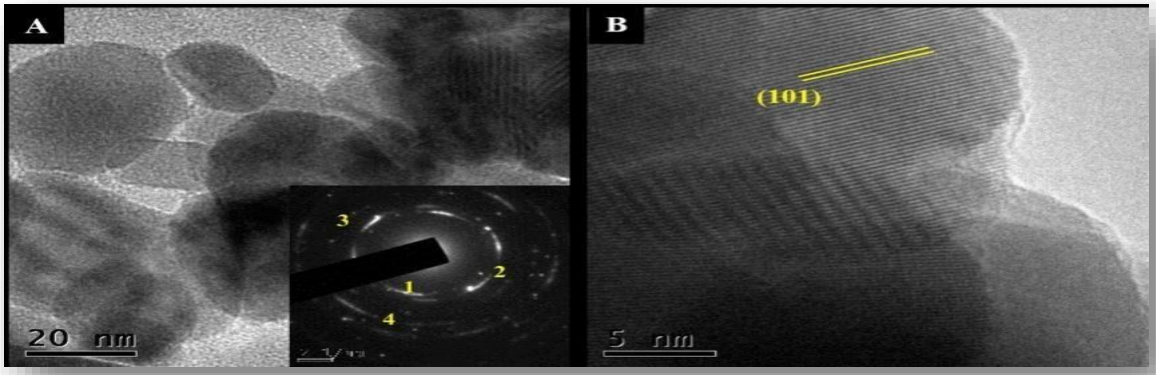


Figure 11 shows the DLS (right side) and the TEM of a sample of ZnONPs (left side) prepared fromethanolic extract of *Leonotisnepetaefolia*. The zeta potential determined (Figure 12) with the zeta meter for thesynthesized nanoparticles in sample A has a value of -36 mV, sufficient to prevent the nanoparticlesfrom agglomerating. Through this technique, the average size of the zinc oxide nanoparticles isdetermined,aswell as their size distributionand their zetapotential.If the zetapotential is in therange between -30 and +30 μV, it indicates that the particles can agglomerate. However, if it is lessthan-30 μV or more significant than +30, the particles will not agglomerate.

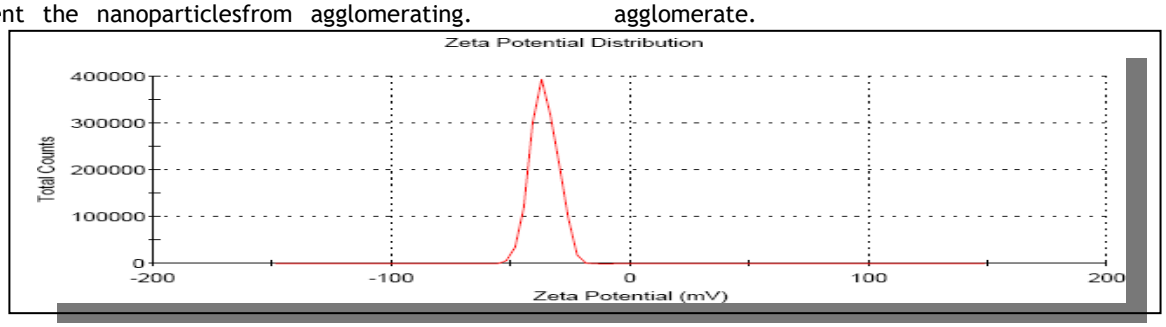
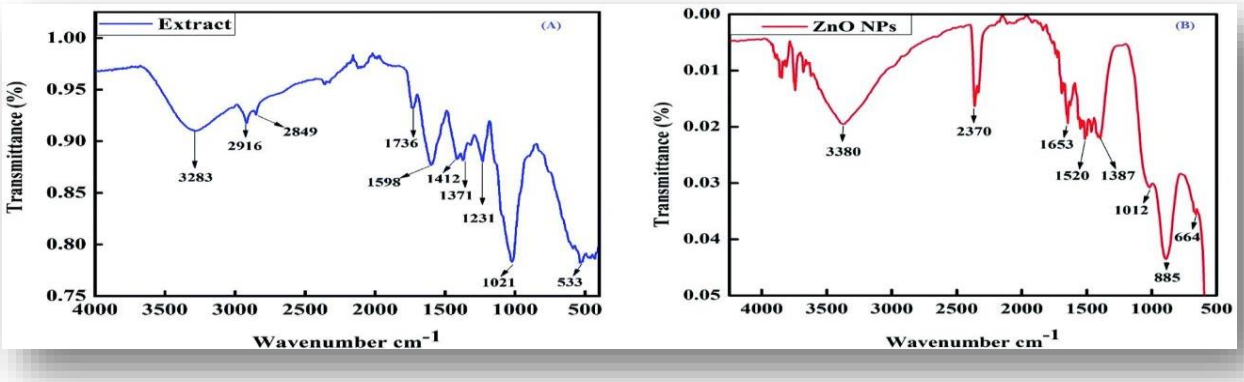


Figure12:ZetapotentialofZnOnanoparticlessynthesizedfrom*Leonotisnepetaefolia*extract.

Figure 13: FT-IR spectra and functional groups of ZnO nanoparticles synthesized at 300°C and Leonotisnepetaefolia extract. The C-N bond stretches between 1021 and 1012 cm-1. The prior bands and the one at 885 cm-1 suggest that phytochemicals (amines, ketones, alcohols, carboxylic acid, polyols, and terpenoids) produce ZnO.



FT-IR analysis

Figure 12 shows the results obtained from the lyophilized extract of *Leonotisnepetaefolia* compared with the spectra of the NPs obtained at 300 °C; the characteristic bands associated with the OH elongations areobserved at 3283 cm^{-1} of the hydroxyl groups (overlapping with –CH< at approximately 2933 cm^{-1})and >C=O at 1604 cm^{-1}, in addition at 1396 cm^{-1} a stretching of the carbonyl group. Also, bandsrelated to the stretching and bending of C-O bonds between 1000 and 1100 cm^{-1} are observed. Theband at 1604 cm^{-1} is related to the presence of alkene groups corresponding to terpenes, monoterpenesand sesquiterpenes reported in ethanolic extracts of *Leonotisnepetaefolia*

leaves. Additionally, in the spectrum, apeak located between 400 and 500 cm^{-1} is observed that corresponds to the vibration of the Zn-O bond of the ZnONPs.

Ahmad *et al*. (2022) reported FT-IR results of *Leonotisnepetaefolia* extracts with different solvents, with the same functional groups reported in this work: hydroxyl, carbonyl, and amines.

Biological studies

In vitro viability assays: Table 2 shows the values determined in assays in 96-well plates with minimalM9 medium:

Table 2: Comparison between the MIC of the nanoparticles in tests carried out in a liquid medium against *Micrococcus luteus and Escherichia coli*

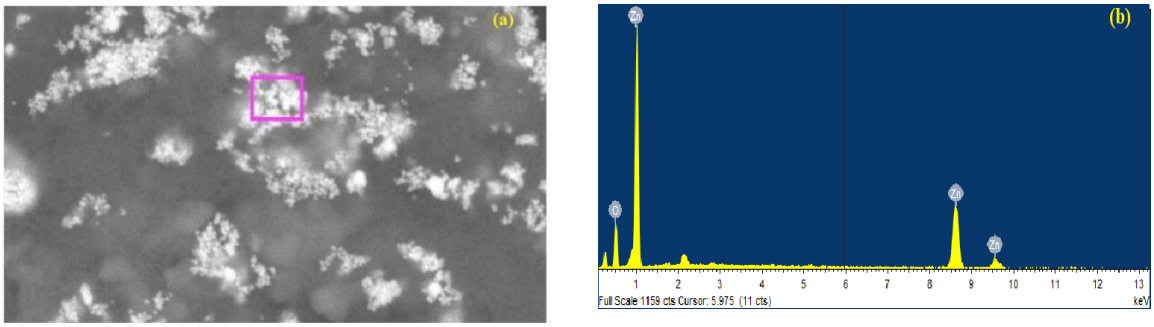
Microorganism	MICofZn-ONPinanaqueousmedium	MICofZn-ONPonsolidmedium
Micrococcus luteus	MIC7.0 µg/ml	MIC1.0 µg/ml
Escherichia coli	MIC5.5 µg/ml	MIC2.0 µg/ml

Cytotoxicity of zinc oxide nanoparticles provided by *Leonotisnepetaefolia* extract in liquid medium against *Micrococcus luteus* ATCC 29213 and *Escherichia coli* ATCC 15442. Zinc oxide nanoparticles presented inhibitoryactivity against bacteria, especially in a solid medium, since the MICs were much smaller than thoseobservedin aliquidmedium.

Bio film analysis by scanning electron microscopy

Bacteria were found to create a biofilm that collected the NPs during testing with a larger volume of the aqueous medium; therefore, it was chosen to investigate this material to determine whether or not it contained traces of the metals in question. The elemental analysis (Figure 14 b) showed that the carbon-based molecule, likely organic, contained ZnO NP. The biofilm reduces the number of bacteria exposed to the NPs, making the MIC higher in liquid medium. It's common knowledge that bacteria produce a layer of defense called exopolysaccharides to ward off predators and chemicals like antibiotics.

Figure 14: SEM analysis of the exopolysaccharides obtained after exposing *Micrococcus luteus* AgNP. (A)shows the micrograph of the biofilm produced by the bacteria after exposure to treatment. (B) shows the elemental analysis confirming the composition ofthe material captured in the biofilm.



Discussion

Since antiquity, researchers have recognized that plants are a natural and limitless source of information. Both metallic (MNPs) and non-metallic (NPs) nanoparticles may be extracted from plants and plant extracts, but they are done so in different ways. The manufacture of nanoparticles from plant parts and their extracts is straightforward and cheap compared to other methods utilized for comparable applications. GC/MS analysis was used to assess the quality and quantity of Leonotisnepetaefolia leaf extracts prepared using the Soxhlet extraction technique. Nearly twenty significant compounds were discovered. According to [12], adjusting the pH of the synthesis solutions might affect the number of ZnO nuclei and the development of the units, which in turn affects the shape of the nanoparticles. It has been shown that a neutral pH is appropriate for the green production of ZnO nanoparticles. The UV-Vis spectra of ZnONPs produced using Leonotisnepetaefolia alcoholic extracts are displayed in Figure 4. Figure 6 shows that the solution turned a light cherry color and the characteristic surface plasmon resonance absorption band (SPR) was obtained in the 320–370 nm range (depending on the particle size) when the E. serpens extract/metal concentration ratio was at least 3:7. On top of that, the Zinc oxide's and other elements' presences were verified. Figure 8a shows SEM micrographs of the ZnO sample, which has a hemispherical, scattered shape with few clusters and less surface area than the other samples, which have materials that take up more space as the concentration of the extract goes up. Three peaks were seen in the EDS spectrum, with the strongest one occurring at 1 keV, indicating the presence of zinc (Figure 8b). There shouldn't be any impurity peaks because the oxygen-to-zinc stoichiometric ratio is 60:30. Stabilizing compounds made from the plant extract are often used as a "cap" on man-made nanoparticles. This could explain why carbon was found in the spectra. It has been determined that Zn EDS spectra always show a peak in the same place.The average particle size of the ZnONPs powdered sample was found to be 20 nm using Transmission Electron Microscopy (TEM), and this size was confirmed using the dynamic light scattering test by scattering 0.001 grams of the powdered sample in 100 ml of reagent-grade acetone and sonicating for 15 seconds. Particles are monodispersed between 20 and 50 nm, as seen by the DLS size distribution and the DLS hydrodynamic diameter, which is larger than that found in the TEM. They have a round shape and are not clustered together. They are filtered out, washed three times in ethanol, and then dried in the natural environment for reuse. an innovative strategy for expanding effective, high-quality health care for human welfare through the green manufacturing of ZnO nanoparticles for antibacterial and vegetative growth applications. Published online ahead of print on December 2, 2020; DOI: 10.1016/j.sjbs.2020.12.02. Absorption bands between wave numbers 694-660 cm-1 may correspond to frequencies in the linkages Zn-O, as described by Saeed et al. , which again suggests the synthesis of ZnO. In plant extract, the region between 6094 and 660 cm1 shows no peak. As a result, it was established that plant extract does not contain Zn-O. Because of the biofilm, bacteria in liquid media have a higher MIC than those in solid media since they are less exposed to NPs. Bacteria frequently use the release of exopolysaccharides as a well-known defense mechanism against environmental and chemical agents, including antibiotics.

Conclusion

Using as a reducing agent the bio components of the ethanolic extract of Leonotisnepetaefolia leaves allowed for the green synthesis of ZnONPs with a particle size of less than 40 nm. This technology is a cost-effective, straightforward, and environmentally responsible approach. According to the findings of this research, ZnONPs are hazardous to the microorganisms that were investigated. According to the findings, the cellular structures of the various bacteria each possessed a unique set of mechanisms that inhibited their growth, and these processes were distinct from one another. Only in Micrococcus luteus and Escherichia coli that had been treated with ZnONPs was structural damage observed and validated. It's probable that enzymes have a role in the inhibitory mechanism of some other kinds of bacteria. The creation of biofilms is a defense strategy that microorganisms use to protect themselves against ZnONPs. This was proven by the fact that ZnONPs had less inhibitory action when they were in the presence of exopolysaccharides.

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DECLARATIONS

Conflict of Interest:

The authors declare no potential conflicts of interest.

Ethical approval:

This Article does not contain any studies with human participants or animals performed by the author.

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