Ministry of Higher Education
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Department of pharmacy

Green synthesis of zinc oxide nanoparticles using beetroot extract

A graduation Project

Submitted to the department of pharmacy in Partial Fulfillment of the Requirements for the Degree of Bachelor in Science of pharmacy

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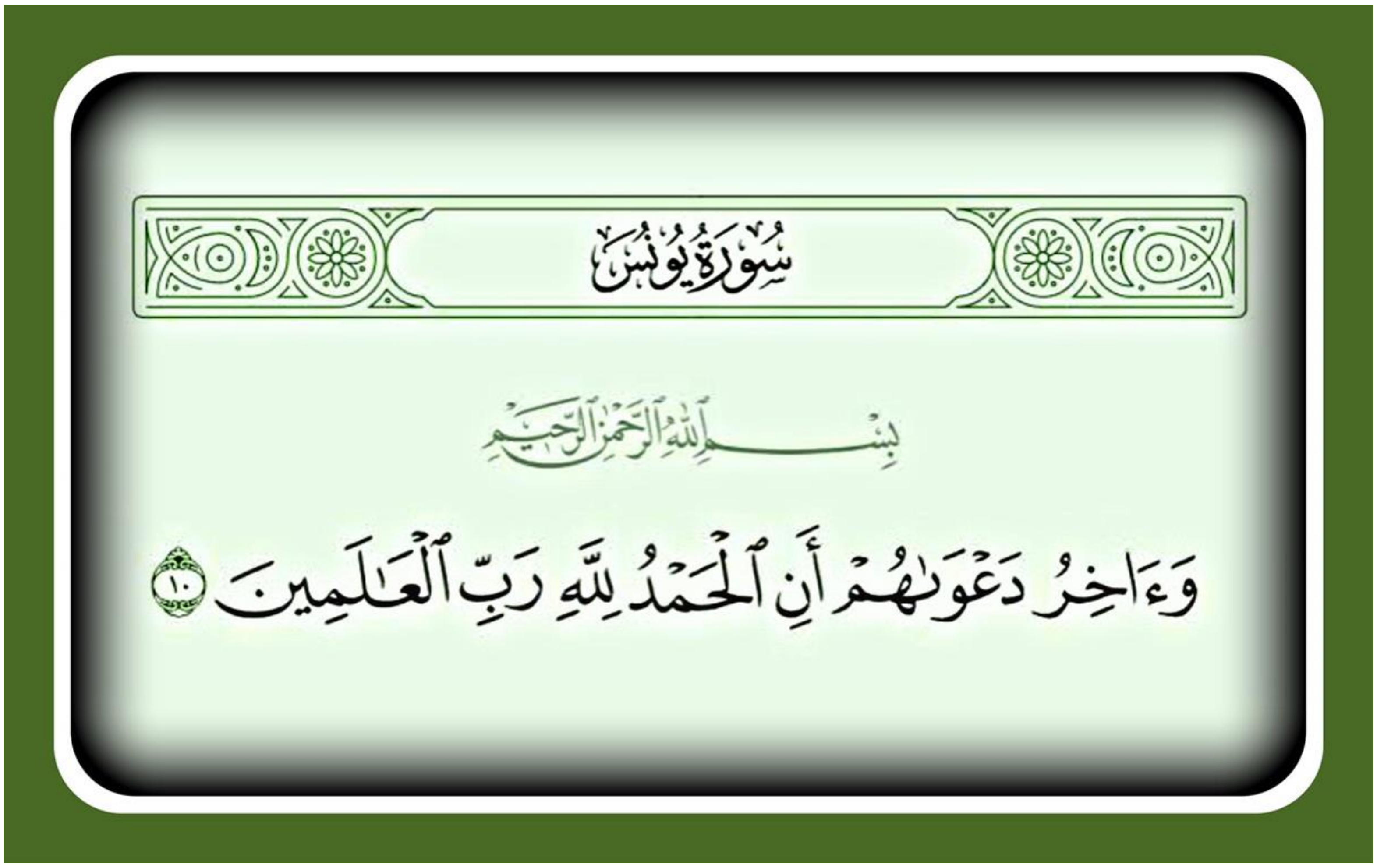

سُورَةُ يُونُسَ

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

وَءَاخِرُ دَعۡوَىٰهُمۡ أَنِ ٱلۡحَمۡدُ لِلَّهِ رَبِّ ٱلۡعَٰلَمِينَ ١٠

سورة يونس (اية 10)

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من قال أنا لها "نالها

لم تكن الرحلة قصيرة ولا ينبغي لها أن تكون، لم يكن الحلم قريبا و لا الطريق كان محفوفا بالتسهيلات

لكني فعلتها و نلتها

الحمد لله حبا و شكرا و امتنانا، الذي بفضله ها أنا اليوم أنظر إلى حلما طال انتظاره و قد أصبح واقعا أفتخر به...

إلى ملاكي الطاهر، وقوتي بعد الله داعمتي الأولى و الأبدية "امي" أهديك هذا الإنجاز الذي لولا تضحياتك لما كان له وجود، ممتنة لإن

الله قد اصطفاك لي من البشر أما يا خير سند و عوض.

إلى من دعمني بلا حدود و أعطاني بلا مقابل

"أبي ".

إلى من قيل فيهم

(سَنَشُدُّ عَضُدَكَ بِأَخِيكَ)

إلى من مد يده دون كلل ولا ملل وقت ضعفي

أخي" أدامك الله ضلعا ثابتا لي، إلى من آمنت بقدراتي و أمان أيامي أختي

الى رفاق الطريق ومؤنسيه الذين لم يقبضوا يدهم يوما عن مساعدتي شكرا لكم

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ABSTRACT

Numerous methodologies have historically been employed for the synthesis of nanoparticles (NPs). However, many of these approaches have proven to be expensive, yielding toxic byproducts and necessitating measures to mitigate chemical and physical contamination. Consequently, the adoption of green nanoparticle synthesis utilizing plant extracts has emerged as a sustainable alternative within nanotechnology, boasting applications across diverse fields.

In this study, beet root extract was utilized for the synthesis of ZnO nanoparticles. Through the utilization of scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FT-IR), we investigated and corroborated the presence of ZnO nanoparticles. The findings of this investigation underscore the efficacy of beet root extract in synthesizing ZnO nanoparticles, as evidenced by SEM and FT-IR analysis.

In conclusion, our study demonstrates the feasibility and effectiveness of utilizing Beet root extract for the eco-friendly synthesis of ZnO nanoparticles. By harnessing the power of nature, we pave the way for sustainable nanotechnology solutions with far-reaching implications across various scientific And and industrial domains.

Chapter one

INTRODUCTION

1.Background

Nanomaterials (NMs) have gained prominence in the 21st century owing to their ability and flexibility in addressing global research problems in fields of energy [1], water and water sanitation [2], medicine [3], agriculture [4], material science [5], and cosmetic industry [6]. In 1959, Richard Feynman's phrase [7], "There's plenty of Room at the Bottom" emerged as a versatile and flexible platform capable of delivering efficient, cost-effective, and environmentally friendly solutions to humanity's sustainability challenges.

The term NMs refers to materials with a diameter ranging between 1 and 100 nm.The large surface area and size of NMs result in their unique properties, which are tuneable to suit their application. Furthermore, the distinct size, shape, and structure of NMs influence their reactivity, toughness, and other properties. These properties make them good candidates for a wide range of industrial and household uses, including environmental, imaging, medical, energy, catalysis, cosmetics, and medical applications [8].

Metal oxide nanoparticles (NPs) such as copper oxide (CuO), tin oxide (SnO2), zinc oxide (ZnO), and titanium dioxide (TiO2) are among the most studied NMs due to their numerous diverse properties and applications. Zinc oxide is one such metal oxide that has received extensive research due to its chemical stability, non-toxic nature, cost-effectiveness, biocompatibility, and biodegradable nature [9,10]. To date, a number of different chemical and physical synthesis methods, including laser ablation, vapour deposition, electrodeposition, sol-gel, spray pyrolysis, hydrothermal, and microwave-assisted methods, have been employed to prepare ZnO NPs. However, some of these synthesis methods are hampered by high costs and the usage of hazardous chemicals and solvents that are harmful to the environment, as well as by high energy consumption. [10,11]. As a result, new synthesis methods for the fabrication of ZnO NPs that are both safe and cost-effective are required. Thus, attention has been drawn toward green/biological synthesis as a substitute to conventional chemical and physical synthesis methods.

Plants, bacteria, yeast, and algae have successfully been used to synthesise ZnO NPs, with plants showing great potential. This is due to plant-mediated synthesis being easily scalable for industrial fabrication, simple, environmentally friendly, cost-effective, and time-effective. Plants are known to possess secondary metabolites known as phytochemicals, which include phenols, saponins, tannins, alkaloids, flavonoids, and carbohydrates that can be used as alternatives to toxic organic and inorganic chemicals, which are 2ynthesi as reducing or 2ynthesized agents in conventional preparation of ZnO NPs [12,13].

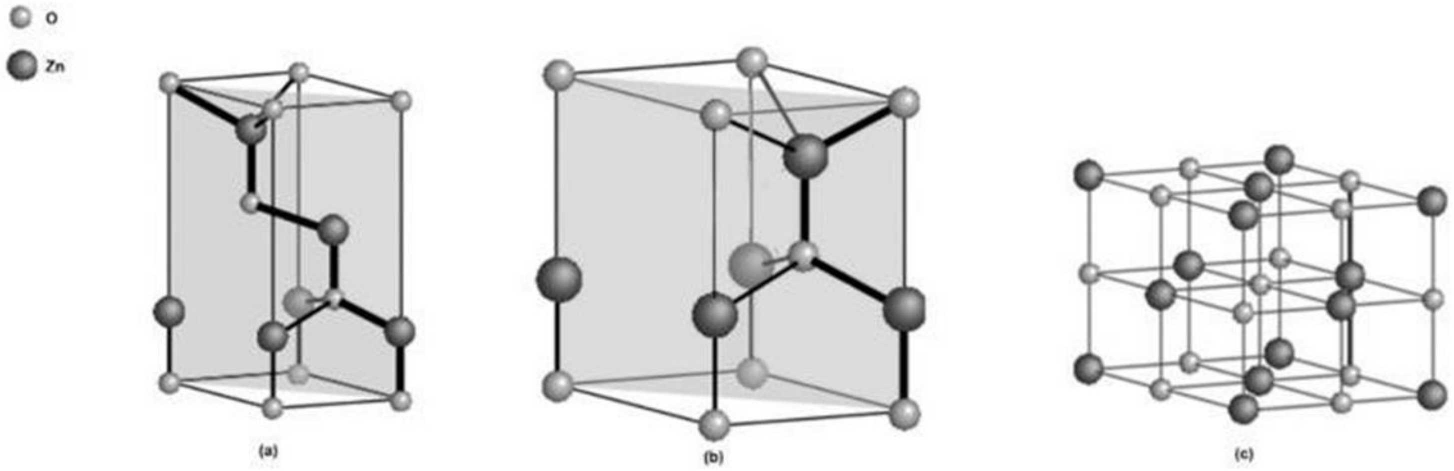
Cassia fistula [16], Euphorbia hirta [14], Rosa indica [15], Costus pictus [16], and Solanum nigrum [17] are medicinal plants that have been successfully employed in the fabrication of ZnO NPs. South Africa, found in southern Africa, is not only home to a unique and diverse plant species, with some of these species having medicinal properties, but it is also mostly endemic [18]. However, there have been few reports on the use of traditional medicinal plants from southern Africa in the preparation of ZnO NPs. As a result, there is a need to investigate more medicinal plants for the preparation of ZnO NPs.

the majority of ZnO NPs created from medicinal plants have been investigated for biological uses such as antibacterial and anticancer properties. This is not shocking considering that NPs produced by utilising plant-mediated synthesis were found to have superior biological characteristics in vitro compared to NPs made using traditional chemical techniques and plant extracts. In addition to biological applications, the varistor application and photodegradation of organic dyes have been investigated for the ZnO NPs synthesised utilising local medicinal plants. This demonstrates the versatility of ZnO NPs produced by plants.

Agathosma betulina [19], Plumbago auriculata [20], Monsomia burkeana [21], Lessertia 3ynthes [22], Lessertia frutescens [23], Tulbaghia violacea [24], Aspalathus linearis [25], Dovyalis caffra [26,27], and Athrixia phylicoides DC [28] are some of the traditional medicinal plants that are endemic to South Africa and southern Africa that have been investigated for the fabrication of ZnO NPs and the as-synthesised ZnO NPs explored for various applications. This synthesis will focus on *Beetroot extract* that have been explored for the preparation of ZnO NPs. It will focus on this *extract* overview, synthesized ion. We believe that this project will draw more attention to the green synthesis of ZnO NPs using medicinal plants and lead to more exploration of the vast diversity of plant species in Iraq.

1.1. Properties of ZnO NPs

The hexagonal wurtzite, cubic zinc blende, and rock salt shown in (Fig.1)are the three known crystalline phases of ZnO. ZnO is commonly found in the wurtzite phase because it is stable under ambient conditions. Both the rock salt and the zinc blende are cubic and unstable under ambient conditions. Growing the zinc blende on cubic substrates makes it stable, whereas the rock salt phase can be obtained by converting ZnO under relatively high pressure [29]. The wurtzite phase belongs to the P63mc space group and has a hexagonal geometry with two interconnecting sublattices of Zn2+ and O2− such that each Zn ion is surrounded by four tetrahedral O2− ions and vice-versa. This geometry lacks inversion symmetry along the hexagonal axis giving rise to a polarity symmetry and resulting in piezoelectric and spontaneous 3ynthesized3 properties [30].ZnO NP is a low-cost n-type bandgap semiconductor that is dielectric, transparent, and piezoelectric. It has a wide bandgap of 3.37 eV at room temperature (RT), large exciton binding energy of 60 meV and high thermal conductivity. This allows its application in optoelectronics, gas sensors, photocatalysis, aerospace, light-emitting diodes, and photovoltaics. ZnO NPs are biocompatible, anti-inflammatory, anticancer, and have wound healing properties, which make them suitable for biomedical applications [31,32].



Figur-1: Crystal structure models of ZnO: (a) zinc blende, (b) wurtzite and (c) rock salt. [1]

1.2. Synthesis of ZnO NPs

An ideal synthesis method for the preparation of NPs needs to be low-cost, eco-friendly, able to produce high-quality NPs of desired morphology and size to suit applications of the NPs. Physical, chemical, and biological synthesis methods have been employed in the preparation of NPs, and these can be classified into top-down and bottom-up approaches, as illustrated in (Fig.2). The top-down approach includes both physical and chemical synthesis methods, which generally involve using chemicals or force to break down bulk material into smaller particles. The top-down approach methods include ball milling, laser ablation, chemical etching, laser pyrolysis, mechanochemical, solid state, etc. [10,33].

The chemical synthesis methods that fall under the bottom-up approach include sol-gel, hydrothermal, microwave irradiation, spray pyrolysis, chemical precipitation, solvochemical, microemulsion, etc. This approach involves nucleation of ions, atoms or molecules in solution followed by aggregation of the nanoparticles. It is the approach that has been extensively reported for the preparation of NPs. Both physical and chemical conventional synthesis processes mentioned above have been reported for the preparation of ZnO NPs. However, some of these conventional synthesis routes are limited by high energy consumption and high pressure and require complex equipment, resulting in a high overall cost [33,34].

Moreover, these methods can also be limited by toxic chemicals used during synthesis, which can be harmful to the environment and the person handling the chemicals. These toxic chemicals are mostly used as reducing agents or capping agents and include sodium borohydride, hydrazine, ethylene glycol, dimethylformamide, cetyltrimethylammonium bromide (CTAB), pyridine, polyethylene glycol, etc. [35]. Therefore, safer and cost-effective synthesis methods are needed for the preparation of NPs. Thus, green synthesis using microorganisms or plant extracts can be a substitute to conventional chemical and physical synthesis methods.

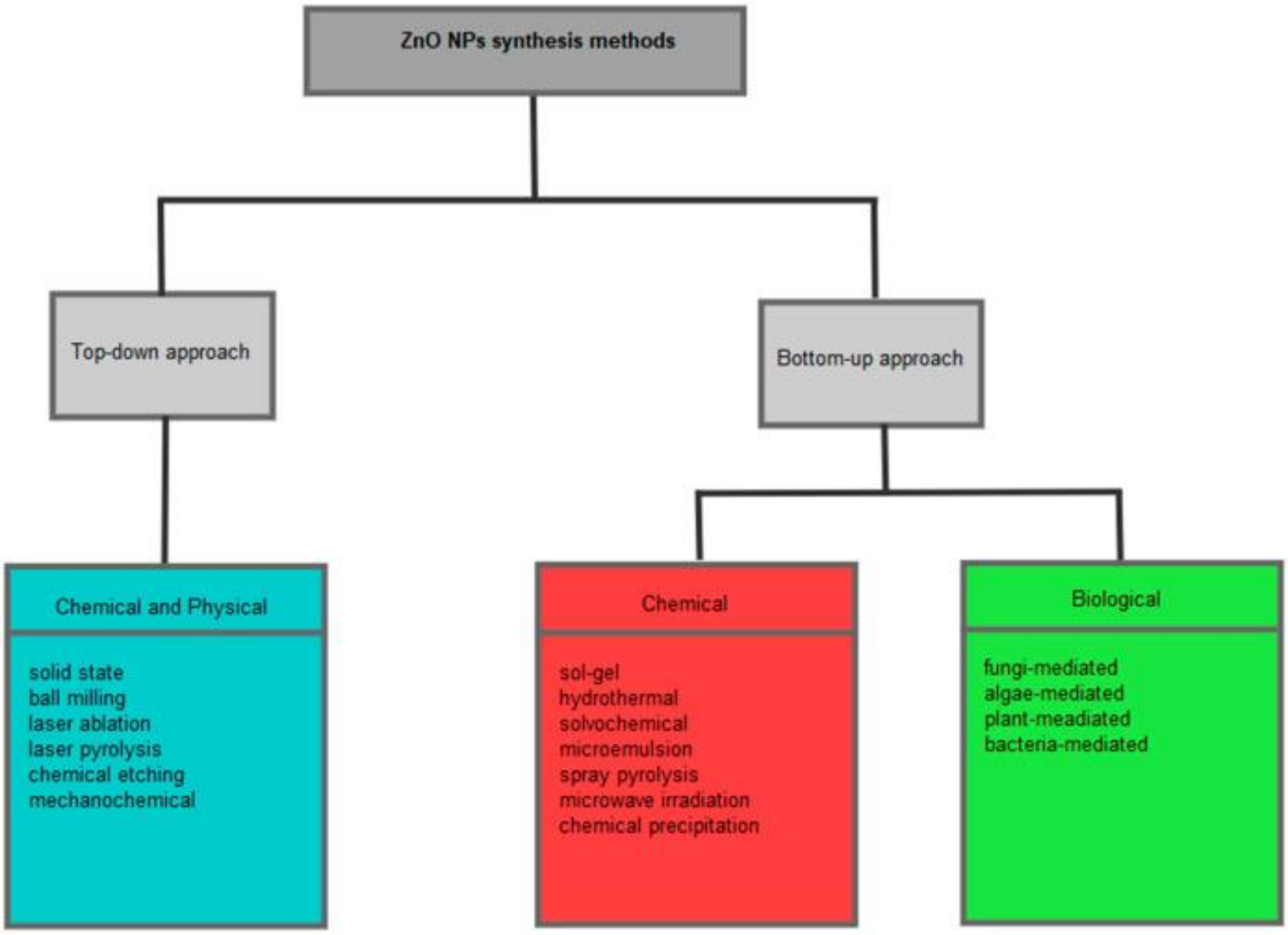


Figure-2: Overview of some of the methods used for ZnO NPs preparation[2].

1.3.Green Synthesis

Biological synthesis is a bottom-up approach that synthesi plants, fungi, bacteria, algae, etc., for the preparation of NPs. This green synthesis approach eliminates the need for reducing and capping agents during the synthesis of NPs. This is due to the ability of microbes and plants to reduce metal salt precursors while also synthesized the synthesized NPs using naturally occurring substances such as enzymes and secondary metabolites. Thus, making biological synthesis a safer and low-cost alternative to conventional NPs synthesis methods. Suba and associates [36] successfully synthesized ZnO NPs using bacteria isolated from cow milk, and the synthesized ZnO NPs exhibited good antibacterial and anticancer activity.

The ZnO NPs were hexagonal in structure and spherical in shape and the average particle size was reported to be 32 nm. Additionally, Hefny et al. [41] successfully synthesized ZnO NPs using five fungal cultures isolated from five fungal species (Aspergillus niger, Aspergillus tubulin, Aspergillus fumigatus, Penicillium citrinum, and Fusarium oxysporum) and the synthesized ZnO NPs also exhibited good antibacterial activity. The synthesized ZnO NPs were crystalline with a hexagonal wurtzite structure, which is the typical structure of ZnO, and the particle size was between 30 and 100 nm. These studies demonstrate that ZnO NPs can be synthesized using microbe-assisted synthesis. However, isolation and growth of the microbes can be time-consuming, and contamination should be strictly avoided during the process; thus, it requires very skilled personnel to handle the process [37]. This has led to research efforts more focused on plant-mediated synthesis as plants are readily available, and their extracts can be extracted with simpler processes. (fig.3)

Chapter two

2.1. Materials

$Zn(CH_3COO)_2 . 2H_2O$ (AR grade), calcium chloride (AR grade) and NaOH (AR grade) were purchased from SRL (India). Rhodamine B & Methylene blue dyes (AR grade) were procured from Sigma-Aldrich (USA). All of the chemicals used were not purified any further. The experiments were carried out at optimum pressure and temperature conditions. The reaction water used was distilled doubly.

2.2. Method

2.2.1. Preparation of beetroot extract

Beetroots were purchased from the local market and washed with distilled water(DW) to remove dust particles. 20 g were grinded in a beaker and boiled with distilled water (100 ml) for 15 min. Then we kept the beaker for cooling. After cooling, the solution was filtered to remove the insoluble part. The wine-colored solution was then stored in glass bottles and kept in the fridge for further use.

Figure-4: liquid beet root extraction

Figure-5: filtering liquid beet root extraction

2.2.2. Prepration of zinc oxide nanoparticles

To synthesise ZnO nanoparticles, 40 ml of beetroot extract was transferred in a 100 CC beaker having a magnetic bead. The solution was heated to 100 °C followed by the addition of 4.5 g of zinc acetate (fig.6). The pH of the solution was maintained between 8.0. It was stirred at this temperature and pH for 40 min. till the formation of pale-yellow; the whole nanoparticles formation process is demonstrated in (Fig.7) The resulting solution was centrifuged and washed using double distilled water at 80 °C in the oven were dried.

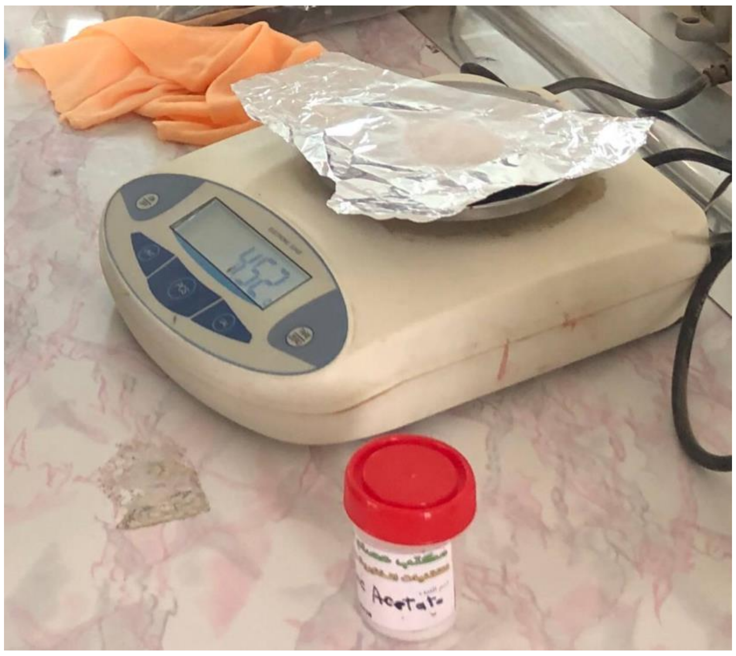


Fig-6: weight of zinc acetate by balance.

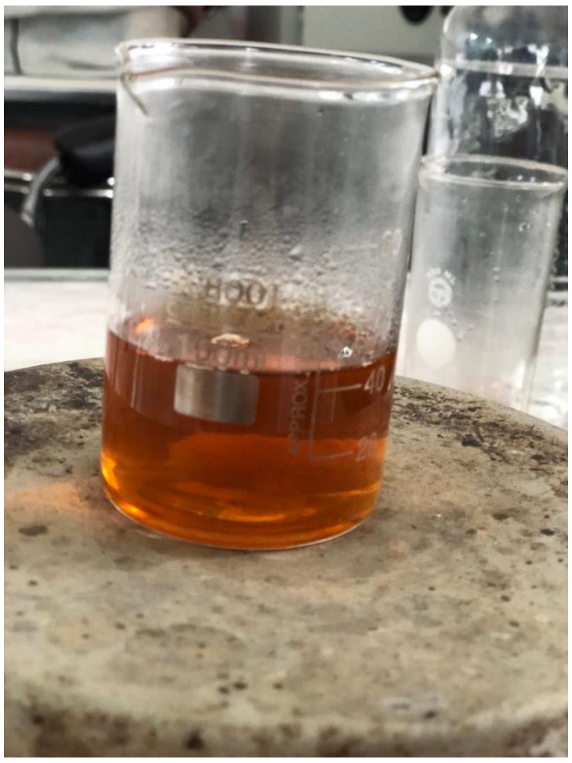


Fig-7: formation of ZnO nanoparticles.

2.2.3. FT-IR Method

In our analyses, we have performed two methods; the first method is the FT-IR method.

To perform FT-IR spectroscopy on ZnO nanoparticles synthesized using Beet root extract (ZnB nanoparticles), several steps are involved:

- **Sample Preparation:** In this, we have prepared the synthesized ZnB nanoparticles for analysis. Typically, that involves dispersing the nanoparticles in a suitable solvent or medium to form a uniform suspension or thin film. We have ensured that the sample preparation method does not alter the structural or chemical properties of the nanoparticles.
- **Instrument Setup:** We have configured the FT-IR spectrometer properly for the analysis, such as calibrating the instrument, setting the appropriate spectral range, and selecting the desired resolution and scan parameters.
- **Baseline Correction:** Before acquiring the spectrum of the ZnB nanoparticles, we performed the following baseline correction to account for any contributions from the solvent or medium. This step ensures that the observed spectral features are primarily attributed to the nanoparticles and not interference from the surrounding environment.

- $$\frac{1000 * w}{50 * 256.41}$$

  $$=0.6$$

- **Acquisition of Spectrum:** Once the instrument is calibrated and the baseline corrected, the FT-IR spectrum of the ZnB nanoparticles can be acquired. This involves illuminating the sample with infrared radiation over a range of wavelengths and measuring the intensity of the transmitted or reflected light as a function of wavelength.

- **Interpretation of Spectrum:** After acquiring the spectrum, it needs to be analyzed to identify characteristic peaks and features. Peaks corresponding to specific molecular vibrations or functional groups are assigned based on known spectral patterns and literature references. Peaks in the fingerprint region are particularly informative for identifying organic compounds from the beet root extract and any surface functional groups on the ZnB nanoparticles.
- **Data Analysis:** Once the spectrum is interpreted, we performed the data analysis to quantify the intensity of specific peaks, assess peak shifts or changes compared to reference spectra, and extract relevant information about the composition and structure of the ZnB nanoparticles.
- **Reporting and Interpretation:** Finally, the results of the FT-IR analysis are reported and interpreted in the context of the synthesis process, the role of Beet root extract as a bio- template, and the potential implications for the properties and applications of the ZnB nanoparticles as shown in the result section.

2.2.4.SEM of ZnO nanoparticles Method

Here are the steps that are involved in acquiring Scanning Electron Micrographs (SEM) of ZnO nanoparticles:

- **Sample Preparation:** In this step, we have prepared the ZnO nanoparticles for SEM analysis. This typically involves dispersing the nanoparticles onto a suitable substrate. Depending on the specific requirements of the analysis, the nanoparticles may be dispersed in a solvent and drop-cast onto a conductive substrate such as a silicon wafer or a carbon-coated grid. Care should be taken to ensure that the nanoparticles are evenly distributed on the substrate to facilitate accurate imaging.

- **Instrument Setup:** In this step, we have adjusted parameters in the SEM instrument such as accelerating voltage, beam current, working distance, and aperture size to optimize imaging conditions for the specific sample being analyzed.
- **Sample Loading:** The prepared sample is then loaded into the SEM chamber and positioned on the sample stage.
- **Vacuum Pumping:** Before imaging, we evacuated the SEM chamber to create a high-vacuum environment, which helps us to minimize scattering of the electron beam by air molecules and ensures optimal imaging conditions.
- **Electron Beam Scanning:** Once the vacuum is established, the electron beam is scanned across the surface of the sample in a raster pattern. As the electron beam interacts with the sample, various signals are generated, including secondary electrons, backscattered electrons, and characteristic X-rays.
- **Image Formation:** The signals generated by the interaction of the electron beam with the sample are collected by detectors within the SEM chamber and used to form an image of the sample surface as shown in our result.
- **Image Analysis:** After acquiring the SEM images, they could be analyzed using specialized software to extract quantitative data such as particle size, shape, and distribution. Image analysis techniques such as particle counting, size measurement, and morphological characterization can provide valuable insights into the properties of the ZnO nanoparticles.
- **Interpretation and Reporting:** Finally, the SEM images and analysis results are interpreted in the context of the synthesis process, sample preparation methods, and the properties of the ZnO nanoparticles.

Chapter three

3.1. Result and discussion

Scanning Electron micrographs (SEM) of ZnO nanoparticles show that the surface morphology of the SEM images depicts the nanoparticles as, aggregated and dense rock- shaped flakes.

The uniform distribution of ZnO nanoparticles was observed on the entire surface as shown in (Fig-8) A, B, and C (40, 20, 10, respectively). Therefore, that can be represented as an improvement of the ability of beet root extraction to be a good method for ZnO nanoparticle creation.

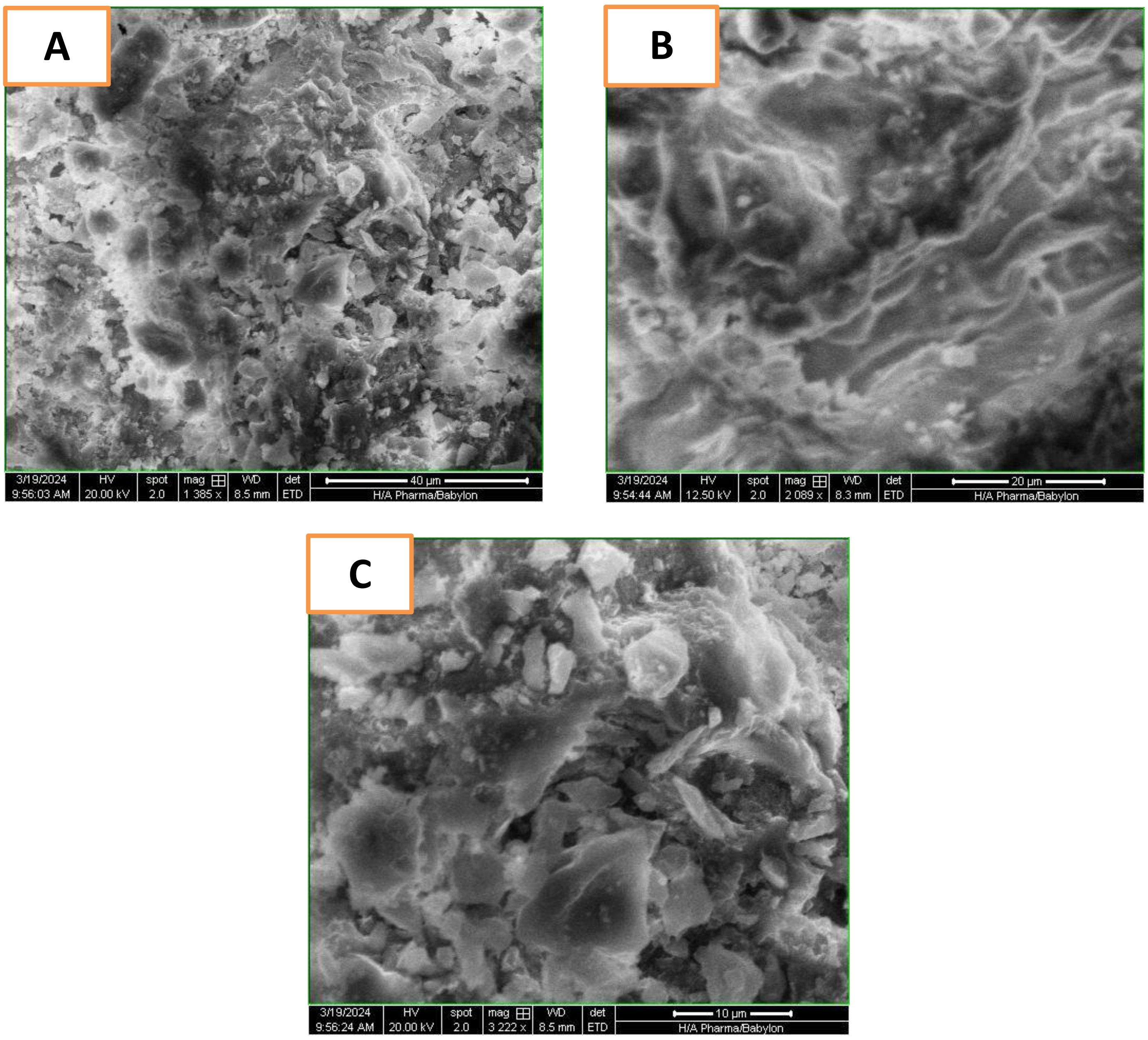


Fig-8: The SEM and Elemental composition of the synthesized zinc oxide nanoparticles, a (40µm), b (20 µm), c (10 µm)

The nanoparticles appear predominantly as small and uniform spheres, characterized by their smooth surfaces and relatively homogeneous size distribution. The nanoparticles in Fig.8(C) appear to have a variety of shapes, including spherical, quasi- spherical, and irregular shapes. Moreover, a scale bar indicating a length of 30 µm, reveals a diverse array of morphologies and shapes. The nanoparticles exhibit predominantly spherical and quasi-spherical shapes, characterized by smooth surfaces and relatively uniform size distributions as shown in Fi.8(A). Therefore, that can be represented as an improvement in the ability of Beet root extraction to be a good method for ZnO nanoparticle creation.[39]

Moreover, the uniform distribution of particles over the whole surface can be observed. The aggregation could be due to the interactions and Van der Waals forces between the ZnONPs [40]. Researchers also reported that larger grains could be attributed to the Oswald ripening process, with limited porosity and crystallinity [41].

Another result has been reported using the FT-IR method, Fourier transform infrared spectroscopy (FT-IR) was used to identify the possible biomolecules that are responsible for the reduction and capping of ZnO NPs. Fig.9 presents the FT-IR spectra of MgO NPs synthesized using the Aloe Vera herb extract. The spectra show bands at 3415, 1458, 1063, 868, 667 and 438 cm−1. The strong infrared band near 3415 cm−1 was observed for the O–H bond vibrations of the hydroxy group, A study by *Bindhu et al. (2014*) on green synthesis of ZnO nanoparticles using plant extracts reported similar absorption peaks associated with hydroxyl groups [42].

The most intense band at 1458 cm−1 represents vibrations C=O, The absorption peak at this wavenumber indicates C-H bending vibrations, particularly associated with alkyl groups. Organic compounds present in Beet root extract may contribute to this peak, acting as stabilizing agents during nanoparticle synthesis. Some researchers on the synthesis of silver nanoparticles using Beet root extract reported similar absorption peaks at around 1460 cm-1, attributed to the stretching vibrations of C-H bonds in aromatic compounds present in the plant extract [43].

Similar peaks have been reported in studies on the synthesis of metal oxide nanoparticles, confirming the presence of ZnO. The absorption at 869cm-1 is due to C-N stretching in amines, potentially originating from organic compounds in the Beet root extract. A study on green synthesis of ZnO nanoparticles using plant extracts observed similar absorption peaks associated with organic functional groups [44].

The (fig-9) below shows the strong sharp absorption at 1458 cm-1 implies the presence of an aromatic ring and in-plane bending of C-H and C-C bonds of the alkyl groups and aromatic ring. The absorption at 869cm-1 is due to C-N stretching in amines.

The band at 668 and 438 cm-1 confirmed the metal oxide linkage (ZnO). The broad and intense band at 3415 cm-1 indicates the presence of stretching vibrations of O–H groups in water.

Further characterization techniques such as Raman spectroscopy could provide additional insights. Additionally, we can confirm these observations that FT-IR spectroscopy is a powerful tool for characterizing ZnO nanoparticles synthesized using beet root plant extract. The observed absorption bands reveal insights into the chemical composition and surface functionalization of the nanoparticles. Green synthesis methods utilizing plant extracts offer sustainable approaches for producing functional nanoparticles with diverse applications.

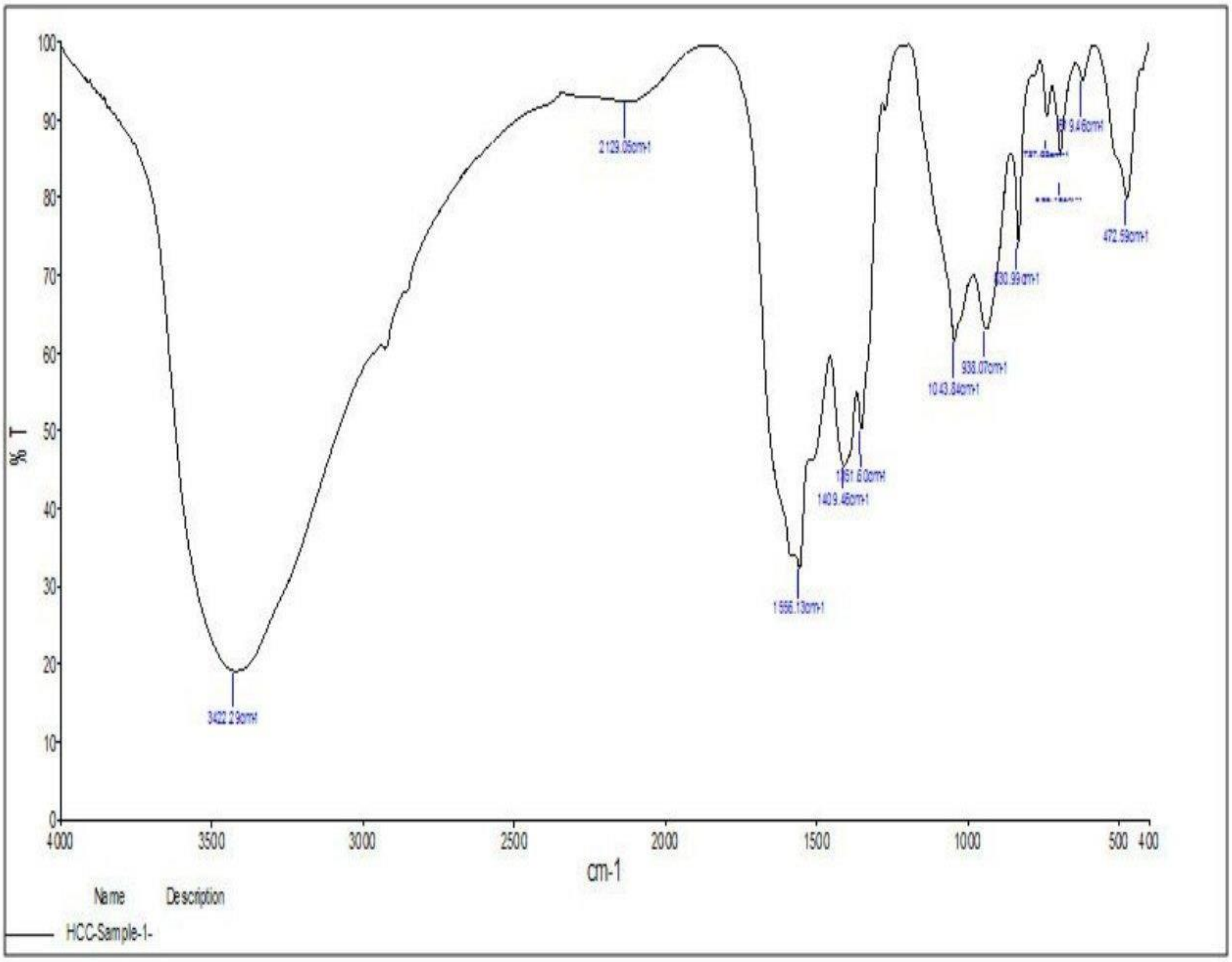


Figure-9: FT-IR of Beta vulgaris aqueous extract[4].

3.2.Conclusion

In conclusion, the findings of our study underscore the remarkable feasibility and efficacy of leveraging beet root extract in the eco-friendly synthesis of Zinc oxide (ZnO) nanoparticles. Through this innovative approach, we not only achieve successful nanoparticle synthesis but also significantly reduce the environmental footprint associated with traditional chemical methods. By harnessing the inherent properties of Beet root, a natural and renewable resource, we unlock a pathway towards sustainable nanotechnology solutions. This pioneering research not only showcases the potential of bio-inspired synthesis techniques but also highlights the importance of interdisciplinary collaboration between biology, chemistry, and materials science. The implications of our work extend far beyond the confines of the laboratory, offering promising avenues for applications in diverse scientific and industrial sectors. From biomedicine to environmental remediation, the utilization of Beet root for nanoparticle synthesis opens doors to environmentally conscious innovations with the potential to revolutionize numerous fields. Embracing nature's versatility and resilience, we chart a course towards a more sustainable future, where the principles of green chemistry intersect with cutting-edge nanotechnology to address pressing global challenges.

الخلاصة

في الختام، تؤكد نتائج دراستنا على الجدوى والفعالية الملحوظة للاستفادة من مستخلص جذر البنجر في التوليف الصديق للبيئة لجسيمات أكسيد الزنك (ZnO) النانوية. ومن خلال هذا النهج المبتكر، لا نحقق تخليقًا ناجحًا للجسيمات النانوية فحسب، بل نحد أيضًا بشكل كبير من البصمة البيئية المرتبطة بالطرق الكيميائية التقليدية. ومن خلال تسخير الخصائص المتأصلة لجذر البنجر، وهو مورد طبيعي ومتجدد، فإننا نفتح الطريق نحو حلول تكنولوجيا النانو المستدامة.

لا يعرض هذا البحث الرائد إمكانات تقنيات التوليف المستوحاة من الحياة فحسب، بل يسلط الضوء أيضًا على أهمية التعاون متعدد التخصصات بين علم الأحياء والكيمياء وعلوم المواد. تمتد آثار عملنا إلى ما هو أبعد من حدود المختبر، مما يوفر طرقًا واعدة للتطبيقات في مختلف القطاعات العلمية والصناعية.

من الطب الحيوي إلى المعالجة البيئية، فإن استخدام جذر البنجر لتخليق الجسيمات النانوية يفتح الأبواب أمام ابتكارات صديقة للبيئة مع إمكانية إحداث ثورة في العديد من المجالات. ومن خلال احتضان تنوع الطبيعة ومرونتها، فإننا نرسم مسارًا نحو مستقبل أكثر استدامة، حيث تتقاطع مبادئ الكيمياء الخضراء مع تكنولوجيا النانو المتطورة لمواجهة التحديات العالمية الملحة.

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