Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

The Role Of Cucurbitaceae Family "Pumpkin" Seed Aqueous Extract In The Synthesis Of Zinc Oxide Nanoparticles And Their Potential Applications As Antimicrobial Agents

Hayat Zerrouki[1*], Khadra Mokadem[1], Mohammed Said Nedjimi[1], Salah Tlili[2], Abdelhakim Medjdoub[1]

[1] VPRS Laboratory , Faculty of Mathematics & Matter Sciences University of Kasdi Merbah, , B.P. 511, 30000, Ouargla, Algeria.
[2]LARENZA Laboratory , Faculty of Mathematics & Matter Sciences Kasdi Merbah Ouargla University, , 30000, Ouargla, Algeria
Corresponding Authors: Hayat Zerrouki, zerroukih2020@gmail.com
Received : 25/05/2024 ; Accepted : 16/09/2024 ; Published : 04/11/2024

Zinc oxide nanoparticles (ZnO-NPs) have wide applications in various fields. Synthesis of nanoparticles using plants is an alternative to conventional physical and chemical methods. Due to its ease, eco-friendliness, broad antimicrobial activity and diverse applications in many health, agricultural fields etc., in this study, we present the synthesis of zinc oxide nanoparticles (ZnO-NPs) using pumpkin seed aqueous extract. The prepared ZnO-NPs were characterized using UV-Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis. In addition, this study determined the antimicrobial activity of zinc oxide nanoparticles synthesized using a reference strain of Candida albicans ATCC 10231 and four bacterial strains, including two Gram-negative strains, Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922, and two Gram-positive strains, Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 25973. These nanoparticles showed effective against most strains, with negligible activity against E. coli and no activity against Candida albicans. They are variable in their activity against the rest of the strains studied, e.g. Pseudomonas aeruginosa (15 mm at 80 µg/ml compared to 33 mm in CIP), Staphylococcus aureus (14.6 mm at 80 µg/ml compared to 33 mm in CIP), and no activity against most strains when using the 10 µg/ml concentration of ZnO nanoparticles.

Keywords: Zinc oxide nanoparticles, Biosynthesis, Pumpkin, antibacterial activity.

1. Introduction

In recent years, the scientific community has shown interest in nanoscale materials with unique physical, chemical and biological properties .[1] Zinc oxide is widely used with a wide range of applications in a number of fields such as catalyst, sensor materials, ceramics, thermoelectric materials, glass, superconducting materials and antimicrobial activity [2]. Nanoscale Zinc oxide is very interesting due to its potential applications in many fields such as heterogeneous catalysts, antimicrobials, antioxidants, imaging agents and drug delivery agents in biomedicine [3]. The size of nanostructures typically ranges from 1 to 100 nm as compared to large biological molecules such as enzymes, receptors etc. Nanoparticles (NPs) can interact with biomolecules and have utility in cancer diagnosis and therapy [4,5].

The potential risks associated with chemical synthesis methods, coupled with growing environmental concerns, have shifted the focus towards green and sustainable approaches for nanoparticle synthesis. The use of biological materials, especially plant extracts and microorganisms, provides a compelling argument for developing eco-friendly, cost-effective and safe alternatives [6,7].Biosynthesis can be carried out using plant parts, secondary metabolites of plants (secondary metabolites of leaves, roots, stems, fruits and flowers), and microbes and their secondary metabolites [8]. In recent years, metals such as copper (Cu), silver (Ag), zinc (Zn), iron (Fe), gold (Au), silicon (Si), nickel (Ni), and platinum (Pt) have been used in the green synthesis of nanoparticles for biomedical applications [9]. It also takes advantage of the bioactive compounds present in these natural resources to enhance the miniaturization and stability of nanoparticles. Zinc oxide (ZnO) is considered one of the best photocatalysts for the degradation of organic pollutants due to its high photosensitivity and non-toxic nature [10–12] . for the synthesis of . Zinc oxide nanoparticles (ZnO NPs). The synthesis of ZnO nanoparticles using plant extracts appears to be a preferred and environmentally friendly approach in the field of nanotechnology [13]. ZnO nanoparticles exhibit exceptional properties and a wide range of applications in many scientific and industrial fields, such as catalysis, electronics, energy storage, and biomedicine [14]. In recent years, the use of plant extracts as reducing and stabilizing agents for the green synthesis of nanoparticles has gained significant attention due to their sustainable and environmentally friendly nature [15]. Conventional methods for producing zinc oxide nanoparticles often involve the use of hazardous chemicals and high temperatures,
In this research, we aim to study the effect of starting materials on the structural properties of ZnO nanostructures synthesized by green synthesis of ZnO nanoparticles using aqueous extract of **pumpkin seeds** and their potential applications as antimicrobial activity. The prepared precipitates were analyzed using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) techniques. Furthermore, the potential antimicrobial properties of the synthesized ZnO nanoparticles were evaluated against different bacterial strains, such as Escherichia coli (ATCC 25922) ,Pseudomonas aeruginosa(ATCC 27853), Staphylococcus aureus (ATCC 25932), and Bacillus subtilis (ATCC 25973).

2. Presentation of plants

The origin of the word "squash" has been attributed to the native American word "Wata hti" (true squash) - "Wata miha sned" (long squash), etc. Fewkes [16]; according to whom one of the southern sides of the Hopi Indian people is identified with the Patun family, originated from the equines seeds that were found in the mortuary bowls.[17]Squashes and pumpkins, **(Fig.1)** with a strong preference for gourds are the fruits most frequently depicted in ancient iconography (Whitaker and Davis 1962); only maize, of course, surpasses them in the riches of relics found. The bottle gourd dates back thousands of light years and was one of the first agricultural plant crop farming of the Incas as well as the Mexicans. Europeans thought that it was a native to the old world which is considered to be Africa **,** Cucurbita moschata is well known archaeologically in both Mexico (5000 B.C.) and Peru (3000 B.C.).[18] The **(Fig.2)** shows a distribution map of wild cucurbit species with their occurrences based on 7,000 records from GBIF.

Fig.1: Pumpkin fruits

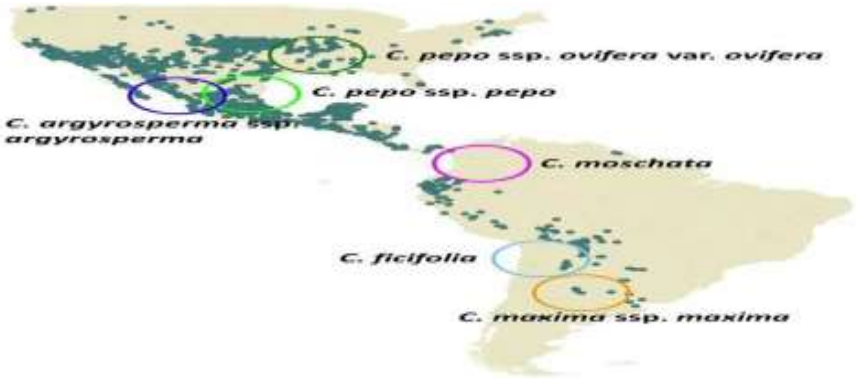

Fig. 2: . Distribution map of wild species of Cucurbita with occurrences based on 7000 records from GBIF

(www.gbif.org, accessed on 15 November 2020). Hypothesized origins of domestication for the five Cucurbita species are indicated by open circles. C pepo was split into the two cultivated spp. C. pepo ssp. ovifera: dark green; C. pepo ssp. pepo: light green; C. argyrosperma ssp. argyrosperma: blue; C. moschata: magenta; C. ficifolia: light blue; C. maxima ssp. maxima: orange Copyright 2021 Kates, Lopez-Anido, Sánchez-de la Vega, Eguiarte, Soltis and Soltis[19]

3. Chemical composition

Compounds and the whole range of nutrient profiles of the pumpkin depend on the type of the species or the variety. The moisture content of the fried pumpkin pulp ranged between 75.81 and 91.33% while the crude protein was between 0.2 and 2.7%, 0.47 and 2.1% crude ash, and 3.91 and 13% carbohydrate [20]. Pumpkin-based products or fruits are filled with plenty of different nutrients such as polysaccharides, proteins, important amino acids, provitamin A preparations, carotenoids and minerals. Seeds of pumpkin are very fat rich, and it may be because of their broad genetic diversity which may vary the oil content. Pumpkins can save on oil costs due to its high nutritive values (in table 1) and may also serve as a good source of protein. This type of oil is almost uniquely suited for enriching the nutritional values of other foods because of having a high content of iron, zinc, and linoleic and alpha-linoleic acids.[21]

4. Pumpkin Seeds

Pumpkin seeds contain phytoestrogens (lignans and other plant compounds) that have estrogenic properties. They are also rich in fatty acids, proteins , and vitamins A&E. The **(table. 1)** shows the biologically active components and their percentage in pumpkin seeds (nutritional value per 100 grams).[21] Thus, the benefits are manifold, to include anti-inflammatory properties. The seeds contain alkaline and are used in a variety of food products which is directly we can add sweetness in our food items. Moreover, they have mainly easily digested by the digestive system of the the body. Pumpkin seeds come in green color and they are wrapped with protective white coverings. The outcomes of which different research has become evident some health benefits of the seeds such as lowering risk or type 2 diabetes, weight loss and cholesterol level, and improving brain healthy. These polyunsaturated fatty acids are abundant in this food, and they can reduce blood pressure levels. Nowadays, the scientific studies of the health protective value of protein and oil from the seeds and polysaccharides from the pulp of the pumpkin fruit are contributory to the interest of such food processing industries as feed, pharmaceutical, and even the pumpkin fruit producers to the pumpkin derived products in the modern times.[22]

Table .1: Bioactive components and their percentage in Pumpkin seed (nutritive value per 100 g)

Components	nutritive value	Percentage of RDA	Components	nutritive value	Percentage of RDA
Energy	559 kcal	28	Electrolytes		
Carbohydrates	10.71 g	8	Sodium	7 mg	0.5
Protein	30.23 g	54	Potassium	809 mg	17
Total fat	49.05 g	164	Minerals		
Cholesterol	0 mg	0	Calcium	46 mg	4.5
Dietary fibre	6 g	16	Copper	1.343 mg	159
Vitamins			Iron	8.82 mg	110
Folate	58 µg	15	Magnesium	592 mg	148
Niacin	4.987 mg	31	Manganese	4.543 mg	19
Pa ntothenic acid	0.750 mg	15	Phosphorus	1,233 mg	176
Pyridoxine	0.143 mg	11	Selenium	9.4 µg	17
Pyridoxine	0.143 mg	11	Zinc	7.81 mg	71
Riboflavin	0.153 mg	12	Phytonutrients		
Thiamine	0.27 mg	23	Carotene-b	9 µg	-
Vitamin A	16 IU	0.5	Cryptoxanthin-b	1 µg	-
Vitamin C	1.9µg	3	Lutein–zeaxanthin	74 µg	-

5. Sample collection and preparation:

5.1.Washing and drying:

Pumpkin fruits were obtained from the daily market of the city of Metlili, Ghardaia province, and it is known that it is from an agricultural area in the city of Hassi El Fahel. All its seeds were collected and washed well with water and then with distilled water and dried in the open air for 15 days.

5.2.Extract preparation:

After drying the sample, the extract was prepared by soaking 10 grams of seed residue in 100 ml of distilled water twice. The solution was shaken at 80 °C for 25-28 minutes at a speed of 400 rpm. After shaking, the solution was left to cool at room temperature, The **(fig.3)** shows this protocol for preparing the seed extract. then filtered and the extract was kept in the refrigerator at 4 °C for later use.

Fig. 3: :seed extract preparation protocol

6. Green synthesis of nanomaterials:

6.1.reparation of Zinc Acetate Solution:

To prepare a solution with a molarity of 0.5 M, 9.17 grams of zinc acetate were dissolved in 100 ml of double-distilled water. The solution was then transferred to a securely sealed 250 ml volumetric flask for storage.

6.2. Preparation of Zinc oxide nanoparticles:

Zinc nanoparticles:Take 150 ml of zinc acetate with a concentration of 0.5 mol. Add 60 ml of the extract. Then add some NaOH to raise the pH to about 6.5-7. Mix the solution at 40 °C for 1 hour at 400 rpm. Then separate the precipitate from the solution by centrifugation, and then at 4000 rpm for 10 minutes. Wash it with distilled water twice and dry it at 100 °C. Then burn it in the oven at 550 °C. After the nanoparticles are formed, grind it with a mortar and pestle., yielding Zinc oxide nanoparticles, as illustrated in **(Fig. 4).** The **(Fig. 5)** represents a picture showing the color change during the preparation of: ZnO NPs by seed extract

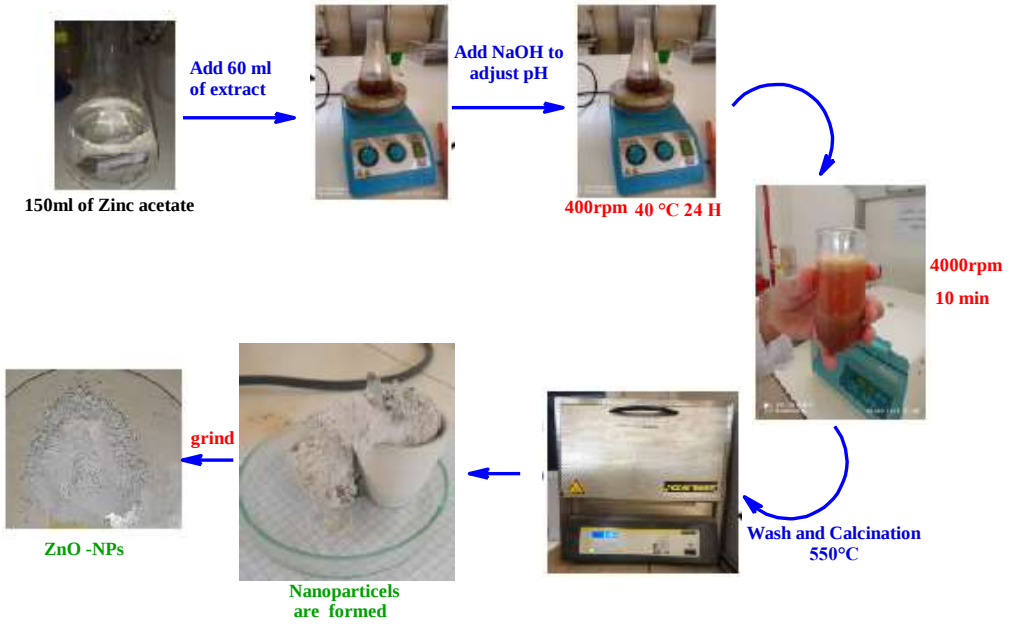

Fig4: ZnO- Nps synthesis protocol

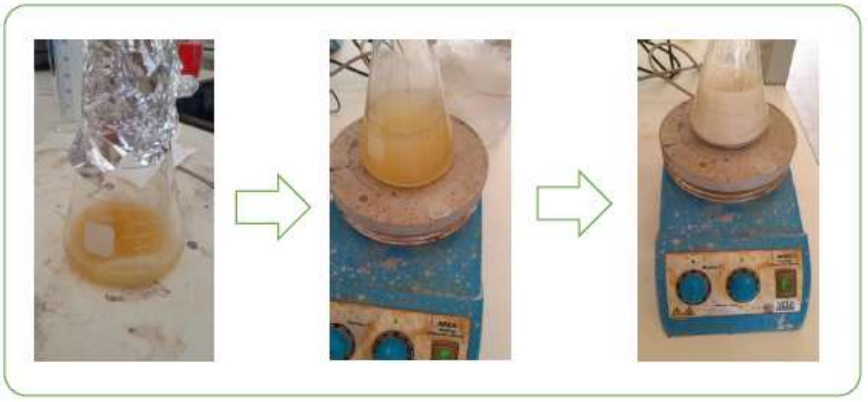

Fig. 5: Photo showing the color change during preparation for: ZnO NPs seeds ex

7. Characterization of Synthesized Zinc oxide Nanoparticles

The initial characterization of Zinc nanoparticles was conducted using ultraviolet– visible (UV-Vis) spectroscopy (model: UV- 1800, Make: Shimadzu, Kyoto, Japan) in a scanning wavelength range of 200–800 nm as a preliminary confirmation of Zinc ion reduction. FT-IR (Shimadzu IR Spirit, Kyoto, Japan) was performed in the range from 450 to 4000 cm−1. This analysis was helpful for detecting the biomolecules of the whole-plant **"Pumpkin"** seeds extract in the aqueous extract of plants responsible for the formation of ZnO-NPs. Scanning electron microscopy combined (ZEISS, Gemini SEM 360, Jane, Germany) with EDX (Carl Zeiss Microscopy Germany, model: EVO 18) was used to identify the surface morphology, size, and elemental composition of **"Pumpkin"** seeds extract-mediated ZnO NPs. X-ray diffraction (XRD) analysis was carried out using a Bruker D8 Advance Diffractometer (Bruker, Ettlingen, Germany) with a copper tube operating at 40 kV and 40 mA. The diffractograms were recorded using monochromatic CuKα radiation (λ = 0.1542 nm) across a 2θ range of 5°–90°, with a step size of 0.05° and a measurement time of 1 second per step. The XRD patterns provided information on the crystalline structure and phase purity of the ZnO-NPs. The average crystalline size of the nanoparticles was calculated using the **Debye-Scherrer** equation, **(Equation Nº1)** offering insights into the crystalline properties of the synthesized material.

8. Results and Discussion

A sample of zinc oxide nanoparticles (ZnO NPs) prepared using pumpkin seeds was subjected to a wide range of characterizations in order to determine their morphology, structure, surface chemistry, particle size, and thermal properties.

8.1.UV-Visible Spectrum Analysis

The figure shows the UV-Vis absorption spectrum of zinc oxide nanoparticles (ZnO NPs) for pumpkin seeds**(Fig. 6.A)** , where the absorption peak appears at a wavelength of 373 nm. Zinc oxide nanoparticles have an absorption peak in the range of 200-400 nm[23]. Comparing this with the absorption spectrum of the extract**(Fig. B)** , we observe a difference in wavelength, where the absorption peak of the extract reaches 373 nm.

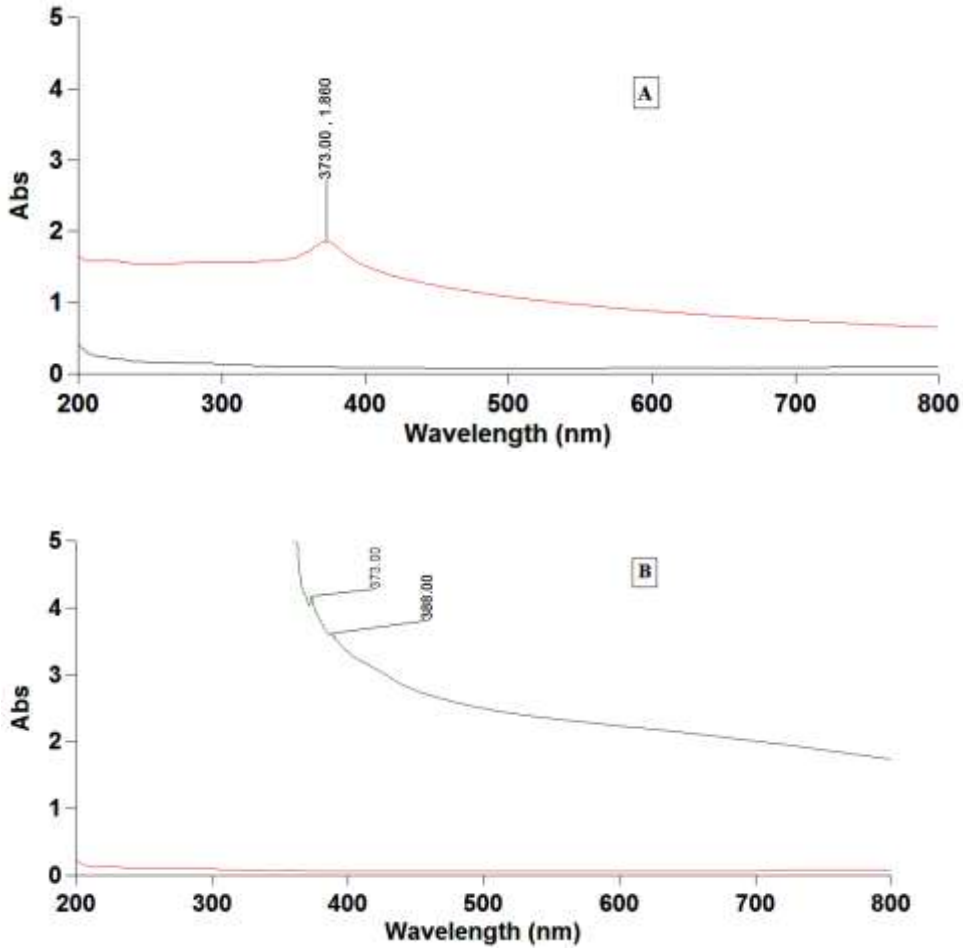

Fig. 6: UV-vis spectra of ZnO NPs Seeds**(A)** seeds extract**(B)**

8.2.FT-IR Analysis

The results were further enhanced by Fourier transform infrared analysis, which showed shifts and differences in the peak areas. With this technique, it is possible to identify the

biomolecules in the plant extracts that play a crucial role in the reduction processes and stabilization of the green nanoparticle structure .[24] **(Fig. 7)** shows the IR spectrum of the sample. It is observed that the bands are present at 3245 cm-1, 2168 cm-1, 1383 cm-1, 1599 cm-1, 1076 cm-1, 780 cm-1, and 515 cm-1. The Fourier transform infrared spectrum of ZnO nanoparticles was recorded in the range of 500-4000 cm-1. The peak in the region between 400 and 600 cm-1 is assigned to ZnO .[25] Also, the band located near 515 cm-1 is assigned to the ZnO stretching vibration. The bands present at 3245 cm-1 and 1599 cm-1 are characteristic of the hydroxyl group (O-H). The peaks at 1383 cm-1 and 1076 cm-11 can be attributed to -C-O and -C-O-C stretching modes. The band at 2168 cm-1 is due to C=C stretching.

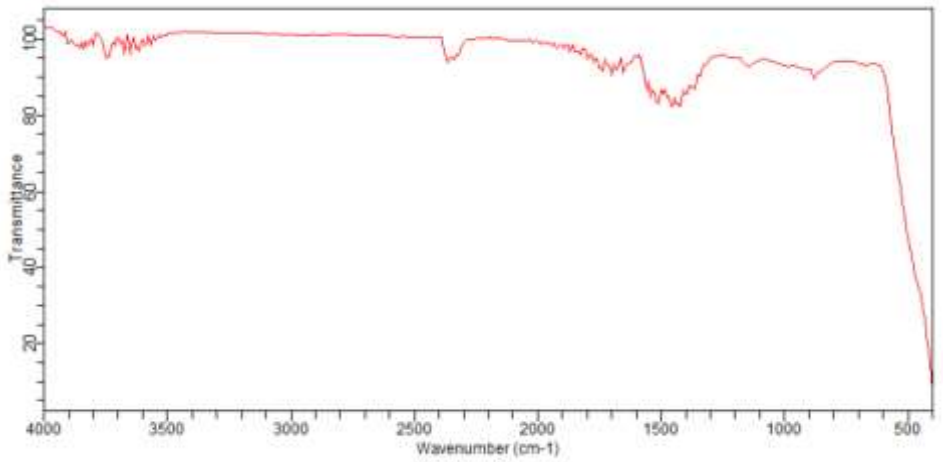

Fig. 7: FTIR spectra of ZnO NPs seeds extract

8.3.SEM and EDX Analysis

The morphology of the nanostructures was studied using scanning electron microscopy (SEM). **(Fig.8)** present the SEM images of the obtained ZnO nanoparticles. The synthesized ZnO nanoparticles were agglomerated with a particle size ranging from below 100 to 190 nm.. To gain further insight into the features of ZnO nanoparticles, the analysis of the sample was performed using EDS **(Fig.9)** techniques. The energy dispersive spectra of the samples obtained from the SEM-EDS analysis show that the sample prepared by the above route has pure ZnO phases [26].

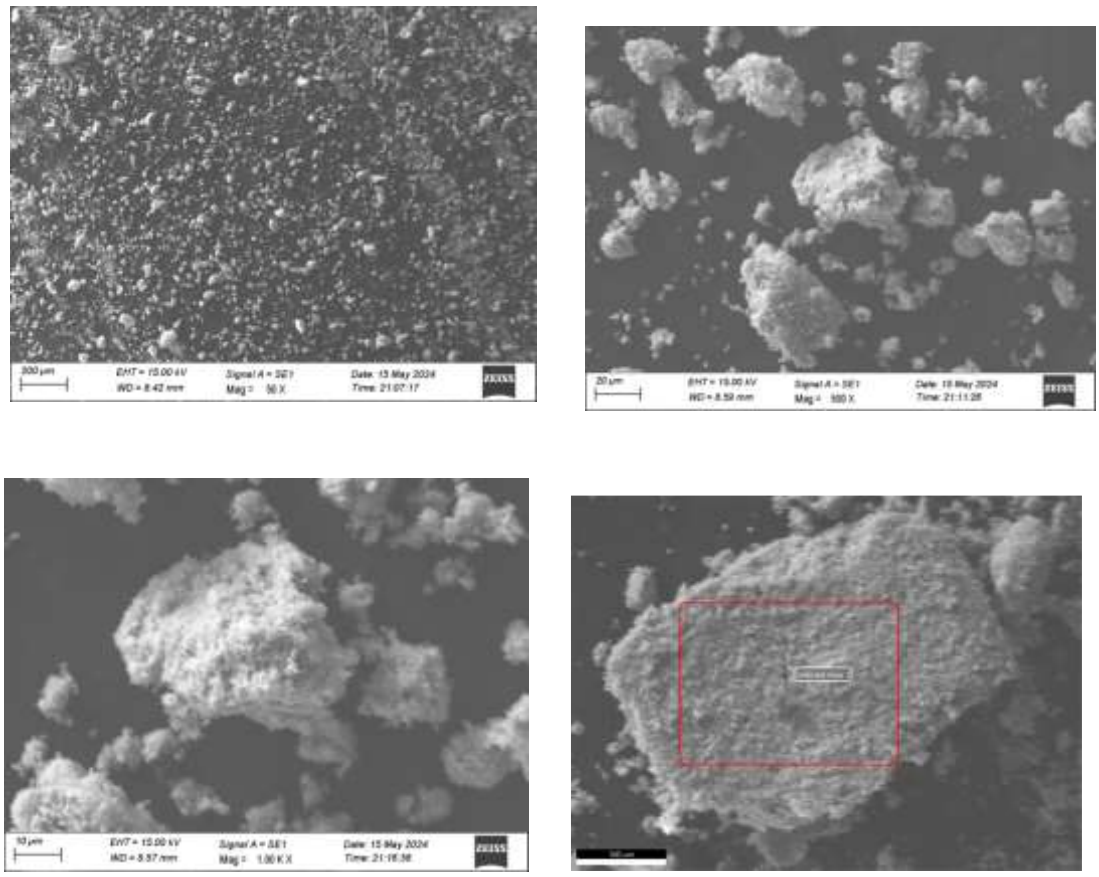

Fig. 8:images taken with a EDX/SEM of ZnO NPs seed extracts

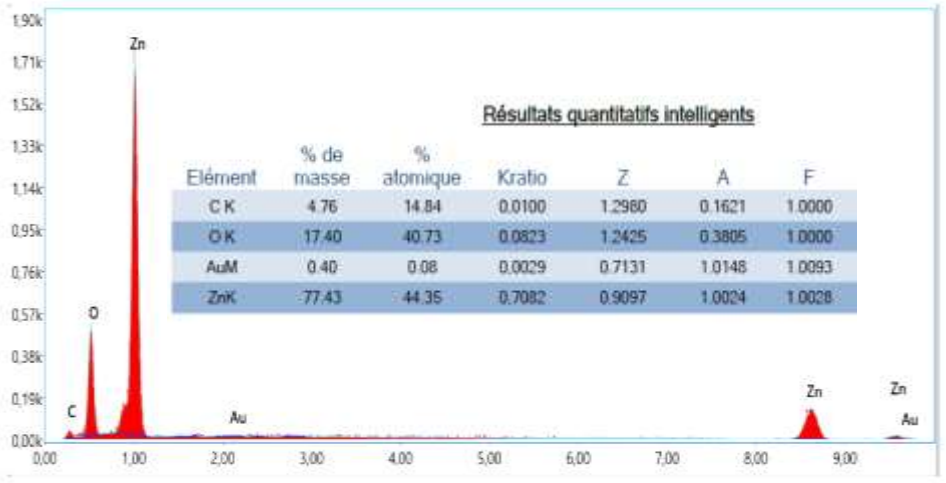

Fig. 9 : EDX of ZnO NPs seeds extract

8.4.XRD Analysis

X-ray diffraction was taken to further confirm the zinc oxide phase of the nanoparticles. The XRD pattern of zinc oxide nanoparticles is shown in **(Fig.10)**. The XRD peaks were identified as (100), (002), (101), (012), (110), (013), (200), (112), (004) and (202).The narrow and strong diffraction peaks indicate well crystalline nature of zinc oxide. The size of ZnO nanoparticles was obtained by Debye-Scherrer's formula given by the **(Equation Nº1)**

where:

$$D = k\lambda \Big/ \beta \frac{1}{2} \cos\theta \ldots\ldots\ldots (1)$$

$D -$ the crystal size,

$\lambda -$ the wavelength of the X-ray radiation (λ = 0.15406 nm) for $CuK\alpha$

$K -$ usually taken as 0.89,

$\beta -$ the line width at half-maximum height [27].

The Debye-Scherrer's formula was used to calculate the particle sizes and was found to be in the range of 70–90 nm. XRD study confirmed the presence of even smaller particles than the SEM examination. The larger nanoparticles of ZnO (about 200 nm) in the sample result from the agglomeration of smaller nanoparticles, whose presence is confirmed by X-ray diffraction (XRD). The XRD method allowed for the identification of smaller sizes of nanoparticles. The
agglomeration of smaller nanoparticles occurs due to the fact that we are dealing with biological material.

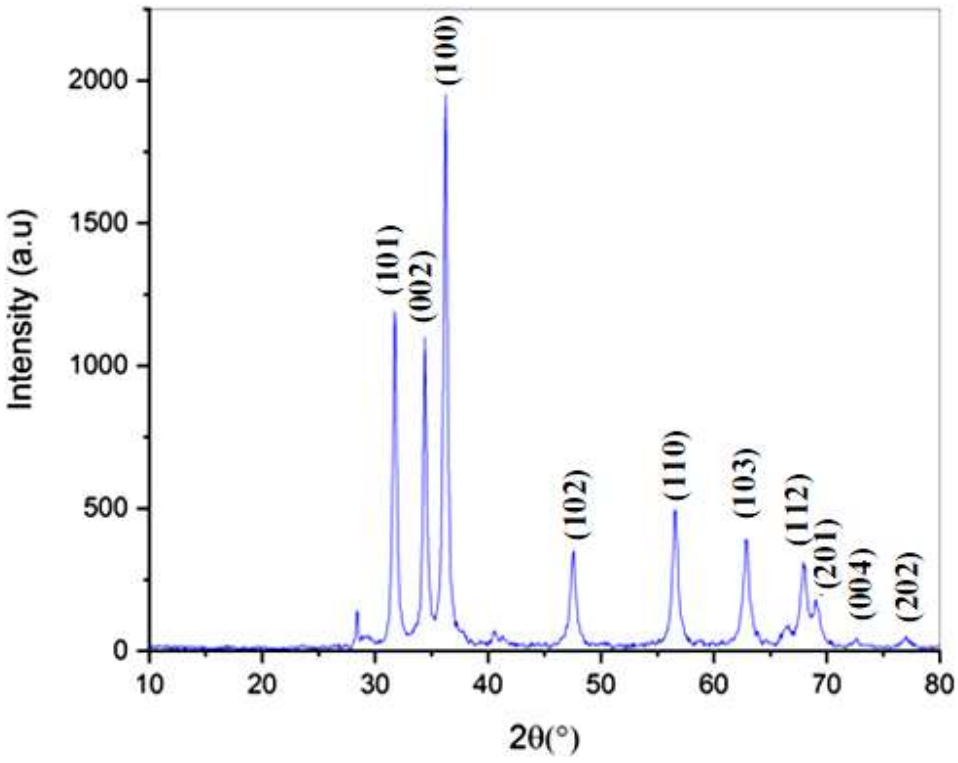

Fig.10 :FTIR spectrom of ZnO leaves and seeds Extracts

9. Antibacterial efficacy study of ZnO-NPs

9.1.Biological Materials

We used a reference strain of Candida albicans ATCC 10231 and four bacterial strains, including two Gram-negative strains, Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922, and two Gram-positive strains, Staphylococcus aureus ATCC 25923 and Bacillus subtilis ATCC 25973**.**

9.2. Agar Diffusion Method (Well Method)

The agar diffusion method, specifically the well method, is a fundamental technique used to evaluate the antimicrobial activity of a substance. To begin, Petri dishes are prepared with Sabouraud dextrose agar supplemented with 2% glucose for yeasts, and Mueller-Hinton agar for bacteria. These dishes are then aseptically inoculated with a suspension containing 1.5×108 CFU/mL, standardized using McFarland Standard No. 0.5. The suspension is

prepared using a VITEK® DENSICHEK® device and a mixture of sterile physiological saline solution (0.9% NaCl) and sterile distilled water.
Once the agar surfaces have dried, wells are created at the center of each dish using the upper part of a Pasteur pipette. These wells are subsequently filled with 50 µL of an aqueous solution of the test compound (ZnO NPs seeds) at concentrations of 80, 40, 20, and 10 µg/mL, prepared with 5% DMSO to ensure proper dissolution of the compounds.
The inoculated plates are incubated at 37°C for 48 hours for yeasts and 24 hours for bacteria. Antimicrobial activity is indicated by forming a clear zone of inhibition around the wells, where microbial growth has been prevented. The diameter of these inhibition zones is measured, and a product is considered to exhibit antimicrobial activity if the zone of inhibition is greater than 6 mm [28,29].

9.3.Results and Discussion of Antibacterial Activity of Zinc Oxide Nanoparticles (ZnO-NPs)

We note that ZnO nanoparticles are effective against most strains, with negligible activity against E. coli and no activity against Candida albicans. They are variable in their activity against the rest of the strains studied, e.g. Pseudomonas aeruginosa (15 mm at 80 µg/ml compared to 33 mm in CIP), Staphylococcus aureus (14.6 mm at 80 µg/ml compared to 33 mm in CIP), and no activity against most strains when using the 10 µg/ml concentration of ZnO nanoparticles. **(Table 2)** Shows the results of antimicrobial tests of (ZnO-NPs) prepared with aqueous extract of pumpkin seeds.

Table 2: Results of antimicrobial tests: **ZnO** NPs **seeds**

Strains used	**Average zone of inhibition (mm)**					
	Blank disc (–	**Microbial inhibition ZnO NPs**				**Ciproflo xacin (CIP) 5µg**
		80µg/ ml	**40µg/ ml**	**20µg/ ml**	**10µg/ ml**	
Escherichia coli	**No zone**	**08**	**No**	**No**	**No**	**33**
Pseudomonas	**No zone**	**15**	**10**	**08**	**No**	**33**
Staphylococcus aureus	**No zone**	**14,6**	**12**	**07**	**No**	**31**
Bacillus subtilis ATCC	**No zone**	**12**	**10**	**No**	**No**	**33**
	Anti-Candida activity					
Candida albicans	**No zone**	**No**	**No**	**No**	**No**	**/**

10. Conclusions

The green synthesis of nanoparticles (NPs) is a modern field in preparing NPs using organic molecules as reducing agents. . In this study, we focused on the green synthesis of nanocompounds (zinc oxide and silver) from pumpkin seeds by following a clean and environmentally friendly method. These compounds were characterized based on color change and UV-Vis, FTIR, EDX, and XRD spectroscopy. The larger nanoparticles of ZnO

resulted from the agglomeration of smaller nanoparticles. Moreover, the synthesized ZnO nanoparticles exhibited high activity against E. coli ATCC 25922, P. aeruginosa ATCC 27853, S. aureus ATCC 25932 and Bacillus subtilis ATCC 25973. Also, the green synthesis of ZnO nanoparticles using Extract of seed plant Cucurbitaceae family "Pumpkin" be an alternative to chemical methods.In conclusion, through our research and work, we have demonstrated that it is possible to exploit the biomass from plants, bacteria, and viruses in the synthesis of nanomaterials, reducing the environmental damage caused by the use of toxic chemicals. These materials have various applications in several fields and hold promising future prospects caused .

11. .References

1. Azam, A., Ahmed, A. S., Oves, M., Khan, M. S., & Memic, A. (2012). Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and-negative bacterial strains. International journal of nanomedicine, 3527-3535.
2. Nedjimi, M. S. (2023). Eco-Friendly synthesis of Zinc Oxide Nanoparticles Using Aqueous Extracts of Different Types of Dates (Phoenix Dactyliferal) Grown in the Oases of Southern Algeria. Tobacco Regulatory Science (TRS), 5345-5361.
3. Yallappa, S., Manjanna, J., Sindhe, M. A., Satyanarayan, N. D., Pramod, S. N., & Nagaraja, K. (2013). Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using T. arjuna bark extract. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 110, 108-115.
4. Seigneuric, R., Markey, L., SA Nuyten, D., Dubernet, C., TA Evelo, C., Finot, E., & Garrido, C. (2010). From nanotechnology to nanomedicine: applications to cancer research. Current Molecular Medicine, 10(7), 640-652.
5. Liu, Z., Kiessling, F., & Gätjens, J. (2010). Advanced nanomaterials in multimodal imaging: design, functionalization, and biomedical applications. Journal of Nanomaterials, 2010(1), 894303.
6. Dejen, K. D., Kibret, D. Y., Mengesha, T. H., Bekele, E. T., Tedla, A., Bafa, T. A., & Derib, F. T. (2023). Green synthesis and characterisation of silver nanoparticles from leaf and bark extract of Croton macrostachyus for antibacterial activity. Materials Technology, 38(1), 2164647.
7. Prakash, M., Kavitha, H. P., Abinaya, S., Vennila, J. P., & Lohita, D. (2022). Green synthesis of bismuth based nanoparticles and its applications-A review. Sustainable Chemistry and Pharmacy, 25, 100547.
8. Nthunya, L. N., Mbakop, S., Makgabutlane, B., Matlou, G., Mhlanga, S., & Richards, H. (2023). Fungal synthesis of copper nanoparticles and their applications in agri-food, environmental, and biomedical sectors. In Fungal Cell Factories for Sustainable Nanomaterials Productions and Agricultural Applications (pp. 91-114). Elsevier.
9. Abed, A. S., Khalaf, Y. H., & Mohammed, A. M. (2023). Green synthesis of gold nanoparticles as an effective opportunity for cancer treatment. Results in Chemistry, 5, 100848.
10. Rathnasamy, R., Thangasamy, P., Thangamuthu, R., Sampath, S., & Alagan, V. (2017). Green synthesis of ZnO nanoparticles using Carica papaya leaf extracts for photocatalytic and photovoltaic applications. Journal of Materials Science: Materials in Electronics, 28, 10374-10381.
11. Siripireddy, B., & Mandal, B. K. (2017). Facile green synthesis of zinc oxide nanoparticles by Eucalyptus globulus and their photocatalytic and antioxidant activity. Advanced Powder Technology, 28(3), 785-797.

12. Zheng, Y., Fu, L., Han, F., Wang, A., Cai, W., Yu, J., ... & Peng, F. (2015). Green biosynthesis and characterization of zinc oxide nanoparticles using Corymbia citriodora leaf extract and their photocatalytic activity. Green Chemistry Letters and Reviews, 8(2), 59-63.
13. Cuong, H. N., Pansambal, S., Ghotekar, S., Oza, R., Hai, N. T. T., Viet, N. M., & Nguyen, V. H. (2022). New frontiers in the plant extract mediated biosynthesis of copper oxide (CuO) nanoparticles and their potential applications: A review. Environmental Research, 203, 111858.
14. Salih, A. M., Al-Qurainy, F., Khan, S., Tarroum, M., Nadeem, M., Shaikhaldein, H. O., ... & Alfarraj, N. S. (2021). Biosynthesis of zinc oxide nanoparticles using Phoenix dactylifera and their effect on biomass and phytochemical compounds in Juniperus procera. Scientific reports, 11(1), 19136.
15. Adil, S. F., Assal, M. E., Khan, M., Al-Warthan, A., Siddiqui, M. R. H., & Liz-Marzán, L. M. (2015). Biogenic synthesis of metallic nanoparticles and prospects toward green chemistry. Dalton Transactions, 44(21), 9709-9717..
16. Isara, R. D. S., & Gunathilaka, M. D. T. L. (2023). Pumpkin seeds and leaves as an alternative medicine for the treatment of hyperglycemia. University of Colombo Review, vol. 4, pp. 31–47, Oct. 2023.
17. Castetter, E. F. (1930). Species crosses in the genus Cucurbita. American Journal of Botany, 17(1),41-57.
18. Heiser, C. B. (1965). Cultivated plants and cultural diffusion in nuclear America. American Anthropologist, 67(4), 930-949.
19. López-Anido, F. S. (2021). Cultivar-groups in Cucurbita maxima Duchesne: Diversity and possible domestication pathways. Diversity, 13(8), 354.
20. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. Molecular pharmaceutics, 5(4), 505-515.
21. Dar, A. H., Sofi, S. A., & Rafiq, S. (2017). Pumpkin the functional and therapeutic ingredient: A review. International Journal of Food Science and Nutrition, 2(6), 165-170.
22. Kamarubahrin, A. F., Haris, A. B., Daud, S. N. B. M., Kefeli, Z. B., Ahmad, N. B., Muhamed, N. A. B., & Shukor, S. B. A. (2018). The potential of pumpkin (Cucurbita moschata Duschene) as commercial crop in Malaysia. Pertanika Journal of Scholarly Research Reviews, 4(3).
23. M. Yass, A. Al-Haddad, M. J. M. Ali, A. Jaafar, and M. Veres, "Effectiveness of Green Synthesized Zinc Oxide Nanoparticles against Extensively Drug-resistant Klebsiella pneumoniae," Biomedical and Biotechnology Research Journal (BBRJ), vol. 7, no. 3, 2023,
24. Senthilkumar, S. R., & Sivakumar, T. (2014). Green tea (Camellia sinensis) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. Int. j. pharm. pharm. sci, 6(6), 461-465.
25. Yuvakkumar, R., Suresh, J., Saravanakumar, B., Nathanael, A. J., Hong, S. I., & Rajendran, V. (2015). Rambutan peels promoted biomimetic synthesis of bioinspired zinc oxide nanochains for biomedical applications. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 137, 250-258.
26. Kumar, S. S., Venkateswarlu, P., Rao, V. R., & Rao, G. N. (2013). Synthesis, characterization and optical properties of zinc oxide nanoparticles. International Nano Letters, 3, 1-6.
27. Vijayalakshmi, R., & Rajendran, V. (2012). Synthesis and characterization of nano-TiO2 via different methods. Arch. Appl. Sci. Res, 4(2), 1183-1190.
28. Kiehlbauch, J. A., Hannett, G. E., Salfinger, M., Archinal, W., Monserrat, C., & Carlyn, C. (2000). Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York state laboratories. Journal of clinical microbiology, 38(9), 3341-3348.

29. Bonev, B., Hooper, J., & Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Journal of antimicrobial chemotherapy, 61(6), 1295-1301..