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BIOSYNTHESIS OF ZnO NANOPARTICLES USING *OCIMUM BASILICUM* LEAVES AND DETERMINATION OF ITS ANTIBACTERIAL ACTIVITY

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Abstract

In this study, zinc oxide nanoparticles were prepared in an easy, simple, safe and cheap way known as green synthesis. This was done by the interaction between a solution of zinc acetate dihydrate and an aqueous extract of basil leaves, and the important bioactive compounds present in the extract were detected, and the synthesis of zinc oxide nanoparticles were characterized by UV-Vis and FT-IR spectrum. FTIR characterization of plant extract and ZnO nanoparticles depicted strong presence of important bioactive components in plant extract while weak presence or absence observed in ZnO nanoparticles supernatant layer, NPs IR spectrum showed a prominent sharp peak at 688cm^{-1} of hexagonal ZnO that was totally absent in the extracted spectrum. Moreover, the antimicrobial activity of synthesized nanoparticles was measured against bacteria *Staphylococcus aureus*, and *Escherichia coli*, and the results showed a high inhibition rate of zinc oxide nanoparticles against the growth of bacteria compared to the leaves extract. Thus a cheap and ecofriendly plant mediated complete method was used that can be exploited on a large scale.

Keywords: green synthesis, ZnO nanoparticles, Ocimum basilicum, zinc acetate dihydrate

1. Introduction

Nanotechnology is one of the most important technologies in multiple fields. It depends on synthesis particles with nano dimensions, and these particles have different characteristics of the metals that are based on the engineering of mineral molecules in various forms and sizes. In recent years, attention to the production of nano mineral materials has increased by its uses in various fields such as vital medical, agricultural, environmental and industrial [1,2]. The importance of nanoparticles is due to their small size and high surface to volume ratio. Various physical and chemical methods were used to manufacture metal nanoparticles. However, these methods have short comings; as they are more time consuming, use toxic and biodegradable chemicals that are dangerous for biological systems. Therefore, plant mediated synthesis has a prominent position for NPs synthesis as it is an inexpensive, nontoxic and eco-friendly alternative [3, 4].

Zinc oxide nanoparticles (ZnO NPs) have received considerate attention due to their unique antibacterial, antifungal, UV filtering properties, and high catalytic and photochemical activity. Increasing awareness of green chemistry has led to the development of an ecofriendly approach to the synthesis of metal oxide NPs [5].

Recently, there has been a great desire to use medicinal plants in the treatment of many pathogens, because most of them are devoid of side effects, easy to handle and contain many bioactive phytochemicals. The fact that the plant contains many chemical compound made it of medical importance in the treatment of many diseases, by using it directly in the form of solutions, juices, powders and compresses, or indirectly by using the active compounds present in the plant in a pure form for medical and therapeutic purposes, including aromatic plants containing volatile oils that are used in different economic and medical fields [6].

Ocimum basilicum, also called great basil or Saint Joseph's wort, belongs to the family Lamiaceae (mints). Basil is possibly native to India [7]. The genus Ocimum has a number of species that have been used to treat different types of ailments from ancient times, especially the species Ocimum basilicum. It has numerous potent activities due to the metabolites present in it. Because of its virulent metabolites, it is used in traditional medicine [8].

Fig 1: *Ocimum basilicum L.* leaves

Fig 2: *Ocimum basilicum L.* plant

2. Material and Methods

Ocimum basilicum L. leaves were collected during the period of June-Spetmeaper from garden, and were washed well with distilled water to remove dirt particles, then dried under shade for 2 weeks, ground with electric mill, and stored.

Preparation of plant extract: 5.00g of basil dried leaves in 50.00ml of deionized water was heated for 2 hours at 80°C with continuous stirring, then cooled at room temperature and filtered. A clear extract was obtained after centrifugation at 4000rpm for 10min [4].

Synthesis of ZnO NPs: Zinc acetate dihydrate (0.2M) [Zn(CH3COO)2.2H2O] was prepared by dissolving 4.38g in 100ml of deionized distilled water, and then 11.5ml of it was added to 5.00ml of *Ocimum basilicum* leaves extract at room temperature (25°C). Brown-orange ZnO NPs formed instantly, and became thicker within minutes [4].

Characterization: the purpose of the phytochemical analysis is to detect the bioactive phytochemicals such as coumarins, alkaloids, flavonoids, saponins, carbohydrate, phenol, tannis, glycosides and resins [9-19]. The synthesis of zinc oxide nanoparticles was characterized by UV-Vis and FT-IR spectrum [20, 21].

Antibacterial studies: the ZnO NPs and basil leaves extract was tested for their antibacterial activity against Staphylococcus aureus, Escherichia coli by the disk diffusion method aka filter paper disk agar diffusion. In this test, a number of small, sterile filter paper disks that have each been impregnated with different concentration of ZnO NPs and basil leave extract, then placed on the surface of an agar plate previously inoculated with the bacteria. The plates were maintained at room temperature for 30 minutes to allow the diffusion of the solutions and then incubated at 37°C for 24 hours. Finally, the inhibition zone around the disk was measured [22].

3. Results and Discussion

Green synthesis of metal oxide nanoparticles using biomass materials is desirable because these approaches are simple, low-cost and eco-friendly The present study was done by adding 11.5ml of zinc acetate dihydrate (0.2M) into 5.00 ml of basil leaf extract at room temperature, and the color change of the mixture solution to orange-brown is due to the occurrence of ZnO NPs production (figure 3), and the longer the reaction time, the darker the color of the solution.

$Zn(CH_3COO)_2.2H_2O$

Fig 3: Color change of ZnO NPs production.

Characterization Well-known qualitative tests were performed to determine the presence or absence of important bioactive compounds in basil leaf extract as shown in Table 1. The qualitative test shows the presence of alkaloids, flavonoids, phenols, carbohydrates, tannins, resins, glycosides, coumarins and saponins. The obtained results were in agreement with those of other researchers [23-26].

Table 1: The qualitative phytochemical analysis.

Phytochemicals	Reagent	Results
Coumarins	NaOH	+
Flavonoids	Isoamyl alcohol	+++
Alkaloids	Wagner's test	++
	Mayer's test	+
Resins	HCl	++
Saponins	Foam test	+++
Glycosides	H_2SO_4, KOH, Fehling	+++
Tannis	Lead acetate test	++
Phenol	Ferric chloride test	+
Carbohydrate	Molisch's test	+++
	Benedict's test	+++
	Fehling's test	+++

(+++): highly presence, (++): medium presence, (+): weak presence, (-): absence.

UV-Vis study: The UV-Vis spectrum shows an absorption peak around 370-450 nm, which did not appear in the extract before the process of adding zinc acetate, which indicates the formation of ZnO NPs, and that is consistent with the previously reported result [21]. This absorption appeared at low concentrations of the extract and was difficult to notice in high concentrations (Figure 4, 5).

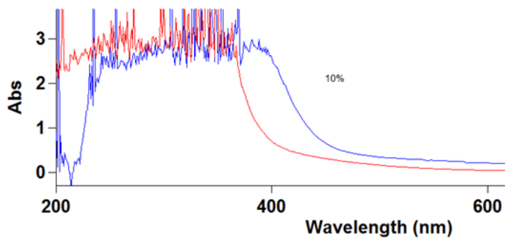

Fig 4: UV-Vis spectrum before and after the addition of zinc acetate to extract 10%

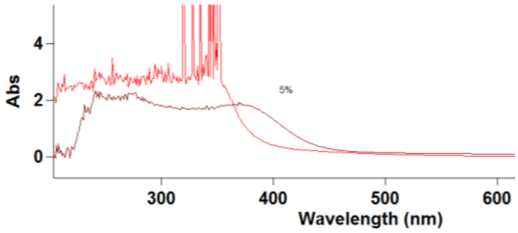

Fig 5: UV-Vis spectrum before and after the addition of zinc acetate to extract 5%

FT-IR analysis: FTIR study was conducted to examine the function groups of basil leaf extract and ZnO NPs, and different pattern of peaks were observed in each of them as shown in figures (6, 7). The results were consistent with those reported earlier [4, 26, 27].

There is a broad stretch between 3000-3500cm^{-1} with absorption maxima at 3246.73cm^{-1} that ascribed the stretching frequencies of amino and hydroxyl of amine, alcohols and phenols. When this peak is compared with the IR spectrum of ZnO NPs, this stretching become narrow with the decrease of peak broadening in NPs spectrum and absorption maxima are shifted to 3085.68cm^{-1}, that's because these these functional groups are used to reduce Zn^{+2}. Further by comparing both spectrums, visible difference between absorption maxima and stretching frequencies were found. As ZnO NPs spectrum has a prominent sharp peak at 688.5cm^{-1} of hexagonal ZnO that is totally absent in the leaves extract spectrum. Moreover, a peak at 1590.84cm^{-1} (carbonyl functional group in amide I and II) and a peak at 1387.51cm^{-1} (C-N stretching frequencies of amide I) in leaves extract, reducibly appeared with absorption maxima at 1549.13cm^{-1} and 1397.97 cm^{-1} in NPs spectrum. A prominent peak at 1045.25cm^{-1} corresponded to C-O vibrational stretching frequencies of alcohol and amino acids in the leaves spectrum whereas two reduced weak peaks appeared at 1018.52cm^{-1} and 953.08cm^{-1} in the ZnO NPs spectrum. The presence of some sharp and prominent peaks in the extract spectrum and the absence or weak presence in the ZnO NPs spectrum suggested that those functional groups of active ingredients were working as stabilizing agents for NPs.

Antibacterial studies: This study proved that NPs are very active against gram-positive bacteria S. aureus and gram-negative bacteria E. coli. The NPs and the leaf extract were more effective on the positive bacteria than the negative bacteria. After 24 hours of incubation, clear inhibition appeared as shown in figure 8. The inhibition zone of ZnO NPs prepared by basil extract is shown in Table 2. These results are consistent with the previous reported outputs [4, 26].

Table 2: The inhibition zone of Ocimum basilicum ZnO NPs and Ocimum basilicum leaves extract against bacteria E. coli and S. aureus.

Type of bacteria	Substance	Concentration	Inhibition zone (mm)
E. coli	**ZnO NPs of basil leaves**	100%	2.4
		50%	1.8
		25%	1.6
	Basil leaves	100%	no zone
		50%	no zone
		25%	no zone
S. aureus	**ZnO NPs of basil leaves**	100%	2.9
		50%	2
		25%	1.7
	Basil leaves	100%	1.7
		50%	no zone
		25%	no zone

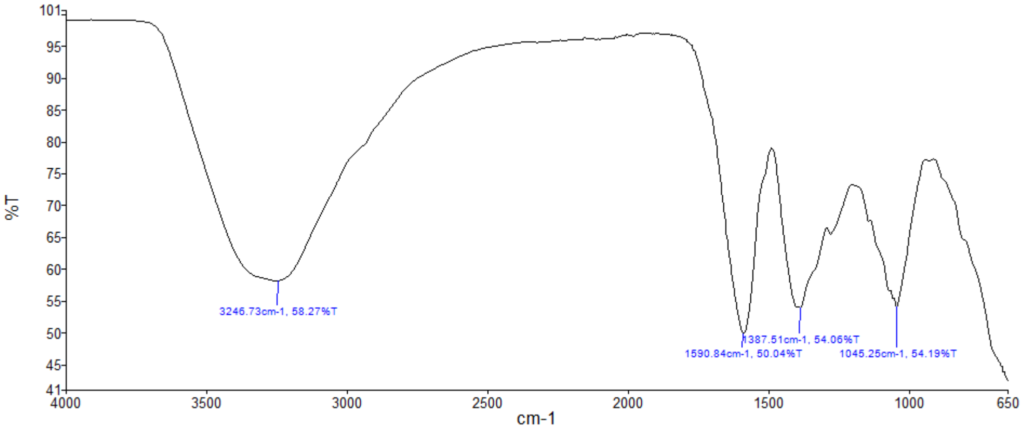

Fig 6: IR spectrum of basil leaves extract

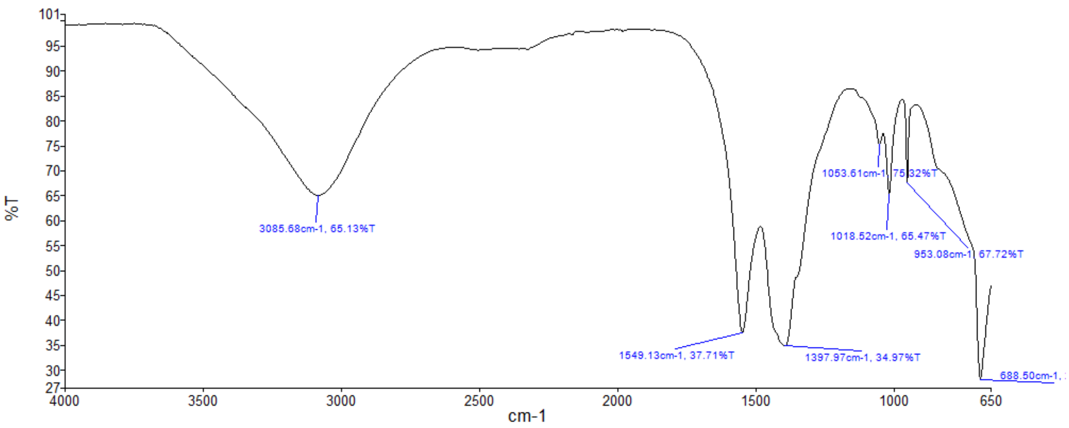

Fig 7: spectrum of ZnO

Fig 8: (A) *S. aureus* inhibition zone of *Ocimum basilicum*. (B) *E. coli* inhibition zone of *Ocimum basilicum* ZnO NPs. (C) *S. aureus* inhibition zone of *Ocimum basilicum* ZnO NPs.

Conclusion: Biosynthesis of ZnO NPs from Ocimum basilicum leaves extract is considered an easy, fast, cheap, ecofriendly and safe method, and extracted phytochemicals act as reducing and stabilizing agents during the formation of the ZnO NPs. In this study, it was proven that basil ZnO NPs are an effective antibacterial agent against bacteria S. aureus and E. coli, and they can be used as a treatment rather than medicines that have side effects.

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