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Green Synthesis of Zinc Oxide Nanoparticles by Cardiospermum

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Abstract

Zinc is an essential trace element that widely exists in human body and play a major role in nucleic acid synthesis, protein metabolism etc. Nano zinc can be easily absorbed by the human biological system. In this study, green synthesis of zinc oxide nanoparticles was achieved by *Cardiospermum* leaf extract which act as reducing and capping agent. The synthesized silver nanoparticles were characterized by UV-Visible spectroscopy, TEM-SAED, XRD, FTIR. UV Visible spectra shows the maximum absorbance peak at 320nm which confirms the formation of zinc oxide nanoparticles. TEM-SAED analysis reveals the hexagonal shape of zinc oxide nanoparticles and size of about 37nm. The XRD spectra reveals the (fcc) cubic crystal structure of the zinc oxide nanoparticles. The peak at 553.57c m−1 in FTIR analysis depicts the characteristic ZnO NPs synthesis. The Cardiospermum mediated Zinc oxide nanoparticles shows substantial antibacterial, antifungal, antioxidant activity in dose dependent manner.

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BACKGROUND

Nanotechnology signify advancement and encourages the stage to manufacture novel nanomaterials for a wide scope of natural and biomedical applications [1]. Green synthesis of nanoparticles is getting progressively mainstream as more secure, practical, simple to utilize, timesaving, free from toxics and contaminations, what's more, basic without numerous natural concerns. It is another option to the standard physical and chemical synthesis methods [2]. Zinc oxide nanoparticles (ZnO NPs), as one of the most significant metal oxide nanoparticles, are prevalently utilized in different fields due to their unconventional physical and chemical properties [4,5]. ZnO NPs are initially used in the rubber industry as they can give wearproof of the elastic rubber composite, improve execution of high polymer in their sturdiness and power and antiaging, and different capacities [6,7]. Due to the solid UV assimilation properties of ZnO, they are progressively utilized in close to home consideration items, for example, beauty care products and sunscreen [8]. Likewise, ZnO NPs have unrivaled antibacterial, antimicrobial, and incredible UV-blocking properties. Therefore, in textile industry, the completed fabrics are exposed to ZnO NPs to attain alluring elements of bright and visible light resistance, antibacteria, and antiperspirant properties [9]. Aside from the applications referenced above, zinc oxide can likewise be utilized in other parts of industry, including electrotechnology, hardware, concrete production, photocatalysis and so on [6,10]. *Cardiospermum halicacabum* L. has a place with family Sapindaceae. This herbaceous plant is widely scattered in tropical and subtropical zones of the world. Cardiospermum plants can be found in the fields of Africa, America, Bangladesh, India and Pakistan [11,12]. The plant based natural items like gel, cream, cleanser, shampoo and so on are available in the market and are useful in dry irritated skin and scalp [13]. The aim of the present study is to focus, examine and reveal the antibacterial, antifungal, antioxidant, anti-inflammatory and anti-diabetic properties of *Cardiospermum* intervened zinc oxide nanoparticles.

MATERIALS AND METHOD

Preparation of plant extract

Fresh leaves of *Cardiospermum* and were collected from garden in Katpadi town, Vellore. The *Cardiospermum* leaves were washed and removed all the contaminants present on the leaves' skin with soap water followed by deionised water. The washed leaves were then air dried well to remove moisture from the leaves. The dried leaves were then crushed finely with the help of mortar and pestle to make it a fine powder. 1g of dried powder was weighed and dissolved in 100mL of distilled water by slowly adding into the fine powder. The extract is heated at 80 degree C for about 20 min using a heating mantle. The extracts were filtered using No: 1 Whattman filter paper. The extracts were filtered twice. The extracts were stored at cool and dry place for future usage.

Synthesis of zinc oxide nanoparticles

A total 3g (10mM) of Zinc Nitrate was dissolved in 50ml of deionized water. 50mL of *Cardiospermum* extract was added to 50mL of zinc nitrate aqueous solution to make 100ml. The mixture is kept on continuous stirring for more than 48 hours. The mixture is then centrifuged for 20 minutes at 8000rpm. The collected pellet was washed twice with distilled water and kept in hot air oven at70 °C for 2 hours. The powdered *cardiospermum* mediated zinc oxide nanoparticles were stored in air tight vials for further studies.

Characterization of ZnO nanoparticles

These bio active nanomaterials can be examined by using UV-Vis Spectrometer, X-ray diffraction, Fourier-transform infrared spectroscopy (FTIR), and Transmission electron microscope.

Biomedical applications of ZnO nanoparticles

Antibacterial activity

The antibacterial activity of *cardiospermum* mediated zinc oxide nanoparticles was determined by adopting agar well diffusion method. Mueller Hinton agar was prepared and sterilized using autoclave *at* 121°C for 15-20minutes.After pouring the medium on the surface of sterile petriplates, the plates were allowed for solidification. After solidification, the pathogens such as *Staphylococcus aureus, Escherichia coli, Bacillus* were swabbed using sterile cotton swabs. The wells were made using a sterile 5mm polystyrene tip. Among four wells per plate wells were loaded with *Cardiospermum* mediated Zinc oxide nanoparticles pellet solution in the concentration range of 25µL, 50µL, 100µL, 150 µL and the standard antibiotic amoxyrite disc was placed in the center of the petriplate. The plates were incubated at 37°C for 24 hours. After incubation, the Petriplates were observed and measured for Zone of inhibition around the nanoparticle and antibiotic disc loaded area.

Antifungal activity

To determine the antifungal activity same agar well diffusion method was adopted. Sabouraud's Dextrose agar was prepared, sterilized using autoclave for 15 minutes at 121°C.The sterilized medium was poured and allowed for solidification. After that, the fungal pathogens such as *Aspergillus*

fumigatus, Aspergillus flavus, Aspergillus niger were swabbed using cotton sterile swabs. Four wells per plate were made using 5mm sterile polystyrene tip. Among four wells per plate wells were loaded with *Cardiospermum* mediated Zinc oxide nanoparticles pellet solution in the concentration range of 25µL,50µL,100µL,150 µL and the standard fungal antibiotic (Voricanazole) disc was placed in the center of the Petriplate.

The plates were incubated at 25 to 28°C for 2-3 days. The zone of inhibition around the nanoparticle loaded wells were measured. The zone of inhibition measured around the antibiotic disc were considered as the standard value.

Antioxidant activity

The antioxidant activity of biosynthesized ZnO NPs were decided utilizing DPPH technique that was reported in [14]. Diverse concentrations (2-10 µg/ml) of *Cardiospermum* leaf extract interceded zinc oxide nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control. The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Anti-inflammatory activity

The anti-inflammatory activity of *Cardiospermum* mediated zinc oxide nanoparticles was done by the method reported in [15]. 2mL of 1% Bovine serum albumin was mixed with 400 µL of biosynthesized zinc oxide nanoparticles in different concentrations (500–100 µg/mL) such as 10 µL,20 µL,30 µL,40 µL,50 µL . The pH was suggested to 6.8 using 1N HCL. The mixture was incubated at room temperature for 20 minutes and then heated at 55°C for 10 minutes in a water bath. After cooling, the absorbance values were noted at 660nm.Dimethyl sulphoxide is used as a control. Diclofenac sodium in various concentrations was used as standard. The percentage inhibition was calculated using the following formula,

$$\% \text{ inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Anti-diabetic activity

The inhibition of alpha-amylase was determined by calculating the quantity of maltose liberated during the process which was modified from that of [16]. By using alpha amylase activity the antidiabetic activity of bio-synthesized zinc oxide nanoparticles were determined. Different concentrations of the sample was taken and 500µl of phosphate buffer which contains alpha amylase solution is added to the solution. The solution is then incubated for 10 minutes in room temperature. Add 500ul of starch (1%) that contains phosphate buffer solution and then incubate it for 10 minutes. 1 ml of DNSA is added to the mixture and kept in water bath for 5 minutes. The mixture is then made up to 5ml with deionized water and OD value was observed at 540nm.Acarbose was used as positive control.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

UV-visible spectroscopy

The Fig 1 represents the UV-Visible spectra of *Cardiospermum* mediated zinc oxide nanoparticles. The UV-Visible spectra wavelength range from 250-650nm. The maximum absorption peak at 320 nm indicates the formation of zinc oxide nanoparticle and confirms the reducing ability of *Cardiospermum* leaf extract. This was in agreement with the previous studies where zinc oxide nanoparticles was synthesized using *Cassia alata* [17].

FTIR

The FTIR spectra of bio synthesized zinc oxide nanoparticles are shown in Fig 2. The peak range showed in the FT-IR spectra of *Cardiospermum* mediated zinc oxide nanoparticles is 500-4000 cm^{-1}. The ZnO nanoparticles synthesized using *cardiospermum* shows peak at 3479.58cm-1 which is due to the N-H stretch and the presence of amine group. And the 3238.48 cm-1 is due to the presence of C-H stretch and amine group. 1620.21 and 1309.67 band is due to the presence of C=C band and the presence of aromatic The peaks at 1143.79 cm-1 and 1047.35 cm-1 is due to the presence of C-F bond and presence of aldehyde compounds. The FTIR spectra of *Cardiospermum* mediated zinc oxide nanoparticles shows peak at 553.57which indicates the probable existence of C-Alkyl chloride and Hexagonal phase ZnO [22]. Also, a few researchers reported that alcohol, aliphatic amine, phenols and carboxylic acids are the functional groups linked with nanoparticles [18-21].

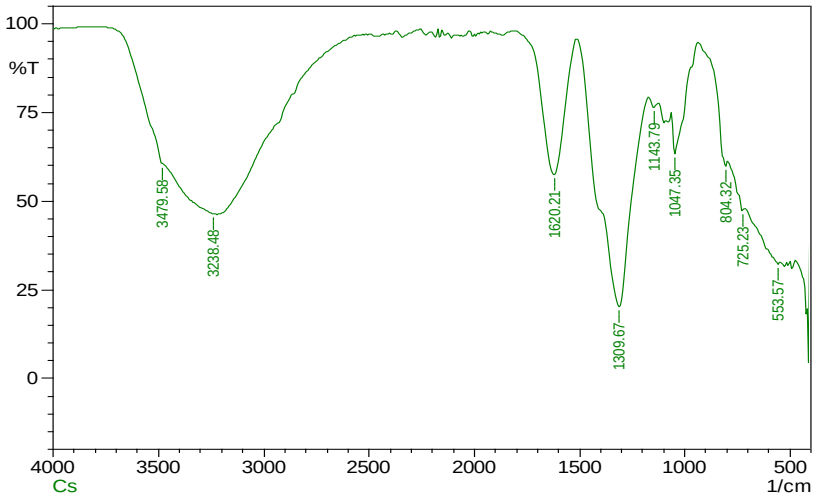

Fig 2: FTIR spectra of Cardiospermum leaf extract mediated zinc oxide nanoparticles.

XRD analysis

XRD Spectra provides an insight about the crystallinity of nanoparticle. Fig 3 depicts XRD spectra of ZnO NPs synthesized using *Cardiospermum* leaf extract.Size of the nanoparticle was determined utilizing Debye–Scherrer equation. X-ray diffraction peaks obtained at 24.96°,28.76°,37.15°,43.83°,50.46°,58.89°,63.42° corresponded to the lattice plane of (4 6 9), (4 6 7),(4 0 4),(3 8 9),(2 3 4),(2 1 7),(1 9 8) suggesting the face –centered cubic (fcc) crystal structure of the nanoparticle. The XRD results of existing works also corresponds to the crystalline nature of the zinc oxide nanoparticles [23].

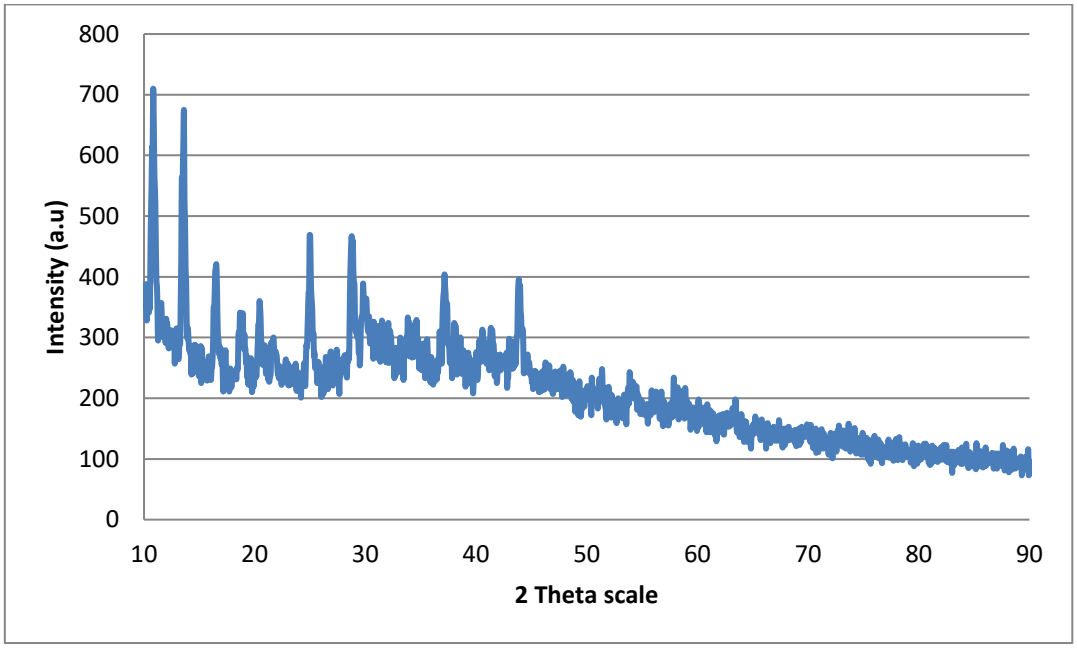

Fig 3: XRD spectra of ZnO nanoparticles.

TEM ANALYSIS

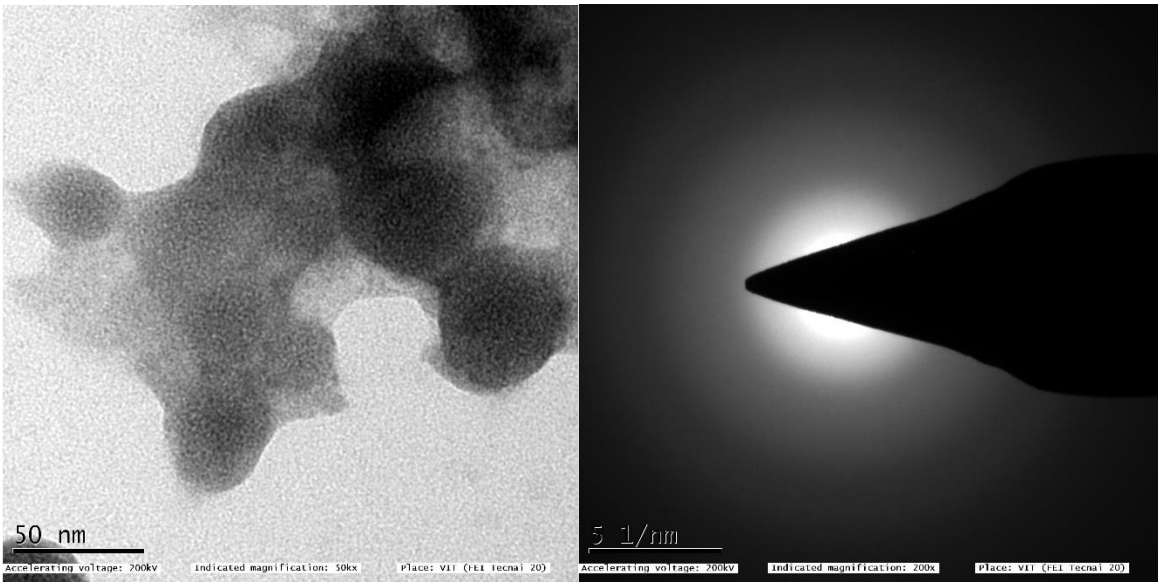

Fig 4: (a) TEM image of ZnO NPs (b) SAED image.

Fig 4 represents the TEM image of biosynthesized zinc oxide nanoparticles. The TEM results reveals the hexagonal shape and size of zinc oxide nanoparticles of about 37nm.The SAED pattern corresponds to the XRD results. Previous studies states most of the zinc oxide nanoparticles as spherical, hexagonal, triangle, radial, rectangle in shape [24].

Antibacterial activity

Agar well diffusion technique was implemented to perform the assay. Antibacterial effect of *Cardiospermum* mediated Zinc oxide nanoparticles was visualized against pathogens such as *Bacillus,Escherichia coli, Staphylococcus aureus*. Amoxyrite was used as a control. Results demonstrates that the antibacterial effect of biosynthesized zinc nanoparticles in dose dependent manner. Maximum zone of inhibition was observed in gram positive organism *Staphylococcus aureus*. Minimum in zone of inhibition was observed in gram negative organism *E.coli*. Diverse mechanism of activity of nanoparticle against gram positive and gram negative organisms has been reported in [25,26]. Zone of inhibition attained using zinc oxide nanoparticle was much lower than the amoxyrite disc (Standard antibiotic) used which represents the further research in zinc oxide nanoparticle to obtain desirable effects.Recent works also reported that *Cassia alata* mediated zinc oxide nanoparticles reveals intense antibacterial activity in dose dependent manner [17].

Antifungal activity

The antifungal effect of the biosynthesized zinc nanoparticles was performed by employing agar well diffusion technique. The fungal pathogens such as *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* has been employed as model organisms to test the antifungal activity. Maximum zone of inhibition was observed in *Aspergillus fumigatus* and this correlates with the result obtained in Basker et al., 2017 stating that maximum zone of inhibition was noted in *Aspergillus fumigatus* by synthesizing zno nanoparticles using *Aspergillus terreus* [27]. Minimum zone of inhibition was observed in *Aspergillus flavus* which indicates the resistance action against zinc oxide nanoparticles. *Aspergillus niger* also observed to be sensitive against zinc oxide nanoparticles.

Antioxidant activity

The free radical scavenging activity of *Cardiospermum* intervened zinc oxide nanoparticles was performed by using DPPH assay. DPPH is a stable free radical, which gains an electron or hydrogen from the antioxidants, it reacts and results in reduction from 1, 1- diphenyl-2- picrylhydrazyl to identical hydrazine [28,29]. Ascorbic acid is used as a standard antioxidant to compare the antioxidant effect of the biosynthesized zinc nanoparticles. The IC50 value was 324.42 which is obtained at 100µL/mL concentration. The antioxidant effect of the zinc oxide nanoparticles increases in dose dependent manner. A substantial result was observed at 200µL/mL, which is a bit lesser than the standard ascorbic acid. Similar result was revealed in previous studies stating the ability of the nanoparticles in proliferation of the antioxidant property [30]. Recently Rajeshkumar et al also synthesized zinc oxide nanoparticles using *Mangifera indica* leaves and obtained substantial antioxidant activity in dose dependent manner [5].

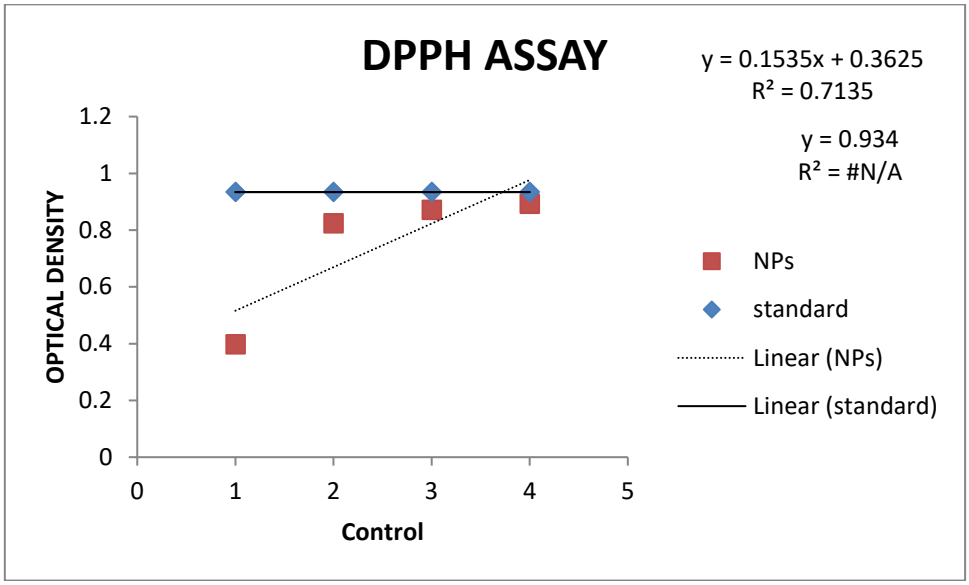

Fig 5: Antioxidant activity of the Cardiospermum mediated Zinc oxide nanoparticles

Table 1: Antioxidant activity of ZnO (CS) Nanoparticles

Concentration	Standard	Control	NPs	scavening activity	IC 50
50ug/ml	0.084	0.934	0.397	57.49464668	
100ug/ml	0.095	0.934	0.824	11.77730193	324.42
150ug/ml	0.102	0.934	0.871	6.745182013	
200ug/ml	0.108	0.934	0.893	4.389721627	

CONCLUSION

Green synthesis of zinc oxide nanoparticles using fresh leaf extract of *Cardiospermum* provides an eco-accommodating, quick, basic, non-harmful and efficient means for the synthesis of nanoparticles. Synthesized ZnO NPs were further characterized using UV–Vis absorption spectroscopy, XRD, TEM, FTIR spectroscopy. UV–Visible spectra shows maximum absorption peak at 320 nm confirming the zinc oxide nanoparticles synthesis. TEM micrograph demonstrated the presence of hexagonal nanoparticles with a size range of 37 nm. The crystallite size was confirmed using XRD and SAED pattern analysis. FTIR spectra represents the peak at 553.57cm^{-1} which is the characteristic peak for zinc oxide nanoparticles synthesis. This study also revealed the substantial anti-bacterial, antifungal activity of zinc oxide nanoparticles. The biosynthesized zinc nanoparticles also shows potential antioxidant activity in dose dependent manner. These biosynthesized zinc oxide nanoparticles can be used as an alternative for chemically derived substances in pharmaceutical industry, cosmetic industry, and also in agriculture.

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