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GREEN SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLE USING CENTELLA ASIATICA LEAF EXTRACT

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Abstract: Development of plant based nanoparticles has many advantages over conventional physico-chemical methods and has various applications in medicine and biology. In present study, two zinc oxide (ZnO) nanoparticles (NPs) were synthesized using leaf extracts of Centella Asiatica (Family: Apiaceae). medicinal plants using zinc nitrate and. 0.01 M zinc acetate dihydrate was used as a precursor. The structural characterization of synthesized Nanoparticles was carried out using UV and FT – IR SEM and anti-bacterial studies; the results showed that both the Zinc Oxide Nanoparticles have the ability to inhibit the growth of various pathogenic microorganisms. Furthermore, this study also evaluated the antibacterial activity of the synthesised Zinc oxide nanoparticles against strains of Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa by the disc diffusion method. The synthesized Zinc oxide Nanoparticles can be used for various applications due to its eco – friendly, non-toxic and compatibility for pharmaceutical and other applications.

Keywords**: Zinc oxide, Centella Asiatica, Zinc nitrate, Zinc acetate, antimicrobial activity.**

I. INTRODUCTION

India is rich in medicinal plant diversity all known types of agro-climatic, ecologic and edaphic conditions are met within India. India is rich in all three levels of biodiversity, such as species diversity, genetic diversity and habitat diversity [1]. *Centella asiatica* L., a creeping plant in the family of Apiaceae, is found almost all over the world, including China, India and Thailand. This plant has been used in traditional folk healing for the ailments of a variety of conditions including headache, body ache, insanity, asthma, leprosy, eczemas, ulcers and wound healing [2, 3]. This plant also provides numerous beneficial effects for the nervous system such as anxiolytic [4] and anti- depression [5]. Recent studies both in animals and in human volunteers demonstrated that this medicinal plant could enhance cognitive function [6– 9], and is having antioxidant activity [10]. Nanoparticles are a particular group of materials with unique features and extensive applications in diverse fields [11]. To explain the influence of the “green precursors” during the synthesis stages of the nanostructured compounds, several mechanisms are proposed, such as reported by the authors Makarov and co-workers [12], who consider plant extracts and theirfunctional groups in the role of ligands to the metallic ions responsible for the chemical reduction of the metal atoms and stabilizing the particles. In other studies, the chemical group’s carbonyl, thiol, and Amina are associated with the potential for the reduction of metallic ions and the favoring of the growth of the crystals [13].

Plant mediated synthesis of nanoparticles (NPs) is a revolutionary technique that has wide range of applications in agriculture, food industry and medicine. NPs synthesized via conventional methods have limited uses in clinical domain due to their toxicity. Due to the physico-chemical properties of plant based NPs, this method also offer an added advantage of increased life span of NPs that overcome the limitations of conventional chemical and physical methods of NPs synthesis [14–16]. Plants possess rich genetic variability with respect to number of biomolecules and metabolites like proteins, vitamins, coenzymes based intermediates, phenols, flavonoids and carbohydrates. More specifically, flavonoids contain several functional groups and it is believed that -OH group of flavonoids is mainly considered responsible for the reduction of metal ions into NPs [17]. These molecules not only help in bioreduction of the ions to the nano scale size, but they also play a pivotal role in the capping of the nanoparticles which is important for stability and biocompatibility[18]. Reducing agents such as phenolic compounds, sterols and alkaloids can reduce metal ions into NPs in a single reaction [19].

Several metals such as silver (Ag), copper (Cu), gold (Au) and many others have been widely used for the bio- synthesis of NPs using plant extracts of various plant species [20-22]. However their higher toxicity to animals and humans pose a serious limitation for use in medical industry. ZnO is an inorganic compound which occurs rarely in nature. It is generally found in crystalline form. Naturally occurring ZnO has manganese impurities that give it a typical red or orange color appearance [23]. When purified, ZnO appears as white crystalline powder which is nearly insoluble in water. Due to their low toxicity and size dependent properties, ZnO NPs have been widely used for various applications in textiles, cosmetics, and diagnostics and even in micro-electronics. Because ZnO is generally recognized as safe (GRAS) and exhibits antimicrobial properties, ZnO NPs hold greater potential to treat infectious diseases in humans and animals [24]. ZnO has been found to be potentially useful and efficient than other metals for biosynthesis of NPs for clinical purposes. Several studies have demonstrated the synthesis of ZnO NPs using different plant extracts. For example, flower extract of the medicinal plant Cassia auriculata [25] and leaf extract of Hibiscus rosasinensi [26] were used as reducing agents for zinc nitrate to synthesize ZnO NPs.In recent, green synthesis of zinc oxide nanoparticles achieved by using microorganisms, show potential antimicrobial effects against infectious organisms such as Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Due to its UV filtering properties, it has been extensively used in cosmetics like sunscreen lotions [27]. It has a wide range of biomedical applications like drug delivery, anti- cancer, anti- diabetic, antibacterial, antifungal and agricultural properties [28-32]. Although ZnO is used for targeted drug delivery, it still has the limitation of cytotoxicity which is yet to be resolved [33]. ZnO NPs have a very strong antibacterial effect at a very low concen- tration of gram negative and gram positive bacteria as confirmed by the studies, they have shown strong antibacterial effect than the ZnO NPs synthesized chemically [34-36].

In the present work, synthesis and characterization of the Zincoxide nanoparticles using centella asiatica leaf extracts was carried out. Further zinc oxide nanoparticles were optical characterized using UV-VIS and FT-IR spectrometer, structural characterization using SEM and antimicrobial activities.

II. Materials and Methods

2.1 Chemicals and Reagents

Zinc nitrate(Zn $Zn(NO_3)_2$) (Sigma Aldrich), Zinc acetate (Sigma Aldrich), Sodium hydroxide (Merk India), and ethanol was purchased from Merck Chemical Reagent Co. Ltd. India. All the glasswares used were washed with distilled water and dried in an hot air oven before use.

2.2 Preparation of the leaf extract

Centella asiatica plant leaves were collected from the surroundings of Kanyakumari. Plants were washed several times with double distilled water to remove any debris or par ticulates then shade dried at room temperature. Aerial parts were finely ground into a fine powder. The aqueous extract was prepared by the cold maceration method [37]. The dried leaves were cut and ground for powder; 5gms of dried leaves were powdered in 250 ml glass beaker along with 100ml of double distilled water. The mixture was then boiled for 60 minutes until the colour of the aqueous solution changes from watery to light yellow by using magnetic stirrer. The extract was cooled to room temperature and filtered using filter paper. The extract was stored in a refrigerator in order to be used for further experiments

2.3 Synthesis of ZnO nanoparticles from Zinc nitrate

Biogenic synthesis of ZnO.NPs was carried out according to the method of Elumalai et al.[38] For the synthesis of nanoparticles, 50ml of centella leaves extract was taken and boiled to 60-80 degree Celsius using a stirrer-heater. Zinc nitrate (0.02Mol, 1.6g) and sodium hydroxide (0.08Mol, 3.2g) solutions were prepared in 100ml separately. Both are added to the extract under constant stirring using magnetic stirrer. The solution was allowed to settle, the solution was filtered and the white precipitate was dried at 110oC for 6 hours.

2.4 Synthesis of ZnO nanoparticles from Zinc acetate

Zinc acetate dehydrate (0.02Mol, 2g) and sodium hydroxide (4g) were prepared in 100ml water separately. Zinc acetate solution and Sodium hydroxide solution was added to the extract with constant stirring using magnetic stirrer for 2 hours. The solution was allowed to settle, the solution was filtered and washed with little distilled water and ethanol to get free of the impurities and the white precipitate was dried at 110°C in air oven for 6 hours

III. Characterization techniques

3.1 U.V. spectrophotometric analysis

UV absorption spectrum of ZnO.NPs samples were studied by different absorbance against different wavelength using a UV- absorption spectrophotometer from SHIMADZU Corporation, Japan; Model: UV -1601, With in the range 200–800 nm.

3.2 FTIR spectroscopic analysis

Then FTIR was used to identify the functional groups and various physicochemical constituents involved in the reduction and stabilization of the synthesized nanoparticles. FTIR was carried out using the attenuated total reflectance (ATR) mode with a Jasco FTIR 4100 spectrophotometer (India). The results recorded in the range of 4000–400 cm^{-1}

3.3 SEM analysis

The prepared samples were carried out Scanning electron microscopic (SEM) analysis was performed with the Hitachi S4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by simply dropping a very minute amount of the sample on the grid, with excess solution being removed using blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min. The electron interacts with the sample producing various signals and give information about surface morphology of the synthesized nanoparticles.

3.4 Microbial studies

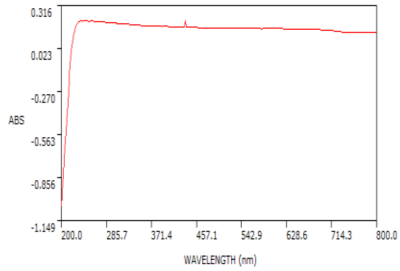
Bacterial adhesion and antimicrobial activity were evaluated by agar diffusion method. For bacterial adhesion study samples of 1 mgm samples were used and for antimicrobial activity spherical disc of 10 mm diameter were used. Both test samples were sterilized by autoclaving before performing the test and finished by agar diffusion method. The test was done in triplicates. Gentamycin (10 μ/ disc) of positive control was used for antimicrobial activity testing. The bacterial adhesion study was carried out ubing the Gram-negative and Gram-positive of Pseudomonas aeruginosa, Staphylococcus aureus and Eschericia coli, wasselected as the typical organisms.

IV RESULTS AND DISCUSSION

4.1 U.V. spectrophotometric analysis.

Zno nanoparticles prepared using the extract obtained from the dry leaf powder of Centella Asiatica is subjected to recored UV-Vis spectroscopy The absorption spectrum of green synthesized ZnO.NPs was initially confirmed by UV-vis spectroscopy within the range 200–800 nm. The absorption peaks is obtained at the wavelength 224 nm and 234nm for ZnO particles prepared from leaf of Centella Asiatica with *$Zn(NO_3)_2$* and $ZnC_4H_6O_4$ from the UV graph, the direct band gap (E_g) calculated was 3.32 eV and 4.53eV using the formula,

$$E_g = \frac{1240}{\lambda \max ev}$$



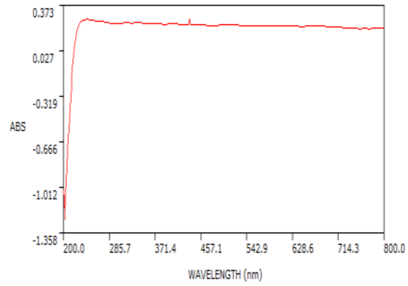


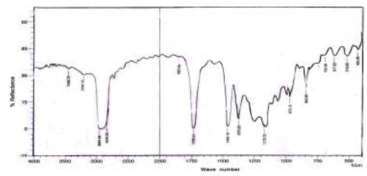
Fig: 1.a
UV-Visible Spectrum of ZnO NPs from $Zn(NO_3)_2$

Fig: 1.b
UV-visible spectrum of ZnO NPs from $ZnC_4H_6O_4$

A sharp peak in the UV-VISIBLE Spectrum is due to the Surface Plasmon Absorption of ZnO nanoparticles. The surface Plasmon absorption in the metal oxide nanoparticles is due to the collective band electrons which are excited by the incident electromagnetic radiation. The reduction of Zn nitrate into ZnO.NPs, which informed through the change in color, may be attributed to excitation of surface plasmon vibrations of nanoparticles which results in Surface Plasmon Resonance [39]

4.2 FTIR Spectra Analysis

FTIR is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range [40]. FTIR is used as a confirmatory technique to the nanoparticle formation and offers an impression to the vibrational and rotational modes of the existing molecules; hence it helps to identify the functional and possible phytochemical molecules involved in the reduction and stabilization of ZnO.NPs. Fig.2a, 2b representing the FTIR spectra of ZnO.NPs synthesized by the green approaches using Zn $(NO_3)_2$ and $ZnC_4H_6O_4$. Peaks showed at 432 $cm^{-1}$and 439$cm^{-1}$was corresponding to the hexagonal ZnO symmetric bending vibration and a peaks at 842 cm-[1] and 810 cm-[1] due to weak vibration of ZnO. The wide peaks present at 3400 cm^{-1} and 3414 cm^{-1} reflect the presence of OH stretching vibration and peak at 1172 cm^{-1} and 1165 cm^{-1} due to C—OH stretching vibrations 0f ZnO nps. Other smaller bond vibration peaks including 2954 cm^{-1} and 2905cm^{-1} denotes the C—H stretching vibration,



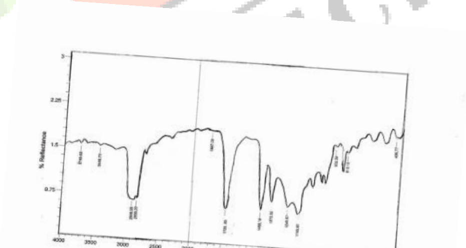


Fig: 2.a

Fig: 2.b

FTIR spectrum of synthesis of ZnO NPs using Zinc Nitrate

FTIR spectrum of ZnO NPs using Zinc acetate

4.3 SEM analysis

The size and morphology of the prepared ZnO NPs have been determined by using scanning electron microscopy. The SEM image shows random distribution of the ZnO NPs having semi spherical shape and diameter in the range of 25– 40 nm. The average size of the nanoparticles is ~100 nm, and the shapes were spherical and cubic. In fig.3.a and 3.b shows SEM photograph of prepared ZnONps all particles size are good agreement with spectral analysis.

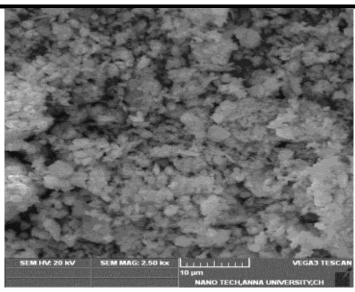


Fig 3.a
SEM image Zinc oxide NPs using Zinc Nitrate

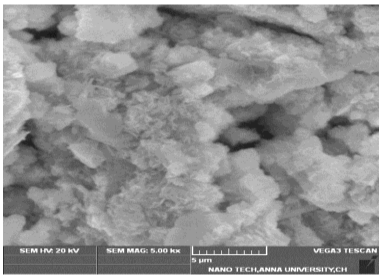


Fig 3.b
SEM image Zinc oxide NPs using Zinc acetate

4.4Antimicrobial activity

The newly prepared ZNO Nps have been studied against bacteria and fungi strains .The ZNO Nps of Zinc Nitrate and Zinc acetate showed potential anti bacterial and anti microbial activity against micro organisms (Fig4.a, 4.b).Anti microbial activity of Zinc Nitrate and Zinc acetate Nps .

Fig 4.a
Antimicrobial activity of ZnONPs using Zinc Nitrate

Fig 4.b
Antimicrobial activity of ZnONPs using Zincacetate

S. No	Source	Inhibition Zone (mm)		
		E. Coil	Staph.Aureus	Pseudo. Aeruginosa
1	Zinc nitrate	10	08	12
2	Zinc acetate	-	-	11

Table 1 Antimicrobial activity of ZnoNps

.V. CONCLUSION

In the present work, we report the simple method for synthesis of ZnO nanoparticles by green method using aqueous extract of centella asiatica dry leaf. The UV-Visible Spectra showed a sharp peak located at 246.8 nm for ZnO nanoparticles. The ZnO nanoparticles obtained from zinc nitrate is greater, we concluded that the particles size is lower when compared with the ZnO obtained from the other source. From FT-IR studies we confirmed the presence of Alkynes, alcohols, alkenes in the ZnO nanoparticles. The SEM image shows formation of spherical shape of zinc oxide nanoparticle.The antibacterial activities showed that ZnO nanoparticles obtained from Centella asiatica dry leaf extract posses good inhibition against both Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria. This method was best against conventional method and is less expensive and eco-friendly. ZnO Nanoparticles can be used in different industries like rubber, electronic, textile industry, Pharamaceutical industry due to their amazing properties like antibacterial. Thus, the ZnO nanoparticles synthesized using aqueous dry leaf extract of Centella Asiatica find use in medical applications and environmental science.

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