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Research Article

Green Synthesis of Zinc Oxide Nanoparticles Using Hydro Methanolic Extract of *Flueggea leucopyrus* Willd Fruit

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Abstract

Biosynthesis of nanoparticle is a vital role in the field of nanotechnology. In the present study zinc oxide nanoparticles synthesis were mediated by green synthesis using hydro methanolic extract of *Flueggea leucopyrus* Willd fruit. The green synthesized ZnO nanoparticles were analysed by UV-Vis, FT-IR Spectroscopy, X-Ray Diffraction and Scanning Electron Microscopy. The results of the various techniques confirmed the formation of ZnO-NPs. The UV-Vis spectra exhibited the absorption maximum at 360 nm corresponds to pure ZnO-NP. FT-IR spectra revealed the presence of stretching vibrations of -O-H, C-H, C-N, C=O and Zn-O involved in the reduction and stabilisation of nanoparticles. The SEM and XRD confirmed the synthesised ZnO-NPs is hexagonal (Wurtzite) shaped and size about 87.59 nm. The antioxidant property was reported using DPPH Radical Scavenging activity. The maximum concentration of ZnO-NPs was shown a promising antioxidant value of 71.22 ± 1.61 comparable with the standard vitamin-C. The current study is an attempt to describe an effective, simple and eco-friendly method of ZnO-NPs and to appraise its potential for various therapeutic applications in future.

Keywords: *F. leucopyrus* Fruit; ZnO-NP; XRD; SEM; DPPH Radical

Introduction

Nanotechnology is one of the emerging fields in 21st century. Nanoparticles are very small sized particles with enhanced catalytic reactivity, thermal conductivity, non-linear optical performance and chemical steadiness owing to its large surface area to volume ratio [1]. Transition metal oxide with nano structure and semiconductors with nano dimensions have attracted considerable interest in numerous areas of science and technology as next generation technologies [2-4]. Nowadays Zinc Oxide nanoparticles are very much essential in various streams of science. Zinc oxide is the most promising inorganic oxides that are extensively used owing to its suitable magnetic, electrical and optical properties [5]. ZnO has been listed as "Generally Recognized as safe" (GRAS) by the US Food and Drug Administration (FDA 21CFR182.8991) [6]. It crystallizes in two main forms, hexagonal Wurtzite and cubic Zinc blende [7]. Traditionally, ZnO nanoparticles are synthesized using physical and chemical processes, which offer higher production rate and produce the better-controlled size of nanoparticles. Nonetheless, these methods are considered unfavourable due to high capital cost, high energy requirements and involve the use of toxic and hazardous chemicals. Consequently, these features result in secondary pollution to the environment. Moreover, a study dem-

onstrated that the chemical synthesis of nanoparticles is toxic and less biocompatible [8]. Biological fabrication of ZnO nanoparticles using plants, microorganisms, algae and enzymes are ecologically favourable and sustainable compared to physical and chemical approaches [9]. *Flueggea leucopyrus* Willd (Family: Euphorbiacea) is one of the medicinally used bushy weed [10]. A bergenin found in hydro methanolic extract of the *Flueggea leucopyrus* leaves has shown antioxidant and immunomodulatory activities *in vitro* [11]. The Present study was aimed at green synthesis of Zinc Oxide nanoparticles using hydro-methanolic extract of *Flueggea leucopyrus* willd Fruit. This use of plant extract has some benefits such as been safe, cost effective, environment friendly, non-hazardous, biocompatible, and large-scale production is plausible [12-15].

Material and Methods

Collection of plant materials

Healthy disease-free fruits of *Flueggea leucopyrus* Willd were collected from foot hills of Western Ghats, Sivanthipuram, Tamil Nadu, India. The voucher specimen (XCH 26879) was deposited in St. Xavier's College Herbarium, Palayamkottai.

Extract preparation

About 60g of air-dried, coarsely powdered *F. leucopyrus* Willd fruits were extracted by green synthesis using hydro-methanolic cold extraction method. The extraction was carried out about 72 hours. The extracts were concentrated using vacuum evaporator and analysed for further analysis.

Synthesis of zinc oxide nanoparticles

About 0.22g of zinc acetate dihydrate was weighed accurately and dissolved in 100ml of distilled water. 20ml of the fruit extract of *F. leucopyrus* Willd was slowly added to the Zinc acetate dihydrate solution. This mixture was constantly stirred about four hours using magnetic stirrer. The extract was filtered using Whatman filter paper No.1 and centrifuged to remove any undissolved debris. Then the reaction mixture was kept for a day. The change in colour indicates the formation of zinc oxide nanoparticles. The pure Zinc oxide nanoparticles were collected and kept in air tight bottles in a desiccator for further studies [16].

Results and Discussion

The reaction between the hydro-methanolic extract of *Flueggea leucopyrus* fruit and zinc acetate dihydrate solution leads to the formation of ZnO nanoparticle. The bioactive compounds present in the *Flueggea leucopyrus* fruit can play an important role in the reduction and stabilization of ZnO-NPs. Various analytical methods were used for the characterization of the ZnO-NPs to evaluate their properties like size, shape, morphology and composition.

Visual assessment

The stage by stage colour change exposes the formation of Zinc oxide Nanoparticles by plant materials. This is one of the significant tests for checking the formation of ZnO-NPs. The colour of the reaction mixture changes from dark brown to pale yellow indicating the synthesis of ZnO-NPs. The pale coloured residue obtained was dried in hot air oven to yield amorphous zinc oxide nano particle.

Figure 1: Formation of ZnO-NPs using F. leucopyrus fruit.

(a) Zinc acetate dihydrate solution. (b) Methanolic extract of Flueggea leucopyrus fruit. (c) Immediate Colour Change. (d) Formation of Zinc oxide nanoparticles.

UV-visible spectral analysis

The UV-Visible spectral studies of the green synthesized ZnO-NPs. were examined using Shimadzu Make UV Spectrophotometer UV-1700 with range of 200-900 nm. The absorption spectrum of the synthesized ZnO NPs using *Flueggea leucopyrus* fruit extract are shown in figure 2. The absorption maximum at 360 nm supports the formation of Zinc oxide nanoparticles. Strong absorption bands of the biosynthesized samples were observed from UV-Visible spectra in the range of 360-363 nm which corresponds to the characteristic band of ZnO nanoparticles [17]. Absence of any other absorbance peak in the spectra confirms that the synthesized products are pure ZnO-NPs. Furthermore, it is reported that the peak positions of UV-Visible spectra are related with size of nanoparticles and blue shifted as the crystal size of the nanoparticles decreased [18].

Figure 2: UV-V is spectrum of ZnO-NPs.

Figure 3: FT-IR spectrum of green synthesized ZnO-NPs.

FT-IR analysis

The FT-IR spectra of green synthesized ZnO-NPs of *Flueggea leucopyrus* Fruit were shown in figure 3. The FTIR spectrum of zinc oxide nano particles with absorption peaks situated between 4500 cm^{-1} and 400 cm^{-1}. A broad Band observed at 3448.12 cm^{-1} assigned to the stretching of phenolic O-H group vibrations. The bands observed at 2362.80 cm^{-1} could be allocated to-C-C- stretching. A sharp band at 1714.72 cm^{-1} corresponding to -C=O group. Two absorption peaks observed at 1543.05 and 1506.41 cm^{-1} are assigned to the stretching of C =C groups. The peaks at 1064.71 cm^{-1} result from C-N stretching of aliphatic amines. The intense absorption peak at ~ 800 to 400 cm^{-1} indicates that the stretching vibrations of Zn-O bond. The band formed at 472.56 cm^{-1} highly supports the presence of Zinc Oxide Nano Particles free from functional groups associated with bioorganic compounds of *F. leucopyrus* fruit [19].

X-ray diffraction data

The powder XRD pattern of the prepared ZnO nanoparticles is recorded with X-Ray Diffractometer (D8 Advance ECO XRCD Systems with SSD160 1 D Detector) (Bruker) at wavelength 1.54060 A°. The sample is scanned over 2θ range 20°-90°. Table 1. Showed the XRD studies of biosynthesized ZnO-NPs using *Flueggea leucopyrus* fruit. The average size of ZnO-NP is calculated using Debye-Scherrer equation.

$$D = K\lambda/\beta \cos \theta$$

Where D is crystalline size, K is shape factor (0.9), λ is wavelength of X-ray (1.5406 Å), β is full width half maxima, θ is Bragg angle. The mean particle size of the pure ZnO-NPs was found around 87.59 nm, which shows some agglomeration of ZnO-NPs.

No. of peaks	Planes	2^{θ} (deg)	θ (rad)	FWHM, β (rad)	Size (nm)
1	100	30.2020	0.2636	0.1557	93.0109
2	002	34.2354	0.2988	0.2279	63.5447
3	101	42.7018	0.3726	0.1796	80.6344
4	102	45.1220	0.3938	0.1503	96.3538
5	110	50.8293	0.4436	0.3441	42.0868
6	103	60.4054	0.5271	0.1780	81.3609
7	200	66.1052	0.5769	0.1305	110.9760
8	201	70.6888	0.6169	0.1176	123.1504
9	202	77.2106	0.6738	0.1490	97.1989
Average Size					87.5908

Table 1: The ZnO NPs particle size calculation using Debye- Scherrer's equation and data.

Scanning Electron Microscopy Analysis

SEM analysis was performed to visualize the shape of nanoparticle. The surface morphology of biosynthesized ZnO-NPs obtained from the *Flueggea leucopyrus* Fruit were studied by SEM and the results are reported in figure 4. The particles are having beautiful hexagonal shape as like wurtzite type of structure.

DPPH radical scavenging activity

In Pharmaceutical science besides nanoscience and technology antioxidant activity plays a key role was carried out by William's method using DPPH assay to evaluate the percentage of scavenging

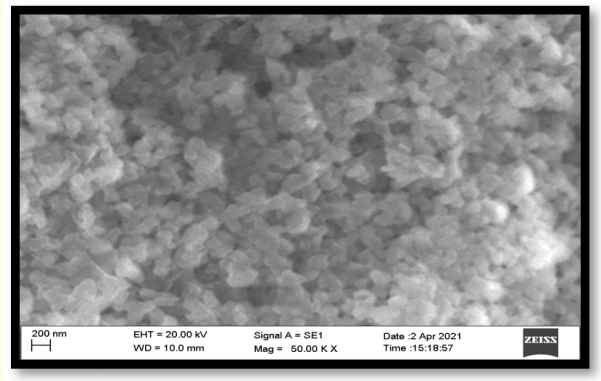

Figure 4: SEM Analysis of ZnO-NPs.

activity [20]. The DPPH radical scavenging activity of green synthesized ZnO-NPs of *Flueggea leucopyrus* fruit was tabulated in table 2. These measurements revealed that the antioxidant effect increased with increase in the concentration of ZnO nanoparticles.

Percentage of DPPH Scavenging Inhibition = A_o - A_t/A_o X100

Where

A_o = Absorption value of control (DPPH) sample

A_t = Absorption value of test sample.

Concentration	Percentage of anti-oxidant effect on DPPH	
	Vitamin C	ZnO-NPs
10**µg**	90.16 ± 1.76	48.16 ± 1.56
20**µg**	92.82 ± 0.98	52.75 ± 2.99
30**µg**	95.44 ± 1.75	58.82 ± 1.65
40**µg**	99.99 ± 1.65	67.15 ± 1.97
50**µg**	99.99 ± 1.24	71.22 ± 1.61

Table 2: Scavenging effect of ZnO-NPs and Vitamin C on DPPH free radical.

All the samples were tested triplicate or constant results. The unstable DPPH solution turns stable by the addition of different dosage of antioxidants ZnO-NPs. This is due to the donation of an electron from Oxygen which is present in the ZnO-NPs to the nitrogen atom of the DPPH molecule. The 50 **µg** of ZnO-NPs shows maximum inhibition value of 71.22 ± 1.61 comparable with the standard vitamin-C. From the result we conclude that maximum concentration of ZnO-NPs inhibits the DPPH free radical to yield a stable DPPH molecule.

Conclusion

In this study, a simple and eco-friendly, biological process have been developed to synthesise ZnO-NPs. It has been characterized using UV-Visible, FT-IR spectral analysis the secondary metabolites present in the hydro-methanolic extracts of *F. leucopyrus* fruit involved in the formation of ZnO-NPs. The biosynthesised ZnO-NPs have hexagonal structures and mean particle size about 87.59 nm determined from SEM an XRD analysis respectively. The antioxidant behaviour of ZnO-NPs shown this as a potential drug over DPPH radical scavenging activity. This green synthesis of ZnO-NPs using hydro-methanolic extracts of *F. leucopyrus* fruit paved a route for the development of novel drug in the field pharmaceutical and biomedical applications.

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