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Supporting Information

for

Green synthesis of ZnO-NPs using sugarcane bagasse waste: Phytochemical assessment of extract and biological study of nanoparticles

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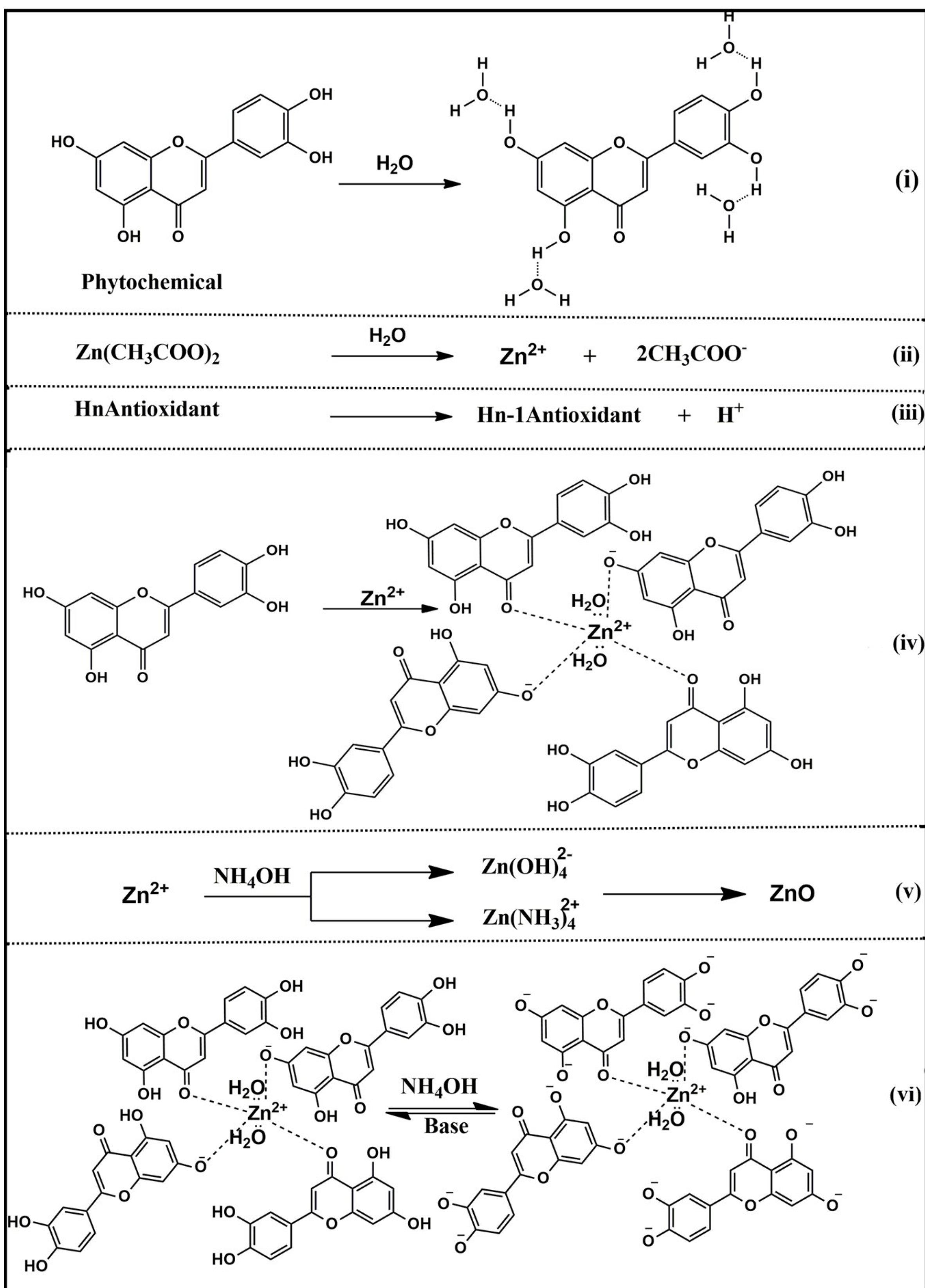

Fig. S1: The proposed of formation mechanism of ZnO-NPs mediated by aqueous sugarcane bagasse extract.

Phytochemical analysis of ASCBE

Total phenolic content (TPC)

A total phenolic content (TPC) was investigated as gallic acid equivalent (GAE) using the Folin–Ciocalteu method [1]. Briefly, the procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 1M Na_2CO_3 was added and incubated at room temperature (25 °C) for 20 min in the dark. At the end of incubation, the resulting blue complex color was measured at 630 nm. Data are represented as means ± SD.

Total flavonoid content (TFC)

The total flavonoid content (TFC) was determined as rutin equivalent (RE) using the aluminum chloride method [2] with minor modifications to be carried out in microplates. Briefly, 15 µL of sample/standard was placed in a 96-well microplate, then 175 µL of methanol was added, followed by 30 µL of 1.25 % c. Finally, 30 µL of 0.125 M $C_2H_3NaO_2$ was added and incubated for 5 min. At the end of incubation, the resulting yellow color was measured at 420 nm. Data are represented as means ± SD.

Antioxidant assays

DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out according to Trolox equivalent (TE) using the method of Arnao et al [3] adopting the modifications of Elkholy et al. [4]. Briefly; 100 µL of freshly prepared DPPH reagent (0.1% in methanol) was added to 100 µL of ASCBE sample in 96-well plates (n=3). The reaction was incubated at room temperature for 30 min. in the dark. At the end of the incubation time, the resulting reduction in DPPH color intensity was measured at 540 nm. Data are represented as means ± SD according to the following equation:

$$Percentage\ inhibition = [(Average\ absorbance\ of\ blank - Average\ absorbance\ of\ the\ test) \div (Average\ absorbance\ of\ blank)] \times 100$$

FRAP assay

FRAP (Ferric Reducing Antioxidant Power) assay was carried out as Trolox equivalent (TE) according to the method of Arnao et al. [3], with minor modifications to be carried out in

microplates. A freshly prepared TPTZ reagent (300 mM Acetate Buffer (PH=3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$, in a ratio of 10:1:1 v/v/v, respectively). 190 µL of the freshly prepared TPTZ reagent were mixed with 10 µL of ASCBE sample in 96 wells plate (n=3); the reaction was incubated at room temperature for 30 min in the dark. At the end of the incubation period, the resulting blue color was measured at 593 nm. Data are represented as means ± SD.

ABTS assay

ABTS (The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation-based assays measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase] assay was carried out as Trolox equivalent (TE) according to the method of Arnao et al. [3], adopting the modifications of Elkholy et al. [4], to be carried out in microplates. Briefly, 192 mg of ABTS were dissolved in distilled water and transferred to a 50 mL volumetric flask then the volume was completed with distilled water. 1mL of the previous solution was added to 17 µL of 140 mM potassium per-sulphate, and the mixture was left in the dark for 24 hours. After that, 1mL of the reaction mixture was completed to 50 mL with methanol to obtain the final ABTS dilution used in the assay. 190 µL of the freshly prepared ABTS reagent were mixed with 10 µL of ASCBE sample compound in 96 wells plate (n=6), and the reaction was incubated at room temp for 30 min in the dark. At the end of the incubation time, the decrease in ABTS color intensity was measured at 734 nm. Data are represented as means ± SD.

Characterization techniques

ZnO samples were characterized using various techniques. To characterize the crystalline phase and relative crystalline nature of fabricated ZnO and -NPs, an X-ray diffraction (XRD) pattern was performed using a D8 advance X-ray diffractometer [Bruker AXS D8, Germany] equipped with Cu-K radiation (λ =0.154056 nm). A high-resolution transmission electron microscope (HR-TEM) with an acceleration voltage of up to 200 keV [TEM, JEOL-JEM-2100, Japan] was used to study the particle size and shape. The organic groups within the sample were examined using a Fourier transform infrared FTIR-JASCO 3600-spectrometer via KBr tablets in the range (400–4000 cm^{-1}) at room temperature. UV-visible absorption spectroscopy was used to record the absorption spectra of the NPs using a UV/VIS-JASCO V-770 spectrophotometer. Thermogravimetric analysis (TGA) was accomplished on the synthesized ZnO-NPs using a Q50

analyzer to evaluate their thermostability and change of weight %. The photoluminescence (PL) spectra of the samples were provided using a Spectro fluorophotometer (Shimadzu RF-5301PC) at 25° C with an excitation wavelength of 320 nm. The conditions were fixed to compare the PL intensities.

References

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