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Biological Activity and Toxicological Profile of Zinc Oxide Nanoparticles Synthesized by *Portulaca oleracea (L)* Leaves Extract

Sara Chetehouna[1], Ouidad Atoussi[1] and Samir Derouiche[1,2*]

*[*1]Department of Cellular and Molecular Biology, Faculty of Natural Sciences and Life, University of El Oued, Algeria,*

[2]Laboratory of Biodiversity and Application of Biotechnology, Faculty of Natural Sciences and Life, University of El Oued, Algeria.

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ABSTRACT

The objectives of this present study were to use by an eco-friendly method the mixture of aqueous extracting *P. oleracea L* leaves and the initiating material zinc sulfate to produce Zinc oxide nanoparticles (ZnNPs). Also, the identification of their characterizes and biological ability. Ultraviolet-Visible Spectroscopy, showed a peak of ZnNPs at 230 nm, the used of SEM confirmed that the synthesized of ZnNPs have a size relatively less than 37.5 nm. The Fourier Transform Infrared (FTIR) spectrum presented an appeared peak in the rang 400-700 cm^{-1}. Furthermore, the biological activity revealed that the ZnNPs and aqueous extract of *Portulaca oleracea L* leaves had anti-inflammatory activities with IC50 values 50.552 and 60.727 mg/l respectively, an efficient protective effect of RBCs hemolysis with different concentration and antioxidant prosperity used by FRAP assay, which characterized by IC50 73.54 for ZnNPs, in conjunction to the leaves aqueous extract withe 79.65 mg/l values. In this study, the toxicity test showed no mortality or behavioral change up to 20 mg / kg of albino Wistar rats ZnNPs and purslan aqueous extract. We concluded that *Portulaca oleracea L* has potential properties as biocatalyst or natural stabilizers for ZnNPs synthesis. This efficient activity of synthesized ZnNPs might be utilized in several biological applications.

Keywords: ZnONPs, *Portulaca oleracea L*, SEM, Biological activity

INTRODUCTION

Due to the environmental benefits of biological bio fabrication over conventional chemical and physical methods [1], the application of nanotechnology in biology requires further studies for the development of new materials in the nanosized range [2]. The Green synthesis of nanoparticles is a novel way to synthesize nanoparticles by using biological sources. It is gaining attention and attracted the scientists from different fields due to its simplicity and environment-friendly nature [3]. Zinc oxide nanoparticle is considered as one of the most promising and novel magic materials because of its unique catalytic, electrical, electronic, optical and antimicrobial properties as well as its low cost and extensive applications in diverse areas [4]. Known as "vegetable for long life" in Chinese folklore [5], *Portulaca oleracea L* has a wide cosmopolitan distribution which gave it different names in various geographical locations commonly it's known as purslane in English [6], "Ma-Chi-Xian" in China, "rigla" in Egypt, "Pourpier" in France [7], it is listed in the World Health Organization as one of the most used medicinal plants.

The water extracts of *P. oleracea* show no cytotoxicity or genotoxicity and have been certified safe for daily consumption as a vegetable [8]. Purslane possesses a wide spectrum of pharmacological properties such as neuroprotective, antidiabetic, antioxidant, anti-inflammatory, and anticancer activities [9]. These effects have been demonstrated to result from various active components, including alkaloids, fatty acids, flavonoids, polysaccharides, and terpenoids [10].The aim of this study was to use the ecofriendly method in order to synthesize the ZnNPs by leaves aqueous extract of *P. oleracea L* as a stabilizing

Corresponding author: Derouiche Samir, Faculty of Natural Sciencesand Life, University of El Oued, El-Oued 39000, Algeria, Tel: 00213552285235, E-mail: dersamebio@gmail.com

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agent, to investigate their characterizes and the exploration of biological activities.

MATERIALS AND METHODS

Chemicals

analytical-grade zinc sulfate, sodium hydroxide and other reagents used were obtained from Sigma Aldrich.

Plant materials

The plant of *Portulaca oleracea* were collected in August from a village in Touggourt of Ouargla state, Algeria. The leaves were washed with distilled water, then dried at room temperature, then grind to powder and stored at room temperature until use.

Portulacae oleracea L. leaves extract preparation

The extraction methods described by Zebidi et al. (2018) [11]. The aqueous extract was prepared by adding 50 ml of distilled water to 5 g dry leave powder of *Portulaca oleracea L*. After 24 h of maceration at room temperature, the mixture was filtered by filter paper and then dried in a stove.

Green synthesis of ZnONPs

In order to prepare Zn NPs, 200 ml of aqueous zinc sulfate was mixed with 20 ml of the aqueous leaf extract of *Portulacae oleracea L*, and subsequently treated with 1.0 M sodium hydroxide (10 ml). The ions, which initiated the reaction, were afforded by the zinc sulfate in de-ionized water. The reaction mixture was incubated with constant stirring in the dark at 60°C to avoid photo-catalysis. An observed off-white color marked the formation of Zn NPs at the end of 24 h. The resultant product was further purified by centrifugation and washed in double-distilled water and ethanol, respectively, dried and kept in an amber-colored sample bottle until use [12].

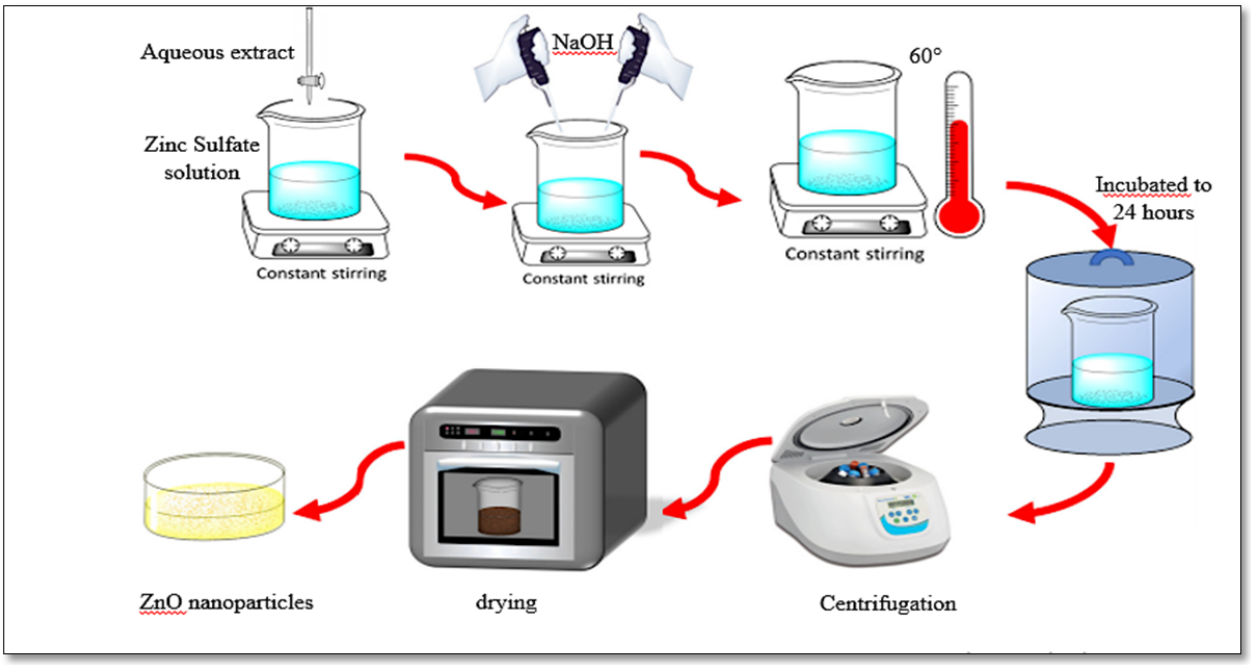

Figure 1. The method of zinc oxide nanoparticles green synthesis from the mixture of zinc sulfate and purslan aqueous extract.

Characterization

The characterization of ZnNPs were identified by UV-Vis, FTIR spectroscopy, XRD and SEM analysis. UV-Vis spectrum of zinc nano-colloidal solution was recorded in the range of 200-500 nm. SEM analysis were performed in order to determine the average particle size.

BIOLOGICAL ACTIVITIES

Anti-inflammatory activity

The anti-inflammatory activity of the *Portulaca oleracea* and synthesized NPs as a measure of protein denaturation inhibition was studied through in vitro assay. Bovine serum albumin (BSA) solution (1%) was incubated at room temperature for 30 min with or without different concentrations (10-50 µg ml^{-1}) of sample. The pH of the solution was adjusted to 2 using drop-wise addition of concentration HCl. After incubation, the mixture was heated at 72°C for 30 min. Finally, all tubes were cooled for 10 min and the turbidity was measured at a wavelength of 660 nm. Diclofenac was used as standard. The percentage inhibition

[(A0-A1/A0) × 100] was calculated and the results was expired by IC50 [13].

Hemolysis assay

The Hemolysis assay was done as described by Vinjamuri S.et al. [14], 5 mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40°C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min. Washed erythrocytes were stored at 4°C and used within 6 h for the hemolysis assay. 50 µL of 10 dilutions (100 µL Erythrocytes suspension: 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples (*extract of Portulaca oleracea* and synthesized NPs) (20-80 µg/mL), 100 µL of 1XPBS was used as negative control and 100 µL of 1% SDS as positive controls. Reaction mixture was incubated at 37°C water bath for 60 min. The volume of reaction mixture was made upto 1 mL by adding 850 µL of 1XPB. Finally, it was centrifuged at 300 rpm for 3 min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin. The percentage hemolysis was calculated as % Hemolysis inhibition= 100-[Sample / Control] x 100.

Antioxidant activity (Ferric reducing ability power "FRAP")

The ferric reducing antioxidant power of the extracts was determined following previous report [15,16]. A standard curve was constructed from different concentrations of FeSO4 solution and absorbance values at 593 nm. The FRAP value was reported as mmol Fe^{2+}/g dry weight (mmol Fe^{2+}/g DW).

FRAP Value = 100- [(control abs/Sample abs) × 100]

Sub-Acute toxicity studies

The test was performed using healthy albino rats of Wistar strain weighing between 154 and 181 g. The animals were divided into three groups for each one of them tow rats, which administered 0, 25 & 50 mg/kg of ZnNPs and 0, 2000 & 4000mg/Kg of leaves aqueous extract of *P.oleracea L* orally. Animals were observed after dosing at least once during the first 30 min, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter for a total of 14 consecutive days [17].

RESULTS & DISCUSSION

Results presented in **Figure 2** show a solution color change from white to off white. The hydrolysis phase is the initiated step of ZnNPs formation wherein the zinc sulfate dihydrate precursor react with reducing agent OH- in the purslan leaves aqueous extract environment [18]. The color change was due to excitation of surface plasmon vibrations in nanoparticles [19].

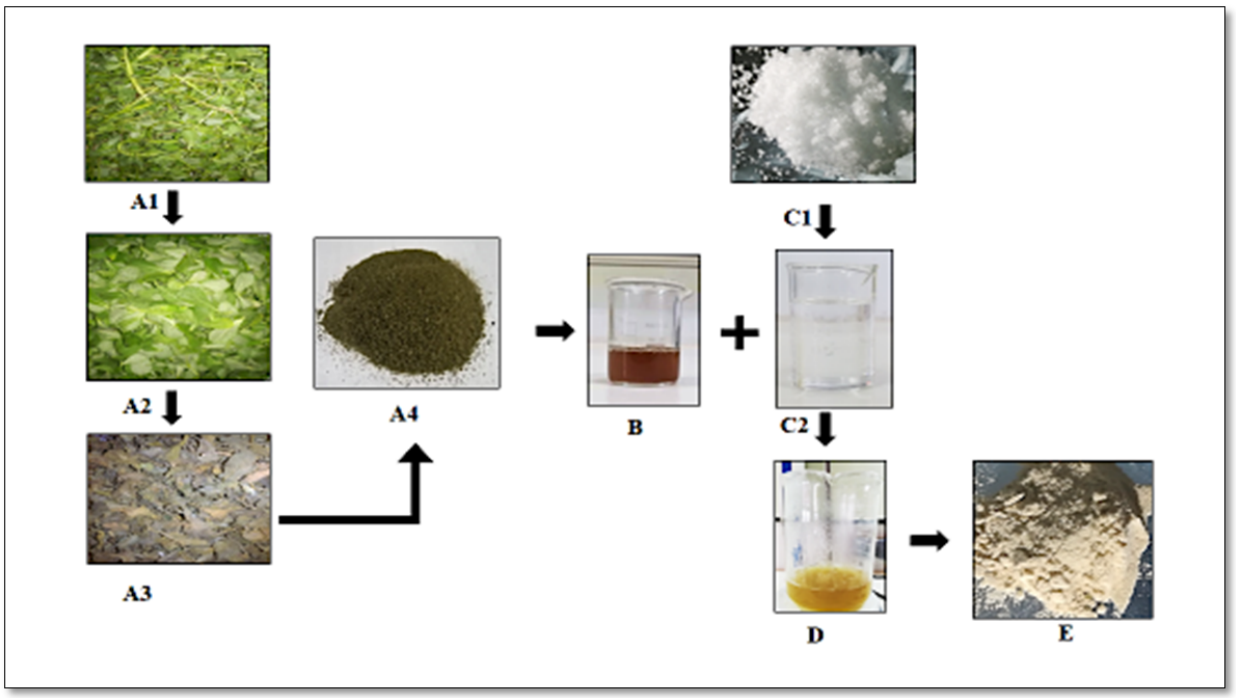

Figure 2. Visual observation of *P.oleracea L* (A1), *P.oleracea L* leaves (A2), Dried leaves of *P.oleracea L* (A3), Grinded leaves of *P.oleracea L* (A4), Leaves extract of *P.oleracea L* (B), Zinc sulfate powder (C1), Zinc sulfate solution (C2), solution of ZnNPs synthesized (D), powder of ZnNPs synthesized (E).

UV-Vis spectroscopy

To confirm the synthesis of ZnNPs we usually used spectroscopy uv-visible, as showing in **Figure 3**, the sample absorbe the radiation in the rang 200 to 250 nm. Whether the pic was appeared in 298 nm as what reported by Moharram et al. [20].

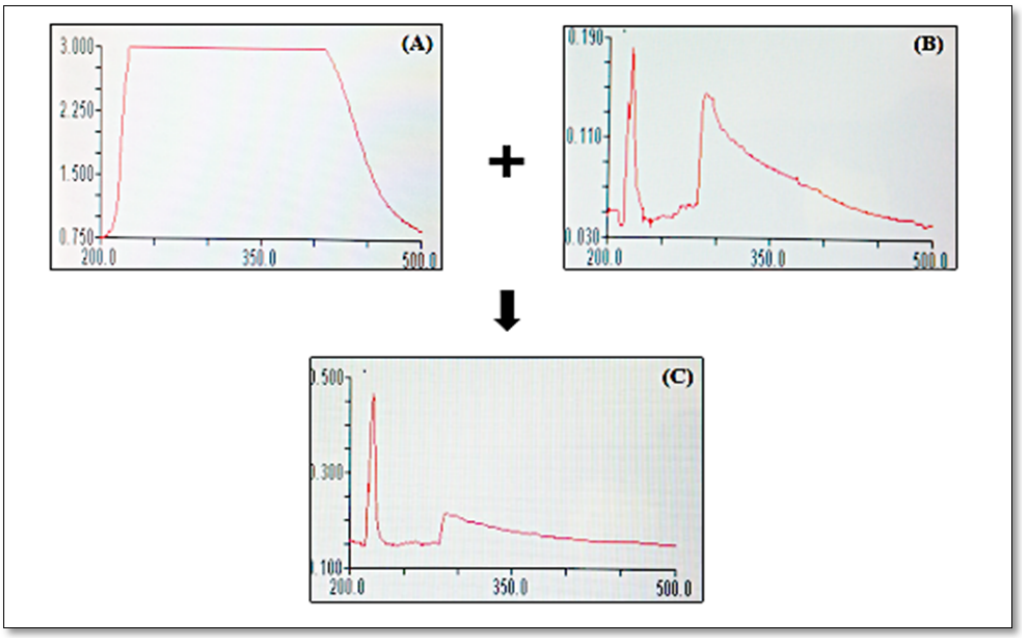

Figure 3. UV-Vis spectra of purslane aqueous extract (A), Zinc sulfate (B) and ZnNPs biosynthesized by purslane aqueous extract (C).

SEM analysis

SEM image **(Figure 4)** show the biosynthesized Zinc nanoparticles powder by *P. oleracea L* aqueous extract have a size relatively less than 37.5 nm, while the Zinc nanoparticle synthesized using *Melia azedarach* leaf with a size range 33-96 nm [21].

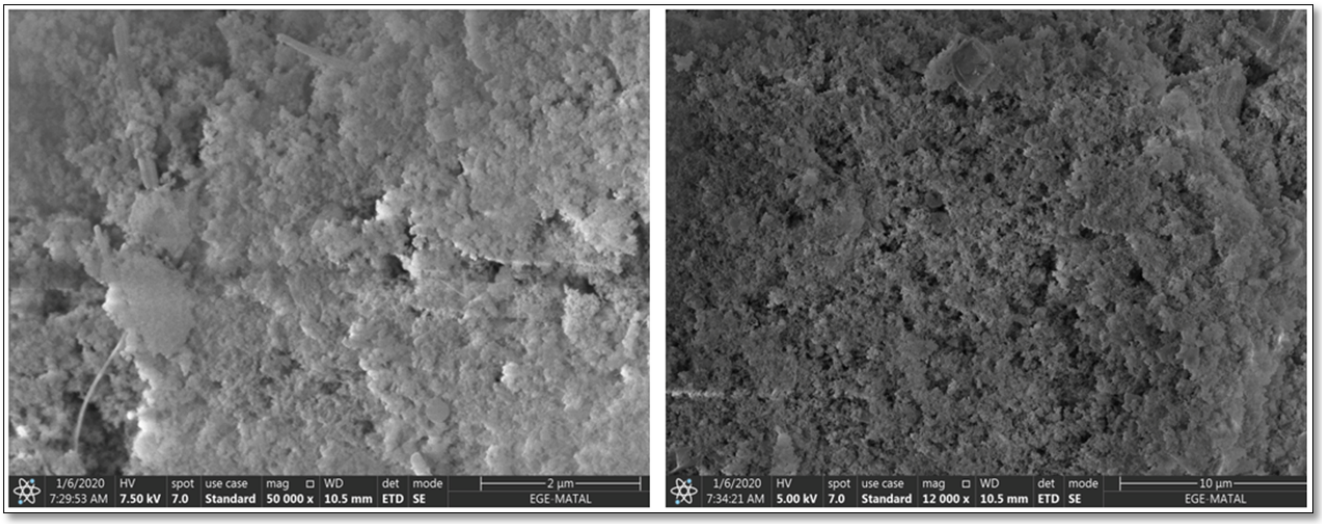

Figure 4. Scanning electron microscopy image of the biosynthesized ZnO nanoparticles from purslan aqueous extract.

FT‐IR analysis

To confirm the successful preparation of ZnNPs, we used the FT-IR spectroscopy. As shown in **Figure 5** the presence of peak in the rang 400-700 cm^{-1}, as what reported by Sharma et al. [22].

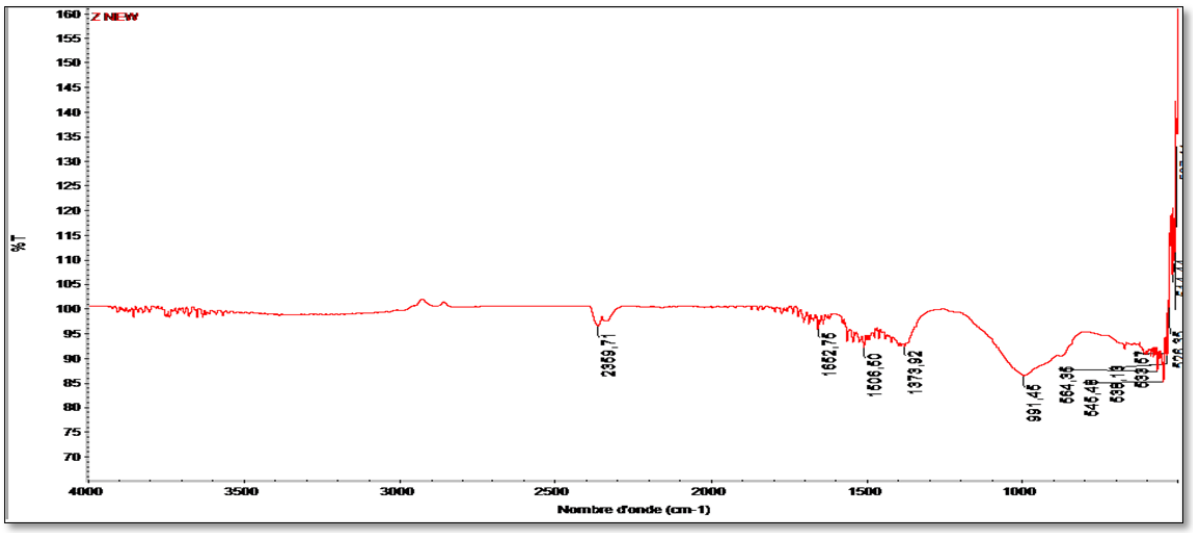

Figure 5. FTIR spectrum of Zn nanoparticles synthesized by *Portulaca oleracea L* leaves aqueous extract.

BIOLOGICAL ACTIVITIES

Antioxidant activity "Ferric reducing antioxidant power (FRAP)"

The antioxidant activity determined by FRAP assay [23], the antioxidant agents present in the sample are involved in the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), this reducing power expresses the antioxidant activity of these agents [24]. Our results in **Figure 6** was indicated that Zinc nanoparticles and *Portulaca oleracea L* aqueous extract have antioxidant activity when the FRAP values were 73,547 µg/ml and 79,675 µg/ml respectively. This result is reported by several studies [25,26] while the ZnNPs synthesized by *C.abyssinica* tuber extract has antioxidant activity that determined by DPPH assay. ZnNPs has no known mechanism of action as an antioxidant [27]. The vitamin C, glutathione and the total phenolic content present in *P.oleracea L* [28]are the responsible of the antioxidant potential of this plant [29].

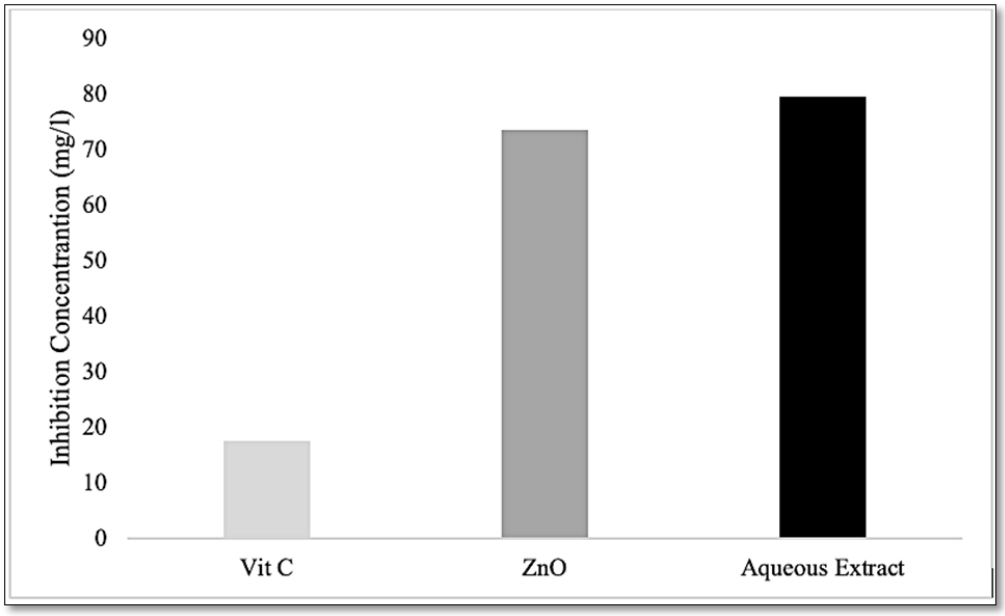

Figure 6. FRAP scavenging assay of ZnO, *P.oleracea L* aqueous extract and vitamin C.

Anti-inflammatory activity

Our results showed in figure 7 that the ZnNPs and leaf aqueous extract of *P.oleracea L* presented an anti-inflammatory activity with IC50 values 50,552 and 60,727 mg/l respectively compared to diclofenac sodium as the positive control with IC50 value 27,2306 mg/l. The anti-inflammatory activity of ZnO nanoparticles synthesized

using *Polygala tenuifolia* root extract reveled in Nagajyothi et al. [30], while it reduces the mRNA and protein expressions of IL-6, TNF-α and iNOS on PLS induced inflammation in RAW 264.7 macrophage. Probably, the same effect on the same cell line demonstrate by aqueous extract of *P.oleracea L* [31]. This is due to the alkaloids present in *P.olerecea L* such as oleracimine, oleracimine A, oleracone A, oleracone B and β carboline [32].

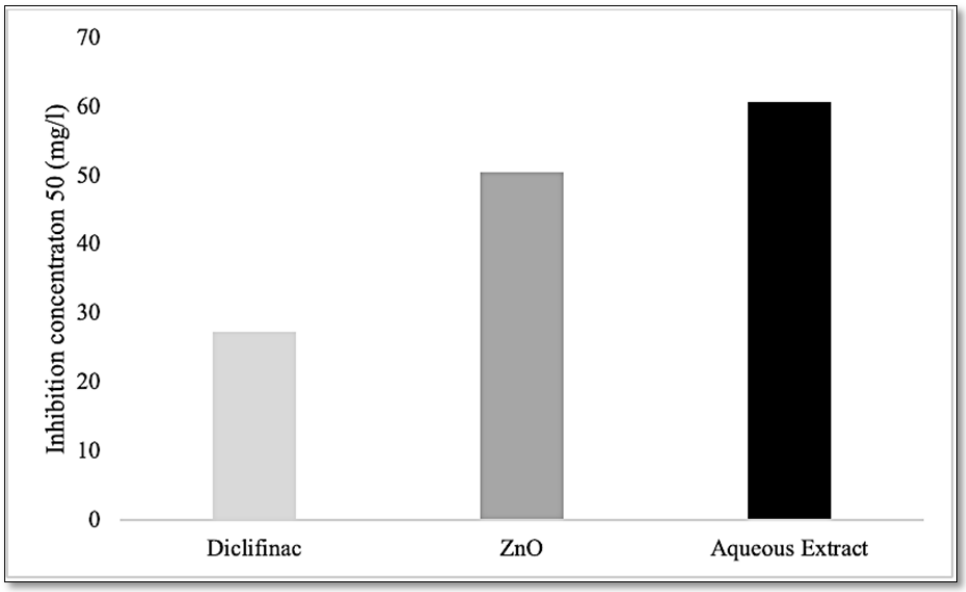

Figure 7. IC50 levels of anti-inflammatory activity of ZnNPs, *P.oleracea L* aqueous extract, and diclofenac.

Hemolysis assay

The detection of RBCs hemolysis by measuring the concentration of Hb in blood serum or plasma [33]. **Figure 8** demonstrated the protective effect of ZnNPs and aqueous extract of *P.oleracea L* against Red Blood Cells (RBCs) hemolysis at different concentration. The protective effect of ZnNPs is similar to what found by Preedia Babuet al. [34], whether the interaction of nanoparticles with red blood cells can be caused by their small size and their physicochemical properties [35]. Also, the results of aqueous extract of *P.oleracea L* as confermed by Karimiet et al. [36], through the antioxidants, in particular the phenolic compounds are responsible for protecting RBCs against the hemolysis process by the phenolic hydrogen atom [37].

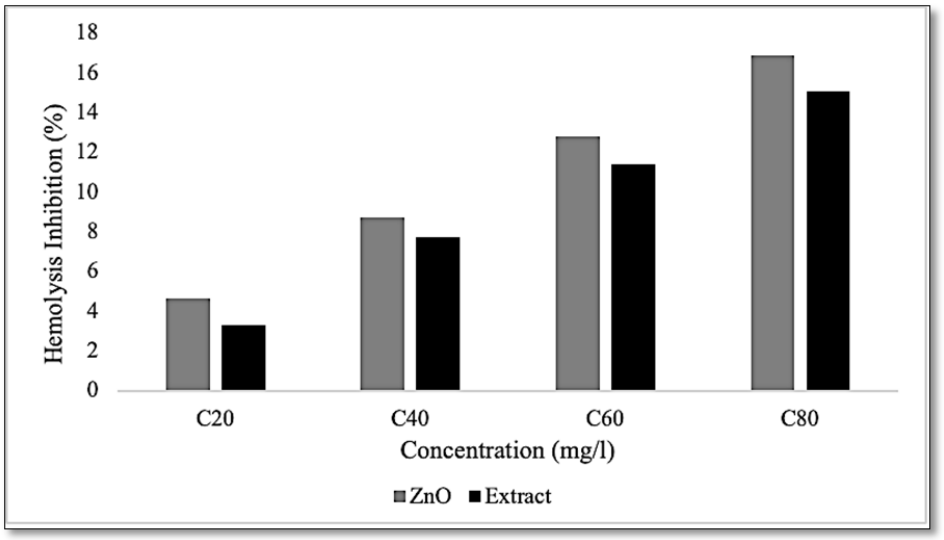

Figure 8. Effects of ZnNPs and *P.oleracea L* aqueous extract on Red Blood Cells (RBCs) hemolysis.

SubAcute toxicity study

The results obtained from this test (**Table 1**) showed that normal physiological parameters during the experimental period as the eyes, sleep and diarrhea of the albino Wister rats were treated by different dose of ZnNPs 0, 25 and 50 mg/kg either the purslane leaves aqueous extract 0, 2000 and 4000 mg/kg. In addition, there was no abnormal symptom or adverse effects and no mortality case were noted before 14 days.

Table 1. Subacute toxicity test of ZnNPs and leaves aqueous extract of *P. oleracea L*on physiological parameters of Wister albino rats.

Parameters	0 h		3 h		24 h		Day- 7		Day-14	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Dead rats	0	0	0	0	0	0	0	0	0	0
Eyes	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N

Test, ZnNPs and leaves aqueous extract of *P. oleracea L* which respectively (25& 50; 2000 & 4000 mg/kg bw rats) administered rats, N, Normal.

CONCLUSION

The present study demonstrates the low cost and eco-friendly approach in biogenic synthesis of ZnO nanoparticles using aqueous extracts of *P. oleracea*. Prepared herbal extract demonstrated valuable biological effects including anti-inflammatory and antioxidant activities. In addition, ZnO nanoparticles developed in this work promised as potentially useful preparations, which were able to stimulate anti-inflammatory property and to protect from oxidative stress according to FRAP assay models without any toxicity in the studied concentrations. Further, in vivo studies are warranted to understand the biological application of ZnNPs.

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CONFLICT OF INTERESTS

All authors have approved the manuscript with no conflict of interest.

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