fraction provide the stabilization of casein micelles in the aqueous medium. Such structure of casein does not allow to receive film materials, they have low technological characteristics, very low durability and elasticity, high fragility. Moreover, they are easily soluble in water.

Studies have shown that on the basis of casein it is possible to obtain polymer films, provided the use of a polymer composition, in which besides casein there is a modified polysaccharide. To obtain such a composition, carboxymethylcellulose was used, which also has biodegradable properties, however, unlike casein, it is capable of forming sufficiently strong polymer films. The polymer composition was synthesized in two steps: first, an aqueous solution of carboxymethyl cellulose was prepared, to which freshly precipitated casein with a moisture content of 20%, precipitated from skim milk, was added. The composition of the polymer composition was enriched with a number of additives serving as plasticizers, and structure-forming. The triatomic alcohol glycerol and diphenylamine were added as plasticizers too. In order to regulate the structure of the aqueous dispersion medium and prevent the processes of aggregation of casein micelles, calcium chloride solution was injected in the composition. The given polymer composition after polymerization in the air-drying process makes it possible to obtain sufficiently strong composite polymer films, the transparency of which depends on the type of plasticizer selected and the presence of $CaCl_2$.

Key words: biodegradable polymeric materials, casein, copolymers, carboxymethylcellulose, films, tensile strength.

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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES FROM THE AYURVEDIC HERBS AND THEIR ANTIRADICAL POTENTIALS

Green synthesis of nanoparticles using environmental-friendly reducing agents is in the focus. We applied green technology for the synthesis of zinc nanoparticles (nZnO) using leaves extract of *Catharanthus roseus* and fruits extract of *Momordica charantia (Karela)*. The development and advance of nZnO biosynthesis from leaves and fruits extracts of *target medicinal plants* was observed by UV/VIS spectroscopy. The peaks were determined at 355 and 365 nm for nZnO synthesized from the leaf extracts of *M. charantia* and *C. roseus* correspondingly. Synthetized nanoparticles have demonstrated antiradical capacity against 2,2-diphenyl-1-picrylhydrazyl and towards 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) in physiologically relevant concentration. Synthesized nZnO using *Catharanthus roseus* and *Momordica charantia* extracts reflected the same optical, antioxidant and antihyperglycemic (Horyn et al., 2019) characteristics and could be applied in the fields of medical and pharmaceuticals for formulation of new drugs.

Key words: Green synthesis, nano zinc oxide, Catharanthus roseus, Momordica charantia, antiradical capacity.

Nowadays, metal-containing nanoparticles, and particularly nanozinc oxide (nZnO), is going shares deeply in broad range of industrial fields namely electronics, photonic devices, biomedical and pharmaceutical sector [9]. However, the potential adverse effects of metal oxide nanoparticles in biomedical and pharmaceutical fields needs to be properly evaluated. Numerous studies have demonstrated that nZnO should provoke adverse effects to both animals and human [8, 28, 30]. We have shown in particular that nZnO provoked up-regulation of stress-related, metal-binding proteins, metallothioneins in the liver and Zn-carrying vitellogenin-like proteins in the blood plasma of frog *P. ridibundus* [1]. Also, it was capable to stimulate lysosomal protease cathepsin D activity, oppress caspase-3 activity and modulate the multixenobiotic-resistance protein activity and HSP70, HSP72 and HSP60 in the digestive gland and in the gills of bivalves mollusks [8, 12]. nZnO has affected not only animals, but also human. In particular, nZnO significantly reduced alveolar A549 cell viability [30], altered cellular morphology, cytoskeletal arrangement, lysosomal stability and mitochondrial membrane potential in human primary astrocytes as well as stimulated apoptosis [27].

The synthesis of nZnO was grounded on several physical and chemical methods namely spray-pyrolysis, chemical reduction, laser ablation, electrolysis, sol gel, hydrothermal, but most of these methods being costly, and/or wanting the use of toxic solvents [26]. Indeed, the above mention approaches not only use toxic and expensive reagents, but it is highly likely by-products of reactions make nZnO so formed inappropriate for use in biomedical applications. To overcome these challenges, the popularity of 'green' chemistry technology is rapidly arising. The most useful in green synthesis of nanoparticles are plant, bacteria, fungi, enzymes and algae [19-21]. The biosynthetic nZnO provide numerous benefits of eco-friendliness and compatibility for biomedical and pharmaceutical applications.

Catharanthus roseus and Momordica charantia (Karela) belong to important medicinal herbs listed in Aurveda, enriched with more than 100 alkaloids and bioactive compounds including phenolic compounds and other enzymatic and non-enzymatic antioxidants [4]. It was shown that C. *roseus contains* terpenoid indole alkaloids such as vinblastine and vincristine (natural anticancer drugs), ajmalicine (antihypertensive) and serpentine (sedative) and *M. charantia* contains charantin, charine, cryptoxanthin etc used in therapy for treating different diseases. However, few reports have addressed to the development of the environmental-friendly nZnO synthesis based on *Catharanthus roseus and Momordica charantia* as reducing and capping agents [15]. Hence, present work aimed to elaboration of zinc oxide nanoparticles biosynthesis using *C. roseus* and *M. charantia* and evaluation its antiradical activity. The process can be also applied for production of other metallic nanocrystalline with biomedical properties.

Materials and methods

*Phyto*extracts preparation

The ground powdered leaves of *Catharanthus roseus* were boiled in the distilled water for 45 min at 100 °C. The dark brown extract was filtered using 0.45-µm sintered glass funnel to remove insoluble fractions and macromolecules. The resulting extract reached the polyphenols and aminoacids which acted as the reducing agent for nZnO biosynthesis.

Green-synthesis technology

Green synthesis of ZnO nanoparticles using a leaf extract of *Catharanthus roseus* and commercial form of *Momordica charantia* fruits (Swanson® Bitter Melon) carried out from $ZnSO_4$ solution. For synthesis of nanoparticles, 25 ml of 2.25% plant extract treated with 1.0 M sodium hydroxide (10 ml) was taken and heated to 60°C in the dark to avoid photo-catalysis. Then 100 ml zinc sulphate solution (0.0025, 0.05, 0.075, 0.01, 0.0155, 0.02 M) was added and the mixture was boiled for 15-120 min (15, 30, 60, 120 min) until it diminished to dark-yellow paste. The resultant product was further washed in double-distilled water and ethanol. The paste was then collected in a ceramic mortar and heated at 200 oC for 2 minutes up to the state of light yellow powder. The resulting powder was used to antiradical and antihyperglycemic activity determination. The preparation of nZnO was characterized by UV/Vis spectrophotometer (U-LAB 100UV) in the wavelength range of 200–500 nm.

Evaluation of the antiradical activity

Antiradical activity of synthetized nZnO against $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl, *Sigma*) according to Carrasco-Pozo et al., 2008 [5]. The assay is based on the measurement of the reducing ability of the thiols toward $DPPH^{\bullet}$. Briefly, the studied samples were mixed with 30 µM DPPH in 80% methanol and allowed to react during 40 min at 30 ^{o}C. The absorbance was read within 40 min at 517 nm. Glutathione reduced was used as a standard compound.

ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid, *Sigma*) free radical scavenging activity was analyzed according to Shabestarian et al. (2017) [19]. The reaction mixture contained 1 mL of the ABTS working solution and 1mL of 10, 100 and 200 μM of synthetized nanoparticles. After incubation for 1 h at room temperature in dark, absorbance was registered at 734 nm. Glutathione reduced was used as a standard compound.

The experiments were done in triplicate and results were expressed as mean ± SD. All statistical calculations were performed with Statistica v. 12.0 and Excel for Windows-2013. Differences were considered significant if the probability of Type I error was less than 0.05.

Results and discussion

Our results have proven that *C. roseus* and *M. charantia* are the good sources for the green synthesis of nZnO. The synthesized nZnO were characterized by UV/Vis spectrophotometric technique (Fig. 1). The absorption peaks were registered between 355 and 365 nm and located in the specific absorbance range (320-390 nm) for biosynthesized nZnO based on different herbal extracts as reducing and capping agents [15] due to their large excitation binding energy at room temperature. It is well known from absorption spectroscopy that the band gap increases on decreasing particle size. The high blue shift absorption for the synthesized nZnO in comparison with the bulk ZnO (around 385nm) can be due to a high decrease in particle size [21]. Then we can sum that the particle size of synthetized nZnO using *M. charantia* was smaller than for *C. roseus*.

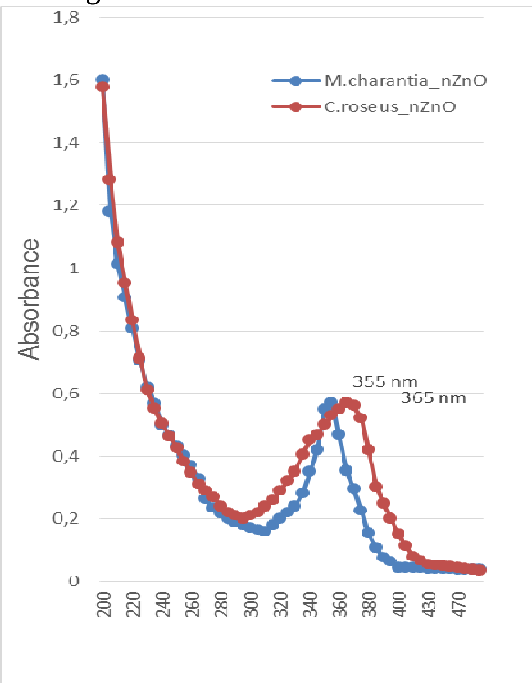
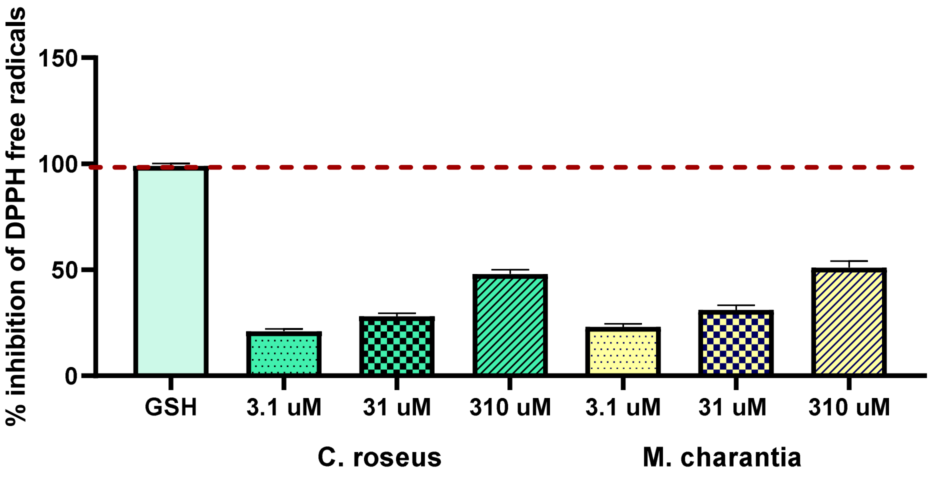


Figure 1. UV–Visible spectrum of the synthesized ZnO nanoparticles by (A) zinc sulphate and *Momordica charantia* and *Catharantus roseus* extracts as precursors

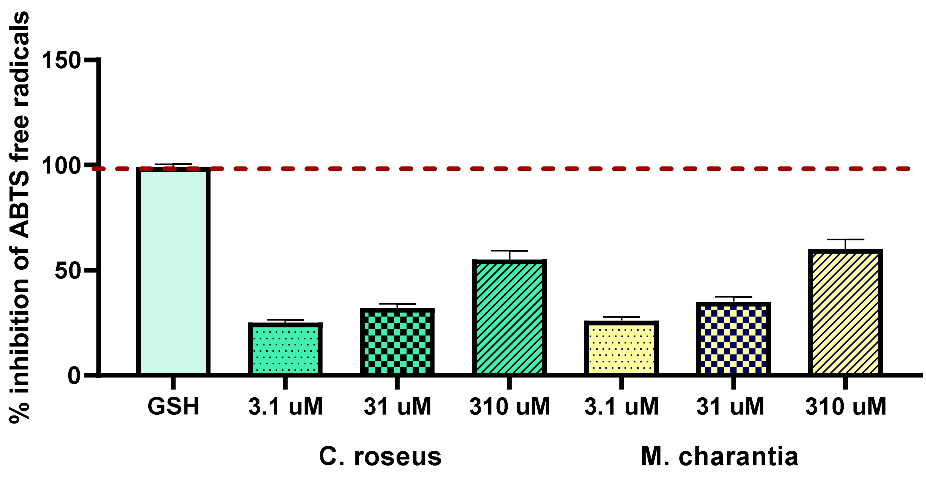
To estimate how the nZnO green synthesis is affected by the concentration of zinc sulphate and time of the reaction as the most important parameters we have carried the reaction out with constant concentration of herbal extracts, temperature and pH, but variable salt concentration or reaction time. As had predicted, the nZnO formation was increased while increasing the zinc sulphate concentration and the concentration of 0.0155 M was chosen as the most appropriate within 0.0025-0.02 M range according to the best ratio of $D_{355\text{-}365}/D_{300\text{-}310}$ equal to 3.5. Meanwhile no absorption peaks were observed at 0.0025M and 0.005M. Similar results were observed with flower extract of *Nyctanthes arbortristis*, where increasing concentrations of zinc acetate (0.0025–0.01 M) were used to optimize the synthesis of ZnO NPs [23].

It was proven that the reaction time is an important factor for the nanoparticles synthesis. The reaction time should depend on the nature of metals and reducing agent namely herbal extracts. In the present experiment no absorption peak was observed in 15 min and 30 min. It was appeared with increasing the reaction time up to 60 and 120 min, but no principal difference in the nZnO absorbance was registered between 60 and 120 min of reaction. Then, 60 min of reaction was stated as the most appropriate for both *C. roseus* and *M. charantia* extracts. Our result is in a good agreement with the previously published studies on *C. roseus*, where the most appropriate reaction time was also determined in the range of 1-3 h [10].

The synthetized nZnO possessed dose dependent free radical scavenging activity against both DPPH and ABTS in the same range for nZnO prepared based on *C. roseus* and *M. charantia* extracts. Meanwhile antiradical activity to ABST was slightly, but significantly higher than to DPPH (Fig. 2). Many kinds of antioxidants of the extract could perform synergistically. Obviously, during the synthesis of the nZnO, bioactive compounds of medicinal plants extracts, among them vinblastine, vincristine, alkaloids ajmalicine, serpentine, phenolic compounds, are adsorbed onto the surface of the synthetized nanoparticles and displace high tendency to interact with and reduce ABTS and DPPH with high efficiency according to the high surface area to volume ratio of nanoparticles.



A



B

Figure 2. Scavenging capacity of synthetized ZnO nanoparticles on DPPH (A) and ABTS (B) free radicals as compared to glutathione as reference compound

It was proven that nanoparticles can penetrate biological membranes and damage living tissues and organs due to their small size, unique composition and high activity. Nevertheless nZnO are used in different fields of science and industry, among them pharmacy, health care and foods, it has been classified by EU hazard classification as ecotoxic (N; R50-53) [27]. nZnO exhibits higher toxic effects than other metallic nanoparticles and this is likely because of their ion-shedding ability [27]. The design of ecofriendly green synthetic protocols for nZnO allows omitting toxic by-products and, which is vitally important, dressing nZnO with antibacterial [14] and, putatively, antiradical properties. The present study results showed that nZnO synthetized using both *C. roseus* and

M. charantia extracts were capable to inhibit DPPH and ABTS free radicals. Due to our knowledge, there is no data in regards to radical-scavenging activity of nZnO based on selected plants. Meanwhile, our results are in a good agreement with previous reports devoted to gold and silver nanoparticles [19, 20, 25]. In particular, it was estimated that DPPH radical-scavenging activity of gold nanoparticles obtained from *S. monoica* was 43% at 1 mg/mL [25].

Thus, a green synthetic method using *C. roseus* and *M. charantia* extracts can be used as a safe, simple and economical process for production of ZnO nanoparticles, without requirement of any chemical reductant or capping agent. The bioactive compounds presented in both *C. roseus* and *M. charantia* primary should potentially served as an electron donor system and ligand agents to form stabilized nanoparticles. Tested *C. roseus* and *M. charantia* extracts seem to be perspective alternatives in large-scale metallic nanoparticles manufacturing without generating any toxic byproducts. Synthetized nZnO possess promising antiradical and antidiabetic activities that allows using them for biomedical and pharmaceutical purposes.

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ЗЕЛЕНИЙ СИНТЕЗ НАНОЧАСТИНОК ЦИНК ОКСИДУ З ВИКОРИСТАННЯМ АЮРВЕДИЧНИХ ТРАВ ТА ЇХ АНТИРАДИКАЛЬНИЙ ПОТЕНЦІАЛ

Технології зеленого синтезу наночастинок з використанням екологічно чистих відновників набувають все більшої популярності. Ми застосували технологію зеленого синтезу для отримання наночастинок цинк оксиду (nZnO), на основі екстракту листя катарантуса *Catharanthus roseus* та екстракту плодів момордіки *Momordica charantia* (карела). Успішність процесу біосинтезу nZnO з екстрактів листя та плодів лікарських рослин оцінювали спектрофотометрично. Інтенсивність піків визначали при 355 і 365 нм для nZnO, синтезованих з фітоекстрактів *M. charantia* і *C. roseus* відповідно. Синтезовані наночастинки володіють антирадикальними властивостями щодо 2,2-дифеніл-1-пікрилгідразилу та до 2,2'-азино-біс (3-етилбензотіазолін-6-сульфонової кислоти) у фізіологічно релевантних концентраціях. Синтезовані nZnO з використанням екстрактів *Catharanthus roseus* та *Momordica charantia* характеризуються подібними оптичними, антиоксидантними та антигіперглікемічними характеристиками (Horyn et al., 2019) та можуть бути застосовані у медичній та фармацевтичній галузях для розробки нових лікарських препаратів.

Ключові слова: зелений синтез, нано-цинк оксид, Catharanthus roseus, Momordica charantia, антирадикальні властивості.

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INFLUENCE OF HEAVY METALS IONS ON THE CONTENT OF PROTEINS AND NUCLEIC ACIDS IN THE ORGANISM OF FRESHWATER FISH

From the launched research we obtained the aggregate data, that not only confirm and broaden our concept of the important role of protein and nucleic metabolism in the processes of detoxication of heavy metals ions and formation of resistance to them, but also allow making an integral estimation of biochemical reaction of carp organism to chronic intoxication.

Key words: freshwater fish, proteins, nucleic acids, heavy metals.

Contamination of water reservoirs by heavy metals is one of the limiting factors of aquatic ecosystems functioning and their biological productivity. Being part of many organic substances, or engaging them in the interaction, they influence many biochemical processes in aquatic organisms. The ions of metals can form strong connections in the tissues along with various biologically active centres, including the sulphur-containing ligands, that may be enclosed in proteins and amino acids. Their activity is related to the enzymes that contain metal ions in their composition or are actuated by them [6, 10].

One of the basic principles of biochemical adaptation of an organism is to maintain the structural and functional integrity of macromolecules. Much of this is applied to proteins and nucleic acids – biopolymers, that perform an extremely important role in the adaptation of aquatic lives to environmental conditions [10].

Materials and methods

The object of the given research was carp – *Cyprinus carpio* L. For the experiment the 2 year old fish with the mass of 250-300 grams were rummaged from the natural stews of Ternopil region (Zalistsi fish-breeding complex). The experiments were carried out in 200 litre aquariums filled with the precipitated water from the local water supply system under constant gas and temperature operating conditions. During the process the fish were not fed. The effect of Mg, Zn, Cu and Pb ions in two concentrations are complied with 2 and 5 maximum permissible concentrations (MPC) [1]. The period of acclimation was 14 days.