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Taraxacum officinale extracts (flower, stem and seed)-incorporated nanoflowers with their peroxidase-like and antimicrobial activities

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Abstract

In this study, organic-inorganic hybrid nanoflowers (NFs) using *Taraxacum Officinale (T. officinale)* extracts (flower, stem and seed) and copper II ions (Cu^{2+}) were synthesized. In terms of *T. officinale* NFs formation, while *T. officinale* extracts were used as organic components, Cu^{2+} acted as an inorganic component. We demonstrated how extracts of flower, stem and seed parts of *T. officinale* plant affect morphologies of *T. officinale* NFs. The NFs were characterized by SEM and EDX analysis. We also investigated peroxidase-like and antimicrobial activities of *T. officinale* flower extract NF (TOF@NF), *T. officinale* stem extract NF (TOSt@ NF) and *T. officinale* seed extract NF (TOS@NF) in the presence of the hydrogen peroxide (H_2O_2) via Fenton reaction mechanism. The catalytic activities of all NFs were tested towards guaiacol used as a substrate and the NFs showed antimicrobial activities against model microorganisms like *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, Candida albicans ATCC 1023. We claim that *T. officinale* extracts can be considered as promising organic components for synthesis of flower-shaped hybrid nanostructures.

Keywords: *Taraxacum officinale*, *taraxacum officinale* nanoflower, phytochemicals, antimicrobial activity, catalytic activity

Introduction

In recent years, the use of plant extracts in the synthesis of nanoparticles (NPs) has attracted great interest. The combination of many different plants with different metal salts has opened up new avenue for synthesis of various single and multi-component NPs [1-4]. The fact that these NPs are kind of biocompatible and they can be produced in one step. The organic-inorganic hybrid structures called "Nanoflower (NF)" have become received great attention in recent years due to their large surface area, easy-synthesis procedure and non-toxicity [5]. The NFs are formed by using organic and inorganic components in buffer solutions.

Taraxacum officinale (*T. officinale*) plant has a rich composition with flavonoids, phenolic compounds, polyphenols and inulin in its structure [6,7]. With its known properties, *T. officinale* increases the permeability of the membrane by binding to the bacterial cell membrane with the phenolic acid and flavonoids contents. Thus, the ion balance of the bacterial cells is disrupted and leaks occur in their membranes. On the other hand, it inhibits enzymes that are critical for protein synthesis and energy metabolism of bacterial and fungal organisms with the terpenoids in its structure. It also affects the inhibition of the microorganism in the same way. The fact that *T. officinale* extract gives such impressive results alone directly affects its nano-sized properties. Owing to presence of active biological agents in *T. officinale* extract, it acts as an effective agent for the reduction and stabilization of metal ions in the synthesis of various metal NPs [8-10]. However, there has been no NF study conducted with *T. officinale* reported up to now.

In this study, we synthesized *T. officinale* extract obtained from its flower, stem and seed part of incorporated-nanoflowers called T. officinale flower extract NF (TOF@NF), T. officinale stem extract NF (TOSt@ NF) and T. officinale seed extract NF (TOS@NF), respectively. The all NFs were characterized with several characterization techniques, and their peroxidase like and antimicrobial activities were examined.

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Material and Methods

An ethics committee decision is not required in NP synthesis of plant extracts [2,4,6].

Chemicals and reagents

T. officinale, Copper sulfate pentahydrate ($CuSO_4.5H_2O$), Guaiacol, Hydrochloric acid (HCl), Sodium Hydroxide (NaOH) and Hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich. Sodium Chloride (NaCl), Potassium Chloride (KCl), Disodium Hydrogen Phos¬phate (Na_2HPO_4) and Potassium Dihydrogen Phosphate (KH_2PO_4) were obtained from ISO LAB Chemicals for the preparation of phosphate buffered salt solution (PBS). *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 25922, *Candida albicans* (*C. albicans*) ATCC 90030 strains were obtained from Erciyes University, Faculty of Pharmacy Pharma¬ceutical Microbiology Research Laboratory.

Synthesis and characterization

Preparation of Taraxacum officinale (Flower, stem and seed) extract

T. officinale plant was collected from Kayseri province of Turkey. *T. officinale* was divided into three parts as flowers (TOF), stem (TOSt) and seeds (TOS). Plant parts were dried by waiting for 7 days at room temperature (RT). After drying process, 100 ml of distilled water was heated to boiling point and dried flowers were dropped into boiling distilled water as 10 mg. After 5 minutes of boiling period, the mixture was filtered with (Whatman paper no:1). The same process was applied to stem and seeds. After the process, the obtained extracts were stored at +4 C° to be used in the following applications.

Synthesis of *T. officinale* extract nanoflowers

Firstly, PBS was prepared and made ready for synthesis. Then, 120 mM $CuSO_4.5H_2O$ stock solution was prepared in distilled water. In the next step, Cu^{2+} solution (final concentration will be 0.08 mM) was added to PBS (10 mM, pH: 7.4) buffer. Finally, 0.01 mg/ml each *T. officinale* extract solution was added and mixed quickly for 1 minute (min). The same procedure was applied separately for all *T. officinale* TOS, TOSt and TOS extract. Each mixture was incubated at +4 C° for 72 hours (hrs). After incubation, blue precipitates were observed and collected by centrifugation (5000 rpm, 5 minutes). The obtained precipitates were completely dried in oven at 50 °C. Then, they were stored at RT for characterization and applications.

Characterization of *T. officinale* extract nanoflower

Synthesized NFs were characterized by Scanning Electron Microscopy (SEM, LEO 440) to monitor their morphologies, Energy-dispersive X-ray spectroscopy (EDX, LEO 440) was used for determination metal in the NFs.

Catalytic activity assay

A previously reported method was used for the catalytic activity [11]. This method is performed to show the how NFs catalyze the oxidation of guaiacol in the presence of H_2O_2. First, in 0.01 mg/ml of each NF was dissolved in PBS solution. 1 ml of 22.5 mM H_2O_2 and 1 ml of 45 mM guaiacol were added to each reaction tube including NFs. The oxidation of guaiacol induced new absorbance at a wavelength of 470 nm, and then which was measured every 3 min.

Antimicrobial activity assay

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 1023 strains were obtained at -20 °C and grown in corresponding nutrient broth for bacteria and yeast broth for fungi. The concentrations of each microorganism was fixed to be 1×108 CFU mL-1 for bacteria and 1×106 CFU mL-1 for fungi. In terms of antimicrobial activities, The growth of each microorganism in microdilution plates exposed to each plant part extract and NF (300 µg/mL) was analized [12-14].

Results

The all NFs like TOF@NF, TOSt@NF and TOS@NF were produced with reaction of each *T. officinale* extract with Cu^{2+} ions in PBS at RT in 72 hrs. The each extract react with $Cu_3(PO_4)_2$ primary crystals to form seeds. The growth of seeds induce formation of *T. officinale* extract-$Cu_3(PO_4)_2$ petals. The combinations of petals finally lead to formation of the NFs. The peroxidise-like and antimicrobial activities of each NFs were evaluated via Fenton like reaction. The potential formation mechanism of the *T. officinale* NF was illustrated in Figure 1.

Photograph of *T. officinale* and SEM images of TOF@NF, TOSt@

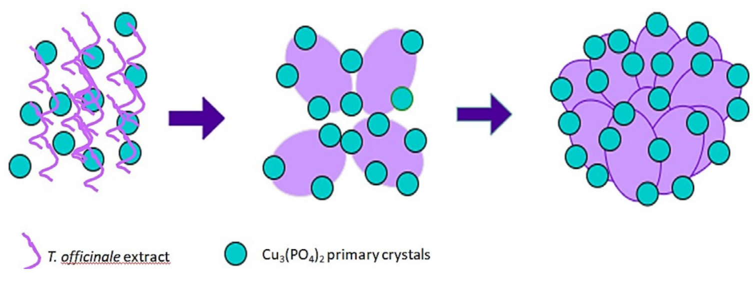

Figure 1. The potential formation mechanism of the T. officinale NF

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NF and TOS@NF were given in Figure 2. The Figure 2a gave photograph of *T. officinale* in nature, the morphologies of TOF@NF, TOSt@NF and TOS@NF were presented by SEM images in Figure 2b, 2c and 2d, respectively. Each NF has spherical, monodispersed and uniformed morphology. In addition to these, NFs have also porous and quite compact structures.

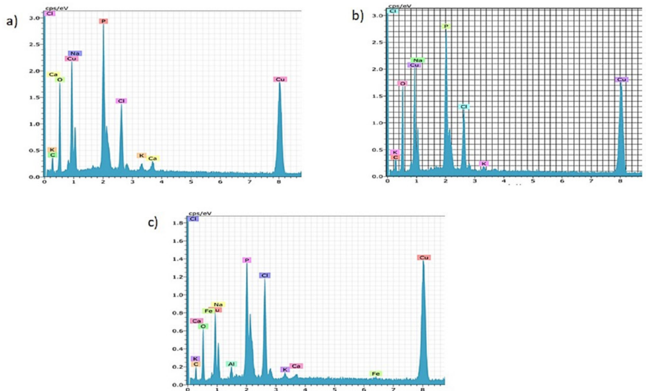

Figure 2. a) Photo of *T. officinale* in nature. SEM analysis of b) TOF@NF, c) TOSt@NF and d) TOS@NF

The elemental composition of NFs was determined by EDX analysis. In Figures 3a, 3b and 3c, the presence of Cu, P and O confirmed the formation of $Cu_3(PO_4)_2$ crystals in the NFs. It is well documented that there is no formation of the NFs in the absence of Cu metal or $Cu_3(PO_4)_2$ crystals. We proved that Cu metal or $Cu_3(PO_4)_2$ crystals can be considered backbone for formation of NFs.

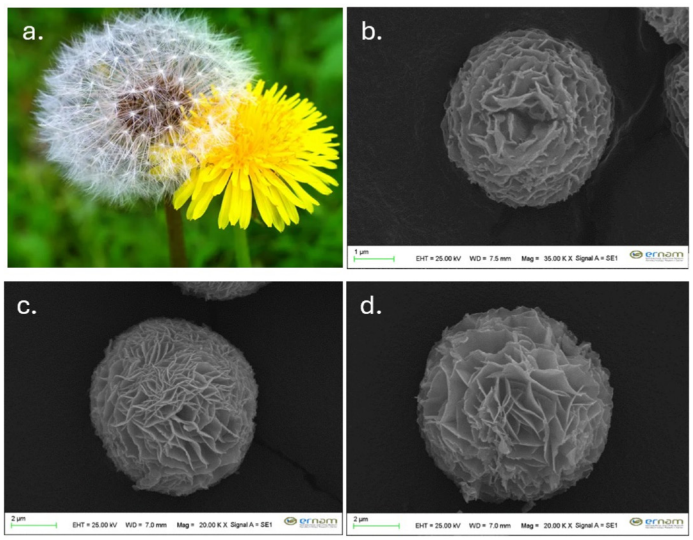

Figure 3. EDX analysis of a) TOF@NF, b)TOSt@NF c)TOS@NF

Peroxidase-like catalytic activity of *T. officinale* extract nanoflower

In addition to characterization studies, the catalytic activities of TOF@NF, TOSt@NF and TOS@NF were investigated. The absorbance values measured during the reaction time were shown in the graph to compare the catalyst activities of NFs. In the study, it was determined that all NF structures showed peroxidase-like activity in the presence of H_2O_2. As seen in Figure 4, it was determined that TOF@NF showed higher activity than other NFs.

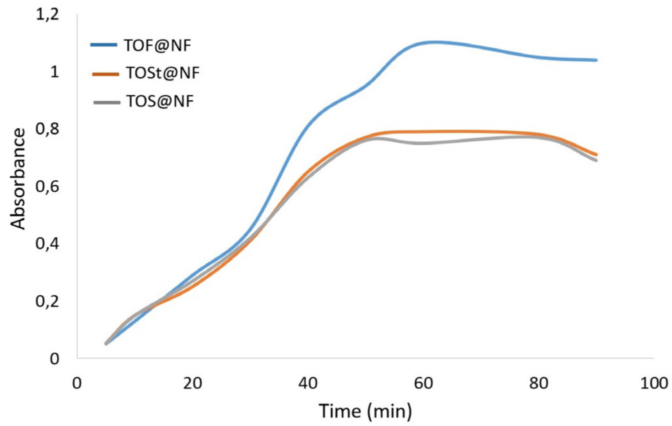

Figure 4. Comparison of catalytic activities of TOF@NF (blue line), TOSt@NF (orange line), and TOS@NF (gray line)

Antimicrobial activities of *T. officinale* extract nanoflowers

TOF@NF, TOSt@NF, TOS@NF and direct each *T. officinale* extracts were tested with the same microorganisms. The analysis results are presented comparatively in Figure 5. It was determined that TOF@NF has higher activity against for both the extract and the other parts. TOS@NF showed a more effective activity against S. aureus and E. coli with a very small difference after TOF@NF. While TOF@NF showed the most successful result against C. albicance, TOSt@NF showed better activity than TOS@NF. When the antibacterial activities of *T. officinale* extracts and NFs were compared, it was determined that NFs were at least 5 fold more effective. Similar results were obtained to the antimicrobial activity study previously conducted with *T. officinale* extracts. Confirming the catalytic activity data determined in Figure 4, TOF@NF showed much effective antibacterial activity against all microorganisms.

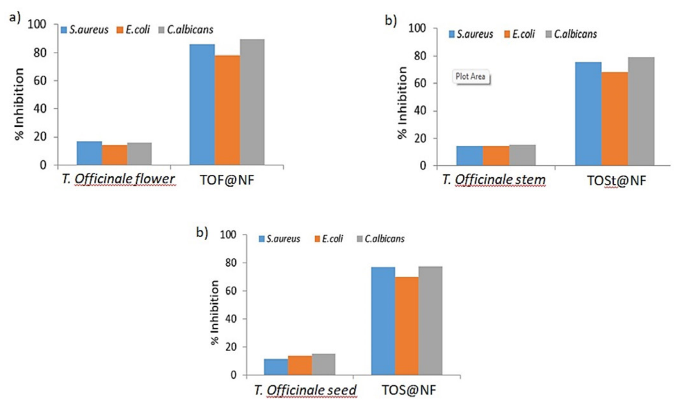

Figure 5. Antimicrobial activity of a) TOF@NF, b)TOSt@NF c)TOS@NF

Discussion

We discussed that both peroxidase like and antimicrobial activities of all NFs relied on Fenton-like reaction. We claim that redox reaction on Cu^{2+} ions is responsible for Fenton-like reaction in the presence of H_2O_2. Briefly, Cu^{2+} in each NFs is reduced to Cu^{1+} ions in the presence of H_2O_2, then formation of highly reactive hydroxyl radicals was produced. The free hydroxyl radical is continuously generated by cascade like -redox cycle reaction, Cu^{1+} ions was turned to Cu^{2+} ions which cause oxidation of guaiacol and bacterial cell membrane for bacterial death the NFs, respectively. The Fenton-like reaction was given in Figure 6.

$$Cu^{2+} + H_2O_2 \longrightarrow Cu^{+} + HOO\cdot + H^{+}$$

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + HO\cdot + OH^{-}$$

Figure 6. Fenton-like reaction mechanism of the NFs

Conclusion

We have reported formation of *T. officinale* extracts from flower, stem and seed-based NFs and examined their peroxidase-like and antimicrobial activities. We showed how part of *T. officinale* plant extracts influence the morphology of each NF. The TOF@NF, TOSt@NF and TOS@NF exhibited both peroxidase-like and antimicrobial activities of via Fenton reaction mechanism. The NFs produced highly reactive hydroxyl radicals in the presence of H_2O_2 which played a critical role for catalytic activity by oxidizing guaiacol and antimicrobial activities for oxidizing membrane of target microorganisms. We offer that NF formation can be carried out without use of protein and these NFs can act as nanobiocatalysts for catalytic activities.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
Ethical committee approval was unnecessary for this study.

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