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Research Article

Biosynthesis of Naringenin@Cu (II), Zn (II) Hybrid Nanoflower: Anticancer Activity Assay process

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Abstract

In the study, the synthesis of organic-inorganic hybrid nanoflowers, which have a flower-like structure and are called nanoflowers, was carried out. For this purpose, hybrid nanoflower synthesis was carried out using naringenin as the organic part and different metal ions (Cu and Zn) as the inorganic part. Various analyzes were carried out to characterize the synthesized nanoflowers (such as SEM, EDX, FTIR, XRD). In addition, the anticancer activities of hybrid nanoflowers were tested on the MCF7 (breast cancer) cell line. Naringenin-ZnhNFs killed MCF7 cells at a rate of approximately 76%, while Naringenin CuhNFs caused cell death at a rate of 68%. Naringenin caused 45% cell death. It was concluded that there was a good increase in anticancer activity when naringenin was converted into hybrid nanoflower form. In particular, the anticancer activity of hybrid nanoflowers synthesized with Zn metal ion was quite excellent. This means that Zn hybrid nanoflowers may be a safer therapeutic alternative than others.

Keywords: nanoflower, anticancer, naringenin

Naringenin@Cu (II), Zn (II) Hibrit Nanoçiçeğinin Biyosentezi: Antikanser Aktivite Testi süreci

Öz

Çalışmada çiçeğe benzer bir yapıya sahip olan ve nanoçiçek olarak adlandırılan organik-inorganik hibrit nanoçiçeklerin sentezi gerçekleştirildi. Bu amaçla organik kısım olarak naringenin, inorganik kısım olarak ise farklı metal iyonları (Cu ve Zn) kullanılarak hibrit nanoçiçek sentezi gerçekleştirilmiştir. Sentezlenen nanoçiçekleri (SEM, EDX, FTIR, XRD gibi) karakterize etmek için çeşitli analizler yapıldı. Ayrıca hibrit nanoçiçeklerin antikanser aktiviteleri MCF7 (meme kanseri) hücre hattı üzerinde test edildi. Naringenin-ZnhNF'ler MCF7 hücrelerini yaklaşık %76 oranında öldürürken Naringenin CuhNF'ler %68 oranında hücre ölümüne neden oldu. Naringenin %45 hücre ölümüne neden oldu. Naringenin hibrit nanoçiçek formuna dönüştürüldüğünde antikanser aktivitesinde iyi bir artış olduğu sonucuna varıldı. Özellikle Zn metal iyonu ile sentezlenen hibrit nanoçiçeklerin antikanser aktivitesi oldukça mükemmeldi. Bu, Zn hibrid nano çiçeklerinin diğerlerinden daha güvenli bir terapötik alternatif olabileceği anlamına gelir.

Anahtar Kelimeler: nanoçiçek, antikanser, naringenin

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1. Introduction

Flower-shaped nanoflowers, which are the most striking member of nanoparticles today, are called nanoflowers because they have a flower-like structure. Hybrid nanoflowers consist of two parts: organic and inorganic. While proteins and enzymes containing nitrogen groups are preferred as the organic part, various metal ions (such as Zn(II), Cu(II), Mn(II) and Co(II)) are preferred as the inorganic part. However, today, it has been proven in many studies that nanoflower synthesis can be achieved with organic molecules or plant extracts containing hydroxyl and carboxyl groups other than nitrogen groups. For example, Somtürk et al. (2024) synthesized hybrid nanoflowers using *Tribulus Terrestris L.* plant extract as the organic part and different metal ions (Cu, Co and Zn) as the inorganic part, and examined the anticancer activities of the nanoflowers they synthesized. [1]. Uras et al. synthesized hybrid nanoflowers using Aspergillus terreus extract as the organic part and examined the antimicrobial activities of the hybrid nanoflowers they synthesized. [2]. Dadi and colleagues (2020) synthesized hybrid nanoflowers using gallic acid and examined its peroxidase-like activity [3]. Hybrid nanoflowers have excellent properties. These can be listed as having good stability, high stability and very good activity. The reason for this may be due to its morphology. Because it has a flower structure, it has a very large surface area. This is why it has such unique features. Although the formation mechanism of hybrid nanoflowers has not yet been fully elucidated, it can be explained as follows when the literature is examined. It can only be said that metal ions primarily form metal phosphate crystals in a buffered environment. Then, the metal phosphate crystals are bonded with the nitrogen groups in the enzyme or protein in the environment with a coordination bond, and it is predicted that hybrid nanoflowers are formed in this way. Naringenin, a flavanone, was preferred in the study. Naringenin has anticancer, antimutagenic, anti-inflammatory and antiatherogenic activities. Naringenin (5,7,4'-trihidroksiflavanonis) is found in orange, grapefruit, grape, tangerine and lemon peel [4-7]. Moreover, it is easily soluble in solvents such as ethanol and dimethyl sulfoxide. However, it has very little solubility in aqueous buffer systems. However, the poor water solubility of naringenin limits its use [8-12]. To overcome such limitations, strategies such as nanosized tools have been developed. In this context, organic-inorganic hybrid nanomaterials may be promising candidates [13-17]**.** There are not many studies on the anticancer activities of nanoflowers. In recent years, the anticancer effectiveness of hybrid nanoflowers was evaluated by Somturk and his group (2024) and they observed that the effectiveness of nanoflowers was very good [1]. Based on these studies, our study aimed to synthesize hybrid nanoflowers by considering the naringenin molecule, which has anticancer activity. Hybrid nanoflower synthesis was carried out using naringenin as the organic part and various metal ions (Cu and Zn) as the inorganic part. After characterization, anticancer activities were evaluated on the MCF7 cell line.

2. Material and Methods

Synthesis of Naringenin-CuhNFs

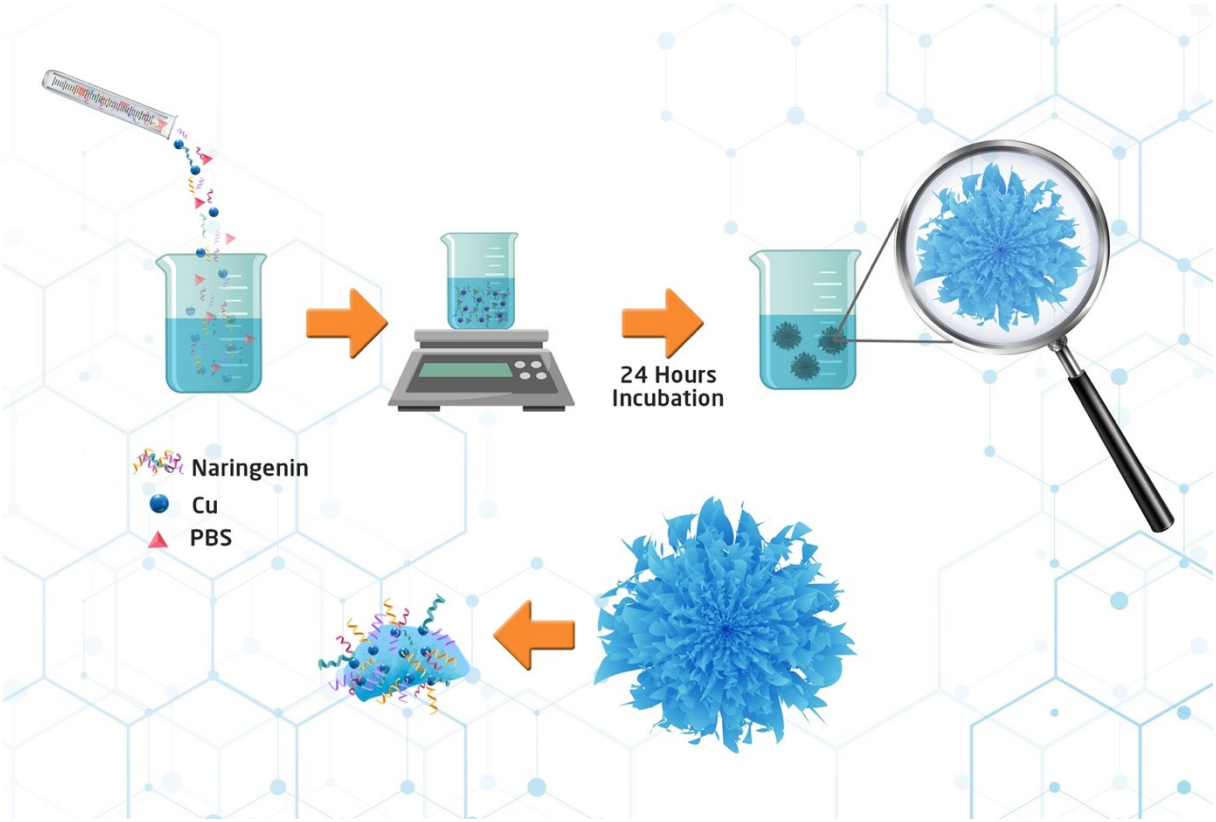


Figure 1. Naringenin-CuhNFs synthesis scheme

Synthesis of Naringenin-Cu(II) hybrid nanoflowers was synthesized using a method previously reported by Somturk et al. [18-20]. In this method, first 333 microliters of copper sulfate solution was added into 50 mL of PBS buffer. Then, Naringenin was added to the medium at a certain concentration. It is then centrifuged at 10,000 rpm for 25 minutes and left to dry. Naringenin-CuhNFs synthesis scheme is given in Figure 1.

Synthesis of Naringenin-ZnhNFs

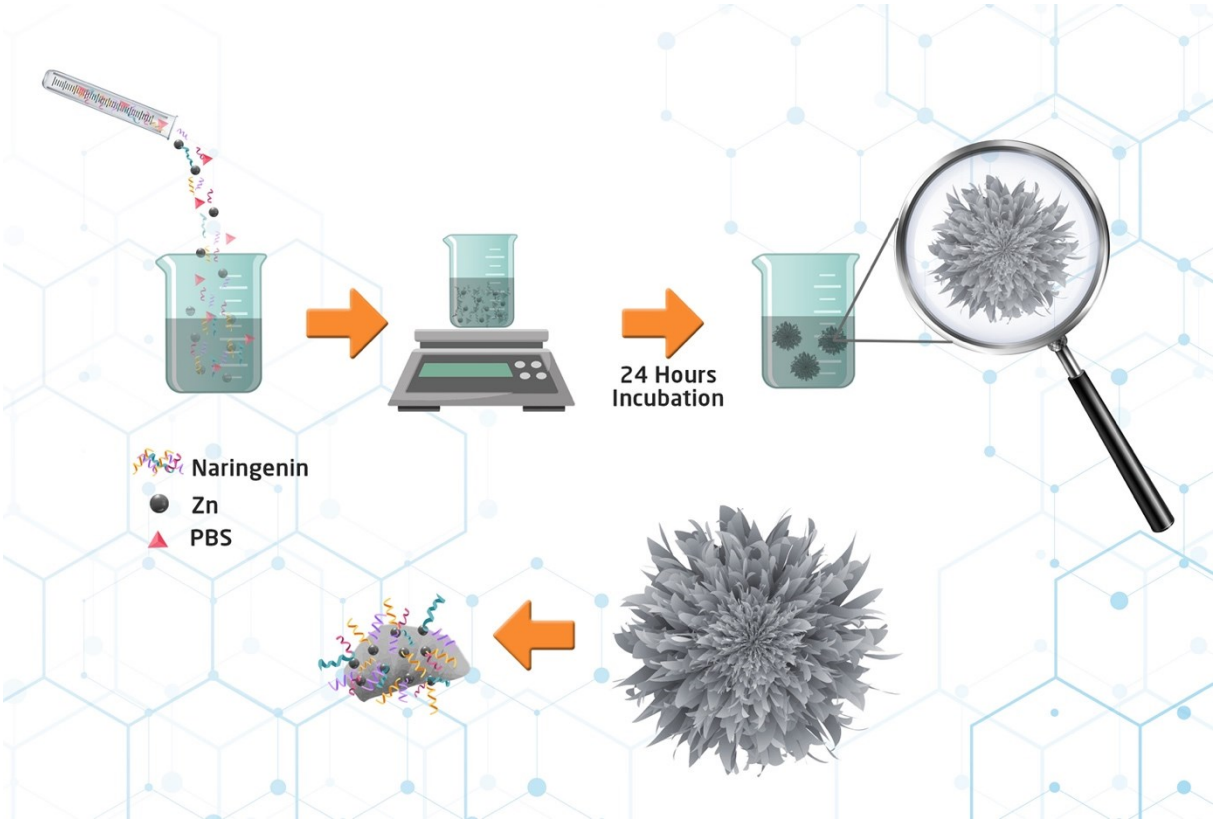


Figure 2. Naringenin-ZnhNFs synthesis scheme

Synthesis of Naringenin-Zn(II) hybrid nanoflowers was synthesized according to a method previously reported by Somturk et al. [21]. In this method, 1.6 mL of zinc acetate solution is added into 20 mL of phosphate buffer saline and then naringenin is added to the medium at a certain concentration. It is then centrifuged at 10,000 rpm for 25 minutes and left to dry. Naringenin-ZnhNFs synthesis scheme is given in Figure 2.

Characterization of Naringenin-CuhNFs and Naringenin-ZnhNFs

The morphologies of the hNFs were determined using the SEM device (FESEM, Zeiss GeminiSEM 500). Additionally, the metal ions contained in the hybrid nanoflowers were illuminated with EDX. While the crystal structures were determined using XRD, the chemical structure of the hNFs was determined by FTIR.

Cytotoxicity assessment (MTT assay)

The anticancer activity of Naringenin, Naringenin-CuhNFs and Naringenin-ZnhNFs were determined using the MCF7 (breast cancer) cell line and the MTT method. MCF7 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM)/ High Glucose supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in an atmosphere of 5% CO_2 at 37 °C. For cell culture analysis, stock solutions of Naringenin, Naringenin-CuhNFs and Naringenin-ZnhNFs were prepared and applied at 7 dilutions to cell lines grown in 96-well plates. It was then incubated for 24 hours. MTT([3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltrazolium bromide]) test was applied to reveal the possible cytotoxic effects of synthesized hybrid nanoflowers and to determine the concentrations that can be used in genotoxicity studies. For this purpose, MCF-7 cell was seeded at a density of 5000 cells/well and incubated overnight. Concentrations used in the MTT test were gradually reduced starting from the highest concentration. In this context, Naringenin, Naringenin-CuhNFs and Naringenin-ZnhNFs in the concentration range of 7.875-500 µg/mL were added to the culture medium. It was then subjected to incubation and after the staining process was performed, absorbance was read at 572 nm in a microplate reader (FilterMax F5, Molecular Devices, USA).

3. Results and discussion

SEM analysis

The morphologies of hNFs synthesized at pH 7.4 at +4 ^{o}C were determined by FESEM. As seen from the SEM images, the structures were confirmed to be flower-like (Figure 3). As the metal ion content changed, there was a visible difference in nanoflower formation. It was observed that the leaves were lighter in morphology when synthesized with Cu metal ion, and the formation of spiny leaves was observed when synthesized with Zn metal ion.

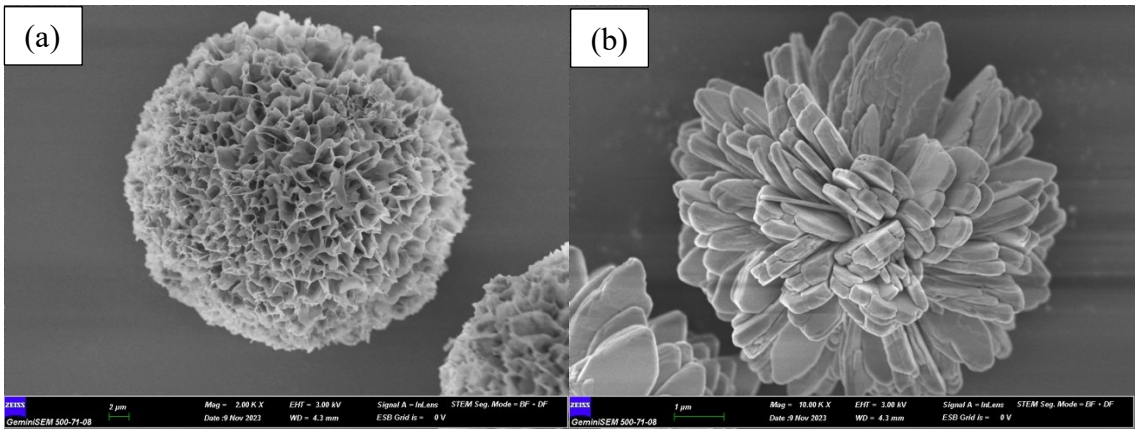


Figure 3. SEM images of Naringenin hybrid nanoflowers a) naringenin-Cu(II) hybrid nanoflower (2.00KX), b) naringenin-Zn(II) hybrid nanoflower (10.000 KX)

EDX Analysis

EDX analysis was performed to elucidate the metal content in hybrid nanoflowers. The EDX spectrum of synthesized hNFs is given in Figures 4-5. The elements Cu, Zn, O and P found in the spectrum come from the $Cu_3(PO_4)_2 \cdot 3H_2O$ and $Zn_3(PO_4)_2 . 4H_2O$ nanocrystalline.

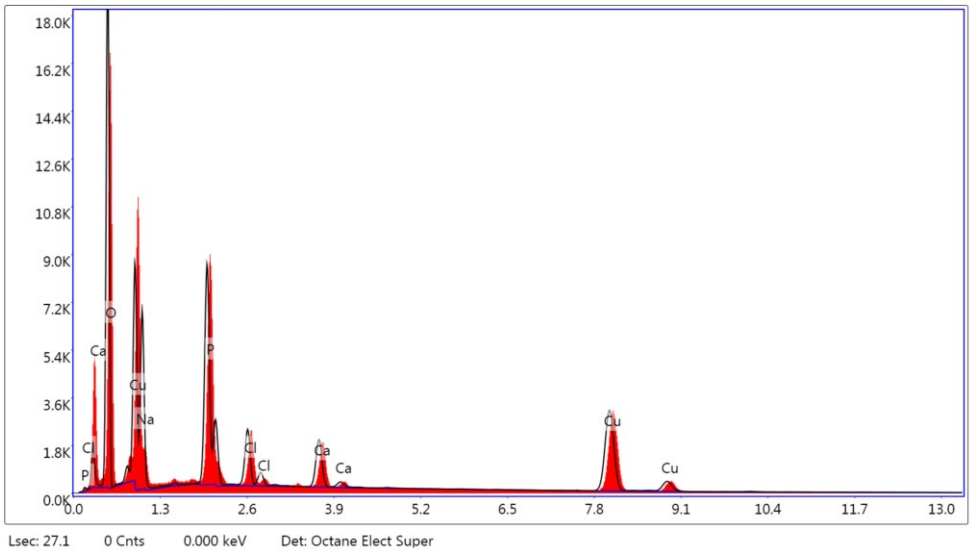


Figure 4. EDX analysis of Naringenin-CuhNFs

Table 1. Weight and atomic percentage of Naringenin-CuhNFs

Element	Weight %	Atomic %	Net Int.
O K	36.73	56.58	4173.98
NaK	17.81	19.09	1534.62
P K	11.9	9.47	2631.34
ClK	3.13	2.18	708.69
CaK	3.9	2.4	739.25
CuK	26.53	10.29	1840.83

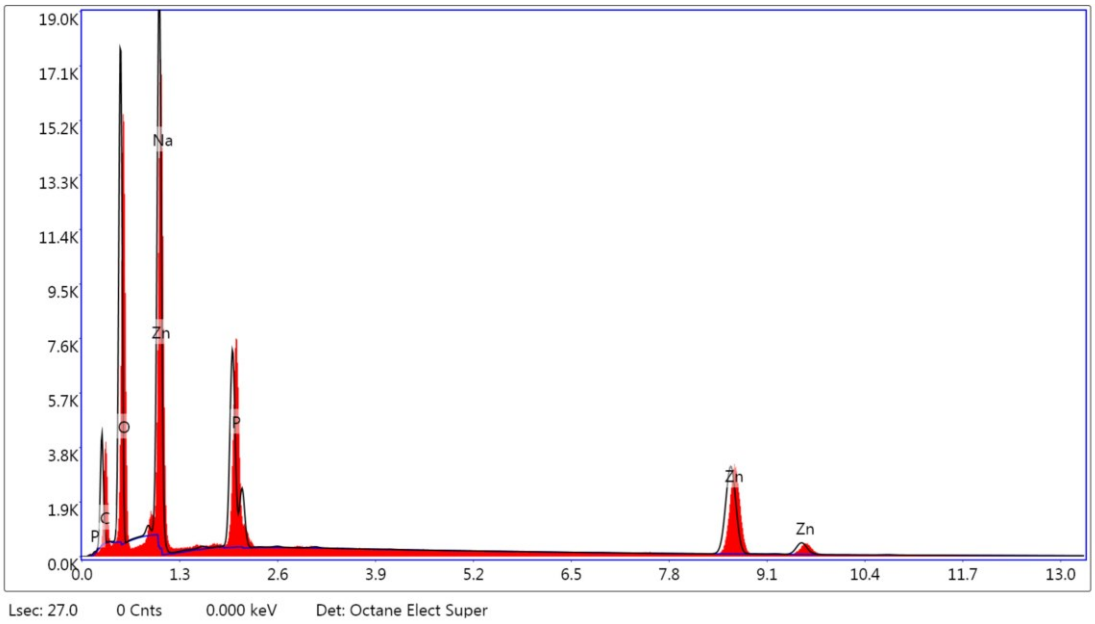


Figure 5. EDX analysis of Naringenin-ZnhNFs

Table 2. Weight and atomic percentage of Naringenin-ZnhNFs

Element	Weight %	Atomic %	Net Int.
C K	19.16	32.29	721.69
O K	27.61	34.93	3812.58
NaK	24.56	21.63	4644.73
P K	6.62	4.33	2149.98
ZnK	22.05	6.83	1899.62

Elemental mapping

Base mapping of Naringenin-CuhNFs and Naringenin-ZnhNFs were carried out. The homogeneous distribution of the metals was equal as seen in Figures 6-7.

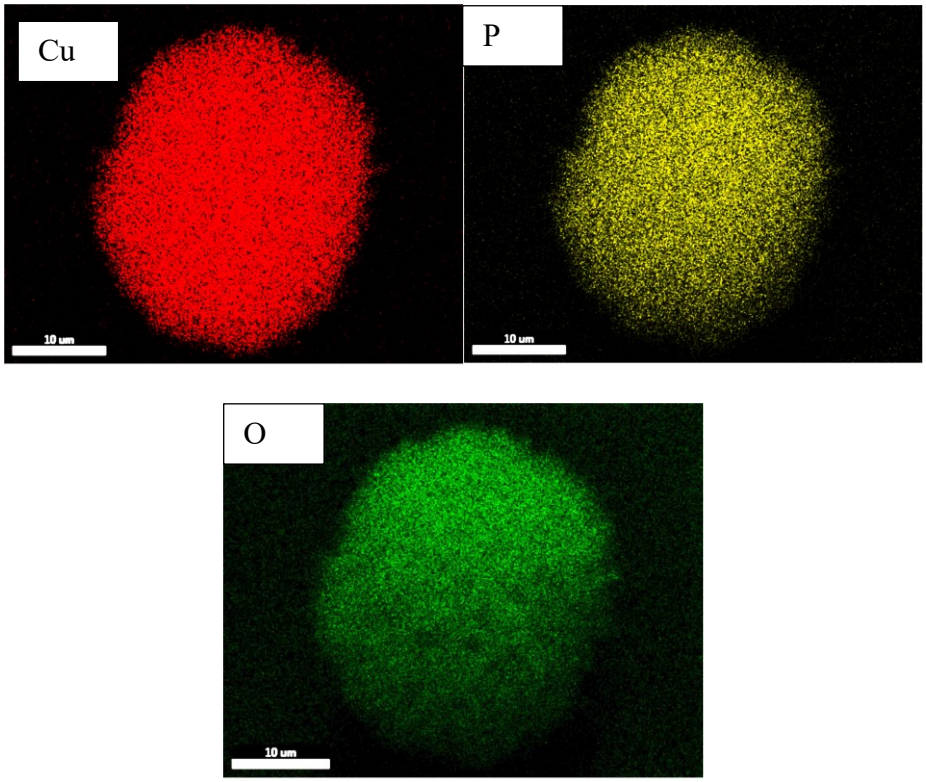


Figure 6. Elemental mapping (Cu,P, O) of Naringenin-CuhNFs.

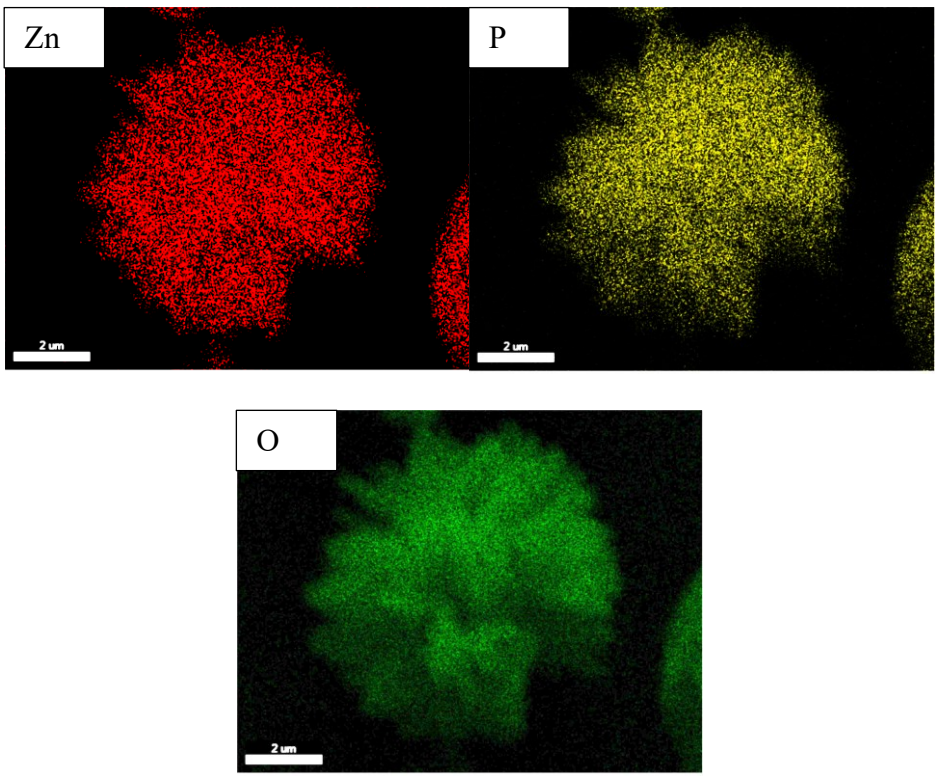
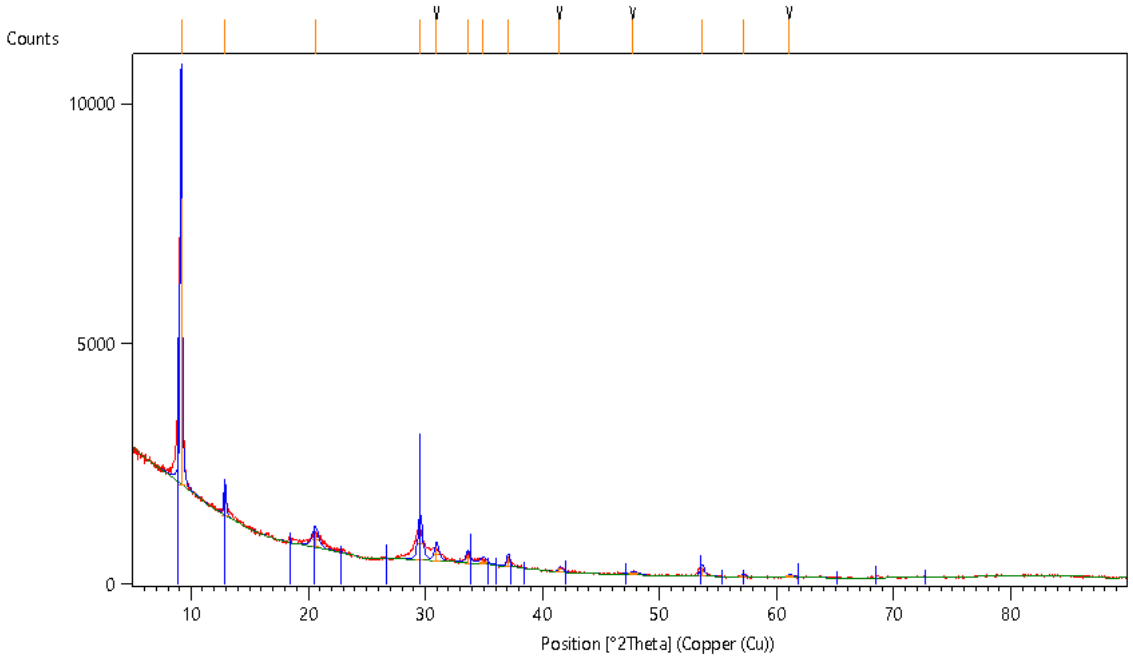


Figure 7. Elemental mapping (Zn, P, O) of Naringenin-ZnhNFs.

XRD analysis

XRD analysis was carried out to determine the crystal structure of hNFs. (Figure 8). The peaks for hNFs as follows: for Naringenin-CuhNFs, XRD: 9.10°, 12.96°, 18.79°, 20.87°, 27.38°, 29.48°, 30.64°, 31.74°, 33.71°, 37.21°, 41.59°, 45.48°, 47.74°, 53.46°, 56.48°, 61.10°, 63.57°, 66.22°, 68.19°, 71.42°, 75.26°, 79.06°, 83.95° in Fig. 8(a) in comparison with JCPDS (00−022−0548), and for Naringenin-ZnhNFs, XRD: 9.68°, 16.83°, 17.54°, 18.35°, 19.45°, 20.21°, 22.21°, 22.99°, 24.55°, 25.83°, 26.36°, 27.46°, 28.67°, 31.48°, 33.95°, 34.45°, 35.79°, 37.18°, 38.49°, 39.73°, 41.20°, 41.99°, 43.12°, 45.47°, 46.92°, 50.07°, 52.99°, 54.23°, 55.06°, 56.37°, 57.87°, 59.11°, 60.60°, 61.34°, 66.12°, 69.35°, 71.42°, 75.07°, 77.07°, 82.11° in Figure 8(b) in comparison with JCPDS (01−076−0896)

(a)



(b)

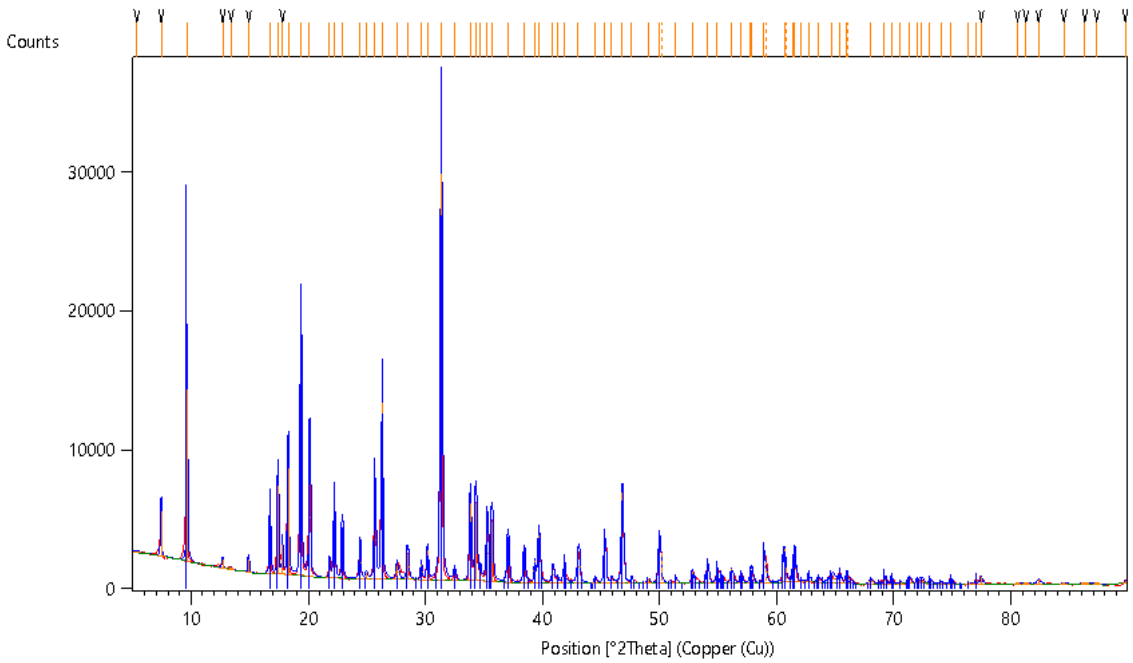


Figure 8. XRD analysis of Naringenin-CuhNFs (red line). This analysis complies with the JCPDS (00−022−0548) standard (blue line), b) XRD analysis of Naringenin-ZnhNFs (red line). This analysis complies with the JCPDS (01−076−0896) standard (blue line)

FTIR analysis

As seen in Figure 9, the peaks of hybrid nano flowers are as follows: For Naringenin- CuhNFs, FT-IR (cm^{-1}): 3592 (N–H and O–H stretching), 3311 (Ar–H and C–H stretching), 2939 (C–H, stretching), 2018, 1674, 1556, 1176 (P=O), 1088, 975 (P–O), 645, 568(O=P=O) . For Naringenin-ZnhNFs, FT-IR (cm−1): 3758 (N–H and O–H stretching), 2968 (Ar–H, stretching), 2653 (Ar–H, stretching), 2346, 2043 (C–H, stretching), 1548, 1335, 1139, (P=O), 993 (P=O), 930 (P–O), 648, 587 (O=P=O).

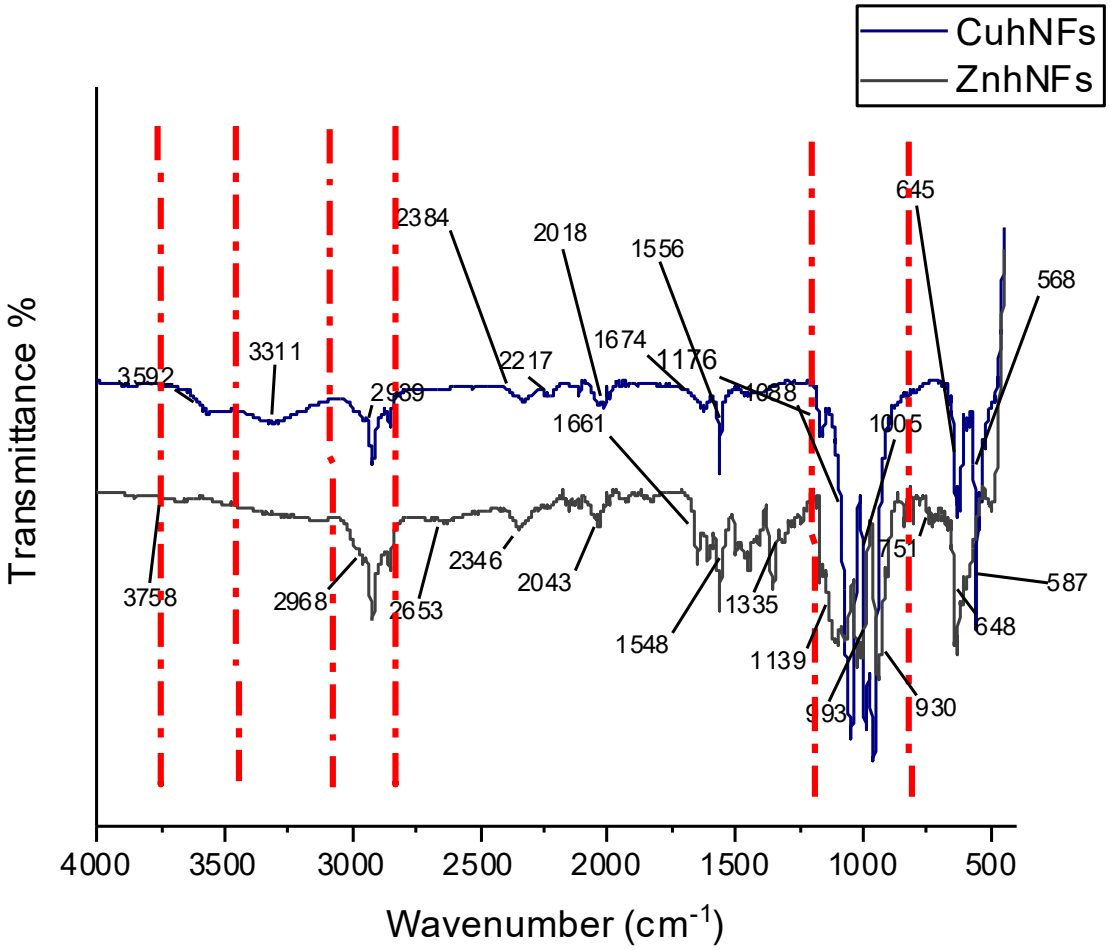


Figure 9. FT-IR spectrum of Naringenin-CuhNFs, Naringenin-ZnhNFs

Cytotoxicity assessment

The cytotoxic effect of Naringenin, Naringenin-CuhNFs and Naringenin-ZnhNFs on the MCF7 cell line is given in Figure 10. As seen in Figure 10, it was observed that Naringenin had a very good anticancer activity on the MCF7 cell line when converted into hybrid nanoflower form. As can be seen, Naringenin-ZnhNFs killed MCF7 cells at a rate of approximately 76%, while Naringenin CuhNFs caused cell death at a rate of 68%. Naringenin caused 45% cell death. As can be concluded from the results, when Naringenin was converted into hybrid nanoflower form, its effectiveness in the MCF7 cell line was greatly increased. CuhNFs synthesized according to ISO 10993-5 criteria were classified as non-cytotoxic

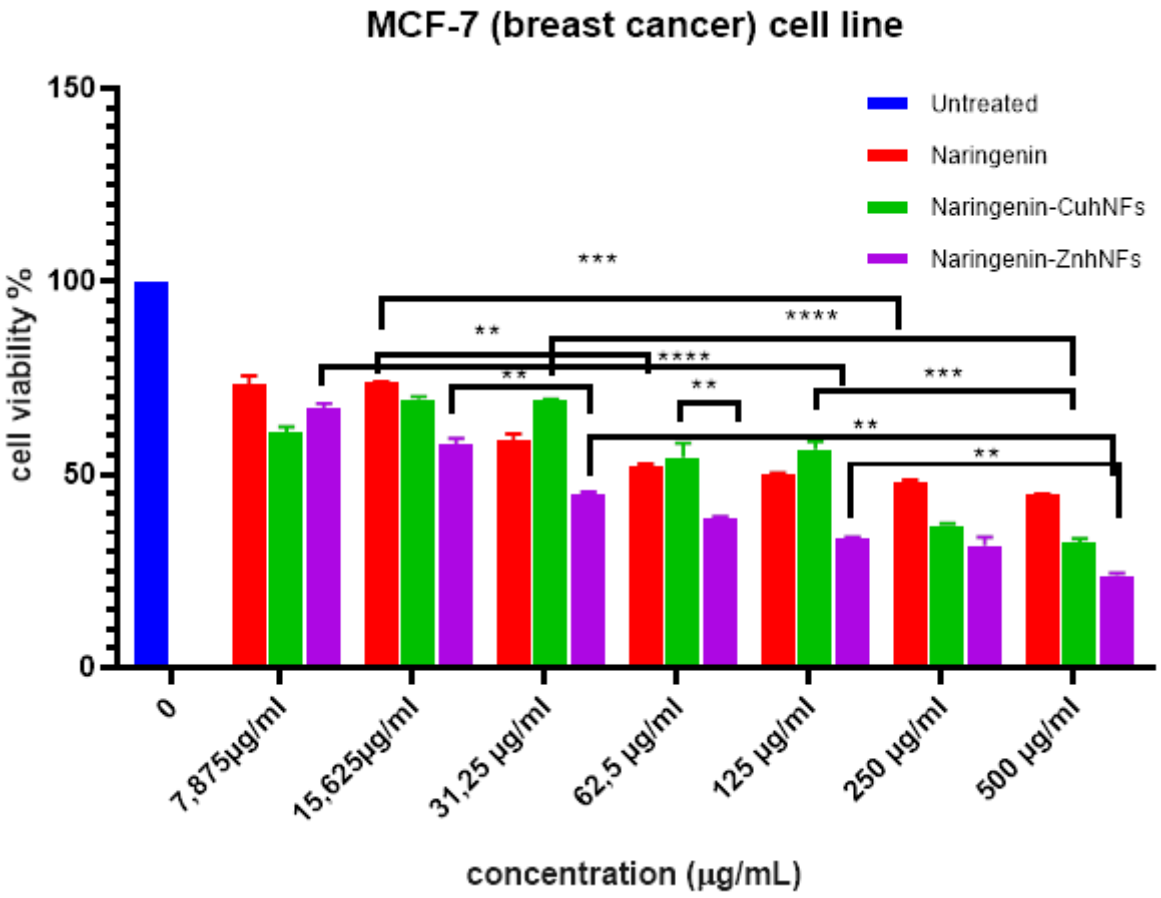


Figure 10. Cytotoxicity of CuhNFs, ZnhNFs and naringenin on MCF7cells lines. Data were presented as mean ± SD. $^{**}p < 0.01$,$^{***}p < 0.001$, $^{****}p < 0.0001$

Many studies have been conducted on plants and similar hybrid nanoflowers in the literature. Bor et al. [22] synthesized Cu and Zn hybrid nanoflowers using the *Persea americana Mill.* leaves extract. They applied the cytotoxic effects of the metal nanoflowers they synthesized to the L929-mouse fibroblast cell line. They found that the anticancer activity of hybrid nanoflowers synthesized with Cu was higher than that of plant extract. Somturk et al. [1] synthesized hybrid nanoflowers using *Tribulus Terrestrist L* extract and Cu, Co and Zn metal ions. The anticancer activities of these synthesized hybrid nanoflowers were applied to the A549 cell line. They observed that when the plant extract was converted into a hybrid nanoflower, its anticancer activity was greatly increased. They concluded that the effectiveness of hybrid nanoflowers especially synthesized with Co metal ion was better than others.

4. Conclusion

As a result, nanoflower synthesis was carried out using Naringenin as the organic part and Cu (II) and Zn (II) metal ions as the inorganic part. The structures of the synthesized nanoflowers were elucidated using SEM, EDX, FTIR, XRD and elemental mapping. Next, cytotoxic effects were evaluated using MCF7 cells. When the CuhNFs, ZnhNFs and Naringenin were applied to the MCF7 cell line, an increase in cell death occurred depending on the concentration. Additionally, an increase in cell death occurred when naringenin was converted into its hybrid

nanoflower form. In particular, it was observed that hybrid nanoflowers synthesized with Zn metal ion killed breast cancer cells by 76%. It was observed that hybrid nanoflowers synthesized with Cu metal ion caused 68% cell death. This means that it can be said that Zn metal ion is more effective than naringenin and the hybrid nanoflower synthesized with Cu metal ion.

Author Contributions

B.S.Y. performed all experiments. B.S.Y. conceived the original idea and designed the project. B.S.Y. wrote the manuscript.

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