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Article

Antimicrobial and catalytic activity of citrus fruits peels mediated nano-flowers

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In this work, plant extract–metal ions hybrid nanoflowers (HNFs) were synthesized and their catalytic and antimicrobial properties were examined. While the sweet and sour lemon peel extract (acquired in boil water) and its primary elements were engaged as organic elements in the creation of NFs, copper (II) ions (Cu^{2+}) were the inorganic element. Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray (EDX) spectroscopy, and Fourier Transform Infrared Spectrometry (FT-IR) were examined to characterize the structures of the NFs. The structural examination showed that the existence of Cu–O and Cu–N bonds in NFs can be a sign of the creation of NFs. Antimicrobial activities of the NFs were systematically studied against *Candida albicans* (ATCC 90028), *Staphylococcus aureus* (ATCC 25923), and *Escherichia coli* (ATCC 25922) with broth microdilution and short time-kill assay. The peroxidase-mimicking activity based on the NFs' Fenton-like response mechanism was assessed against guaiacol in the presence of H_2O_2. The findings have shown that plant extract-based hybrid NFs technology is promising and can find prospective applications in numerous technical areas.

Keywords: sweet lemon, nanotechnology, catalytic activity, lemon peel, antimicrobial activity

1. Introduction

Vitamin C (also known as L-ascorbic acid) is found in all living tissues [1]. The richest sources of this vitamin, which is very common in nature, are fresh fruits and vegetables [1]. Among the fruits, most of them contain ascorbic acid; lemon, orange, grapefruit, kiwi, pineapple, strawberry and currant. Of these fruits, especially citrus fruits (lemon, orange, grapefruit), kiwi and tomato outer parts (husk) is rich in ascorbic acid. Studies show that lemon juice is a source of antioxidants as well. Polyphenols, vitamins, minerals, fiber, carotenoids and essential oils in lemon peel protect against many diseases [2-12].

In the 1970s, studies by Cameron, Campbell and Pauling suggested that high doses of vitamin C had positive effects on quality of life and survival in terminal cancer patients [13], [14]. However, some later studies did not support these findings [10], [15]. In advanced cancer patients, vitamin C administered intravenously can maximize plasma levels and have toxic effects on cancer cells. In

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another work, trials in mice suggest that vitamin C may give hope for the treatment of difficult-to-treat tumors [16-18]. High concentrations of vitamin C act as pro-oxidants and hydrogen peroxide, with selective toxicity to cancer cells, may occur [16]. These findings and the presence of several patients with advanced cancer who have reached a very long life after administration of high-dose IV vitamin C support the re-evaluation of the use of high-dose IV vitamin C as a medication for cancer treatment [16-28].

In recent years, interest in hybrid functional nanostructures formed by the interaction of metal ions and organic / biomolecules has increased greatly due to their many advantages such as their simple synthesis, high efficiency, and enzyme stabilizing ability. In particular, the increase in the stability and activity of the organic / biomolecules used has been proven by many studies used in applications for a host of devices such as solar cells, sensors, detectors, energy generators, as well as artificial structures for tissue engineering, water treatment system, medicine, pharmaceutical science and industry (textile, food, etc) [29-33]. In this study, lemon and sweet lemon peel extracts containing high percentage of ascorbic acid and copper (Cu^{2+}) ions were combined in phosphate buffer to produce single-dispersed, porous and high surface-volume ratio flower-like hybrid nano structures. In the second stage, characterization of flower-like hybrid nano structures was performed. In the last stage, antimicrobial activities of flower-like hybrid nano structures obtained from extracts were investigated systematically because of their unique morphology and metal ions.

2. Experimental methods

2.1. Preparation of hybrid nanoflower

The synthesis of hybrid nanoflowers (HNFs) was accomplished using a slightly modified method [34]. First; 120 mM $CuSO_4$ stock solution in the ultrapure water was freshly prepared. Then, 60 µL of $CuSO_4$ stock solution was added to 9 mL of 10 mM PBS solution (pH 7.4), containing 0.2 mL of plant extract (lemon and sweet lemon extract). This final mixture was vigorously agitated and was then left undisturbed for incubation for 72 hours at room temperature (RT=20°C). After incubation, the growth process of HNFs was completed and the mixture was centrifuged to obtain a blue-colored precipitate. The collected HNFs powder was washed at least 3 times with water and centrifugation at 12000 rpm for 10 min in order to remove unreacted components. Finally, the HNF precipitates were dried under vacuum at room temperature.

2.2. Catalytic activity measurement

In all for activity measurement experiments, the HNFs (prepared from 0.2 mL plant extract and 0.8 mM Cu^{2+}) were used. The activities of HNFs were determined by colorimetric and spectroscopic methods using guaiacol as a chromogenic substrate [35], [36]. In activity measurements with HNFs a standard protocol (pH 6.8, 0.1 M KH_2PO_4, 25°C) was applied. First, HNFs were dissolved in 1mL of PBS in separate reaction tubes and then 1 mL of 22.5 mM hydrogen peroxide (H_2O_2) and 1 mL of 45 mM guaiacol were added to each reaction tube. The change in color was visually observed with naked eyes and the changes in absorbance values were also monitored for 3 min at 470 nm at RT using a UV-Vis spectrophotometer.

2.3. Antibacterial activity

For determining the antibacterial activity of HNFs, the agar disk diffusion method was applied, as previously reported [37-39]. Briefly, HNFs at 0.5 μg/mL were used to prevent *E. coli* (ATCC 25922), *Candida albicans* (ATCC 90028) and *S. aureus* (ATCC 25923) growth. Bacterial cultures at a concentration of 1.5×10^8 CFU/mL were inoculated with different concentrations of NFs. After incubating the culture plates at 37°C for 24 h, the bacterial growth inhibition zone was measured in millimeters. For each bacterial strain, independent experiments were performed as triplicate.

3. Results and discussion

Morphology evolution with two sources of organic part (lemon and sweet lemon extract) and the mechanism underlying catalytic activity in terms of HNFs morphology were systematically studied. It is well known that reaction parameters, including precursor concentrations, reaction temperature and reaction time, can influence the morphology of all types of nanomaterials and their physical and chemical properties. In addition, the precursor concentration ratio has a significant impact on the formation of hybrid nanomaterials by controlling the number of nucleation centers [35], [40-42]. In this study, the HNFs formation mechanism is described using different organic sources like extracts of sour lemon and sweet lemon peels.

I chose citrus fruits as organic structure for this study, owing to its wide catalytic activity, high sensitivity and substrate specificity. It has been widely used in many applications, including removal of phenols from polluted waters, organic synthesis, biosensor design, medical and micro-analytical studies [36], [43-45].

In this study, synthesis of NFs from extracts of lemon and sweet lemon peel, which is the first target, was successfully performed. When the SEM images of the HNFs were examined, the shape and size were found to be interestingly different. This was interpreted as the content of all extract species and the interaction of Cu^{2+} ions and extracts in phosphate buffer in the formation of NF. When SEM images obtained from lemon peel extracts were examined, it was found that NFs were generally more tight petals, compact and spherical (Figure 2). In the hybrid structures obtained from sweet lemon peel extract, the morphological structure was observed as circular and compact with more loose petals (Figure 3). The dimensions of lemon and sweet lemon nano-flower structures were approximately ~ 5.9 and 12.9 μm, respectively.

In the study, it was found that the both hybrid NFs synthesized from the lemon and sweet lemon extracts in the catalytic activity test were much lower level when compared to work of Ildız *et al*. (2016) [46]. In their study, *Viburnum opulus* L. fruit methanol extract-Cu^{2+} nanoflower hybrid structures were synthesized and compared to the extract alone reported a high increase in antimicrobial and catalytic activity [46]. In another study, *Ficus benghalensis* L. nanoflowers were synthesized from leaf extracts and these structures caused an increase in catalytic degradation of methylene blue [47]. Baldemir *et al*. [48] focused on a work related that green tea (*Camellia sinensis* L.) with ethanol and water extracts contained caffeine and catechin substances by synthesizing HNFs structures. They also obtained enhancement on the antimicrobial and catalytic activity. In this study, there is no significant increase in catalytic activity of HNFs. I observed that slight increase in the catalytic activity until 40 min. and stabilization from 40 min. to 250 min. for both extracts HNFs (Figure 4). There is a clear inhibition zone for each types of bacteria as shown in photograph (Figure 5). However, we reported that antimicrobial activity for both extract HNFs were promising for the future and supports literature (Figure 6 and 7).

As a result of the analysis, FTIR spectra of all plant extracts HNFs were found to be similar and FTIR spectra of extract-nanoflowers were interpreted (Figure 8). According to this, the flexural bands of the PO_4^{3-} groups were contained in the plant extracts. This shows that PO_4^{3-} groups are integrated in the structure of the NF. Vibration bands when examined ~ 1621 cm^{-1} and ~ 1622 cm^{-1}, NH_2 groups were more prominent in lemon-extract NFs, while sweet lemon-ex.-NFs was weaker in hybrid structure. This may be the result of some NH_2 groups partially embedded in the NF. Finally, the tensile bands attributed to the CH_2 and CH_3 groups in the range of about 2880 cm^{-1} and 2987 cm^{-1}; the two peaks at about 1393 cm^{-1} and about 3673 cm^{-1}, represent the C=O and O-H groups of the phenolic compounds in the plant-extract. Plant extracts showed much lower catalytic activity compared to HNFs due to negatively charged groups. In contrast, low catalytic activities were observed by negatively charged groups and NFs with Cu^{2+} ions due to mimicking Fenton-like reaction.

It can oxidize the substrate by producing Cu^{+} ions in NFs. On the other hand, EDX analysis showed that copper metal is necessary for the formation of plant extract-Cu^{2+} hybrid nano structure and that it is present in the structure of plant-HNFs (Figure 9).

Figure 1. Photo of lemon peel and sweet lemon peel and their extracts

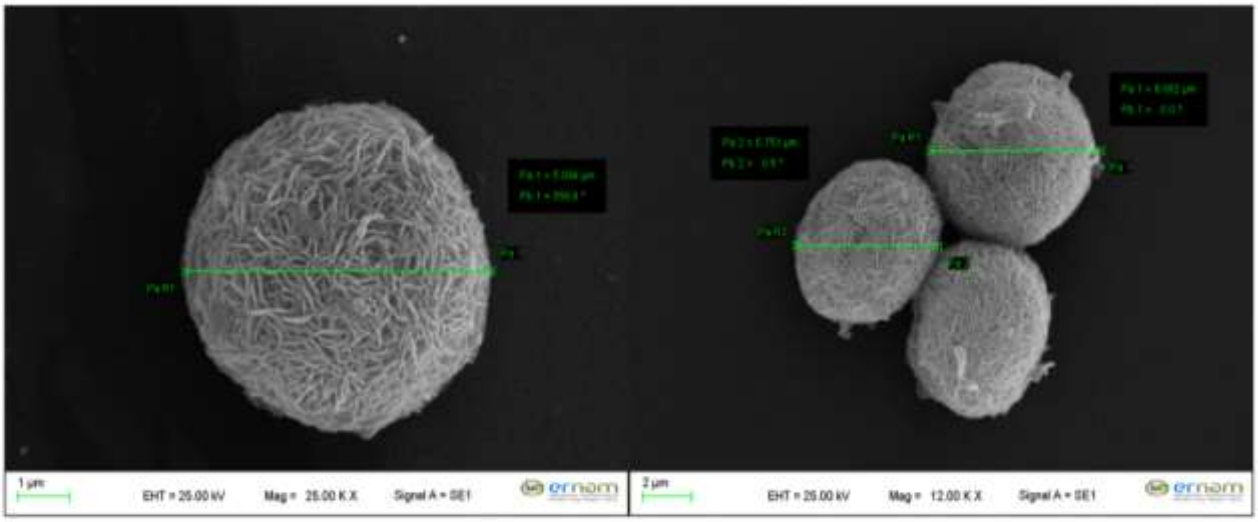

Figure 2. SEM images of HNFs using sour lemon peel flowers

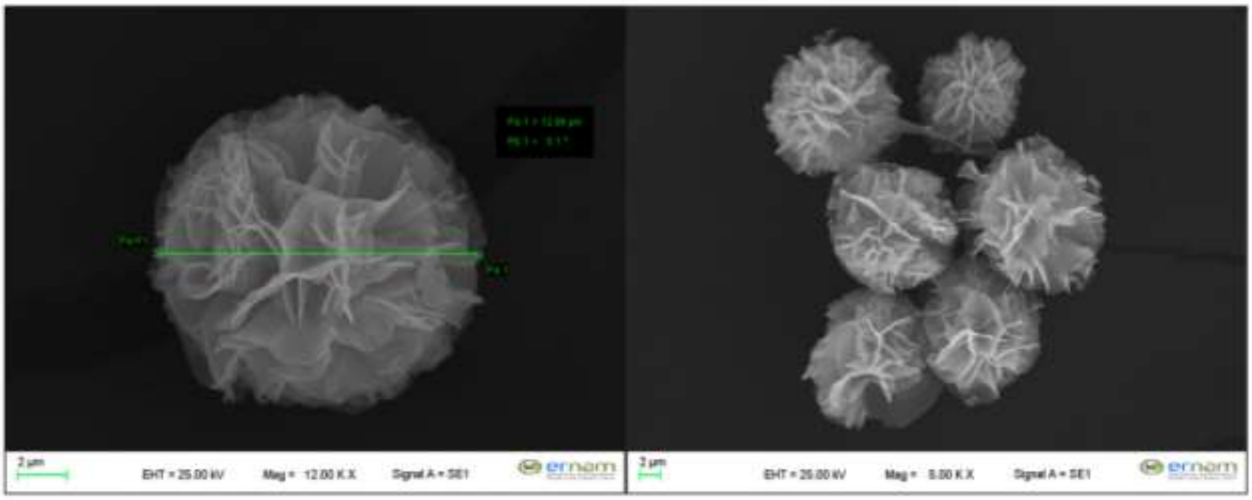

Figure 3. SEM images of sweet lemon peel nanoflowers

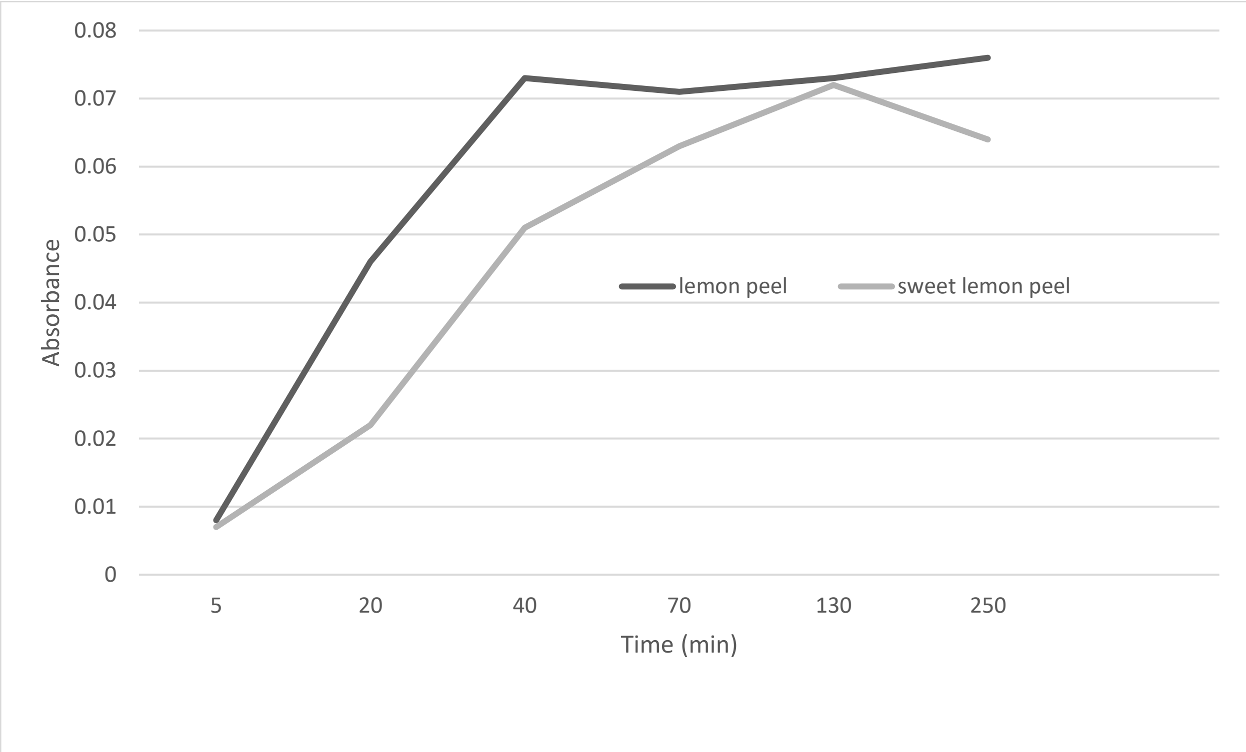

Figure4. Catalytic activity of lemon peel and sweet lemon peel nanoflowers in 5, 20, 40, 70, 130 and 250 minutes.

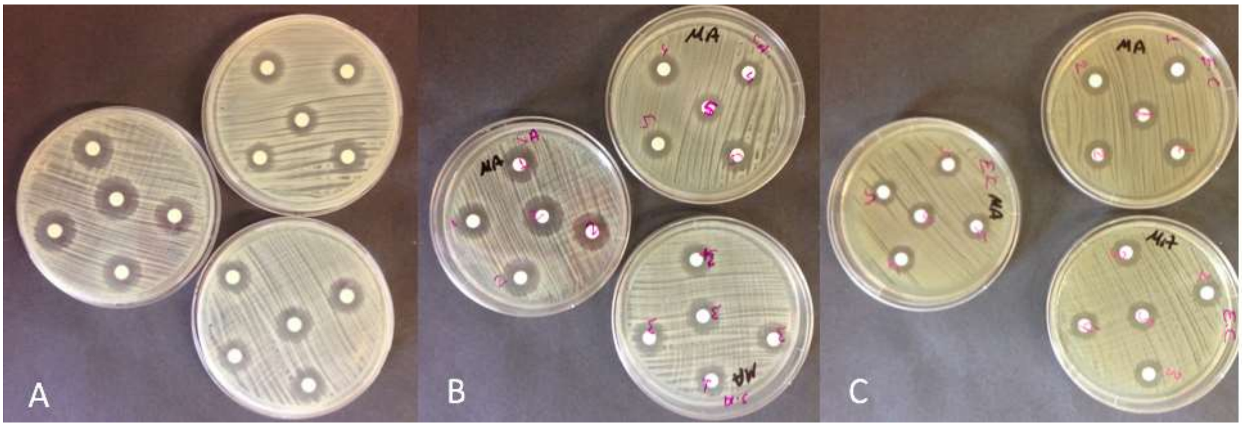

Figure5. Photo of results for *Candida albicans* (ATCC 90028) (A), *Staphylococcus aureus* (ATCC 25923) (B), *Escherichia coli* (ATCC 25922) (C), disc diffusion method.

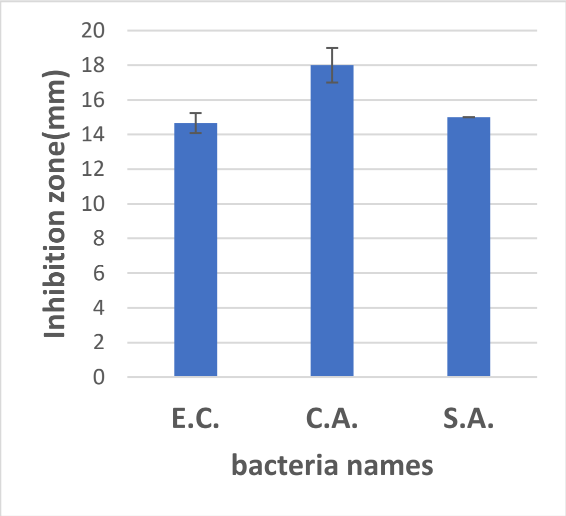

Figure6. Inhibition zone (mm) of sour lemon peel nanoflowers on E.C. (*Escherichia coli* (ATCC 25922), C.A. (*Candida albicans* (ATCC 90028), S.A. (*Staphylococcus aureus* (ATCC 25923).

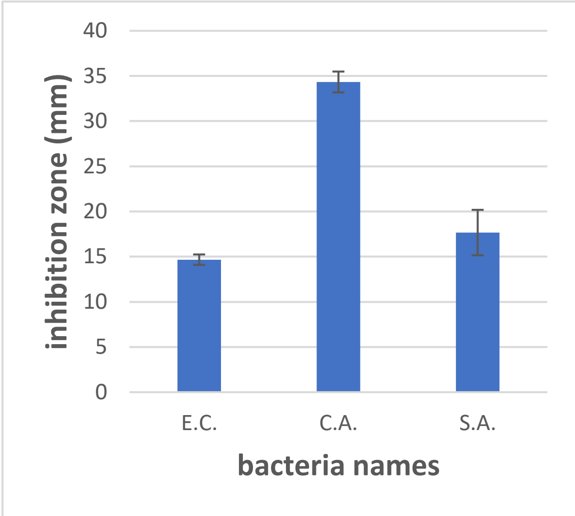

Figure7. Inhibition zone (mm) of sweet lemon peel nanoflowers on E.C. (*Escherichia coli* (ATCC 25922), C.A. (*Candida albicans* (ATCC 90028), S.A. (*Staphylococcus aureus* (ATCC 25923).

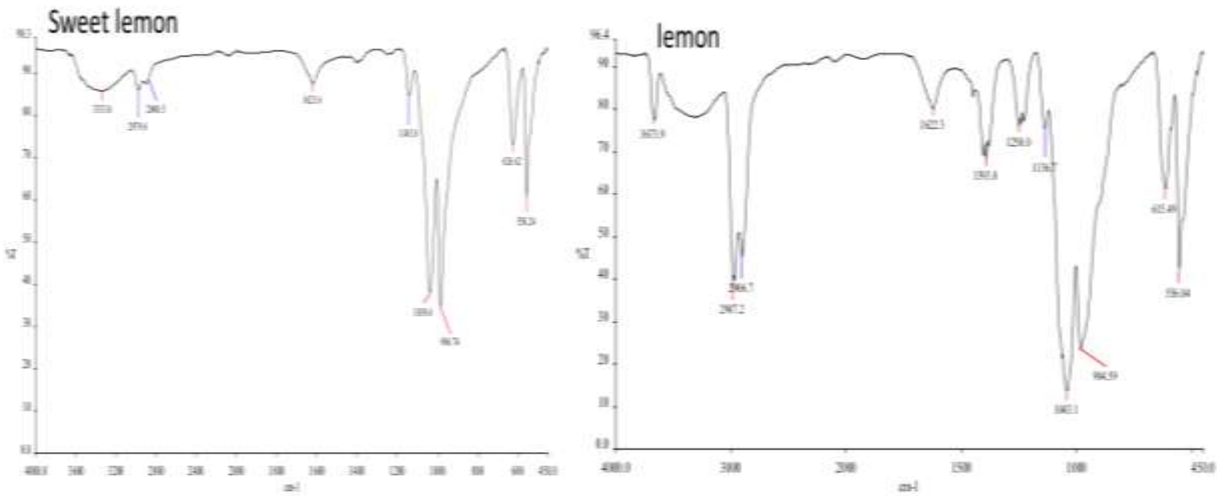

Figure8. FT-IR Results of sweet lemon and lemon peels nanoflowers

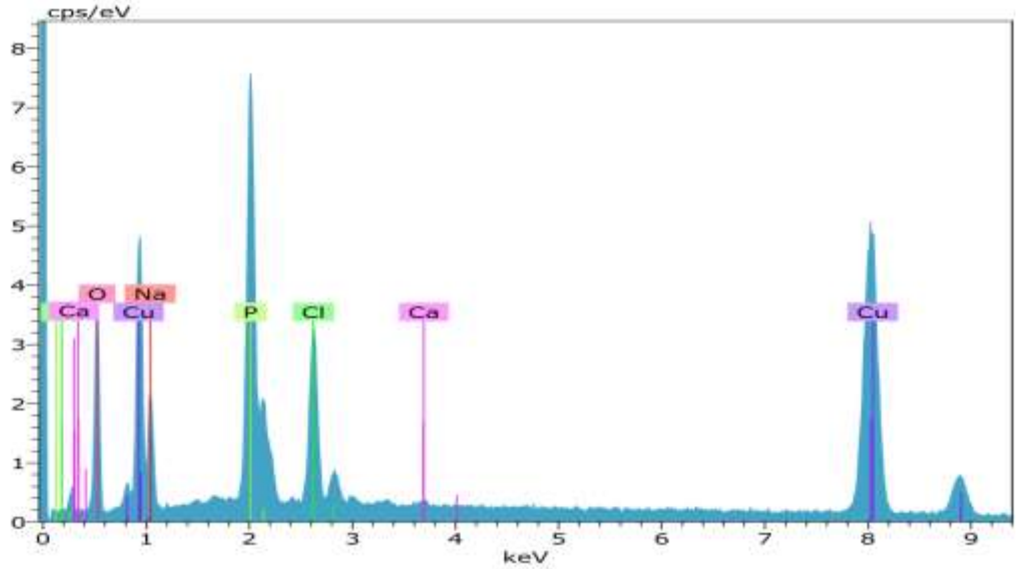

Figure9. EDX analysis of hybrid-nano structures showing the presence of Cu metal

4. Conclusion

Nanoflowers were successfully synthesized by solution reduction method in aqueous media at an ambient condition. The synthesized both lemon and sweet lemon peels nanoflowers were highly pure and uniform in size. As a result, it was found that both lemon and sweet lemon peel extracts HNFs showed antimicrobial activity approximately two times more effective on *Candida albicans* than on the other bacteria. There is no significant observation related to catalytic activity of nanoflowers synthesized using both sweet and sour lemon. In the future studies, it is aimed to standardize the HNFs. Thus, the nanomaterial synthesized from the plant extract can be used as a natural antibacterial agent for any application.

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