Middle Black Sea Journal of Health Science / Mid Blac Sea J Health Sci
May 2023; 9(2): 245-255

RESEARCH ARTICLE
DOI: 10.19127/mbsjohs.1223340

Synthesis, Characterization and Investigation of Antimicrobial Activity of Orthophtaldehyde Nanoflowers

Gülten Can Sezgin[1(ID)] Nilay Ildız[2(ID)]

[1]Department of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
[2]Pharmaceutical Microbiology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey.

Received: 23 December 2022, Accepted: 29 March 2023, Published online: 31 May 2023

Abstract

Objective: Endoscopy procedures are frequently performed in gastroenterology clinics, and disinfection cost, corrosion and toxicity are important problems in this area. For this purpose, hybrid nanoflowers at various pHs were synthesized with orthophtaldehyde (OPA), which has an important in disinfection in these clinics, and the hybrid nanostructures obtained by reducing the amount of orthophtaldehyde use were aimed to also increase the effectiveness of the disinfectant.

Methods: OPA nanoflowers (OPA NFs) were synthesized and their effective diameters (hydrodynamic diameters) and surface charges were determined by dynamic light scattering (DLS) and Zeta potential (ZP) measurements, respectively. Antimicrobial activities of orthophtaldehyde and OPA NFs against *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 35218 and *Candida albicans* (*C. albicans*) ATCC 90028 standard strains were evaluated by the liquid microdilution method using percent inhibition method.

Results: OPA-based OPA-Cu^{2+}hybridnano flowers (OPANF) were synthesized successfully at different pH values (pH 7.4, 9 and 11). The most effective antimicrobial activity was observed in the nanoflowers synthesized at pH=7.4 for all tested microorganisms. Although the antimicrobial activity decreased as the pH value increased, NFs activity was higher than OPA alone at all pH values ($p<0.001$). In this study, NFs synthesized with 0.02 mg/ml OPA were found to be 5.2 times more effective for *C.albicans*, 5.75 times for *E.coli* and 4.4 times for *S.aureus* than OPA(0.02 mg/ml).

Conclusion: NFs showed very high antimicrobial activity compared to OPA and also promise to be a preferred agent in medical device disinfection providing a high level of disinfection with the use of a few amounts of OPA that use in clinics.

Key words: disinfection, endoscopy, nanoflower, orthophthaldehyde.

Suggested Citation: Can Sezgin G, Ildız N. Synthesis, Characterization and Investigation of Antimicrobial Activity of Orthophtaldehyde Nanoflowers. Mid Blac Sea Journal of Health Sci, 2023;9(2):245-255.

Address for correspondence/reprints:

Nilay Ildız

Telephone number: +90 (352) 207 66 66

E-mail: nilaygucluer@erciyes.edu.tr

INTRODUCTION

Endoscopic procedures are widely used medical procedures all over the world. During these procedures, patients are exposed to some risks. A number of infectious pathogens can be transmitted to patients during the procedure. Devices used in endoscopy are classified as semi-critical devices. These devices come into contact with damaged or intact mucous membranes and skin (1). Endoscopy devices are cleaned with high level disinfection (HLD) procedures to reduce the risk of microorganisms transfer from endoscopes. The HLD process removes all microorganisms, only a small number of bacterial spores may remain (2,3). Cleaning of endoscopes requires cleaning of interior, exterior and all surfaces and the use of effective microbicidal agents (4). Flexible gastrointestinal endoscopic devices are heat labile, so chemicals are often used in HLD. Low temperature sterilization methods can also be used, but there is no low temperature sterilization method approved by the United States Food and Drug Administration (FDA). Orthophtaldehyde (OPA) is a highly effective and important chemical substance used for this purpose. The use of OPA is a chemical agent used for HLD, approved by the FDA in October 1999. In vitro studies show that it has excellent microbicidal activity (5). In United States, more than 10 million gastrointestinal endoscopy procedures are performed annually (6). Therefore, sterilization of these devices is of great importance.

Recently, hybrid nanostructures in the form of organic inorganic flowers called “nanoflowers (NFs)” were first discovered by Zare et al. using Cu^{2+} as an inorganic molecules and protein/enzyme as organic compounds and were described to support catalytic activity and stability (7). NFs are also important for industrial areas such as enzyme purification processes, drug delivery, vaccine studies, and possible applications for environmental protection (8-11). Altınkaynak et al. (2016) observed that lactoperoxidase derived NFs show more efficient catalytic activity than free lactoperoxidase (12). Since the discovery of NF, plant extracts are among the most commonly used organic compounds (13-18).

In this context, orthophtaldehyde, which is used as a HLD were firstly synthesized as NFs. It is aimed to evaluate the antimicrobial activity of this newly created substance on bacteria and fungi and to compare it with the use of OPA alone.

METHODS

Microorganisms:

In the study, Candida albicans (C. albicans) ATCC 90028, Staphylococcus aureus (S. aureus) ATCC 25923, Escherichia coli (E. coli) ATCC 35218 were used as standard strains. Microorganisms are stored from -80 °C and expected to dissolve, and bacteria were grown on tryptic soy agar and fungi were cultivated on yeast extract agar after an overnight incubation, and the incubated microorganisms were used in antimicrobial activity experiments. The strains used for this study were obtained from the collection of Erciyes University Faculty of Pharmacy, Department of Pharmaceutical Microbiology.

Synthesis of OPA Nanoflower:

OPA Copper nanoflower syntheses were made with phosphate buffer saline (PBS) solution prepared at three different pH.

For 1000 ml of synthesis

20 mg of OPA is weighed on a precision balance so that the final concentration in the solution is 0.02 mg/ml. It is mixed thoroughly in the magnetic stirrer for about 5 minutes until it is well dissolved in 20 ml of distilled water. 6.66 ml of the pre-prepared 120 mM Copper sulfate solution is added and mixed approximately for one minute so that the final concentration in the solution is 0.8 Mm/L. The solution is transferred to a one liter balloon jug. The volume is made up to 1 liter with freshly prepared PBS with pH adjusted to 7.4.

For pH 9:

The pH of PBS is adjusted to pH 9 with 0.1 M NaOH and used for nanoflower synthesis.

For pH 11:

The pH of PBS is adjusted to pH 11 with 0.1 M NaOH and used for nanoflower synthesis (14).

Antimicrobial activity method:

The minimum inhibitory concentrations were determined by using broth microdilution method. Mueller Hinton broth and RPMI 1640 were used respectively for bacteria and fungus. Microplates were incubated for 24 hours (590 nm) for bacteria and 48 hours (600 nm) for fungi. Results were evaluated spectrophotometrically (Azure Ao, Biosystem, France) and inhibition percentages were calculated. The study was performed in three replications for each microorganism (19,20).

Statistical analysis

In the data analysis, Graphpad Prism software version 8.0.1. was used. The conformity of the data to the normal distribution was examined using KolmogorovSmirnov tests. At least three experiments were repeated in the measurements. The data is presented as mean value ± SD value.and its statistically evaluated with Mann-Whitney U and One-Way ANOVA tests (Tukey's multiple comparison test). One-way ANOVA procedure was used to perform the analysis of variance. The p value less than 0.01 were considered significant

RESULTS

Ortophitaldehyde hybrid nanoflowers and their characterization:

OPA-based OPA-Cu^{2+} hybrid nanoflowers (OPA NFs) were synthesized by incubating OPA and copper (II) (Cu^{2+}) ions in PBS at different pH values (pH 7.4, 9 and 11). First, the synthesis of OPA NFs were completed and characterized by adjusting the buffer solution to pH 7.4 (Figure 1). As seen in Figure 1A, the OPA NF has a spherical, porous and compact structure with a scanning electron microscope. The mean diameter of the OPA NF was determined as 15 µm. Hydrodynamic diameter and surface charge of OPA NFs are seen as dynamic light scattering and zeta potential of 1.5 µm and -30 mV, respectively. The main reason why the effective diameter is much smaller than the electron microscope diameter is that OPA NFs precipitate after a certain period of time and smaller leaf-like structures suspended in solution are measured. When the zeta potential value is considered, it is an indicator that the surface charge is negative and that the OPA molecules have lost protons.

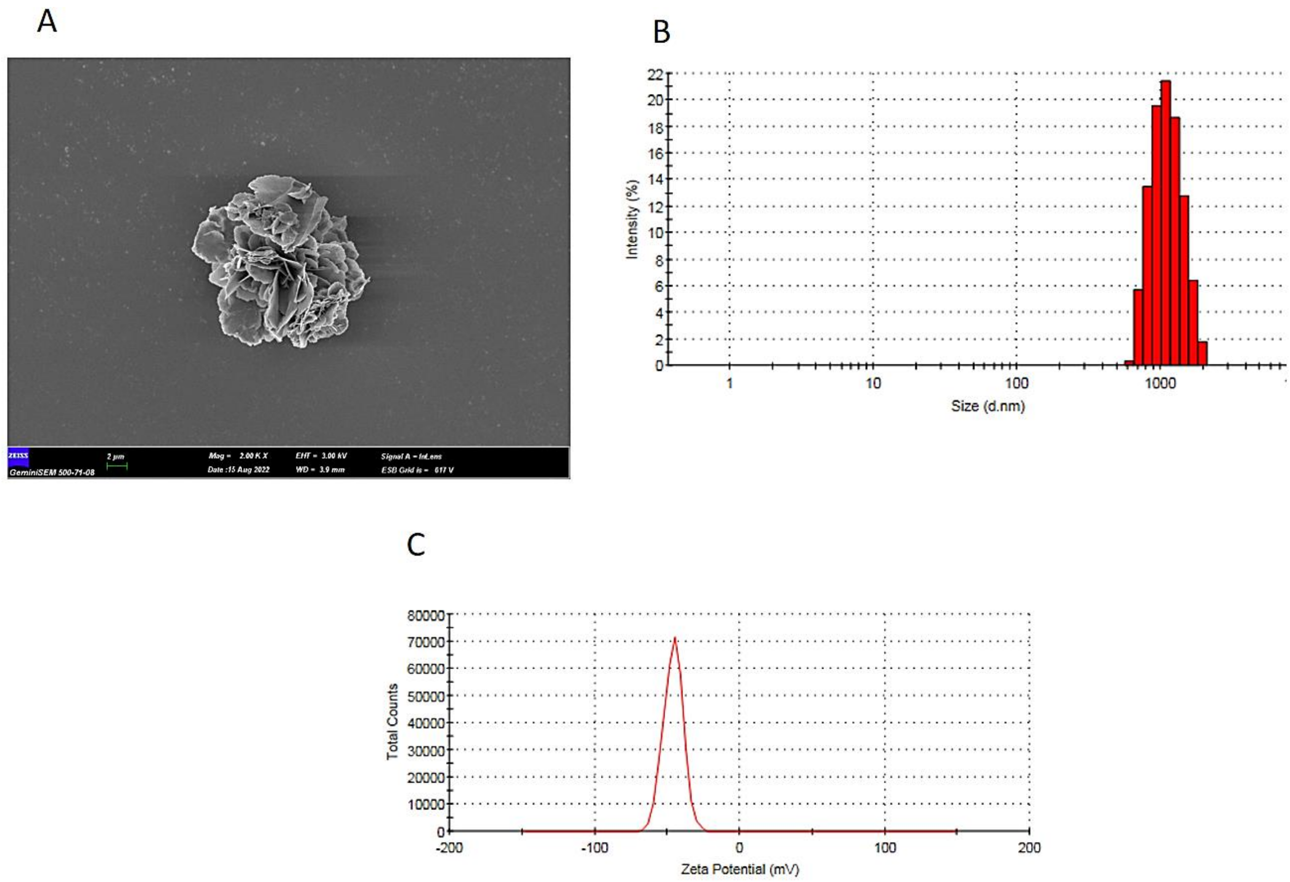


Figure 1. Characterization of OPA NF (pH 7.4): A) Electron microscope image (2000 KX), B) Hydrodynamic diameter spectrum and C) Zeta potential spectrum

The same synthesis protocol was carried out at pH 9 and 11, respectively, and the resulting OPA NF like structures were characterized as seen in Figure 2 and Figure 3. For the formation of OPA NF at pH 9, according to the scanning electron microscope image in Figure 2A, leaves with an average size of 5-10 µm were formed to form OPA NF and entered the growth process, but they did not combine to form a flower-like morphology. Structures in square-like morphology are seen in the additional enlarged image in Figure 2A. Hydrodynamic diameter and surface charge, dynamic light scattering and zeta potential of OPA NF like structures are seen as 3.5 µm and -35 mV in Figures 2B and 2C, respectively. In the measurement of the effective diameter, the diameter of the leaves were measured in general, and when the zeta potential value is considered, the surface charge being more negative compared to Figure 1C is an indicator of higher proton loss of OPA molecules.

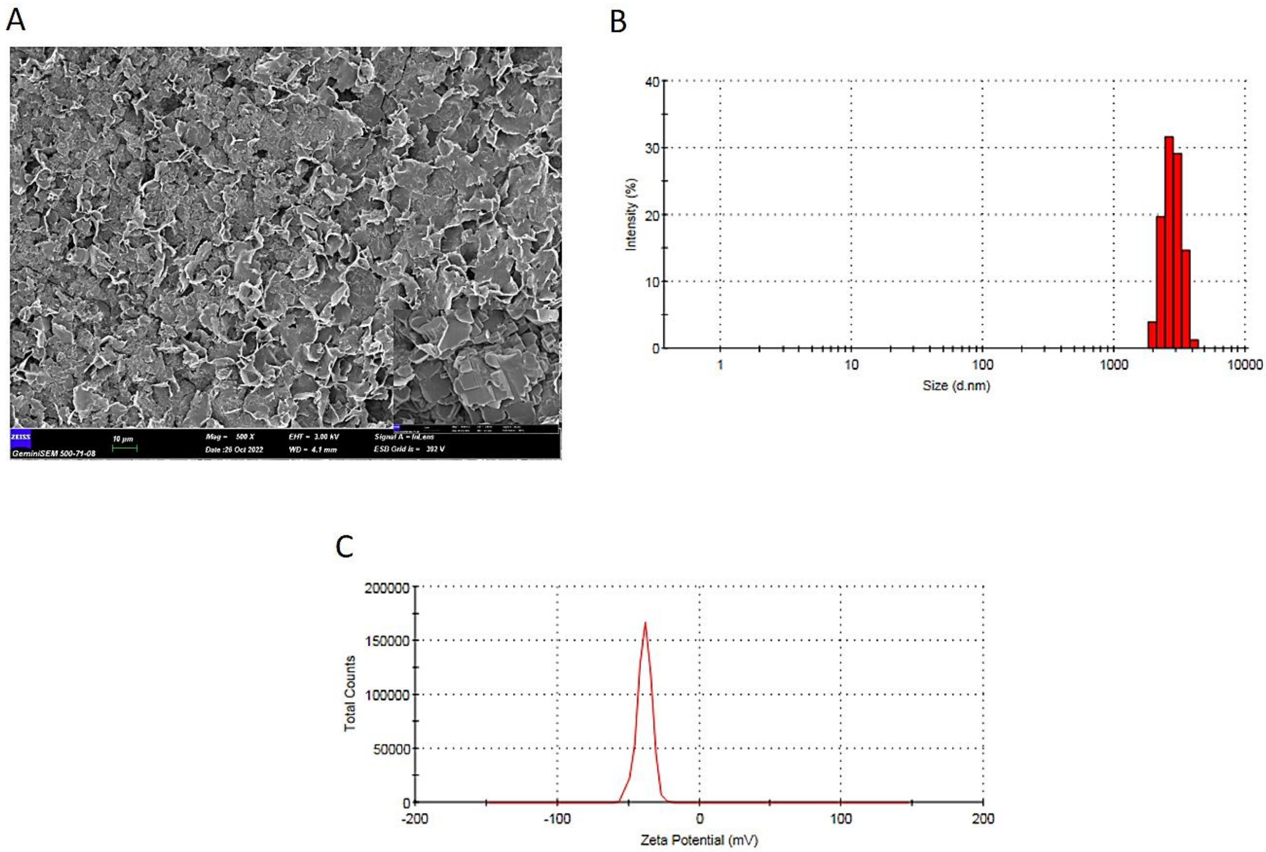


Figure 2. Characterization of OPA NF (pH9): A) Electron microscope image (500 KX), enlarged image that is in the lower right corner of figure 2A (10000 KX). B) Hydrodynamic diameter spectrum and C) Zeta potential spectrum

It was seen that spherical Cu-based structures were formed instead of OPA NF according to the scanning electron microscope image in Figure 3A for the formation of OPA NF at pH 11. Hybrid structures have been obtained in different size, from nano-size to micro-size, where the reaction medium is highly alkaline. The hydrodynamic diameters of the OPA-Cu hybrid structures showed polydispersity as dynamic light scattering at 200 nm, 1 µm and 3.5 µm (Figure 3B). The surface charges of these structures, on the other hand, give a high negative zeta potential of approximately -50 mV (Figure 3C).

OPA NFs were also synthesized by incubating OPA and copper (II) (Cu^{2+}) ions in PBS at acidic pH values (pH 5 and 3). However, both OPA NF formation and reaction no precipitate was obtained as a result. Since OPA carries a positive or nearly positive charge in an acidic environment, a repulsive force is formed between the Cu^{2+} ions in the reaction and the OPA molecules, thus preventing their interactions.

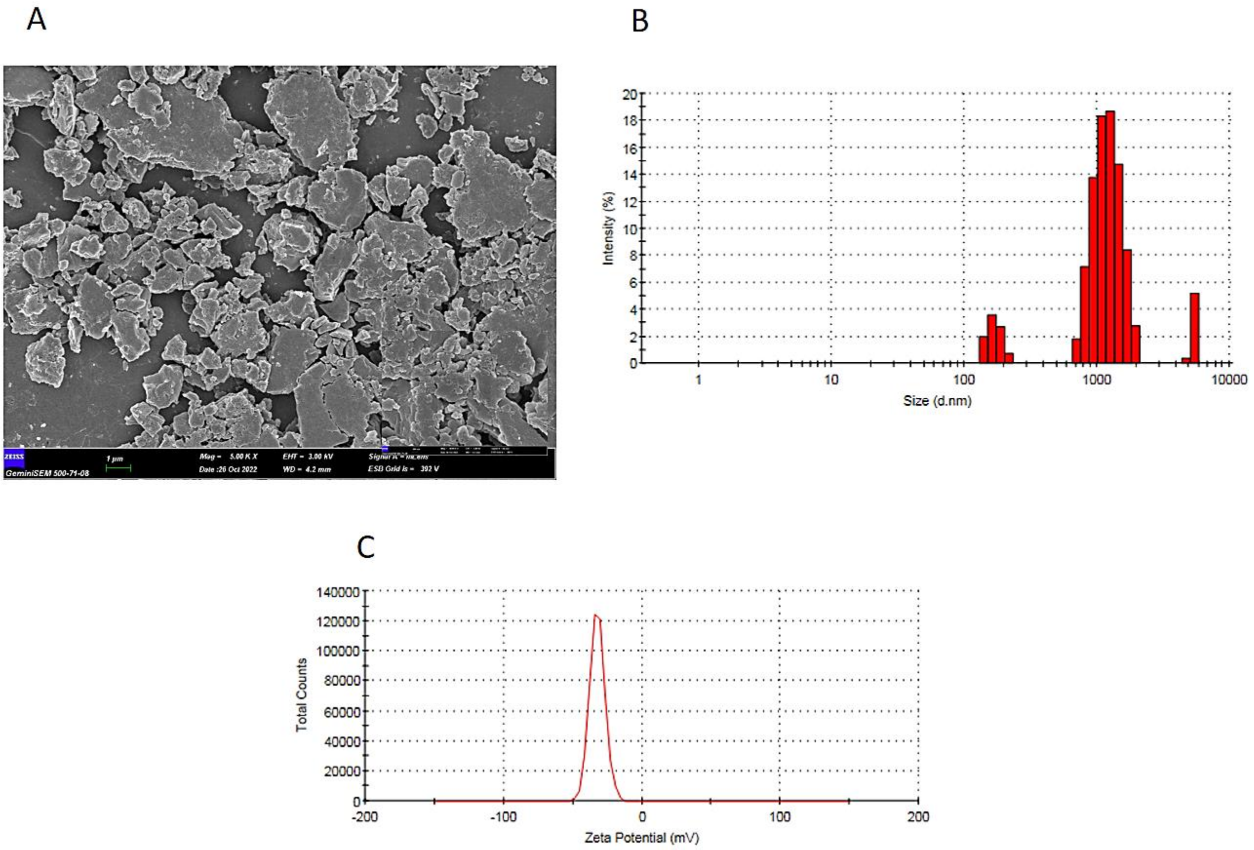
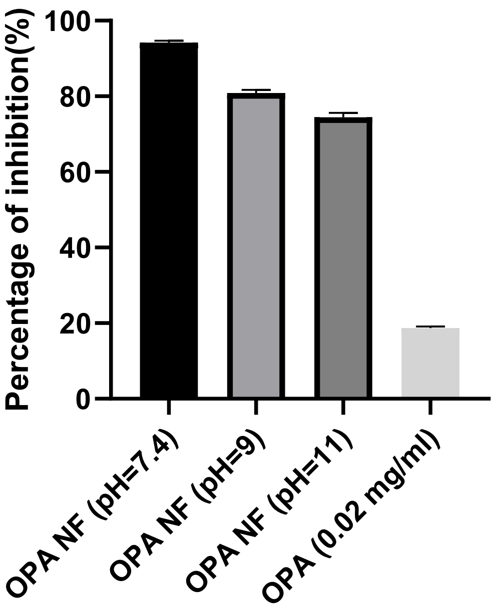
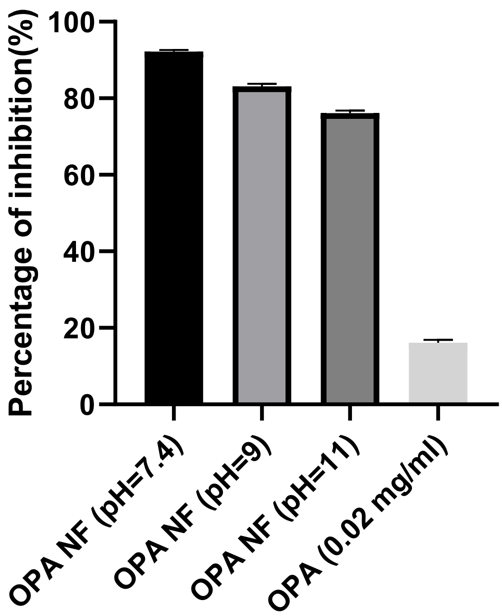


Figure 3. A) Characterization of OPA NF (pH11): A) Electron microscope image (5000 KX). enlarged image that is in the lower right corner of figure 3A (30000 KX). B) Hydrodynamic diameter spectrum and C) Zeta potential spectrum.



Graphic 1. Percentage inhibition of OPA NFs (pH=7.4, 9 and 11) and OPA (0.02 mg/ml) against *C.albicans*



Graphic 2. Percentage inhibition of OPA NFs (pH=7.4, 9 and 11) and OPA (0.02 mg/ml) against *E.coli*

According to the results of the study, the most effective antimicrobial activity was observed in the NF synthesized at pH=7.4 for all tested microorganisms. Although the antimicrobial activity decreased as the pH value increased, OPA NF activity was higher than OPA alone at all pH values. However, the percent inhibition achieved at pH 7.4 was over 80% and these synthesized OPA NFs have minimum inhibitory effect for all tested microorganisms. These values were found to be statistically significant (p<0.001).

The highest antimicrobial activity was determined in *C.albicans*, a fungus (~94 % graphic1), followed by *E.coli* (~92 % graphic, 2) which is Gram-negative bacteria *and S.aureus* (~88 % graphic, 3), which is Gram-positive.

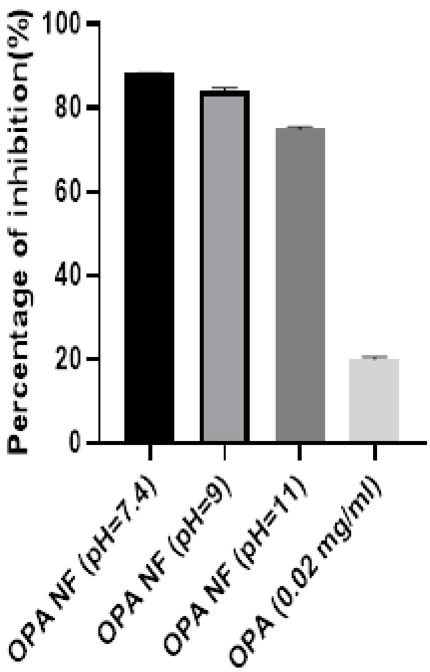


Table.3. Percentage inhibition of OPA NFs (pH=7.4,9 and 11) and OPA (0.02 mg/ml) against *S. aureus*

DISCUSSION

Today, although endoscopy procedures are widely used all over the world, the field of application and the variety of devices are increasing day by day. A high level disinfection process is applied for the disinfection of these devices. Problems such as cost, toxicity and effectiveness of chemicals used for HLD have become more important with increasing microorganism resistance. This study has unique value as it is the first study to investigate disinfectant-based nanoflower synthesis and efficacy. According to the findings, NFs-based OPA synthesized with a small amount of OPA showed highly effective antimicrobial activity. The amount of OPA used by the FDA in the routine has been specified as 0.55% (21). In this study, NFs synthesized with 0.02 mg/ml OPA were found to be 5.2 times more effective for *C.albicans*, 5.75 times for *E.coli* and 4.4 times for *S.aureus* than OPA(0.02 mg/ml).

Several toxicity studies performed indicate that OPA can be a chemical irritant and sensitizer as an adjuvant for other allergens (22,23). OPA concentrations ranging from 1.0 to 13.5 ppb have been detected in air samples taken from the endoscope cleaning unit of a hospital using OPA as the primary disinfectant (24-26). These findings demonstrate that OPA has the potential to induce respiratory sensitization following inhalation exposure. By reducing the amount of OPA that used with OPA NF, high antimicrobial activity will be achieved and toxicity will be reduced, thus reducing the amount of inhalation on healthcare workers and the exposure of patients.

There are several types of disinfectants, each with their own advantages and disadvantages. Short contact time is an important consideration in countries with a high frequency of endoscopy procedures and device limitations. For this reason, disinfectants that require shorter contact times are used, such as OPA and formulas of peracetic acid and hydrogen peroxide. However, these disinfectants are more expensive than the glutaraldehyde. Given the growing need for endoscopy worldwide, the cost of endoscope reprocessing is a major concern (27). Most endoscopy units in China have used glutaral aldehyde (GA) as the disinfectant of choice because of its costeffectiveness. OPA is seen as the best alternative according to GA, the cost of OPA is almost prohibitive than GA (USD, 6.20$ per endoscope). Therefore, very few endoscopy units in China have been able to chosen OPA (28). In this study, both high-efficiency and low-cost disinfectants were obtained by reducing the standard usage dose of OPA with synthesizing OPA NFs.

As a result of OPA NF (pH 7.4) acting as Fenton agents in the presence of hydrogen peroxide (H_2O_2), OPA NF oxidized the model substrate by showing enzyme-like activity through the Fenton reaction mechanism. In general, the Fenton reaction Cu^{2+} ions in the structure of OPA NFs are reduced to Cu^{1+} ions by reacting with H_2O_2, and these Cu^{1+} ions react with H_2O_2 again to form highly reactive hydroxyl radicals, and the free hydroxyl radicals produced by the Fenton reaction, oxidizing the model substrate, cause enzyme-like activity (13,14).

We hypothesized that abundant negatively charged groups in the cell promote the interaction between NFs and the bacterial cell membrane, leading to inhibition of cell replication and eventual cell death due to deformation of the cell membrane. Also, NFs are thought to act as Fenton-like agent based on Fenton chemistry to generate Cu^{1+} ions and various radicals that can cause cell death through oxidative stress and membrane damage.

Limitations:

This study has some limitations. Although the diversity of microorganisms is in terms of fungi and bacteria, however virus and parasitic activity can be investigated with further studies.

CONCLUSIONS

In this study, It is thought that the peroxidase like activity of NFs synthesized increases the antimicrobial activity. NFs synthesized for this purpose promises to be a preferable agent in medical device disinfection with further studies, by providing high level disinfection with the use of a few amount of OPA, being more environmentally friendly and less toxic.

Ethics Committee Approval: Ethics committee approval was not obtained for this study as it was carried out only with standard strains

Peer-review: Externally peer-reviewed.

Author Contributions: Concept: G.C.S, N.I; Design: G.C.S, N.I; Literature search: G.C.S, N.I; Data Collection and Processing: G.C.S, N.I; Analysis or Interpretation: GCS, NI; Writing: G.C.S, N.I

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study hasn't received any financial support.

REFERENCES

1. Spaulding EH. Chemical disinfection of medical and surgical materials. In: Disinfection, Sterilization, and Preservation. Lawrence, C.A., Block, S.S., Eds.; Leaand Febiger: Philadelphia, PA, USA, 1968. p. 517.
2. Beilenhoff U, Neumann CS, Rey JF, Biering H, Blum R, Cimbro M, et al. ESGE-ESGENA Guideline: Cleaning and disinfection in gastrointestinal endoscopy. Endoscopy 2008; 40: 939-957.
3. Petersen BT, Cohen J, Hambrick RD, 3rd Buttar N, Greenwald DA, Buscaglia JM, et al. Multisociety guideline on reprocessing flexible GI endoscopes: 2016 update. Gastrointest Endosc 2017;85: 282-294.
4. Marchese V, Di Carlo D, Fazio G, Gioè SM, Luca A, Alduino R, et al. Microbiological Surveillance of Endoscopes in a Southern Italian Transplantation Hospital: A Retrospective Study from 2016 to 2019. Int J Environ Res Public Health 2021;18(6): 3057.
5. Rutala WA, Weber DJ. Healthcare Infection Control PracticesAdvisory Committee. Guideline for disinfection and sterilization in healthcare facilities, 2008. Available from: URL: http://www.cdc.gov/hicpac/pdf /guidelines/disinfection_nov_2008.pdf.
6. Weber DJ. Managing and preventing exposure events from in appropriately reprocessed endoscopes. Infect Control Hosp Epidemiol 2012;33 (7):657-660.
7. Ge J, Lei JD, Zare RN. Functional Protein-Organic/Inorganic Hybrid Nanomaterials. Nat Nanotechnol 2012; 7: 428-432.
8. Lee SW, Cheon SA, Kim M, Park TJ. Organic–inorganic hybrid nanoflowers: types, characteristics, and future prospects. J of Nanobiotech. 2015; 13(1): 1-10.
9. Shaalan M, Saleh M, El-Mahdy M, El-Matbouli M. Recentprogress in applications of nanoparticles in fishmedicine: a review. *Nanomedicine:* Nanotech Biol Med. 2016; 12(3): 701-710.
10. Shende P, Kasture P, Gaud RS. Nanoflowers: The future trend of nanotechnology formulti applications. Artif Cells, Nanomed Biotech. 2018; 46(1): 413-422.

11. Dar AH, Rashid N, Majid I, Hussain S, Dar MA. Nanotechnology interventions in aqua culture and sea food preservation. Crit Rev Food Sci Nutr. 2020;60(11):1912-1921.
12. Altinkaynak C, Tavlasoglu S, Özdemir N,Ocsoy I. A new generation approach in enzyme immobilization: Organic-inorganic hybrid nanoflowers with enhanced catalytic activity and stability. Enzyme Microb Technol. 2016; 93: 105-112.
13. Baldemir A, Kose NB, Ildiz N, Ilgun S, Yusufbeyoglu S, Yilmaz V,Ocsoy I. Synthesis and characterization of gren tea (*Camellia sinensis* (L.) Kuntze) extract and its major components-based nanoflowers: a new strategy to enhance antimicrobial activity. RSC Adv. 2017; 7: 44303.
14. Ildiz N, Baldemir A, Altinkaynak C, Özdemir N, Yilmaz V,Ocsoy, I. Self assembled snowball-like hybrid nanostructures comprising *Viburnum opulus* L. extract and metal ions for antimicrobial and catalytic applications. Enzyme Microb Technol. 2017;102: 60-66.
15. Vinayagam R, Pai S, Varadavenkatesan T, Pugazhendhi A,Selvaraj R. Characterization and photocatalytic activity of ZnO nanoflowers synthesized using Bridelia retusa leaf extract. Appl Nanosci. 2021; 1-10.
16. Agarwal M, Singh Bhadwal A, Kumar N, Shrivastav A, RajShrivastav B, Pratap Singh M, et al. Catalytic degradation of methylene blue by biosynthesised copper nanoflowers using *F. benghalensis* leaf extract. IET nanobiotech. 2016;10(5): 321-325.
17. Badgujar HF, Bora S, Kumar U. Eco-benevolent synthesis of ZnO nanoflowers using Oxalis corniculata leaf extract for potential antimicrobial application in agriculture and cosmeceutical. Biocatal Agricul Biotech. 2021; 38:102216.
18. Koca-Caliskan U, Dönmez C, Eruygur N, Ayaz F, Altinkaynak C, Ozdemir N. Synthesis and Characterization of Copper-Nanoflowers with the Utilization of Medicinal Plant Extracts for Enhanced Various Enzyme Inhibitory Activities. Chem Biodiver. 2022;19: 1-12.
19. Clinical Laboratory Standard Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI standard M07, 2018 11th ed. Wayne, PA.
20. Clinical Laboratory Standard Institute (CLSI). Reference method for broth dilution antifungalsusceptibilitytesting of yeasts; ApprovedStandards-Second Edition, in CLSI document M07-A10, 2012 CLSI Pennsylvania, USA
21. Gregory AW, Schaalje GB, Smart JD, Robison RA. The mycobactericidal efficacy of ortho-phthalaldehyde and the comparative resistances of *Mycobacterium bovis*, *Mycobacterium terrae*, and Mycobacterium chelonae. Infect Control

Hosp Epidemiol. 1999;20: 324-330.
22. Anderson SE, Umbright C, Sellamuthu R. Irritancy and allergic responses induced by topical application of orthophthalaldehyde. Toxicol Sci. 2010; 115(2): 435-443.
23. Morinaga T, Hasegawa G, Koyama S, Ishihara Y, Nishikawa T, Acute inflammation and immunoresponses induced byortho-phthalaldehyde in mice. Arch Toxicol. 2010;84(5): 397-404.
24. Marena C, Lodola L, Maestri L, Alessio A, Negri S, Zambianchi L. Monitoring air dispersed concentrations of aldehydes during the use of ortho-phthalaldehyde and glutaraldehyde for high disinfection of endoscopes. G Ital Med Lav Ergon. 2003; 25(2):131-136.
25. Tucker Samuel P. Determination of ortho-phthalaldehyde in airand on surfaces. J Environ Monit. 2008; 10(11): 1337-1349.
26. Fujita H, Masanori O, Yoko E. A case of occupational bronchial asthma and contact dermatitis caused by ortho-phthalaldehyde exposure in a medical worker. J Occup Health. 2006;48(6): 413-416.
27. Zhang XL, Kong JY, Tang P. Current status of cleaning and disinfection for gastrointestinal endoscopy in China: a survey of 122 endoscopy units. Digest Liver Dis. 2011;43(4):305-308.
28. Seo HI, Lee DS, Yoon EM, Kwon MJ, Park H, Jung YS, et al. Comparison of the efficacy of disinfectants in automated endoscope reprocessors for colonoscopes: tertiary amine compound (Sencron2®) versusortho-phthalaldehyde (Cidex® OPA). Intest Res. 2016; 14(2): 178.