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Some biological potential of silver nanoparticles synthesized from *Ocimum basilicum* L.

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Abstract

In our study, the ability to produce silver nanoparticles (AgNP) by green synthesis method using *Ocimum basilicum* L. plant was investigated. The color of the extract changed from yellow to brown, proving the presence of AgNPs. Scanning electron microscopy (SEM) and energy dispersed spectra (EDS) were used to characterized AgNPs. AgNPs ranged from 15 nm to 22 nm in size and were mostly spherical. The antimicrobial potential of AgNPs was tested against four bacterial and one yeast cultures by disc diffusion and minimum inhibitory concentration (MIC) methods. AgNPs significantly inhibited all test cultures. The highest antimicrobial and antibiofilm activities of plant extract and AgNP were found against *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231, respectively. In free radical scavenging activity (DPPH), the higher antioxidant activity were found 56.31±0.73% (plant extract) and 30.71±1.35% (AgNP). The higher 2,2'- Azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) ($ABTS^{\bullet+}$) cation radical scavenging acitivty were also determined 55.87±1.38% and 39.56±1%, respectively.

Keywords: *Ocimum basilicum* L; Silver nanoparticle; Antibiofilm; DPPH

1. Introduction

Nanotechnology is a dynamic and multidisciplinary science that basically aims to synthesize nanoparticles with antimicrobial and antioxidant properties [1]. The nanoparticles that can be synthesized by plants, there are various metals such as silver (Ag), gold (Au), magnesium (Mg), titanium (Ti), zinc (Zn) and copper (Cu). Silver nanoparticles (AgNPs), in particular, provide the advantage of being used in most industries [2]. It was found that AgNPs inhibited the growth of gram-negative bacteria as well as gram-positive bacteria, in studies [3]. Synthesis of nanoparticles using chemical compounds that cannot be broken down in the biological cycle in nature creates negative effects on the ecosystem and biota [4]. In addition the green synthesis method used in the synthesis of metal nanoparticles is environmentally friendly; it is an advantage that no toxic chemicals, high temperature or pressure are used [5]. Synthesis of metal nanoparticles is possible using *Ricinus communis*, *Caesalpinia pulcherrima*, *Azadirachta indica*, olive leaf and herbal teas [3,6,7]. Biosynthesis using plant extracts helps the formation of stable nanoparticles, while at the same time providing faster reduction of metal ions [8].

Biofilm is a complex matrix containing extracellular polymeric substance, proteins, extracellular DNA, various enzymes and the microorganism. Biofilm has emerged as an industrial and medical problem for years. Eradication of the biofilm layer is one of the most important problems of the modern age [9]. Microorganisms associated with biofilms, which adapt and develop rapidly in harsh environmental conditions, cause material and moral damages in many areas. Bacterial strains, material surface properties and various environmental factors are effective in the formation and development of biofilm [9,10]. Biofilms are highly resistant to chemical and physical processes, and disinfectant residues used in removal are harmful to both the environment and living things. Therefore, there is a need for safe

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biofilm inhibitors for use in various industries. Unlike traditional methods, natural resources such as plants and products produced from plants are used in the development of biological antibiofilm agents that do not have negative effects on health and the environment [10,11].

Ocimum basilicum L. is a valuable essential oil and spice plant from the Lamiaceae family. There are about 35 species that spread mostly in tropical and warm climatic regions of the old world [12]. These plants are called 'basilic' in French, 'basilicum' in German, and 'basil' in English [13]. The economically used part of *O. basilicum* L. is its leaves, and 0.05-1% essential oil is obtained from this region by steam distillation. The most important main components of *O. basilicum* L. essential oil are linalool, methylcavicol, eugenol and 1,8-cineol. However, a wide variety of chemotypes with main components such as camphor, citral, osimene, methylcinnamate, methyleugenol, trans-β-osimene, β-caryophyllene and β-bisabolene have been detected in germplasms of the world. *O. basilicum* L. species is methylcavicol, linalool, eugenol and richer in methylcinnamate. *O. basilicum* L. essential oil is widely used as perfume, cosmetics, aromatherapy, traditional medicine and food flavoring. It has sedative, stomachic, diuretic, expectorant and gas-digesting antimicrobial, insecticidal, nematicidal, fungistatic, herbicidal and antioxidant effects [12].

The aim of this study is to determine the potential of *O. basilicum* L. leaves to synthesis of AgNPs. The characterization, antimicrobial, antibiofilm and antioxidant activities of AgNP were also determined.

2. Material and methods

2.1. Experiment Materials

The plant *O. basilicum* L. was obtained from transfusion and stored at +4 °C until further analysis. Silver nitrate salt (Merck) was purchased commercially with a purity of 99.98%. All solutions used in the study were freshly prepared using ddH_2O and kept in the dark.

2.2. Green Synthesis of AgNP

Dried leaves of *O. basilicum* L. were ground and extracted with 100 mL of sterile distilled water at 70 °C for 1 hour. After cooling at room temperature, filtration was carried out with filter paper. For AgNP synthesis, 1mM 500 mL silver nitrate ($AgNO_3$) aqueous solution prepared in advance and 125 mL ground *O. basilicum* L. leaves were added and allowed to react in a flask at room temperature under constant conditions. The dark solution formed by the reduction of silver ions was centrifuged at 10.000 rpm for 5 minutes and the upper liquid phase was removed and the remaining solid part was washed several times with distilled water. The obtained solid part (AgNP) was left to dry in an oven at 65 °C for 48 hours [14].

2.3. Characterization of AgNPS

Scanning electron microscopy (SEM) is a surface imaging method that can clearly determine the dimensions of nanoparticles and the surface morphology of the synthesized nanoparticles at the micro and nano scale [15]. Energy-dispersive spectra (EDS) analysis is used to determine the surface element composition of nanoparticles. The combination of SEM with EDS can be used to study the morphology of AgNPs and also to perform chemical composition analysis [16]. EDS and SEM analysis was carried out as service procurement at Kastamonu University Central Laboratory (MERLAB).

2.4. Antimicrobial Activity Assays

Disk diffusion and microdilution (MIC) methods were used to determine the antimicrobial effect of AgNPs on bacteria and yeast cultures [17]. In antimicrobial studies, a total of 5 microorganisms, *Staphylococcus haemolyticus* ATCC 43252, *Staphylococcus aureus* ATCC 6538P, *Acinetobacter baumanii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 were used. Test cultures were suspended on Mueller Hinton Agar (MHA), sterile discs were placed on the medium, and 20 µL of AgNP was added to each disc. After 24 hours of incubation, zone formation around the discs was examined. The MIC testing was performed in a 96-well round-bottom microtiter plate using the standard broth microdilution method. Test cultures and AgNPs prepared with McFarland 0.5 turbidity standard were then added for each culture and incubated overnight at 37°C. The lowest concentration value without microbial growth was accepted as the MIC value [18]. Penicillin, streptomycin and nystatin were used as positive controls.

2.5. Antibiofilm Activity Assay

The microplate biofilm method [19] was used to evaluate the inhibition of biofilm formation of *O. basilicum* L extract (OBE) and AgNP against the tested microorganisms. Cultures were incubated in 5 mL of Tryptic Soy Broth (TSB)

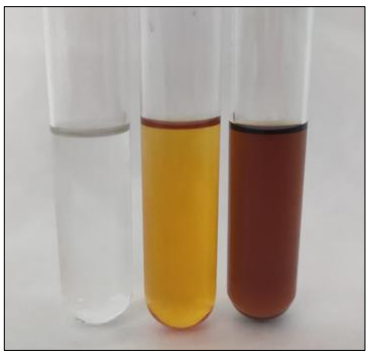
medium containing 5% glucose. Cultures were diluted 1:100 in TSB and loaded into each well in 4 sterile microplates. Yeast extracts were prepared at 50%, 25, 12.5 concentrations and transferred to each microplate well. After 48 hours of incubation at 37 ± 0.1 °C, bacteria in the wells were removed and the wells were washed twice with distilled water. 200 µl of a 0.1% crystal violet solution was added to each well and incubated for 20 minutes. The crystal violet-bound extracts were poured off and washed until the crystal violet finally came out. The microtiter plates were inverted and the remaining liquid was filtered and dried at room temperature. Finally, the adherent biofilm bound crystal violet was separated in ethanol (95%) and absorbance was measured at 550 nm using an automated Elisa reader (BioTek, UK). All experiments were repeated in duplicate. Calculation of the antibiofilm effect of the extract and AgNP was done by reducing formulation percentage.

2.6. Antioxidant Activity Assay

Free radical scavenging activity (DPPH) method and 2,2'-Azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) $ABTS^{\bullet+}$ methods were used to determine the antioxidant activities of OBE and AgNPs [20, 21]. The % inhibition of the samples was calculated and synthetic antioxidant butylated hydroxytoluene (BHT) was used as a standard.

3. Results and discussion

In the interaction of OBE with silver nitrate, a color change is observed with the reduction of Ag^{+} ions to AgNPs. The color of the fresh OBE was yellow. However, after treatment with silver nitrate and incubation at 70 °C for 2 hours, the color of the mixture turned dark brown (Figure 1). The result obtained is proof that *O. basilicum* L. is a good reducing agent for AgNPs.



(a) (b) (c)

Figure 1 Color changes of synthesized AgNP. (a) Extract of $AgNO_3$ (b) Aqueous extract of *O. basilicum* L. (c) Synthesized AgNP

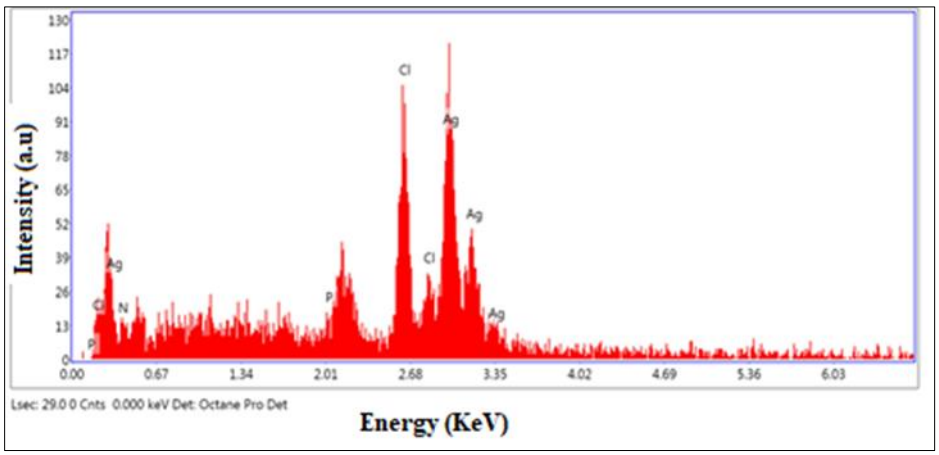


Figure 2 EDS analysis of AgNPs

The presence of Ag elements in the nanoparticle was confirmed by EDS analysis. It was determined that there was a silver ion at the peak at 3 keV in the EDS spectrum. Other peaks in the EDS spectrum were observed to belong to chlorine (Cl) (Figure 2). It is reported that these peaks result from phenolic and flavonoid components in the plant [22].

SEM analysis proved the existence of AgNPs of the same shape and size. The results of the SEM analysis revealed that the AgNPs produced by the green synthesis method were spherical with sizes ranging from 15 nm to 22 nm (Figure 3).

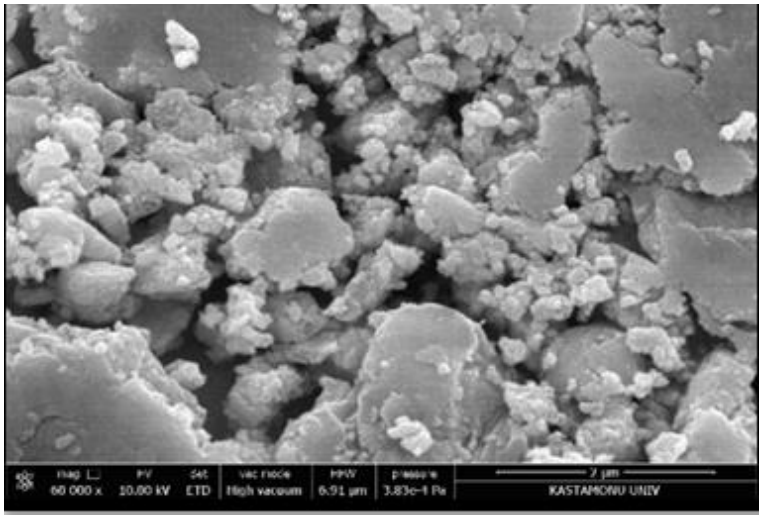


Figure 3 The SEM images of synthesized AgNP

Malapermal et al., (2017) [23], More et al. (2022) [24] were reported that synthesized AgNPs by *Ocimum* species were found to be crystalline and relatively spherical. Melkamu et al. (2022) [25] reported that as a result of SEM analysis of *Ocimum sanctum*-AgNP had a various sizes including spherical, cubic and flake-shaped (20 nm). According to the SEM and EDS analysis results, it was determined that AgNP production could be made from *O. basilicum* L. plant extracts.

The antimicrobial activity findings of OBE and AgNP were given in Table 1.

Table 1 Antibacterial activity result of OBE and AgNPs

Test cultures	**Methods**							
	Disc Diffusion (mm)				**MIC (µg mL^{-1})**			
	Extracts		**Control antibiotics**		**Extracts**		**Control antibiotics**	
	OBE	**AgNP**	**P10**	**NY100**	**OBE**	**AgNP**	**ST**	**NY100**
S. haemolyticus ATCC 43252	8.00	10.00	16.00	NT	500	500	5.0	NT
S. aureus ATCC 6538P	9.00	9.00	13.00	NT	500	500	4.0	NT
A. baumanii ATCC 19606	10.00	9.00	8.00	NT	500	500	2.0	NT
P. aeruginosa ATCC 27853	11.00	10.00	10.00	NT	500	500	1.0	NT
C. albicans ATCC 10231	10.00	12.00	NT	9.00	500	250	NT	5.0

OBE: *O.basilicum* L. extract; P10: Penicillin (10 ug/disc); ST: Streptomycin (10 ug/disc); NY100: Nystatin (100 ug/disc), Nt: not tested

The highest inhibition zone diameter values obtained from OBE and AgNP were found to be 11.00 mm and 12.00 mm, respectively. OBE and AgNP extracts showed higher antagonistic effects against *A. baumanii* ATCC 19606, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231 than comparison antibiotics. The MIC values were also determined as 500 µg mL^{-1} and 250 µg mL^{-1}, respectively, for OBE and AgNP.

The antimicrobial and antidiabetic activities of AgNPs of *O. basilicum* and *O. sanctum* were investigated. The highest inhibition values obtained for *O. sanctum* L. and *O. basilicum* L. were determined as 6.33 mm for *S. aureus* and 4.16 mm for *Escherichia coli*, respectively [23].

Silver and platinum nanoparticles were synthesized from *O. basilicum* L. leaf extracts by green synthesis method and their antibacterial activity was investigated by using them separately and in combination. The MIC value for the AgNPs was determined as 3.12 µg mL^{-1} and 6.25 µg mL^{-1} for gram positive and negative bacteria, respectively [24]. The antibacterial effect of AgNPs which is formed by combining *O. basilicum* and *Hibiscus sabdariffa* L. plant extracts were investigated, and the MIC value was determined as 6.25 µg mL^{-1} [26].

The results indicated that OBE and AgNPs extracts reduced metabolic activity of cells in biofilm all test cultures, showing an inhibition percentage range of 20.01- 55.01% and 25.21- 65.07%, respectively (Table 2). By comparison, OBE and AgNPs extracts were the most effective in inhibiting formation and growth of *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231 biofilm by 55.01% and 65.07%, respectively.

Table 2 Antibiofilm activities (% inhibition rates) of OBE and AgNPs

Test cultures	OBE		AgNP	
	MIC	MIC/2	MIC	MIC/2
S. haemolyticus ATCC 43252	35.21±0.01	20.01±0.11	48.01±0.12	25.21±0.02
S. aureus ATCC 6538P	40.01±1.22	35.32±0.31	42.58±4.01	30.05±1.11
A. baumanii ATCC 19606	50.21±2.01	30.01±1.11	48.11±0.51	26.02±0.1
P. aeruginosa ATCC 27853	55.01±1.11	30.08±4.02	52.01±1.11	29.12±0.21
C. albicans ATCC 10231	53.01±1.11	26.01±2.14	65.07±0.22	60.01±1.33

In DPPH, the maximum inhibition values at 200 µg mL^{-1} were 56.31±0.73% and 30.71±1.35%, respectively (Figure 4).

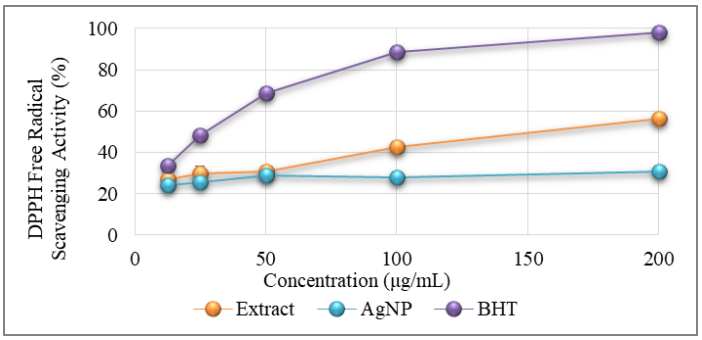


Figure 4 DPPH Activity (%) of OBE and AgNPs

For $ABTS^{\bullet+}$ activity at 200 µg mL^{-1}, which is the highest concentration of the extract and nanoparticle, were determined to be 55.87±1.38% and 39.56±1.47%, respectively (Figure 5).

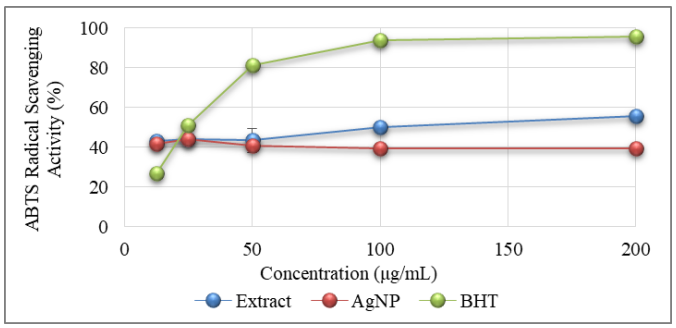


Figure 5 $ABTS^{\bullet+}$ Activity (%) of OBE and AgNPs

Antioxidant potential of leaves of the *O. sanctum* and OS-AgNP were found 38.22% and 63.20%, respectively [25]. Malik et al. (2022) [27], were also investigated the antioxidant activity of zinc nanoparticles produced from the leaf extract of

O. basilicum. DPPH activities of nanoparticles treated with grapen oxide and pure zinc nanoparticles were also determined as 69% and 64.56%.

As a result of the metabolic activity of the cell, free radicals, which are harmful to body, are produced. By giving free radicals to biomolecules, they cause cancer, mutagenesis and dementia-like damage [28]. It has been suggested that compounds with antioxidant properties of natural origin will contribute to the treatment of diseases and degenerative processes caused by free radicals [29]. Therefore, in this study, the antioxidant activity of both the OBE and the AgNPs were evaluated. It was determined that AgNPs produced using the leaf extract of *O. basilicum* L. had more antioxidant potential than the OBE. these results showed that AgNPs coulbu used for antioxidants obtained from plants in the treatment of various oxidative stresses.

4. Conclusion

In this study, AgNP synthesis was performed using *O. basilicum* L. plant leaves with an eco-friendly method. These AgNPs were characterized by SEM-EDS techniques and their antimicrobial, antibiofilm and antioxidant activities were investigated. Biosynthesized AgNPs showed high antimicrobial, antibiofilm and antioxidant activities compared to plant extracts and standard controls. Therefore, AgNPs synthesized from *O. basilicum* L. and similar plants can be combined with medicinal phytochemicals, leading to the discovery of industrial raw materials with higher activity. Our results reveal that biologically synthesized AgNPs exhibited multifunctional properties and could be used as antimicrobial and antioxidant agents.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

[1] Nazem A., Mansoori G. A. Nanotechnology solutions for Alzheimer's disease: advances in research tools, diagnostic methods and therapeutic agents. Journal of Alzheimer's disease. 2008; 13(2): 199-223.

[2] Wijnhoven S. W., Peijnenburg W. J., Herberts C. A., Hagens W. I., Oomen A. G., Heugens E. H., Geertsma R. E. Nano-silver–a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology. 2009; 3(2): 109-138.

[3] Mintiwab A., Jeyaramraja P. R. Evaluation of phytochemical components, antioxidant and antibacterial activities of silver nanoparticles synthesized using Ricinus communis leaf extracts. Vegetos. 2021; 34(3): 606-618.

[4] Phull A. R., Abbas Q., Ali A., Raza H., Zia M., Haq I. U. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of Bergenia ciliata. Future Journal of Pharmaceutical Sciences. 2016; 2(1): 31-36.

[5] Willner I., Baron R., Willner B. Growing metal nanoparticles by enzymes. Advanced Materials. 2006; 18(9): 1109-1120.

[6] Ahmed S., Ahmad M., Swami B. L., Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences. 2016; 9(1): 1-7.

[7] Moteriya P., Chanda S. Biosynthesis of silver nanoparticles formation from Caesalpinia pulcherrima stem metabolites and their broad spectrum biological activities. Journal of Genetic Engineering and Biotechnology. 2018; 16(1): 105-113.

[8] Moteriya P., Padalia H., Chanda S. Characterization, synergistic antibacterial and free radical scavenging efficacy of silver nanoparticles synthesized using Cassia roxburghii leaf extract. Journal of Genetic Engineering and Biotechnology. 2017; 15(2): 505-513.

[9] Akçelik N., Akçelik M. Bacterial biofilms and their interactions with the host defense system. Journal of Erciyes University Graduate School of Natural and Applied Sciences. 2017; 33(1): 15-28.

[10] Devaraj A., Buzzo J.R., Mashburn-Warren L., Gloag E.S., Novotny L.A., Stoodley P., Bakaltz L., Goodman S.D. The extracellular DNA lattice of bacterial biofilms is structurally related to Holliday junction recombination intermediates. Proceedings of the National Academy of Sciences. 2019; 96(6): 50-60.

[11] Gün İ., Ekinci F.Y. Biofilms: Microbial life on surfaces. Food Journal. 2009; 34(3): 165-173.

[12] Baydar H. The science and technology of medicinal and aromatic plants," Süleyman Demirel University, Turkey; Publication number: 51, Isparta, 2013; 206-208.

[13] Ceylan A. Medicinal Plants II (Essential oil plants). Ege University, Turkey; Publication number: 481, İzmir, 1996; 202-206.

[14] Bayğu G. Determination of genotoxic effect of silver nanoparticle obtained by green synthesis method using Cimin grape leaf by wing speckle test. Erzincan Binali Yıldırım University, Turkey, Master's thesis, 2020.

[15] Lin P.C., Lin S., Wang P.C., Sridhar R. Techniques for physicochemical characterization of nanomaterials. Biotechnology Advances. 2014; 32: 711–726.

[16] Zhang X.F., Liu Z.G., Shen W., Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International Journal of Molecular Sciences. 2016; 17(9): 1534.

[17] Bauer A.W., Kirby M.M., Sherris J.C., Turck M.D. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45(4): 493–496.

[18] Elshikh M., Ahmed S., Funston S., Dunlop P., McGaw M., Marchant R., Banat I. M. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnology Letters. 2016; 38(6): 1015-1019.

[19] Zmantar T., Kouidhi B., Miladi H. A microtiter plate assay for Staphylococcus aureus biofilm quantification at various pH levels and hydrogen peroxide supplementation. New Microbiology. 2010; 33: 137-145.

[20] Blois M.S. Antioxidant determinations by the use of a free free radical. Nature. 1958; (181): 1199- 1200.

[21] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999; 26: 1231–1237.

[22] Sukweenadhi J., Setiawan K.I., Avanti C., Kartini K., Rupa E.J., Yang D.C. Scale-Up of green synthesis and characterization of silver nanoparticles using ethanol extract of Plantago major L. leaf and Its antibacterial potential: A Recent study. South African Journal of Chemical Engineering. 2022; 38; 1-8.

[23] Malapermal V., Botha I., Krishna S.B.N., Mbatha J.N. Enhancing antidiabetic and antimicrobial performance of *Ocimum basilicum*, and Ocimum sanctum (L.) using silver nanoparticles. Saudi Journal of Biological Sciences. 2017; 24(6): 1294-1305.

[24] More P.R., Zannella C., Folliero V., Foglia F., Troisi R., Vergara A., Galdiero M. Antimicrobial applications of green synthesized bimetallic nanoparticles from *Ocimum basilicum*. Pharmaceutics. 2022; 14(11): 2457.

[25] Melkamu Z., Jeyaramraja P.R. Optimization of the synthesis of silver nanoparticles using the leaf extract of Ocimum sanctum L. and evaluation of their antioxidant potential. Nano Express. 2022; 3(3): 035006.

[26] Abdelsattar A.S., Hakim T.A., Rezk N., Farouk W.M., Hassan Y.Y., Gouda S.M., El-Shibiny A. Green synthesis of silver nanoparticles using *Ocimum basilicum* L. and Hibiscus sabdariffa L. extracts and their antibacterial activity in combination with phage ZCSE6 and sensing properties. Journal of Inorganic and Organometallic Polymers and Materials. 2022; 32(6): 1951-1965.

[27] Malik A.R., Sharif S., Shaheen F., Khalid M., Iqbal Y., Faisal A., Botmart T. Green synthesis of RGO-ZnO mediated *Ocimum basilicum* leaves extract nanocomposite for antioxidant, antibacterial, antidiabetic and photocatalytic activity. Journal of Saudi Chemical Society. 2022; 26(2): 101438.

[28] Ren X., Yang L., Liu J., Su D., You D., Liu C., Shen J. A novel glutathione peroxidase mimic with antioxidant activity. Archives of Biochemistry and Biophysics. 2001; 387(2): 250-256.

[29] Acker C.I., Brandão R., Rosário A.R., Nogueira C.W. Antioxidant effect of alkynylselenoalcohol compounds on liver and brain of rats in vitro Environ. Toxicology and Applied Pharmacology. 2009; 28(2): 280-287.