GeoPatterns, Vol. VI (2021), pp. 6-10, https://doi.org/10.5719/GeoP.6/1

Green-synthesized Ag-MnO_2 nanoparticles as plausible non-invasive antimicrobial treatment of cultural heritage

ALEXANDRA CIORÎȚĂ[1, 2,]*, ROBERT GUTT[2], ILDIKO LUNG[2], MARIA LOREDANA SORAN[2], MARCEL PÂRVU[1]

[1]"Babeș-Bolyai" University, Faculty of Biology and Geology, Cluj-Napoca, Romania
[2]National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania
alexandra.ciorita@ubbcluj.ro

Abstract. Green-synthesized Ag-MnO_2 nanoparticles were tested for their antimicrobial capacity. Two important plant extracts were used for synthesis: *Vinca minor* and *Chelidonium majus*, with well-known pharmacological activities. After the determination of the minimal inhibitory concentration against two bacterial strains (one Gram-negative and one Gram-positive model), the plant extracts were used to form three types of metal nanoparticles. The antimicrobial effect of the nanoparticles was assessed against *E. coli*, *Staphylococcus aureus*, and the yeast *Candida albicans*. The results indicated that the Ag-MnO_2 nanoparticles synthesized with *V. minor* plant extract were the most efficient against all tested pathogens. As a future perspective, these nanoparticles are suited to design a non-invasive applicator to treat biodeteriorated cultural heritage, such as archives, sculptures, or paintings.

Keywords: *Ag-MnO_2*, green synthesis, antimicrobial effects, image processing, cultural heritage

INTRODUCTION

The rapid ascension of nanomaterial production as a response to the increased demand, attracted numerous funds at industrial levels (Cvjetko et al., 2017). Consequently, the environmental impact was major due to the pollution generated by the industrial processes required for mass production. An alternative solution to this could be 'green'-synthesis of nanoparticles (NPs) with the help of plant extracts (Ahmed et al., 2016; Kamran et al., 2019).

NPs are new generation, technological tools with various applications from biomedical to food industry or cosmetics. Together with the fast development of NP synthesis, the demand for alternative treatment of diseases increased as well (Jyoti et al., 2018).

Microbial diseases are widespread, and they affect not only living organisms, such as plants or mammals, but they deteriorate the cultural heritage as well (Borrego et al., 2018; Doud et al., 2020). It is important mention that art objects, especially old ones, are prone to deteriorate due to several physical, chemical, and biological factors. Apart from poor handling and storage conditions, that lead to a physical degradation of paintings, archives, or sculptures, biodeterioration is a real concern. If not inhibited properly, bacteria or fungi present on the cultural heritage recur and cause irreparable damages (Karbowska-Berent et al., 2011; Kavkler et al., 2015).

Although prevention is the optimal solution, non-deteriorating techniques should be employed for the treatment of existing patrimony. Such an alternative could be provided with the help of antimicrobial NPs that can be applied both prior the use of materials (paper, clay, cloth, etc.) and for the treatment of infested art objects (Gutarowska et al., 2012).

The current work explored the alternative of using previously obtained NPs through green-synthesis as antimicrobial agents. In this preliminary study, the Ag-MnO_2 NPs were tested against *E. coli*, *Staphylococcus aureus*, and *Candida albicans*. The obtained NPs have a great antimicrobial potential and a possible solution to treat the already affected cultural heritage is to develop a non-invasive applicator with targeted treatment of microbial infestations, without affecting the art objects.

MATERIALS AND METHODS

Nanoparticle synthesis

Three types of nanoparticles were obtained as described in a previous study (Ciorîță et al., 2020). Briefly, MnO_2 nanoparticles were synthesized from $KMnO_4$ in presence of *Vinca minor* and/or *Chelidonium majus* plant extracts. The obtained nanoparticles were combined with $AgNO_3$ and the same plant extracts to obtain core-shell Ag-MnO_2 nanoparticles: Ag-MnO_2-Vm (obtained with *V. minor* plant extract), Ag-MnO_2-Cm (obtained with *C. majus* plant extract), and Ag-MnO_2-M (1:1 mix of plant extracts).

Preliminary antibacterial effect of the plant extracts

The effect of the *V. minor* and *C. majus* plant extracts against *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were assessed through scanning electron microscopy (SEM) and through the microdilution method, according to EUCAST protocols and to a previous study (EUCAST, 2020; Ciorîță et al., 2021).

A computational method was employed to determine the effect of the extracts against the length of *E. coli* bacilli using a MATLAB script, according to (Belean et al., 2020). Based on the scale bar, the software estimated the bacterial dimensions (length and/or width), after a rigorous determination of the bacterial contour.

Antimicrobial effects of the Ag-MnO2 nanoparticles

The antimicrobial effect of the nanoparticles was assessed against *E. coli*, *S. aureus*, and *Candida albicans* (ATCC 90028) through the agar diffusion method, according to EUCAST protocols.

Statistical analyses

Each experiment was conducted in triplicate and the mean and standard deviation were calculated. One Way ANOVA, Tukey, and Student's t test were used to determine the level of significance. The difference was considered significant at values of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The chosen plant extracts had a great antibacterial effect, inhibiting both *E. coli* and *S. aureus* (Figure 1 a). The morphology of the bacterial cells was not significantly affected as indicated by the SEM analyses (Figure 1 b). However, the length of the *E. coli* bacterial cells measured with the help of a MATLAB script (Figure 1 c) was significantly smaller compared to untreated control (Figure 1 c).

Once the antibacterial effect was established, the reducing capacity of the plant extracts was assessed. Hence, three types of Ag-MnO_2 nanoparticles were obtained and their polygonal aspect was observed thorough transmission electron microscopy (Figure 2 a-c). The antimicrobial effects of the nanoparticles were assessed through the agar diffusion method (Figure 2 d).

The antimicrobial properties of *Vinca minor* and *Chelidonium majus* had been previously reported, and these results are consistent with our findings (Gilca et al., 2010; Özçelik et al., 2011; Pârvu and Pârvu, 2011; Grujić et al., 2015). Moreover, the inhibitory effect of silver against bacterial or fungal strains are thoroughly documented (Du et al., 2018). The plant extracts present on the surface of the synthesized Ag-MnO_2 NPs lead to a synergistic activity of the nanomaterials against the tested strains (Ciorîță et al., 2020).

Although *C. majus* had a slightly increased inhibitory capacity against *E. coli* (MIC = 5%) and *S. aureus* (MIC = 10%), compared to *V. minor* (MIC = 15% and 10%, respectively), Ag-MnO_2-Vm were significantly more potent against the tested strains.

After this initial assessment of the green synthesized Ag-MnO_2 NPs against microbial strains, the NPs obtained with *V. minor* plant extract are suited for further investigations. Therefore, a non-invasive applicator could be developed where NPs are left to interact with the microbiome present on cultural heritage for 24h and inhibit the development of biodeteriogens, without affecting the integrity of the art objects.

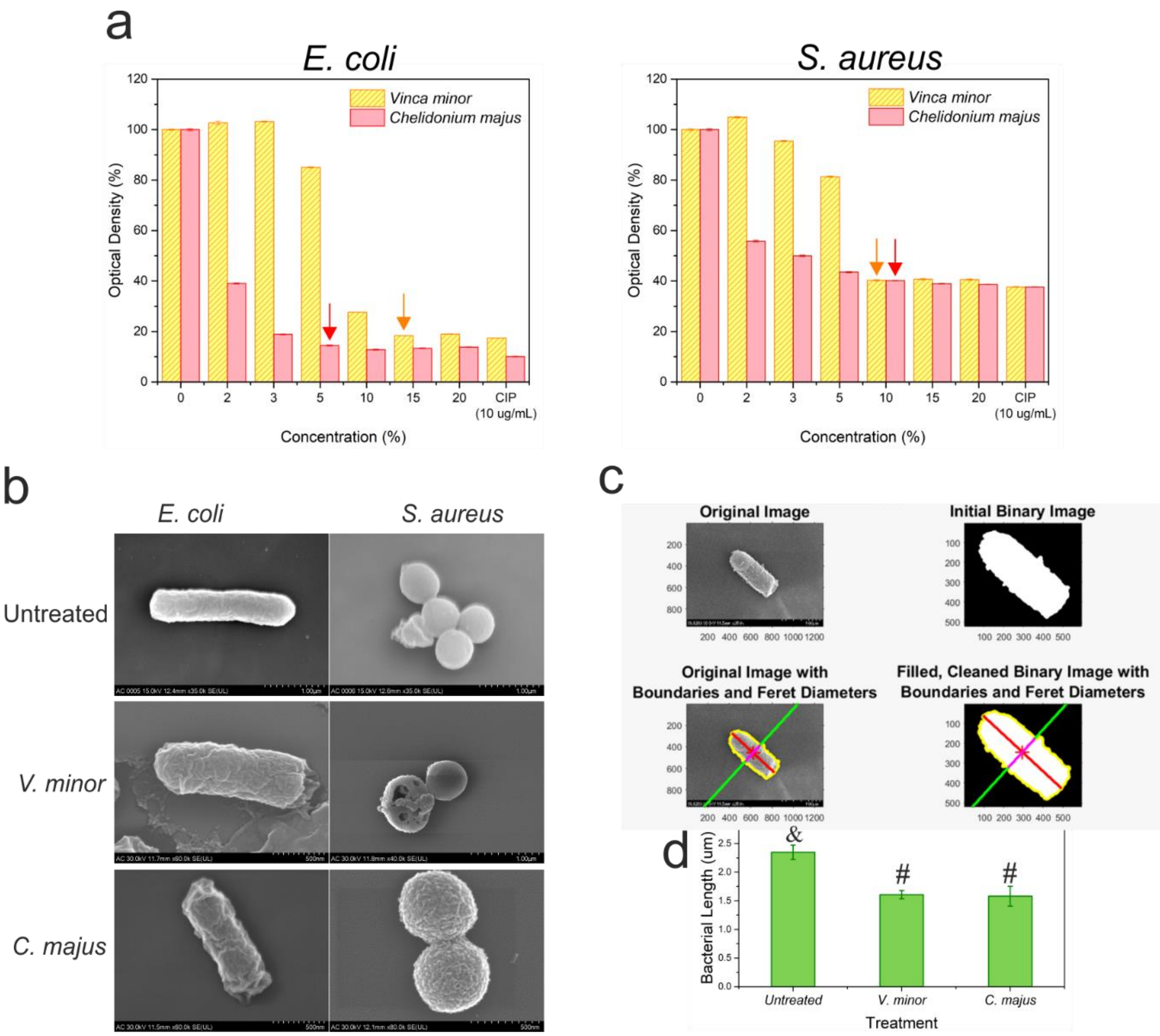

Figure 1. The antibacterial effect of the V. minor and C. majus plant extracts against *E. coli* and *S. aureus*. *The minimal inhibitory concentration (MIC) was determined thorough the microdilution method (a); the arrows indicate the MIC in %. Morphological examination of bacterial cells treated with plant extracts and compared to untreated control (b); no significant alterations were observed. Length measurement of the E. coli cells with the help of a MATLAB script (c) and its graphical representation (d); The columns marked with the same symbol are significantly different than the control at significance level of p <0.05, according to the One Way ANOVA and Tukey's tests*

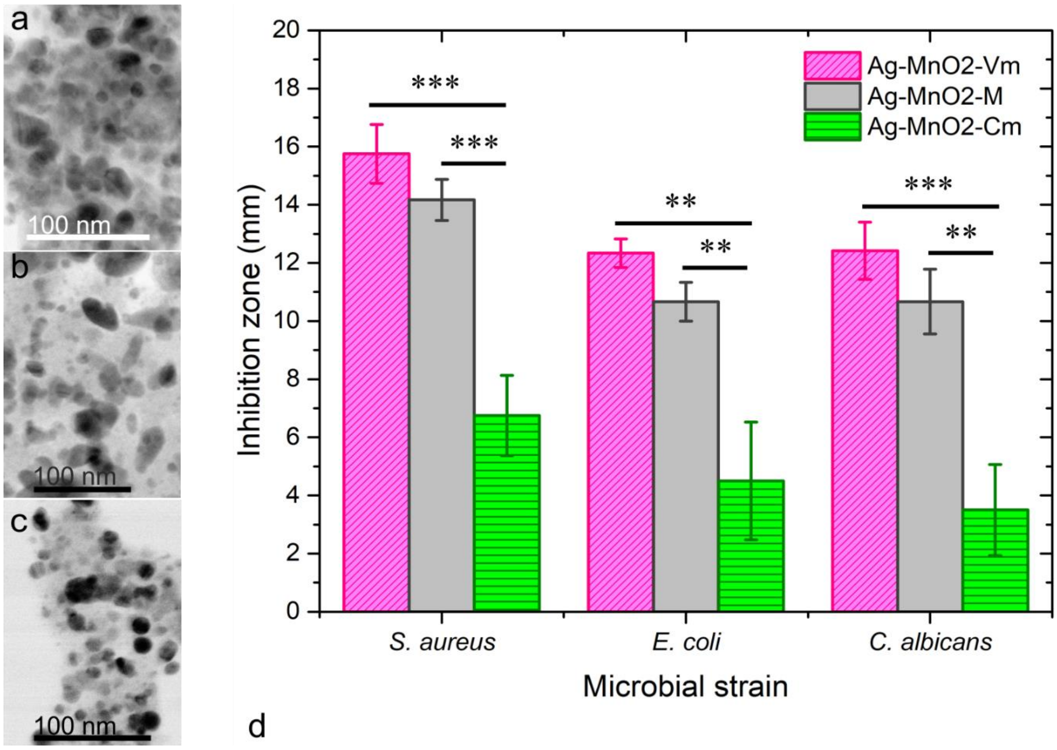

Figure 2. Morphological examination of Ag-MnO$_2$ nanoparticles synthesized with V. minor (a), C. majus (b), and a 1:1 mix (c) plant extracts. The agar diffusion method examination of antimicrobial effects of nanoparticles against E. coli, S. aureus, and the yeast Candida albicans;
Student's t test was performed and the significance levels are scaled as follows:
**** $p<0.001$, ** $p<0.01$, *$p<0.05$*

ACKNOWLEDGMENT

The author Alexandra CIORÎȚĂ would like to acknowledge the project POC A1-A1.2.3-G-2015, UBB-TeMATIC-Art - ID P-40-374 - „Partnership for transfer of innovative technologies and advanced materials in visual arts (production, conservation, restoration)".

REFERENCES

Ahmed, S., Ahmad, M., Swami, B.L., and Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. Journal of Advanced Research 7**,** 17-28. doi: 10.1016/j.jare.2015.02.007.

Belean, B., Gutt, R., Costea, C., and Balacescu, O. (2020). Microarray Image Analysis: From Image Processing Methods to Gene Expression Levels Estimation. IEEE Access 8, 159196-159205. doi: 10.1109/ACCESS.2020.3019844.

Borrego, S., Guiamet, P., Vivar, I., and Battistoni, P. (2018). Fungi involved in biodeterioration of documents in paper and effect on substrate. Acta Microscopica 27(1), 37-44.

Ciorîță, A., Suciu, M., Macavei, S., Kacso, I., Lung, I., Soran, M.-L., et al. (2020). Green synthesis of Ag-MnO$_2$ nanoparticles using *Chelidonium majus* and *Vinca minor* extracts and their *in vitro* cytotoxicity. Molecules 25(4), 819. doi: 10.3390/ molecules 25040819.

Ciorîță, A., Zăgrean-Tuza, C., Moț, A., Carpa, R., and Pârvu, M. (2021). The phytochemical analysis of *Vinca* L. species leaf extracts is correlated with the antioxidant, antibacterial, and antitumor effects Molecules 26(10). doi: https://doi.org/10.3390/ molecules26103040.

Cvjetko, P., Milošić, A., Domijan, A.-M., Vrček, I.V., Tolić, S., Štefanić, P.P., et al. (2017). Toxicity of silver ions and differently coated silver nanoparticles in *Allium cepa* roots. Ecotoxicology and Environmental Safety 137, 18-28. doi: 10.1016/j. ecoenv.2016.11.009.

Doud, D.F.R., Bowers, R.M., Schulz, F., De Raad, M., Deng, K., Tarver, A., et al. (2020). Function-driven single-cell genomics uncovers cellulose-degrading bacteria from the rare biosphere. *Isme j* 14(3), 659-675. doi: 10.1038/s41396-019-0557-y.

Du, J., Tang, J., Xu, S., Ge, J., Dong, Y., Li, H., et al. (2018). A review on silver nanoparticles-induced ecotoxicity and the underlying toxicity mechanisms. Regulatory Toxicology and Pharmacology 98, 231-239. doi: 10.1016/j.yrtph.2018.08.003.

EUCAST (2020). *Antimicrobial susceptibility testing.* European Committee on Antimicrobial Susceptibility Testing.

Gilca, M., Gaman, L., Panait, E., Stoian, I., and Atanasiu, V. (2010). *Chelidonium majus* – an integrative review: Traditional knowledge versus modern findings. Forsch Komplementmed 17**,** 241–248. doi: 10.1159/000321397.

Grujić, S.M., Radojevic, I.D., Vasic, S.M., Comic, L.R., and Topuzovic, M.D. (2015). Antimicrobial and antibiofilm activities of secondary metabolites from *Vinca minor* L. Applied Biochemistry and Microbiology 51(5)**,** 572–578. doi: 10.1134/S0003683815050087.

Gutarowska, B., Skora, J., Zduniak, K., and Rembisz, D. (2012). Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles. International Biodeterioration & Biodegradation 68**,** 7-17. doi: 10.1016/j.ibiod.2011.12.002.

Jyoti, K., Baunthiyal, M., and Singh, A. (2018). Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. Journal of Radiation Research and Applied Sciences 9**,** 217-227. doi: 10.1016/j.jrras.2015.10.002.

Kamran, U., Bhatti, H.N., Iqbal, M., Jamil, S., and Zahid, M. (2019). Biogenic synthesis, characterization and investigation of photocatalytic and antimicrobial activity of manganese nanoparticles synthesized from *Cinnamomum verum* bark extract. Journal of Molecular Structure 1179**,** 532-539. doi: 10.1016/j.molstruc.2018.11.006.

Karbowska-Berent, J., Górny, R.L., Strzelczyk, A.B., and Wlazlo, A. (2011). Airborne and dust borne microorganisms in selected Polish libraries and archives. Building and Environment 46**,** 1872-1879. doi: 10.1016/j.buildenv.2011.03.007.

Kavkler, K., Gunde-Cimerman, N., Zalar, P., and Demsar, A. (2015). Fungal contamination of textile objects preserved in Slovene museums and religious institutions. International Biodeterioration & Biodegradation 97**,** 51-59. doi: 10.1016/j.ibiod.2014.09.020.

Özçelik, B., Kartal, M., and Orhan, I. (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharmaceutical Biology 49(4)**,** 396-402. doi: 10.3109/13880209.2010.519390.

Pârvu, M., and Pârvu, A.E. (2011). Antifungal plant extracts, 1055-1062, In: *Science against microbial pathogens: communicating current research and technological advances*, Antonio Méndez-Vilas (Ed.), Publisher: Formatex Research Center, Badajoz, Spain. http://www.formatex.info/microbiology3/book/1055-1062.pdf).

