SYNTHESIS AND CHARACTERIZATION OF AGAR-AGAR – CHITOSAN COMPOSITE FILMS INCORPORATED WITH GREEN SYNTHESIZED SILVER NANOPARTICLES

Nevena V. Ilić[1*], Andrej M. Kukučka, Marija D. Milić[2], Milica D. Milutinović[2], Miona G. Miljković[2], Slađana Z. Davidović[2]

[1]*University of Belgrade, Innovation Center of Faculty of Technology and Metallurgy, Belgrade, Serbia*
[2]*University of Belgrade, Faculty of Technology and Metallurgy, Belgrade, Serbia*

**Corresponding author: nilic@tmf.bg.ac.rs*

Abstract

Antimicrobial properties of silver nanoparticles (Ag NPs) are well known and widely exploited. Various approaches have been applied for preparation of Ag NPs. Nowadays, „green" methods are attracting more attention since they are eco-friendly and generally cheap. Moreover, incorporation of Ag NPs into various natural polymers enables preparation of biocompatible antimicrobial materials with controlled releasing of Ag NPs. Polymeric material also provides long term stability of Ag NPs. The aim of this study was to develop antimicrobial materials based on silver nanoparticles using green approach. Reduction of silver was performed by plant extracts of horsetail (Equisetum arvense L.) and cocoa (Theobroma cacao); the obtained nanoparticles were designated as EA Ag NPs and TC Ag NPs, respectively. The effect of extract concentration on particle size distribution and antimicrobial activity against Gram-negative bacteria Escherichia coli and Gram-positive bacteria Staphylococcus aureus were investigated. The smallest particles with the narrowest size distribution (between 46 nm and 91 nm) were obtained with the lowest tested concentration (10% v/v) of plant extract for both horsetail and cacao. These samples also showed the highest antibacterial activities, so they were selected for preparation of nanocomposite films based on agar-agar and chitosan. It has been shown that incorporation of both EA and TC Ag NPs significantly improved antibacterial properties of the films against E. coli (≈80% higher inhibition in comparison to the pristine agar-agar/chitosan films). In the case of S. aures, TC Ag NPs incorporated in agar-agar/chitosan films enhanced inhibition of the film for 60%. On the other side, incorporation of EA Ag NPs into agar-agar/chitosan film increased the growth inhibition for only 20%. This might be due to the contribution of cocoa extract itself, i.e. some active components that inhibit the growth of S. aureus. Our results show that agar-agar/chitosan films with incorporated green synthesized Ag NPs have potential application in the areas where usage of biodegradable and biocompatible materials with high antibacterial activity is desired, such as food package, wound healing, coatings for medical devices etc.

Key words: *Silver nanoparticles, antibacterial activity, nanocomposite films*

Introduction

Nanotechnology involves fabrication, production, manipulation, characterization, and application of structures, devices and systems by controlling shape and size at nano-scale (1–100 nm). Due to the high surface to volume ratio nanoparticles possess unique physical, chemical and mechanical characteristics that are exploited in all branches of industry (Hassanien and Khatoon, 2019). Among them, particularly attractive are silver nanoparticles (Ag NPs) because of their antimicrobial properties against wide range of microorganisms including viruses, Gram-negative and Gram-positive bacteria, and fungi. Ag NPs are used in plastics, food packaging, textiles, cosmetics, healthcare products, building facades, electronic devices, and wastewater treatment (Abd-Alla et al., 2016; Schlich et al., 2013; Yan and Chen, 2019).

For preparation of Ag NPs with desired properties various physical and chemical methods are commonly employed (Jorge de Souza et al., 2019). However, these production methods are usually expensive, require high energy consumption and are potentially dangerous to the environment and living organisms due to the use of toxic chemicals and the creation of hazardous by-products. Therefore, nowadays the research focus is shifting toward "green synthesis" of NPs as an environmentally benign approach. Numerous methods of green synthesis of metal nanoparticles have been described in the literature, which include the use of microorganisms, plants or plant extracts, as well as natural polymers (Shukla and Iravani, 2017). The main advantage of using plant extracts lies in the fact that they contain medically valuable phenolic compounds (polyphenols, tannic acid, flavonoids, terpenoids, etc.). The use of plants and their different parts as reducing as well as stabilizing agents in synthesis of nanomaterials is especially encouraged for biomedical, pharmaceutical and food-packaging applications (Alomar et al., 2020). Moreover, Ag NPs can be incorporated into various polymeric materials to obtain coatings with better biocompatibility and greater antimicrobial activity as well as controlled releasing of Ag NPs (Eby et al., 2009; Sur et al., 2010).

Horsetail (*Equisetum arvense* L.) is a perennial fern from the *Equisetaceae* family, well known as culinary and medicinal herb. It has been shown that horsetail extract exhibits many biological effects, such as antioxidant, anti-inflammatory, antibacterial, antifungal, vasorelaxant, neuro and cardio protectors, and antiproliferative properties (Pallag et al., 2018). These activities of horsetail are related to the content of several classes of phytochemical compounds including flavonoids, phenolic acids, alkaloids, phytosterols, tannins, and triterpenoids (Cetojević-Simin et al., 2010).

Cacao tree (*Theoboma cacao*) is a tropical plant indigenous to the equatorial regions of America. This plant contains elevated amounts of various flavonoids (catechin, epicatechin…), tannins, saponin, cardiac glycosides, terpenoids and alkaloids. The anti-oxidative properties of cacao allow its application in medicine treatment for various diseases such as cancer, malaria, diabetes, cough etc. In addition, cacao has significant influence on the cardiovascular system, blood pressure and hummoral immunity (Baharum et al., 2016; Ishaq and Jafri, 2017).

Chitosan is a modified carbohydrate polymer derived from chitin. It is biocompatible, biodegradable, antibacterial and inexpensive natural polymer with good film forming ability (El-Hefian et al., 2012; Hu et al., 2016). The composite chitosan films with other film forming substance show better properties compared to the single component films (Glucomannan and Starch, 2021). They have been extensively applied in agriculture, food industry, biotechnology and biomedicine (Hu et al., 2016). Agar-agar is linear hydrophilic polysaccharide derived from red sea weed (El-Hefian et al., 2012; Roy and Rhim, 2019). It is thermally reversible and stable at low pH, so it can be used to make clear, elastic and flexible films. The application of agar-agar films is limited due to

its relatively low mechanical properties and low water vapor barrier properties. Thus, agar is combined with different organic and inorganic substances to obtain films with advanced properties (Roy and Rhim, 2019). In the combination with chitosan, agar contributes by improving gelation and swelling properties of the films. The agar-agar – chitosan films are characterized by hydrogel nature, higher thermal stability due to strong intermolecular hydrogen bonding between amino groups of chitosan and hydroxyl groups of agar. However, reduced tensile strength and elongation are consequence of high agar content in the agar chitosan films (El-Hefian et al., 2012).

The aim of this study was to develop biomaterials with high antimicrobial properties. First, the reduction of Ag NO_3 was performed by green method using the aqueous extracts of horsetail (*Equisetum arvense* L.) and cocoa (*Theobroma cacao*). The obtained colloids were then mixed with chitosan and agar-agar. Finally, the antimicrobial potential of nanocomposite films was examined.

Materials and methods

Plant material

In this study two different plant materials were used. The horsetail was obtained from the Institute of Medicinal Plant Research "Dr Josif Pancic", Belgrade, Serbia. The obtained horsetail was a dust waste (approximately size 0.5-1.2 mm) produced during industrial processing of the herb. The other plant material was organic cocoa beans bought in local market. The cocoa beans were ground and sieved. Fraction of size 0.5-1.2 mm was used for extraction.

Extract preparation

The extraction was performed as follows: 5 g of plant material (horsetail or cocoa) was placed into a 250 ml Erlenmeyer flask, and 95 ml of distilled water was added. Extraction was performed using a household microwave oven (Samsung MS23F301TAK) at a microwave power of 100 W for 1 min, followed by 2 min at a power of 180 W. After extraction the liquid phase was separated from the solid phase by filtration using a vacuum pump (V-700, Büchi labortechnik AG, Fanil, Switzerland). The freshly prepared extract was used in all experiments.

Synthesis and characterization of silver nanoparticles

First, the pH of the extracts was adjusted by the addition of 1M NaOH, until an alkaline medium is reached (pH value ~ 10). Then, the obtained extracts were diluted with distilled water to obtain three different concentrations: 10% 20% and 50% v/v. Thus prepared extracts were used for reduction of $AgNO_3$. For that purpose, 10 ml of 2 mM $AgNO_3$ was added to 90 ml of each extract. The samples were vigorously mixed for 2 hours using magnetic stirrer and left in the dark for the next 24 h. The formation of colloidal silver was indicated by a color change from very light yellow to yellow–brown. The obtained colloids were designated as EA Ag NPs and TC Ag NPs based on plant extract used for reduction (horsetail and cocoa, respectively).

For monitoring of nanoparticles formation and obtaining preliminary information on their size and shape, the UV-Vis spectroscopy (Ultrospec 3300 pro „Amersham Biosciense") was used. Antimicrobial activity of nanoparticles was determined by agar plate method. The optimal concentration of plant extract of 10% v/v (for both horsetail and cocoa extracts) was selected based on the UV-Vis absorption spectra and antimicrobial activity. Ag NPs synthesized using this concentration of extracts, were used in further experiments. The nanoparticles size distribution was determined by laser diffraction (Mastersizer 2000, Malvern Instruments Ltd.).

Preparation of agar-agar chitosan nanocomposite films

For preparation of composite films chitosan and agar-agar were used. First 1 % solutions of polysaccharides were made. Agar-agar was suspended in distilled water and autoclaved for 15 min at 120 °C to achieve its complete dissolution. Chitosan was dissolved in 20 % citric acid for 24 h, at 60 ºC on the magnetic stirrer. After cooling to 40 ºC, agar-agar and chitosan were mixed (9 mL of each) and then 2 mL of Ag colloid was added under constant stirring. Three different samples were made, two of which contained EA and TC Ag NPs, respectively, while the third one served as control, thus contained 2 mL of distilled water instead Ag NPs. Glycerol was added to all three samples and stirring was continued for 30 min. Afterwards the mixtures were poured into molds and let at dark to dry at room temperature during 48 h.

Antimicrobial activity

Antimicrobial activity of Ag NPs and nanocomposite films was examined against Gram negative bacterium *Escherichia coli* ATCC 25922 and Gram positive bacterum *Staphylococcus aureus* ATCC 25923. The inocula were prepared by growing the bacteria in the triptic soy broth supplemented with yeast extract during 18 h at 37 °C. Overnight cultures were diluted in saline to obtain number of viable cells of the order of magnitude 10^6 CFU/ml (adjusted using McFarland turbidity standard). To assess antimicrobial activity of green synthesized Ag NPs, 1 mL of colloid solution was added to 9 mL bacterial suspension. After 4 h of incubation at 37 °C viable cell numbers were determined in the samples and in the control (bacterial suspension without Ag NPs) using agar plate method. Antimicrobial activity of nanocomposite films was determined in the same manner, using films' pieces of size 1x1 cm. Viable cell numbers were determined after 24 h of incubation at 37 °C. The percentage of viable cell reduction (R %) was calculated according to equation:

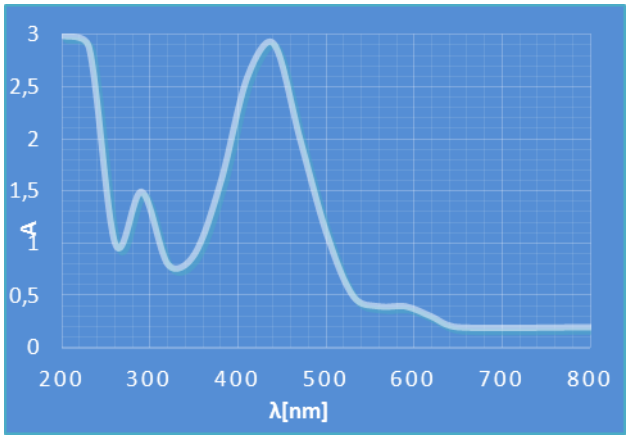
$$R(\%) = \left[\frac{C_0 - C}{C_0} \right] \times 100$$

where C_0, is the number of microorganisms in controls and C is the number of microorganism colonies of the samples.

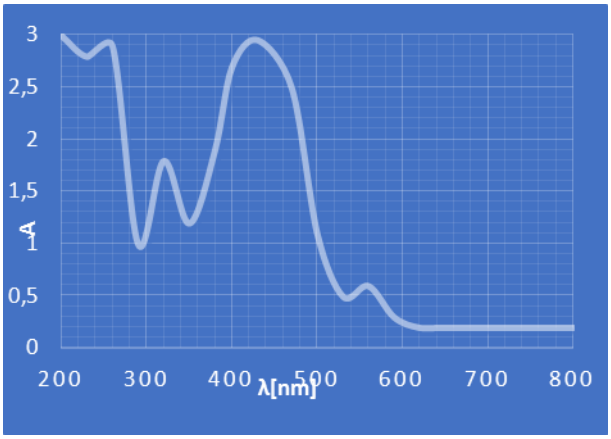
Results and discussion

Characterization of silver nanoparticles

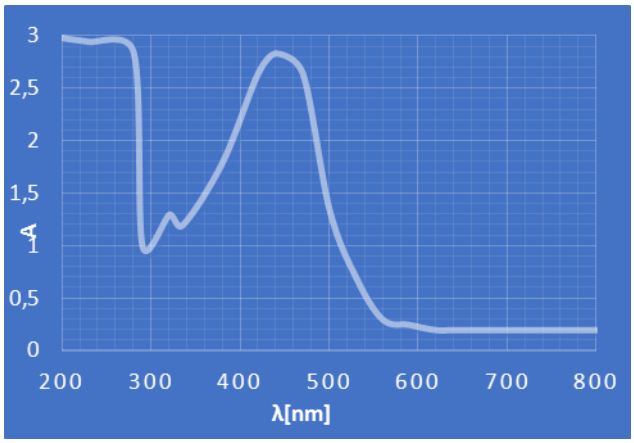
The green method using two different plant extracts (horsetail and cocoa) has been used for synthesis of silver nanoparticles. The effect of extract concentration was examined, while other synthetic parameters were kept constant (pH 10 and temperature 25 °C). The absorption spectra were measured 24 h after initiation of the reaction. The influence of cocoa extract concentration on the formation of colloidal silver is presented in Fig. 1. The absorption maxima around 420-435 nm can be observed in all three spectra. They are attributed to the plasmon resonance effect of the AgNPs formed by reduction of $AgNO_3$. Generally, a typical plasmon resonance band of AgNPs has been observed in the range of 400 – 450 nm (Kelly et al., 2003). The presence of plasmon resonance band in all three spectra confirmed that tested concentration of cocoa extract led to the formation of Ag NPs. However, based on the shape of the surface plasmon resonance band (Figure 1a), it was assumed that the smallest particles with the narrowest size distribution were obtained with 10% v/v. The spectra of EA Ag NPs had the same appearance (data not shown).



A



B



C

Figure 1. Absorption spectra of TC Ag NPs obtained by reduction with different concentrations of cocoa extract: 10% (A), 20% (B) and 50% (C)

The Ag NPs obtained with 10% v/v of EA and TC extracts were analyzed by laser diffraction. The results are presented in Figure 2.

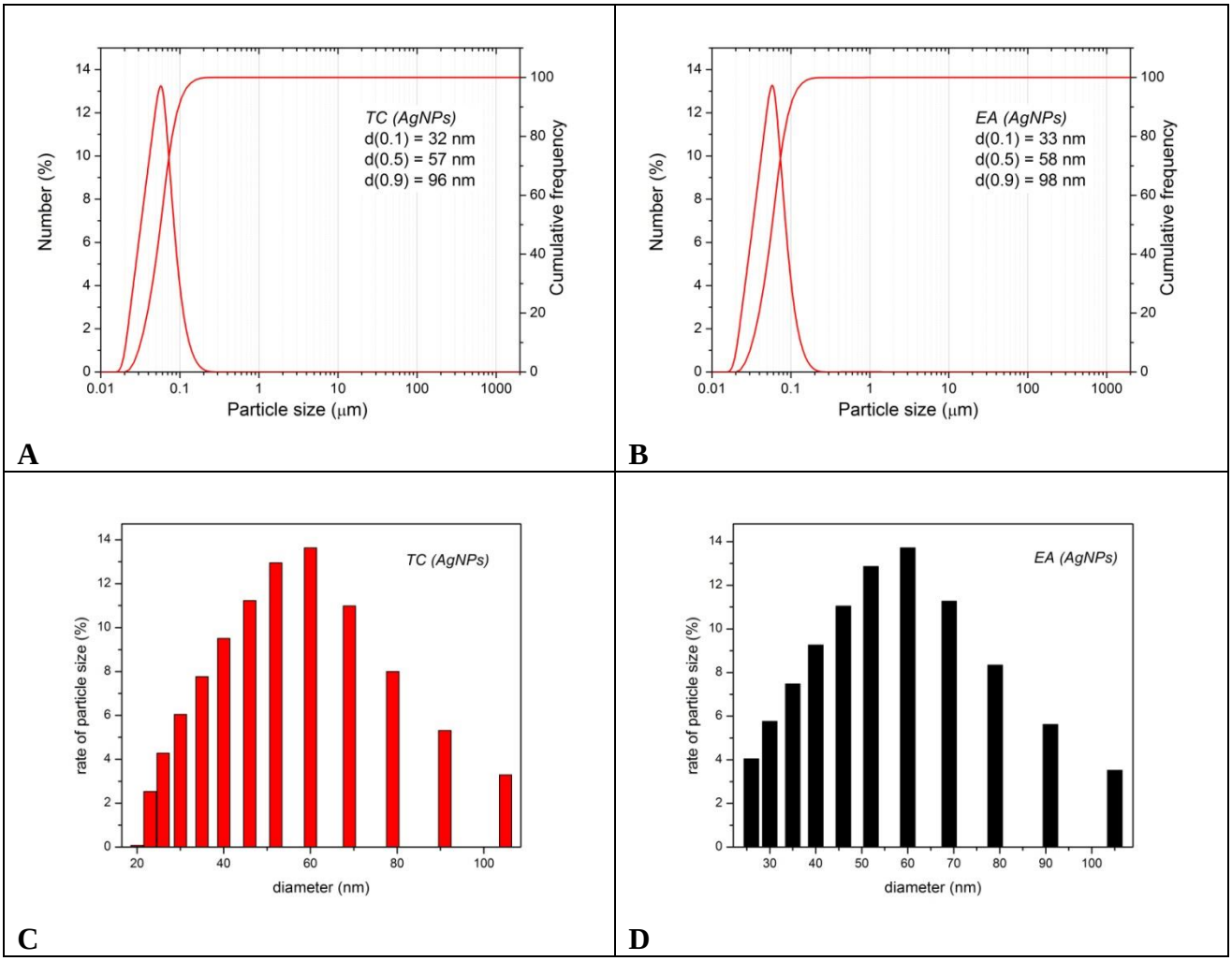


Figure 2. Particle size distribution

The samples were measured on a mastersizer one week after biosynthesis. On the logarithmic scale of the diagram obtained by the analysis of particle sizes for EA Ag NPs, with reference to its distribution, it was observed that the most of the particles (~ 70%) were grouped between the range 0.046 μm and 0.091 μm. The wider distribution (~ 98% of the sample particles) is between 0.026 μm and 0.120 μm. The average specific surface area of the particles is 35m^2/g.

For TC Ag NPs, similar results for distribution of particle size were obtained. More than 50% of the particles (~ 62%) were grouped between the range 0.046 μm and 0.091 μm. The largest number of particles (even ~ 95% of the sample) was within the range between 0.026 μm and 0.120 μm. The average specific surface area of the particles is 67m^2/g.

Antimicrobial activity of EA and TC Ag NPs was examined against *Escherichia coli* and *Staphylococcus aureus*, as representatives of Gram negative and Gram positive bacteria, respectively. Both green synthesized nanoparticles exhibited antimicrobial activity against tested bacteria with some differences in inhibitory effect (Table 1). However, some common features can be observed. First, Ag NPs synthesized by higher concentration of plant extract were less effective in bacterial growth inhibition. This result is in correlation with data obtained by UV-vis spectroscopy. Namely, the antimicrobial activity of Ag NPs is consequence of their small size,

dispersion, greater persistence, less aggregation ability and specific surface area (Thatikayala et al., 2019). The surface area of nanoparticles is crucial element of antimicrobial activity, because it is reservoir for releasing the Ag ions. Generally, the positively charged ions can interact with negatively charged biomacromolecular components of cell structures and nucleic acids, causing structural changes and deformation in the bacterial cell walls and membranes, which can cause bacterial death. In addition, free radicals derived from the nanoparticles surface can damage cells membrane, increase membrane permeability and lead to cell death (Balalakshmi et al., 2020; Rhim et al., 2013).

It is assumed that agglomeration of Ag NPs occurred when 20% and 50% plant extracts were used for Ag NPs synthesis. The larger agglomerate size, comparing to more dispersed NPs, makes it difficult for them to enter the microbial cell and exhibit harmful effect.
The other common characteristic of TC Ag NPs and EA Ag NPs is their higher inhibitory effect on *E. coli* than on *S. aureus*. The similar result has been reported in the literature (Sedaghat and Omidi, 2018) and can be explained by differences in the cell walls between Gram positive and Gram negative bacteria. It is well-known that thinner peptidoglycan layer of Gram negative bacteria allows easier passage in the cytoplasm through cell membrane, leading to protein damage and bacterial killing. Because of that, Gram negative bacteria are more sensitive than Gram positive bacteria (Lateef et al., 2016; Thatikayala et al., 2019).

Table 1. Antimicrobial activity of green synthesized silver nanoparticles

Concentration of extract	Percentage of viable cell reduction (%)			
	E.coli		*S.aureus*	
	TC Ag NPs	EA Ag NPs	TC Ag NPs	EA Ag NPs
10%	51.88	68.7	44.8	19.8
20%	41.33	54.3	31.2	11.6
50%	12.2	21.1	8.8	4.1

Antimicrobial activity of agar-agar – chitosan nanocomposite films

The nanoparticles synthesized by 10 % concentration plants' extracts showed the highest inhibitory effect on bacterial growth and because of that, those Ag NPs were used for preparing nanocomposite films. The results of antimicrobial potential of the films are presented in Table 2. The composite films without Ag NPs showed growth inhibition against both *E. coli* and *S. aureus*. This antibacterial activity is ascribed to chitosan. There are several proposed mechanisms of chitosan antimicrobial activity, yet the certain mechanism of action is still unclear. Positively charged amino groups in chitosan react with negative charged microbial cells membranes altering the membrane properties that can cause cell death. According to other assumption the low molecular –weight chitosan can penetrate into cells and bind with DNA, resulting in modified synthesis of proteins, while the high molecular-weight chitosan can make layer on the cell surface and block the entry of nutrients which cause potential bacteria death. Furthermore, in the acidic conditions, interaction between positively charged amino groups in chitosan and negatively charged microbial cell membrane are stronger (Kumar et al., 2020).

Incorporation of Ag NPs significantly improved antibacterial properties of the films. In addition, nanocomposite films expressed higher antimicrobial activity than nanoparticles. Incorporation of Ag NPs into polymer matrix enables their good dispersion without agglomeration (Kraśniewska et al., 2020). Also, controllable release of silver ions is probably achieved. Both these factors contributed

to enhanced antibacterial properties of nanocomposite films.

The high antibacterial activity of ~93% was achieved on *E. coli* with both nanocomposite films. *S. aureus* as Gram-positive bacterium was less susceptible to inhibitory actions of the films. However, the film incorporated with TC Ag NPs reduced the growth of *S. aureus* for 77.4%. On the other side, incorporation of EA Ag NPs into agar-agar-chitosan film led to the growth inhibition for only 37%. This higher inhibition obtained with TC Ag NPs could be attributed to the contribution of cocoa extract itself, i.e. some active components that inhibit the growth of *S. aureus* (Todorovic et al., 2017)

Table 2. Antimicrobial activity of agar-agar – chitosan nanocomposite films

	Percentage of inhibition (%)		
	Control film	Film + TC Ag NPs	Film + EA Ag NPs
E.coli	12.4	92.8	93.6
S.aureus	17.8	77.4	37.1

Conclusions

In this study, the potential of horsetail and cocoa extracts to serve as reducing and stabilizing agents for the synthesis of Ag NPs was explored. Ag NPs with the most desirable properties were obtained with 10% of plant extract. Incorporation of EA Ag NPs and TC Ag NPs into agar-agar – chitosan films enhanced the antimicrobial activity of the films against both *E. coli* and *S. aureus*. According to our preliminary results agar-agar-chitosan films with incorporated green synthesized Ag NPs have potential application in the areas such as food package, wound healing, coatings for medical devices etc. where usage of biodegradable and biocompatible materials with high antibacterial activity is desired.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-9/2021-14/200287 and 451-03-9/2021-14/200135).

References

Abd-Alla, M. H., Nafady, N. A., & Khalaf, D. M. (2016). Assessment of silver nanoparticles contamination on faba bean-Rhizobium leguminosarum bv. viciae-Glomus aggregatum symbiosis: Implications for induction of autophagy process in root nodule. *Agriculture, Ecosystems and Environment*, *218*, 163–177.

Alomar, T. S., AlMasoud, N., Awad, M. A., El-Tohamy, M. F., & Soliman, D. A. (2020). An eco-friendly plant-mediated synthesis of silver nanoparticles: Characterization, pharmaceutical and biomedical applications. *Materials Chemistry and Physics*, *249*.

Baharum, Z., Akim, A. M., Hin, T. Y. Y., Hamid, R. A., & Kasran, R. (2016). Theobroma cacao: Review of the Extraction, Isolation, and Bioassay of Its Potential Anti-cancer Compounds. *Tropical Life Sciences Research*, *27*(1), 21–42.

Balalakshmi, C., Alharbi, N. S., Kadaikunnan, S., & Khaled, J. M. (2020). *Development of chitosan / agar - silver nanoparticles - coated paper for antibacterial application*. 751–759.

Cetojević-Simin, D. D., Canadanović-Brunet, J. M., Bogdanović, G. M., Djilas, S. M., Cetković, G. S., Tumbas, V. T., & Stojiljković, B. T. (2010). Antioxidative and antiproliferative activities of different horsetail (Equisetum arvense L.) extracts. *Journal of Medicinal Food*, *13*(2), 452–459.

Eby, D. M., Luckarift, H. R., & Johnson, G. R. (2009). Hybrid antimicrobial enzyme and silver nanoparticle coatings for medical Instruments. *ACS Applied Materials and Interfaces*, *1*(7), 1553–1560.

El-Hefian, E. A., Nasef, M. M., & Yahaya, A. H. (2012). Preparation and characterization of chitosan/agar blended films: Part 2. Thermal, Mechanical, and surface properties. *E-Journal of Chemistry*, *9*(2), 510–516.

Glucomannan, K., & Starch, C. (2021). *Characterization of Chitosan Films Incorporated with Different*.

Hassanien, A. S., & Khatoon, U. T. (2019). Synthesis and characterization of stable silver nanoparticles, Ag-NPs: Discussion on the applications of Ag-NPs as antimicrobial agents. *Physica B: Condensed Matter*, *554*(October 2018), 21–30.

Hu, Z., Hong, P., Liao, M., Kong, S., Huang, N., Ou, C., & Li, S. (2016). Preparation and characterization of chitosan-agarose composite films. *Materials*, *9*(10), 1–9.

Ishaq, S., & Jafri, L. (2017). Biomedical Importance of Cocoa (Theobroma cacao): Significance and Potential for the Maintenance of Human Health. *Matrix Science Pharma*, *1*(1), 1–5.

Jorge de Souza, T. A., Rosa Souza, L. R., & Franchi, L. P. (2019). Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity. In *Ecotoxicology and Environmental Safety* (Vol. 171, pp. 691–700).

Kelly, K. L., Coronado, E., Zhao, L. L., & Schatz, G. C. (2003). The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment. *Journal of Physical Chemistry B*, *107*(3), 668–677.

Kraśniewska, K., Galus, S., & Gniewosz, M. (2020). Biopolymers-based materials containing silver nanoparticles as active packaging for food applications–A review. *International Journal of Molecular Sciences*, *21*(3).

Kumar, S., Mukherjee, A., & Dutta, J. (2020). Chitosan based nanocomposite films and coatings: Emerging antimicrobial food packaging alternatives. *Trends in Food Science and Technology*, *97*(December 2019), 196–209.

Lateef, A., Azeez, M. A., Asafa, T. B., Yekeen, T. A., Akinboro, A., Oladipo, I. C., Azeez, L., Ojo, S. A., Gueguim-Kana, E. B., & Beukes, L. S. (2016). Cocoa pod husk extract-mediated biosynthesis of silver nanoparticles: its antimicrobial, antioxidant and larvicidal activities. *Journal of Nanostructure in Chemistry*, *6*(2), 159–169.

Pallag, A., Filip, G. A., Olteanu, D., Clichici, S., Baldea, I., Jurca, T., Micle, O., Vicas, L., Marian,

E., Soriţău, O., Cenariu, M., & Mureşan, M. (2018). Equisetum arvense L. extract induces antibacterial activity and modulates oxidative stress, inflammation, and apoptosis in endothelial vascular cells exposed to hyperosmotic stress. *Oxidative Medicine and Cellular Longevity*, *2018*.

Rhim, J. W., Wang, L. F., & Hong, S. I. (2013). Preparation and characterization of agar/silver nanoparticles composite films with antimicrobial activity. *Food Hydrocolloids*, *33*(2), 327–335.

Roy, S., & Rhim, J. W. (2019). Agar-based antioxidant composite films incorporated with melanin nanoparticles. *Food Hydrocolloids*, *94*(February), 391–398.

Schlich, K., Klawonn, T., Terytze, K., & Hund-Rinke, K. (2013). Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. *Environmental Sciences Europe*, *25*(1), 1–14.

Sedaghat, S., & Omidi, S. (2018). Batch process biosynthesis of silver nanoparticles using Equisetum arvense leaf extract. *Bioinspired, Biomimetic and Nanobiomaterials*, *8*(3), 190–197.

Shukla, A. K., & Iravani, S. (2017). Metallic nanoparticles: green synthesis and spectroscopic characterization. In *Environmental Chemistry Letters* (Vol. 15, Issue 2, pp. 223–231).

Sur, I., Cam, D., Kahraman, M., Baysal, A., & Culha, M. (2010). Interaction of multi-functional silver nanoparticles with living cells. *Nanotechnology*, *21*(17).

Thatikayala, D., Jayarambabu, N., Banothu, V., Ballipalli, C. B., Park, J., & Rao, K. V. (2019). Biogenic synthesis of silver nanoparticles mediated by Theobroma cacao extract: enhanced antibacterial and photocatalytic activities. *Journal of Materials Science: Materials in Electronics*, *30*(18), 17303–17313.

Todorovic, V., Milenkovic, M., Vidovic, B., Todorovic, Z., & Sobajic, S. (2017). Correlation between Antimicrobial, Antioxidant Activity, and Polyphenols of Alkalized/Nonalkalized Cocoa Powders. *Journal of Food Science*, *82*(4), 1020–1027.

Yan, A., & Chen, Z. (2019). Impacts of silver nanoparticles on plants: A focus on the phytotoxicity and underlying mechanism. *International Journal of Molecular Sciences*, *20*(5), 23–25.