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Green synthesis of silver nanoparticles by using aqueous mint (*Mentha piperita*) and cabbage (*Brassica oleracea* var. *capitata*) extracts and their antibacterial activity

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Abstract: *Green synthesis of silver nanoparticles by using aqueous mint (*Mentha piperita*) and cabbage (*Brassica oleracea *var.* capitata*) extracts and their antibacterial activity*. The objective of this study was the synthesis of silver nanoparticles (Ag-NP) using leaves of mint and cabbage extracts as the reducing and stabilising agents. The presence of nanoparticles was initially confirmed by the obtained colour and next by transmission electron microscope (TEM). Analysis of TEM of obtained Ag-NP indicated that their size ranged 5–50 nm for mint and 10–150 nm for cabbage. The antibacterial activity of nanoparticles against pathogenic strains *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* were assessed by evaluation of metabolic activity, using the PrestoBlue and XTT test. The higher inhibition of bacterial viability was observed against Gram-negative (*E. coli*, *S. enterica*) than Gram-positive (*S. aureus*) bacteria.

Key words: silver nanoparticles, green synthesis, bioreduction, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*

INTRODUCTION

In recent years, an increasing number of studies considering alternative and more eco-friendly processes for the synthesis of nanoparticles has been observed. The main methods used for silver nanoparticles (Ag-NP) synthesis are physical, chemical and biological methods (Prabhu and Poulose 2012). The problem with physiochemical methods is that the synthesis is expensive (necessity to use high pressure, energy and temperature) and involve the use of toxic and harsh chemicals (Kalishwaralal et al. 2008).

Green synthesis of nanomaterials is based on extracts from biological organisms such as plants, bacteria, yeasts, fungi and algae. Silver nanoparticles can be synthesised using different parts of plants and their extracts such as leaf (*Euphorbia hirta*), seed (*Jatropha curcas*), fruit (*Carica papaya*), root (*Morinda citrifolia*), flower (*Tagetes erecta*), steam and peel (Kuppusamy et al. 2016). Plant extracts are natural sources of non-toxic reducer/stabiliser agents, so they may be useful for manufacturing of nanoparticles. *Mentha piperita* is also good sources of menthol, limonene, pulegone, carvophyllene and pinene. Menthol reacts in many aspects as a normal secondary alcohol and also can be useful in biosynthesis of Ag-NP by alcohol reduction of $AgNO_3$ (Kamatou et al. 2013). Cabbage is a good source of vitamins, especially

ascorbic acid (30–36 mg in 100 g), minerals, electrolytes, sulforaphane, indoles, zea-xanthin and lutein (Tamileswari et al. 2015).

Silver nanoparticles have high antibacterial, antimalarial, antidiabetic, antioxidant and anticancer activity (Jeyaraj et al. 2013, Chung et al. 2016). They were also used to explore their antibacterial potential against resistant pathogens (Chung et al. 2016). The objective of this study was to determine the efficiency of Ag-NP synthesis, using mint and cabbage leaves extracts, and to characterise obtained nanoparticles. Furthermore, the effect of pH and temperature on the size and shape of nanoparticles as well as their antibacterial activity were evaluated.

MATERIAL AND METHODS

Preparation of plant extract

Commercially available fresh leafs of *Mentha piperita* and *Brassica oleracea* were purchased from a local market in Warsaw (Poland). Powder of $AgNO_3$ was obtained from Sigma (Saint Louis, USA). Leaves of *M. piperita* and *B. oleracea* were washed thoroughly four-fold with distilled water, dried and cut into small pieces. 2.5 g of mint leaves and 25 g of cabbage were flooded with 25 and 150 ml of distilled water respectively, and boiled for 10 min. Next, the plants extracts were filtered through filter paper round ø 125 mm FILTRAK 388.

Preparation of silver nanoparticles

We assumed that all amount of used/introduced silver nitrate was reduced to Ag-NP and the final concentration of the obtained Ag-NP corresponds the ratio of silver nitrate (μg) to the solution volume (ml). The assumption has been confirmed by transmission electron microscopy (TEM, JEOL, Japan). For the biosynthesis of Ag-NP using *M. piperita*, 2.5, 5 and 12.5 ml of filtered *M. piperita* extract solutions were added to the 50 ml of silver nitrate water solution (170 μg/ml) to obtain after synthesis: 162, 148 and 136 μg/ml concentrations of mint-Ag-NP. For the biosynthesis of cabbage-Ag-NP using white cabbage, 10 ml of filtered cabbage extract was added to 50 ml of 340 μg/ml silver nitrate water solution. The procedure was performed in triplicate to obtain tree separate mix plant extract – silver nitrate solutions. Next, each of $AgNO_3$ – plant extract mix solutions were heated under different temperatures: 30°C, 60°C and 90°C. After bioreduction the concentration of cabbage-Ag-NP was 283 μg/ml. Synthesised Ag-NP were sterilised in autoclave (Prestige Medical, UK) and sonicated for 15 min in ultrasonic bath (Bandelin Electronic, Germany) to avoid agglomeration. All Ag-NP characterisations were performed in triplicate, using TEM and zeta potential analyser (Malvern, UK) according to the procedures described by Sawosz et al. (2010).

Evaluation of the antibacterial effect of nanoparticles

Salmonella enterica subspecies *enterica* serovar Enteritidis (ATCC 13076), *Escherichia coli* (ATCC MP-26) and *Staphylococcus aureus* subspecies *aureus* (ATCC 12600) were obtained from LGC Standards (Łomianki, Poland). The bacteria were then grown on nutritive agar (2.8%) with the addition of NaCl

(Bio-Rad, Warsaw, Poland). Sterilisation of media was carried out at 121°C for 30 min (Tuttnauer 2450EL, Tuttnauer Ltd., Jerusalem, Israel). Next, bacterial cultures were prepared overnight. Volumetric flasks, which contained 10.75 ml of peptone water (Biocorp, Warsaw, Poland), were filled with 50 µl of night culture in six repetitions for each species of bacteria. Subsequently, mint-Ag-NP and cabbage-Ag-NP with the smallest diameter were added (solution 1 for mint and cabbage). The final concentrations in volumetric flasks were 1, 8 and 16 µg/ml for the mint-Ag-NP and 1, 14 and 28 µg/ml for the cabbage-Ag-NP. Each species of bacteria had its own control, which contained water instead of nanoparticles. During the night, the cultures were shaken up at 100 rpm and 37°C. Then, the cultures were seeded at 5×10^5 cells per well in a sterile 96-well plate. The assessment of antibacterial activity of Ag-NP against *E. coli*, *S. aureus* and *S. enterica* were evaluated by metabolic assays – PrestoBlue (Life Technologies, USA) and XTT test (Roche Protocol, Germany). PrestoBlue and XTT cell viability assays are based on the ability of metabolically active cells to reduce and form a coloured product (PrestoBlue – pink product, XTT – orange product). The reducing environment within viable cells converts PrestoBlue reagent into pink dye. Only living cells are capable to reduce tetrazolium salt (XTT) to formazan by trans-plasma membrane electron transport at the cell surface. Cell viability was expressed as the percentage (ODtest – ODblank) / (ODcontrol – ODblank), where "ODtest" is the optical density of cells exposed to Ag-NP, "ODcontrol" is the optical density of the control sample, and "ODblank" is the optical density of wells without bacterial cells.

Statistical analysis

Statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's post-test using Statgraphics Plus 4.1 (StatPoint Technologies, Warrenton, VA, USA). Differences at $P \leq 0.05$ were defined as statistically significant.

RESULTS AND DISCUSSION

Formation of Ag-NP was confirmed by the change in colour of solution (Fig. 1). The fresh suspensions of mint and cabbage extracts were yellowish-green and pale yellow respectively. After addition of $AgNO_3$ solution, the colours change was observed within 60 min. After 60 min of the incubation at room temperature, the mint solution had almost black (1 solution), dark (2 solution) and pale brown (3 solution) colour while cabbage solution had almost black (solution 1) and dark brown (solutions 2 and 3) colour.

The addition of 5 ml mint extract was most effective for the synthesis of nanoparticles. Menthol can enhance penetration of other agents and have great cooling taste and smell properties. The cooling sensation results from the ability to chemically activate the cold-sensitive transient receptor potential cation channel (TRPM8). Kamatou et al. (2013) reported that when 0.02% menthol solutions held in the mouth, solutions above 37°C seemed warmer than water without menthol of the same temperature (warmth enhancement). However, menthol solutions below 37°C seemed cooler than water of the same temperature (cold

FIGURE 1. Colour change after incubation for 20 and 60 min at room temperature: A – mint-nanosilver, B – cabbage-nanosilver

enhancement). Menthol treatments of silver nitrate solution resulted in the conversion of menthol and Ag^+ to menthone and Ag^0 (Kamatou et al. 2013).

The synthesis of cabbage-Ag-NP was most efficient at 30°C. Thermal treatments of cabbage at 30°C and 60°C resulted in the conversion of L-ascorbic acid (L-AA) and Ag^+ to dehydroascorbic acid (DHHA) and Ag^0 but treatments at 90°C retained vitamin C as L-AA. The conversion is made possible by ascorbic acid oxidase (AAO) – thermolabile enzyme (EC 1.10.3.3) (Munyaka et al. 2010).

Figure 2 shows representative TEM images of obtained Ag-NP. The shape of newly synthesised Ag-NP was regular and rounded, and hydrocolloids showed a high density of Ag-NP. The pictures showed Ag-NP but absence of crystals salts. Nanoparticles prepared using the mint extract had a smaller diameter (from 5 to 50 nm) than cabbage (from 10 to 100–150 nm). The nanoparticles of smaller diameter can easily pass through the membrane channel of the bacteria (Kuppusamy et al. 2015). The mean zeta-potential for the most stable samples of mint-Ag-NP was –11.5 mV and cabbage-Ag-NP was –18.6 mV. Results showed that use of biological methods allows obtaining stable nanoparticles without stabiliser.

During green synthesis, pH is an important factor for regulating the size and shape of Ag-NP. The pH induces the reactivity of plant extract with silver ions (Vanaja et al. 2013). Fayaz et al. (2009) reported that at low pH nanoparticles of large size were formed. At alkaline pH,

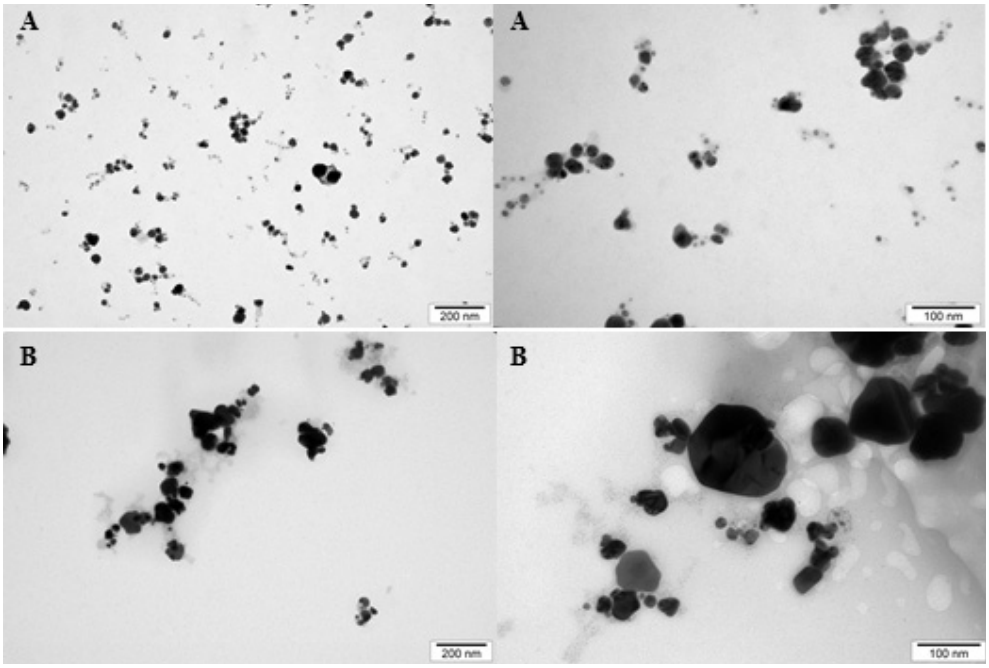

FIGURE 2. Transmission electron microscopy (TEM) images of silver nanoparticles. Bar scale 200 and 100 nm. AgNPs obtained using the aqueous plant extracts: A – mint, B – cabbage

a large number of Ag-NP with the small surface area are present due to the bioavailability of functional groups (Fayaz et al. 2009). Similarly, Vanaja et al. (2013) reported that alkaline pH is suitable for synthesis of Ag-NP.

The influence of mint-Ag-NP and cabbage-Ag-NP on the growth of *E. coli*, *S. aureus* and *S. enterica* is presented in Figures 3–5. The results showed that bacterial cells treated with Ag-NP had decreased metabolic activity compared to the control cells. Experiments demonstrated that the nanoparticles prepared using the mint extract had higher antimicrobial activity than cabbage-Ag-NP. The smaller diameter may be a reason of a high antibacterial activity of nanoparticles obtained from mint. The cabbage-Ag-NP did not show significant antibacterial activity. According to the PrestoBlue assay, the lowest bacterial viability was observed for *S. enterica* cells, irrespective of the type of nanoparticles (mint-Ag-NP: 5–8%, cabbage--Ag-NP: 35–36%). XTT assay showed a lower viability of both used species of Gram-negative than Gram-positive bacteria, probably due to the thin peptidoglycan layer in the cell wall and presence of beta barrel proteins called porins (Shukla and Vankar 2012). The XTT is more sensitive than PrestoBlue test because the reduction process occurs mainly on the cell surface or plasma membrane with the transmembrane electron transport chain (on the cell membrane of bacteria). Only living cells (possessing an intact cell membrane, active dehydrogenase) are capable to reduce XTT to formazan.

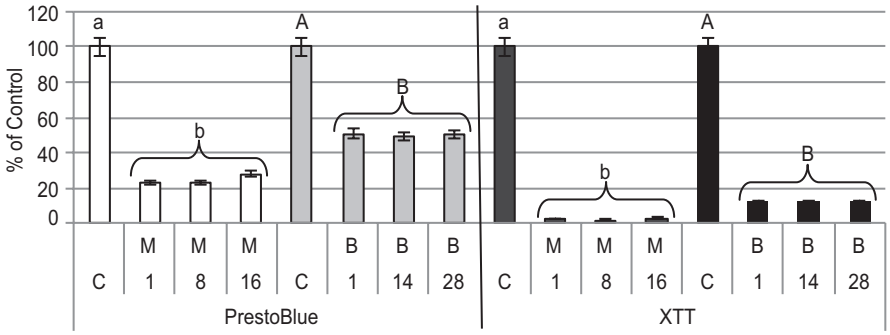

FIGURE 3. Mean viability of *Escherichia coli* cells evaluated for two tests, i.e. PrestoBlue and XTT, after treatment with: C – control (no treatment Ag-NP), M – *Mentha piperita* (1, 8, 16 µg/ml of mint-Ag-NP), B – *Brassica oleracea* (1, 14, 28 µg/ml of cabbage-Ag-NP). The differences between the treated group Ag-NP and non-treated control group were statistical significance ($P \leq 0.05$). Different letters (a, b and A, B) indicate significant differences between the groups (different colours show groups). ANOVA, Tukey post-test

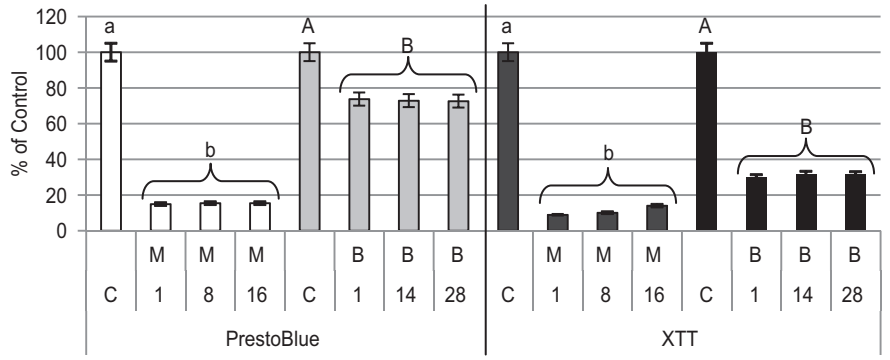

FIGURE 4. Mean viability of *Staphylococcus aureus* cells evaluated for two tests, i.e. PrestoBlue and XTT, after treatment with: C – control (no treatment Ag-NP), M – *Mentha piperita* (1, 8, 16 µg/ml of mint-Ag-NP), B – *Brassica oleracea* (1, 14, 28 µg/ml of cabbage-Ag-NP). The differences between the treated group Ag-NP and non-treated control group were statistical significance ($P \leq 0.05$). Different letters (a, b and A, B) indicate significant differences between the groups (different colours show groups). ANOVA, Tukey post-test

Therefore, the concentration of the dye is proportional to the number of metabolically active cells. Consequently, antibacterial activity of Ag-NP is bacteria species dependent.

Most studies used disc diffusion method and measured zone of inhibition. Tamileswari et al. (2015) synthesised of Ag-NP using cabbage extract. Their results showed good antibacterial and antifungal activity against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pneumocystis* sp. (Kappusamy 2015, Tamileswari et al. 2015). Some researchers reported that the mint-Ag-NP have

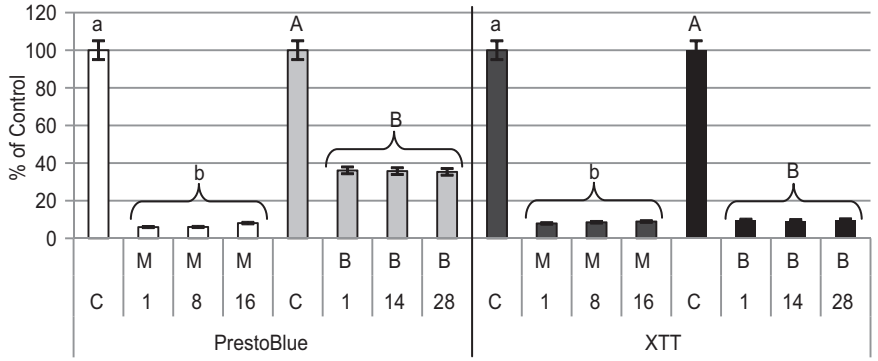

FIGURE 5. Mean viability of *Salmonella enterica* cells evaluated for two tests, i.e. PrestoBlue and XTT, after treatment with: C – control (no treatment Ag-NP), M – *Mentha piperita* (1, 8, 16 µg/ml of mint-Ag-NP), B – *Brassica oleracea* (1, 14, 28 µg/ml of cabbage-Ag-NP). The differences between the treated group Ag-NP and non-treated control group were statistical significance ($P \leq 0.05$). Different letters (a, b and A, B) indicate significant differences between the groups (different colours show groups). ANOVA, Tukey post-test

antimicrobial effect against *E. coli* (the maximum zone of inhibition), *S. aureus* and additionally *Pseudomonas aeruginosa* and *Bacillus subtilis* (MubarakAli et al. 2011, Sarkar and Paul 2017). Saikia et al. (2015) reported that antibacterial activity of Ag-NP is plant extract dependent.

Antibacterial activity of Ag-NP can have several causes e.g. nanoparticles penetration inside and binding DNA, hampering the normal replication, loss of cell viability by modulating tyrosine phosphorylation, attack on the respiratory chain and finally resulting in cell death (Sarkar and Paul 2017).

The aqueous extract of *M. piperita* and *B. oleracea* showed the presence of carbohydrates, amino acids, tannins, flavonoids, terpenoids, quinones, phenols, proteins and coumarins (Satya Prasad et al. 2015, Patil et al. 2016). Secondary metabolites act as interfering protein synthesis agents (tannins) and inhibitors of the extracellular enzymes (required for microbial growth and oxidative phosphorylation) (Satya Prasad et al. 2015).

Al-Sum et al. (2013) reported that aqueous *Mentha* species extract was active against six pathogenic bacteria, i.e.: *Bacillus fastidiosus* (the highest inhibitory effect), *Proteus mirabilis*, *P. vulgaris*, *Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Serratia odorifera* except for *Staphylococcus aureus*. According to Patil et al. (2016), the aqueous mint extract showed inhibitory effect against *Proteus vulgaris* and *Staphylococcus aureus* but *Bacillus cereus* and *Salmonella typhimurium* did not show a zone of inhibition. Extracts from *Brassica oleracea* exhibit distinct zones of inhibition towards bacterial strains like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermis*, *Salmonella typhimurium* and *S. paratyphi* (Satya Prasad et al. 2015). Ethanol extract has a greater activity and protein content than the other

extracts. This extract is the most effective solvent for extracting a broad spectrum of antibacterial compounds from plant origin (Satya Prasad et al. 2015). Antibacterial nature of plants and their secondary metabolites could be useful to improve the efficiency of nanoparticles.

CONCLUSION

Silver nanoparticles were successfully synthesised from silver nitrate solution using mint and cabbage extracts. Green synthesis using mint and cabbage leaves extracts provides an eco-friendly, stable, simple, cheap and efficient route of Ag--NP synthesis. The pH and temperature play a major role in size control of the Ag-NP. Mint with silver nanoparticles complex had a smaller diameter than cabbage with silver nanoparticles complex probably due to the higher pH and lower temperature of mint extract used in nanoparticle synthesis. The obtained nanoparticles showed powerful antibacterial activity against human pathogens, i.e. *E. coli*, *S. aureus* and *S. enterica*, indicating that Ag-NP are good candidates for their usage as antibacterial agents. These nanoparticles had higher antibacterial activity against Gram-negative than Gram-positive bacteria. In the future nanoparticles synthesised from plants may be a better alternative for elimination of multidrug resistance microorganism than commercial antibiotics.

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REFERENCES

Al-SUM B.A., Al-ARFAJ A.A., 2013: Antimicrobial activity of the aqueous extract of mint plant. Sci. J. Clin. Med. 2: 110–113.

CHUNG I.M., PARK I., SEUNG-HYUN K., THIRUVENGADAM M., RAJAKUMAR G., 2016: Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications. Nanoscale Res. Lett. 11: 1–14.

FAYAZ A.M., BALAJI K., KALAICHELVAN P.T., VENKATESAN R., 2009: Fungal based synthesis of silver nanoparticles – an effect of temperature on the size of particles. Colloids Surf. B. 74: 123–126.

JEYARAJ M., REJESH M., ARUN R., MUBARAKALI D., SATHISHKUMAR G., SIVANANDHAN G., DEVA G.K., MANICKAVASAGAMA M., PREMKUMAR K., THAJUDDINC N., GANAPATHI A., 2013: An investigation on the cytotoxicity and caspase-mediated apoptotic effect of biologically synthesized silver nanoparticles using *Podyphyllum hexandrum* on human cervical carcinoma cells. Colloids Surf. B. 102: 708–717.

KALISHWARALAL K., DEEPAK V., RAMKUMARPANDIAN S., NELLAIAH H., SANGILIYANDI G., 2008: Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. Mater. Lett. 62: 4411–4413.

KAMATOU G.P.P., VERMAAK I., VILIOEN A.M., LAWRENCE B.M., 2013: Menthol: A simple monoterpene with remarkable biological properties. Phytochem. 96: 15–25.

KAPPUSAMY P., ICHWAN S.J.A., REDDY PARINE N.R., YUSOFF M.M., MANIAN G.P., GOVINDAN N., 2015: Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of *Brassica oleracea* L. and studies on their physicochemical and biological properties. J. Environ. Sci. 29: 151–157.

KUPPUSAMY P., YUSOFF M.M., MANIAM G.P., GOVINDAN N., 2016: Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report. Saudi Pharm. J. 24: 473–484.

MUBARAKALI D., THAJUDDIN N., JEGANATHAN K., GUNASEKARAN M., 2011:

Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf. B. 85: 360–365.

MUNYAKA A.W., MAKULE E.E., OEY I., Van LOEY A., HENDRICKX M., 2010: Thermal stability of L-ascorbic acid and ascorbic acid oxidase in broccoli (*Brassica oleracea* var. *italic*). J. Food Sci. 75: 336–340.

PATIL S.R., PATIL R.S., GODGHATE A.G., 2016: *Mentha piperita* Linn: Phytochemical, antibacterial and dipterian adulticidal approach. Int. J. Pharm. Pharm. Sci. 8: 352–355.

PRABHU S., POULOSE M.K., 2012: Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int. Nano Lett. 2: 1–10.

SAIKIA D., GOGOI P.K., PHUKAN P., BHUYAN N., BORCHETIA S., SAIKIA J., 2015: Green synthesis of silver nanoparticles using Asiatic pannywort and *Bryophyllum* leaves extract and their antimicrobial activity. Adv. Mater. Lett. 6: 260–264.

SARKAR D., PAUL G., 2017: Green synthesis of silver nanoparticles using *Mentha asiatica* (mint) extract and evaluation of their antimicrobial potential. Int. J. Curr. Res. Biosci. Plant Biol. 4: 77–82.

SATYA PRASAD M., SUMAN JOSHI D.S.D., NARENDA K., NADIYA S.K., MASTHANI S.K., PADMAJA PHANI N., KRISHNA SATYA A., 2015: A comparative study of phytochemical analysis and in vitro antimicrobial activity of three important vegetables from *Brassicaceae* family. Int. J. Res. Ayurveda Pharm. 6: 767–772.

SAWOSZ E., CHWALIBOG A., SZELIGA J., SAWOSZ F., GRODZIK M., RUPIEWICZ M., NIEMIEC T., KACPRZYK K., 2010: Visualization of gold and platinum nanoparticles interacting with *Salmonella* Enteritidis and *Listeria monocytogenes*. Int. J. Nanomedicine 5: 631–637.

SHUKLA D., VANKAR P.S., 2012: Synthesis of plant parts mediated gold nanoparticles. Int. J. Green Nanotechnol. 4: 277–288.

TAMILESWARI R., HANIFF NISHA M., JESURANI Sr.S., 2015: Green synthesis of silver nanoparticles using *Brassica oleracea* (cauliflower) and *Brassica oleracea capitata* (cabbage) and the analysis of antimicrobial activity. IJERT 4: 1071–1074.

VANAJA M., RAJESHKUMAR S., PAULKUMAR K., GNANAJOBITHA G., MALARKODI C., ANNADURAJ G., 2013: Kinetic study on green synthesis of silver nanoparticles using *Coleus aromaticus* leaf extract. Adv. Appl. Sci. Res. 4: 50–55.

Streszczenie: *Zielona synteza nanocząstek srebra przy użyciu wodnych ekstraktów z mięty (*Mentha piperita*) i kapusty (*Brassica oleracea *var.* capitata*) oraz ich aktywność przeciwbakteryjna.* Celem pracy była synteza nanocząstek srebra (Ag-NP) przy użyciu ekstraktów z liści mięty i kapusty jako czynników redukujących i stabilizujących. Obecność nanocząstek była początkowo stwierdzona przez zmianę koloru i następnie przez transmisyjną mikroskopię elektronową (TEM). Analiza TEM uzyskanych Ag-NP wykazała, że ich rozmiary były w przedziale wielkości 5–50 nm dla mięty i 10–150 nm dla kapusty. Aktywność przeciwbakteryjną nanocząstek przeciwko patogennym szczepom *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enterica* oceniano przez oszacowanie aktywności metabolicznej z użyciem testu PrestoBlue i XTT. Większą inhibicję żywotności bakteryjnej obserwowano przeciwko Gram-ujemnym (*E. coli*, *S. enterica*) niż Gram-dodatnim (*S. aureus*) bakteriom.

Słowa kluczowe: nanocząstki srebra, zielona synteza, bioredukcja, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*

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