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# *Lavandula angustifolia* PROPAGATED IN *IN VITRO* CULTURES ON MEDIA CONTAINING AgNPs AND AuNPs – AN ALTERNATIVE TO SYNTHETIC PRESERVATIVES IN COSMETICS

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**Abstract.** We determined the preservation properties of *Lavandula angustifolia* propagated on media with gold or silver nanoparticles with a particle size of 13 and 30 nm. Cosmetic emulsions prepared by using lavender tissue that was propagated on media containing AuNPs and AgNPs showed increased preservative capacities when compared with the control ones. In the case of control cosmetic emulsions, which had no added plant tissues or dehydroacetic acid and benzoic acid (DHA BA), bacterial and fungal colonies appeared after the second week of the experiment. The addition of lavender tissue propagated on media without AuNPs or AgNPs protected the tasted samples from microbial contamination; in this case, bacterial contamination was detected after 4 weeks and fungal contamination after 6 weeks. The addition of lavender tissue propagated on medium containing AgNPs with a particle size of 13 nm at a concentration of 1 mg · $dm^{-3}$ prolonged the time of detection of bacteria colonies to 8 weeks (0.9) and this result was close and comparable to the effect of DHA BA. Higher concentrations of AgNPs in the culture medium, as well as a larger particle diameter (30 nm), resulted in the decreased preservative capacity of plant tissues. The presence of AuNPs in the culture media showed a positive effect on the antimicrobial activity of lavender; however, to a lesser degree than in the case of AgNPs. Disintegrated fragments of lavender tissue propagated on media containing 1 mg · $dm^{-3}$ AgNPs with particle size of 13 nm can be used to preserve short shelf life cosmetic emulsions.

**Key words:** cosmetic emulsion, metal nanoparticles, silver, gold, micropropagation, elicitation.

## INTRODUCTION

The global cosmetic production is one of the most rapidly developing personal care industries. By the end of 2022, the global cosmetic market is expected to reap a profit of approximately $429.8 billion, registering a compound annual growth rate (CAGR) of 4.3% during the forecast period 2016–2022 (Allied Market Research 2016). The fulfillment of increasing market demand requires continuous multidimensional control, namely, to monitor toxic ingredients and microbial contamination (Halla et al. 2018). Inhibiting the growth of microorganisms is one of the most important aspects of production of cosmetics. Microbial contamination of a cosmetic product decreases its shelf life and increases the chances of potential infection to the consumer. The application of a contaminated product might cause

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irritation to the skin and/or damage the skin thereby threatening the consumer's health. Furthermore, it may contribute to the spread and development of various infections (Groot et al. 1995). Furthermore, microbial contamination of a cosmetic product leads to the decomposition of the active ingredients contained therein, which might decrease its therapeutic activity (Behravan et al. 2005). Modern-day cosmeceuticals contain water, which is the perfect environment for the microorganisms to grow. Microbial contamination in cosmetics and in personal care products might occur during the process of manufacturing or during their use by the customer. In general, these products are exposed to temperature variations. Furthermore, once a cosmetic product is opened, it is highly possible, until it is discarded, that the contamination is introduced or accelerated by consumer use (e.g. by dipping with nonsterile fingers and contact with nonsterile bodies) (Kerdudo et al. 2016). Therefore, it is impossible to mass-produce cosmetics free of preservatives.

According to the definition provided by the European Parliament and Council of the European Union, a preservative is a substance of natural origin or is synthetic, which when added to a cosmetic product inhibits the development of microorganisms during the production stage and prevents repeated contamination in the later stages of its use. Numerous studies have indicated that the presence of chemical preservatives in cosmetics cause allergies and skin diseases (Orton et al. 2004; Mario et al. 2012). Some of the synthetic preservatives available in the market are considered carcinogenic or mutagenic. Recent studies have confirmed that parabens cause breast cancer and affect the development of malignant melanoma (Golden et al. 2005). There are more than 12,000 synthetic chemicals that are used in the manufacture of cosmetics, among which less than 20% are considered completely safe (O'Dell et al. 2016). World Health Organization regulates the use of approved synthetic chemicals by the cosmetic industries thereby considerably reducing the number of chemicals being used during the production. Therefore, in recent years, there is an increasing demand for natural cosmetics that contain at least 95% of substances derived from natural origin (Fonseca-Santos et al. 2015).

Many companies prefer to use natural alternatives instead of synthetic chemicals in their cosmetics. Chemicals of plant origin act as active ingredients (e.g. moisturizers), excipients (e.g. surfactants), and additives (e.g. preservatives) in a single formula (Kusumawati et al. 2013; Kerdudo et al. 2016; Andrys et al. 2018). Because of the antimicrobial and antifungal nature of plant-based essential oils, cosmetics such as creams, gels, and ointments do not necessarily require addition of chemical preservatives if they already contain essential oils or those that contain a single compound as an active agent (Sticher et al. 2015).

Narrow leaf lavender (*Lavandula angustifolia*) (family *Lamiaceae*), originating from the Mediterranean region, has been successfully used in the cosmetic industry as an antimicrobial agent in various cosmetics and in personal care products (Upson et al. 2004). This ornamental and medicinal plant is considered one of the most valuable species because of its widespread use in the perfume, flavoring, cosmetics, and soap industries. Its use can be dated back to ancient Rome and Greece (Wornouk et al. 2011; Brailko et al. 2017). Apart from being used in aromatherapy, lavender is also used in the pharmaceutical industry and in medicine. Due to the characteristic aroma and antiseptic properties of the essential oil, it has been used in the production of cleaning agents (e.g. soaps and shampoos) and perfumes (Cavanagh et al. 2005). Lavender oil shows antimicrobial and antifungal activity; its effect has been

demonstrated in some of the antibiotic-resistant bacteria (Schwiertz et al. 2006). Lavender oil has been shown to be effective against *Staphylococcus aureus*, *Enterococcus faecalis* (Cavanagh et al. 2005), *Candida albicans* (D´auria et al. 2005), and *Botrytis cincerea* (Adam et al. 1998). Because of its antimicrobial property, lavender oil is used to alleviate skin inflammation caused by acne and psoriasis vulgaris. Furthermore, lavender oil has been used to treat wounds, burns, and purulent-states (Jopke et al. 2017).

The composition of the essential oil mainly depends on, among others, the plant's genotype, environmental conditions, and tissue propagation methods (Demissie et al. 2011). Tissue culture is a method of obtaining a high amount of tissues of medicinal plants for the production of secondary metabolites (Gonçalves et al. 2013). Through *in vitro* cultures, it is possible to produce genetically identical individuals that are free from contaminants, which are of particular importance. Medicinal plants propagated through *in vitro* techniques are characterized by a unique composition of essential oils than that of the plants cultivated on fields (Amoo et al. 2012; Jakowijević et al. 2015). The addition of elicitors (i.e. stress-inducing factors) to the media results in a considerable change in the composition of secondary metabolites, including essential oils (Wesołowska et al. 2019). The change in the composition of essential oils and other bioactive compounds may influence, among others, the change in the antioxidative or microbiological activity of essential oils isolated from them. *L. angustifolia* (Andrys et al. 2018), *Ocimum basilicum* (Bais et al. 2002; Złotek et al. 2016), *Dionaea muscipula* and *Dionaea campensis* (Królicka et al. 2008), and *Coleus blumeli* (Szabo et al. 1999) cultures have shown increased antimicrobial activity under the influence of elicitor in the culture media.

The latest, currently studied elicitors are metal nanoparticles (NPs) (Vanisree et al. 2004; Shakeran et al. 2015; Moharrami et al. 2017; Golkar et al. 2019). It has been proven that metal NPs are highly reactive toward plants, and due to their small size, they can easily penetrate the cell membrane and get accumulated intracellularly (Jamshidi et al. 2014). Wesołowska et al. (2019) demonstrated that the accumulation of nanoparticles in tissues of plants propagated through *in vitro* culture technique, depending on, among others, particle size and diameter, may contribute to increased antimicrobial activity.

Typically, essential oils of various plants are used as preservatives in cosmetics (Aburjai et al. 2003; Nostro et al. 2004; Herman et al. 2013). Fresh tissues and dried plants are far less commonly used as preservatives. This might be because of the contamination of the material growing in natural conditions, low content of secondary metabolites, and process issues associated with the usage, such as problems with disintegration due to the presence of high content of phloem. Sterile plant tissues without the thick epidermal tissue, plant tissue propagated through *in vitro* techniques, those with a high content of secondary metabolites may be an alternative to plants growing under natural conditions, as long as they provide a suitable level of sterility to the products. Therefore, in this study, we aimed to verify the preservative properties of disintegrated true lavender tissues propagated *in vitro* on media enriched with gold and/or silver nanoparticles (AuNPs and AgNPs, respectively) with a different particle size.

## MATERIAL AND METHODS

### *In vitro* cultures

Narrow leaf lavender (*Lavandula angustifolia* cultivar Munstead) constituted the plant material. Shoot explants were placed in the media with a mineral composition according to Murashige and Skoog (1962) containing 2 mg · $dm^{-3}$ kinetin (KIN), 0.2 mg · $dm^{-3}$ indole-3-acetic acid (IAA), and 1 or 10 mg · $dm^{-3}$ AgNPs or AuNPs with the particle size of 13 or 30 nm (NPIN Poland). Lavender tissue grown on media that did not contain AuNPs or AgNPs constituted the control sample. The pH of the medium was set to 5.7 by using 0.1 M solutions of HCl and NaOH. To the media, 7 g · $dm^{-3}$ of agar, 30 g · $dm^{-3}$ of sucrose, and 0.1 g · $dm^{-3}$ of inositol were added. Subsequently, the media were subjected to 20-minute sterilization in an autoclave at 121°C and a pressure of 1 atm. Tissue propagation was conducted in 200 mL glass jars containing 20 mL medium. At the culture initiation stage, jars with plants were placed in a growth chamber at a temperature of 24°C and relative humidity of 70–80%. The cultures were illuminated with fluorescent light at an intensity of 40 PAR ($\mu E \cdot m^{-2} \cdot s^{-1}$) for 16 h per day After 28 days of culture growth, the experiment was completed, and the tissues of the propagated plants were used to prepare cosmetic emulsions after disintegration in liquid nitrogen.

### Preparation of cosmetic emulsions

Ingredients of the aqueous and oil phase were prepared in separate beakers. The oil phase consisted of 18 g lanolin, 12 g beeswax, 14 g shea butter, and 88 mL of jojoba oil. In order to prepare the aqueous phase, 6 g D-panthenol 75%, 6.4 g glycerin, and 68 mL water were added to a beaker. Both beakers with contents were tightly closed with aluminum foil and were placed in a water bath, where they were kept until all oil-phase ingredients were dissolved. The entire oil phase was added to the beaker containing aqueous phase which was placed in the water bath. Subsequently, the contents of the beaker were stirred for about 1 min, after which the beaker was removed from the water bath. Stirring was continued until the cream reached a temperature of 40°C.

The cosmetic emulsions were divided into 5 mL portions and were supplemented with the dehydroacetic acid and benzoic acid (DHA BA) as the chemical preservative or the material obtained from tissue propagation. During the study, 12 types of cosmetic emulsions were prepared (Table 1). To each of the variants, 0.1 g fresh plant tissue, which was ground in liquid nitrogen, was added, which constituted 2% plant material. The negative control contained 12.5 µL of DHA BA (Esent, Poland), containing 7% Dehydroacetic Acid (DHA) and 83% Benzyl Alcohol (BA) as the preservative, which constituted to 0.25%. The final cosmetic emulsions were placed in sterile Petri dishes and stored in a refrigerator maintained at 4°C. Emulsions without the addition of preservatives and plant tissues constituted the control samples. One of the control samples was stored in a refrigerator, and the other was stored at 24°C.

### Microbial purity test

After a period of 4 weeks, 0.1 g of cosmetic emulsion was dissolved with 0.9 mL of saline using Vortex (IKA). Subsequently, 0.1 mL of the test material was placed on a microbiological

medium and a surface culture test was performed. For this experiment, Sabouraud medium with chloramphenicol and Brain Heart Infusion Agar medium (BHI) (Biomaxima SA, Poland) were used.

Table 1. Types of cosmetic emulsions and the preservatives added to them

| Designation | Type and concentration of the preservative | Storage temperature [°C] |
|---|---|---|
| Control 24°C | None | 24°C |
| Control 4°C | None | 4°C |
| Control DHA BA | 0.25% DHA BA | |
| Variant 1 | 2% of lavender tissue propagated on media without the addition of nanoparticles | |
| Variant 2 | 2% of lavender tissue propagated on media with addition of 1 mg · $dm^{-3}$ AgNPs, size 13 nm | |
| Variant 3 | 2% of lavender tissue propagated on media with addition of 10 mg · $dm^{-3}$ AgNPs, size 13 nm | |
| Variant 4 | 2% of lavender tissue propagated on media with addition of 1 mg · $dm^{-3}$ AgNPs, size 30 nm | |
| Variant 5 | 2% of lavender tissue propagated on media with addition of 10 mg · $dm^{-3}$ AgNPs, size 30 nm | |
| Variant 6 | 2% of lavender tissue propagated on media with addition of 1 mg · $dm^{-3}$ AuNPs, size 13 nm | |
| Variant 7 | 2% of lavender tissue propagated on media with addition of 10 mg · $dm^{-3}$ AuNPs, size 13 nm | |
| Variant 8 | 2% of lavender tissue propagated on media with addition of 1 mg · $dm^{-3}$ AuNPs, size 30 nm | |
| Variant 9 | 2% of lavender tissue propagated on media with addition of 10 mg · $dm^{-3}$ AuNPs, size 30 nm | |

Explanations: AuNPs – gold nanoparticles, AgNPs – silver nanoparticles.

Each culture test was performed with six replicates. Cultures on the BHI medium were incubated at 37°C and the cultures on the Sabouraud medium were incubated at 25°C. The cultures were incubated for up to 24 h (bacteria) and 48 h (fungi). After this, the colonies were counted.

**Statistical analyses**

The results of all the experiments were statistically analysed using one-way analysis of variance. To evaluate the significance of the differences between treatments, Tukey's test was performed at $p = 0.05$.

## RESULTS

Microbial contamination may occur during the manufacturing process (primary contamination) and/ or during consumer use (secondary contamination) (Mitsui T. 1997). Most cosmetic products are multi-use products that are also required to maintain low levels of contamination during consumer use, which means that their preservation systems need to be effective against contaminants that come in contact with the product after opening. An ideal preservative should protect the product from microbial contamination, both in its original closed packaging until use and in an open container throughout its use (Pitt et al. 2015). To estimate the level of microorganisms in a sample of a cosmetic product, it is required to select the appropriate conditions of each culture (e.g. culture medium, dilution, temperature, and period of incubation). These conditions encourage the microorganisms to grow, resulting in the inactivation of the preservative system present in the sample (Office of Regulatory Affairs, 2015). In this study, we investigated the preservative properties of true lavender tissue that was cultivated *in vitro* on media supplemented with AuNPs or AgNPs at a particle size of 13 and 30 nm and ground in liquid nitrogen. Tables 2 and 3 present the results of this analysis.

In this study, only the addition of synthetic preservative (DHA BA) resulted in complete inhibition of growth of bacterial and fungal colonies for up to 8 weeks of the experiment. The most rapid development of microorganisms was observed for control cosmetic emulsions, to which no plant tissues and DHA BA were added.

Table 2. Number of bacterial colonies in tested cosmetic emulsions per dish (±SD)

| Cream preservation method | | | Storage temperature | Bacterial colony count/dish | | | |
|---|---|---|---|---|---|---|---|
| Preservative | Type and concentration of NPs in the medium | | | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
| None | | | 24°C | $0.6^{b} \pm 0.47$ | $2.3^{b} \pm 0.47$ | $4.5^{b} \pm 0.71$ | $20.9^{b} \pm 0.96$ |
| None | | | 4°C | $5.2^{a} \pm 0.68$ | $14.7^{a} \pm 1.37$ | $35.4^{a} \pm 2.06$ | $62.3^{a} \pm 1.97$ |
| DHA BA | | | | $0^{c}$ | $0^{c}$ | $0^{e}$ | $0^{f}$ |
| 2% tissue of *in vitro* plant | Control medium without NPs | | | $0^{c}$ | $0^{c}$ | $3.5^{bcd} \pm 0.32$ | $8.5^{c} \pm 0.75$ |
| | 13 nm AgNPs | 1 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $0^{e}$ | $0.9^{ef} \pm 0.69$ |
| | | 10 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $1.4^{de} \pm 0.47$ | $3.9^{de} \pm 1.07$ |
| | 30 nm AgNPs | 1 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $1.8^{cde} \pm 0.68$ | $4.5^{de} \pm 0.96$ |
| | | 10 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $2.2^{bcde} \pm 0.37$ | $4.9^{d} \pm 1.34$ |
| | 13 nm AuNPs | 1 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $3.0^{bcd} \pm 0.57$ | $7.1^{cd} \pm 1.21$ |
| | | 10 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $2.9^{bcd} \pm 0.69$ | $6.1^{cd} \pm 1.21$ |
| | 30 nm AuNPs | 1 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $4.5^{b} \pm 0.74$ | $.6.9^{cd} \pm 1.57$ |
| | | 10 mg · dm$^{-3}$ | | $0^{c}$ | $0^{c}$ | $4.0^{bc} \pm 0.58$ | $5.5^{d} \pm 1.21$ |
| LSD $_{0,05}$ | | | | 0.10 | 0.12 | 2.35 | 2.31 |

Explanations: NPs – nanoparticles, AuNPs – gold nanoparticles, AgNPs – silver nanoparticles, LSD – least significant difference, DHA BA – dehydroacetic acid and benzoic acid.

A significant effect of temperature on the appearance of bacterial colonies was observed in the case of samples collected from variants stored at 24°C than those stored at 4°C. After 8 weeks of storage at 24°C, the samples showed a three-fold greater count of bacterial colonies (62.3) than those stored at 4°C (20.9). However, storage temperature did not have any effect on the count of fungal colonies – in the case of variants stored at 24°C, 5.2 colonies were observed, whereas in the case of one stored at 4°C, 5.8 colonies were observed.

Table 3. Number of fungal colonies in tested cosmetic emulsions per dish (±SD)

| Cream preservation method | | | Storage temperature | Fungal colony count/dish | | | |
|---|---|---|---|---|---|---|---|
| Preservative | Type and concentration of NPs in the medium | | | 2 weeks | 4 weeks | 6 weeks | 8 weeks |
| None | | | 24°C | $0.2^{a} \pm 0.37$ | $0.2^{b} \pm 0.37$ | $0.3^{c} \pm 0.47$ | $5.8^{a} \pm 2.19$ |
| None | | | 4°C | $0.2^{a} \pm 0.37$ | $2.1^{a} \pm 1.34$ | $4.5^{a} \pm 1.80$ | $5.2^{a} \pm 1.34$ |
| DHA BA | | | | $0^{b}$ | $0^{b}$ | $0^{d}$ | $0^{e}$ |
| 2% tissue of *in vitro* plant | Control medium without NPs | | | $0^{b}$ | $0^{b}$ | $0^{d}$ | $2.5^{b} \pm 0.37$ |
| | 13 nm AgNPs | 1 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0^{d}$ | $0.7^{e} \pm 0.47$ |
| | | 10 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0.2^{cd} \pm 0.37$ | $0.9^{e} \pm 0.81$ |
| | 30 nm AgNPs | 1 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0.3^{bc} \pm 0.47$ | $1.3^{de} \pm 1.37$ |
| | | 10 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0^{d}$ | $1.1^{e} \pm 1.11$ |
| | 13 nm AuNPs | 1 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0.6^{b} \pm 0.47$ | $2.9^{b} \pm 1.06$ |
| | | 10 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | 0 | $1.9^{cd} \pm 1.67$ |
| | 30 nm AuNPs | 1 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0^{d}$ | $2.7^{b} \pm 2.67$ |
| | | 10 mg · dm$^{-3}$ | | $0^{b}$ | $0^{b}$ | $0.2^{d} \pm 0.01$ | $3.1^{b} \pm 2.41$ |
| LSD $_{0,05}$ | | | | 0.01 | 0.05 | 0.16 | 0.61 |

Explanations: NPs – nanoparticles, AuNPs – gold nanoparticles, AgNPs – silver nanoparticles, LSD – least significant difference, DHA BA – dehydroacetic acid and benzoic acid.

Addition of lavender tissue propagated on control media that did not contain AuNPs or AgNPs protected the samples against the development of bacteria for 4 and fungi for 6 weeks. After 8 weeks of storage, these variants showed 9.5 bacterial and 1.2 fungal colonies.

In the case of cosmetic emulsions supplemented with tissue-propagated lavender on media enriched with NPs, the appearance of bacterial colonies was observed after 6 weeks of storage. An exception was the variant containing lavender tissue propagated in medium containing 1 mg · dm$^{-3}$ AgNPs with a particle size 13 nm; in this case only a few colonies were observed after 8 weeks of storage (0.9). This result is comparable to the variant preserved with synthetic preservative – DHA BA. In the remaining variants, i.e. lavender tissue propagated on media containing AuNPs or AgNPs, the count of bacterial colonies determined after 8 weeks of storage ranged between 3.9 and 7.1.

In the case of fungal colonies, lavender tissue propagated on media containing AuNPs or AgNPs, inhibited their development up to 4 weeks of storage (independently of the particle size and type). However, after 6 weeks of storage, the appearance of fungal colonies was detected in cosmetic emulsions containing lavender tissue propagated on media that did not contain AuNPs or AgNPs. Fungal colonies were also detected in cultures enriched with 10 mg · $dm^{-3}$ AgNPs with a particle size of 13 nm, 1 mg · $dm^{-3}$ with a particle size of 30 nm AgNPs, and in 10 mg · $dm^{-3}$ AuNPs with a particle size of 13 and 30 nm (Table 3). After 8 weeks of incubation, fungal colonies were observed in samples collected from all the tested variants. The lowest colony count was determined for variants containing lavender tissue propagated on media containing 1 and 10 mg · $dm^{-3}$ AgNPs with a particle size of 13 nm, which was only 0.7 and 0.9 colonies, respectively.

A comparison of antimicrobial activity of plant tissue propagated on media containing AgNPs and AuNPs showed that lavender tissues propagated on media containing AgNPs showed higher activity than that of AuNPs.

## DISCUSSION

In this study, we conducted experiments to determine the possibility of using true lavender tissue propagated on media containing AuNPs or AgNPs as preservatives for cosmetic emulsions. According to our results, the tissue-propagated lavender demonstrated preservative properties. Tissue propagated in the absence of NPs ground in liquid nitrogen and added to cosmetic emulsion protected the cosmetic from the development of microorganisms for up to 4 weeks. Andrys et al. (2018) studied three cultivars of *L. angustifolia* and demonstrated that plants propagated in *in vitro* cultures are characterised by a comparatively higher antimicrobial and antioxidative activity than those cultivated in field conditions. This is probably linked to the content of essential oils in their tissues. Muyima et al. (2002) studied the essential oils of *L. officinalis* plants and demonstrated their efficiency in the reduction of microbiological contamination in an aqueous cream formulation. The bacterial population was markedly restricted up to day 7 of the experiments. Herman et al. (2013) compared the efficacy of synthetic versus essential oils toward inhibition of growth of microorganisms. Their results showed a higher inhibitory activity by essential oils against microorganisms than that of synthetic preservatives in cosmetic emulsion. They tested the essential oils obtained from *L. officinalis*, *Melaleuca alternifolia*, and *Cinnamonium zeylannicum* and methylparaben as the preservative. Our study provides further information on the possibility of using essential oils as preservatives instead of synthetic preservatives.

Lavender tissue propagated on media containing 1 mg · $dm^{-3}$ AgNPs with a particle size of 13 nm was characterised by higher antimicrobial activity than that of control. The bacterial contamination was detected after 6 weeks of incubation, and the inhibition was found to be marked for up to 8 weeks. Kim et al. (2006) demonstrated that methyl jasmonate sprayed on *Ocimum basilicum* L. plants significantly increased the content of rosmarinic acid and caffeic acid, as well as it increased eugenol and linalool. These compounds show strong antimicrobial and antioxidant activity. Poulev et al. (2003) showed a powerful effect of elicitation of stress on increasing the antibacterial and antifungal activity of root extracts

of many plant species. Furthermore, Andrys et al. (2018) confirmed the possibility of using essential oil isolated from *L. angustifolia* elicited with jasmonic acid in *in vitro* cultures as a cosmetic emulsion preservative (Patent Andrys, Kulpa).

In our pervious study we showed that AuNPs and AgNPs have a significant effect on the composition and content of essential oil in true lavender propagated in *in vitro* cultures. AuNPs and AgNPs increased notably the content of antimicrobial compounds such as, borneol, γ-cadinene, caryophylleneoxide, τ-cadinol, cadalene, cis-14-nor-muurol-5-en-4-one, and bisabolol oxide A. The percentage increase in these compounds was dependent on the concentration of the nanoparticle used. For instant in case of τ-cadinol, there has been an increase in content up to 37%,for bisabolol oxide A up to 31%, for cis-14-nor-muurol-5-en-4-one up to 66%,and for γ-cadinene up to 22%. (Wesołowska et al. 2019).

Higher concentrations of AgNPs in the culture medium, as well as the particle size of 30 nm, resulted in the decreased preservative capacity of plant tissues used in our experiments. This result may be an outcome of two factors: On the one hand, bigger particle size, which did not allow to penetrate the cell membranes, prevented the accumulation of AgNPs in the tissues. On the other hand, excessive concentration of AgNPs in the culture medium produced excessive oxidative stress, which in turn exhausted the defensive capacities of lavender (it produced lower amounts of secondary metabolites).

The increased antimicrobial activity of lavender tissues propagated on culture media containing AgNPs and AuNPs may be associated with the presence of NPs in plant tissues, which penetrate the cell membranes. Domokos-Szabolcsy et al. (2012) and Lee et al. (2008) demonstrated that NPs penetrate the plant cells during the growth in the media in *in vitro* cultures. The antimicrobial activity of AgNPs and AuNPs is widely known in the literature (Rai et al. 2009; Gong et al. 2007; Zhang et al. 2015). AgNPs show positive effects on problematic skin suffering from acne because of its antimicrobial properties. It shows healing properties by accelerating wound healing, and it prevents the formation of scars (Gajbhiye et al. 2016). AgNPs is currently used as an additive in a range of beautifying face and body masks, as well as in products for improving skin conditions and in mouthwashes (Pardeike et al. 2009). The introduction of NPs along with plant tissues into a cosmetic product may be favorable – they can have a synergistic effect on the skin.

When considering the use of nanoparticles as elicitors or adding plant tissues grown on nutrients with them to cosmetics, careful attention should be paid to the health aspects associated with them. In a study by Asha Rani et al. (2009) it was found that AgNPs with a diameter penetrated inside human cells and induced DNA changes. However, the research was carried out by adding silver nanoparticles to the media in which the lung fibroblast cells (IMR-90) and human glioblastoma cells (U251) were multiplied, instead of treating living organisms with a protective layer with them. The toxicity of AuNPs towards *Arabidopsis thaliana* root cells was also indicated by Taylor et al. (2009).

Most of the published research results indicate the lack of toxicity of nanoparticles added to cosmetics. It is assumed that NPs are safe for the consumer in the cosmetic industry. At minimal and reasonable concentrations of NPs, there are no side effects on human health. According to a previous study, NPs in the current cosmetics do not penetrate the human skin, even in cases when the skin is damaged (Gajbhiye et al. 2016). Kokura et al. (2010) showed

that Ag nanoparticles are not able to penetrate human skin. However, when the barrier function of human skin is disrupted, Ag nanoparticles on the skin surface may penetrate the skin. It may be possible that 0.2% to 2% of Ag nanoparticles could penetrate the skin (0.002–0.02 ppm). At these levels Ag nanoparticles did not show any toxicity. Zang (2013) believes that the penetration of AuNPs depends on the particle size. Nanoparticles with a diameter less than 10 nm could reach the deeper layer of the *stratum corneum*, while NP larger than 40 nm could only reach 5–8 μm into the *stratum corneum*. Campbell et al. (2012), who stated that nanoparticles (20 to 200 nm) contacting intact or partially damaged skin cannot penetrate skin barrier and permeate to lower strata making them safe as cosmeceuticals.

The presence of AuNPs in culture media also had a positive effect on the antimicrobial activity of lavender; however, the effect was lesser than that of AgNPs. Lavender propagated on media containing AuNPs independently of the used concentration and particle size protected the cream from bacterial growth up to 4 weeks and fungal growth, depending on the particle diameter, for up to 4–6 weeks. This result is in-line with the results obtained by Wesołowska et al. (2019), who examined essential oils isolated from *L. angustifolia* tissue elicited with AuNPs and AgNPs determined that AgNPs has a stronger impact on the change of the composition of essential oils than AuNPs.

## CONCLUSIONS

Narrow leaf lavender tissue propagated *in vitro* showed preservative properties. The addition of AuNPs and AgNPs to the culture media increases the preservative potential of lavender in various forms of cosmetic products; however, the effect depends on the particle size of the metal and its concentration in the medium. Disintegrated fragments of narrow leaf lavender propagated *in vitro* on media containing 1 mg · $dm^{-3}$ AgNPs with a particle size of 13 nm can be used to preserve cosmetic emulsions, particularly those with short shelf life.

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## REFERENCES

**Aburjai T., Natsheh F.M.** 2003. Plants used in cosmetics. Phytother. Res. 17, 987–1000.

**Adam K., Sivropoulou A., Kokkini S., Lanaras T., Arsenakis M.** 1998. Antifungal activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agric. Food. Chem. 46, 1739–1745.

**Allied Market Research.** 2016. https://www.alliedmarketresearch.com/cosmetics-market, access: 15.03.2020.

**Amoo S.O., Aremu A.O., Van Staden J.** 2012. *In vitro* plant regeneration, secondary metabolite production and antioxidant activity of micropropagated *Aloe arborescens* Mill. Plant. Cell. Tiss. Organ. Cult. 111, 345–358.

**Andrys D., Adaszyńska-Skwirzyńska M., Kulpa D.** 2018. Essential oil obtained from micropropagated lavender, its effect on HSF cells and application in cosmetic emulsion as a natural protective substance. Nat. Prod. Res. 32, 849–853.

**Andrys D., Kulpa D., Grzeszczuk M., Białecka B.** 2018. Influence of jasmonic acid on the growth and antimicrobial and antioxidant activities of *Lavandula angustifolia* Mill. propagated *in vitro*. Folia Hortic. 30, 3–13.

**AshaRani P.V., Low Kah Mun G., Prakash Hande M., Valiyaveettil S.** 2009. Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. ACS Nan. 3(2), 279–290.

**Bais H.P., Walker T. S., Schweizer H.P., Vivanco J.M.** 2002. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of *Ocimum basilicum*. Plant. Physiol. Bioch. 40, 983–995.

**Behravan J., Bazzaz F., Malaekeh P.** 2005. Survey of bacteriological contamination of cosmetic creams in Iran (2000). Int. J. Dermatol. 44, 482–485.

**Brailko V.A., Mitrofanova O., Leśnikowa-Sedoshenko N., Chelombit S., Mitrofanova I.V.** 2017. Anatomy features of *Lavandula angustifolia* Mill. and *Lavandula hybrida* rev. plants *in vitro*. J. Agric. For. 63, 111–117.

**Campbell C.S.J., Contreras-Rojas L.R., Delgado-Charro M.B,. Guy R.H.** 2012 Objective Assessment of Nanoparticle Disposition in Mammalian Skin after Topical Exposure. J. Controll. Rel.. 162, 201–207.

**Cavanagh H.M.A., Wilkinson J.M.** 2005. Lavender essential oil: a review. Aust. Infect. Control. 10, 35–38.

**D´auria F.D., Tecca M., Strippoli V., Salvatore G., Battinelli L., Mazzanti G.** 2005. Antifungal Activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form. Med. Mycol. 43, 391–396.

**Demissie Z.A., Sarker L.S., Mahmoud S.S.** 2011. Cloning and functional characterization of β-phellandrene synthase from *Lavandula angustifolia*. Planta 233, 685–96.

**Domokos-Szabolcsy E., Marton L., Sztrik A., Babka B., Prokish J., Fari M.** 2012. Accumulation of red elemental selenium nanoparticles and their biological effects in *Nicotinia tabacum*. Plant. Growth. Regul. 68, 525–531.

**EU Patent. Andrys D., Kulpa D.** 2019. Oil/ wather cosmetic emulsion PLA 41341615.

**Fonseca-Santos B., Correa A.A., Chorilli M.** 2015. Sustainability, natural and organic cosmetics: consumer products, efficacy, toxicological and regulatory considerations. Braz. J. Pharm. Sci. 51, 2175–9790.

**Gajbhiye S., Sakharwade S.** 2016. Solver Nanoparticles in Cosmetics. J. Cosm. Dermatol. Sci. App. 6, 48–53.

**Golden R., Gandy J., Vollmer G.** 2005. A Review of the Endocrine Activity of Parabens and Implications for Potential Risks to Human Health. Crit. Rev.Toxiol. 35, 435–458.

**Golkar P., Moradi M., Garousi A.G.** 2019. Elicitation of Stevia Glycosides Using Salicylic Acid and Silver Nanoparticles Under Callus Culture. Sugar. Tech. 4, 569–577.

**Gonçalves S., Romano A.** 2013. *In vitro* culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnol. Adv. 31, 166–174.

**Gong P., Li H., He X., Wang K., Hu J., Tan W., Ahang S., Yang X.** 2007. Preparation and antibacterial activity of Fe3O4Ag nanoparticles. Nanotechnol. 18, 604–11.

**Groot C., White I.R.** 1995. Textbook of Contact Dermatitis, 2 ed., Berlin, Springer-Verlag, 461.

**Halla N., Fernandes I.P., Heleno S.A., Costa P., Boucherit-Otmani Z., Boucherti K., Rodrigues A.E., Ferreria I.C.F.R., Barrerio M.F.** 2018. Cosmetics Preservation: A Review on Present Strategies. Molecules 23, 1571.

**Herman A., Herman A.P., Domagalska B.W., Młynarczyk A.** 2013. Essential oils and herbal extracts as antimicrobial agents in cosmetic emulsion. Indian. J. Microbiol. 53, 232–237.

**Jakowijević D.Z., Sava M. V., Stanković Čomić L., Topuzović M.D.** 2015. Secondary metabolite content and *in vitro* biological effects of *Ajuga chamaepitys* (L.) *Schreb Subsp Chamaepitys*. Arch. Biol. Sci. Belgrade 67,1195–1202.

**Jamshidi M., Ghanti F., Razaei A., Bemani E.** 2014. Change of antioxidant enzymes activity of hazel (*Corylus avellana* L.) cells by AgNPs. Cytoteh. 68, 525–530.

**Jopke K., Sanders H., White-Traut R.** 2017. Use of Essential Oils Following Traumatic Burn Injury: Acase Study. J. Pedriat. Nurs. 34, 72–77.

**Kerdudo A., Burger P., Merck F., Dingas A., Rolland Y., Michel T., Fernandez X.** 2016. Development of a natural ingredient- Natural preservative: A case study. Comptes. Rendus. Chimie. 19, 1077–1098.

**Kim H.-J., Chen F., Wang X., Rajapakse N.C.** 2006. Effect of Methyl Jasmonate on Secondary Metabolites of Sweet Basil (*Ocimum basilicum* L.). J. Agr. Food. Chem. 54, 2327–2332.

**Kokura S., Handa O., Takagi T., Ishikawa T., Naito Y., Yoshikawa T.** 2010. Silver Nanoparticles as a Safe Preservative for Use in Cosmetic. Nanomed. Nanotech. Biol. Med. 6, 570–574.

**Królicka A., Szpitter A., Gilgenast E., Romanik G., Kaminski M., Lojkowska E.** 2008. Stimulation of antibacterial naphthoquinones and flavonoids accumulation in carnivorous plants grown *in vitro* by addition of elicitors. Enzyme. Microbial. Technol. 42, 216–221.

**Kusumawati I., Idrayanto G.** 2013. Natural Antioxidants in Cosmetics. Stud. Nat. Prod. Chem. 40, 485–505.

**Lee W., An Y., Yoon H., Kweon H.** 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum awstivum*): plant uptake for water insoluble nanoparticles. Environ. Toxicol. Chem. 27, 1915–21.

**Mario P., Carvalho R., Amoro C., Santos R., Cardoso J.** 2012. Contact allergy to methylchoroisothiazoline/ methylisothiazolinone (MCI/MI): findings from a Contact Dermatitis Unit. Cutan. Ocul. Toxicol. 31, 151–153.

**Mitsui T.** (ed.). 1997. Preservation of cosmetics in New Cosmetic Science. Amsterdam, Elsevier, 199–208.

**Moharrami F., Hosseini B., Sharafi A., Farjaminezhad M.** 2017. Enhanced production of hyoscyamine and scopolamine from genetically trans-formed root culture of *Hyoscyamus reticulatus* L. elicited by iron oxide nanoparticles. In Vitro. Cell. Dev. Biol. Plant. 53, 104–111.

**Murashige T., Skoog,F.** 1962. A revised medium for rapid growth and bio assays with Tobacco Tissue Cultures. Physiol. Plantarum. 15, 473–497.

**Muyima N.Y.O., Zulu G., Bhengu T. Popplewell D.** 2002. The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. Flavour. Fragr. J. 17, 258–266.

**Nostro A., Cannatelli M. A., Morelli I., Musolino A. D. Scuderi F. Pizzimenti F., Alonzo V.** 2004. Efficiency of *Calamintha officinalis* essential oil as preservative in two topical product types. J. Appl. Microbiol. 97, 395–401.

**O'Dell L.E., Sullivan A., Periman L.M.** 2016. Beauty does not have to hurt. Adv. Ocul. Care. 7/8, 42–47.

**Office of Regulatory Affairs.** 2015. Pharmaceutical Microbiology Manual, vol. ORA.007. Office of Regulatory Affairs, Silver Spring, Meryland, USA.

**Orton D.I., Wilkinson J.D.** 2004. Cosmetic Allergy. Incidence, Diagnosis, and Management. Am. J. Clin. Dermatol. 5, 327–337.

**Pardeike J., Hommoss A., Müller R.H.** 2009. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int. J. Pharm. 366, 170–184.

**Pitt T.L., McClure J., Parker M.D., Amezquita A., McClure P.J.** 2015. *Bacillus cereus* in personal care products: Risk to consumers. Int. J. Cosmet. Sci. 37, 165–174.

**Poulev A., O'Neal J.M., Logendra S., Pouleva R.B., Timeva V., Garvey A.S., Raskin I.** 2003. Elicitation, a New Window into Plant Chemodiversity and Phytochemical Drug Discovery. J. Med. Chem. 46, 2542–2547.

**Rai M., Yadav A., Gade A.** 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27, 76–83.

**Schwiertz A., Duttke C., Hild J.** 2006. *In vitro* activity of essential oils on microorganisms isolated from vaginal infections. Inter. J. Aromather. 16, 169–174.

**Shakeran Z., Keyhanfar M., Asghari G., Ghanadian M.** 2015. Improvement of atropine production by different biotic and abiotic elicitors in hairy root cultures of *Datura metel*. Turk. J. Biol. 39, 111–118.

**Sticher O., Heilmann J., Zündorf I.** 2015. Hänsel & Sticher Pharmakognosie-Phytopharmazie, 10nd ed. Stuttgart, Germany, Wissenschaftliche Verlagsgesellschaft Press, 673.

**Szabo E., Thelen A., Petersen M.** 1999. Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei.* Plant. Cell. Rep. 18, 485–489.

**Taylor A.F., Rylott E.L., Anderson C.W.N., Bruce N.C.** 2014. Investigating the Toxicity, Uptake, Nanoparticle Formation and Genetic Response of Plants to Gold. PLoS ONE 9(4), e93793.

**Upson T., Andrews S.** 2004. The genus *Lavandula*.1nd ed., Portland, Oregon, Timber Press.

**Vanisree M., Hsin-Sheng T.** 2004. Plant Cell Cultures. An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites. Int. J. Appl. Sci. Eng. 2, 29–48.

**Wesołowska A., Jadczak P., Kulpa D., Przewodowsk W.** 2019. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Essential Oil from AgNPs and AuNPs Elicited *Lavandula angustifolia in vitro* Cultures. Molecules. 24, 606.

**Wornouk G., Dermissie Z., Rheault M., Mahmoud S.** 2011. Biosynthesis and therapeutic properties of *Lavandula* essential oil constituents. Planta. Med. 77, 7–15.

**Zhang M.** 2013. Au Natural Nanoparticles Sun Protection. Cosmet. Toiletries. 128, 440.

**Zhang Y., Dasari T.P.S., Deng H., Yu H.** 2015. Antimicrobial Activity of Gold Nanoparticles and Ionic Gold. J. Environ. Sci. Helath, C. Environ. Carcinog. Ecotoxicol. Rev. 33, 286–327.

**Złotek U., Michalak-Majewska M., Szymanowska U.** 2016. Effect of jasmonic acid elicitation on the yield, chemical composition, and antioxidant and anti-inflammatory properties of essential oil of lettuce leaf basil (*Ocimum basilicum* L.). Food. Chem. 15, 1–7.

## *Lavandula angustifolia* NAMNAŻANA W KULTURACH *IN VITRO* NA POŻYWKACH ZAWIERAJĄCYCH AgNP i AuNPs – ALTERNATYWNE ROZWIĄZANIE DLA SYNTETYCZNYCH KONSERWANTÓW W KOSMETYKACH

**Streszczenie.** Określono właściwości konserwujące lawendy wąskolistnej namnażanej na pożywkach z nanocząstkami złota lub srebra o rozmiarach cząsteczek 13 i 30 nm. Emulsje kosmetyczne, przygotowane przy użyciu lawendowej tkanki namnażanej na podłożach zawierających AuNPs i AgNPs, wykazały zwiększone właściwości konserwujące w porównaniu

z emulsjami kontrolnymi. W przypadku kontrolnych emulsji kosmetycznych, które nie zawierały dodatku tkanek roślinnych ani kwasów dehydrooctowego i benzoesowego (DHA BA), po drugim tygodniu trwania doświadczenia zaobserwowano pojawianie się kolonii bakteryjnych i grzybowych. Dodatek tkanki lawendy namnażanej na pożywkach bez nanocząstek chronił badane próbki przed zanieczyszczeniem mikrobiologicznym; w tym przypadku kontaminacja bakteryjna została zaobserwowana po 4 tygodniach, a grzybowa po 6 tygodniach. Dodatek tkanki lawendy, namnażanej na pożywce zawierającej AgNPs o wielkości cząstek 13 nm, w stężeniu 1 mg · $dm^{-3}$ wydłużył czas pojawiania się kolonii bakteryjnych do 8 tygodni (0,9); wynik ten był bliski i porównywalny z efektem DHA BA. Wyższe stężenia AgNPs w pożywkach, a także większa średnica cząstek (30 nm) spowodowały zmniejszenie aktywności konserwujących tkanek roślinnych. Obecność AuNPs w mediach hodowlanych wykazała pozytywny wpływ na aktywność antybakteryjną lawendy, jednak w mniejszym stopniu niż w przypadku AgNPs. Rozdrobnione fragmenty tkanki lawendy, namnażane na pożywkach zawierających 1 mg · $dm^{-3}$ AgNP, o wielkości cząstek 13 nm, mogą być stosowane do zachowania emulsji kosmetycznych w krótkim okresie trwałości.

**Słowa kluczowe:** emulsja kosmetyczna, nanocząstki metalu, srebro, złoto, mikropropagacja, elicytacja.