Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Denisa Manolescu, Georgiana Uță, Anca Șuțan, Cătălin Ducu, Alin Din, Sorin Moga, Denis Negrea, Andrei Biță, Ludovic Bejenaru, Cornelia Bejenaru, Speranța Avram (2022). Biogenic synthesis of noble metal nanoparticles using *Melissa officinalis* L. and *Salvia officinalis* L. extracts and evaluation of their biosafety potential. *Caryologia* 75(3): 65-83. doi: 10.36253/caryologia-1774

Received: August 02, 2022

Accepted: September 11, 2022

Published: April 5, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Biogenic synthesis of noble metal nanoparticles using *Melissa officinalis* L. and *Salvia officinalis* L. extracts and evaluation of their biosafety potential

Denisa Manolescu[1,2], Georgiana Uță[1,2,*], Anca Șuțan[3], Cătălin Ducu[1], Alin Din[1], Sorin Moga[1], Denis Negrea[1], Andrei Biță[4], Ludovic Bejenaru[4], Cornelia Bejenaru[5], Speranța Avram[2]

[1] *Regional Research and Development Center for Innovative Materials, Products and Processes from Automotive Industry, University of Pitesti, 11 Doaga Street, 110440 Pitesti, Arges, Romania*
[2] *Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, 91-95th Independence Street, RO-050095 Bucharest, Romania*
[3] *Department of Natural Sciences, Faculty of Science, Physical Education and Informatics, University of Pitesti, Targu din Vale Street, 110040 Pitesti, Arges, Romania*
[4] *Department of Pharmacognosy & Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Dolj County, Romania*
[5] *Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Dolj County, Romania*
* Corresponding author. E-mail: georgiana.uta@drd.unibuc.ro

Abstract. In this study we targeted the noble metal nanoparticles (MNPs) biogenic synthesis capacity of two medicinal species with therapeutic potential, namely *Melissa officinalis* L. (lemon balm) and *Salvia officinalis* L. (sage), cultivated in Romania. Plant material was extracted by maceration, microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE). Bright field scanning transmission electron microscopy and energy dispersive X-ray spectroscopy (BFSTEM-EDS) techniques were used in order to investigate particles shape, dispersion and chemical elemental analysis. The total polyphenol content for both simple extracts and nanostructured mixtures was determined using the Folin-Ciocalteu method and antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Identification and quantification of secondary metabolites of *M. officinalis* and *S. officinalis* were performed by ultra-high performance liquid chromatography (UHPLC). The *Allium* assay was used to evaluate the potential cytogenotoxic activity, for both simple and nanostructured phytochemical complexes, in the case of *S. officinalis* L. species being performed for the first time. Spherical shaped MNPs with diameters of about 20 nm were biosynthesised in lemon balm extracts. Larger AuNPs were phytosynthesized in sage extract obtained by UAE. Compared to the simple extracts, the antioxidant capacity as well as the amount of total polyphenols in the nanostructured extracts decreased, substantiating the involvement of bioorganic material in the reduction of metal ions. Low frequency of chromosomal aberrations corresponding to crude extracts and extracts supplemented with MNPs, suggest the cytoprotective, antigenotoxic, and safe use of these plant species as potential therapeutic forms in various diseases.

Keywords: lemon balm, sage, metal nanoparticles, antioxidant, cytotoxicity.

INTRODUCTION

Melissa officinalis L. (lemon balm) and *Salvia officinalis* L. (sage) are representatives medicinal plant species family Lamiaceae, whose remarkable therapeutic effects have been attested since ancient times (Shakeri et al. 2016; Ghorbani and Esmaeilizadeh 2017).

The bioactivity of natural compounds of *M. officinalis* has been noted especially for the treatment of neuropsychiatric disorders such as Alzheimer's and Parkinson's diseases, epilepsy, psychosis, depression or anxiety (Gomes et al. 2009; Shakeri et al. 2016; Avram et al. 2017; Udrea et al. 2018). As for *S. officinalis*, this plant species has been cultivated both as a medicinal plant, used therapeutically by humans for the treatment of various diseases such as gout, hyperglycemia, paralysis, rheumatism, cancer, bronchitis, and not least in the relief of symptoms of neurodegenerative diseases (Garcia et al. 2016; Šulniūtė et al. 2016), for decorative purposes (Kintzios 2000) and food or spice (Longaray Delamare et al. 2007).

However, the use of plant extracts in medicine is quite limited, mainly due to the inability of therapeutic plant compounds to penetrate the target, affected structures of organisms. This phenomenon occurs because of the large size of phytochemicals compared to the size of the target structures. It is now well known that due to the nanometric size of noble metal nanoparticles (MNPs), biocompounds embedded in such "capsules" or attached to their surface show both higher bioavailability and stability (Pandey et al. 2003).

At present, the literature abounds with information on the advantages of using different types of metal nanoparticles synthesised using plant extracts in therapy, making phytosynthesis a promising and sustainable alternative to conventional chemical or physical methods (Azeez et al. 2020; Naikoo et al. 2021; Shelembe et al. 2022). These nanostructured phytocomplexes are also only toxic at extremely high concentrations, doses which are not currently used for therapeutic purposes (Badmus et al. 2022).

The biosynthesis of MNPs aims, along with the fragmentation of phytochemicals, to embed various therapeutic plant compounds, or even certain drugs, in nanosized "metal envelopes" that allow their penetration and release into all structures of the target organism (Sun et al. 2008; Kumari et al. 2010; Parveen et al. 2012).

In addition, in recent decades several technologies have emerged to deliver along with NPs conventional drugs, recombinant proteins or even vaccines or nucleotides needed to treat cancer or other diseases (Parveen et al. 2012).

Since the antibacterial and anti-inflammatory action of silver nanoparticles (AgNPs) due to Ag ions is well known worldwide (Kirsner et al. 2001; Tripathy et al. 2008; Yilmaz Öztürk 2019), recently, the attention of researchers has been directed towards obtaining silver chloride nanoparticles (AgClNPs) which have been shown to exhibit identical or even improved properties compared to AgNPs (Eugenio et al. 2018). Moreover, AgClNPs have attracted considerable attention because they are easier to synthesize and also exhibit strong antimicrobial activity (Hu et al. 2009).

Gold (Au) nanorods have become some of the most important and commonly used materials in drug delivery and nanomedicine. The main reason for the use of AuNPs is to facilitate targeted drug transport, particularly in cancer therapy. To this end, a system using AuNPs conjugated with tumour necrosis factor (TNF) molecules has been designed, that has the effect of efficiently destroying only tumour cells while having low cytotoxicity to healthy cells (Mocellin and Nitti 2008; Das et al. 2011).

In addition, the pharmacological properties of lemon balm and sage are due to the content in secondary metabolites, such as polyphenols, alkaloids, triterpenes or sterols (Jaimez Ordaz et al. 2018; Uță et al. 2021) which are usually found in quite low concentrations, their recovery in a higher concentration being a challenge. One of the most important factors affecting the quality of bioactive compounds obtained from plant sources is the extraction method, also considered as a sample preparation technique, playing a vital role on the overall yield and final result. The conventional extraction methods, e.g. maceration, Soxhlet extraction, have been intensively used in recent decades (Zhang et al. 2018), but they have a number of drawbacks like time-consuming and use of a large amount of solvent. The latter not only increases process costs but is also associated with a negative environmental impact (Sasidharan et al. 2011). Therefore, numerous studies have aimed at developing efficient and environmentally friendly extraction techniques. Among the extraction techniques that have been successfully applied in obtaining active phytocompounds are microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) (Wang and Weller 2006; Grosso et al. 2015). Studies in the literature highlight that the amount of polyphenols extracted from this medicinal plant varies depending on certain factors such as: extraction method, solvent range, solvent:plant ratio, temperature, extraction time (Hernández et al. 2009; Zhang et al. 2018), stages of its primary processing (drying, grinding), harvesting area of plant material (Dent et al. 2017), and harvesting period (Francik et al. 2020).

Phytotherapy based on plant extracts has gained worldwide popularity because it is not addictive, it does not have harmful side effects and a high risk of toxicity like synthetic medicines, and last but not least it is cheap (Avram et al. 2005; Andrade et al. 2019; Lin et al. 2019).

Thus, based on this information, this study was focused on the biogenic synthesis of noble metal nanoparticles, namely AgClNPs and AuNPs, using the medicinal species *M. officinalis* and *S. officinalis*, correlated with the determination of the optimal method for extracting the highest possible concentrations of phytocompounds from the plant species, the analysis of the antioxidant capacity of simple extracts and extracts supplemented with MNPs, as well as *in vivo* testing of the cytotoxic activity of the extracts obtained and of nanostructured phytochemical complexes, in order to highlight the safety of these systems as potential therapeutic forms.

MATERIALS AND METHODS

Reagents and chemicals

The 96.9% pharmaceutical ethyl alcohol used for the extraction processes was purchased from SC. Coman Product S.A.; 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, 99.5% absolute ethyl alcohol, distilled water, Na_2CO_3 powder, gallic acid, Trolox, glacial acetic acid, absolute ethyl alcohol, 1N HCl and orcein were purchased from Carlo Erba Reagents S.A.S; acetonitrile, methanol and water were purchased from Merck. Reference compounds such as protocatechuic acid, ferulic acid, p-coumaric acid, caffeic acid were obtained from Merck, while quercetin, rutin and rosmarinic acid were purchased from Sigma-Aldrich and chlorogenic acid was purchased from Alfa Aesar.

The plant material

Aerial parts of *M. officinalis*, *S. officinalis* leaves and *Allium cepa* L. bulbs, were collected from a local producer, Arges county, in September 2020. The authentication of the plant material was performed by Assoc. Prof. Ph.D. Anca Sutan, and kept in paper bags, protected from moisture and sunlight, until primary processing. Primary processing of lemon balm and sage consisted of drying the plants in an oven at 40°C, a temperature designed not to denature the phenolic compounds of interest, and grinding the plant material in a Retsch Grindomix laboratory mill at the following parameters: 3 min pulse grinding at 4,000 RPM and 30 sec continuous grinding at 10,000 RPM (Manolescu et al. 2022).

Extraction procedures

The extraction of secondary metabolites was performed by maceration as classical method and two non-conventional methods, MAE and UAE, respectively. Two different ratios of pharmaceutical ethyl alcohol and distilled water were used as solvent mixtures: 70:30 v/v and 50:50 v/v.

For plant maceration, 1 g of dry sample, ground and weighed on an analytical balance to 4 decimal places, was immersed in 10 ml solvent (pharmaceutical ethyl alcohol:distilled water). Maceration was carried out at room temperature, shielded from sunlight, for 7 days; the first 4 days with continuous stirring for 6 hours at 30 RPM on the Biosan mini-rotator, and the next 3 days without stirring (Dent 2015).

The same binary solvents and the same 1:10 plant to solvent ratio were used for MAE. Initially the plant material was hydrated in the solvent for 1 hour and then subjected to microwave irradiation for 3, 5 and 10 minutes at a maximum power of 250 W. Microwave-assisted extraction was performed using the NEOS-GR equipment, Milestone. The final temperature range of the samples was between 54-78°C (Dent 2015).

UAE was carried out using a Hielscher UP200St ultrasonic extraction system under a working amplitude equal to 80% of the maximum rated output power of the device. In order to avoid overheating of the experimental samples and possible destruction of phytocompounds we used a cooling system, extracts were obtained at temperatures below 45°C (Dent 2015; Žlabur et al. 2016).

All experimental variants (Table 1) were then centrifuged twice (10 min total time) at 6,000 RPM. The supernatants obtained were subjected to vacuum filtration through Pall Flex Membrane Filters QRY:100; MM: 47 filter paper on a Rocker model filtration system: VF6. Pending analysis of total polyphenol content, antioxidant activity, HPLC analysis, MNPs synthesis and evaluation of cytogenotoxicity, samples were kept in glass vials at -18°C.

Determination of the total polyphenol content of the obtained plant extracts and nanostructured phytochemical complexes

Quantitative determination of polyphenolic structure compounds in the obtained extracts was carried out by the Folin-Ciocalteu spectrophotometric method (Sutan et al. 2018). From each experimental variant, diluted beforehand until a dilution factor of 600 was reached, a volume of 500 µL extract was taken over which 2.5 ml Folin-Ciocalteu reagent 10% (aqueous mixture of phosphomolybdate and phosphotungstate) was

Table 1. Experimental variants used in this study.

No	Sample code	Plant species	Extraction procedure	Pharmaceutical ethyl alcohol:distilled water ratio (v/v)	Extraction parameters
1	M_M_50	*M. officinalis*	Maceration	50:50	7 days; room temperature; in the dark
2	M_M_70		Maceration	70:30	7 days; room temperature; in the dark
3	M_MAE_3_50		MAE	50:50	3 min; 250 W
4	M_MAE_5_50		MAE	50:50	5 min; 250 W
5	M_MAE_10_50		MAE	50:50	10 min; 250 W
6	M_MAE_3_70		MAE	70:30	3 min; 250 W
7	M_MAE_5_70		MAE	70:30	5 min; 250 W
8	M_MAE_10_70		MAE	70:30	10 min; 250 W
9	M_UAE_3_50		UAE	50:50	3 min; 80% Amp
10	M_UAE_5_50		UAE	50:50	5 min; 80% Amp
11	M_UAE_10_50		UAE	50:50	10 min; 80% Amp
12	M_UAE_3_70		UAE	70:30	3 min; 80% Amp
13	M_UAE_5_70		UAE	70:30	5 min; 80% Amp
14	M_UAE_10_70		UAE	70:30	10 min; 80% Amp
15	S_M_50	*S. officinalis*	Maceration	50:50	7 days; room temperature; in the dark
16	S_M_70		Maceration	70:30	7 days; room temperature; in the dark
17	S_MAE_3_50		MAE	50:50	3 min; 250 W
18	S_MAE_5_50		MAE	50:50	5 min; 250 W
19	S_MAE_10_50		MAE	50:50	10 min; 250 W
20	S_MAE_3_70		MAE	70:30	3 min; 250 W
21	S_MAE_5_70		MAE	70:30	5 min; 250 W
22	S_MAE_10_70		MAE	70:30	10 min; 250 W
23	S_UAE_3_50		UAE	50:50	3 min; 80% Amp
24	S_UAE_5_50		UAE	50:50	5 min; 80% Amp
25	S_UAE_10_50		UAE	50:50	10 min; 80% Amp
26	S_UAE_3_70		UAE	70:30	3 min; 80% Amp
27	S_UAE_5_70		UAE	70:30	5 min; 80% Amp
28	S_UAE_10_70		UAE	70:30	10 min; 80% Amp

added. The tubes were kept at room temperature for 5 minutes and then 2 mL sodium carbonate solution (7.5%) was added. The tubes were shaken vigorously and kept in the dark at room temperature for 1 hour. Total polyphenol content (TPC) analysis was performed on the Ocean Optics HR2000+ UV-VIS spectrophotometer at 765 nm wavelength. Distilled water was used as blank instead of extract. Polyphenol concentration was expressed as mg gallic acid equivalent/g plant (mg GAE/g) based on the calibration curve constructed for different concentrations of the etalon, i.e. for 7 points of concentrations from 10 to 70 μg/mL gallic acid ($y = 0.0115x + 0.0094$; $R^2 = 0.9995$). TPC values, expressed in mg gallic acid equivalent/g plant were obtained according to the formula (Phuyal et al. 2020):

$$TPC = \frac{\left(\frac{C}{M} x DF\right)}{1000} \quad (1)$$

Where C is the concentration measured from the calibration curve, M is the dry plant mass and DF is the dilution factor.

The quantitative determination of compounds with polyphenolic structure in all phytochemical complexes supplemented with MNPs was also carried out by the Folin-Ciocalteu spectrophotometric method, also used for simple extracts (Sutan et al. 2018).

Determination of the Trolox Equivalent Antioxidant Capacity (TEAC) of simple extracts and nanostructured phytochemical complexes using the DPPH method

The free radical scavenging activity of the extracts and nanostructured mixtures was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The analysis was determined according to Shimamura et al. (2014), but with some modifications.

For the preparation of the standard, 2.50 mg Trolox was weighed on a microbalance and placed in a 10 mL volumetric flask. 5 mL of 99.5% ethanol was added and sonicated for complete dissolution, after which the solvent was made up to 10 mL. This was the stock solution from which the dilution range of 10-70 µg/mL was prepared, which was necessary to carry out the calibration curve ($y = 1.4028x + 2.4888$; $R^2 = 0.9991$).

The DPPH reagent used for both the standard and all experimental variants was prepared as follows: 3.2 mg of 2,2-diphenyl-1-picrylhydrazyl was placed in a 100 mL volumetric flask to which 50 mL of 99.5% ethanol was added; it was then sonicated and made up to 100 mL with solvent. The DPPH solution was prepared fresh and stored at room temperature, protected from light.

From all 28 experimental variants of simple extracts, only those samples with the highest polyphenol content for each extraction procedure were chosen to show DPPH free radical scavenging activity and to assess the biogenic synthesis capacity of noble MNPs. Simple extracts were diluted to obtain 6 different concentrations (250 µg/mL; 500 µg/mL; 1,000 µg/mL; 5,000 µg/mL; 10,000 µg/mL; 100,000 µg/mL), and those supplemented with MNPs were diluted to obtain 3 different concentrations (500 µg/mL; 1,000 µg/mL; 5,000 µg/mL). From each sample of different concentration, 250 µL simple extract/nanostructured mixture was taken over which 1,750 µL DPPH was added. The systems were kept in the dark at room temperature for 30 minutes and then for each sample, the absorbance at 517 nm wavelength was read after 5 minutes of stabilization under UV influence.

The inhibition percentage of DPPH (%IP) was calculated according to the formula (Adebiyi et al. 2017):

$$\%IP = \frac{A0-A1}{A0} \times 100 \quad (2)$$

Where A0 is the control sample absorbance and A1 is the sample absorbance.

To determine the half maximum inhibitory concentration (IC50), two concentration points of each sample were selected for which the inhibition ratio had a value around 50% (one < 50% and one > 50%) and the regression curve (Y = AX + B) was drawn. The IC50 value (sample concentration - X) was calculated by replacing Y by 50 (Shimamura et al. 2014).

The half maximum inhibitory concentration values are required for the determination of the antioxidant activity calculated in Trolox equivalent according to the formula:

$$TEAC = \frac{IC50Trolox}{IC50Sample} \quad (3)$$

UHPLC Analysis. Sample preparation

A stock solution of 0.1 mg/mL from all standard compounds was prepared by dissolving 10 mg of each reference in 100 mL methanol. This stock solution was kept refrigerated at 4°C and used when needed. To obtain the solutions for the calibration curve the stock solution was diluted with a mixture of the first gradient line of the mobile phase. The dilution factors were 2000, 1000, 500, 250 and 100, respectively.

UHPLC-PDA-MS analysis

Separation of polyphenols was carried out on a Waters Arc System coupled with a Waters 2998 PDA detector and a Waters QDa mass detector. The column used was a Waters Cortecs C18 (4.6 × 50 mm, 2.7 µm) eluting with solvent A (0.1% formic acid in water), solvent B (0.1% formic acid in methanol) and solvent C (0.1% formic acid in acetonitrile). Solvent B was set at 1% during the entire separation. The gradient was as follows: 0–4 min 3%-14% C, 4–7.5 min 14% to 29% C, 7.5–13 min 29% to 89% C, 13–15 min 89% to 3% C. The flow rate of the mobile phase was set at 1.0 mL/ min. The column temperature was equilibrated to 35°C. The injection volume was 5 µL. All samples were kept at 20°C during the entire analysis (Velamuri et al. 2020).

Eluted compounds were analysed using a Waters PDA 2998 and a QDa mass detector equipped with electrospray ionization (ESI) source. Capillary voltage was maintained at 0.8 kV, cone voltage was kept at 20 V and the mass spectra spectra were recorded in negative ion mode in the range 100–800 m/z. Quantification was established in selected ion recording (SIR) mode for each compound (as shown in Table 2) using external calibration curves prepared for each standard. Also, the retention times for all reference compounds are presented in Table 3.

Biogenic synthesis of noble metal nanoparticles mediated by plant extracts

For the biosynthesis of MNPs using extracts of lemon balm and sage, the experimental variant that was found to contain the highest content of polyphenols was used from each extraction procedure, since literature data show that phytochemicals, especially polyphenols, present in plant extracts have the strongest reducing properties of silver and gold ions and also confer the highest stability of the nanoparticles (Swilam and Nematallah 2020). For the synthesis of AgClNPs, a 1mM

Table 2. Calibration curve statistics of reference compounds.

Calibration curve standard	Fit Type	Equation	R^2
Protocatechuic acid	Quadratic (2nd Order)	Y = -3.98e+003 X^2 + 1.41e+005 X + 5.19e+003	0.998221
Chlorogenic acid		Y = -9.88e+003 X^2 + 1.40e+005 X + 1.83e+004	0.994078
Caffeic acid		Y = -1.98e+004 X^2 + 3.63e+005 X + 2.73e+004	0.997099
p-Coumaric acid		Y = -3.79e+003 X^2 + 1.01e+005 X + 2.14e+003	0.997983
Ferulic acid		Y = -4.99e+002 X^2 + 2.01e+004 X - 2.72e+002	0.998986
Rutin		Y = -1.52e+003 X^2 + 7.64e+004 X + 4.40e+003	0.998255
Rosmarinic acid		Y = -5.96e+001 X^2 + 5.35e+004 X + 3.51e+005	0.994586
Quercetin		Y = -2.50e+004 X^2 + 7.79e+005 X - 5.95e+001	0.999115

Table 3. Retention times for all reference compounds.

Peak no.	Compound name	Coding	m/z	Retention time [min]
1	Protocatechuic acid	PRO	153	1.667
2	Chlorogenic acid	CHL	353	3.082
3	Caffeic acid	CAF	179	3.332
4	*p*-Coumaric acid	COU	163	4.459
5	Ferulic acid	FER	193	5.152
6	Rutin	RUT	609	5.580
7	Rosmarinic acid	ROS	359	6.715
8	Quercetin	QUE	301	7.757

silver nitrate ($AgNO_3$) solution obtained by weighing 16.98 mg $AgNO_3$ salt was used as a precursor and made up to 100 mL volume with distilled water.

Tetrachloroauric acid ($HAuCl_4$) solution of 1mM concentration, obtained by adding 35.80 mg $HAuCl_4$ to 100 mL of distilled water, was the precursor for the phytosynthesis of gold nanoparticles. The simple extracts were then mixed with the specific precursors in volume ratios of 1:1 and incubated for 24 h at room temperature (25°C). The colour change of the extracts after the addition of the 2 types of precursor solutions was noticeable within the first 2 min, which was a first confirmation of the formation of noble MNPs (Sutan et al. 2019).

BFSTEM-EDS analysis of nanostructured phytochemical complexes

Bright field scanning transmission electron microscopy (BFSTEM) and energy dispersive X-ray spectroscopy (EDS) were used to investigate particles size, shape and dispersion and perform chemical elemental analysis. These analyses were carried out using the FESEM-HITACHI SU8230 microscope. Prior to these analyses, samples were homogenized for 1 minute in an ultrasonic bath (Kerry Guyson) for a better dispersion. Then, one drop of each sample was spread on a copper grid with formvar and the grid was kept for 24 hours in the exicator to evaporate the solvent.

Coating Cu grids with formvar film

Cu grids with thin formvar films are primarily used for transmission electron microscopy for sampling and analysis of ultra-thin sections (Shields 1999).

The formvar film also acts as a support for various suspensions or powders to be analysed by SEM/BFSTEM.

To obtain Cu grids with formvar film, the ~1% formvar solution in 1,2-dyclorethane was poured into a tall covered container. A glass microscope slide was cleaned with distilled water, but not insistently. The glass slide was inserted into the container with the formvar solution and left for 2-3 minutes. The glass slide was then removed from the formvar solution and drained, after which the slide was left at room temperature for a further 2-3 minutes after which the excess solution was removed. After drying the film, it was cut on the edge of the glass blade using a razor blade. A container was filled with clean distilled water and the glass blade was inserted at an angle of 45° to loosen the formvar film. The Cu grids were placed face down over the formvar film on the surface of the water. The formvar grids were collected using another pre-cleaned glass slide, after which the grids were left at room temperature covered with the lid of a petri dish to dry and adhere the film to the Cu grid (Sherman 2014).

In vivo testing of the cytogenotoxic activity of simple extracts and nanostructured phytochemical complexes

Root tip cells were obtained by placing bulbs of *Allium cepa* L. with discoidal stem in contact with distilled water for 48 h, in the dark. The bulbs were transferred to

simple extracts and nanostructured mixtures (24 h).

After 24 h the root tip meristematic cells were removed and subjected to fixation using Farmer's reagent (glacial acetic acid:absolute ethyl alcohol, 1:3 v/v) overnight and then transferred to 70° ethyl alcohol for long-term preservation. For each experimental variant a number of 5 roots were subjected to attenuated hydrolysis with 1N HCl for 18 minutes at 60°C. The fixed and macerated roots were stained with 2% aceto-orcein solution for 15 minutes at 60°C. From the stained meristematic tips, microscopic preparations were made by the squash technique.

Microscopic slides were analysed under the Mshot Trinocular ML 11-II biological microscope at 400× magnification. Microscopic analysis consisted of determining the number of cells at different stages of mitosis, the frequency of chromosomal and nuclear aberrations, based on approximately 3,000 cells per experimental sample. The mitotic index (MI) was determined as the percentage ratio of the number of cells in mitosis to the total number of cells analysed (Tedesco and Laughinghouse 2012). Based on the total number of cells in mitosis, the percentage ratio of cells in prophase, metaphase, anaphase or telophase was determined. The frequency of chromosomal aberrations and nuclear abnormalities was determined by relating them to the appropriate stage of the cell cycle, i.e. mitosis.

Statistical interpretation of the results

The results of the experimental analyses were expressed as mean values ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Šídák's multiple comparisons test was used to analyse differences between mean values. A probability of $p<0.0001$ was considered highly significant. Statistical analysis was performed using GraphPad Prism 9.0.0.0 software.

For cytogenetic analysis, statistical processing of data was performed using IBM SPSS Statistics 20 software. Statistical significance and significant differences between variables were determined using analysis of variance (one way ANOVA) and Duncan's test for multiple comparisons, respectively. Values of $p \leq 0.05$ were considered statistically significant. Graphs and tables were compiled based on mean values ± standard error of several independent experiments.

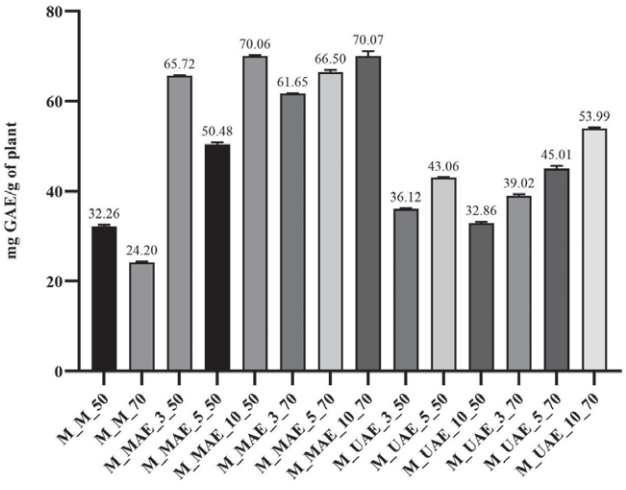

Figure 1. Total polyphenol content of *M. officinalis* extracts (Data are expressed as mean ±SD values from independent triplicate experiments).

RESULTS AND DISCUSSIONS

Determination of the total polyphenol content of the obtained plant extracts and nanostructured phytochemical complexes

As can be seen in Figure 1, the highest amount of polyphenols, i.e. 70.07±1.07 mg GAE/g plant, was recorded for the lemon balm extracts obtained by microwave-assisted extraction technique, in solvent with a volume ratio of pharmaceutical ethyl alcohol and distilled water of 70:30, and after 10 minutes of microwave action on the extraction mixture. For macerates, the highest amount of total polyphenols (32.26±0.26 mg GAE/g plant) was recorded for those obtained in the solvent with equal ratio of water and solvent. For the ultrasound-assisted extraction of aerial parts from lemon balm plants with solvent with a volume ratio of pharmaceutical ethyl alcohol and distilled water of 70:30, there is a directly proportional increase in the amount of total polyphenols with the time of ultrasound action, with the highest amount of these compounds (53.98±0.16 mg GAE/g plant) obtained after 10 minutes of extraction.

A variation from 2.816 to 7.796 mg/mL of phenolic compounds was reported by Papoti et al. (2019) in aqueous preparations, respectively: infusion, decoction, maceration, ultrasound-assisted extraction. Total polyphenols ranging from 18.17±0.04 to 64.17±0.52 mg GAE/g dry plant was obtained by Petkova et al. (2017) when infusions made from lemon balm plants cultivated in Bulgaria were analysed.

Of the two sage extracts obtained by maceration, the highest polyphenol content was determined for the one with a ratio of 70:30 pharmaceutical ethyl alcohol:distilled water (v/v) (25.30±0.96 mg GAE/g plant), data illustrated in Figure 2.

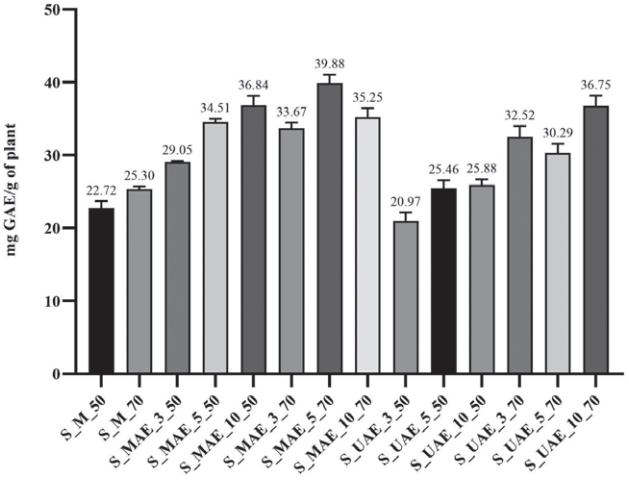

Figure 2. Total polyphenol content of *S. officinalis* extracts (Data are expressed as mean ±SD values from independent triplicate experiments).

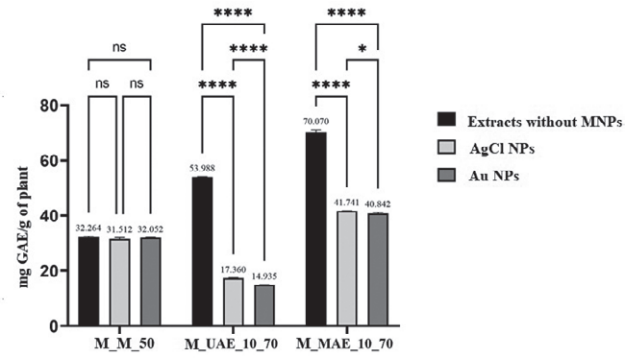

Figure 3. Total polyphenols content of simple extracts and nanostructured phytochemical complexes of *M. officinalis* (Data are expressed as mean ± SD values from independent triplicate experiments and p values were calculated by one-way ANOVA followed by Šídák's multiple comparisons test; ****p < 0.0001; *p = 0.0472; ns p =0.1058; 0.8170; 0.2920).

In contrast, Pop et al. (2015) doubling the extraction time by maceration and using 80% EtOH obtained lower TPC values, 19.49 mg GAE/g dry plant, which shows that a prolonged extraction time may not always lead to a higher phytocompound concentration. This is also confirmed by the results obtained by Osmić et al. (2019), who using a 40% aqueous ethanol solution and the same plant:solvent ratio used by us, obtained from the leaves of *S. officinalis* L. a polyphenol content of 137.11 mg GAE/g in only 60 minutes of maceration at room temperature. However, there are experimental studies in which maceration resulted in much lower TPC values than the present study, namely 13.6±0.4 mg GAE/g (Proestos et al. 2005); 4.25 mg-5.95 mg GAE/g (Roby et al. 2013). Moreover Gîrd et al. (2014) using the same solvent, ethanol 70% managed to extract from one gram of sage leaves only a minimum TPC of 3.26 mg GAE/g and a maximum of 6.32 mg GAE/g.

The extracts obtained using MAE showed a total amount of polyphenolic compounds between 29.05±0.16-39.88±1.19 mg GAE/g plant, the maximum of 39.88±1.19 mg GAE/g plant being obtained after irradiating the plant material with electromagnetic waves for 5 minutes, also at a higher concentration of alcohol, at a temperature of 75°C and a power of 250W. Similar values were also recorded in the experimental study conducted by Dragović-Uzelac et al. (2012), where the range of TPC values obtained was between 31.7-47.0 mg RAE/g, the maximum being determined in the sample irradiated for 9 minutes at a power of 500W.

For the samples subjected to sonication, in order to reach a maximum TPC of 36.75±1.41 mg GAE/ g plant, it was necessary to prolong the acoustic cavitation phenomenon to a maximum of 10 minutes, an alcohol concentration of 70% and a temperature of 45°C. This polyphenol content is much higher compared to Pop et al. (2015) 19.06 mg GAE/g plant and Brindisi et al. (2021), 18.7-35.3 mg CA/g. In contrast, there are studies attesting the recovery of higher concentrations of polyphenolic compounds from sage, such as 67.75 mg GAE/g (Dent 2015); 99.03 mg GAE/g (Zeković et al. 2017); 61.3-143.6 mg GAE/g (Veličković et al. 2011).

A possible explanation for the differences between the TPC values in the literature, and those obtained by us, except for the extraction technique and parameters, would be the time at which the plant material was harvested, Farhat et al. (2014), demonstrating that the highest polyphenol content was recorded for sage plants harvested at the fruiting stage. The results could also be attributed to the drying protocol of the plants (Hamrouni-Sellami et al. 2012) as well as the geographic area of cultivation (Farhat et al. 2014; Dent et al. 2017).

As can be seen in Figure 3, the highest amount of phenolic compounds for lemon balm extracts supplemented with MNPs was recorded for those obtained by MAE technique, i.e. 41.741±0.052 mg GAE/g plant for M_MAE_10_70_AgCl and 40.842±0.343 mg GAE/g plant for M_MAE_10_70_Au.

For the macerates, it can be seen that there are statistically insignificant differences between the TPC values of extracts and mixtures with AgClNPs and AuNPs.

Strongly statistically significant higher values were observed for extracts obtained by using UAE without MNPs comparing with the extracts supplemented with MNPs, the TPC content ranging from 53.988±0.166 mg

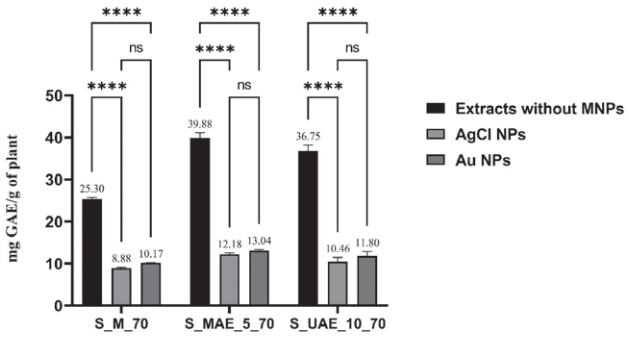

Figure 4. Total polyphenols content of simple extracts and nanostructured phytochemical complexes of *S. officinalis* (Data are expressed as mean ± SD values from independent triplicate experiments and p values were calculated by one-way ANOVA followed by Šídák's multiple comparisons test; ****p < 0.0001; ns p = 0.1310; 0.1497; 0.4079).

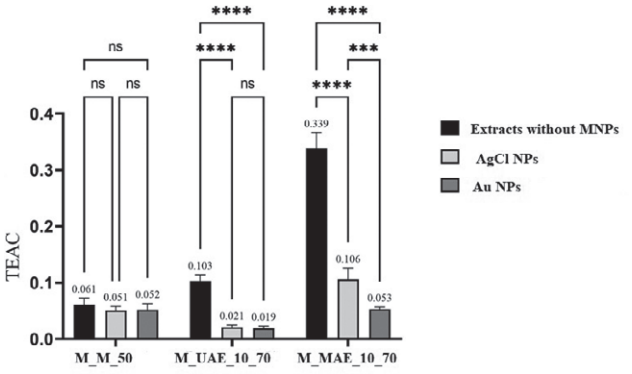

Figure 5. Trolox equivalent antioxidant capacity of simple extract and nanostructured phytochemical complexes of *M. officinalis* (Data are expressed as mean ± SD values from independent triplicate experiments and p values were calculated by one-way ANOVA followed by Šídák's multiple comparisons test; ****p < 0.0001; ***p = 0.0003; ns p = 0.6144; 0.6707; 0.9953; 0.9814).

GAE/g plant to 17.360±0.069 mg GAE/g plant for M_UAE_10_70_AgCl and 14.932±0.044 mg GAE/g plant for M_UAE_10_70_Au.

A statistically significant decrease was also observed for extracts obtained by MAE technique with AgClNPs (41.741±0.052 mg GAE/g) and AuNPs(40.842±0.343 mg GAE/g) compared to extracts without MNPs (70.070±1.070 mg GAE/g plant).

It is important to highlight unlike lemon balm extracts enriched with MNPs where the highest amount of polyphenols was obtained for extracts with AgClNPs, for sage, extracts with AuNPs were found to exhibit this characteristic (Figure 4). Moreover, in the case of nanostructured phytochemical complexes of sage, the maximum total amount of polyphenols was also recorded for the experimental variant using the extract obtained after microwave irradiation of the plant material, i.e. 13.04±0.26 mg/g GAE for sample S_MAE_5_70_70_Au.

However, there are no significant differences between the amount of polyphenols obtained for sage extracts with AgClNPs versus those with AuNPs, for these extracts enriched with MNPs the amount of total polyphenols remained relatively constant regardless of the extraction technique used, with TPC values varying only from 8.88±0.2 mg GAE/g plant for the AgClNPs macerate and 13.04±0.26 mg GAE/g plant for the extract obtained by MAE and with AuNPs.

Making a comparison between the TPC values of simple extracts and phytochemical complexes, in the case of both medicinal species it can be observed that following the phytosynthesis of MNPs, the amount of polyphenols was decreased. An explanation for this is provided by the experimental study conducted by Dzimitrowicz et al. (2016), which highlights that following the reduction reaction of metal ions by a plant extract, a large part of the compounds of polyphenolic nature are oxidized.

Determination of the Trolox Equivalent Antioxidant Capacity (TEAC) of simple extracts and nanostructured phytochemical complexes using the DPPH method

From Figure 5 it can be seen that the best ability of the lemon balm extracts with MNPs to behave as hydrogen atom or electron donors for the conversion of the purple free radical DPPH- to its reduced yellow form DPPH-H was for extracts obtained by the MAE technique, the TEAC values were 0.106±0.019 for M_MAE_10_70_AgCl and 0.053±0.003 for M_MAE_10_70_Au. Also, for these types of extracts a highly significant statistical difference is observed compared to extracts without metal nanoparticles whose TEAC values were 0.339±0.027.

Similar to the TPC values, the statistical differences of TEAC values are insignificant between macerates without MNPs and those supplemented with MNPs. A highly significant statistical difference can also be observed for the TEAC values of the extract obtained by ultrasound action on plant material without MNPs (0.103±0.011) compared to extracts with AgClNPs (0.021±0.004) and AuNPs (0.019±0.004).

For both simple extracts and systems consisting of sage extracts and MNPs, the highest antioxidant activity was recorded for the experimental variant S_MAE_5_70 (Figure 6).

At the opposite pole, the lowest antioxidant activities were recorded for the samples subjected to macera-

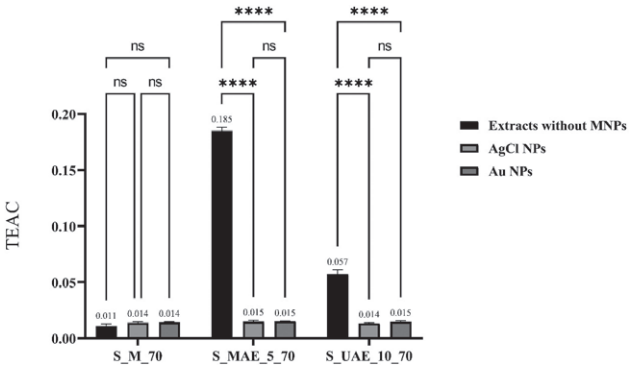

Figure 6. Trolox equivalent antioxidant capacity of simple extract and nanostructured phytochemical complexes of *S. officinalis* (Data are expressed as mean ± SD values from independent triplicate experiments and p values were calculated by one-way ANOVA followed by Šídák's multiple comparisons test; ****p < 0.0001; ns p = 0.1727; 0.1231; 0.6421; 0.9794; 0.9908).

tion and sonication processes. In contrast Zeković et al. (2017), using other extraction parameters, demonstrate that their simple extracts obtained by the cavitating phenomenon show a slight improvement in antioxidant activity as opposed to those subjected to microwaving, but the difference is not a noticeable one.

Comparing the antioxidant activity of simple extracts with that of nanostructured phytochemical complexes, both *M. officinalis* L. and *S. officinalis* L. simple extracts have the highest free radical scavenging capacity, as they also have the highest concentrations of polyphenols.

The results of the evaluation of the antioxidant capacity as well as the amount of total polyphenols in the nanostructured extracts obtained are in agreement with the existing data in the literature, data which show that the reduction in the amount of total polyphenols correlated with a decrease in the antioxidant activity of extracts with MNPs compared to simple extracts is attributed to the fact that the bioorganic material, especially the polyphenols, participates in the reduction of metallic ions as well as in the formation of the coating halo that stabilizes the nanoparticles (Csakvari et al, 2021; Nayeri et al., 2021; Siakavella et al., 2020).

UHPLC Analysis

To identify the compounds obtained by the 3 different extraction methods, 6 experimental variants were subjected to UHPLC-PDA-MS analysis (M_M_50; M_MAE_10_70; M_UAE_10_70; S_M_70; S_MAE_5_70; S_UAE_10_70), namely those variants with the highest TPC.

Thus, according to the data presented in Table 4, maceration of the aerial parts of lemon balm resulted in significant amounts of rosmarinic acid (227,120 µg/mL), the most abundant compound in this medicinal species (Kim et al. 2010). However, modern extraction methods have allowed the recovery of much higher concentrations of it. Thus, UAE revealed a quantity of 263.805 µg/mL of rosmarinic acid, and the MAE method yielded the highest amount of rosmarinic acid, i.e. 1,266.89 µg/mL. Similar value (17.03 mg/g) indicating a significant amounts of rosmarinic acid was obtained by Caniova and Brandsteterova (2001) using the liquid extraction technique (methanol and water in a volume ratio of 60:40) of *M. officinalis* L. plants grown in Slovakia.

The highest concentrations of protocatechuic acid and rutin were obtained from maceration of sage leaves. However, MAE proved to be the optimal method to obtain the highest amounts of all other compounds. As mentioned above, the final temperature range of the samples subjected to sonication and microwaves, was in the case of UAE between 37-45°C, and in MAE between 54-75°C, the final temperature of 75°C being reached in sample S_MAE_5_70. Based on this result, we can also see that at higher temperatures, the solvent's solubilizing power of dissolved substances increases due to the decrease in viscosity and surface tension, thus facilitating wetting and matrix penetration (Paré et al. 1991; Chen et al. 2006; Hayat

Table 4. The amount of natural compounds (µg/mL) obtained from *M. officinalis* and *S. officinalis* by the three extraction techniques.

Sample/Compound name	PRO	CHL	CAF	COU	FER	RUT	ROS	QUE
M_M_50	25.640	15.960	131.650	5.280	2.805	27.735	227.120	0.450
M_MAE_10_70	34.675	49.190	77.585	3.530	1.400	31.645	1266.890	0.230
M_UAE_10_70	24.245	39.795	40.010	2.245	1.000	28.265	263.805	0.190
S_M_70	35.930	12.125	27.845	3.465	6.895	30.295	502.540	0.000
S_MAE_5_70	35.845	16.140	48.935	3.790	12.520	10.240	593.715	0.000
S_UAE_10_70	9.560	11.610	44.045	3.035	6.450	26.195	687.260	0.225

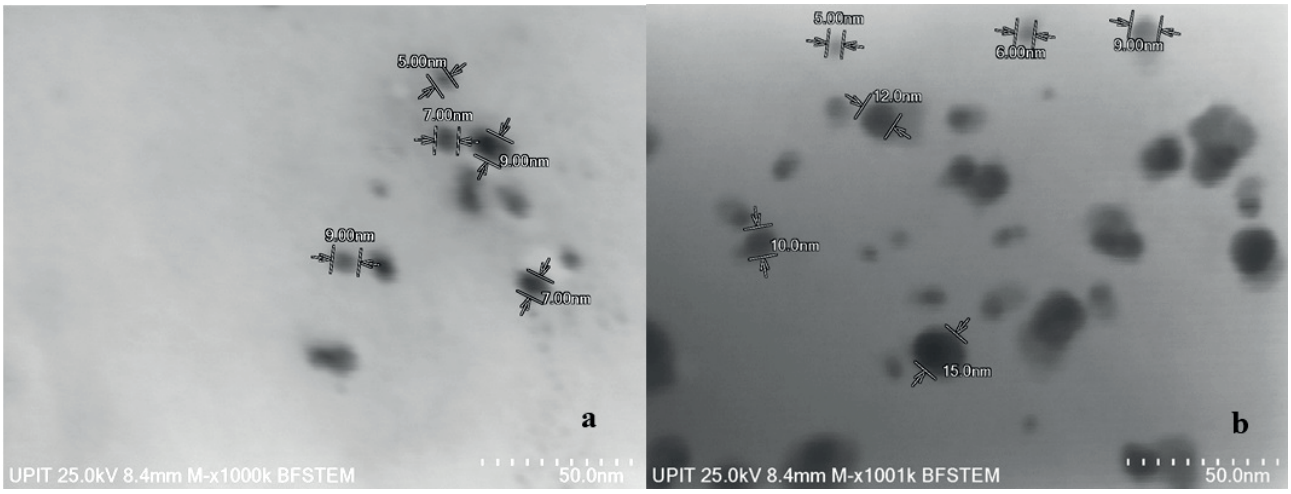

Figure 7. Dispersion dimensional analysis of AgClNPs for M_UAE_10_70_AgCl (a) and for S_M_70_AgCl (b) in BFSTEM at 1,000,000 (x1000k) magnification.

et al. 2009). Consistent with the results in Table 4, it can be seen that depending on the compound targeted for extraction, one extraction technique may be more advantageous than another. Thus, if in order to extract a maximum concentration of rosmarinic acid of 687.26 µg/mL from sage leaves, the phenomenon of acoustic cavitations is the most beneficial, the same cannot be said in the case of protocatechuic acid where the exposure of plant material to sound waves allowed the recovery of the lowest amounts of this phenolic acid compared to the other extraction techniques. Similar results regarding the discrepancy between the values of recovered compounds from this medicinal species were also recorded in the study conducted by Zeković et al. (2017).

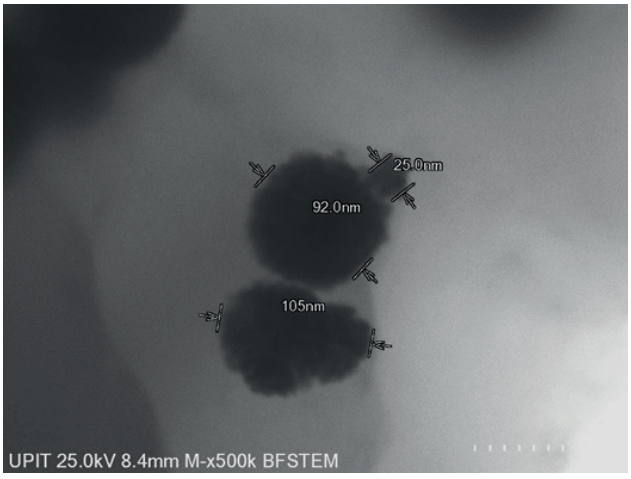

Figure 8. Dimensional analysis of AuNPs aggregates obtained in sage ethanolic extracts.

BFSTEM-EDS analysis of nanostructured phytochemical complexes

From the dimensional analysis on AgClNPs dispersion in BFSTEM at 1,000,000 magnifications for the lemon balm extracts, it is observed that all these extracts showed the ability to phytosynthesize spherical shaped MNPs with diameters of about 20 nm, most of them having sizes just below 10 nm (5 nm, 6 nm, 7 nm and 9 nm). AgClNPs phytosynthesized in sage extracts are also spherical in shape, ranging in size from 5 mm to 20 mm. These aspects illustrated in Figure 7.

For the AuNPs phytosynthesized in all three types of extracts, a reduced dispersion was observed, being found as agglomerates, in which, AuNPs show sizes of about 10 nm. However, larger AuNPs were phytosynthesized in sage extract obtained by UAE, Figure 8 showing 3 agglomerates of AuNPs with sizes of 105 nm, 92 nm and 25 nm.

The aggregation phenomenon of AuNPs may be due to the low concentration of the precursor salt (i.e. $HAuCl_4$), but also to the pH of the solution or even the age of the plants (Teimouri et al. 2018; Boruah et al. 2021).

EDS analysis allowed us to superimpose the spectra generated for the formvar film-coated copper grids and those with nanostructured phytochemical complexes. In Figure 9 it can be seen that AgClNPs and AuNPs are present only in these phytocomplexes.

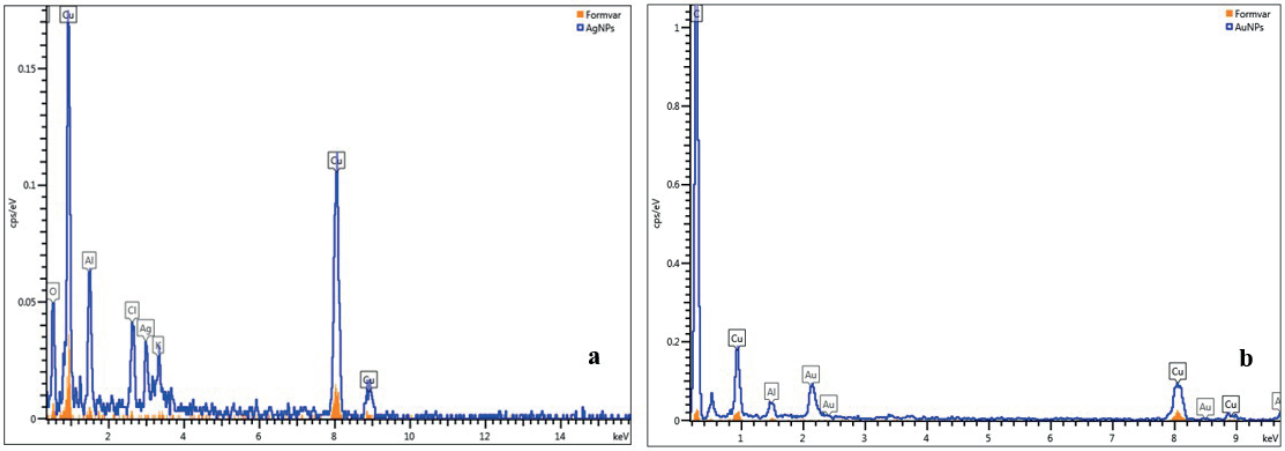

Figure 9. Superimposed EDS spectra obtained for Cu grid with formvar and M_MAE_10_70_AgCl with AgClNPs on Cu grid with formvar (a) and Cu grid with formvar and S_MAE_5_70_Au with AuNPs on Cu grid with formvar (b).

In vivo testing of the cytogenotoxic activity of simple extracts and nanostructured phytochemical complexes

The *Allium cepa* L. test is widely used to determine the benefits and especially the adverse effects of medicinal plants, which are increasingly used nowadays. This is because the test is a very good indicator of toxicity and mutagenicity (Tedesco and Laughinghouse 2012). In our study, the *Allium* assay was used to evaluate the possible cytogenotoxicity of crude or supplemented extracts with MNPs. While for *M. officinalis* L. there is some data on the use of this test, in the case of *S. officinalis* L. it is performed for the first time.

The variation of the MI in onion root tip meristematic cells subjected to treatment with ethanolic extracts of *Melissa officinalis* L. before and after MNPs phytosynthesis is shown in Figure 10.

These results revealed that for *M. officinalis* L. extracts, the highest percentage value of the MI (11.126%) corresponded to the sample M_M_50. Likewise, for the control, the highest MI was recorded for roots incubated in solvent with a ratio of 96% pharmaceutical ethyl alcohol and 50:50 distilled water (8.643%).

Phytosynthesis of AgClNPs and AuNPs inhibited the mitostimulatory action of ethanolic extracts. Thus, there was a statistically significant reduction in the percentage of dividing cells. The sample defined by the extracts supplemented with MNPs, showed a MI values close to those determined for the corresponding concentrations of alcohols, except for the sample M_MAE_10_70_Au for which the frequency of dividing cells was significantly higher (9.613%).

The inhibition of mitotic activity, in the experimental variant M_MAE_10_70_AgCl compared to controls, may be correlated with the ion imbalance that can be induced at the cellular level by the extracts tested (Chakraborty et al., 2009).

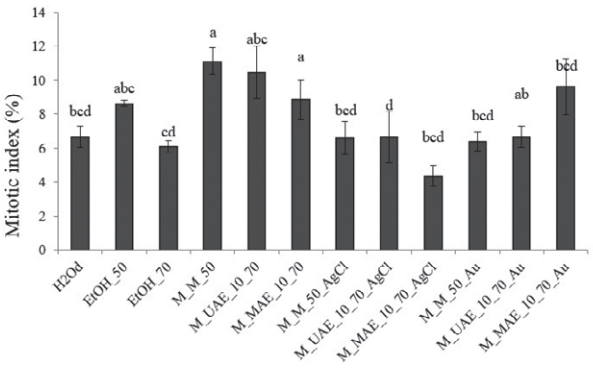

Figure 10. MI (%) of simple extracts of *M. officinalis* and nanostructured phytochemical complexes (a–d: interpretation of the significance of the differences, by means of the Duncan test, $p < 0,05$).

Hsin et al. (2008) showed that AgNPs stimulate intracellular production of reactive oxygen species (ROS), which stimulates cell cycle progression while causing oxidative stress at the DNA level (Carlson et al., 2008).

Statistical analysis of the results on the distribution of mitosis phases (Figure 11) indicates a significant increase of prophase index that the decrease in for sample M_M_50_Au compared to the control. However, a distribution of mitosis phases similar to the control variants was noted for sample M_MAE_10_70_AgCl. Moreover, a significantly higher frecvency of metaphases were defining for thesamples M_UAE_10_70_AgCl and M_M_50_AgCl, suggesting the interference ofAgClNP-

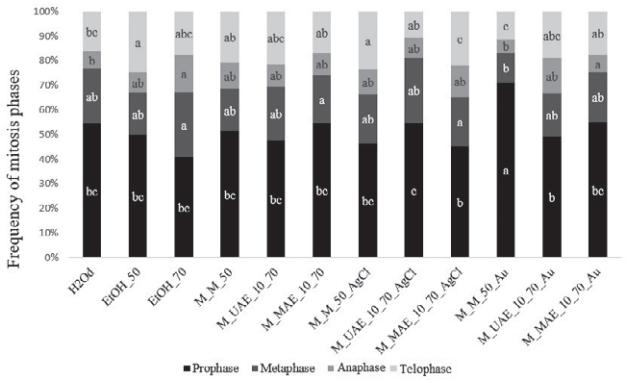

Figure 11. Frequency of mitosis phases (%) in simple extracts of *M. officinalis* and nanostructured phytochemical complexes (a–c: interpretation of the significance of the differences, by means of the Duncan test, $p < 0,05$).

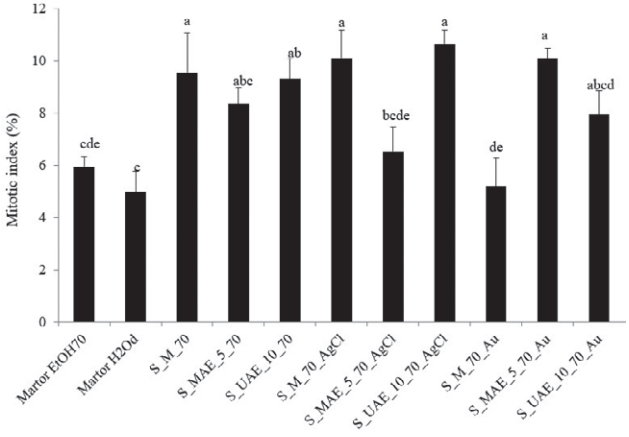

Figure 12. MI (%) of simple extracts of *S. officinalis* and nanostructured phytochemical complexes (a–d: interpretation of the significance of the differences, by means of the Duncan test, $p < 0,05$).

son the formation and/or functioning of the mitotic spindle.

Results of cytogenotoxicity analysis show that extracts of *S. officinalis* and those enriched with MNPs induced statistically significant variation of MI compared to distilled water, and to the control with the equivalent concentration of pharmaceutical ethyl alcohol (Figure 12).

Thus, treatment of onion root meristems with these samples resulted in a statistically significant increase of MI, with a maximum value of 10.01% for the experimental variant S_M_70_AgCl, in contrast to control samples, which showed mitoinhibitory effects.

The distribution of mitosis phases has been investigated and is shown in Figure 13. The highest prophase frequency was observed in the S_M_70_Au variant,

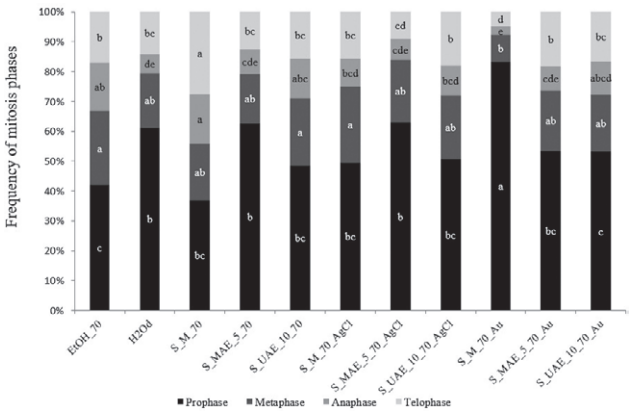

Figure 13. Frequency of mitosis phases (%) in simple extracts of *S. officinalis* and nanostructured phytochemical complexes (a–e: interpretation of the significance of the differences, by means of the Duncan test, $p < 0,05$).

which was associated with the lowest anaphase and telophase indices.

The *Allium* test allows the assessment of the toxic potential of substances both by significant variation of MI and by analysing the type and frequency of chromosomal aberrations. Statistical interpretations of the results on the frequency of chromosomal aberrations (Figure 14) such as vagrant chromosomes, laggard chromosomes, sticky chromosomes, metaphase chromosomes (C-mitosis), fragmented chromosomes, pole-to-pole metaphases, bridge cells or multipolar anaphases are presented in Table 5 and Table 6, as well as nuclear aberrations (Figure 15) such as binucleated cells, budded nuclei, irregularly shaped nuclei, ghost cells and giant cells found in the *Allium cepa* L. root tip meristematic cells exposed to the action of controls, ethanolic extracts of lemon balm and sage and mixtures with MNPs for 24 hours.

Vagrant chromosomes represent the chromosomal aberrations found with a high frequency in all control samples but also in all experimental variants with the exception of those defined by *S. officinalis* macerate and ethanolic extracts of *M. officinalis* obtained by MAE technique supplemented with AgClNPs and AuNPs. These types of chromosomal aberrations occur as a result of "weak spindle formations" (Onwuamah et al. 2014).

Laggard chromosomes were observed in all samples defined by the ethanolic extracts of lemon balm and sage extracts obtained by using UAE and in those with MNPs, with the exception of sage extracts with AuNPs. It is believed that the formation of lagging chromosomes is the result of the disruption of the division spindle for-

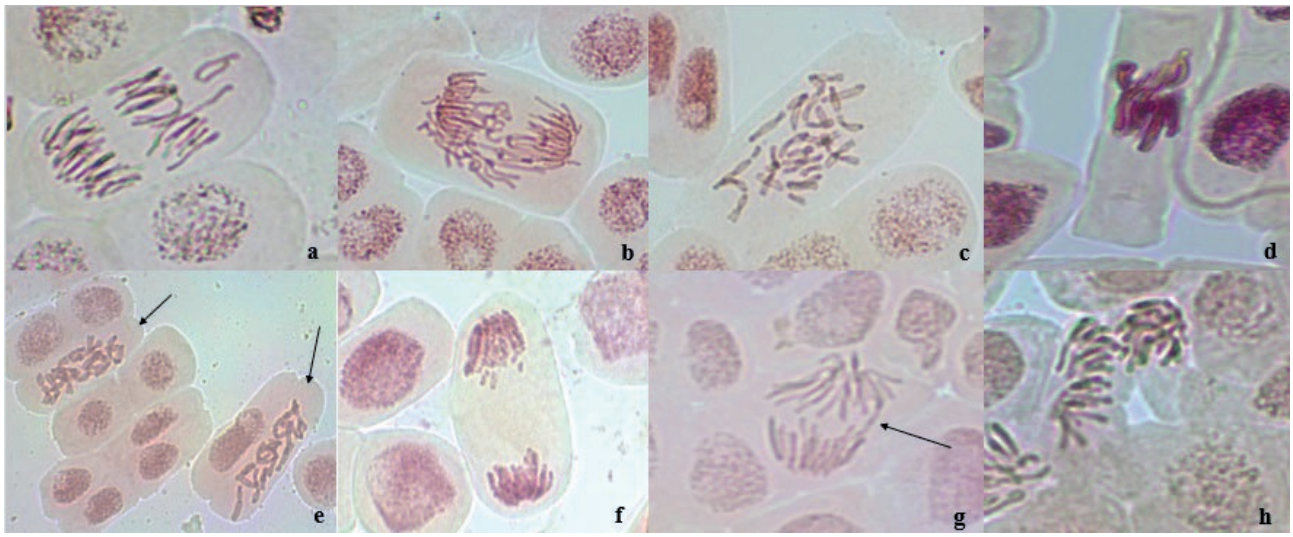

Figure 14. Chromosomal aberrations found in *A. cepa* root tip meristematic cells following treatments with simple extracts and with extracts supplemented with MNPs (a – Vagrant in EtOH_50; b – Laggards in M_UAE_10_70_AgCl; c – C-mitosis in M_UAE_10_70_AgCl; d – Sticky chromosomes in M_MAE_10_70_AgCl; e – Pole-to-pole metaphase in S_UAE_10_70_Au; f – Cell with fragmented chromosomes in S_MAE_5_70; g – Bridges in S_MAE_5_70_Au; h – Multi-polar anaphase cell in M_M_50).

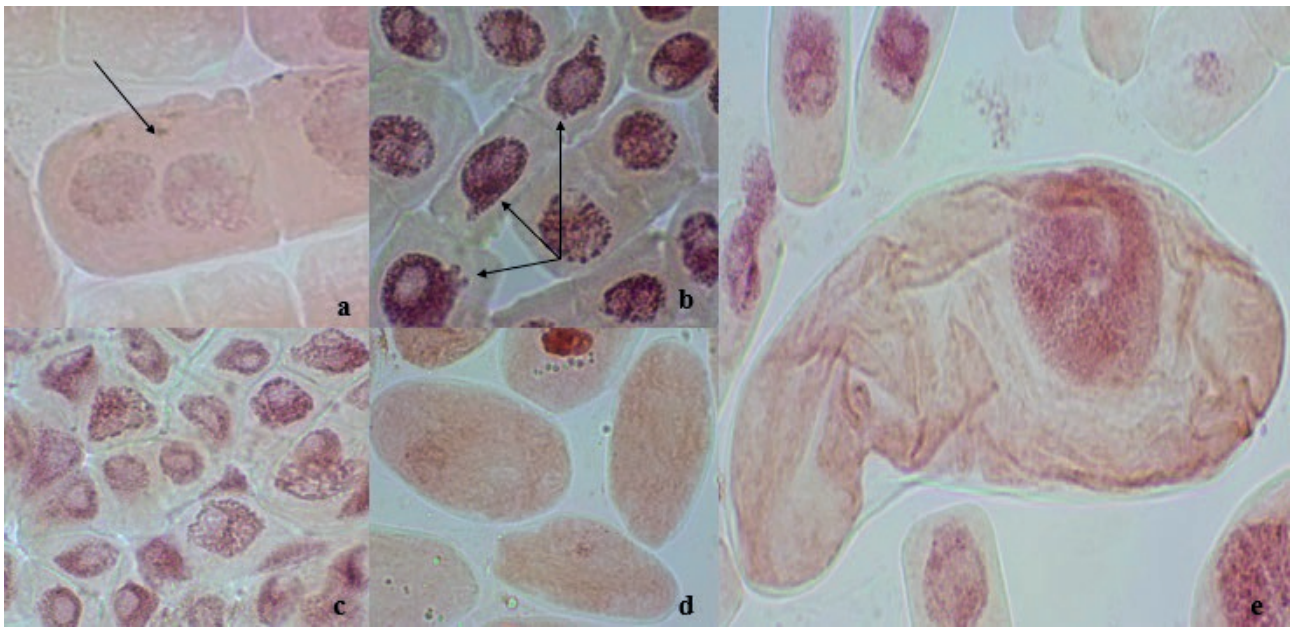

Figure 15. Nuclear aberrations found in *A. cepa* root tip meristematic cells following treatments with simple extracts and with extracts supplemented with MNPs (a – Binucleate cells in M_MAE_10_70; b – Nuclei buds in M_M_50_AgCl; c – Irregularly shaped nuclei in H2Od; d – Ghost cells in M_M_50_AgCl; e – Giant cell in EtOH_70).

mation process under the action of toxic agents (Haliem, 1990).

The frequency of sticky chromosomes significantly decreased in cells treated with lemon balm ethanolic extracts supplemented with AgClNPs and AuNPs regardless of the extraction technique used. Stickies may be the consequence of subchromatid bonds between chromosomes (Liman et al., 2010). Although present at a moderate frequency in the control variants, but also in some experimental variants, the frequency of cells with nucleic buds

Table 5. Chromosomal and nuclear aberrations observed in *A. cepa* root tip meristematic cells treated with ethanolic extracts and nanostructured phytochemical complexes of *M. officinalis*.

SAMPLE	Cells with vagrant chromosomes	Laggard chromosomes	C-mitosis	Sticky chromosomes	Pole-to-pole metaphase	Cells with fragmented chromosomes	Bridges	Multi-polar anaphase cells	Binucleate cells	Nuclei buds	Irregularly shaped nuclei	Ghost cells	Giant cells	TOTAL
H2Od	3.51±1.76 ab	0.00±0.00 c	0.00±0.00 b	12.83±6.43 ab	0.00±0.00 b	1.85±1.00 a	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	33.69±6.06 a	0.00±0.00 b	0.06±0.06 a	32.71±5.52 a
EtOH_50	36.47±19.57 a	0.00±0.00 c	0.00±0.00 b	9.44±3.88 b	0.00±0.00 b	0.00±0.00 b	1.11±1.11 b	0.00±0.00 b	0.00±0.00 b	0.03±0.03 b	0.4±0.30 b	0.00±0.00 b	0.00±0.00 a	0.98±0.29 b
EtOH_70	14.16±3.81 ab	0.00±0.00 c	0.00±0.00 b	4.16±4.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	33.82±16.59 a	0.00±0.00 b	0.33±0.33 a	32.85±15.58 a
M_M_50	10.92±1.44 ab	0.00±0.00 c	0.00±0.00 b	13.42±4.63 ab	0.00±0.00 b	0.00±0.00 b	2.75±1.42 ab	8.51±5.96 a	0.00±0.00 b	0.00±0.00 b	4.46±2.84 b	0.00±0.00 b	0.07±0.07 a	4.87±2.69 b
M_MAE_10_70	27.62±19.52 ab	0.00±0.00 c	0.00±0.00 b	12.40±8.58 ab	0.00±0.00 b	0.00±0.00 b	0.61±0.60 b	1.38±1.30 b	0.23±0.15 a	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.36±0.30 a	1.26±0.67 b
M_UAE_10_70	15.07±8.15 ab	3.05±1.54 c	0.00±0.00 b	24.30±9.30 ab	0.23±0.19 a	0.00±0.00 b	3.72±2.93 ab	0.00±0.00 b	0.00±0.00 b	0.09±0.06 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 a	1.28±0.15 b
M_M_50_AgCl	6.66±5.00 ab	0.00±0.00 c	0.00±0.00 b	9.44±3.88 ab	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	2.08±0.63 a	0.00±0.00 b	1.83±1.83 a	0.09±0.09 a	3.86±2.15 b
M_MAE_10_70_AgCl	0.00±0.00 a	0.00±0.00 c	0.00±0.00 b	33.88±9.64 a	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.25±0.12 b	0.00±0.00 b	0.25±0.20 b	0.34±0.21 a	1.17±1.15 b
M_UAE_10_70_AgCl	16.66±9.62 ab	12.29±7.84 ab	47.66±12.46 a	1.33±1.33 b	0.00±0.00 b	0.00±0.00 b	1.66±1.50 ab	0.00±0.00 b	0.00±0.00 b	0.08±0.08 b	0.20±0.20 b	0.00±0.00 b	0.12±0.12 a	1.90±0.20 b
M_M_50_Au	5.00±2.88 ab	0.74±0.5 c	0.00±0.00 b	3.88±3.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	2.52±0.90 b	0.00±0.00 b	0.00±0.00 a	2.56±0.89 b
M_MAE_10_70_Au	11.11±7.34 ab	1.11±1.11 c	0.00±0.00 b	11.57±8.93 ab	0.00±0.00 b	0.00±0.00 b	9.24±5.79 a	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	1.06±0.36 b	0.00±0.00 b	0.00±0.00 a	1.32±0.42 b
M_UAE_10_70_Au	0.00±0.00 a	0.00±0.00 c	0.00±0.00 b	19.23±12.42 ab	0.00±0.00 b	0.00±0.00 b	6.33±4.66 ab	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.75±0.51 b	0.00±0.00 b	0.02±0.02 a	1.36±0.42 b

Table 6. Chromosomal and nuclear aberrations observed in meristematic root cells of *A. cepa* treated with ethanolic extracts and nanostructured phytochemical complexes of *S. officinalis*.

SAMPLE	Metaphase with vagrant chromosomes	Anaphase with vagrant chromosomes	Laggard chromosomes	Sticky chromosomes	Pole-to-pole metaphase	Cells with fragmented chromosomes	Bridges	Multi-polar anaphase cells	Binucleated cells	Irregularly shaped nuclei	Giant cells	Elongated nucleus cells	Other aberrations	TOTAL
EtOH70	6.50±3.71 ab	10.83±6.51 a	0.00	1.67±1.67 cd	0.00	0.00	0.00	0.00	0.00	29.69±12,39 b	0.38±0.38 a	0.00	0.00	28.95±11.82 a
H2Od	2.78±2.78 b	6.67±6.67 a	1.33±1.33 b	6.67±3.33 bcd	0.00	0.00	0.00	0.00	0.00	28.66±4.04 b	0.06±0.06 a	0.00	0.00	27.75±3.64 a
S_M_70	0.00	3.24±1.67 a	0.00	0.00	0.00	0.00	2.72±1.36 c	0.00	0.00	0.00	0.00	0.00	0.06±0.06 a	0.21±0.10 b
S_MAE_5_70	14.94±5.76 a	4.76±4.76 a	0.00	3.70±3.70 cd	0.00	20.63±9.15 a	1.85±1.85 c	2.38±2.38 a	0.09±0.09 a	0.00	0.00	0.00	0.08±0.04 a	1.27±0.25 b
S_UAE_10_70	7.10±5.16 ab	0.00	1.04±1.04 b	10.95±3.43 abc	0.00	6.94±4.22 b	17.65±5.71 abc	2.78±2.78 a	0.04±0.04 a	0.00	0.00	1.37±0.73 a	0.15±0.10 a	2.51±1.21 b
S_M_70_AgCl	0.00	0.00	0.00	0.00	6.25±3.68 ab	3.89±3.89 b	7.5±3.82 a	10.78±3.01 abc	0.00	16.27±12.34 abc	0.00	0.00	0.12±0.09 a	0.98±0.21 b
S_MAE_5_70_AgCl	0.00	0.00	0.00	4.3±2.08 c	0.00	0.00	10.48±5.79 a	0.00	0.00	10.00±5.77 abc	2.38±2.38 a	0.00	0.06±0.03 a	4.34±2.04 b
S_UAE_10_70_AgCl	0.00	0.00	0.00	49.00±16.44 a	0.33±0.33 b	0.67±0.67 b	0.00	3.67±0.88 cd	0.33±0.33 a	6.00±0.58 bc	0.00	0.00	0.00	5.59±1.67 b
S_M_70_Au	0.43±0.43 b	0.00	0.00	19.47±3.88 a	0.00	0.00	21.74±3.55 abc	0.00	0.06±0.06 a	0.00	0.00	0.00	0.00	2.62±1.51 b
S_MAE_5_70_Au	4.44±2.42 ab	0.00	0.00	14.93±4.90 ab	0.00	0.00	31.11±7.47 a	0.00	0.00	0.04±0.04 a	0.00	0.00	0.04±0.04 a	1.44±0.26 b
S_UAE_10_70_Au	6.35±6.35 ab	0.00	4.52±2.43 a	6.77±4.01 bcd	1.19±1.19 b	0.00	24.86±13.15 ab	0.00	0.00	0.00	0.00	0.00	0.05±0.05 a	2.94±0.85 abc

was null in all sage extracts and in lemon balm extracts supplemented with AuNPs, regardless of the extraction method used, suggesting the protective action of these MNPs on the organization and function of nuclei in onion root cells. Giant cells and cells with irregularly shaped nuclei characterized the control variants, being encountered with much decreased frequency in the experimental variants, including those with AgClNPs and AuNPs.

CONCLUSIONS

Our experimental study demonstrated that the two species of medicinal plants, namely *M. officinalis* L. and *S. officinalis* L., can be considered valuable sources of bioactive compounds, being likely to design novel functional products with different therapeutic properties. According to BFSTEM analysis the biogenic synthesis process of noble metal nanoparticles, was successfully carried out. The AgClNPs particle sizes under 10 nm were obtained in both lemon balm and sage extracts. Irrespective of the species tested, a direct proportionality between the total content of polyphenols of the extracts and nanostructured mixtures and their antioxidant activities was noticed. The most efficient method for obtaining polyphenols with the highest antioxidant activity was found to be microwave-assisted extraction, both for extracts and nanostructured mixtures. Mitosis was slightly inhibited in nanostructured phytochemical complexes of lemon balm compared to those of sage. The controls showed the highest frequency of chromosomal aberrations compared both to samples of simple extracts and extracts supplemented with MNPs, suggesting the cytogenoprotective, antigenotoxic, and the safety of using these bioformulations as therapeutic alternatives.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research, Innovation and Digitization, CNCS– UEFISCDI, project number PN-III-P4-ID-PCE-2020-0620, within PNCDI III and by a grant of the University of Pitesti, project number 2242/28.02.2022 (Internal competition for research projects, Code: CIPCS-2021).

REFERENCES

Adebiyi OE, Olayemi FO, Ning-Hua T, Guang-Zhi Z. 2017. *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. Beni-Seuf Univ J Appl. 6:10-14.

Andrade S, Ramalho MJ, Loureiro JA, Pereira M do C. 2019. Natural compounds for Alzheimer's Disease therapy: A systematic review of preclinical and clinical studies. Int J Mol Sci. 20:2313-2313.

Avram S, Bologa C, Flonta ML. 2005. Quantitative structure-activity relationship by CoMFA for cyclic urea and nonpeptide-cyclic cyanoguanidine derivatives on wild type and mutant HIV-1 protease. J Mol Model. 11:105-115.

Avram S, Mernea M, Bagci E, Hritcu L, Borcan LC, Mihailescu DF. 2017. Advanced structure-activity relationships applied to *Mentha spicata* L. *subsp. spicata* essential oil compounds as AChE and NMDA ligands, in comparison with donepezil, galantamine and memantine – new approach in brain disorders pharmacology. CNS Neurol Disord - Drug Targets. 16:800-811.

Azeez MA, Durodola FA, Lateef A, Yekeen TA, Adubi AO, Oladipo IC, Adebayo EA, Badmus JA, Abawulem AO. 2020. Green synthesized novel silver nanoparticles and their application as anticoagulant and thrombolytic agents: A perspective. IOP Conference Series: Materials Science and Engineering 805: 012043.

Badmus JA, Oyemomi SA, Fatoki JO, Yekeen TA, Adedosu OT, Adegbola PI, Azeez MA, Adebayo EA, Lateef A. 2022. Anti-haemolytic and cytogenotoxic potential of aqueous leaf extract of *Annona muricata* (L.) and its bio-fabricated silver nanoparticles. Caryologia. 75(1):3-13.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, et al. 2018. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia. 71:191-209.

Boruah JS, Devi C, Hazarika U, Bhaskar Reddy PV, Chowdhury D, Barthakur M, Kalita P. 2021. Green synthesis of gold nanoparticles using an antiepileptic plant extract: *in vitro* biological and photo-catalytic activities. RSC Adv. 11:28029-28041.

Brindisi M, Bouzidi C, Frattaruolo L, Loizzo MR, Cappello MS, Dugay A, Deguin B, Lauria G, Cappello AR, Tundis R. 2021. New insights into the antioxidant and anti-Inflammatory effects of italian *Salvia officinalis* leaf and flower extracts in lipopolysaccharide and tumor-mediated inflammation models. ANTIGE. 10:311.

Caniova A, Brandsteterova E. 2001. HPLC Analysis of phenolic acids in *Melissa officinalis*. J Liq Chromatogr Relat Technol. 24:2647-2659.

Carlson C, Hussain SM, Schrand AM, Braydich-Stolle LK, Hess KL, Jones RL, Schlager JJ. 2008. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J. Phys. Chem. A. 112:13608-13619.

Chakraborty R, Mukherjee AK, Mukherjee A. 2009. Evaluation of genotoxicity of coal fly ash in *Allium cepa* root cells by combining comet assay with the Allium test. Environ. Monit. Assess. 153:351-357.

Chen L, Ding L, Zhang H, Li J, Wang Y, Wang X, Qu C, Zhang H. 2006. Dynamic microwave-assisted extraction coupled with on-line spectrophotometeric determination of safflower yellow in *Flos Carthami*. Anal Chim Acta. 580:75-82.

Csakvari AC, Moisa C, Radu DG, Olariu LM, Lupitu AI, Panda AO, Pop G, Chambre D, Socoliuc V, Copolovici L, et al. 2021. Green synthesis, characterization, and antibacterial properties of silver nanoparticles obtained by using diverse varieties of *Cannabis sativa* leaf extracts. Molecules. 26(13):4041.

Das M, Shim KH, An SSA, Yi KD. 2011. Review on gold nanoparticles and their applications. Toxicol Environ Health Sci. 3:193-205.

Dent M, Bursać Kovačević D, Bosiljkov T, Dragović-Uzelac V. 2017. Polyphenolic composition and antioxidant capacity of indigenous wild dalmatian sage (*Salvia officinalis* L.). Croat Chem Acta. 90(3):451-459.

Dent M. 2015. Comparison of conventional and ultrasound-assisted extraction techniques on mass fraction of phenolic compounds from sage (*Salvia officinalis* L.). Chem Biochem Eng Q. 29:475-484.

Dragović-Uzelac V, Elez Garofulić I, Jukić M, Penić M, Dent M. 2012. The influence of microwave-assisted extraction on the isolation of sage (*Salvia officinalis* L.) polyphenols. Food Technol Biotechnol. 50:377-383.

Dzimitrowicz A, Jamróz P, diCenzo GC, Sergiel I, Kozlecki T, Pohl P. 2016. Preparation and characterization of gold nanoparticles prepared with aqueous extracts of *Lamiaceae* plants and the effect of follow-up treatment with atmospheric pressure glow microdischarge. Arab J Chem. 12:4118-4130.

Eugenio M, Campanati L, Müller N, Romão LF, de Souza J, Alves-Leon S, de Souza W, Sant'Anna C. 2018. Silver/silver chloride nanoparticles inhibit the proliferation of human glioblastoma cells. Cytotechnology. 70(6):1607-1618.

Farhat MB, Chaouch-Hamada R, Sotomayor JA, Landoulsi A, Jordán MJ. 2014. Antioxidant potential of *Salvia officinalis* L. residues as affected by the harvesting time. Ind Crops Prod. 54:78-85.

Fernandes TCC, Mazzeo DEC, Marin-Morales MA. 2007. Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pestic Biochem Phys. 88:252-259.

Francik S, Francik R, Sadowska U, Bystrowska B, Zawiślak A, Knapczyk A, Nzeyimana A. 2020. Identification of phenolic compounds and determination of antioxidant activity in etracts and infusions of Salvia leaves. Mater. 13:5811.

Garcia CS, Menti C, Lambert AP, Barcellos T, Moura S, Calloni C, Branco CS, Salvador M, Roesch-Ely M, Henriques JA. 2016. Pharmacological perspectives from Brazilian *Salvia officinalis* (*Lamiaceae*): antioxidant, and antitumor in mammalian cells. AABC. 88:281-292.

Ghorbani A, Esmaeilizadeh M. 2017. Pharmacological properties of *Salvia officinalis* and its components. J Tradit Complement Med. 7:433-440.

Gîrd CE, Nencu I, Costea T, Duţu LE, Popescu ML, Ciupitu N. 2014. Quantitative analysis of phenolic compounds from *Salvia officinalis* L. leaves. Farmacia. 62:649-657.

Gomes NGM, Campos MG, Órfão JMC, Ribeiro CAF. 2009. Plants with neurobiological activity as potential targets for drug discovery. Prog Neuropsychopharmacol Biol Psychiatry. 33:1372-1389.

Grosso C, Valentão P, Ferreres F, Andrade PB. 2015. Alternative and efficient extraction methods for marine-derived compounds. Mar Drugs. 13:3182-3230.

Haliem AS. 1990. Cytological effect of the herbicide sencorer on mitosis of *A. cepa*. Egypt. J. Bot. 33:93-104.

Hamrouni-Sellami I, Rebey IB, Sriti J, Rahali FZ, Limam F, Marzouk B. 2012. Drying sage (*Salvia officinalis* L.) plants and its effects on content, chemical composition, and radical scavenging activity of the essential oil. Food Bioproc Tech. 5:2978-2989.

Hayat K, Hussain S, Abbas S, Farooq U, Ding B, Xia S, Jia C, Zhang X, Xia W. 2009. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity *in vitro*. Sep Purif Technol. 70:63-70.

Hernández Y, Lobo MG, González M. 2009. Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. Food Chem. 114:734–741.

Hsin YH, Chen CF, Huang S, Shih TS, Lai PS, Chueh PJ. 2008. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol. Lett. 179:130-139.

Hu W, Chen S, Li X, Shi S, Shen W, Zhang X, Wang H. 2009. In situ synthesis of silver chloride nanoparticles

into bacterial cellulose membranes. Mater. Sci. Eng. C. 29(4):1216-1219.

Jaimez Ordaz J, Martínez Hernández J, Ramírez Godínez J, Castañeda Ovando A, González Olivares LG, Contreras López E. 2018. Bioactive compounds in aqueous extracts of lemon balm (*Melissa officinalis*) cultivated in Mexico. Arch Latinoam Nutr. 68:268-279.

Kim S, Yun EJ, Bak JS, Lee H, Lee SJ, Kim CT, Lee JH, Kim KH. 2010. Response surface optimised extraction and chromatographic purification of rosmarinic acid from *Melissa officinalis* leaves. Food Chem. 121:521-526.

Kintzios SE. 2000. Sage: The Genus Salvia. In: Kintzios SE, editor. Botany. Amsterdam: CRC Press, p. 10-11.

Kirsner R, Orsted H, Wright B. 2001. Matrix metalloproteinases in normal and impaired wound healing: a potential role of nanocrystalline silver. Wounds. 13:5-10.

Kumari A, Yadav SK, Yadav SC. 2010. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B. 75:1-18.

Liman R, Akyil D, Eren Y, Konuk M. 2010. Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/Salmonella and Allium test. Chemosphere. 80(9):1056-1061.

Lin SR, Chang CH, Hsu CF, Tsai MJ, Cheng H, Leong MK, Sung PJ, Chen JC, Weng CF. 2019. Natural compounds as potential adjuvants to cancer therapy: preclinical evidence. Br J Pharmacol. 177:1409-1423.

Longaray Delamare AP, Moschen-Pistorello IT, Artico L, AttiSerafini L, Echeverrigaray S. 2007. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem. 100:603–608.

Manolescu DȘ, Uță G, Din A, Avram S. 2022. The particle size influence of *Melissa officinalis* L. powder on TEAC and TPC correlated with the *in silico* study of one of the antioxidants: caffeic acid. Rom J Biophys. 32:1-16.

Mocellin S, Nitti D. 2008 TNF and cancer: the two sides of the coin. Front Biosci. 13:2774-2783.

Naikoo GA, Mustaqeem M, Hassan IU, Awan T, Arshad F, Salim H, Qurashi A. 2021. Bioinspired and green synthesis of nanoparticles from plant extracts with antiviral and antimicrobial properties: A critical review. J. Saudi Chem. Soc. 25(9): 101304.

Nayeri FD, Mafakheri S, Mirhosseini M, Sayyed R. 2021. Phyto-mediated silver nanoparticles via *Melissa officinalis* aqueous and methanolic extracts: synthesis, characterization and biological properties against infectious bacterial strains. Int. J. Adv. Biol. Biomed. Res. 9(3):270-285.

Onwuamah CK, Ekama SO, Audu RA, Ezechi OC, Poirier MC, Odeigah PGC. 2014. Exposure of *Allium cepa* root cells to zidovudine or nevirapine induces cytogenotoxic changes. PLoS ONE. 9:e90296.

Osmić S, Begić S, Mićić V, Petrović Z, Avdić G. 2019. Effect of solvent and extraction conditions on antioxidative activityof sage (*Salvia officinalis* L.) extracts obtained by maceration. Technologica Acta. 11:1-8.

Pandey R, Zahoor A, Sharma S, Khuller GK. 2003. Nanoparticle encapsulated antitubercular drugs as a potential oral drug delivery system against murine tuberculosis. Tuberculosis (Edinb). 83:373-378.

Papoti V, Totomis N, Atmatzidou A, Zinoviadou K, Androulaki A, Petridis D, Ritzoulis C. 2019. Phytochemical content of *Melissa officinalis* L. herbal preparations appropriate for consumption. Processes. 7:8-88.

Paré JRJ, Sigouin M, Lapointe J. 1991. Microwave-assisted natural product extraction. US Patent 5 002:784.

Parveen S, Misra R, Sahoo SK. 2012. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomed: Nanotechnol Biol Med. 8:147-166.

Petkova N, Ivanov I, Mihaylova D, Krastanov A. 2017. Phenolic acids content and antioxidant capacity of commercially available *Melissa officinalis* L. teas in Bulgaria. Bulg Chem Commun. 49:69-74.

Phuyal N, Jha PK, Raturi PP, Rajbhandary S. 2020. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. Sci World J .16:1-7.

Pop AV, Tofană M, Socaci SA, Vârban D, Nagy M, Borș MD, Sfechiș S. 2015. Evaluation of antioxidant activity and phenolic content in different *Salvia officinalis* L. extract. Bulletin UASVM Food Science and Technology. 72:210-214.

Proestos C, Chorianopoulos N, Nychas GJE, Komaitis M. 2005. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. J Agric Food Chem. 53:1190-1195.

Roby MHH, Sarhan MA, Selim KAH, Khalel KI. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. Ind Crops Prod. 43:827-831.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha YL. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 8:1-10.

Shakeri A, Sahebkar A, Javadi B. 2016. *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol. 188:204-228.

Shelembe B, Mahlangeni N, Moodley R. 2022. Biosynthesis and bioactivities of metal nanoparticles mediated by *Helichrysum aureonitens*. J Anal Sci Technol. 13:8.

Sherman D. 2014. Preparation of formvar film-coated grids, DS Imaging, LLC. https://www.emsdiasum.com/microscopy/technical/techtips/formvar_film-coated_grids.aspx. [Accessed 10 January 2022].

Shields JP. 1999. Using a formvar-coated bridge to apply formvar support film to TEM grids. MTO. 7:18-19.

Shimamura T, Sumikura Y, Yamazaki T, Tada A, Kashiwagi T, Ishikawa H, Matsui T, Sugimoto N, Akiyama H, Ukeda H. 2014. Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives – inter-laboratory evaluation study. Anal Sci. 30:717-721.

Siakavella IK, Lamari F, Papoulis D, Orkoula M, Gkolfi P, Lykouras M, Avgoustakis K, Hatziantoniou S. 2020. Effect of plant extracts on the characteristics of silver nanoparticles for topical application. Pharmaceutics. 12(12):1244.

Šulniūtė V, Ragažinskienė O, Venskutonis PR. 2016. Comprehensive evaluation of antioxidant potential of 10 Salvia species using high pressure methods for the isolation of lipophilic and hydrophilic plant fractions. Plant Foods Hum Nutr. 71:64-71.

Sun C, Lee JSH, Zhang M. 2008. Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliv Rev. 60:1252-1265.

Sutan NA, Manolescu DS, Fierascu I, Neblea AM, Sutan C, Ducu C, Soare LC, Negrea D, Avramescu SM, Fierascu RC. 2018. Phytosynthesis of gold and silver nanoparticles enhance *in vitro* antioxidant and mitostimulatory activity of *Aconitum toxicum* Reichenb. Rhizomes alcoholic extracts. Mater. Sci. Eng. C. 93:746–758.

Sutan NA, Vilcoci DȘ, Fierascu I, Neblea AM, Sutan C, Ducu C, Soare LC, Negrea D, Avramescu SM, Fierascu RC. 2019. Influence of the phytosynthesis of noble metal nanoparticles on the cytotoxic and genotoxic effects of *Aconitum toxicum* Reichenb. leaves alcoholic extract. J Clust Sci. 30:647-660.

Swilam N, Nematallah KA. 2020. Polyphenols profile of pomegranate leaves and their role in green synthesis of silver nanoparticles. Sci Rep. 10:14851.

Tedesco SB, Laughinghouse HD. 2012. Bioindicator of genotoxicity: the *Allium cepa* test. In: Srivastava JK, editor. Environmental Contamination. Rijeka: InTech Publisher; p. 137-156.

Teimouri M, Khosravinejad F, Attar F, Saboury AA, Kostova I, Benelli G, Falahati M. 2018. Gold nanoparticles fabrication by plant extracts: synthesis, characterization, degradation of 4-nitrophenol from industrial wastewater, and insecticidal activity – A review. J. Clean. Prod. S0959652618306000.

Tripathy A, Chandrasekran N, Raichur AM, Mukherjee A. 2008. Antibacterial applications of silver nanoparticles synthesized by aqueous extract of *Azadirachta indica* (Neem) leaves. J Biomed Nanotechnol. 4:1-6.

Udrea AM, Puia A, Shaposhnikov S, Avram S. 2018. Computational approaches of new perspectives in the treatment of depression during pregnancy. Farmacia. 66:680-687.

Uță G, Manolescu DȘ, Avram S. 2021. Therapeutic properties of several chemical compounds of *Salvia officinalis* L. in Alzheimer's Disease. Mini Rev Med Chem. 21:1421-1430.

Velamuri R, Sharma Y, Fagan J, Schaefer J. 2020. Application of UHPLC-ESI-QTOF-MS in phytochemical profiling of sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*). Planta Med. 7:133-144.

Veličković DT, Karabegović IT, Stojičević SS, Lazić ML, Marinković VD, Veljković VD. 2011. Comparison of antioxidant and antimicrobial activities of extracts obtained from *Salvia glutinosa* L. and *Salvia officinalis* L. Hem Ind. 65:599-605.

Wang L, Weller CL. 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Technol. 17:300-312.

Yilmaz Öztürk B,2019. Intracellular and extracellular green synthesis of silver nanoparticles using *Desmodesmus* sp.: their Antibacterial and antifungal effects. Caryologia. 72(1): 29-43.

Zeković Z, Pintać D, Majkić T, Vidović S, Mimica-Dukić N, Teslić N, Pavlić B. 2017. Utilization of sage by-products as raw material for antioxidants recovery—Ultrasound versus microwave-assisted extraction. Ind Crops Prod. 99:49-59.

Zhang QW, Lin LG, Ye WC. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chin. Med. 13:20.

Žlabur JŠ, Voća S, Dobričević N, Pliestić S, Galić A, Boričević A, Borić N. 2016. Ultrasound-assisted extraction of bioactive compounds from lemon balm and peppermint leaves. Int Agrophys. 30:95-104.