



## IN VITRO EVALUATION OF ANTIOXIDATIVE ACTIVITIES OF THE EXTRACTS OF PETALS OF *PAEONIA LACTIFLORA* AND *CALENDULA OFFICINALIS* INCORPORATED IN THE NEW FORMS OF BIOBASED CARRIERS

Petar M. Batinić<sup>\*1</sup>, Milena D. Milošević<sup>2</sup>, Milan M. Lukić<sup>1</sup>, Željana S. Prijić<sup>1</sup>, Stefan V. Gordanić<sup>1</sup>, Vladimir M. Filipović<sup>1</sup>, Aleksandar D. Marinković<sup>3</sup>, Branko M. Bugarški<sup>3</sup>, Tatjana Lj. Marković<sup>1</sup>

<sup>1</sup>Institute for Medicinal Plants Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia

<sup>2</sup>University of Belgrade, Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia, Njegoševa 12, 11000 Belgrade, Serbia

<sup>3</sup>University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, 11000 Belgrade, Serbia

**Abstract:** In this study, the petals collected from peony hybrid *Paeonia lactiflora* "Bowl of Beauty" and *Calendula officinalis* L. were extracted using an ethanol-water mixture assisted with microwave and ultrasonic treatment. The isolation of *Calendula officinalis* L. essential oil was done by hydrodistillation as well. The total phenolic and flavonoids content in the extracts and oil were determined and their antioxidant activity was evaluated. The highest total phenolic content was found for the extracts of hybrid *P. lactiflora* and *C. officinalis* L. obtained by ultrasound extraction (83.16 and 114.47 mg GA/g, respectively), while the flavonoid content obtained by microwave-assisted extraction was relatively high (123.48 and 65.29 mg QE/g, respectively). The highest antioxidant activity was obtained in DPPH<sup>•</sup> and ABTS<sup>•+</sup> assay for the microwave-assisted extraction of hybrid *P. lactiflora* (79% and 83%) and ultrasound-assisted extraction of *C. officinalis* L. (45% and 49%), respectively. To improve antioxidant activity of both types of examined analytes (extracts and essential oil), the pectin biopolymer film (as a carrier) was prepared in the process of enzymatically assisted catalysis. Optical microscopy and FTIR spectroscopy were used for the characterization of obtained materials. The films, with essential oil of *C. officinalis* L. and gallic acid, showed significantly increased percentage inhibition in DPPH<sup>•</sup> and ABTS<sup>•+</sup> test (91% and 95%, respectively) after 10 minutes. The results, also, showed that all formulations of pectin biopolymer film, modified with gallic acid, can be successfully applied as a carrier for both types of ingredients.

**Key words:** *antioxidative assays, microwave-assisted extraction, ultrasonic-assisted extraction, nanoemulsion, pectin film*

## INTRODUCTION

In recent years many studies have reported that the consumption of phytochemical-rich food, on one side, and the use of different pharmaceutical products based on medicinal plants, on the other side, have extremely important benefits for human health (Dienaitė et al.,

2019). Polyphenols and their derivate (flavonoids, carotenoids, anthocyanins, and tannins) can be defined as a group of pharmaceutical and biological active micronutrients, all of them being widely investigated. Some investigations present the use of phytoche-

micals in pharmaceutical products in the prevention of skin diseases and cancer (Dienaitė et al., 2019). Reactive oxygen species (ROS) can act as signal transduction molecules that disturb normal processes in human cells (Dienaitė et al., 2019) and also intensify protein and lipid oxidation (Dienaitė et al., 2019; Ray, Huang & Tsuji, 2012).

Some of the acute and chronic health problems, such as hypertension, diabetes, and skin ageing, occur as a consequence of disturbed metabolism. Under physiological conditions, the leakage of electrons from the mitochondrial electron transport chain is the major intracellular source of ROS, although there are substantial controversies regarding the exact amounts of ROS produced at the different sites of the respiratory chain. In living cells, ROS are produced in many cellular sites including the plasma membrane, endoplasmic reticulum, mitochondrial membrane, lysosome, and peroxisome.

The cytosol is one of the ROS-generated places. In the cytosol, some of the active components participating in redox reactions include thiols, hydroquinones, catecholamines, and flavins as a consequence of the production of cellular ROS (Freeman & Crapo, 1982).

A large spectrum of bioactive substances found in medicinal plant species may be responsible for their medicinal, pharmacological, and biological activities.

*Paeonia lactiflora* is a Chinese traditional medicinal plant that has had a wide range of applications in medicine for more than 1200 years for the treatment of rheumatoid arthritis, painful menstruation, acute and chronic hepatitis, *lupus erythematosus disseminates*, etc. (He & Dai, 2011).

The main components are paeoniflorin, oxy-paeoniflorin, benzoylpaeoniflorin, albiflorin, paeonoflorigenone and lactiflorin. Literature data suggested that paeoniflorin has a protective effect on cells against oxidative stress. Namely, this active molecule significantly protected primary cultures of rat cortical cells exposed to oxidative stress induced by hydrogen peroxide (Kim & Ha, 2009; He & Dai, 2011). Also, some basic medicinal studies confirm that paeoniflorin and its derivate have a neuroprotective effect on the neurotoxicity induced by glutamate. Due to its analgesic properties, *Paeonia lactiflora* is the main con-

stituent in the different pharmaceutical active forms. Generally, acute dysmenorrhea can be treated by different types of vaginal suppositories that contain medicinal pharmacological substances isolated from *Paeonia lactiflora*.

The plant species *Calendula officinalis* L. is an annual herb originating from South Europe, the Mediterranean region (Ramos et al., 1998). Its common name is Pot Marigold and belongs to the *Asteraceae* family. In the past, its name use to be associated with healing stews used for the prevention of skin inflammation and wounds (Ramos et al., 1998).

According to Gazim, Rezende, Fraga, Svidzinski and Cortez (2008), the herb is abundant in carotenoids (xanthophylls), flavonoids, glycosides, as well as in some other liposoluble bioactive compounds, while its essential oil is abundant in  $\alpha$ -cadinol (24,2%), though  $\alpha$ -muurolol, *d*-cadinene, and 1,3,5-cadinatrien, also use to represent major oil constituents. *Calendula officinalis* L. found its application in pharmaceutical technology, cosmetology, medicine, and perfumery, and holds both, GRAS and FDA status.

Pectin is a natural polymer material that is commonly applied for emulsifying, stabilizing, and thickening purposes in food and cosmetics. It is a biodegradable, biocompatible, and non-toxic material. Due to its mucoadhesive properties, it is suitable for coating nanoemulsions and liposomes (Klemetsrud, Jonassen, Hiorth, Kjøniksen & Smistad, 2013; Zhang, Zheng, Huang & Fei, 2020).

In the present study, microwave and ultrasonic-assisted extraction of the petals of hybrid *Paeonia lactiflora* "Bowl of Beauty" and *Calendula officinalis* L. were applied to examine the antioxidative potential of the obtained extracts; to better understand their antioxidative potential, total phenolic and flavonoid contents were determined.

In addition, the gallic acid-modified pectin film, containing nanoemulsion of the essential oil of *Calendula officinalis* L. was prepared to improve its antioxidative capability.

## MATERIALS AND METHODS

### Chemicals

Anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), Folin-Ciocalteu reagent, gallic acid (GA), aluminium chloride ( $\text{AlCl}_3$ ), quercetin (QE), L-ascorbic acid, 1,1-diphenyl-

2-picryldrazil (DPPH<sup>•</sup>), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>) were purchased from Sigma-Aldrich. Ethanol is obtained from Fisher Chemical. All chemicals and reagents were of p.a. grade and used without further purification. Filter paper CM (12-15 µm) was supplied from CHMLAB GROUP.

### Plant material

Fresh petals of hybrid *Paeonia lactiflora* "Bowl of Beauty" (PLh) were collected from the plants cultivated in the Institute's medicinal plants collection in Pančevo, Serbia. The PLh were left to air-dry at room temperature (21 °C) for three weeks. Petals of *Calendula officinalis* L. (CO) were obtained from a local producer who cultivated this plant species near Pančevo. The CO petals were already air-dried according to standard procedure (40 °C). Prior to the extraction procedure, the petals of PLh and CO were pulverized (to the average particle size of approximately 3 mm), with the use of a laboratory mill (IKA M20 Universal mill).

### Preparation of extracts and essential oil

#### *Microwave-Assisted (MWA) extract preparation*

The pulverized petals (1 g) of the CO plant were extracted with a 30 ml mixture of 97% ethanol and distilled water (at 50:50 volume ratio), at 80 °C for 10 minutes using Microwave Synthesis Reactor – Monowave 300, Anton Paar, GmbH, Germany (Fig. 1). The same extraction procedure was applied for the petals of the PLh plant.

The collected raw extracts of CO (COE\_MWA) and PLh (PLhE\_MWA) from five extractions were filtered using laboratory filter paper, poured into dark glass bottles, and left in the refrigerator at 4 °C, until analysis.

#### *Ultrasound-Assisted (USA) extract preparation*

The pulverized CO petals (1 g) were extracted with a 30 ml mixture of 97% ethanol and distilled water (at 50:50 volume ratio), 60 °C for 30 minutes (3x10 minutes) using the Ultrasonic Transducer, APC, International, Ltd. method (Fig. 1). The same procedure of extraction was applied for the PLh petals. The collected raw extracts of CO (COE\_USA) and PLh (PLhE\_USA) from five extractions were filtered using laboratory filter paper, then

poured into the dark glass bottles and left in the refrigerator at 4 °C, until analysis.

### Isolation of essential oil

The essential oil was isolated from the CO plant material according to following procedure: 500 g of air-dry flower heads was added to flask with 1.5 L of distilled water (DW) and then it has been subjected to 4-hour hydro-distillation (HD) using the Clevenger-type apparatus (Fig. 1). Then, the essential oil of the CO plant material (COO\_HD) was collected in a rounded bottom flask and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, measured on the analytical balance (Mettler, USA, Type AE 200; ±0.00001 g) and left to freeze at -18 °C, until analysis. The essential oil content was calculated using the following formula:

$$\text{Essential oil content \%} = \frac{\text{weight of essential oil}}{\text{weight of dry sample}} \times 100$$

### Preparation of nanoemulsion (NE) with encapsulated COO\_HD

The method of Almasi et al. (2020) was used for the preparation of nanoemulsion (NE) with some modifications. In short, the coarse emulsion was prepared by gradually adding sunflower oil (SO) (1%) and Tween 80 (10% wt. of SO) to distilled water with agitation at 2000 rpm for 10 minutes. Also, 1% of the COO\_HD was added to coarse emulsion prior to the agitation process. Then ultrasonication was used to convert coarse emulsion to NE. An ultrasonic bath operating at 20 kHz and 200 W was used for sonication. The treatment lasted 15 minutes.

### Particle size and stability of NE

NE was analysed using Malvern Zetasizer Nano ZS (Malvern Instruments, Worcester-shire, UK). The average size of NE, index of polydispersity (PDI), and zeta potential (ZP) were measured immediately following the preparation of NE. NE was diluted with deionized water 1000 times before each measurement. Prior to analysis, NE was stored in the refrigerator at 4 °C.

### Preparation of pectin film containing NE

The film was prepared according to the method by Zhang et al. (2020) with some modifications. In short, 7.5 g of pectin (PE) was dissolved in 200 ml distilled water (DW).

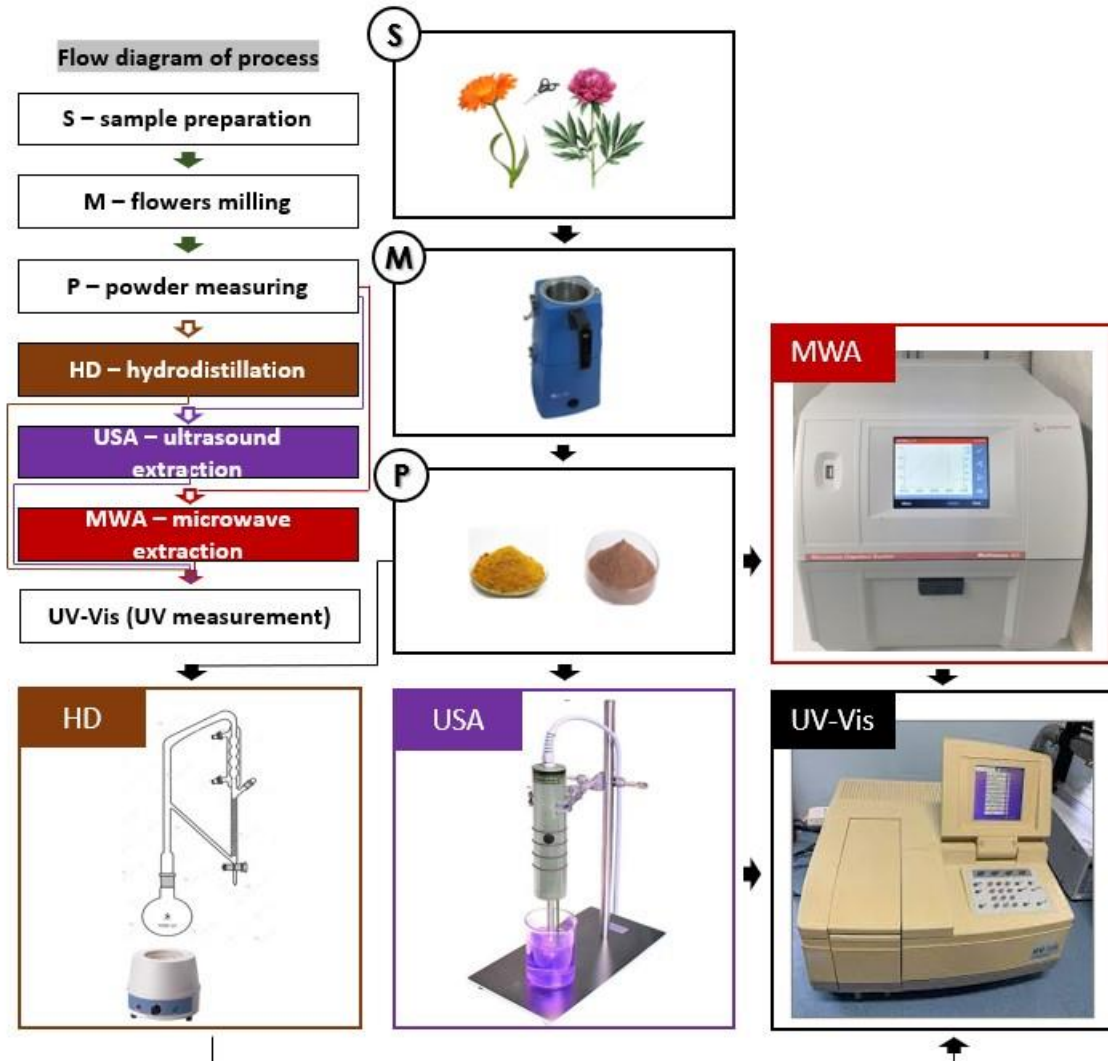


Figure 1. Schematic diagram of MWA and USA extraction of CO and PLh petals, and hydrodistillation of CO plant material

The pectin/nanoemulsion film (PE-NE) was prepared by mixing the solution of PE and NE in a mass ratio of 1:1 at room temperature for 60 min using a magnetic stirrer. The PE-NE film containing GA was prepared as follows: 2.5 g GA was dissolved in 50 ml DW and then mixed with a solution of PE (7.5 g in 200 ml DW) and 0.01 g lipase as a catalyst. The mixture was stirred at 45 °C for 24 h to provide PE-GA (covalently modified with GA, Fig. 2). Rotary evaporation was used to eliminate the residual solvent. Then, 3x30 ml of ethyl alcohol was added to the gel mixture to dissolve the acylating agent. The mixture was centrifuged three times at 4000 rpm for five minutes, at 4 °C. The supernatant was removed and the precipitate was collected. This operation was repeated three times until the

residual acylating agent (GA) was removed. Then, 100 ml of DW was added to pectin to obtain pure biopolymer hydrogel. After that 2.5 ml of NE and 2.5 ml of PE-GA in DW were mixed on a magnetic stirrer at 25 °C for 4 hours, to obtain homogenous dispersion.

Finally, the hydrogel was poured out on the Petri dish and dried at room temperature to obtain the dry film. Biopolymer films, named PE-NEGA (pectin film containing NE and GA) were stored in a dark place until further analysis (schematic illustration is given in Fig. 2). The same procedure was used for the preparation of the pectin containing PLh\_MWA extract (PE-PLh) and COE\_USA extract (PE-COE) and the film was modified with GA (PE-PLhEGA and PE-COEGA).

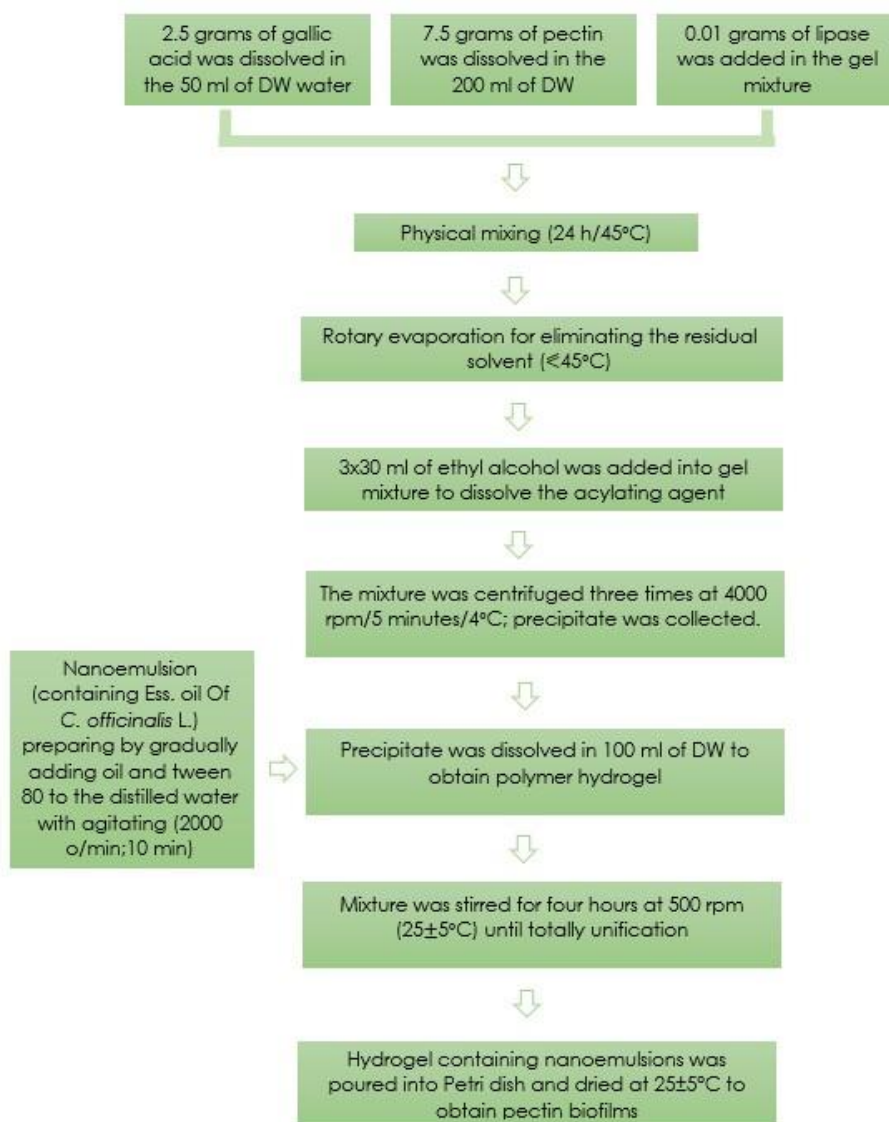


Figure 2. Schematic illustration of the procedure of the preparation of PE-GA and PE-NEGA films

### Determination of total phenols content of the extract

Total phenolic content (TPC) in the extracts was determined using the Folic-Ciocalteu assay (Singleton, Orthofer & Lamuela-Raventos, 1999), with modifications. In short, 0.1 ml of the extract, 0.5 ml of Folin-Ciocalteu reagent and 6 ml of DW were transferred into a 10 ml flask and mixed thoroughly. After five minutes, 1.5 ml of  $\text{Na}_2\text{CO}_3$  was added, followed by DW up to 10 ml. After 2 h of standing, the absorbance was measured at 725 nm using UV/vis spectrophotometer. GA was used as a standard (0.03 – 0.5 mg ml<sup>-1</sup>) and the results of TPC were expressed as mg gallic acid (GA) per gram of the dry extract (mg GAE/g). All measurements were performed in

duplicate using a Shimadzu 1700 UV/vis spectrophotometer (Shimadzu, Japan).

### Determination of total flavonoids content of the extract

Total flavonoid content (TFC) in the extracts was analysed using the modified Patricia do Rocio Smolinski Savi method (Savi, Dos santos, Gonçalves, Biesek & De Lima, 2017). In short,  $\text{AlCl}_3$  was mixed with 2 ml of ethanol-water extract. The absorbance was determined using a Shimadzu UV/vis spectrophotometer (Japan) at 425 nm after 10 minutes against a blank (2 ml of  $\text{AlCl}_3$  + 2 ml of  $\text{H}_2\text{O}$ ). Total flavonoid content was determined using a standard quercetin (QE) curve with a concentration ranging from 1 – 25  $\mu\text{g ml}^{-1}$ . Results were expressed as mg quercetin (QE) per gram of dry

extract (mg QE/g). All assays were performed in duplicate using a Shimadzu 1700 UV/vis spectrophotometer (Japan).

#### Determination of *in vitro* DPPH<sup>•</sup> and ABTS<sup>•+</sup> free radical assay

These assays are based on the reduction of stable DPPH<sup>•</sup> or ABTS<sup>•+</sup> radical by antioxidants measuring the changes in the absorbance of DPPH<sup>•</sup> at 517 nm / (ABTS<sup>•+</sup>) at 734 nm, according to the procedure of Prior, Wu and Schaich (2005), modified by Milošević et al. (2020). In short, 0.2 ml of the sample was mixed with 2.8 ml of DPPH<sup>•</sup> ethanolic solution (7 mM) or ABTS<sup>•+</sup> ethanolic working solution (7.8 mM ABTS<sup>•+</sup> stock solution and 2.45 mM solution K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>); the antioxidant capacity was evaluated by measuring absorbance at 517 nm and 734 nm for DPPH<sup>•</sup> and ABTS<sup>•+</sup> free radical assay, respectively. The same procedure (0.2 ml of pure ethanol in 2.8 ml of radical solution) for control was used. Antioxidant assays were measured using Shimadzu 1700 UV/vis spectrophotometer. The tests were performed in duplicate. Ascorbic acid was used as a control standard solution. Radical scavenging activity was evaluated using the following equation:

$$\text{RSA (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

where  $A_{\text{sample}}$  and  $A_{\text{control}}$  are absorbances at 517 nm of DPPH<sup>•</sup> and 734 nm of ABTS<sup>•+</sup> in the sample and the control solutions, respectively.

#### Film characterization

##### Fourier-Transfer Infrared Spectroscopy (FT-IR) analysis

FT-IR spectra of the film were recorded using a Thermo Scientific Nicolet 6700 spectrometer (Thermo Fisher Scientific, USA) in the attenuated total reflectance (ATR) mode. The range of wavenumber was 4000 - 500 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> at room temperature.

##### Optical microscopy

The surface morphology of modified pectin films was observed using an optical microscope (Olympus CX41, Japan) with reflected light equipped with a CCD digital camera (magnification of 50 times). The obtained micrographs were analysed by Image J software (Olympus Viewer 3 Image Management Soft-

ware) to access the average diameter of the samples.

## RESULTS

### Particle size and stability of NE containing essential oil of *C. officinalis*

The obtained measurements proved high stability of nanodroplets (high negative charge, ZP = -39), with the average droplet's diameter of 404.6 nm. The polydispersity index was 0.5 (the system was monodispersed, PDI ≤ 0.5, Table 1). Also, essential oil content (%) was calculated using formula defined in the theoretical part of this paper.

### Total phenolic and flavonoid content of the extracts

The TPC and TFC contents in the extracts were determined by constructing a standard curve with GA and quercetin (QE), respectively, and the results were presented in Table 2. The values of TPC in MWA extracts were lower compared to that of USA-assisted extraction. It varied from 18 mg GA/g dry extract (COE\_MWA) to 104.47 mg GA/g (COE\_USA), suggesting that the phenolic compounds could be better extracted via ethanol-water from COE\_USA. Previous data for *C. officinalis* showed that TPC ranged from 29.79% mg/g (Veličković, Dimitrijević, Mitić, Mitić & Kostić, 2014) to 72.91% mg/g (Sabir, Khan, Rocha, Boligon & Athayde, 2015), while the TFC was 24.67 mg/g (Deuschle et al., 2015), or 28.3 mg/g (Gunes et al., 2020). We believe that the time of harvest and given climatic conditions in which the plants grew, influenced the polyphenolic profile of the studied extracts. Actually, in a previous study on *C. officinalis* it was reported that TFC reaches its highest content about three days following the full anthesis (Honório et al., 2016). However, data concerning TPC are missing. In addition, the content of flavonoids in all extracts obtained by extraction using the MWA transducer was much higher than that obtained by the USA treatments; TFC ranged from 13.51 mg (COE\_USA) to 123.48 mg QE/g (PLhE\_MWA). The lower extractability of flavonoids in ultrasound treatment might be explained by the fact that the ultrasound radiation probably causes the destruction of certain macromolecules in the plant thus providing more efficient extraction of the phenols bonded to the cell wall of polysaccharides.

**Table 1.**

Particle size and stability of nanoemulsion (NE) containing *Calendula officinalis* L. essential oil obtained by hydrodistillation (COO\_HD)

Sample –day of analysis	Zeta potential (mV)	Particle size (nm)	Polydispersity index	Essential oil content (%)
NE – 1 <sup>st</sup>	-39.0±0.3	404.6±5.1	0.5±0.1	0.155
NE – 10 <sup>th</sup>	-41.0±0.9	412.6±2.8	0.5±1.2	
NE – 20 <sup>th</sup>	-39.0±0.6	400.6±2.0	0.5±0.8	
NE – 30 <sup>th</sup>	-40.0±0.3	404.6±0.7	0.5±1.9	

**Table 2.**

Total phenolic content (TPC) and total flavonoid content (TFC) of the *Paeonia lactiflora* hybrid extract (PLhE) and *Calendula officinalis* L. extract (COE) and essential oil of *Calendula officinalis* L. (COO\_HD)

Sample	TPC <sup>a</sup> (mg GA/g)	TFC <sup>b</sup> (mg QE/g)
PLhE_MWA <sup>c</sup>	54.45	123.48
PLhE_USA <sup>d</sup>	83.16	54.69
COE_MWA	18.00	65.29
COE_USA	104.47	13.51
COO_HD <sup>e</sup>	5.17	18.62

<sup>a</sup>TPC (total phenolic content expressed as mg gallic acid (GA) per gram of dry extract)

<sup>b</sup>TFC (total flavonoid content expressed as mg quercetin (QE) per gram of dry extract)

<sup>c</sup>MWA (microwave-assisted extraction)

<sup>d</sup>USA (ultrasonic-assisted extraction)

<sup>e</sup>HD (hydrodistillation)

**Table 3.**

Radical scavenging activity (RSA) of ethanol-water extracts of the petals of *Paeonia lactiflora* and *Calendula officinalis*, and *Calendula officinalis* essential oil (%)

Extracts/Essential oil	RSA <sub>DPPH</sub> <sup>*</sup> (%)	RSA <sub>ABTS</sub> <sup>++</sup> (%)
<sup>a</sup> PLhE_MWA	79	83
<sup>b</sup> PLhE_USA	70	75
<sup>c</sup> COE_MWA	39	41
<sup>d</sup> COE_USA	45	49
<sup>e</sup> COO_HD	32	37
<sup>f</sup> PE-PLhE	86	88
<sup>g</sup> PE-COE	51	56
<sup>h</sup> PE-PLhEGA	93	98
<sup>i</sup> PE-COEGA	92	97
<sup>j</sup> PE-NE	45	51
<sup>k</sup> PE-NEGA	91	95
L-ascorbic acid	95	99

<sup>\*</sup>Radical scavenging activity (RSA) expressed as a percentage of inhibition of DPPH<sup>\*</sup> or ABTS<sup>++</sup> radical by phenolic compounds isolated from extract or essential oil;

<sup>a</sup>PLhE\_MWA – *Paeonia lactiflora* hybrid extract\_microwave-assisted; <sup>b</sup>PLhE\_USA – *Paeonia lactiflora* hybrid extract\_ultrasound-assisted; <sup>c</sup>COE\_MWA – *Calendula officinalis* extract\_microwave-assisted; <sup>d</sup>COE\_USA – *Calendula officinalis* extract\_ultrasound-assisted; <sup>e</sup>COO\_HD – *Calendula officinalis* essential oil\_hydrodistillation; <sup>f</sup>PE-PLhE – Pectin-*Paeonia lactiflora* hybrid extract; <sup>g</sup>PE-COE – Pectin-*Calendula officinalis* extract; <sup>h</sup>PE-PLhEGA – Pectin-*Paeonia lactiflora* hybrid extract modified by gallic acid; <sup>i</sup>PE-COEGA – Pectin-*Calendula officinalis* extract modified by gallic acid; <sup>j</sup>PE-NE – Pectin containing nanoemulsion with encapsulated essential oil of *Calendula officinalis*; <sup>k</sup>PE-NEGA – Pectin containing nanoemulsion with encapsulated essential oil of *Calendula officinalis* modified by gallic acid

On the other side, microwave radiation quickly achieves high temperatures which shortens extraction time and it uses moderate energy during the extraction procedure. Thus, the extraction of flavonoids not bonded to polysac-

charides prove to be more efficient. In addition, a short extraction time eliminates the risk of degradation of some heat-sensitive molecules (Rajbhar et al., 2015; Chen, Xiao, Zheng & Liang, 2015; Wu et al., 2021).

## Antioxidant activity

Antioxidant activity of extracts and essential oil, analysed using two radical assays, is presented as a percentage RSA of DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals (Table 3).

The results obtained using the DPPH<sup>•</sup> assay for the extracts showed good antioxidant properties, presented in the following order: PLhE\_MWA>PLhE\_USA>COE\_USA>COE\_MWA. Both extracts, PLhE\_MWA and PLhE\_USA, displayed more than 50% of inhibition of free radicals (Table 2), which is comparable to that of the standard, L-ascorbic acid. ABTS<sup>•+</sup> assay revealed that all extracts exerted antioxidant activity similar to that obtained in the DPPH<sup>•</sup> assay, which is presented in the following order: PLhE\_MWA>PLhE\_USA>COE\_USA>COE\_MWA (Table 2). Essential oil of COO\_HD with scavenging activities of 32% and 37% for DPPH<sup>•</sup> and ABTS<sup>•+</sup>, respectively, showed moderate to weak activity relative to L-ascorbic acid, while the activity of the most biopolymer films was more than 50% of inhibition of free radicals.

## FTIR analysis of film

The FT-IR is a useful technique which is used to explain the nature of the functional groups, confirming the presence of PE in the system and elucidate intermolecular interactions of modified PE films (Fig. 3). FT-IR analysis showed the absorption peaks of the phenol group of GA at 3280, 3310, and 3520 cm<sup>-1</sup>. The main characteristic bands of the C=O stretching vibration appeared at 1697 cm<sup>-1</sup> which is in agreement with Singh, Rawat, Semalty and Semalty (2011). The region 1612-1436 cm<sup>-1</sup> relates to the C=C skeletal deformation vibration in aromatic structures, while the bands at 1337, 1230, and 1210 cm<sup>-1</sup> are assigned to C-O stretching vibrations of hydroxyl group. The C-H deformations vibration in aromatic structures appeared at 860 and 672 cm<sup>-1</sup> (Rocha et al., 2019).

In the case of pectin film, the broadband at 3280 cm<sup>-1</sup> is assigned to hydroxyl stretching vibration. Peaks at 2935 and 2886 cm<sup>-1</sup> were assigned to the aliphatic C-H groups asymmetric and symmetric stretching vibrations, respectively. The band at 1730 cm<sup>-1</sup> was assigned to C=O stretching vibrations of the ester carbonyl groups, while the peak at 1612 cm<sup>-1</sup> was assigned to the asymmetric stretching vibration of the carbonyl group of the car-

boxylate ion (Ognyanov et al., 2019). The peak at 1436 cm<sup>-1</sup> describes the presence of the (O=C-O) vibration of non-esterified groups in pectin (Demir, Ceylan, Göktürk & Bölgen, 2020). In addition, all peaks in the region from 1137 to 1029 cm<sup>-1</sup> indicate the presence of C-O stretching vibration from carboxylic acids, alcohols, and esters groups. The peak at 1106 cm<sup>-1</sup> confirms the presence of C-C stretching vibrations (Wathoni et al., 2019). In the case of PE-NE and PE-NEGA spectra, some changes were observed. The spectral region of the O-H bond shows a slightly sharp shape after modification of pectin with NE and GA.

Further, the band for C=O ester groups was moved to a high frequency at 1743 cm<sup>-1</sup>. In the methylene groups, a stretching vibration similar pattern was observed. Except for the methyl ester group from pectin, additional absorption comes from the emulators used in the emulsification process. Lipase provides a catalytic effect to the esterification of GA with pectin hydroxyl, and thus new ester group vibration appeared in the spectrum (Zhang et al., 2020).

Finally, in all samples, there are no significant changes in the region from 552 cm<sup>-1</sup> to 1230 cm<sup>-1</sup>, for C-H bonds in aromatics (Rocha et al., 2019). The results of FTIR analysis have confirmed the presence of a functional group of PE, GA in a film containing pectin and NE, and pectin fil 7m with NE modified by GA.

## Optical microscopy analysis

Optical microscopy was used to characterize produced film. The success of emulsification of the PE-NE and PE-NEGA was evaluated according to the emulsion morphology, size, and stability. As shown in Fig. 4a there is a significant number of uneven nanodroplets of different sizes (in the range of submicron to several tens of micrometres). Visually, it represents a noticeable clear border between the oil-water phases.

On the contrary, nanodroplets were uniform and entirely dispersed in pectin film. As can be seen in Fig. 4b the nanodroplets were homogenous and compact, indicating that the presence of galloyl influenced the emulsion properties of the PE-NEGA pectin film. This phenomenon is a consequence of the high hydrophobicity of galloyl, which could be oriented to the oil phase of nanodroplets (Zhang et al., 2020).



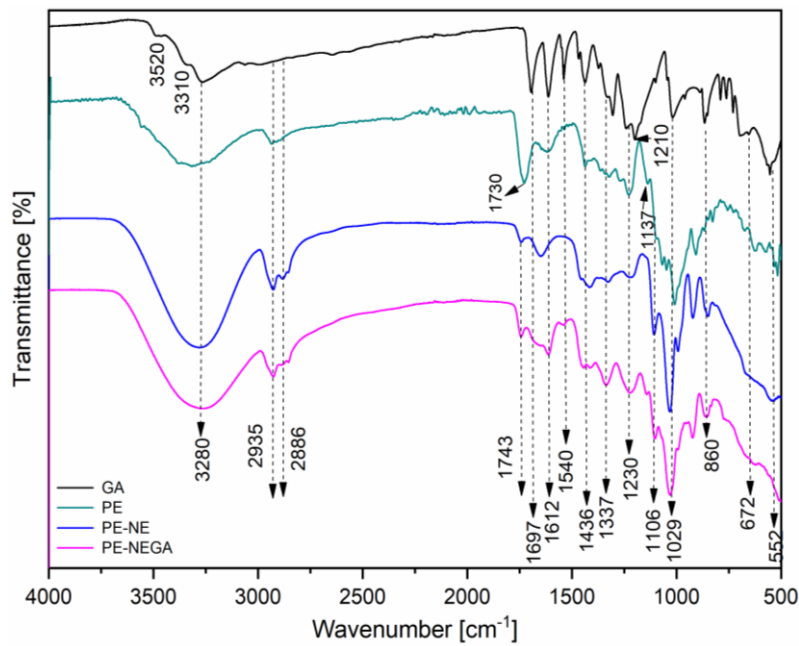


Figure 3. FT-IR analysis of pure GA, PE, PE-NE, and PE-NEGA

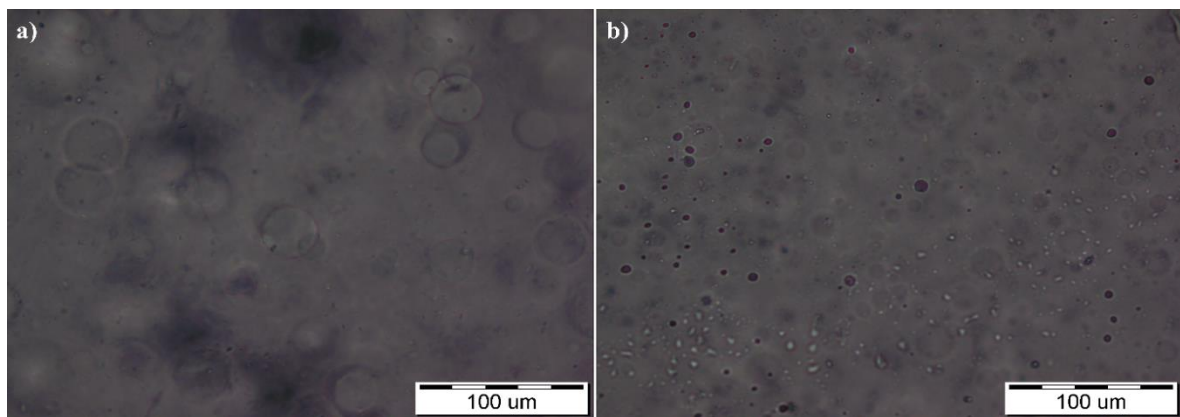


Figure 4. Optical microscopy of a) PE-NE, b) PE-NEGA

## DISCUSSION

Plants contain different natural phenolic structures with various properties that contribute to their various antioxidant activities. Previous research confirmed that some antioxidant compounds may be strongly bound to the insoluble plant matrix thus being unavailable for any solvent or solvents mixture without pre-treatment (Saura-Calixto, 2012). TPC and TFC values are variable and depend on many factors, such as plant variety, growing conditions, method and period of harvesting, climatic factors, plant organ (leaf, flower, root), extraction procedure and similar. The extraction of CO

flowers heads or PLh petals were performed using both, MWA and USA methods. For the extraction of polyphenols and flavonoids a system of two polar solvents, water and ethanol was applied in both mentioned techniques. According to Dienaitė et al. (2019), the system of two solvents in different ratios shows better extraction properties and higher efficacy. The solvents used in this study, ethanol and water are considered desirable as they are non-toxic and do not produce health risks. Considering total flavonoid content, the obtained results suggest that the extraction of selected plant materials using the MWA method was more efficient than the USA method

(Table 1). Probably, the temperature treatment greatly affected extraction efficiency. Namely, the microwave reactor generates a higher temperature than the ultrasonic transducer which has a temperature limitation of 60 °C. The samples of ethanol-water PLhE have high activity in DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical assay. On the other hand, both ethanol-water COE extracts and COO\_HD essential oil showed moderate activity. A previous study already presented the flower extracts of CO plant obtained by infusion and decoction, which exhibited moderate antioxidant activity in DPPH<sup>•</sup>, ABTS<sup>•+</sup>, CUPRAC and FRAP (Petkova, Mihaylova, Denev & Krastanov, 2020).

A comparative analysis of the achieved antioxidant activities of both types of extracts from the petals of CO and PLh dry plant material revealed that the MWA gave better results for PLhE extract while the USA for COE extracts. The trend in the activity of all extracts in the ABTS<sup>•+</sup> assay is the same as for the DPPH<sup>•</sup> assay, but statistically insignificant antioxidant activity in the DPPH<sup>•</sup> assay was obtained. The outcomes of this study showed that the ethanol-water extracts of PLhE and COE contain a component capable to donate electrons and/or hydrogen in a free-radical reaction.

A recent study, performed using HPLC–DPPH<sup>•</sup> scavenging method, showed that phenolic acids and GA and its derivatives are the most abundant in PLh plants, which significantly contribute to the overall antioxidant potential of the ethanol extracts of *P. lactiflora* (Yichao Wu et al., 2021). Also, theoretical calculations additionally confirmed that GA and its derivative can donate a hydrogen atom (hydrogen atom transfer or HAT mechanism) in the gas phase or donate the first electron and then proton (single electron transfer or SET mechanism) to a radical in water and ethanol (Chen et al., 2015). Taking into account the displayed data in previous research for phenolic compounds, and extraction in a polar solvent (ethanol and water) it could be assumed that the active components responsible for the activity of the PLh extract interact with free radicals via the following single electron transfer-proton transfer (SET-PT) mechanism (Chen et al., 2015).

Due to that covalently bonded GA was used in the production of the pectin-based film of COE\_USA and COO\_HD was introduced as nanoemulsion as a carrier to improve antioxi-

dative activity. The PE films containing the PE-PLhE (PE-COE) showed antioxidant activity by 86% (RSA<sub>DPPH<sup>•</sup></sub>) and 88% (RSA<sub>ABTS<sup>•+</sup></sub>); 51% (RSA<sub>DPPH<sup>•</sup></sub>) and 56% (RSA<sub>ABTS<sup>•+</sup></sub>) after 1h, while film PE-NE (pectin, nanoemulsion of COO\_HD) showed activity by 45 and 51% after 1h in DPPH<sup>•</sup> and ABTS<sup>•+</sup> assays. According to Wang, Hu, Nie, Yu and Xie (2016), pectin shows certain antioxidative potential which could be attributed to OH and COOH groups.

Considering the results of antioxidant activity of the COE\_USA in DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical assay, the weak increase of the time-dependent inhibition of PE-COE film could be attributed to the antioxidant activity of pectin. The films PE-PLhEGA (PE-COEGA) (pectin, PLhE\_MWA (COE\_USA) extract, GA) and PE-NEGA (pectin, COO\_HD NE, and GA), containing covalently bonded GA, showed significantly better antioxidant activity. The PE-PLhEGA (PE-COEGA) films, with the extract and GA, showed a slow increase of antioxidant activity to 93% (RSA<sub>DPPH<sup>•</sup></sub>) and 98% (RSA<sub>ABTS<sup>•+</sup></sub>); 92% (RSA<sub>DPPH<sup>•</sup></sub>) and 97% (RSA<sub>ABTS<sup>•+</sup></sub>) after 60 minutes in DPPH<sup>•</sup> and ABTS<sup>•+</sup> assays. Finally, film PE-NEGA (a film with nanoemulsion and GA), showed high initial antioxidant activity of 91% and 95% after 10 minutes and steadily in-creased the activity to 92% and 96% after 60 min in DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging assay, respectively. These values are significantly higher than for pectin film with essential oil COO\_HD 13% and 14% for 10 min, and 21% and 31% for 60 min in DPPH<sup>•</sup> and ABTS<sup>•+</sup>, respectively. The results showed the significance of the synergetic effect of the phenolic group from bonded GA and NE containing essential oil of *C. officinalis* in increasing the antioxidant activity.

## CONCLUSIONS

In the present research, we demonstrated that PLh is a moderate source of antioxidant polyphenolic and flavonoids content. For this extract, the better antioxidant activity was obtained using ethanol and water mixture in a ratio of 50:50 ethanol/water using MWA than ultrasound treatment. Also, the extracts from COE show moderate content of TPC and TFC. Considering DPPH<sup>•</sup> and ABTS<sup>•+</sup> radical scavenging assay, results indicate that both extracts COE showed moderate activity. SET

(SET-PT) is the possible antioxidant mechanism for ethanol-water extracts. The highest inhibitory activity of the examined samples showed the extract of pectin film with incorporated NE-GA, in both tests (DPPH<sup>•</sup> and ABTS<sup>•+</sup>). Accordingly, further study will be devoted to the incorporation (encapsulation) of the different extract materials into the biobased carrier to achieve the synergetic antioxidant and antibacterial effect as crucial parameters for further research and applications in the food or drug industry.

### ACKNOWLEDGMENTS

The authors acknowledge the financial support from the Ministry of Education, Science and Technological Development of Serbia - project title: "Resource protection and efficiency utilization of the endangered functional plants originated in China and Serbia", contract number: 451-03-1202/2021-09, 451-03-68/2022-14/200026.

### REFERENCES

- Almasi, H., Azizi, S., & Amjadi, S. (2020). Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (*Origanum majorana* L.) essential oil. *Food Hydrocolloids*, 99, 105338. <https://doi.org/10.1016/j.foodhyd.2019.105338>
- Chen, Y., Xiao, H., Zheng, J., & Liang, G. (2015). Structure-thermodynamics-antioxidant activity relationships of selected natural phenolic acids and derivatives: An experimental and theoretical evaluation. *PLoS ONE*, 10(3), 1-20. <https://doi.org/10.1371/journal.pone.0121276>
- Demir, D., Ceylan, S., Göktürk, D., & Bölgen, N. (2020). Extraction of pectin from albedo of lemon peels for preparation of tissue engineering scaffolds. *Polymer Bulletin*, 78, 2211-2226. <https://doi.org/10.1007/s00289-020-03208-1>
- Deuschle, V. C. K. N., Deuschle, R. A. N., Piana, M., Boligon, A. A., Bortoluzzi, M. R. B., Dal Prá, V., Dolwish, C. B., Lima, F. O., Carvalho, L. M. & Athayde, M. L. (2015). Phytochemical evaluation and in vitro antioxidant and photo-protective capacity of *Calendula officinalis* L. leaves. *Revista Brasileira de Plantas Medicinai*s, 17, 4(1), 693-701. [https://doi.org/10.1590/1983-084X/14\\_055](https://doi.org/10.1590/1983-084X/14_055)
- Dienaitė, L., Pukalskienė, M., Pukalskas, A., Pereira, C. V., Matias, A. A., & Venskutonis, P. R. (2019). Isolation of strong antioxidants from *Paeonia officinalis* roots and leaves and evaluation of their bioactivities. *Antioxidants*, 8(8). <https://doi.org/10.3390/antiox8080249>
- Freeman, B. A., & Crapo, J. D. (1982). Biology of disease: free radicals and tissue injury. *Laboratory Investigation, a Journal of Technical Methods and Pathology*, 47(5), 412-426.
- Gazim, Z. C., Rezende, C. M., Fraga, S. R., Svidzinski, T. I. E., & Cortez, D. A. G. (2008). Antifungal activity of the essential oil from *Calendula officinalis* L. (Asteraceae) growing in Brazil. *Brazilian Journal of Microbiology*, 39(1), 61-63. <https://doi.org/10.1590/S1517-83822008000100015>
- Gunes, A., Zengin, G., Sinan, K. I., Mahomoodally, M. F., Picot-Allain, M. C. N., Cakir, O., Bensari, S., Yilmaz, M. A., Gallo, M., & Montesano, D. (2020). A comparative bio-evaluation and chemical profiles of *Calendula officinalis* L. extracts prepared via different extraction techniques. *Applied Sciences*, 10(17), 5920. <https://doi.org/10.3390/app10175920>
- He, D-Y. & Dai, S-M. (2011): Anti-inflammatory and immunomodulatory effects of *Paeonia lactiflora* Pall., a traditional Chinese herbal medicine, *Frontiers in Pharmacology*, 2(10), 1-5. <https://doi.org/10.3389/fphar.2011.00010>
- Honório, I. C. G., Bonfim, F. P. G., Montoya, S. G., Casali, V. V. D., Leite, J. P. V. & Cecon, P. R. (2016). Growth, development and content of flavonoids in calendula (*Calendula officinalis* L.). *Acta Scientiarum - Agronomy*, 38(1), 69-75. <https://doi.org/10.4025/actasciagron.v38i1.25976>
- Kim, I. D. & Ha, B. J. (2009): Paeoniflorin protects RAW 264.7 macrophages from LPS-induced cytotoxicity and genotoxicity. *Toxicology in Vitro*, 23(6), 1014-1019. <https://doi.org/10.1016/j.tiv.2009.06.019>
- Klemetsrud, T., Jonassen, H., Hiorth, M., Kjøniksen, A. L., & Smistad, G. (2013). Studies on pectin-coated liposomes and their interaction with mucin. *Colloids and Surfaces B: Biointerfaces*, 103, 158-165. <https://doi.org/10.1016/j.colsurfb.2012.10.012>
- Milošević, M., Marinković, A., Petrović, P., Klaus, A., Nikolić, M., Prlainović, N., Cvijetić, I. (2020). Synthesis, characterization and SAR studies of bis(imino)pyridines as antioxidants, acetylcholinesterase inhibitors and antimicrobial agents. *Bioorganic Chemistry*, 102, 104073. <https://doi.org/10.1016/j.bioorg.2020.104073>
- Ognyanov, M., Georgiev, Y., Petkova, N., Ivanov, I., Vasileva, I., & Kratchanova, M. (2018). Isolation and characterization of pectic polysaccharide fraction from in vitro suspension culture of *Fumaria officinalis* L. *International Journal of Polymer Science*. 2018, 5705036, 13 pages. <https://doi.org/10.1155/2018/5705036>
- Petkova, D., Mihaylova, D., Denev, P., & Krastanov, A. (2020). Antioxidant activity of some edible flowers water extracts from Bulgaria. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology*, 77(1), 54-61. <https://doi.org/10.15835/buasvmcn-fst:2019.0025>
- Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290-4302. <https://doi.org/10.1021/jf0502698>
- Rajbhar, K., Dawda, H., & Makundan, U. (2015). Polyphenols: method of extraction. *Scientific Reviews & Chemical Communications*, 77(1), 1-6.
- Ramos, A., Edreira, A., Vizoso, A., Betancourt, J., López, M., & Décalo, M. (1998). Genotoxicity of an extract of *Calendula officinalis* L. *Journal of Ethnopharmacology*, 61(1), 49-55. [https://doi.org/10.1016/S0378-8741\(98\)00017-8](https://doi.org/10.1016/S0378-8741(98)00017-8)

- Ray, P. D., Huang, B-W., & Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cellular Signalling*, 24(5), 981-990.  
<https://doi.org/10.1016/j.cellsig.2012.01.008>
- Rocha, J. E., Guedes, T. T. A. M., Bezerra, C. F., Costa, M. do S., Campina, F. F., de Freitas, T. S., Souza, A. K., Sobral Souza, C. E., de Matos, Y. M. L. S., Pereira-Junior, F. N., da Silva, J. H., Menezes, I. R. A., Teixeira, R. N. P., Colares, A. V., & Coutinho, H. D. M. (2019). Identification of the gallic acid mechanism of action on mercury chloride toxicity reduction using infrared spectroscopy and antioxidant assays. *International Biodeterioration and Biodegradation*, 141, 24-29.  
<https://doi.org/10.1016/j.ibiod.2018.07.002>
- Sabir, S. M., Khan, M. F., Rocha, J. B. T., Boligon, A. A., & Athayde, M. L. (2015). Phenolic profile, antioxidant activities and genotoxic evaluations of *Calendula officinalis*. *Journal of Food Biochemistry*, 39(3), 316-324.  
<https://doi.org/10.1111/jfbc.12132>
- Saura-Calixto, F. (2012). Concept and health-related properties of nonextractable polyphenols: The missing dietary polyphenols. *Journal of Agricultural and Food Chemistry*, 60(45), 11195-11200.  
<https://doi.org/10.1021/jf303758j>
- Savi, P. D. R. S., Dos santos, L., Gonçalves, A. M., Biesek, S., & De Lima, C. P. (2017). Análise de flavonoides totais presentes em algumas frutas e hortaliças convencionais e orgânicas mais consumidas na região sul do Brasil. *DEMETRA: Alimentação, Nutrição & Saúde*, 12(1), 275-288.  
<https://doi.org/10.12957/demetra.2017.22391>
- Singh, D., Rawat, M. S. M., Semalty, A., & Semalty, M. (2011). Gallic acid-phospholipid complex: Drug incorporation and physicochemical characterization. *Letters in Drug Design & Discovery*, 8(3), 284-291.  
<https://doi.org/10.2174/157018011794578240>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.  
[http://dx.doi.org/10.1016/S0076-6879\(99\)99017-1](http://dx.doi.org/10.1016/S0076-6879(99)99017-1)
- Veličković, J. M., Dimitrijević, D. S., Mitić, S. S., Mitić, M. N. & Kostić, D. A. (2014). The determination of the phenolic composition, antioxidative activity and heavy metals in the extracts of *Calendula officinalis* L. *Advanced Technologies*, 3(2), 46-51.  
<https://doi.org/10.5937/savteh1402046V>
- Wang, J., Hu, S., Nie, S., Yu, Q., & Xie, M. (2016). Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. *Oxidative Medicine and Cellular Longevity*, 2016, 5692852, 13.  
<https://doi.org/10.1155/2016/5692852>
- Wathoni, N., Yuan Shan, C., Yi Shan, W., Rostinawati, T., Indradi, R. B., Pratiwi, R., & Muchtaridi, M. (2019). Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. *Heliyon*, 13, 5(8), e02299.  
<https://doi.org/10.1016/j.heliyon.2019.e02299>
- Wu, Y., Jiang, Y., Zhang, L., Zhou, J., Yu, Y., Zhou, Y., & Kang, T. (2021). Chemical profiling and antioxidant evaluation of *Paeonia lactiflora* Pall. "Zhongjiang" by HPLC-ESI-MS combined with DPPH assay. *Journal of Chromatographic Science*, 59 (9), 795-805.  
<https://doi.org/10.1093/chromsci/bmab005>
- Zhang, G., Zheng, C., Huang, B., & Fei, P. (2020). Preparation of acylated pectin with gallic acid through the enzymatic method and their emulsifying properties, antioxidation activities and antibacterial activities. *International Journal of Biological Macromolecules*, 165(A), 198-204.  
<https://doi.org/10.1016/j.ijbiomac.2020.09.195>

## IN VITRO PROCENA ANTIOKSIDATIVNE AKTIVNOSTI EKSTRAKTA LATICA PAEONIA LACTIFLORA I CALENDULA OFFICINALIS UGRAĐENIH U NOVE FORME NOSAČA NA BIOBAZI

Petar M. Batinić<sup>\*1</sup>, Milena D. Milošević<sup>2</sup>, Milan M. Lukić<sup>1</sup>, Željana S. Prijjić<sup>1</sup>, Stefan V. Gordanić<sup>1</sup>, Vladimir M. Filipović<sup>1</sup>, Aleksandar D. Marinković<sup>3</sup>, Branko M. Bugarski<sup>3</sup>, Tatjana Lj. Marković<sup>1</sup>

<sup>1</sup>Institut za proučavanje lekovitog bilja "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Beograd, Srbija

<sup>2</sup>Univerzitet u Beogradu, Institut za hemiju, tehnologiju i metalurgiju, Institut od nacionalnog značaja za Republiku Srbiju, Njegoševa 12, 11000 Beograd, Srbija

<sup>3</sup>Univerzitet u Beogradu, Tehnološko-metalurški fakultet, Karnegijeva 4, 11000 Beograd, Srbija

**Sažetak:** U ovom radu prikazana je ekstrakcija latica hibrida *Paeonia lactiflora* „Činija lepote“ i *Calendula officinalis* L. pomoću mikrotalasnog reaktora i ultrazvuka u sistemu etanol-voda, kao i izolacija etarskog ulja latica *C. officinalis* L. hidrodestilacijom. Određen je ukupan sadržaj fenola i flavonoida u ekstraktima i ulju, i procenjena je antioksidativna aktivnost. Antioksidativna aktivnost je određena korišćenjem standardnih, antioksidativnih *in vitro* DPPH<sup>•</sup> i ABTS<sup>•+</sup> testova. Najveći ukupni sadržaj fenola utvrđen je kod ekstrakata *P. lactiflora* i *C. officinalis* L. dobijenih ultrazvučnom ekstrakcijom (83,16 i 114,47 mg GA/g, redom), dok je najveći sadržaj flavonoida dobijen ekstrakcijom uz pomoć mikrotalasnog reaktora (123,48 i 65,29 mg QE/g, redom). Najveća antioksidativna aktivnost za ekstrakt hibrida *Paeonia lactiflora* „Činija lepote“ dobijena je mikrotalasnom ekstrakcijom (79% i 83%), dok je za ekstrakt *C. officinalis* L. dobijena ultrazvučnom ekstrakcijom (45% i 49%) u DPPH<sup>•</sup> i ABTS<sup>•+</sup>, redom. U cilju poboljšanja antioksidativne aktivnosti oba tipa ispitivanih analita (ekstrakta i etarskog ulja), pektinski biopolimerni film, (u formi nosača), u procesu enzimski potpomognute katalize, je pripremljen. Za karakterizaciju dobijenih filmova korišćena je optička mikroskopija i FTIR spektroskopija. Filmovi sa esencijalnim uljem *C. officinalis* L. i galnom kiselinom, pokazali su značajno povećan procenat inhibicije u DPPH<sup>•</sup> i ABTS<sup>•+</sup> testu (91% i 95%, redom) nakon 10 minuta. Rezultati su, takođe, pokazali da se sve formulacije pektinskog biopolimernog filma, modifikovanog galnom kiselinom, mogu uspešno primenjivati kao nosač za oba tipa ingredijenata.

**Ključne reči:** antioksidativna aktivnost, mikrotalasna ekstrakcija, ultrazvučna ekstrakcija, nanoemulzija, pektinski film

**Received:** 18 February 2022 / **Received in revised form:** 06 April 2022 / **Accepted:** 20 April 2022

**Available online:** April 2022



This is an open-access article under the CC BY license (<http://creativecommons.org/licenses/by/3.0>).