In vitro antineurodegenerative activity and *in silico* predictions of blood-brain barrier penetration of *Helichrysum plicatum* flower extract

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This study aimed to assess the antineurodegenerative and antioxidant activity of Helichrysum plicatum flower extract, as well as to identify extract ingredients with acceptable pharmacokinetic parameters such as gastrointestinal absorption, blood-brain barrier permeation, and P-glycoprotein-mediated effusion for optimal therapeutic brain exposure. Antioxidant activity was evaluated by ABTS, FRAP, and β-carotene bleaching assays, while antineurodegenerative activity was tested using acetylcholinesterase (AChE) and tyrosinase (TYR) inhibitory activity assays. In the ABTS test, the dry extract at the highest applied concentration (500 μg/mL) showed better or similar antioxidant activity compared to the standards. In the β-carotene assay, all applied concentrations of the extract showed significantly higher activity than vitamin C. No concentration-dependent activity was observed in the AChE assay, while in the TYR assay the lowest extract concentration (100 μg/mL) showed the highest percentage of inhibition (27.92 %). Pharmacokinetic parameters of compounds were predicted by *in silico* SwissADME online tool in accordance by the rules of drug-likeness. According to the pharmacokinetic properties, we concluded that pentoxymethoxylated flavones may represent CNS drug candidates for further studies.

1. INTRODUCTION

Neurodegenerative disorders such as Alzheimer's and Parkinson's disease have become a significant health problem in modern society. Their increased prevalence is associated with an increase in the life expectancy of the population (Pandey et al., 2020). In complex pathogenesis, persistent oxidative damage is recognized as one of the key factors of neuronal degeneration during aging (Vila, 2019). Namely, due to intensive metabolism, neurons are prone to increased production of highly reactive free radicals. Increased exposure to free radicals and oxidative denaturation of essential biomolecules (membrane lipids, proteins, enzymes, DNA) has led to disease progression (Ritter et al., 2020). The aging-related neuron loss in Parkinson's disease correlates directly with the accumulation of neuromelanin which is produced by tyrosinase (TYR) activity or spontaneous non-enzymatic autoxidation of dopamine (Vila, 2019). Decreased cholinergic transmission due to increased acetylcholine degradation by acetylcholinesterase (AChE) exists in various neurodegenerative

diseases and is associated with memory loss in Alzheimer's patients. Therefore, both processes (oxidative damage and enhanced enzyme activity) may represent a potential target for novel antineurodegenerative therapy. Individual compounds have achieved modest symptomatic efficacy, with no causal action and poor safety that limit their chronic use (Ritter et al., 2020).

The traditional pharmacological approach of "one target - one disease" that addresses only a single target has evolved into the concept of "multitarget drug" (polypharmacology) with the idea that one drug modulates multiple targets simultaneously. In this context, plant extracts as a cocktail of pharmacologically active ingredients that act on multiple targets can realize better efficacy compared to highly specific compounds for individual targets (Morphy and Rankovic, 2008). Optimizing various activities and achieving acceptable distribution at the desired therapeutic target in brain tissue are major challenges in CNS multitarget drug discovery. An important limitation to drug distribution in the CNS is the existence of a blood-

brain barrier (BBB) with its main active efflux gatekeeper, the so-called P-glycoprotein (P-gp) (Morphy and Rankovic, 2008; Ranković, 2015).

Helichrysum plicatum DC. (Asteraceae) is a herbaceous plant native to the Anatolian Peninsulas, Balkan, and Iran with a long tradition of use in the European ethnopharmacology (Bigović et al., 2017). It is traditionally used for wound healing, in the treatment of diabetes, kidney stones as a diuretic and litagogue, and in gastrointestinal and liver diseases as a stimulator of gastric secretion, cholagogue, choleretic, and hepatoprotectant (Aslan et al., 2007; Vujić et al., 2020). In recent years, this species has attracted the attention of scientists, and it has been confirmed that its extracts exhibit urolithic, nephroprotective, antihyperglycemic, antioxidant, antimicrobial, cytotoxic, and spasmolitic activity by various *in vitro*, *in vivo*, and *ex vivo* methods (Apaydin Yildirim et al., 2017; Aslan et al., 2007; Bayir et al., 2011; Bigovic et al., 2010; Bigović et al., 2017; 2011; Sezik et al., 2010). Flavones, flavanones, flavonols, and chalcones are probably the main contributors to the therapeutic activity of the extracts (Vujić et al., 2020).

As *H. plicatum* is a valuable source of pharmacologically interesting compounds with a wide range of activities, this study aimed to assess the neuroprotective and antioxidative potential of its flower extract. Assessment bioassays included three different antioxidant activity tests (ABTS, FRAP, and β-carotene bleaching assay), as well as assays regarding CNS enzymes (AChE and TYR) to assess the neuroprotective potential by different mechanisms of action. Moreover, the aim of the study was to perform *in silico* screening of compounds with acceptable pharmacokinetics (gastrointestinal absorption, blood-brain barrier permeation and P-glycoprotein-mediated effusion) for optimal brain exposure. To the best of our knowledge, this is the first study of antineurodegenerative activity of *H. plicatum* flower extract.

2. MATERIALS AND METHODS

2.1. Extract preparation

Flowers heads of *H. plicatum* were purchased from Institute for Medicinal Plant Research "Dr. Josif Pančić", Belgrade (batch number 29470605). The plant material was comminuted before extraction (degree of comminution 710 μm, according to European Pharmacopoeia 6.0).

The dry extract was prepared according to the previously described procedure (Bigović et al., 2011). Briefly, extraction was performed with 50 % ethanol (drug:solvent ratio 1:5 w/v) at room temperature by triple percolation. Evaporation of the obtained extract on 1/3 of its volume was performed on a vacuum evaporator (Buchi rotavapor R-114) at 80 °C and then cooled to 8 °C. The obtained extract was re-extracted with ethylacetate. The yellow-orange dry extract was obtained after evaporation of ethylacetate at 60 °C.

In the extract prepared by this procedure, Bigović et al. (2011) quantified total phenols and three individual flavone aglycone. Content of total phenolics were 359.8 mg of gallic acid equivalent per dry weight (DW) of plant extract, while content of naringenin, apigenin and kaempferol were 17.4, 16.2, and 3.8 mg/g DW, respectively.

2.2. Evaluation of antioxidant activity

2.2.1. ABTS assay

Radical scavenging activity of the extract was evaluated by ABTS (2,2'-azino-bis (3-ethyl-benzothiazoline-6- sulfonic acid)) assay according to the Miller et al. (1993) procedure with slight modifications. The working solution was prepared 12-16 hours before the experiment by dissolving 7 mM of ABTS$^+$ in 2.46 mM potassium-persulfate and kept in a dark place at room temperature. The working solution of ABTS$^+$ (1000 μL)

was mixed with extract (25 μL) in three different concentrations (100, 200, and 500 μg/mL) or positive controls of BHA, BHT, and ascorbic acid in two different concentrations (50 and 100 μg/mL). The mixtures were incubated for 30 minutes at 30 °C and then the absorbance was measured at 734 nm. The percentage of inhibition of ABTS$^+$ radical was calculated using the equation:

$$\text{Inhibition of ABTS radical (\%)} = \frac{A_c - A_s}{A_c} \times 100\%$$

, where A_c is the absorbance of the control (without test sample) and A_s is the absorbance of the test samples at different concentrations. All analytical measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

2.2.2. Ferric-reducing ability of plasma (FRAP) assay

The total antioxidant capacity of the extract was evaluated by ferric-reducing ability of plasma (FRAP) assay according to the Benzie and Strain (1996) method with slight modifications. The ability of the extract to reduce ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH was measured. FRAP reagent was prepared by mixing sodium acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl, and $FeCl_3 \times 6H_2O$ solution (20 mmol/L) in proportion 10:1:1 (v/v/v), respectively. Extract samples (40 μL) dissolved in methanol (100, 200, and 500 μg/mL) was mixed with 1200 μL of freshly prepared FRAP reagent and absorbance was measured after 4 minutes at 593 nm. The calibration curve was constructed using a standard solution of FeSO4 x 7H2O (0.2-1.6 mmol/L), while as standards were used BHT, BHA, and ascorbic acid in concentrations of 50 and 100 μg/mL. Results were expressed as μmol $FeSO_4 \times 7H_2O$/g dry extract. All analytical measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

2.2.3. β-carotene bleaching assay

The capacity of *H. plicatum* flower extract to reduce oxidative degradation of β-carotene in a β-carotene / linoleic acid emulsion was evaluated according to the procedure of Dapkevicius et al. (1998) with slight modifications. The emulsion was prepared by dissolving β-carotene (1 mg), linoleic acid (50 μL), and Tween 40 (400 mg) in 2 ml of chloroform. This was followed by evaporation of the chloroform and the addition of 200 ml of distilled water with vigorous stirring. Extract samples (140 μL) dissolved in methanol in concentrations of 100, 200, and 500 μg/mL were mixed with 1000 μL of emulsion. BHA, BHT, and ascorbic acid in concentrations of 50 and 100 μg/mL were used as standards. Absorbances were measured at zero time and after an incubation period of 120 minutes at 490 nm. The antioxidant activity of the sample (expressed as a percentage of inhibition) was evaluated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_{120} - C_{120}}{C_0 - C_{120}} \times 100\%$$

, where A_{120} and C_{120} are the absorbances measured in t=120 minutes for sample and control, respectively, while C_0 is the absorbance of control in t=0 minutes. All analytical measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

2.3. Evaluation of antineurodegenerative activities

2.3.1. Acetylacholinesterase (AChE) inhibitory activity assay

Antineurodegenerative activity of the obtained extract was determined using the spectrophotometric AChE inhibitory

Table 1. Antioxidant activity of *Helichrysum plicatum* flowers dry extract (HFE) compared with commercial preservatives

Sample[a]	Concentration [μg/mL]	ABTS[a] [% of inhibition]	FRAP [μmolFe(II)/g]	β-carotene [% of inhibition]
HFE	100	20.36 ± 0.99 a	139.48 ± 5.71 a	29.25 ± 0.94 b
	200	45.66 ± 0.51 d	357.41 ± 6.59 c	32.08 ± 1.89 b
	500	63.84 ± 1.03 f	547.95 ± 6.93 f	45.60 ± 2.23 c
BHA	50	56.18 ± 0.80 e	231.22 ± 4.71 b	50.79 ± 2.76 d
	100	64.95 ± 0.63 f	572.85 ± 5.71 g	57.70 ± 1.91 e
BHT	50	37.47 ± 0.67 c	364.05 ± 5.62 c	48.74 ± 1.79 cd
	100	55.11 ± 0.44 e	413.03 ± 3.13 d	56.29 ± 1.44 e
Ascorbic acid	50	35.14 ± 0.58 b	427.56 ± 5.18 e	1.73 ± 1.19 a
	100	55.70 ± 0.69 e	576.17 ± 7.61 g	2.99 ± 2.13 a

[a] Values with the same letter (a-g) in each column showed no statistically significant difference (P<0.05) according to the *post-hoc* Tukey test
[b] BHA, BHT, Ascorbic acid are commercial preservatives used as positive control

activity assay, previously described by Ellman et al. (1961). The activity of *H. plicatum* flower extract was measured by spectroscopic monitoring of yellow color intensity increase produced from tiocholine after reaction with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) ion. The extract was used in three different concentrations (100, 200, and 500 μg/mL), while alkaloid galantamine (100 μg/mL) was used as a standard. The mixture without AChE solution was used as blank (B), while the control (C) did not contain extract. The reaction was initiated by adding 10 μL of acetylthiocholine iodide after an incubation period of 15 minutes at room temperature. Absorbance was measured using a Tecan Sunrise SN microplate reader equipped with XFluor4 software at 412 nm. The percentage of inhibition of AChE by samples was determined using the equation:

$$\text{Inhibition of AChE (\%)} = \frac{A_C - (A_S - A_B)}{A_C} \times 100\%$$

, where A_S is the absorbance of the samples, while A_B and A_C are absorbances of blank and control solutions. All analytical measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

2.3.2. Tyrosinase inhibitory activity assay

Antineurodegenerative activity of the obtained extract was also determined using the spectrophotometric tyrosinase inhibitory activity assay, previously described by Masuda et al. (2005). The experiment was designed so that wells A contain 120 μL of sodium buffer (0.1 M, pH 7.0) and 40 μL of 46 units/L tyrosinase in the same buffer; wells B contain only buffer; wells C contain 80 μL of a buffer, 40 μL of tyrosinase and 40 μL of extract sample; wells D contain 120 μL of buffer and 40 μL of extract sample. The extract dissolved in sodium phosphate buffer containing 5 % DMSO was used in three different concentrations (100, 200, and 500 μg/mL), while kojic acid (100 μg/mL) was used as a standard. The absorbance was measured at 475 nm after the addition of L-DOPA (40 μL) and an incubation period of 30 minutes at room temperature, using Tecan Sunrise SN microplate reader equipped with XFluor4 software. Percentage of inhibition of tyrosinase was determined using the equation:

$$\text{Inhibition of tyrosinase (\%)} = \frac{(AA - AB) - (AC - AD)}{(AA - AB)} \times 100\%$$

, where AA, AB, AC and AD are absorbances of the mixtures that contained buffer and enzyme; only buffer; buffer, enzyme and sample; buffer and sample, respectively.
All analytical measurements were performed in triplicate and the results were expressed as mean ± standard deviation.

2.4. *In silico* predicting of physicochemical and pharmacokinetic parameters by SwissADME

Physicochemical and pharmacokinetic properties of the compounds as potential drug candidates were screened using the computer online tool SwissADME (http://www.swiss.adme.ch/). Nineteen compounds from the group of flavonoids (chalcones, flavonols, flavanones, flavones) were analyzed. Detailed chemical analysis (Vujić et al., 2020) has shown that these compounds predominate in ethanol extract of *H. plicatum* flowers and are probably the main contributor to the therapeutic activity of the extract. Canonical SMILE of compounds from the PubChem database was entered into the SwissADME software. Pharmacokinetic parameters such as gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeation, and P-glycoprotein-mediated (Pgp-) effusion were predicted by software according to the rules of drug-likeness with physicochemical characteristics of successful CNS drugs candidates (Daina et al., 2017).

2.5. Statistical analysis

All results were reported as the mean ± standard deviation for three replicates in each set of experiments. All the statistical analyses were performed using the SPSS software package (trial version, Dublin, Ireland). Statistical analyses were based on one-way ANOVA and post-hoc Tukey tests. The level of statistical significance is a P-value less than 0.05.

3. RESULTS AND DISCUSSION

3.1. Evaluation of antioxidant activity

The results of antioxidant activity of different concentrations of *H. plicatum* flower extract (HFE) and standards investigated

Table 2. Antioxidant activity of *Helichrysum plicatum* flowers dry extract (HFE) compared with commercial preservatives

Sample	Conc. [μg/mL]	AChE [% of inhib.]	TYR [% of inhib.]
HFE	100	39.53 ± 2.12 a	27.92 ± 2.74 b
	200	40.99 ± 3.42 a	16.45 ± 1.21 a
	500	41.15 ± 1.68 a	19.24 ± 2.26 a
GAL	100	57.11 ± 1.68 b	-
Kojic acid	100	-	51.81 ± 2.55 c

[a] Values with the same letter (a-c) in each column showed no statistically significant difference (P<0.05) according to the *post-hoc* Tukey test
[b] GAL - Galanthamine

in three different *in vitro* assays are shown in Table 1. The use of complementary *in vitro* assays provides better predictability of antioxidant activity in real biological systems. This set of assays was chosen because they enable the assessment of antioxidant activity by various mechanisms: the electron or radical scavenging ability (ABTS and FRAP) and the lipid peroxidation prevention ability (β-carotene bleaching assay). Also, the assays enable to assess the antioxidant activity of the extract ingredients in different reaction media: in the aqueous and lipid phase, only in the aqueous phase and at the two phases boundary (emulsion) using of ABTS, FRAP, and β-carotene bleaching assay, respectively (Moon and Shibamoto, 2009).

Concentration-dependent antioxidant activity was noticed in all assays, and the highest tested extract concentration (500 μg/mL) showed the strongest antioxidant activity. According to the results of the ABTS assay, the HFE at the highest applied concentration showed better or similar antioxidant activity compared to BHA, BHT, and vitamin C at both used concentrations (Table 1). The results of previously published study (Acet et al., 2020) indicate that the ethanol, methanol, and ethylacetate extracts of the aerial parts of *H. plicatum* (with 50 % inhibition concentrations of 51.4, 64.2, and 25.9 μg/mL, respectively) showed better ABTS radical scavenging activity compared to our extract. Ferric reducing antioxidant power of extract of 500 μg/mL was statistically significantly higher than standards, except BHA and vitamin C at a concentration of 100 μg/mLwhere it was slightly lower.

In the β-carotene test, the extract applied in concentration of 500 μg/mL was significantly more active than the vitamin C at both applied concentrations, while it showed lower activity than BHA and BHT. This behavior in the emulsion medium indicates that the active principles of HFE, as well as lipophilic BHA and BHT, can penetrate into formed micelles and prevent lipid peroxidation of linolic acid unlike hydrophilic vitamin C. Tepe (2005) examined β-carotene bleaching activity of a methanol extract of four Helichrysum species (*H. noeanum*, *H. chionophilum*, *H. plicatum* subsp. *plicatum*, and *H. arenarium* subsp. *aucheri*) obtained using Soxhlet apparatus. They reported higher activity of all tested extracts, including *H. plicatum*, with an inhibition rate greater than 50 %. This can be explained by the higher concentration of extracts they used (2000 μg/mL) and the different extraction procedures.

Identified antioxidant activity of the extract could be explained by the relatively high content of total phenolics which are well known highly effective natural antioxidants. In the aforementioned paper, Bigović et al. (2011) showed a close correction between total phenolic content and radical scavenging activity examined by the DPPH method.

3.2. Evaluation of antineurodegenerative activities

Extract of *H. plicatum* was tested for antineurodegenerative activity at three different concentrations (100, 200, and 500 μg/mL), and the percentage of enzyme inhibition was compared with galantamine for AChE inhibitory activity or kojic acid for TYR inhibitory activity at a concentration of 100 μg/mL. The activities of the tested samples in all concentrations were lower than the standards (Table 2). The highest concentration of extract showed the strongest activity (41.15 %), but no statistically significant difference between applied concentrations of extract in AChE inhibitory activity was observed. On the other hand, the lowest concentration of the extract showed significantly better TYR inhibitory activity (27.92 %) than the remaining two concentrations.

In the aforementioned study, Acet et al. (2020) examined the AChE inhibitory activity of ethanol, methanol, and ethylacetate flower extracts of *H. plicatum* from Turkey. The strongest inhibitory activity was shown by the ethylacetate extract (17.75 %), weaker by the ethanol extract (15.3 %), while methanol extract activity was not detected, which indicates that the activity depends on the type of the used extraction solvent. In a recently published paper, Aydin (2020) proved AChE inhibitory activity of flower extract of *H. plicatum* and eight isolated components. To the best of our knowledge, this is the first report on the TYR inhibitory activity of *H. plicatum* flower extract.

The antineurodegenerative activity of the HFE could be attributed to the presence of various pharmacologically active polyphenols. Polyphenols, including naringenin, apigenin, and kaempferol as the major phytoconstituents of the HFE, are recognized as compound that may exhibit significant neuroprotective effects as inhibitors of cholinesterase activity. This potent inhibitory potential can be explained by existence of multiple hydroxyl groups in polyphenol molecules. They can bind the amino acid sequence defining the active site of the enzyme via hydrogen bond, hydrophobic, and π–π interaction and thus interfere with the catalytic activity of the enzyme (Jabir et al., 2018; Athipornchai et al., 2020). Through similar in vitro studies, the antineurodegenerative activity of polyphenol-rich plant drugs has been confirmed (Zdunić et al., 2020; Šavikin et al., 2018).

3.3. Evaluation of physicochemical and pharmacokinetic parameters by SwissADME

Abandonment of drug candidates due to pharmacokinetic weaknesses in the later stage of drug development is relatively often, so it is desirable to perform preliminary screening at an early stage of drug research. In the absence of reliable *in vitro* or *in vivo* experimental models for estimating compound penetration through BBB, computational prediction models represent valid alternatives. The SwissADME software was used to determine the physicochemical descriptors and pharmacokinetic properties of selected compounds from *H. plicatum* flower extracts in accordance by the roles of drug-likeness.

To predict GI absorption, this program uses Lipinski's "rule of five" (Ro5). According to this rule, a compound shows high GI absorption if the n-octanol/water partition coefficient (logP) is $\leq$ 5, the molecular weight (MW) is $\leq$ 500 g/mol, the number of hydrogen bond acceptors (HBA) is $\leq$ 10, the number of hydrogen bond donors (HBD) is $\leq$ 5, and a number of rotatable bonds (NRB) is $\leq$ 10. In addition, it was experimentally determined that the compounds should have a topological polar surface (TPSA) $\leq$ 140 Å^2 (Daina and Zoete, 2016). Analogously, the ability of compounds to cross BBB is predicted based on physicochemical parameters that are more stringent than the prediction of absorption.

Table 3. Predicted physicochemical and pharmacokinetic parameters of selected phytochemical compounds from *Helichrisum plicatum* flower extract using SwissADME software

Compounds	Log P^a (0.4-6.0)	MW (≤450 g/mol)	HBA (≤ 7)	HBD (≤ 3)	NRB (≤ 8)	TPSA (≤ 79 A2)	GI absorption	BBB permeability	P-gp substrate
			Physicochemical properties (criteria for BBB permeability)				Pharmacokinetic parameters		
Standards									
galanthamine	1.91	287.35	4	1	1	41.93	High	Yes	Yes
kojic acid	-0.16	142.11	4	2	1	70.67	High	No	No
Chalcones									
helilupolone	5.97	462.62	4	2	10	74.60	Low	No	Yes
isosalipurposide aglycone	1.83	272.25	5	4	3	97.99	High	No	No
arenariumoside and tomoroside A aglycones	2.68	544.51	10	8	6	195.98	Low	No	No
Flavonols									
quercetin	1.23	302.24	7	5	1	131.36	High	No	No
kaempferol	1.58	286.24	6	4	1	111.13	High	No	No
helichrysoside	1.11	610.52	14	8	8	236.81	Low	No	No
tiliroside	1.52	594.52	14	7	8	216.58	Low	No	No
Flavanones									
naringenin	1.84	272.25	5	3	1	86.99	High	No	Yes
eriodictyol	1.45	288.25	6	4	1	107.22	High	No	Yes
Flavones									
apigenin	2.11	270.24	5	3	1	90.90	High	No	No
luteolin	1.73	286.24	6	4	1	111.13	High	No	No
3,5,6,7,8-pentamethoxyflavone	3.02	372.37	7	0	6	76.36	High	Yes	No
3′,4′,5,6,7-pentamethoxyflavone	1.10	372.37	7	0	6	76.36	High	Yes	No
dihydroxy-dimethoxyflavone	2.20	314.29	6	2	3	89.13	High	No	No
jaceosidin	2.11	330.29	7	3	3	109.36	High	No	No
chrysoeriol	2.18	300.26	6	3	2	100.13	High	No	No
5,7-dihydroxy-3-methoxyflavone	2.56	284.26	5	2	2	79.90	High	No	No
5,7,4′-trihydroxy-3,6-dimethoxy-3′-prenylflavone	3.54	389.41	7	3	5	109.36	High	No	No
4′,5,7-triethoxy-3′,6-dimethoxyflavone	4.08	414.45	7	0	9	76.36	High	No	No

^{a}LogP - Partition coefficient; MW - Molecular weight; HBA - Hydrogen bond acceptor; HBD - Hydrogen bond donor; NRB - Number of rotatable bonds; TPSA - Topological polar surface area.

The ideal candidate for CNS drugs should have a logP from 0.4 to 6.0, MW $\leq$ 450 g/mol, HBA $\leq$ 7, HBD $\leq$ 3, NRB $\leq$ 8, and TPSA $\leq$ 79 Å^2 (Daina and Zoete, 2016; Ranković, 2015). Additional to the fact that BBB is a physical barrier to drug passage (tight junctions between endothelial cells defy drug penetration), it is also a biochemical barrier (P-gp pumps out xenobiotics from CNS). Therefore, the possibility of efflux of selected compounds by P-gp pump was considered. It was found that CNS drugs showed a significantly lower frequency of P-gp-mediated efflux compared to non-CNS drugs, indicating the importance of these transportersin CNS therapy (Ranković, 2015).

Most of the tested compounds showed high GI absorption (Table 3). Two chalcones and two flavonols noted at least one Lipinski's violations, so they were predicted to have low GI absorption in the human body. On the other hand, it is predicted that only two of the nineteen tested compounds will be able to penetrate the blood-brain barrier. Both compounds are from the group of pentoxymethoxylated flavones (3,5,6,7,8-pentamethoxyflavone and 3′,4′,5,6,7-pentamethoxyflavone). It is interesting prediction that naringenin, apigenin and kaempferol as the three major ingredients of HFE have no ability to cross the blood-brain barrier due to the high value of TPSA, while kaempferol also shows a high value of HBD. These results are in line with the statement that the possibility of penetration cross blood-brain barrier is a key obstacle that is eliminatory for many compounds in CNS drug development (Ranković, 2015). It is also promising that these compounds did not exhibit substrate specificity for the P-gp transporter. Interestingly, according to this criterion, the mentioned compounds are better candidates than the galanthamine standard. A favorable circumstance is that most of the other compounds are not a substrate for the P-gp, which may indicate a low potential for interaction with other drugs in this pathway (Ranković, 2015). Namely, P-gp is an active efflux pump on the cytoplasmic membrane that plays a major role in protecting the CNS from xenobiotics. It shows a wide substrate specificity and is, therefore, suitable for drug-drug interaction (Daina and Zoete, 2016; Ranković, 2015).

CONCLUSION

In the present study, we examined the antioxidant and antineurodegenerative activities of Helichrysum plicatum flowers extract using *in vitro* models. The concentration-dependent activity was observed in all antioxidant assays. In the β-carotene assay, all tested concentrations of dry extract were significantly more potent than both concentrations of vitamin C standards. The dry extract showed better acetylcholinesterase (AChE) inhibitory activity compared to tyrosinase (TYR) inhibitory activity at all applied concentrations. Following confirmed activities, *in silico* pharmacokinetic screening of selected extract ingredients was performed to the reveal predictability of gastrointestinal absorption, blood-brain barrier permeation, and P-glycoprotein-mediated effusion. According to the pharmacokinetic parameters determined by *in silico* method, it was concluded that candidates for CNS drugs may be pentoxymethoxylated flavones. Further comprehensive studies in animal models are required to more accurately assess the applicability of this plant extract in treatment of neurodegenerative diseases.

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