

CHEMICAL, ANTIOXIDANT AND *IN VITRO* PERMEATION AND PENETRATION STUDIES OF EXTRACTS OBTAINED FROM *VIBURNUM OPULUS* AND *CRATAEGUS PENTAGYNA*

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Manuscript received: February 2020

Abstract

The purposes of the study were the comparison between the levels of different polyphenol groups in *Crataegus pentagyna* (CP) leaf and *Viburnum opulus* (VO) fruit extracts, the assessment of their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and the *in vitro* determination of their permeability parameters. CP extract had higher total phenolic, flavonoid and proanthocyanidin contents than VO extract (901.12 ± 3.23 vs. 231.15 ± 1.53 mg chlorogenic acid equivalents/g extract, 416.68 ± 2.03 vs. 95.33 ± 0.95 mg catechin equivalents/g extract and 112.7 ± 1.21 vs. 18.40 ± 0.87 mg cyanidin/g extract, respectively), while both extracts showed promising DPPH scavenging activity ($EC_{50} = 15.56 \pm 0.03$ and 47.18 ± 0.12 $\mu\text{g/mL}$, respectively). To investigate whether the sublingual or cutaneous routes are suitable for the administration of CP and VO extracts, permeation and penetration studies were carried out in Franz diffusion cells. The polyphenols of CP and VO extracts had higher transfer across the sublingual porcine membrane (71.93% and 62.65% polyphenols in the receptor compartment, respectively) than across the chicken skin membrane (54.48% and 39.42% polyphenols in the receptor compartment, respectively). The results provide evidence that these extracts can be used to develop oromucosal dosage forms.

Rezumat

Obiectivele studiului au constat în compararea conținutului diferitelor clase de polifenoli din extractele obținute din frunzele de *Crataegus pentagyna* (CP) și fructele de *Viburnum opulus* (VO), evaluarea capacității extractelor de a inactiva radicalul liber DPPH (2,2-difenil-1-picrilhidrazil), precum și determinarea *in vitro* a parametrilor de permeabilitate pentru ambele extracte. Extractul CP s-a remarcat prin conținutul în polifenoli totali, flavonoide și proantocianidoli mai crescut decât al extractului VO ($901,12 \pm 3,23$ vs. $231,15 \pm 1,53$ mg echivalenți acid clorogenic/g extract, $416,68 \pm 2,03$ vs. $95,33 \pm 0,95$ mg echivalenți catechină/g extract și respectiv, $112,7 \pm 1,21$ vs. $18,40 \pm 0,87$ mg cianidol/g extract), însă ambele extracte au avut o acțiune promițătoare de a neutraliza radicalul liber DPPH ($EC_{50} = 15,56 \pm 0,03$ respectiv $47,18 \pm 0,12$ $\mu\text{g/mL}$). Pentru a investiga dacă este adecvată administrarea extractelor CP și VO pe calea sublinguală sau dermică, au fost realizate studii de permeație și penetrație în celule de difuzie Franz. Polifenolii din ambele extracte au dovedit o capacitate de transfer mai mare prin membrana sublinguală porcină (71,93% și respectiv, 62,65% polifenoli în compartimentul receptor) comparativ cu membrana din piele de pui (54,48% și respectiv, 39,42% polifenoli în compartimentul receptor). Rezultatele studiului susțin posibilitatea de formulare a extractelor în forme farmaceutice oromucozale.

Keywords: *Viburnum opulus*, *Crataegus pentagyna*, permeation study, antioxidant activity

Introduction

In the past several years, researchers have shown an increasing interest towards the bioavailability of polyphenols. After oral administration, polyphenols reach the gastrointestinal system where small molecules such as caffeic acid, epicatechin, quercetin, genistein, naringenin, gallic acid or epigallocatechin can be directly absorbed by passive diffusion, while complex structures have a low absorption rate. For instance, studies reported for caffeic acid a percentage of absorption up to 95%, while its ester with quinic acid

(namely chlorogenic acid) reaches an absorption of only 33% [25]. Similar situations are reported for large molecules such as polymers of flavan-3-ol (proanthocyanidins). In case of proanthocyanidins, only the monomers, dimers and trimers are absorbable with very low absorption rates (less than 4%) [17]. Glycosides of polyphenols are usually cleaved by enzymes such as β -glucosidases inside the intestinal cells, resulting in a lipophilic aglycon that can be easily absorbed by passive transport. Moreover, before reaching systemic circulation, within enterocytes, most of the polyphenolic

aglycones are partially inactivated and undergo phase II metabolism pathways (glucuronidation, sulphation, methylation) [26]. As a result, plasma levels of polyphenols in human trials are sometimes too low to exert a biological activity. Nowadays, specific strategies are directed towards increasing the bioavailability of polyphenols by developing novel delivery systems or finding new routes for their administration. Resveratrol was previously reported to be a successful candidate for transmucosal delivery systems. A good passive diffusion through buccal mucosa was determined for this compound, relying on two important characteristics: a low molecular weight and the presence of unionized form at the physiological pH of saliva. Lozenges containing resveratrol formulated with a solubility enhancer such as ribose showed significantly improved pharmacokinetic parameters in human plasma in comparison with resveratrol. The maximum plasma level of resveratrol (328 ± 5 ng/mL) was obtained after only 15 minutes with lozenges placed between gum and cheek, while the oral administration of resveratrol led to maximum plasma levels of 25 - 43.8 ng/mL after an interval of 0.8 to 1 h. Besides the increase in solubility, avoidance of the first pass effect also improves resveratrol pharmacokinetics as it reduces the glycosylation and sulphation rates of resveratrol [5]. Quercetin is another example of a polyphenol whose bioavailability was increased by a novel formulation obtained by coaxial electrospraying. Thus, micro-particles containing polyvinylpyrrolidone (PVP) and quercetin in the core, covered by PVP/sodium dodecyl sulphate/sucralose shells, were able to increase ten times the permeation rate through sublingual mucosa compared to quercetin powder and to release the incorporated quercetin in less than one minute [15]. Such novel sublingual delivery systems can enhance the permeation properties of polyphenols with low bioavailability.

Viburnum opulus L. (European cranberrybush, family *Adoxaceae*) fruits were traditionally used in the treatment of hypertension [28]. We have recently reported that the hydroacetic fruit extract of *V. opulus* can produce vasodilation of the rat aortic rings [6]. Other studies outlined that the juice and ethanolic fruit extract of *V. opulus* possess antimicrobial properties against Gram-positive and Gram-negative bacteria [8]. Previous studies on *Crataegus pentagyna* Waldst. et Kit. ex Willd. (small-flowered black hawthorn, *Rosaceae*) reported the antiarrhythmic activity of the hydro-ethanolic leaf extract [18], *in vivo* anti-hypoxic effect of the hydroacetomethanolic fruit extract [10] and *ex vivo* vasorelaxant properties of the ethyl acetate leaf, flower and fruit extracts [7]. All these effects were mainly correlated with the presence of polyphenols. For example, the hydro-acetic extract of *V. opulus* fruits contains flavonoids (rutin, quercetin rhamnoside), phenolic acids (coumaroyl quinic acid, cryptochlorogenic acid) and proantho-

cyanidins (procyanidin dimer and trimer) [6], while the ethyl acetate extract of *C. pentagyna* leaves is characterised by the presence of flavonoids (rutin, quercetin, apigenin-*C*-hexoside), phenolic acids (caffeoyl-threonic acid, protocatechuic acid) and proanthocyanidins (procyanidin trimer, properlarginidin dimer) [7]. Both *V. opulus* and *C. pentagyna* extracts contain significant amounts of chlorogenic acid (30.89 and 17.57 mg/g, respectively) [6, 7]. However, in case of polyphenol-rich extracts, one of the main challenges regarding the translation of *in vitro* effects to *in vivo* is related to the limited oral bioavailability of polyphenols.

The present study aimed to investigate the total phenolic, flavonoid and proanthocyanidin contents in *C. pentagyna* leaf (CP) and *V. opulus* fruit (VO) extracts and to assess their antioxidant activity. In addition, some pharmacokinetic properties important for the further development of dosage forms were explored by *in vitro* permeation and penetration studies on porcine sublingual membrane and chicken skin. To the best of our knowledge, there are no previous permeation and penetration studies through different biological membranes on either *C. pentagyna* leaf or *V. opulus* fruit extracts. However, there is a study reporting an absorption rate of only 31% for the oligomeric proanthocyanidin fraction isolated from other *Crataegus* species after oral administration in mice [13].

Materials and Methods

Materials

Aluminium chloride, (+)-catechin, dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent, chlorogenic acid, sodium hydroxide, hydrochloric acid, *n*-butanol, sodium nitrite, sodium carbonate, ammonium iron(III) sulphate dodecahydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Steinheim, Germany). Ethyl acetate was acquired from Riedel-de Haën (Seelze, Germany), while the other solvents were purchased from Sigma-Aldrich (Steinheim, Germany).

Plant material and extraction

V. opulus fruits (collected in Suceava County, Romania in October 2017) and *C. pentagyna* leaves (collected in Tulcea County, Romania in May 2016) were extracted as previously reported [6, 7]. In brief, frozen *V. opulus* fruits (without kernels) were subjected to ultrasonic extraction with acetone:water:acetic acid (80:19.5:0.5, v/v/v), delipidated with diethyl ether and concentrated to dryness (yield 11.5%) [6]. Dried *C. pentagyna* leaves were delipidated with hexane and extracted with acetone:water (7:3, v/v). The hydroacetic extract was successively partitioned in chloroform and ethyl acetate. The ethyl acetate fraction was concentrated to dryness (yield 4.25%) [7].

In vitro diffusion study

The *in vitro* diffusion study was performed using Franz vertical diffusion cells of 1.5 cm internal diameter

(Orchid Scientific Ltd., Ambad, India). The assays were performed on both porcine sublingual mucosa/epithelium and chicken skin that were purchased fresh from a local slaughterhouse. The sublingual mucosa was detached from the ventral surface of fresh porcine tongue, after the muscle and connective tissues were removed with a scalpel. The chicken skin was obtained from fresh chicken legs, excised from areas without pores, followed by the removal of fatty tissue. These membranes were cut in areas of 4 cm² and placed in 10% glycerine before being mounted between the donor and receptor compartments. Sublingual membranes were characterized by a thickness of 0.4 ± 0.05 mm, while the mean thickness of chicken skin membranes was 0.9 ± 0.1 mm. Diffusion cells were connected to a thermostated water bath at $36.5 \pm 0.5^\circ\text{C}$ and the homogeneity of receptor fluid was maintained by magnetic stirring. Each receptor compartment was filled with 12 mL of isotonic phosphate buffer pH 7.4 solution and the rotation was set at 100 rpm. During the diffusion process, 500 μL sample aliquots were withdrawn at regular time intervals for a period of 4 hours and replaced with the same volume of fresh medium. The sample aliquots were analysed in terms of total phenolic content using the Folin-Ciocalteu reagent as described below. At the end of experiment, the membranes were removed and placed in DMSO and methanol 1:3 (v/v) for 24 h; further, the filtrate was used to quantify the polyphenols retained in the membrane. The cumulative amount of polyphenols transported across the membrane *per cm²* vs. incubation time was plotted in GraphPad[®]. The steady-state flux, lag time and permeability coefficients were calculated in MatLab[®] according to Fick's first law of diffusion [23, 29]. The experiments were conducted in duplicate.

Total phenolic content

The total phenolic (TP) content of both extracts was determined using the Folin-Ciocalteu reagent [12]. The absorbance was measured at 765 nm using a SPECORD 210 PLUS spectrophotometer (Analytik Jena, Germany) and the TP content was calculated from the calibration curve using chlorogenic acid as standard (2 - 15 $\mu\text{g}/\text{mL}$). This method was used to quantify the total phenolic content in Franz diffusion cell.

In-house validation of the total phenolic quantification method

We used Folin-Ciocalteu assay as a simple and low-cost method to quantify the polyphenols that were able to penetrate and permeate the biological membranes based on chlorogenic acid calibration curve. For validation, we took into consideration the following parameters: linearity (evaluated according to the calibration curve and its correlation coefficient R^2), limit of quantification (LQ) and limit of detection (LD) (calculated according to International Council for Harmonisation (ICH) guidelines: $\text{LQ} = 10 \times \sigma/S$, $\text{LD} = 3.3 \times \sigma/S$, where σ is the standard deviation of

the intercept and S is the slope of the curve), repeatability or intra-day precision (evaluated by measuring the absorbance of six replicates of the concentration of 6 $\mu\text{g}/\text{mL}$), intermediate precision (inter-day precision) (determined by recording the absorbance of three concentrations of chlorogenic acid (3, 6 and 10 $\mu\text{g}/\text{mL}$) in the same laboratory by one person on three different days), accuracy (determined by quantification of total phenolic content of the standard solution (chlorogenic acid) at three different concentration levels of 80, 100 and 120%, namely 6.4, 8 and 9.6 $\mu\text{g}/\text{mL}$). The mean recovery percentages and relative standard deviation (RSD) % were calculated [16, 30].

Total flavonoid content

The quantification of total flavonoid (TF) content was based on the formation of flavonoid-aluminium chloride complexes. The absorbance was recorded at 510 nm and the results were expressed in mg (+)-catechin equivalents/g of dry extract [12, 27].

Total proanthocyanidin content

The total proanthocyanidin (TPA) content was estimated using *n*-butanol/HCl method. The TPA content, expressed in mg cyanidin/g of dry extract, was calculated using the molar absorptivity of cyanidin ($\epsilon = 17.360 \text{ L} / (\text{mol} \times \text{cm})$) [12, 21].

DPPH free radical scavenging assay

The free radical scavenging activity was evaluated by detecting the decrease of the absorbance of a methanolic DPPH solution at 517 nm in the presence of the extract according to a previously described methodology [4]. The results were expressed as effective concentration required scavenging 50% of the amount of DPPH free radical (EC_{50}). Chlorogenic acid and quercetin were used as positive controls.

Statistical analysis

All results were expressed as mean \pm SD. Statistical analysis was performed with Prism[®] (GraphPad software, version 5.03). Plots of the permeation profiles were compared by linear regression analysis. Differences between two values were evaluated by unpaired Student's *t* test. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

As our previous investigations showed potent endothelium-dependent vasorelaxant activity [6, 7] and antiplatelet effects (unpublished data) for *C. pentagyna* leaf and *V. opulus* fruit extracts, we chose these two extracts for further investigations focusing on the development of dosage forms with cardiovascular benefits. As oxidative stress plays a significant role in the pathogenesis of many cardiovascular diseases (atherosclerosis, ischemic stroke, hypertension) [2, 19], we evaluated the antioxidant capacity of both extracts using the DPPH assay.

CP and VO extracts exerted potent DPPH radical scavenging effects as resulted from their low EC_{50}

values (15.56 ± 0.03 and 47.18 ± 0.12 $\mu\text{g/mL}$, respectively), but still less pronounced than those of reference antioxidants such as quercetin and chlorogenic acid ($\text{EC}_{50} = 3.01 \pm 0.01$ and 9.97 ± 0.02 $\mu\text{g/mL}$, respectively). While CP extract was able to reach a maximum scavenging activity of 98.5% at 340 $\mu\text{g/mL}$, VO extract was less potent exerting a maximum scavenging activity of 92.5% at the same concentration (Figure 1). The greater antioxidant potency of CP extract can be ascribed mainly to its higher total phenolic, flavonoid and proanthocyanidin contents in comparison with VO extract (Table I). A hydromethanolic extract of *C. pentagyna* leaves was reported to contain lower total phenolic, flavonoid and proanthocyanidin contents (206.94 mg gallic acid equivalents, 57.08 mg catechin and 68.92 mg cyaniding/g of dry extract, respectively) [12] in comparison with *C. pentagyna* leaf extract investigated in the present study (901.12 mg chlorogenic acid equivalents, 416.68 mg catechin equivalents and 112.7 ± 1.21 mg cyanidin/g of dry extract, respectively). A hydroethanolic extract of *V. opulus* fruits had lower total phenolic, flavonoid and proanthocyanidin contents (3.73 mg of gallic acid equivalents, 2.01 mg of catechin equivalents and 0.52 mg of cyanidin equivalents/g of dry extract, respectively) [20] in comparison with *V. opulus* fruit extract under study (231.15 mg chlorogenic acid equivalents, 95.33 mg catechin equivalents and 18.40 mg cyanidin/g of dry extract, respectively). It is obvious that the extraction and purification procedures

used in the present study led to extracts enriched in polyphenols. A water extract obtained from *V. opulus* fruits showed lower DPPH scavenging activity ($\text{EC}_{50} = 57$ $\mu\text{g/mL}$) [3] than VO extract ($\text{EC}_{50} = 47.18$ $\mu\text{g/mL}$). The juice obtained from the fruits of different *V. opulus* genotypes, having a total phenolic content ranging from 5.47 to 10.61 mg of gallic acid equivalents/g of juice, showed DPPH scavenging effects of 70.2 - 77.5% [14]. However, due to the different experimental protocols, a comparison of these results with the ones obtained in our study is not feasible.

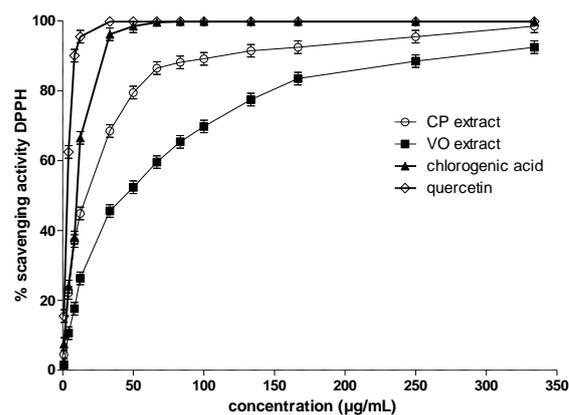


Figure 1.

DPPH scavenging effects of *C. pentagyna* leaf (CP) and *V. opulus* fruit (VO) extracts

Table I

Total phenolic (TP), total flavonoid (TF) and total proanthocyanidin (TPA) contents of *C. pentagyna* leaf (CP) and *V. opulus* fruit (VO) extracts

Sample	TP (mg chlorogenic acid equivalents/g extract)	TF (mg catechin equivalents/g extract)	TPA (mg cyanidin/g extract)
CP extract	901.12 ± 3.23^a	416.68 ± 2.03^c	112.7 ± 1.21^e
VO extract	231.15 ± 1.53^b	95.33 ± 0.95^d	18.40 ± 0.87^f

Values represent mean \pm SD of the determinations performed in triplicate. Values with a statistical significance ($p < 0.05$) are annotated with different letters.

The calibration curve for total phenolic content showed linearity in the concentration range of 2 - 15 $\mu\text{g/mL}$ and the correlation coefficient was higher than 0.999. The detection and quantification limits were 0.985 and 2.987 $\mu\text{g/mL}$, respectively and these values were below the minimum concentration of polyphenols (expressed in chlorogenic acid equivalents) quantified in the Franz diffusion cell. For the intra-day and inter-day precision, RSD (%) were 1.603% and 2.654%, respectively. The mean recovery percentages varied between 98.41% - 103.40%, thus being in the adequate range of 95 - 105%. These data confirm that the method used for the quantification of total phenolic content is precise and accurate.

As shown in Figure 2A, in the first ten minutes, CP extract presented a higher amount of polyphenols permeated *per* unit area of chicken skin (1140.75 $\mu\text{g/cm}^2$) compared to porcine sublingual membrane (568.24 $\mu\text{g/cm}^2$). However, in the next 20 minutes,

the permeability of polyphenols of CP extract through sublingual membrane was notably accelerated; thus, after 30 minutes, the amount of polyphenols permeated across the sublingual membrane (1698.97 $\mu\text{g/cm}^2$) exceeded the one permeated across the skin membrane (1614.94 $\mu\text{g/cm}^2$). In the next stages of the experiment, the permeation speed of polyphenols of CP extract across the sublingual membrane had a relatively constant increase rate (300 - 400 $\mu\text{g/cm}^2$ every 30 minutes), while for the skin membrane, the increase rate was slower (100 - 150 $\mu\text{g/cm}^2$ every 30 minutes). As a consequence of this behaviour, the final results of the *in vitro* permeability test after 240 minutes revealed a superior permeability of polyphenols of CP extract through sublingual (3301.73 $\mu\text{g/cm}^2$) vs. skin (2538.61 $\mu\text{g/cm}^2$) pathway. All these data are supported by the percentage of polyphenols of CP extract in the receptor compartment, as well as by the permeability coefficient and flux across the membranes (Table II).

Moreover, the permeation through sublingual membrane reached faster the steady-state, while in the case of skin membrane; the steady state couldn't be reached within four hours (Figure 2A).

The polyphenols of VO extract had a different permeation profile than the ones of CP extract (Figure 2B). For the first ten minutes, the polyphenols of VO extract presented a higher permeation through sublingual membrane than the one through the skin membrane (473.47 $\mu\text{g}/\text{cm}^2$ vs. 137.35 $\mu\text{g}/\text{cm}^2$). For the next 20 minutes, it has been recorded a more accelerated permeation rate through the sublingual membrane than skin membrane (200 - 400 $\mu\text{g}/\text{cm}^2$ vs. 80 - 130 $\mu\text{g}/\text{cm}^2$ every ten minutes). Thus, at 30 minutes, the

amount of polyphenols permeated *per unit area* of sublingual membrane (1019.65 $\mu\text{g}/\text{cm}^2$) surpassed the amount of polyphenols permeated through skin (249.50 $\mu\text{g}/\text{cm}^2$). For the following stages of the experiment, the permeation rates remained constant for the two membranes and, at the end of the permeation study; we concluded that the permeation through the sublingual membrane was superior to the one through skin. These results were confirmed by other permeation parameters such as the permeability coefficient, percentage of polyphenols in the receptor compartment and values of the flux across membranes (Table II).

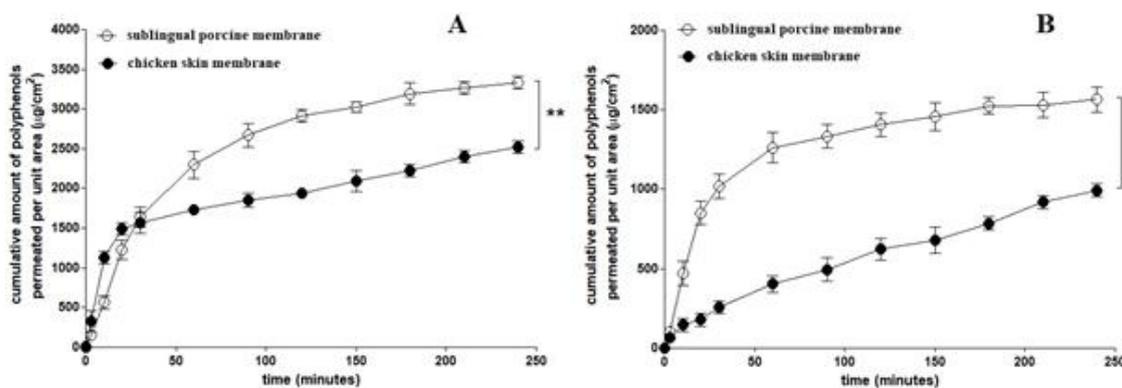


Figure 2.

In vitro permeation kinetics of polyphenols of *C. pentagyna* leaf (CP) extract (A) and *V. opulus* fruit (VO) extract (B) across the porcine sublingual and chicken skin membranes, * $p < 0.5$, ** $p < 0.001$

Table II

Parameters for permeation and diffusion of polyphenols of *C. pentagyna* leaf (CP) and *V. opulus* fruit (VO) extracts through biological membranes

Extract	Biological membrane	Flux ($\mu\text{g}/\text{cm} \times \text{h}$)	Lag time (h)	Permeability coefficient ($\times 10^{-3} \text{cm}/\text{h}$)	Diffusion coefficient ($\times 10^3 \text{cm}^2/\text{h}$)	Maximum percentage of polyphenols in the receptor compartment (%)	Total percentage of polyphenols in the membrane (%)
CP	porcine sublingual membrane	78.54 ± 5.74^a	0.17 ± 0.01^a	4.85 ± 0.51^a	0.86 ± 0.15^a	71.93 ± 2.38^a	14.68 ± 1.31^a
	chicken skin membrane	63.29 ± 4.41^b	0.31 ± 0.02^b	3.92 ± 0.45^b	7.94 ± 0.29^b	54.48 ± 3.51^b	21.16 ± 2.23^b
VO	porcine sublingual membrane	32.13 ± 3.57^c	0.16 ± 0.05^a	3.65 ± 0.21^b	0.74 ± 0.12^c	62.65 ± 2.72^c	18.17 ± 1.52^b
	chicken skin membrane	25.31 ± 2.85^d	0.36 ± 0.04^b	2.87 ± 0.33^c	8.43 ± 0.38^b	39.42 ± 3.35^d	27.50 ± 2.91^c

Values represent mean \pm SD of two different experiments. Statistically significant differences ($p < 0.05$) between values from a column ($p < 0.05$) are annotated with different letters.

According to the results in Table II, the polyphenols tend to accumulate more in the skin layers comparing to the sublingual mucosa suggesting a high penetration through this type of membrane. These results were confirmed by the determination of polyphenol percentages in the membranes at the end of the experiment as well as by the diffusion coefficient. Interaction with the membranes can be explained by the presence of

proanthocyanidins that are known to possess high binding affinity to proteins [9]. Procyanidin dimers were previously tentatively identified in VO and CP extracts [6, 7]. The presence of extract compounds inside the skin is important if extracts will be designed to be used in skin infections; in this case, their persistence could increase their antimicrobial efficacy. Other studies confirmed that polyphenols such as resveratrol, curcumin

and catechin can interact with the phospholipids present in the skin membrane thereby favouring the formation of a skin reservoir [1]. Polyphenols such as epicatechin, chlorogenic acid and quercetin were previously identified and quantified in CP and VO extracts [6, 7]. These polyphenols were reported to exert antimicrobial effects [11].

On contrast, in case of the sublingual administration of extracts, the aim is to have a low lag time with a rapid onset of action and more specifically, a reduced amount of polyphenols retained in the membrane. This can be correlated with a rapid transfer of the active compounds inside the blood stream which, according to our permeability studies, can be accomplished by polyphenols of CP and VO extracts. The sublingual membrane is thinner than the skin membrane and therefore both extracts were able to surpass this membrane in a greater extend compared to skin membrane. Besides, the epithelial characteristics of the sublingual mucosa can favour the fast transfer of polyphenols into the blood stream [24].

The ability of the polyphenols of CP and VO extracts to permeate both membranes might be the consequence of their amphiphilic characteristics. Thus, not only the capacity of the polyphenols to easily penetrate the hydrophobic bilayer of the biological membranes, but also their ability to dissolve into the aqueous receptor medium have contributed to a higher permeation capacity across biological membranes. Another factor that might influence the permeation capacity is linked to the presence of polyphenols in the aglycone forms which are more lipophilic than the glycosylated ones [22].

Conclusions

The extract obtained from *Crataegus pentagyna* leaves was characterized by higher polyphenolic, flavonoid and proanthocyanidin contents than *Viburnum opulus* fruit extract. Both extracts exhibited a potent scavenging activity against DPPH free radical. The permeation profiles offered useful information for pre-formulation studies; both extracts had a good permeability through the sublingual membrane supporting the idea that they are good candidates for the development of oromucosal dosage forms with accelerated release. In addition, *Crataegus pentagyna* leaf extract showed a good, but slow permeability through the skin membrane, this behaviour being appropriate for the formulation of modified release semisolid dosage forms.

Acknowledgement

This research was financially supported by “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania, grant no. 7626/18.04.2019.

Conflict of interest

The authors declare no conflict of interest.

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