https://doi.org/10.31925/farmacia.2023.4.9 **ORIGINAL ARTICLE**

MUCOADHESIVE FILMS BASED ON POLYVINYL ALCOHOL AND BIOACTIVE COMPOUNDS FOR ORAL ADMINISTRATION: STRUCTURAL CHARACTERIZATION AND MUCOADHESIVE PROPERTIES

OANA MIHAELA NEAGU [1], LAURA GRAȚIELA VICAȘ [2]*, ANA-MARIA VLASE [3], LAURIAN VLASE [4], ANNAMARIA PALLAG [2], ALIN TEUȘDEA [5], ILDIKÓ BÁCSKAY [6], ELEONORA MARIAN [2], MARIANA EUGENIA MUREȘAN [7], TÜNDE JURCA [1,2]

[1]*Doctoral School of Biomedical Science, University of Oradea, 1 University Street, 410087, Oradea, Romania*
[2]*Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Oradea, 10 1st December Square, 410073, Oradea, Romania*
[3]*Department of Pharmaceutical Botany, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 8 Victor Babeș Street, 400012, Cluj-Napoca, Romania*
[4]*Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 8 Victor Babeș Street, 400012, Cluj-Napoca, Romania*
[5]*Faculty of Environmental Protection, University of Oradea, 26 General Magheru Avenue, 410087, Oradea, Romania*
[6]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, 98 Nagyerdei Körút, 4032 Debrecen, Hungary*
[7]*Department of Preclinical Discipline, Faculty of Medicine and Pharmacy, University of Oradea, 10 1st December Square, 410073, Oradea, Romania*

**corresponding author: laura.vicas@gmail.com*

Manuscript received: April 2023

Abstract

In this study, two well known plant species, *Matricaria chamomilla* L. and *Viola tricolor* L., were characterized in terms of their polyphenolic and antioxidant profiles. 1:1, 1:2 and 2:1 mixtures ratioswere obtained from the alcoholic extracts. The plant extracts were analysed by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and by Folin-Ciocâlteu, CUPRAC and DPPH methods. Comparing the values of the bioactive principles contained in each mixture, as well as the antioxidant character, the mixture *Matricariae flos/Violae tricolor flos* 2:1, with an inhibition percentage of 84.28 ± 1.36%, was chosen to be incorporated into a mucoadhesive polymeric film. The formulated polymeric films were realized by incorporating 5% and 8% polyvinyl alcohol, 5% and 10% plant extract, respectively. The mucoadhesive films pH ranged between the normal salivary pH values (6.899 ± 0.016 and 7.045 ± 0.017), high folding strength and high tensile strength. According to the performed analyses and the plant extract yield operation from the polymer films, the films incorporating 5% polymer and 10% plant extract, respectively 5% polymer and 5% extract exhibited excelent results.

Rezumat

În acest studiu au fost caracterizate două specii de plante recunoscute, *Matricaria chamomilla* L. și *Viola tricolor* L., din punct de vedere al profilului polifenolic și antioxidant. Din extractele alcoolice au fost obținute amestecuri în raport de 1:1, 1:2 și 2:1. Extractele vegetale au fost analizate prin cromatografie lichidă de înaltă performanță cuplată cu spectrometrie de masă (HPLC-MS) și prin metodele Folin-Ciocâlteu, CUPRAC și DPPH. Comparând concentrațiile de principii bioactive conținute în fiecare amestec, precum și caracterul antioxidant, a fost ales amestecul *Matricariae flos/Violae tricolor flos* 2:1, cu un procent de inhibiție de 84,28 ± 1,36%, pentru a fi încorporat într-un film polimeric mucoadeziv. Filmele polimerice formulate au avut în compoziție 5% și 8% polivinilalcool, respectiv 5% și 10% extract vegetal. pH-ul a fost cuprins între valorile normale ale pH-ului salivar (6,899 ± 0,016 - 7,045 ± 0,017), rezistență mare la pliere și rezistență la tracțiune ridicată. Conform analizelor efectuate și cedării extractului vegetal din filmele polimerice, preparatelecu 5% polimer și 10% extract vegetal, respectiv 5% polimer și 5% extract au prezintat cele mai bune rezultate.

Keywords: antioxidants, polyphenols, *Matriacaria chamomilla* L., *Viola tricolor* L., mucoadhesive film

Introduction

The use of medicinal plants for various topical conditions has been studied since ancient times. Pharmaceutical forms with plant extracts are important because of their antioxidant properties which are able to neutralize free radicals and their effect [16].

Chamomile (*Matricaria chamomilla* L.) is the most widely used species of the genus *Matricaria* for its medicinal purposes. It contains several types of bio-

active compounds such as polyphenols, flavonoids and essential oils [33]. Chamomile flowers are widely known and used in herbal medicine for their anti-inflammatory, disinfectant and spasmolytic properties due to the presence of active principles such as chamazulene and sweet myrrh soft oxide [44].

The anti-inflammatory activity of aqueous chamomile extract and chamomile essential oil has been demonstrated in laboratory animals through the inhibition of PGE2 (prostaglandin E2) and nitric oxide (NO) production, both of which play an important role in cellular inflammation. PGE2 promotes capillary permeability and vasodilation, while NO is a free radical involved in the inflammatory process through the production of pro-inflammatory cytokines [44].

Viola tricolor L., commonly known as the three-headed fritillary or wild pansy, is a well-known species in both literature and traditional medicine. This plant has been used for many centuries for its beneficial effects in respiratory ailments, allergies, skin disorders like acne, eczema, impetigo, cysts, as well as for its diuretic action [16, 34, 40]. Also, in the literature is acknowledged the antimicrobial activity of the alcoholic extract of *Viola tricolor* L. flowers, especially inhibitory activity against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Candida albicans* [35, 43].

Viola tricolor L. is rich in flavonoids, anthocyanins, phenolic acids, salicylic acid and carotenoids [6, 41]. The main flavonoids identified in *Viola tricolor* L. were violanthin and rutin [36, 37], which in particular establishes this species anti-inflammatory character [26, 27]. By incorporating *Violae flos* extract into a topical gel, the anti-inflammatory activity on sunburn induced rats was studied. Thus, anti-inflammatory, antinociceptive and antiedematogenic effects were proven [25].

Polymer films must be flexible and resistant to breakage under the action of mechanical movements, but also must have good bioadhesive strength in order to be retained on the mucosa for the desired effect [18]. For the purpose of using mucoadhesive films for mouth wounds, these films help to protect the mucosal lesion, thus helping to reduce pain and speed up healing of the lesion [17].

Mucoadhesive polymers must possess a number of physicochemical characteristics, such as anionic hydrophilicity with numerous hydrogen bond-forming groups, surface properties suitable for moistening mucosal tissues and flexibility [5]. Polyvinyl alcohol (PVA) has been widely studied as a film former due to its high tensile strength, good flexibility and convenient transparency [30]. In this study, mucoadhesive polymer films were formulated with different concentrations of polyvinyl alcohol and plant extract, respectively. The purpose of combining these twois related to the synergism of action, in order to obtain a higher pharmacological effect. Since this type of pharmaceutical preparation is not acknowledged in the medical practice in Romania, we wanted to evaluate in this study the characteristics of such a product, in order to use it in lesions of the oral cavity. The polymeric films obtained were studied from a technological and physicochemical point of view.

The aim of the research was to determine the optimal concentration of polyvinyl alcohol necessary to obtain the polymeric films that offers the best yield of the active ingredients of the pharmaceutical product, by obtaining a topical product with anti-inflammatory activity, which can be used in diverse oral cavity diseases.

Materials and Methods

Reagents

All reagents used in the study were of analytical grade. The standards used in the chromatographic analysis were purchased from Sigma-Aldrich, Schnelldorf, Germany. Ethyl alcohol 96°, polyvinyl alcohol (PVA), glycerol, phosphate buffered saline (PBS), 1,1-di-phenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocâlteu reagent, methyl alcohol, acetic acid (≥ 99%), sodium carbonate, acetonitrile, aluminium chloride (≥ 98%), sodium nitrite, sodium hydroxide, copper chloride, neocuprein, ammonium acetate buffer were purchased from Sigma-Aldrich, Budapest, Hungary.

Plant material and extraction

Chamomile flowers (M) come from medicinal teas purchased from Fares, Orăștie, Romania. The flowers of heartsease (V) were harvested from the village of Codru, Bihor county, Romania. They were left to dry naturally. For the inclusion of the heartsease flowers in the study, the approval of the Ethics Committee of the Faculty of Medicine and Pharmacy of Oradea was received, with registration number CEFMF/11 from 30.05.2022. A specimen of the species *Viola tricolor* L. can be found in the herbarium of the Faculty of Medicine and Pharmacy, Pharmacy specialty, discipline Pharmaceutical Botany (herbarium registered in NYBG Steere Herbarium, code UOP) with registration number UOP05704. From the dried plant product, by maceration with ethanol, alcoholic extracts of 10% (w/w) concentration and mixtures of *Matricariae flos/Violae tricolor flos* extracts (M/V) in the ratio of 1:1, 1:2 and 2:1 were obtained.

Determination of the content of bioactive components

HPLC profile of plant extracts

A 1100 series HPLC system from Agilent Tehnologies, Santa Clara, California, USA, equipped with a de-agglomerator, binary gradient pump, thermostat, UV detector and Zorbax SB - C18 analytical column (100 x 3.0 mm i.d., with 3.5 µm particles) was used for the chromatographic analyses. The HPLC system was coupled with an Agilent Ion Trap 1100 SL mass

spectrometer and Brucker Ion Trap SL from Brucker Daltonics GmbH, Leipzig, Germany [21].

Identification of polyphenols, methoxylated flavones and tocopherols

Polyphenols, methoxylated flavones and tocopherols were identified by a HPLC-MS (high-performance liquid chromatography coupled to mass spectrometry) method. The standards used for the quantification of polyphenols were: chlorogenic acid, p-coumaric acid, ferulic acid, rutin, quercitrin, quercetol, luteolin, apigenin, syringic acid, vanillic acid, protocatechuic acid and catechin. Results were expressed in µg polyphenol/mL extract. Data were processed with Agilent's Chem-Station and DataAnalysis software [2, 35]. Three standards were used to identify methoxylated flavones: eupatorin, casticin and hispidulin. Results were expressed as ng methoxylated flavone/mL extract [9, 21]. The content of alpha-tocopherol, gamma-tocopherol and delta-tocopherol in the plant matrix was expressed in ng tocopherol/mL plant extract [29].

Determination of total polyphenol content and total flavonoid content

Total polyphenol content (TPC) was determined using Folin-Ciocâlteu reagent by a method described in a previous study. The total polyphenol content of the extracts was expressed as mg gallic acid equivalents (GAE)/100 g dry sample [14]. The total flavonoid content (TFC) was determined by a colorimetric method. The flavonoid content of plant extracts was calculated and expressed as mg quercetin (QE)/mL extract [39].

Determination of antioxidant activity

CUPRAC assay - Reduction of Cu^{2+} ions. The anti-oxidant activity of plant extracts towards copper ions (Cu^{2+}) was determined by the CUPRAC method. The antioxidant capacity of the extracts was expressed in µmol Trolox/100 µL [15].

DPPH assay. The detection and reduction of free radicals *in vitro* was carried out by the DPPH method. The percentage inhibition of DPPH is calculated by the equation below [13]:

$$\%Inhibition = \frac{(A_{Blank} - A_{Sample})}{A_{Blank}} \times 100 \qquad (1)$$

where, A_{blank} is the absorbance of the control and A_{sample} is the absorbance registered for the sample.

Statistical analysis

Data were statistically analysed using the One-Way Analysis of Variance (ANOVA) with a Tukey post-hoc test. Data analysis was performed with Prism software, version 9.3.0 (GraphPad Software, Boston, MA, USA). Results were considered statistically significant for P values less than 0.05.

Obtaining of polymer films

Formulated mucoadhesive polymer films have a composition based on polyvinyl alcohol as polymer and film former, which is dispersed in a solvent composed of bi-distilled water and 96° ethyl alcohol and glycerine as plasticiser and sweetener [7, 19]. The ratio of solvent constituents was water/etyl alcohol/glycerine 5:4:1. The plant extract added to the film matrix was chosen according to the character of the three mixtures preliminarily analysed. Following the DPPH method we selected the M/V 2:1 mixture, having the inhibition percentage of 84.28% ± 1.36. The polymer is dispersed in the solvent mixture under continuous stirring for 8 hours, during the last hour the plant extract is added. The resulting viscous solution is poured into glass vessels and left to dry for 2 days. After this time, the films are cut to the desired size and stored in airtight containers, protected from moisture and heat. By using this method, we prepared polymer films with concentrations of 5% and 8% polyvinyl alcohol, and for each polymer concentration we used vegetable extract with 5% and 10% concentration, respectively, as noted in Table I.

Table I

Composition of polymeric films

	PVA %	Plant extract %	Solvent %
M1	5	-	95
F1	5	5	90
F2	5	10	85
M2	8	-	92
F3	8	5	87
F4	8	10	82

PVA – polyvinyl alcohol; Plant extract – *Matricariae flos/Violae tricolor flos* 2:1; M1, M2, F1, F2, F3, F4 – film abbreviation

Appearance

The appearance of the polymer films was assessed by evaluating the organoleptic properties.

Film samples chromatic and imagistic analysis

The film samples were scanned with Canon CanoScan 9000F optical scanner (Canon Inc., 30-2, Shimomaruko 3-chome, Ohta-ku Tokyo 146-8501, Japan). Before each sample scanning, a colour self-calibration was performed by the scanner. In order to cancel embedding noise, the scanned images were pre-processed with Corel Paint Shop Pro v2021 (Corel Alludo HQ, 333 Preston Street, Suite 700, Ottawa, ON K1S 5N4, Canada). The univariate statistic test, one-way ANOVA (P = 0.05), combined with *post-hoc* Tukey's multiple comparisons test (P = 0.05) was used to compare the film samples images.

Sensory analysis

The hedonic test is a method based on the sensory analysis of a product using questions and presents a high degree of subjectivity. The participants in this test were untrained tasters (consumers). Before each tasting, the participants cleaned their mouths with water. The sample was allowed to dissolve slowly in the oral cavity without being swallowed. The taste of the product was rated on a scale of 1 to 9, where 9 = extremely pleasant and 1 = extremely unpleasant [22].

Loss on drying

The percentage loss of solvent through drying was calculated based on the initial weight of the solution poured into the vessel and the final weight. The solvent

evaporation percentage was calculated with the formula below:

$$\% = \frac{Mi-Mf}{Mi} \times 10, \qquad (2)$$

where, M_i = the amount of solution poured into the glass vessels was initially weighed, M_f = the mass of the film obtained after evaporation of the solvent, weighed after 48 hours.

Mass uniformity

For each sample, three different sections of 1 cm^2 size were selected and weighed on an analytical balance. The mass uniformity was calculated by averaging the weightings [23].

Film thickness

Film thickness was analysed on the Optika B-290 series optical microscope, Ponteranica Italy. Three samples from each film were measured using the graduations on the microscope slide, after which the measurements were averaged [41].

Folding endurance and tensile strength

For each type of film, three sections of 1 cm^2 were selected and folded 100 times through the same place [23]. Tensile strength was evaluated using the Brookfield CT3 Brookfield texture analysis instrument, Middleboro, MA, USA. It contains two load-bearing cell handles, of which the lower one is fixed and the upper one is movable. Samples were cut to size 1 x 0.5 cm, clamped between the cell handles and force was applied incrementally until the film broke. Tensile strength was measured in mN [23].

pH determination

The pH was evaluated with the Sension™ 1 digital pH meter, Hach Company, Loveland, CO, USA, after the dissolution of films in neutral bi-distilled water [30].

In vitro activity of polymeric films

In vitro disintegration time of the polymeric films

Disintegration of polymeric films was carried out under similar conditions to the oral cavity. Samples of 1 cm^2 size were allowed to dissolve in 10 mL phosphate buffered saline (PBS) under stirring at 37°C, registering the time for disintegration [38].

Antioxidant activity of the polymeric films

0.5 mL solutions obtained within the disintegration operation were processed by DPPH technique, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent, in order to evaluate the antioxidant character. Absorbances were read at 517 nm after the samples had been left in the dark for 30 minutes. The percentage inhibition was calculated using the equation (1) [13].

The active substance assay of polymeric films

To estimate the concentration of the active principles in polymer films, samples were taken from the solutions obtained within the disintegration operation and the absorbance was read at 370 nm. The content of active principles was expressed in mmol/L quercetin using the calibration curve. Values were expressed as a percentage using the equation below [5]:

$$\% = \frac{A_{Sample}}{A_{Blank}} \times 100, \qquad (3)$$

where, A_{blank} = is the absorbance of the control and A_{sample} = is the absorbance of the sample.

Plant extract release from polymeric films

In vitro release was performed using the Franz Microette-Hanson diffusion system, model 57-6AS9, USA. The receptor chamber in each cell was filled with phosphate buffer saline mixed with freshly prepared 30% ethanol. Approximately 0.1000 g sample was weighed and then applied to the previously hydrated membrane surface. Synthetic membranes were mounted between the donor and acceptor compartments of the Franz diffusion cell. The system was maintained at 32°C with continuous stirring at 600 rpm. 0.5 mL of receptor solution was extracted at 5, 10, 15, 30, 60, 120, 180, 240, 300, 360 minutes [19]. Absorbances were read 370 nm. Each film was tested in triplicate.

The film extract release data was subjected to non-linear regression with allosteric sigmoidal function. This statistical analysis was performed with GraphPad Prism v5.3 (GraphPad Software, Boston, MA 02110, USA).

Results and Discussion

Identification of polyphenols

Polyphenols are a major source of antioxidants with well-known pharmacological action [14]. The two extracts studied complement each other harmoniously in polyphenol composition, as it can be depicted in the Table II.

Literature data show amounts of polyphenols similar to ours in aqueous extracts obtained from chamomile tea: luteolin 0.04 - 0.13 mg/L, apigenin 2.24 - 2.60 mg/L, caffeic acid 0.41 - 1.53 mg/L, rutin 0.26 - 4. 21 mg/L, chlorogenic acid 0.04 - 4.36 mg/L, p-coumaric acid 0.03 - 0.11 mg/L [30], but also lower amounts than those obtained by us: apigenin (1.388), luteolin (5.113), rutin (6.013), chlorogenic acid (8.180), caffeic acid (1.296), all expressed in µg polyphenol/ 100 µg extract [8]. In another study from the literature, in which a lyophilized extract of *Viola tricolor* L. dissolved in both methanol and acetic acid was studied, a large number of polyphenols were quantified, that we did not identify in our study, such as epicatechin, luteolin, naringenin, myricetin, caffeic acid and sinapic acid. Compared to this study, vanillic acid, protocatechuic acid, quercitrin and quercetol were identified in our extract [14]. The amount of rutin present in our extract is considerable compared to other studies, in which rutin ranged between 33.70 ± 0.81 and 143.57 ± 8.48 mg/kg [16], 124.56 ± 4.54 mg/kg [14], respectively 420 ± 1.17 µg/g [40]. The polyphenolic acids identified in the current study were in lower amounts unlike other studies in which p-coumaric acid (89.23 ±1.24 mg/kg), ferulic acid (77.76 ± 2.35 mg/kg) were quantified [14].

Table II

Content of tocopherols, flavonoids and polyphenols in the extracts of *Matriacariae flos* (M) and *Violae tricolor flos* (V)

	M	V
Polyphenols (µg/mL)*		
Chlorogenic acid	70.686 ± 8.850	0.704 ± 0.08
Luteolin	2.680 ± 0.033	N.D.
Ferulic acid	0.557 ± 0.048	1.214 ± 0.94
Quercitrin	87.301 ± 6.105	3.169 ± 0.47
Quercetol	3.642 ± 0.021	4.633 ± 0.61
Apigenin	33.613 ± 2.057	N.D.
p-Coumaric acid	N.D.	0.441 ± 0.05
Rutin	N.D.	2550.911 ± 261.87
Syringic acid	0.23 ± 0.05	N.D.
Protocatechuic acid	1.54 ± 0.11	0.46 ±0.06
Vanillic acid	2.34 ± 0.13	0.85 ± 0.07
Catechin	N.D.	1.42 ± 0.36
Methoxylated flavones (ng/mL)*		
Eupatorin	394.97 ± 0.11	14.61 ± 1.52
Casticin	30766.19 ± 9.52	N.D.
Hispidulin	1844.71 ± 2.25	144.82 ± 15.7
Tocopherols (ng/mL extract)*		
α-tocopherol	134.50 ± 0.02	33.00 ± 2.82
γ-tocopherol	152.60 ± 0.01	26.50 ± 0.67
δ-tocopherol	27.00 ± 0.01	11.50 ± 0.09

N.D. – not detected, below limit of detection, * concentrations were expressed as mean ± SD, n = 3. M – *Matricariae flos*, V – *Violae tricolor flos*

The use of polyphenols from plant extracts for the prevention and treatment of oral cavity diseases is of particular interest in medical practice due to their considerable antioxidant capacity and lack of adverse effects [37]. The presence of polyphenols in the oral cavity helps to create a redox system, thus contributing to the management of oxidative stress. Research to date suggests that plant extracts have a superior pharmacological effect in oral health as opposed to individual polyphenols, thus maximising the bioavailability of contained polyphenols due to the multifactorial aetiology of oral diseases [12]. There are studies on the efficacy of polyphenols (chlorogenic acid, quercetin, luteolin, apigenin, catechin and rutin) in periodontal disease, that present their biological activity against periodontal pathogens, strengthen the antioxidant activity of saliva and crevicular fluid [24, 37].

Identification of methoxylated flavones

Methoxylated flavones are a subclass of polyphenols and exhibit antioxidant properties [4]. Under the chromatographic conditions presented, three flavones were identified, having the following retention times: 4.2 min for hispidulin, 7.6 min for eupatorine and 8.05 min for casticin, respectively. In the flower extract of three strawberry brothers only two methoxylated flavones were identified as it can be depicted in Table II. Also in this case, the bioactive compounds enriched the chamomile flower extract unlike the second extract. In a previous study, hispidulin was identified in the composition of freeze-dried chamomile extract, estimated at 1.584 ± 0.181 mg/g extract, being a value close to that obtained in our study [31].

Identification of tocopherols

Tocopherols are a class of compounds with strong antioxidant and anti-inflammatory properties. They are known as vitamin E, a compound that increases immune response, inhibits cell proliferation and suppresses tumour angiogenesis. Of the vitamin E compounds, alpha-tocopherol is the predominant form in the human body. β-tocopherol and γ-tocopherol have the same molecular mass, which is why they cannot be separated by chromatography, so their total content is analysed and expressed as γ-tocopherol [30]. Retention times were 3.3 minutes for δ-tocopherol, 4.1 minutes for γ-tocopherol and 5.1 minutes for α-tocopherol, respectively. The extract obtained with *Matricariae flos* was richer in tocopherols unlike the extract obtained with *Violae tricolor flos*, as presented in Table II. By using the ultrasonic extraction method, the amount of α-tocopherol in chamomile extract was 120.46 µg/mL, which is a higher concentration in comparison to the one obtained by us [10].

Determination of total polyphenols, flavonoids and antioxidant activity

The flavonoid content is higher in the extract of *Violae tricolor flos* (V) as opposed to the extract of *Matriacariae flos* (M), as shown in Table III. The total amount of polyphenols is higher in the chamomile extract, but the observed differences are very small. Regarding the DPPH method, the antioxidant character was pronounced while taking into account the chamomile extract. In the

case of the extracts mixtures, it is observed that the M/V 2:1 mixture exhibits the higher percentage of inhibition. Data were compared using the multivariate analysis, but the differences obtained are very small and statistically insignificant. The results are presented in Table III.

Table III

TFC, TPC and antioxidants values

The extracts and their ratios	Method			
	TFC mg QE/mL extract*	TPC mg GAE/100 g DW*	DPPH Inhibition % (TE mg/mL)*	CUPRAC µmol equivalent Trolox/100 µL extract*
M	1.08 ± 0.05	96.28 ± 4.81	88.92 ± 4.44	5.20 ± 0.26
V	1.30 ± 0.26	95.97 ± 1.73	77.14 ± 2.24	7.10 ± 0.79
M/V 1:1	1.30 ± 0.30	96.03 ± 2.12	82.32 ± 6.24	6.70 ± 0.74
M/V 1:2	1.30 ± 0.71	95.80 ± 0.30	80.71 ± 2.07	7.00 ± 0.63
M/V 2:1	1.26 ± 0.36	95.84 ± 1.72	84.28 ± 1.36	6.20 ± 0.24

* Concentrations were expressed as mean ± SD, n = 3. M – *Matricariae flos*, V – *Violae tricolor flos*, TFC – Total Flavonoids content, TPC – Total Polyphenols Content, GAE – gallic acid equivalents, DW – dry weight, QE – quercetin, TE – Trolox equivalents

According to another study in which the ethanolic extract of chamomile flowers was analysed, the TPC was higher than that obtained by us, having a value of 3.5 ± 1.7 mg GAE/g DM [29]. The flavonoid content ranged between 530.9 ± 20 mg QE/100 g DW and 710.7 ± 9 mg QE/100 DW in a methanolic extract of chamomile flowers, harvested from the Southern Italy, being values much higher than those obtained by us [11]. TPC in comparative studies were 21.4 ± 0.327 mg GAE/g for an ethanolic extract of chamomile [1] and 2689.2 ± 15 mg GAE/100 g DW for a methanolic extract, respectively [11]. The percentage inhibition of other previously studied chamomile extracts ranged from 84.2% ± 0.86 and 94.8% ± 0.03 [1], values similar to those obtained in the current study.

The total polyphenol content of *Violae tricolor flos* extract obtained by us was higher than fractions obtained with dichloromethane, ethyl acetate and butanol (12.84 ± 0.072 mg/g), respectively lower compared to a freeze-dried extract (445.03 ± 0.12 mg GAE/100 g DW) [14, 16]. TFC obtained in another study was higher than that obtained by us, being equal to 2.69 ± 0.17 mg QE/mL [14]. The antioxidant activity of *Violae tricolor flos* extract was lower in a comparative study, regardless the used method: DPPH (74.34% ± 2.64) or CUPRAC (2.68 ± 1.74 mmol Trolox/100 g extract) [14].

Characteristics of polymeric films

Appearance

The polymeric films obtained have a homogeneous aspect, having a translucent colour for those without vegetable extract, respectively slightly yellowish for those with vegetable extract. Thus, the conclusion that the colour of the films is provided by the vegetable extract. In the case of similar preparations, incorporating medicinal substances, the films were opaque. The texture of our obtained films was uniform, smooth and non-sticky, unlike other films without active substance which were sticky [20].

Compared to other herbal polymeric films that had citrate odour, our preparations were odourless. According to the Hedonic test results, the polymeric films have a pleasant, sweet taste, respectively those with plant extract exhibited a specific aroma of the plants used. In other studies, films with plant extracts tasted bitter or minty, depending on the composition [23].

The solvent evaporation percentage of the obtained polymer films ranged from 79.022 ± 8.185 to 81.621 ± 9.058 according to the Table IV. The evaporation percentage can then be used to calculate the amount of solution needed to prepare a certain amount of polymer film.

pH determination

The pH of the mucoadhesive films obtained are within the range of the salivary pH (6.3 - 7.3) [30], according to Table IV, denoting their isotonicity with the buccal mucosa [3].

Disintegration time

The results obtained in the disintegration operation are similar, however it is observed that films prepared with a higher PVA content require more time to dissolve completely.

Table IV

Characteristics of polymeric films

Polymeric film	Composition	Solvent loss %*	pH*	Disintegration time (min)*
M1	5% PVA	79.325 ± 8.152	7.015 ± 0.005	225 ± 5
M2	8% PVA	78.816 ± 7.924	7.016 ± 0.010	245 ± 10
F1	5% PVA 5% M/V Extract	81.621 ± 9.058	6.899 ± 0.016	240 ± 5
F2	5% PVA 10% M/V Extract	81.160 ± 9.346	6.949 ± 0.005	235 ± 5
F3	8% PVA 5% M/V Extract	79.022 ± 8.185	6.929 ± 0.008	245 ± 10
F4	8% PVA 10% M/V Extract	79.184 ± 9.382	7.045 ± 0.017	260 ± 10

* Concentrations were expressed as mean ± SD, n = 3. Note: M1 – 5% PVA, M2 – 8% PVA, F1 – 5% PVA 5% extract, F2 – 5% PVA 10% extract, F3 – 8% PVA 5% extract, F4 – 8% PVA 10% extract.

Film samples chromatic and imagistic analysis

Film samples were scanned in reflected light to establish which one had the highest content of active ingredients. The chromatic parameter values were subjected to one-way ANOVA (P = 0.05). In order to emphasize the discrimination between the film samples with extract (F1, F2, F3 and F4) and without extract (M1 and M2), a pairwise multiple comparison with post-hoc Tukey's test (p = 0.05) was performed. According to statistics, F2 sample exhibited the highest content of active ingredients. *Mass uniformity*

According to the weighing average, the weight of a 1 cm^2 film is 0.45 ± 0.05 g. The weight of a film also depends on its thickness. In other studies, films had weights between 0.21 and 0.59 g/cm^2 [5] or less, 63.2 ± 4.7 mg and 73.5 ± 5.2 mg, for a 15 mm diameter film [42].

Film thickness

Since the obtained films were so thin in comparison to their thickness, which could not be estimated with the naked eye, this feature was determined using an optical microscope and evaluated at 0.20 ± 0.02 mm. In a study in which mucoadhesive films based on 10% PVA were analysed, films with thicknesses ranging from 0.58 ± 0.047 mm to 0.91 ± 0.070 mm were obtained, being thinner than our obtained films [20].

Tensile strength and folding endurance

The breaking characteristics of polymer films were evaluated by folding endurance and tensile strength. The polymer films were folded 100 times through the same place without breaking.

Tensile strength was measured in Newtons, following three measurements. The F2 film with 5% polymer showed the highest tensile strength, according to Figure 1. Compared to other studies, the tensile strength of mucoadhesive films based on sodium carboxymethyl cellulose (NaCMC) was similar, but also higher in contrast to our samples, with values ranging from 17.4 ± 2.1 to 568.5 ± 62.6 N/cm^2 [42]. In other studies, the formulated mucoadhesive films had high flexibility without breaking after 100 folds [23].

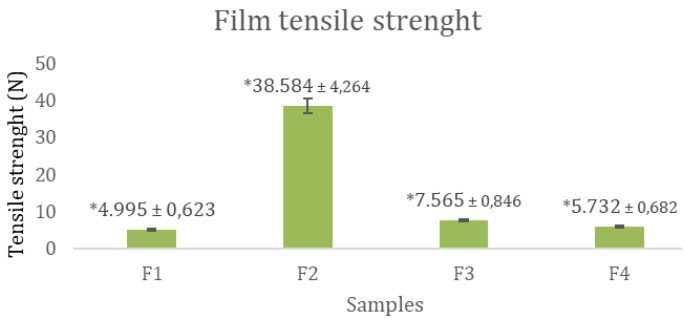

Figure 1.

Tensile strength of the mucoadhesive films

Tensile strength was measured in N, each result representing the mean ± SD, n = 3. F1 – 5% PVA 5% extract, F2 – 5% PVA 10% extract, F3 – 8% PVA 5% extract, F4 – 8% PVA 10% extract. The data was analysed with the Kruskal-Wallis test followed by the Dunn's multiple comparisons test. Significant differences of the formulations were detected between the compositions. Significant differences are marked on the figure with * ($p < 0.05$), showing the significance levels in the case of composition F2 - F1, F2 - F3, F2 - F4

The antioxidant character of polymer films and drug content

As it is a mixture of extracts, the whole phyto-complex contained in the blend is responsible for the antioxidant activity. Polymeric films prepared with 10% plant extract presented higher antioxidant activity than those with 5% extract, as well as a higher content of active ingredients. The films with 10% plant extract and 5% polymer exhibited the highest values for both antioxidant activity and active ingredient content, according to the Table V.

Table V

Antioxidant activity of mucoadhesive films and drug content

Polymeric film	DPPH Inhibition % (TE mg/mL)*	Drug content mmol/L QE*	Drug content %*
F1	17.936	0.43	66.67
F2	18.613	0.51	79.63
F3	10.660	0.46	70.37
F4	13.875	0.50	77.78

* Concentrations were expressed as mean ± SD, n = 3. Note: F1 - 5% PVA 5% extract, F2 – 5% PVA 10% extract, F3 – 8% PVA 5% extract, F4 – 8% PVA 10% extract, QE – quercetin, TE – Trolox equivalents.

Plant extract release

The release of the plant extract from the polymer films occurred gradually, directly proportional to the time required for release, as can be seen in the Figure 2. Thus, at the end of the yield period (360 min), the release rate is maximum and the film is disintegrated.

From a pharmacokinetic point of view, even though the F1 film had the lowest percentage of yield, this film consistently releases the plant extract over the timescale studied. The release of the active substance from the other polymeric studied films was increasing, directly proportional to the release time, with a maximum value at the end of the release time [19].

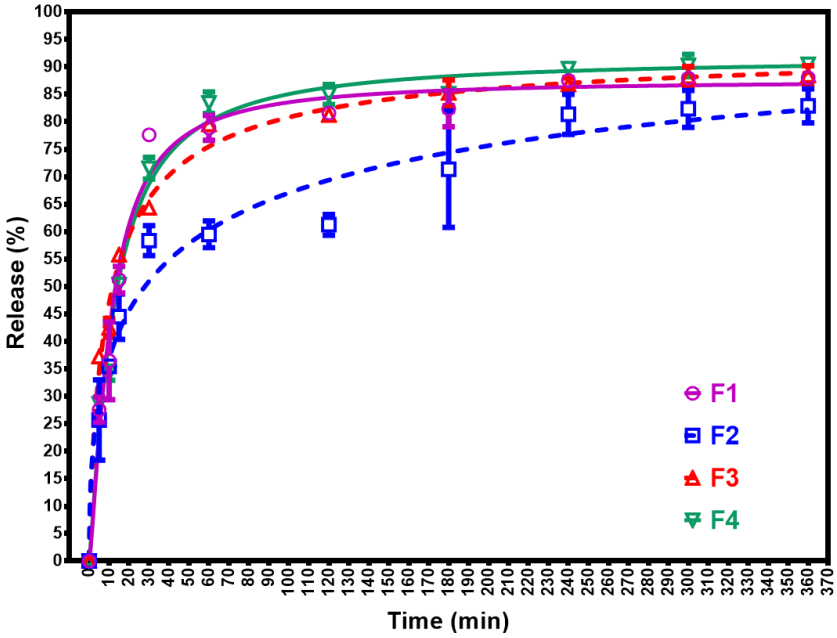

Figure 2.
Film samples extract release (%)
F1 – 5% PVA 5% extract, F2 – 5% PVA 10% extract, F3 – 8% PVA 5% extract, F4 – 8% PVA 10% extract

Conclusions

According to the phytochemical profile, the extract obtained from *Matriacariae flos* was found to be richer in tocopherols, methoxylated flavones and polyphenols compared to the extract of *Violae tricolor flos*. The antioxidant activity was higher compared to the chamomile extract. In the case of the extract mixtures, the percentage of inhibition was higher for the M/V ratio 2:1, which is why we incorporated this mixture into the prepared polymeric films. In terms of appearance and including the Hedonic test, the polymer films represent a product that can be easily accepted by patients. The pH of the mucoadhesive films is neutral, being in accordance with the pH range of saliva. According to the antioxidant activity and the content of active principles the F2 film, containing 5% PVA and 10% plant extract achieved excellent results. As *per* the *in vitro* release of the plant extract in the studied products, the F1 film, containing 5% PVA and 5% plant extract was characterized as the most convenient to be studied and used in oral lesions.

Conflict of interest

The authors declare no conflict of interest.

References

1. Al-Dabbagh B, Elhaty I, Elhaw M, Murali C, Al Mansoori A, Awad B, Amin A, Antioxidant and anticancer activities of chamomile (*Matricaria recutita* L.). *BMC Research Notes*, 2019; 2019, 12(1): 3.
2. Anton A, Moacă EA, Sarau CA, Dinu Ș, Semenescu AD, Macașoi IG, Dehelean CA, Antioxidant and *in vitro* cytotoxic activity of commercial lemongrass, sea buckthorn and basil essential oils, against colorectal cancer cell line HCT 116. *Farmacia*, 2022; 70(4): 683-689.
3. Baliga S, Muglikar S, Kale R, Salivary pH: A diagnostic biomarker. *J Indian Soc Periodontol.*, 2013; 17: 461-465.
4. Berim A, Gang DR, Methoxylated flavones: occurrence, importance, biosynthesis. *Phytochem Rev.*, 2016; 15: 363-390.
5. Bhattacharjee S, Nagalakshmi S, Shanmuganathan S, Formulation characterization and *in-vitro* diffusion studies of herbal extract loaded mucoadhesive buccal patches. *IJPSR*, 2014; 5 (11): 4963-4968.
6. Carnat AP, Carnat A, Fraisse D, Lamaison JL, Heitz A, Wylde R, Teulade JC, Violarvensin, a new flavone di-C-glycoside from *Viola arvensis*. *J Nat Prod.*, 1998; 61(2): 272-274.
7. Chaiprateep E, Khobjai W, Noysang C, *Clinacanthus nutans*-Based Mucoadhesive Films for Oral Ulcers, Research *J Pharm Technol.*, 2018; 11: 5089-5095.
8. Danciu C, Zupko I, Bor A, Schwiebs A, Radeke H, Hancianu M, Cioancă O, Alexa E, Oprean C, Bojin F, Soica C, Păunescu V, Dehelean CA, Botanical Therapeutics: Phytochemical Screening and Biological Assessment of Chamomile, Parsley and Celery Extracts against A375 Human Melanoma and Dendritic Cells. *Int J Mol Sci.*, 2018; 19: 3624.

9. Epure A, E. Parvu AE, Vlase L, Benedec D, Hanganu D, Gheldiu AM, Toma VA, Oniga I, Phytochemical profile, antioxidant, cardioprotective and nephroprotective activity of Romanian chicory extract. *Plants*, 2021; 10(1): 64.
10. Farahmandfar R, Asnaashari M, Asadi Y, Beyranvand B, Comparison of Bioactive Compounds of *Matricaria recutita* Extracted by Ultrasound and Maceration and their Effects on Preventing Sunflower Oil During Frying. *Cur Nutr Food Sci*., 2019; 15(2): 156-164.
11. Formisano C, Delfine S, Oliviero F, Tenore GC, Rigano D, Senatore F, Correlation among environmental factors, chemical composition and antioxidative properties of essential oil and extracts of chamomile (*Matricaria chamomilla* L.) collected in Molise (South-central Italy). *Ind Crops Products.*, 2015; 63: 256-263.
12. Ginsburg I, Koren E, Shalish M, Kanner J, Kohen R, Saliva increases the availability of lipophilic polyphenols as antioxidants and enhances their retention in the oral cavity. *Arch Oral Biol.*, 2012; 57(10): 1327-1334.
13. Jurca T, Vicaș L, Tóth I, Braun M, Marian E, Teusdea A, Vicaș S, Mureșan M, Mineral elements profile, bioactive compound and antioxidant capacity of wild blueberry and of pharmaceutical preparations from blueberry (*Vaccinium myrtillus*). *Farmacia*, 2016; 64(4): 581-587.
14. Jurca T, Pallag A, Marian E, Mureșan ME, Stan RL, Vicaș L, The Histo-anatomical investigation and the polyphenolic profile of antioxidant complex active ingredients from three *Viola* species. *Farmacia*, 2019; 67(4): 634-640.
15. Karaman S, Tutem E, Baskan KS, Apak R, Comparison of total antioxidant capacity and phenolic composition of some apple juices with combined HPLC- CUPRAC assay. *Food Chem.*, 2010; 120(4): 1201-1209.
16. Kieling Gonçalves AF, Friedrich RB, Boligon AA, Piana M, Ruver Beck RC, Athayde ML, Anti-oxidant capacity, total phenolic contents and HPLC determination of rutin in *Viola tricolor* (L) flowers. *Free Radicals and Antioxidants*, 2012; 2(4): 32-37.
17. Laffleur F, Mucoadhesive polymers for buccal drug delivery. *Drug Develop Ind Pharm.*, 2014; 40(5): 591-598.
18. Lahoti SS, Shep SG, Mayee RV, Toshniwal SS, Mucoadhesive Drug Delivery System: A Review. *Indo-Global J Pharmaceut Sci*., 2011; 1(3): 243-251.
19. Miksusanti, Fithri AN, Herlina, Wijaya DP, Taher T, Optimization of chitosan-tapioca starch composite as polymer in the formulation of gingival mucoadhesive patch film for delivery of gambier (*Uncaria gambir* Roxb) leaf extract. *Int J Biol Macromol.*, 2020; 144: 289-295.
20. Mishra SK, Garud N, Singh R, Development and evaluation of mucoadhesive buccal patches of flurbiprofen. *Acta Pol Pharm.*, 2011: 68(6): 955-964.
21. Mocan A, Crișan G, Vlase L, Ivănescu B, Bădărău AS, Arsene AL, Phytochemical investigation on four *Galium* species (*Rubiaceae*) from Romania. *Farmacia*., 2016; 64(1): 95-99.
22. Neagu OM, Ghitea T, Marian E, Vlase L, Vlase AM, Ciavoi G, Fehér P, Pallag A, Bácskay I, Nemes D, Vicaș LG, Teușdea A, Jurca T, Formulation and Characterization of Mucoadhesive Polymeric Films Containing Extracts of *Taraxaci Folium* and *Matricariae Flos*. *Molecules*, 2023; 28(10): 4002.
23. Perez Zamora CM, Michaluk AG, Chiappetta DA, Nuñez MB, Herbal buccal films with *in vitro* antibacterial and anti-inflammatory effects. *J Herb Med.*, 2022; 31.
24. Petti S, Scully C, Polyphenols, oral health and disease: A review. *J Dent.*, 2009; 37(6): 413-423.
25. Piana M, Silva MA, Trevisan G, de Brum TF, Silva CR, Boligon AA, Oliveira SM, Zadra M, Hoffmeister C, Rossato MF, Tonello R, Laporta LV, de Freitas RB, Belke BV, da Jesus R, Ferreira J, Athayde ML, Antiinflammatory effects of *Viola tricolor* gel in a model of sunburn in rats and the gel stability study. *J Ethnopharmacol.*, 2013; 150(2): 458-465.
26. Piana M, Zadra M, de Brum TF, Boligon AA, Kieling Gonçalves AF, da Cruz RC, de Freitas RB, do Canto GS, Athayde ML, Analysis of rutin in the extract and gel of *Viola tricolor*. *J Chromatogr Sci.*, 2013; 51(5): 406-411.
27. Rimkiene S, Ragazinskiene O, Savickiene N, The cumulation of wild pansy (*Viola tricolor* L.) accessions: The possibility of species preservation and usage in medicine, *Medicina (Kaunas)*, (2003); 39(4): 411-416.
28. Roby MHH., Sarhan MA, Selim HAH, Khalel IK, Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). *Industrial Crops and Products*., 2013; 44: 437-445.
29. Rusu ME, Fizeșan I, Pop A, Mocan A, Gheldiu AM, Babotă M, Vodnar DC, Jurj A, Berindan-Neagoe I, Vlase L, Popa DS, Walnut (*Juglans regia* L.) Septum: Assessment of Bioactive Molecules and *In Vitro* Biological Effects. *Molecules*, 2020; 25: 2187.
30. Salehi S, Boddohi S, New formulation and approach for mucoadhesive buccal film of rizatriptan benzoate. *Prog Biomater*., 2017; 6(4): 175-187.
31. Sándor Z, Mottaghipisheh J, Veres K, Hohmann J, Bencsik T, Horváth A, Kelemen D, Papp R, Barthó L, Csupor D, Evidence Supports Tradition: The *in Vitro* Effects of Roman Chamomile on Smooth Muscles. *Front Pharmacol.*, 2018; 9.
32. Sentkowska A, Biesaga M, Pyrzynska K, Effects of brewing process on phenolic compunds and antioxidant activity of herbs. *Food Sci Biotechnol.*, 2016; 25(4): 965-970.
33. Srivastava JK, Gupta S, Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. *Mol Cell Pharmacol.*, 2010; 1(3): 138.
34. Ștefan CS, Chițescu CL, Manolache N, Diaconu C, Elisei AM, Beznea A, Iancu AV, Gurău G, Chiriac ER, Fulga I, The investigation of antimicrobial activity of some extracts from *Momordica charantia* by using as solvent extraction an ionic liquid. *Farmacia*, 2022; 70(1): 144-150.
35. Toiu A, Vlase L, Gheldiu AM, Vodnar D, Oniga I, Evaluation of the antioxidant and antibacterial potential of bioactive compounds from *Ajuga reptans* extracts. *Farmacia*, 2017; 65(3): 351-355.
36. Toiu A, Vlase L, Vodnar DC, Gheldiu AM, Oniga I, *Solidago graminifolia* L. Salisb. (*Asteraceae*) as a valuable source of bioactive polyphenols: HPLC profile,

in vitro antioxidant and antimicrobial potential. *Molecules*, 2019; 24(14): 2666

37. Varoni EM, Lodi G, Sardella A, Carrassi A, Iriti M, Plant polyphenols and oral health: old phytochemicals for new fields. *Curr Med Chem.*, 2012; 19(11): 1706-1720.
38. Vecchi CF, Said dos Santos R, Bassi da Silvia J, Rosseto HC, Sakita KM, Svidzinski TIE, Bonfim-Mendonça PS, Bruschi ML, Development and in vitro evaluation of buccal mucoadhesive films for photodynamic inactivation of *Candida albicans*. *Photodiagnosis Photodyn Ther.*, 2020; 32: 101957.
39. Vicaș L, Teușdea A, Vicaș S, Marian E, Jurca T, Mureșan M, Gligor F, Assessment of antioxidant capacity of some extracts for further use in therapy. *Farmacia*, 2015; 63(2): 267-274.
40. Vukics V, Hevesi Toth B, Ringer T, Ludanyi K, Kery A, Bonn GK, Guttman A, Quantitative and qualitative investigation of the main flavonoids in heartsease (*Viola tricolor* L.). *J Chromatogr Sci.*, 2008; 46(2): 97-101.
41. Vukics V, Kery A, Bonn GK, Guttman A, Major flavonoid components of heartsease (*Viola tricolor* L.) and their antioxidant activities. *Anal Bioanal Chem.*, 2008; 390(7): 1917-1925.
42. Walicová V, Gajdziok J, Pavloková S, Vetchý D, Design and evaluation of mucoadhesive oral films containing sodium hyaluronate using multivariate data analysis. *Pharm Dev Technol.*, 2017; 22(2): 229-236.
43. Witkowska-Banaszczak E, Bylka W, Matławska I, Goślińska O, Muszyński Z, Antimicrobial activity of *Viola tricolor* herb. *Fitoterapia*, 2005; 76(5): 458-461.
44. Wu YN, Yong X, Yao L, Anti-inflammatory and Anti-allergic Effects of German Chamomile (*Matricaria chamomilla* L.). *J Ess Oil Bearing Plants*, 2012; 15(1): 75-83.