EFFECT OF EXTRACTION SOLVENT ON THE COMPOSITION AND ANTIOXIDANT ACTIVITY OF EDIBLE FLOWER EXTRACTS

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Extracts of basil (Ocimum basilicum), chicory (Cichorium intybus), and common sage (Salvia officinalis) were prepared through maceration, using ethanol, methanol, and two mixtures of ethanol and methanol (containing 40% and 60% ethanol) as extraction solvents. Total polyphenol content (TPC), total flavonoid content (TFC), and percentage of inhibition (PI) of edible flower extracts obtained at four levels of ethanol concentration in the extraction solvent (0%, 40%, 60%, and 100%), i.e., TPC0, TPC40, TPC60, TPC100, TFC0, TFC40, TFC60, TFC100, PI0, PI40, PI60, and PI100, were determined. On the one hand, the extracts derived from S. officinalis had significantly higher mean values of TPC0–100 (46.31–61.77 mg GAE/mg DM), TFC0–60 (24.44–34.92 mg QE/mg DM), and PI0–100 (80.45–89.35%) than the extracts obtained from C. intybus (24.98–31.41 mg GAE/mg DM, 9.32–12.51 mg QE/mg DM, and 70.28–72.81%) and O. basilicum (31.70–35.76 mg GAE/mg DM, 19.16–26.02 mg QE/mg DM, and 80.45–89.35%). On the other hand, the extracts prepared from O. basilicum had significantly lower mean values of TFC100 (0.97 mg QE/mg DM) than those derived from S. officinalis (24.40 mg QE/mg DM) and C. intybus (14.85 mg QE/mg DM). The correlations between TPC0–100, TFC0–100, and PI0–100 were generally significant ($0.525 \leq r \leq 0.999$).

Keywords: antioxidant activity, basil (*Ocimum basilicum*), chicory (*Cichorium intybus*), common sage (*Salvia officinalis*), edible flower, extraction, flavonoids, phenolic compounds

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1. Introduction

Plants have been studied since ancient times for their use in both traditional medicine and food industry, with the aim of enhancing the health benefits and sustainability of food products [1,2]. Plant secondary metabolites, including phenolic compounds, terpenoids, and alkaloids, can have important antioxidant, anti-inflammatory, antiallergic, cardioprotective, antidiabetic, and antiviral properties [3,4]. Different aromatic and medicinal plants have been intensively studied recently to prepare various formulations that can be used in the food, cosmetic, and pharmaceutical industries [3].

Basil (*Ocimum basilicum* L.) is an aromatic plant belonging to the Lamiaceae family, which gives foods a distinctive aroma and taste [5]. Its leaves, either fresh or dried, are widely used as a spice [6]. Basil is also a digestive stimulant and has antimicrobial, antibacterial, anticonvulsant, and anticarcinogenic properties [7]. Its leaves are used to treat nausea, dysentery, gastroenteritis, and abdominal cramps [5]. Basil presents a wide range of compounds beneficial to human health, including phenolic compounds (*e.g.*, phenolic acids, flavonoids), vitamins, and minerals [6].

Chicory (*Cichorium intybus* L.) is a perennial plant that belongs to the Asteraceae family and grows spontaneously in any type of soil, from May to October [8,9]. Its aerial parts are used as ingredients in salads and a raw material for the production of fructose and spices [10]. Chicory extracts can be added to alcoholic and non-alcoholic beverages [9]. Antioxidant, hepatoprotective, anti-inflammatory, antimicrobial, antimutagenic, and anticarcinogenic effects of chicory extracts were reported [10,11]. The most important compounds identified in this plant are polyphenols and terpenoids, but it also contains saponins, volatile compounds, amino acids, fatty acids, organic acids, vitamins, and minerals [8,9].

Common sage (*Salvia officinalis* L.) is an annual herb belonging to the Lamiaceae family, widely cultivated in different parts of the world due to its wide use in traditional medicine and cooking [12]. It is a promising antioxidant agent of natural origin and has the ability to improve brain function and memory as well as to delay age-related cognitive problems, making it a potential therapeutic agent for neurodegenerative disorders [13,14]. Common sage is rich in phenolic acids (including caffeic, ferulic, rosmarinic, and vanillic acids), flavonoids (*e.g.*, apigenin, luteolin, quercetin), and terpenes (especially α- and β-thujone, eucalyptol, camphor, α-humulone, viridiflorol, carnosic, oleanolic, and ursolic acids) [15].

Extraction yields of bioactive compounds from plant matrices and antioxidant activity of the extracts depend on different process factors, including extraction method (*e.g.*, maceration with/without stirring, percolation, Soxhlet extraction, ultrasound-assisted extraction, microwave-assisted extraction,

supercritical fluid extraction) and equipment, operating conditions (temperature, pressure, solid/liquid ratio), type of plant and extraction solvent [3,16].

This paper aimed at evaluating the effect of extraction solvent (ethanol, methanol, and mixtures of ethanol and methanol) on the composition and antioxidant activity of extracts of basil (*O. basilicum*), chicory (*C. intybus*), and common sage (*S. officinalis*) obtained by maceration under stirring.

2. Materials and methods

Plant material

Dried aerial parts of *O. basilicum*, *C. intybus*, and *S. officinalis* were provided by Fares (Orastie, Hunedoara, Romania), Stef Mar (Ramnicu Valcea, Valcea, Romania), and Dacia Plant (Bod, Brasov, Romania), respectively. The dried plant material was ground in a grinder.

Chemicals

Ethanol, methanol, Folin-Ciocalteu reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), and quercetin were purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), and ascorbic acid were purchased from VWR International (Vienna, Austria). Distilled water (DW) was obtained using a Milli-Q (Millipore, Merck, Italy) water purification system (Millipore, Merck). All chemicals were of analytical grade.

Extraction procedure

Extracts of edible flowers were prepared through maceration under stirring of dried and ground plant material, using ethanol, methanol, and two mixtures of ethanol and methanol as extraction solvents. Extraction process was performed for 24 h at four levels of ethanol concentration in the extraction solvent (0%, 40%, 60%, and 100%) and a single level of solid/liquid ratio (1/10 g/mL), process temperature (30 °C), and stirring speed (250 rpm). Each extract was filtered using filter paper.

Determination of total phenolic content (TPC) of plant extracts

TPC of plant extracts was determined by the Folin-Ciocalteu method with some modifications [17]. 1 mL of extract was mixed with 9 mL of DW in a 25 mL flask, then 2.5 mL of diluted FCR (1/10 mL FCR/mL DW) was added to the flask. The mixture was stirred for 5 min, then 10 mL of 7.5% Na_2CO_3 solution was added and made up to the mark with DW. This mixture was kept in the dark for 90 min at ambient temperature. The values of sample absorbance were read at a wavelength

of 760 nm with a UV/Vis spectrophotometer (Specord 250 Plus, Analytik Jena, Germany). *TPC* of each sample was determined from the calibration curve and expressed as mg GAE (gallic acid equivalents)/g DM (dry matter). The determinations were performed in five replicates.

Determination of total flavonoid content (TFC) of plant extracts

TFC of plant extracts was determined by the colorimetric method with $AlCl_3 \cdot 6H_2O$ solution [17]. 1 mL of extract was mixed with 4 mL of DW in a 10 mL flask, then 0.30 mL of 5% $NaNO_2$ was added. The mixture was stirred for 5 min, then 0.30 mL of 10% $AlCl_3 \cdot 6H_2O$ solution was added and, after another 5 min of stirring, 2 mL of 1 M NaOH solution was added to the mixture and made up to the mark with DW. The values of sample absorbance were read at a wavelength of 510 nm with the UV/Vis spectrophotometer Specord 250 Plus. *TFC* of each sample was determined from the calibration curve and expressed as mg QE (quercetin equivalents)/g DM. The determinations were performed in five replicates.

Evaluation of antioxidant activity of plant extracts

Evaluation of antioxidant activity of plant extracts was performed according to the DPPH free radical scavenging assay [18]. 1 mL of extract was mixed with 1 mL of 0.1 mM DPPH methanolic solution in a flask. The mixture was kept in the dark for 30 min at ambient temperature, and then the values of sample absorbance were read at a wavelength of 517 nm with the UV/Vis spectrophotometer Specord 250 Plus. The results were expressed as percentage of inhibition (*PI*) defined by equation (1), where A_0 is the absorbance of DPPH solution without extract sample and A_1 the absorbance of DPPH solution with extract sample.

$$PI = 100 \frac{A_0 - A_1}{A_0} \tag{1}$$

Data processing

The values of response variables (*TPC*, *TFC*, and *PI*) obtained at different levels of ethanol concentration in the extraction solvent (0–100%) were processed applying principal component analysis (PCA) [19–21]. One-way analysis of variance (ANOVA) was used to assess whether the mean values of the response variables were significantly different ($p < 0.05$) or not. The strength of the linear correlations between different response variables was evaluated based on the Pearson correlation coefficient (r). Statistical analysis was performed using XLSTAT version 2019.1.

3. Results and discussion

A data matrix with 15 rows (5 replicates for each type of flower) and 12 columns (number of variables, *i.e.*, *TPC*0, *TPC*40, *TPC*60, *TPC*100, *TFC*0, *TFC*40, *TFC*60, *TFC*100, *PI*0, *PI*40, *PI*60, and *PI*100) was used in PCA. Only the first two principal components (PCs) had eigenvalues > 1 (10.20 for PC1 and 1.79 for PC2). PC1 and PC2 explained 99.9% (85.0% + 14.9%) of the total variance, and only these two PCs were further considered in the multivariate analysis. The results shown in Table 1, Fig. 1, and Fig. 2 indicate the following:

- depending on significant levels of factor loadings (highlighted in bold in Table 1), the most important variables are *TPC*0, *TPC*40, *TPC*60, *TPC*100, *TFC*0, *TFC*40, *TFC*60, *PI*0, *PI*40, *PI*60, and *PI*100 for PC1 as well as *TFC*100 for PC2;

Table 1

Factor loadings

No.	Name	Symbol	PC1	PC2
1	Total phenolic content for extraction with 100% methanol (0%Et)	*TPC*0	**0.989**	0.144
2	Total phenolic content for extraction with 60% methanol + 40% ethanol (40%Et)	*TPC*40	**0.944**	0.325
3	Total phenolic content for extraction with 40% methanol + 60% ethanol (60%Et)	*TPC*60	**0.944**	0.327
4	Total phenolic content for extraction with 100% ethanol (100%Et)	*TPC*100	**0.998**	0.050
5	Total flavonoid content for extraction with 100% methanol (0%Et)	*TFC*0	**1.000**	0.013
6	Total flavonoid content for extraction with 60% methanol + 40% ethanol (40%Et)	*TFC*40	**0.997**	-0.077
7	Total flavonoid content for extraction with 40% methanol + 60% ethanol (60%Et)	*TFC*60	**0.744**	-0.668
8	Total flavonoid content for extraction with 100% ethanol (100%Et)	*TFC*100	0.513	**0.858**
9	Antioxidant activity for extraction with 100% methanol (0%Et)	*PI*0	**0.906**	-0.422
10	Antioxidant activity for extraction with 60% methanol + 40% ethanol (40%Et)	*PI*40	**0.977**	0.210
11	Antioxidant activity for extraction with 40% methanol + 60% ethanol (60%Et)	*PI*60	**0.967**	-0.252
12	Antioxidant activity for extraction with 100% ethanol (100%Et)	*PI*100	**0.960**	-0.278

Significant values are highlighted in bold.

- the extracts obtained from common sage (*S. officinalis*) had higher values of *TPC*0–100, *TFC*0–60, and *PI*0–100 than those prepared from chicory (*C. intybus*) and basil (*O. basilicum*) (discrimination on PC1 between *S. officinalis* samples and the other samples in Fig. 1); with one exception, the data presented in Fig. 2 confirm this finding, *i.e.*, the mean values of *TPC*0–100, *TFC*0–60, and *PI*0–100 for *S. officinalis* (*SO*) samples ($TPC0–100_{m,SO}$ = 46.31–61.77 mg GAE/mg DM, $TFC0–60_{m,SO}$ = 24.44–34.92 mg QE/mg DM, and $PI0–100_{m,SO}$ = 80.45–89.35%) were generally significantly higher ($p < 0.05$) than those for *C. intybus* (*CI*) samples ($TPC0–100_{m,CI}$ = 24.98–31.41 mg GAE/mg DM, $TFC0–60_{m,CI}$ = 9.32–12.51 mg QE/mg DM, and $PI0–100_{m,CI}$ = 70.28–72.81%) and *O. basilicum* (*OB*) samples ($TPC0–100_{m,OB}$ = 31.70–35.76 mg GAE/mg DM, $TFC0–60_{m,OB}$ = 19.16–26.02 mg QE/mg DM, and $PI0–100_{m,OB}$ = 80.45–89.35%); only $TFC60_{m,SO}$ (24.44 mg QE/mg DM) was significantly lower than $TFC60_{m,OB}$ (26.02 mg QE/mg DM);

- the extracts obtained from *O. basilicum* had lower values of *TFC*100 than those prepared from the other plants (discrimination on PC2 between *O. basilicum* samples and the other samples in Fig. 1); the data presented in Fig. 2 confirm this finding, *i.e.*, the mean value of *TFC*100 for *O. basilicum* samples ($TFC100_{m,OB}$ = 0.97 mg QE/mg DM) was significantly lower than $TFC100_{m,SO}$ = 24.40 mg QE/mg DM and $TFC100_{m,CI}$ = 14.85 mg QE/mg DM.

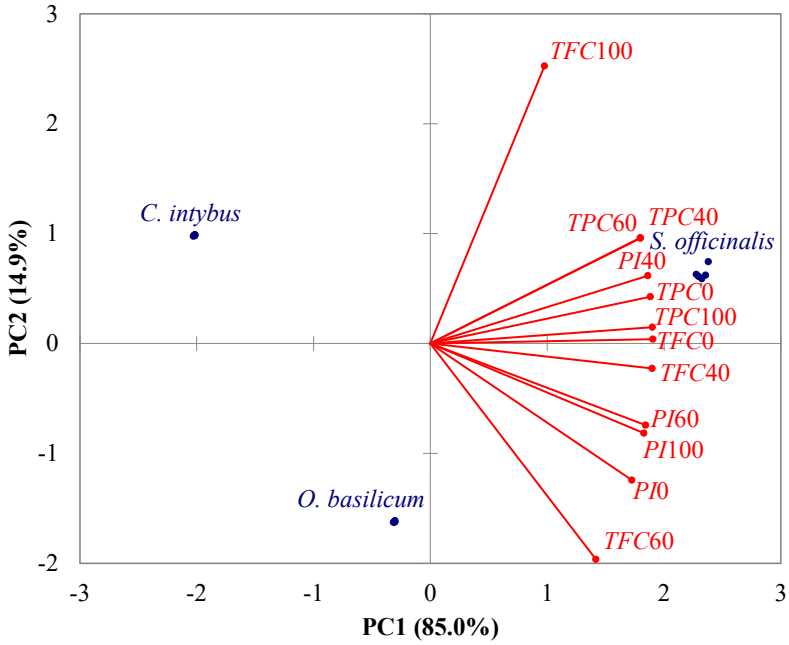
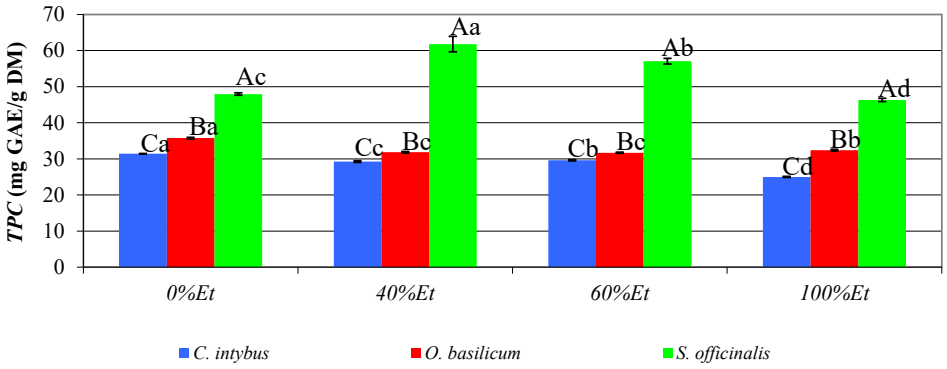
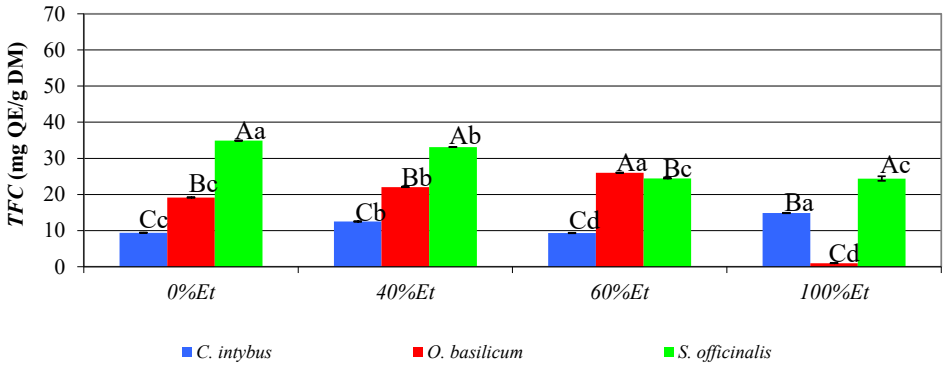


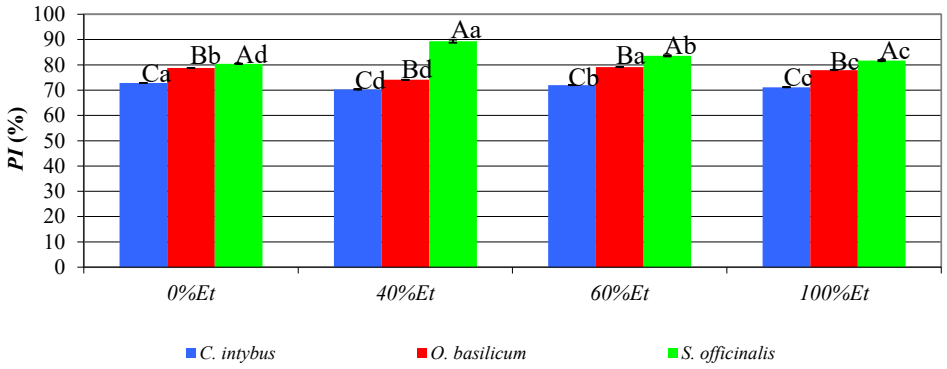
Fig. 1. Projections of variables (*TPC*0–100, *TFC*0–100, and *PI*0–100) and samples (*C. intybus*, *O. basilicum*, and *S. officinalis*) on PC1 and PC2.



(a)



(b)



(c)

Fig. 2. Mean values ± *SD* of total polyphenol content (*TPC*) (a), total flavonoid content (*TFC*) (b), and percentage of inhibition (*PI*) (c) of extracts of edible flowers obtained at different levels of ethanol concentration in a solution consisting of ethanol and methanol (0%, 40%, 60%, and 100%); different uppercase letters indicate significant differences ($p < 0.05$) among flower type for each treatment; different lowercase letters indicate significant differences among treatments for each flower type.

Except for the non-significant correlations between *TFC*60 and *TPC*40, *TFC*60 and *TPC*60, *TFC*100 and *TFC*40, *TFC*100 and *TFC*60, *TFC*100 and *PI*0, *TFC*100 and *PI*60, and *TFC*100 and *PI*100 (-0.191 ≤ r ≤ 0.486), the other correlations between variables were significant (0.525 ≤ r ≤ 0.999) (Table 2).

Table 2

Correlation matrix

Variable	*TPC*0	*TPC*40	*TPC*60	*TPC*100	*TFC*0	*TFC*40	*TFC*60	*TFC*100	*PI*0	*PI*40	*PI*60	*PI*100
*TPC*0	**1**											
*TPC*40	**0.981**	**1**										
*TPC*60	**0.982**	**0.997**	**1**									
*TPC*100	**0.994**	**0.956**	**0.959**	**1**								
*TFC*0	**0.991**	**0.947**	**0.949**	**0.999**	**1**							
*TFC*40	**0.975**	**0.915**	**0.916**	**0.991**	**0.996**	**1**						
*TFC*60	**0.640**	0.486	0.484	**0.709**	**0.735**	**0.793**	**1**					
*TFC*100	**0.631**	**0.763**	**0.764**	**0.556**	**0.525**	0.446	**-0.191**	**1**				
*PI*0	**0.835**	**0.718**	**0.718**	**0.883**	**0.901**	**0.936**	**0.957**	0.103	**1**			
*PI*40	**0.997**	**0.992**	**0.991**	**0.985**	**0.980**	**0.958**	**0.588**	**0.681**	**0.797**	**1**		
*PI*60	**0.921**	**0.831**	**0.831**	**0.953**	**0.964**	**0.984**	**0.888**	0.280	**0.983**	**0.893**	**1**	
*PI*100	**0.910**	**0.815**	**0.816**	**0.944**	**0.957**	**0.979**	**0.900**	0.255	**0.987**	**0.880**	**0.998**	**1**

Values in bold of correlation coefficient (r) are different from 0 with a significance level α = 0.05.

Moreover, the data shown in Fig. 2 highlight the following aspects:

- for *S. officinalis* extract samples, higher values of *TPC* and *PI* were obtained at ethanol concentrations (c_{et}) of 40% (61.67 ± 2.14 mg GAE/mg DM and 89.35 ± 0.63%) and 60% (57.04 ± 0.78 mg GAE/mg DM and 83.56 ± 0.26%) compared to c_{et} = 0% (47.93 ± 0.34 mg GAE/mg DM and 80.45 ± 0.04%) and c_{et} = 100% (46.31 ± 0.47 mg GAE/mg DM and 81.73 ± 0.26%); higher values of *TFC* were obtained at c_{et} = 0% (34.92 ± 0.07 mg QE/mg DM) and c_{et} = 40% (33.13 ± 0.05 mg QE/mg DM) compared to c_{et} = 60% (24.44 ± 0.03 mg QE/mg DM) and c_{et} = 100% (24.40 ± 0.61 mg QE/mg DM); accordingly, operating at c_{et} = 40% is more advantageous;

- for *O. basilicum* extract samples, the highest value of *TPC* was obtained at c_{et} = 0% (35.76 ± 0.20 mg GAE/mg DM) and the highest values of *TFC* and *PI* were obtained at c_{et} = 60% (26.02 ± 0.05 mg QE/mg DM and 79.12 ± 0.02%);

- for *C. intybus* extract samples, higher values of *TPC* were obtained at c_{et} = 0% (31.41 ± 0.06 mg GAE/mg DM), c_{et} = 40% (29.28 ± 0.24 mg GAE/mg DM), and c_{et} = 60% (29.62 ± 0.16 mg GAE/mg DM) compared to c_{et} = 100% (24.98 ± 0.16 mg GAE/mg DM); higher values of *TFC* were obtained at c_{et} = 100% (14.85 ± 0.05 mg QE/mg DM) and c_{et} = 40% (12.51 ± 0.03 mg QE/mg DM) compared to c_{et} = 0% (9.41 ± 0.03 mg QE/mg DM) and c_{et} = 60% (9.32 ± 0.07 mg QE/mg DM);

higher values of *PI* were obtained at c_{et} = 0% (72.81 ± 0.01%) and c_{et} = 60% (71.99 ± 0.01%) compared to c_{et} = 40% (70.28 ± 0.02%) and c_{et} = 100% (71.15 ± 0.01%);

- extract samples prepared from *S. officinalis* generally had significantly higher mean values of *TPC* (46.31–61.77 mg GAE/mg DM), *TFC* (24.40–34.92 mg QE/mg DM), and *PI* (80.45–89.35%) compared to those obtained from *O. basilicum and C. intybus* (only $TFC60_{m,SO}$ = 24.44 mg QE/mg DM was lower than $TFC60_{m,OB}$ = 26.02 mg QE/mg DM); these values are consistent with data reported in the related literature [12,22].

4. Conclusions

Extracts of common sage (*S. officinalis*), chicory (*C. intybus*), and basil (*O. basilicum*) were obtained through maceration, using ethanol, methanol, and two mixtures of ethanol and methanol (containing 40% and 60% ethanol) as extraction solvents. Total polyphenol content (*TPC*), total flavonoid content (*TFC*), and percentage of inhibition (*PI*) of edible flower extracts were determined. The experimental data, which were processed using PCA and one-way ANOVA, indicate the following relevant aspects:

- generally, the extracts obtained from *S. officinalis* had significantly higher mean values of *TPC*0–100 (46.31–61.77 mg GAE/mg DM), *TFC*0–60 (24.44–34.92 mg QE/mg DM), and *PI*0–100 (80.45–89.35%) than those prepared from *C. intybus* (24.98–31.41 mg GAE/mg DM, 9.32–12.51 mg QE/mg DM, and 70.28–72.81%) and *O. basilicum* (31.70–35.76 mg GAE/mg DM, 19.16–26.02 mg QE/mg DM, and 80.45–89.35%);

- the extracts obtained from *O. basilicum* had significantly lower mean values of *TFC*100 (0.97 mg QE/mg DM) than those prepared from *S. officinalis* (24.40 mg QE/mg DM) and *C. intybus* (14.85 mg QE/mg DM);

- the correlations between *TPC*, *TFC*, and *PI* were generally significant ($0.525 \leq r \leq 0.999$).

The extracts obtained could be integrated into cosmetic formulations and/or food products.

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