Research Article

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Evaluation of phenolic compounds and antioxidant activity in some edible flowers

Abstract: Recently, edible flowers (EF) have aroused increased interest because of their aesthetic properties as well as potential health benefits related to the occurrence of some bioactive compounds. Therefore, the aim of this work was to evaluate the total phenolics, anthocyanins, flavonoids, and antioxidant activity (AOA) (following DPPH and ABTS methods) in eleven EF. The samples were subjected to three successive extraction steps using methanol, and these extracts were then analysed for the aforementioned properties using spectrophotometric methods. The obtained extracts were used for the quantification of phenolic composition and AOA. The results indicated that, among the flowers analysed in this study, red rose, pink rose, and red carnation possessed the highest total phenolic contents (27.53, 23.30, and 18.17 mg g^{-1} gallic acid equivalents, respectively), total anthocyanins (3.07, 1.97, and 4.47 mg g^{-1} catechin equivalents [CE], respectively), and AOA (12.07, 15.77, and 12.93 mg g^{-1} TE, respectively, as given by the DPPH method or 8.23, 9.27 and 8.00 mg g^{-1} TE, respectively, as given by the ABTS method). The flowers with highest flavonoids contents were red carnation, Mexican marigold, and pink rose (17.50, 16.90, and 16.57 mg g^{-1} CE, respectively). Cluster analysis grouped the analysed flowers into two groups, those richest in phenolics with AOA and those not so rich. Finally, some important correlations were observed between the total phenolics and the AOA. In conclusion, these flowers could represent a potential source of natural compounds with antioxidant capacity.

1 Introduction

The use of edible flowers (EF) in culinary practices dates back thousands of years. In ancient Romanic and Greek civilizations and also in oriental countries, like China, the tradition linked to the use of EF is because of historic background, and also because they have been using them in food preparation, as aroma enhancers, as flavouring intensifiers, and also for their aesthetic value (Gostin and Waisundara 2019; Vinokur et al. 2006). Later on, in the middle ages some types of aromatic herbs and flowers were grown in gardens, being used as materials for the preparation of infusions or syrups, and also in confectionaries or jams, besides their use as ingredients in food preparations (diversified meals, from soups, to main courses or deserts) (Kaisoon et al. 2011; Mlcek and Rop 2011; Takahashi et al. 2020).

In recent years, we have observed an enlarged interest in using EF in gastronomy, especially among culinary chefs, because of their aesthetic as well as health properties. EF are those that can be safely consumed by humans, i.e. they have no contraindications and therefore can be safely consumed because their components are absorbed when travelling through the intestine without causing toxicity (Felippe 2004; Guiné et al. 2017). Although some flowers can be consumed as a whole, in other cases only some specific portions of the flower are appropriate for human consumption. However, it is important to notice that EF must be organic and free from pesticides. In addition, one must take into consideration that some flowers are safe at appropriate dosages and therefore they can only be consumed in small quantities. So as to prevent possible problems along the digestive tract, it is therefore important to consume EF only in small amounts and preferably without mixing different species when ingesting them for the first time. Nonetheless,

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there are some flowers that can trigger allergic reactions, especially among those who are more sensitive, like people who suffer from hay fever, asthma, or allergies (Chen and Wei 2017a; Cunningham 2015; Kelley et al. 2003; Mlcek and Rop 2011; Pires et al. 2017).

Although the nutritional composition is highly variable according to the type of flower, the most abundant component present in EF is water (variable from 70 to 95% wet basis); however, other macronutrients are also present: carbohydrates (40–90% dry basis), followed by proteins and ash, being low in lipids. In addition, they constitute a source of micronutrients, like vitamins (A, C, B_2, and B_3) and different dietary minerals (K, P, Ca, and Mg are the major components followed by Na, Zn, Mn, and Cu) (Arya et al. 2014; Chen and Wei 2017b; Fernandes et al. 2017; Navarro-González et al. 2014; Petrova et al. 2016a).

Besides nutrition, EF also provide different phytochemical compounds associated with many pharmacological properties. They are a rich source of a wide variety of phenolic compounds, among which flavonoids and organic acids are those most frequently described in the literature. The bioactive compounds present in EF contribute for their positive effect in terms of health benefits, because these compounds have been proven to be associated with antioxidant properties. Besides, EF bioactives may protect against cardiovascular diseases and have beneficial effects against anxiety, cancer, diabetes, and obesity. Other described properties include anti-inflammatory, diuretic, anthelmintic, modulatory of immune response, antimicrobial, and neuroprotective effects (Benvenuti et al. 2016; Chen et al. 2015; Kaisoon et al. 2011; Loizzo et al. 2016; Petrova et al. 2016b; Zare 2019).

There is a lack of information about phenolic composition and antioxidant capacity of Portuguese EF. Therefore, the objective of this work was to analyse some EF to assess their contents in terms of phenolic compounds, namely total phenolics, anthocyanins, and flavonoids, as well as their antioxidant activity (AOA), for being properties with interest from attending to their positive effects on human health. It is expected that the results provide important information about composition of the analysed EF.

2 Materials and methods

2.1 Samples

The flowers used in the development of this study are of edible quality and have been produced in organic farming, i.e. without the use of pesticides, fertilizers, or other blooming intensifiers. They were obtained from a farm in Viseu, collected on the same day that they were transported to the laboratory. They were stored in the refrigerator (at 6–8°C) in closed plastic cuvettes, until the extraction process occurred, 1 day after harvest.

Eleven flowers were used in this study, varying the species and/or colour, as shown in Table 1 and Figure 1.

2.2 Extraction conditions

The quantification of total phenolic compounds (TPC) as well as AOA was made on the extracts that were obtained using methanol as extraction solvent, following an adaptation of the method proposed by Guiné et al. (2015). In this way, the extraction for each of the EF was obtained from a sample of 10 g taken form the grounded and homogenized flower mass. The same mass was used to make three successive extractions, each one lasting 60 min, using a magnetic stirrer. Each of the extracts was ordered: first (E1), second (E2), and third (E3). The obtained extracts were used for the quantification of phenolic composition and AOA. Figure 2 presents a schematic flowchart of the experimental procedure, for easier interpretation.

2.3 Analysis of phenolic compounds

2.3.1 Total phenols

The TPC were evaluated using the Folin–Ciocalteu method (Gonçalves et al. 2012). In this way, 0.125 mL

Table 1: Characteristics and codes of the flowers used in the study

Common name	Scientific name	Colour	Code
Lavender	*Lavandula spica* L.	Lilac	Lav_Li
Carnation	*Dianthus caryophyllus* L.	Red	Car_Re
Sweet William	*Dianthus barbatus* L.	Red/white	SW_ReWh
Sweet William	*Dianthus barbatus* L.	Red	SW_Re
Mexican marigold	*Tagetes erecta* L.	Orange	MM_Or
Blue plumbago	*Plumbago auriculata* Lam.	Blue	BP_Bl
Orchid	*Orchis* L.	Pink	Orc_Pi
Orchid	*Orchis* L.	Yellow	Orc_Ye
Rose	*Rosa* L.	Pink	Ros_Pi
Rose	*Rosa* L.	Red	Ros_Re
Rose	*Rosa* L.	Orange	Ros_Or

Figure 1: Photos of the flowers used in the study.

was taken from each extract and added to 0.75 mL of deionized water, along with the Folin–Ciocalteu reagent (0.125 mL). This mixture was left for 6 min, and after that 2 mL of sodium carbonate solution was added, in a concentration of 5% (m/v). This was then placed undisturbed in the absence of light for 1½ h at room temperature. The absorbance of the resulting solution was measured in a spectrophotometer at 760 nm versus prepared blanks. For the calibration, standard solutions of gallic acid were prepared. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh sample. Analyses were realized in triplicate.

2.3.2 Total flavonoids

Total flavonoid content was determined according to a colorimetric assay (Zhishen et al. 1999). One millilitre of each extract was added to 4 mL of water and 0.3 mL of sodium nitrite solution (5% concentration). Then we

waited for 5 min to add 0.3 mL of aluminium chloride (10% concentration), and after another 6 min, added 2 mL of sodium hydroxide (concentration 1 M). Immediately after that, the final volume was completed with water until reaching 10 mL and mixed. Absorbance of the resulting solution was determined at 510 nm against a prepared blank solution. For the calibration, standard solutions of catechin were prepared. The results were expressed as milligrams of catechin equivalents (CE) per gram of fresh sample. Analyses were performed in triplicate.

2.3.3 Total anthocyanins

Total anthocyanins (TA) were measured using the sulphur dioxide bleaching method (Boulton, 2001). A sample of each extract (1 mL) was added to equal volume of ethanol, which was previously acidified by a diluted solution of hydrochloric acid. Then, 2 mL of this solution was
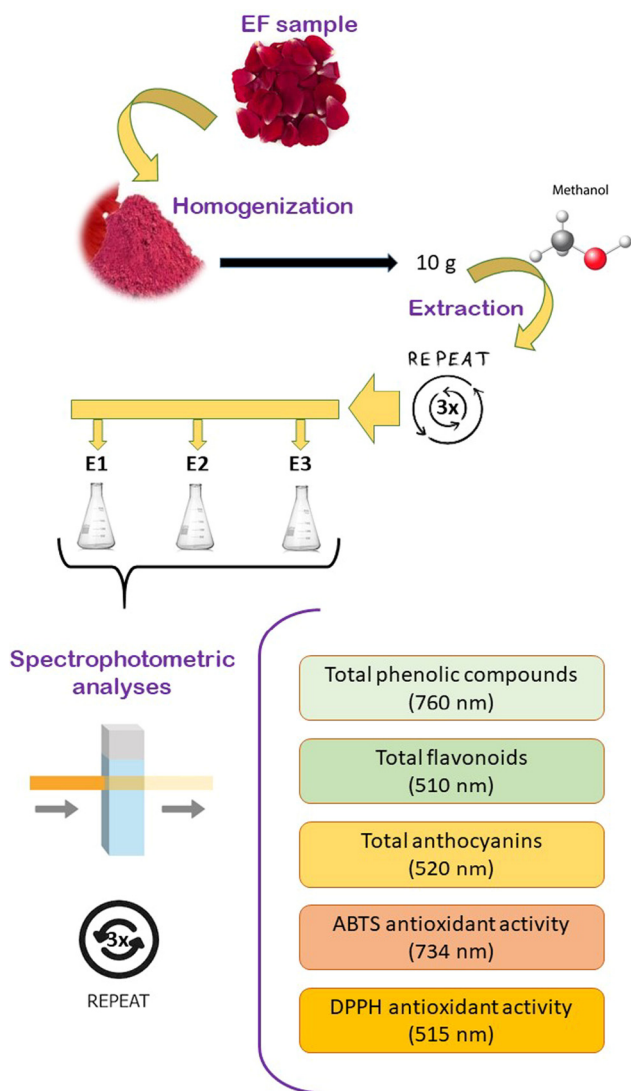
Figure 2: Schematic flowchart of the experimental procedure.

For ABTS method, 1 mL of ABTS radical solution was mixed with ethanol until a final volume of 80 mL was obtained. The initial absorbance was registered and was found to be about 0.700. Then a tube was used to mix 2 mL of ABTS+ solution with 0.1 mL of the extract obtained from each EF. After stirring, the tube was left to stand for a period of 15 min in the dark, after which the absorbance was measured at a wavelength of 734 nm (Santos et al. 2014).

For DPPH, the radical DPPH˙ in a methanol solution $(6 \times 10^{-5}\,\text{mol/L})$ was used, always freshly prepared and kept in the dark. The stability of the radical was ensured by measuring the absorbance throughout the time of analysis. Initial absorbance was found to be near 0.700 for all cases. To prepare the samples for analyses, 0.1 mL of each EF extract was mixed with 2.0 mL of DPPH˙ solution.

This was left to stand for 30 min protected from light, and then the absorbance was measured at 515 nm (Brand-Williams et al. 1995).

In both cases, the percentage of inhibition was calculated according to the equation: % Inhibition = $(1 - A_f/A_0) \times 100$, where A_0 is the absorbance of the blank essay at initial time and A_f is the absorbance measured at the end of reaction. The calibration was achieved through points of experimental measurements made with the standard Trolox. The results were expressed as milligram per gram of Trolox equivalents (TE) per gram of fresh sample.

All evaluations for AOA were replicated thrice for each of the extracts analysed.

added to 0.8 mL of water in one tube (t1), whereas to the other tube (t2) were added 2 mL of the solution and 0.4 mL of sodium hydrogen sulphite solution (at a concentration of 15% w/v). After keeping for 20 min in the dark and at ambient temperature, the absorbance was measured at 520 nm. To calculate the TA, this formula was used: TA = $875 \times (\text{abs}_{t1} - \text{abs}_{t2})$. The final obtained results were expressed as malvidin equivalents (Mv3GlcE). The essays were performed in triplicate.

2.4 Analysis of AOA

To evaluate the AOA, the radicals DPPH (2,2-diphenyil-picrylhydrazyl) and ABTS+ (2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid)) were used.

2.5 Statistical analysis

So as to confirm the results obtained for the mean values calculated from the different replicas, a comparison of means was undertaken by performing analysis of variance (ANOVA) coupled with *post-hoc* Tukey test to identify where the differences are located.

Bivariate correlation analysis was also performed, and the Pearson correlation coefficients were used to assess the strength of the correlations between the properties studied. The reference absolute values considered were: $r = 0$ – no correlation, $r \in\,]0.0,\, 0.2[$ – very weak correlation, $r \in [0.2,\, 0.4[$ – weak correlation, $r \in [0.4,\, 0.6[$ – moderate correlation, $r \in [0.6,\, 0.8[$ – strong correlation, $r \in [0.8,\, 1.0[$ – very strong correlation, and $r = 1$ – perfect correlation (Maroco 2012; Pestana and Gageiro 2014).

Factor analysis (FA) based on principal component analysis (PCA) was undertaken. Before this, the data were evaluated to assess if it were suitable for this type of analysis. To fix the number of components, the Kaiser criterion was used; therefore, the eigenvalues greater than or equal to one was retained. The communalities were calculated to evidence the percentage of variance explained by the factors extracted (Broen et al. 2015).

The cluster analysis was performed through two types of hierarchical methods both based on the measurement of Euclidean distance: a) Average linkage between groups; b) Average linkage within groups.

The software SPSS from IBM Inc. (version 26) was used to perform the data analysis, with a level of significance of 5%.

Table 2: Phenolic compounds and AOA of the flowers analysed

Flower	Total phenols[1] (mg g^{-1})	Total flavonoids[1] (mg g^{-1})	TA[1] (mg g^{-1})	DPPH[1] (mg g^{-1})	ABTS[1] (mg g^{-1})
Lav_Li	7.93^c	3.37^c	0.40^a	10.10^f	4.83^d
Car_Re	18.17^d	17.50^i	4.47^f	12.93^i	8.00^h
SW_ReWh	5.10^b	7.50^e	0.33^a	5.57^d	2.57^c
SW_Re	1.87^a	2.57^b	0.30^a	1.53^a	1.33^{ab}
MM_Or	17.47^d	16.90^h	0.90^c	8.47^e	5.87^e
BP_Bl	18.27^d	3.63^c	0.60^b	11.20^g	7.00^f
Orc_Pi	4.87^b	2.63^b	0.30^a	4.37^c	1.57^b
Orc_Ye	3.57^{ab}	2.20^a	0.30^a	3.57^b	1.10^a
Ros_Pi	23.30^e	16.57^g	1.97^d	15.77^j	9.27^i
Ros_Re	27.53^f	6.00^c	3.07^e	12.07^h	8.23^h
Ros_Or	17.60^d	7.73^f	0.40^a	10.73^g	7.50^g

[1]Values in the same column with the same letter are not statistically significant (Tukey test, $p < 0.05$).

3 Results and discussion

The general phenolic composition and AOA of the flowers used in this work are presented in Table 2. The total phenolic content differed among the different types of plants, being in accordance with Kaissoon (2011). The amount of total phenols for each flower was obtained by the sum of their content in the three extracts. A large range of values was obtained ranging from 1.87 for red sweet William (SW_RE) to 27.53 mg g^{-1} GAE for red rose (ROS_Re). There were significant differences amongst the different varieties of flowers. In general, roses (Ros) presented higher amounts of TPC (17.60–27.53 mg g^{-1} GAE), followed by blue plumbago (BP_Bl), carnation (Car_Re), and Mexican marigold (MM_Or). Despite the different varieties of flowers and extraction conditions, these values can be considered slightly higher than those reported by Li et al. (2014) for 51 edible and wild flowers, whereas the total phenolic contents varied from 0.13 to 11.48 mg GAE g^{-1}.

Flavonoids are a diverse and widespread group of natural compounds, and probably the most important natural phenolics (Prasad et al., 2009). Carnation (17.50 mg g^{-1} of CE), Mexican marigold (16.90 mg g^{-1} of CE), and pink rose (16.57 mg g^{-1} of CE) presented the highest values of total flavonoids from the flowers analysed, with differences statistically significant. Orange and red rose contained 7.73 and 6.00 mg g^{-1} CE, respectively, and blue plumbago 3.63 00 mg g^{-1} CE of total flavonoids. These results showed a higher contribution of flavonoids to TPC in case of carnation and Mexican marigold varieties in opposition with blue plumbago and red rose.

Regarding anthocyanins, they play an important role in flowers, not only for the colour but also for the antioxidant power. Carnation presented the highest value of anthocyanins, 4.47 mg g^{-1} Mv3GlcE, which is 15-fold greater than the anthocyanins' content in the majority of the other studied flowers. Red rose contained 3.07 mg g^{-1} Mv3GlcE, the second highest value. Benvenuti et al. (2016) described the red colour as a good indicator of the anthocyanins content. However, sweet William flowers also presented a red colour, but exhibited a lower amount of these pigments. The composition of products of vegetable origin is highly variable, not only among species but also according to climate or cultivation conditions (Msukwa et al. 2019).

The values of antioxidant capacity determined by DPPH and ABTS methodologies are presented in Table 2. Trolox was used to express the results as milligram per gram of fresh weight. As seen in Table 2, the absolute values obtained by DPPH assay were higher than those obtained by ABTS, for all the flowers analysed. Despite the difference in ranking of antioxidant values, in general the same tendency was observed for both methodologies. The differences in the antioxidant activity are a consequence of the different reactivities that the phenolic compounds present according to the method applied (Wang et al. 2004). ABTS$^+$ and DPPH$^·$ radicals have a different stereochemical structure, because they are generated from different mechanismsms. Therefore, when they react with the antioxidants, they originate a different response to the inactivation of the radical.

Anthocyanins are pigments abundantly present in nature, bearing very important roles in the ecophysiology of plants and in basic plant processes such as
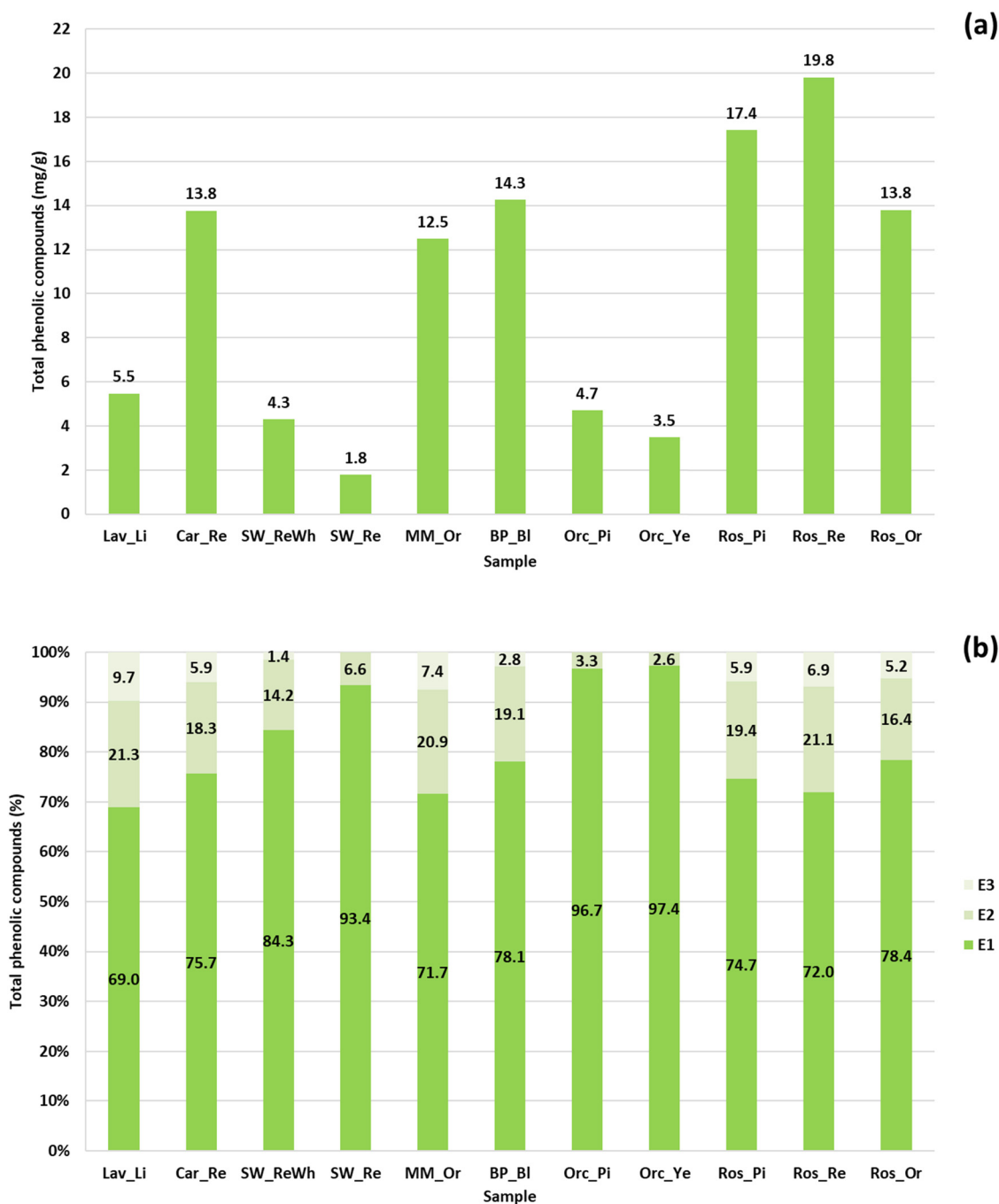
Figure 3: TPC in the first extraction (a) and relative percentage of three extractions (b).

propagation and defence mechanisms against external stress factors. These chemical structures are responsible for a variety of colours in fruits, vegetables, and flowers (Kusmawati and Kusumaningrum 2019). Pink rose was the flower with the highest number of antioxidants, 15.77 mg g^{-1} TE for DPPH and 9.27 mg g^{-1} TE for ABTS. Red carnation and red rose also exhibited high antioxidant power (around 12–13 mg g^{-1} TE for DPPH method

and about 8 mg g^{-1} TE for ABTS method). These flowers are rich in total phenols and anthocyanins, which contributes for their high AOA, because the AOA has been reported to be associated with total phenols (Kaisoon et al. 2011) and anthocyanins content (Benvenuti et al. 2016) in various plants. Marković et al. (2017) described different antiradical activities of monomeric pigments, where delphinidin and pelargonidin showed to be more efficient
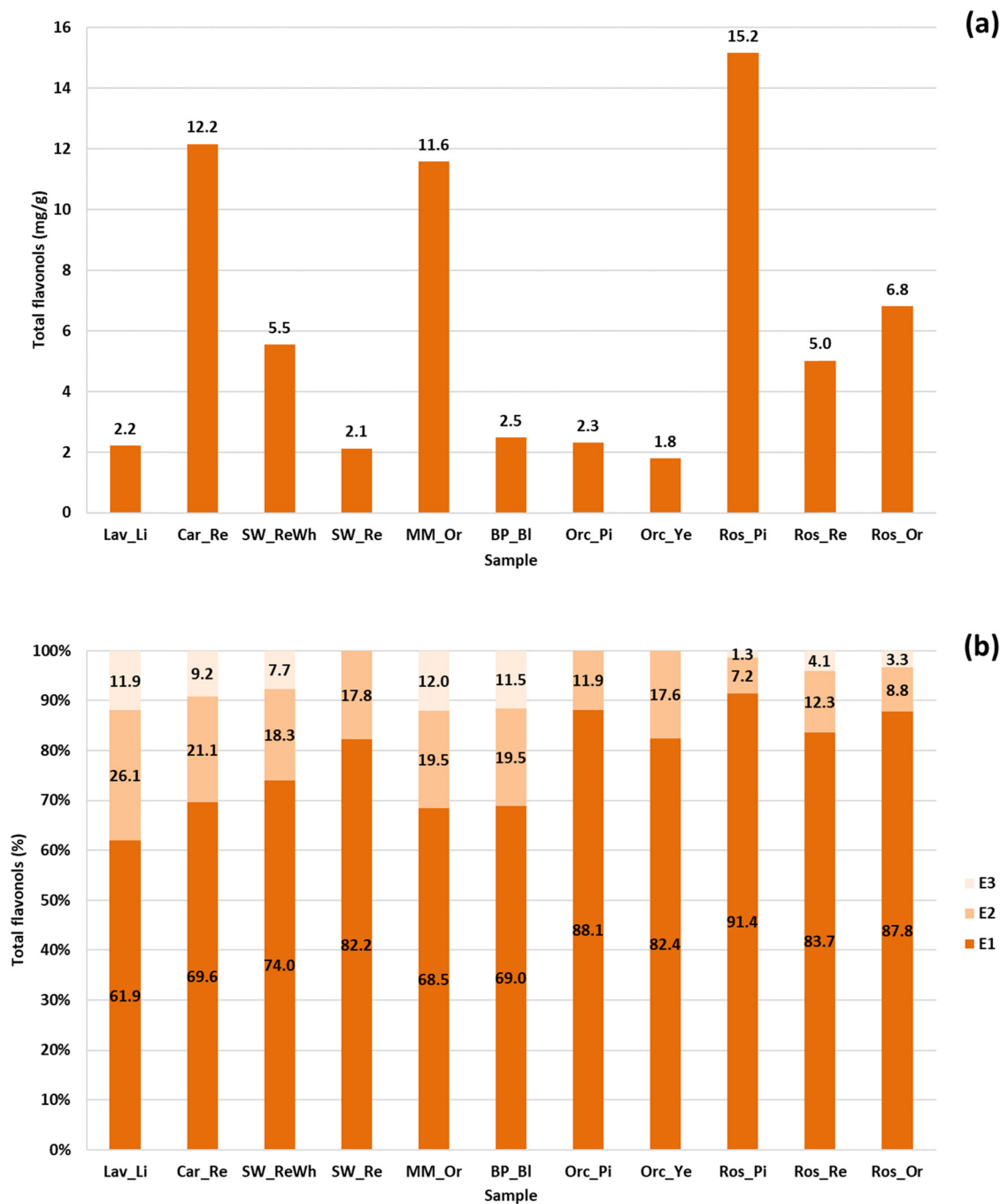
Figure 4: Total flavonoids in the first extraction (a) and relative percentage of three extractions (b).

than malvin. However, some species with a high AOA are not characterized by a high anthocyanins' content, and consequently, in those cases, the antioxidant power is derived also from the other compounds present. Sweet William flowers with red or coloration, usually associated with high AOA, possessed, nevertheless, a low antioxidant value. In addition, differences were observed for species analysed, which is in accordance with Oliveira et al. (2018). The values of AOA obtained by ABTS assay were similar to those obtained by Li et al. (2014) for Chinese flowers (0.4–25 mg g^{-1}). These values were similar to those obtained by Lae et al. (2019) for methanolic and aqueous extracts of stem barks of *Phyllanthus albizzioides*, 23.89 and 20.05 mg g^{-1} GAE, respectively. Pejin
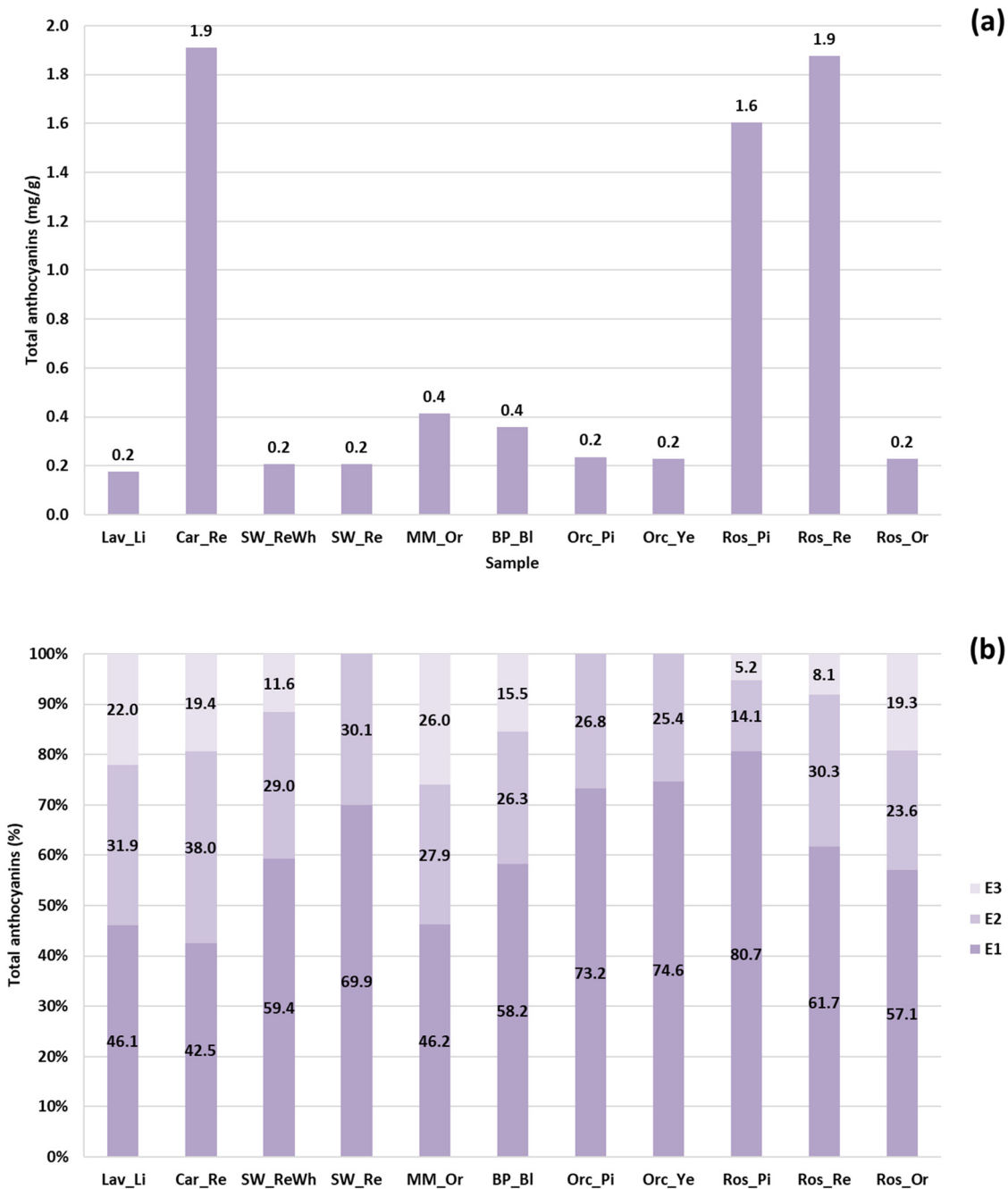
Figure 5: TA in the first extraction (a) and relative percentage of three extractions (b).

et al. (2012) reported the values of ABTS AOA for a related matrix (the moss *Bryum moravicum*) of $84.56 \, \mu g \, g^{-1}$ expressed as ascorbic acid.

The value of TPC in the first extraction, expressed as milligram per gram GAE, and the relative percentage in the three extractions are shown in Figure 3. The flowers with highest value in the first extraction were red rose, pink rose, blue plumbago, orange rose, carnation, and Mexican marigold, containing between 19.8 and $12.5 \, mg \, g^{-1}$

GAE of phenolic compounds in first extract, which represented 71.7–78.4% of the total amount recovered. The second extract comprised, for this set of flowers, 16.4–21.1% of the total phenolics recovered.

Red sweet William (SW_Re) and pink or yellow orchids (Orc_Pi or Orc_Ye) are flowers with a low phenolic content profile. In these cases, no measurable phenolic compounds were obtained in the third extract, which indicates that two extractions were enough for
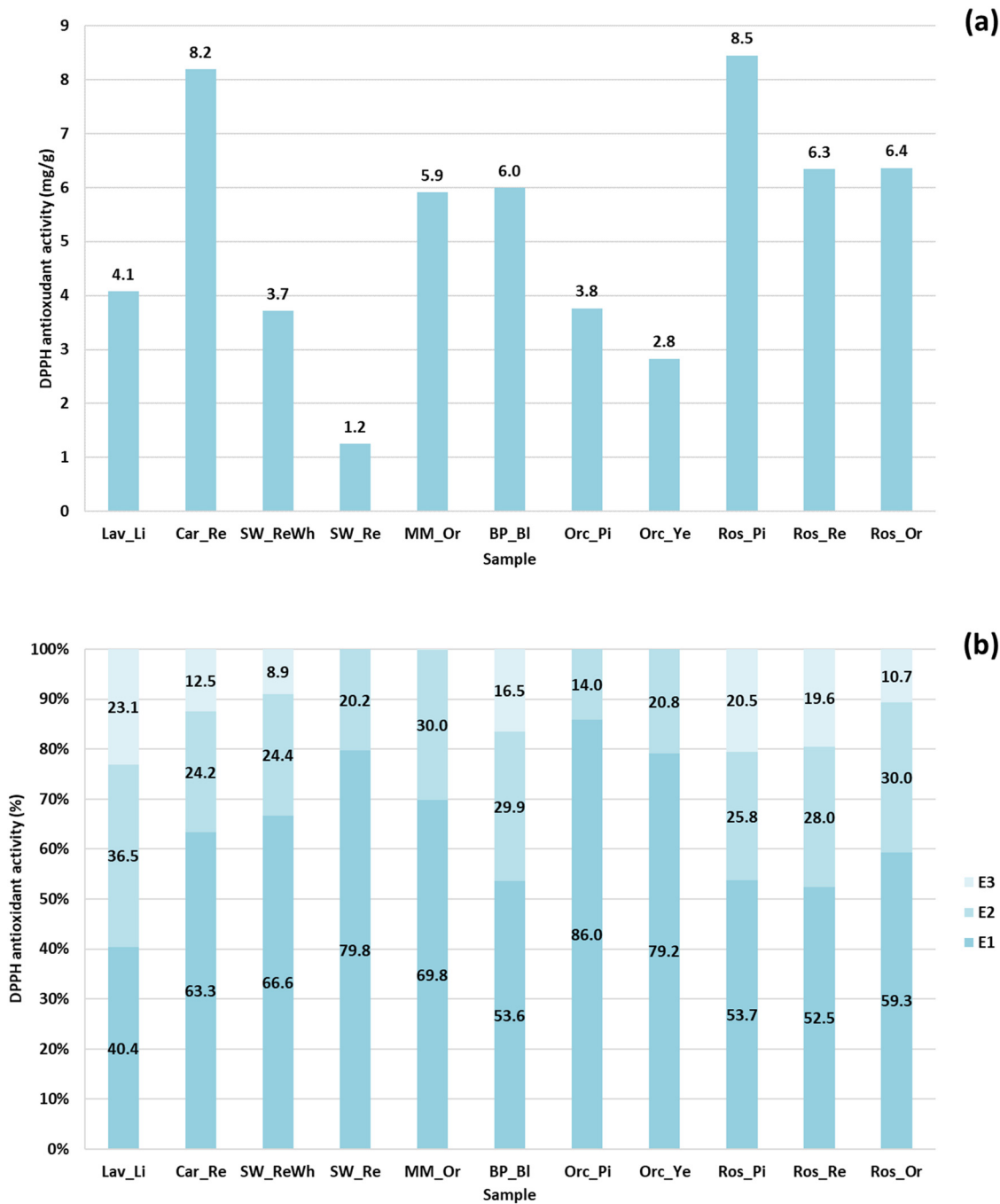
Figure 6: DPPH AOA in the first extraction (a) and relative percentage of three extractions (b).

phenolic extraction. The first extraction recovered 93.4–97.4% of the total quantified.

In case of red sweet William (SW_Re) and pink or yellow orchids (Orc_Pi or Orc_Ye), which are flowers with a low phenolic content profile, the first extraction recovered 93.4–97.4% of the total quantified, whereas no measurable phenolic compounds were obtained in the third extract. This indicates that, in case of flowers poor

in phenolic compounds, the third extraction would be unnecessary.

Figure 4 shows the total flavonoids in the first extraction, expressed as milligram per gram CE, and relative percentage obtained in each one of the three extractions. The first extracts of pink rose (15.2 mg g^{-1} of CatE), carnation (12.2 mg g^{-1} of CatE), and Mexican marigold (11.6 mg g^{-1} of CatE) were rich in flavonoids. The relative percentage of
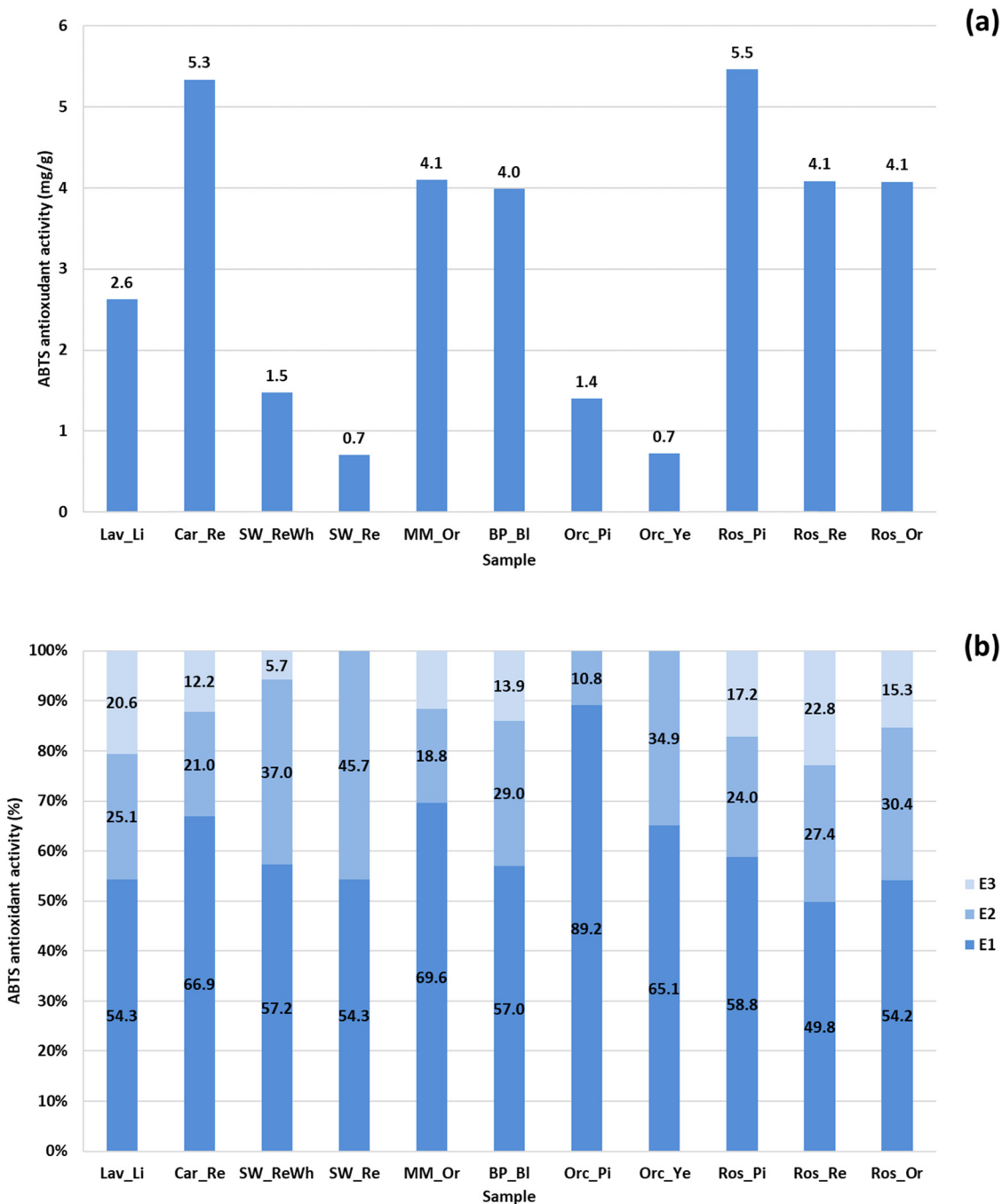
Figure 7: ABTS AOA in the first extraction (a) and relative percentage of three extractions (b).

flavonoids recovered in the first extract for the whole set of flowers analysed (61.9–91.4%) was lower when compared with TPC, with just the exception of pink rose. This may indicate a different kinetic extraction of flavonoids and non-flavonoid compounds. As observed for phenolic compounds, no flavonoids were quantified in the third extract

for red sweet William (SW_Re), pink orchid (Orc_Pi), or yellow orchid (Orc_Ye),

The TA in the first extraction and relative percentage recovered in each one of the three extracts can be observed in Figure 5. The first extracts of carnation (Car_Re) and red rose (Ros_Re) presented 1.9 mg g^{-1} Mv3GlcE, whereas pink

rose (Ros_Pi) contained 1.6 mg g^{-1} Mv3GlcE, being these three flowers those with the highest anthocyanins' contents, way different from all other evaluated. However, the relative percentage of anthocyanins in the different extracts for these three flowers was quite different. For pink rose 80.7% of anthocyanins were obtained in the first extract, higher percentage compared to red rose (61.7%) and carnation (42.5%). The second extract contained 38.0% of TA for carnation, 30.3% for red rose, and 14.1% for pink rose. In the case of carnation, the third extract contained 0.9 mg g^{-1} of anthocyanins, which represented 19.4% of the total amount quantified. Comparing with TPC and total flavonoids, a lower relative percentage of anthocyanins present in the first extract was observed.

The AOA obtained by DPPH assay in the first extraction, expressed in milligram per gram TE, and the relative percentage in each extract is described in Figure 6. Pink rose presented the highest value in the first extract, reaching 8.5 mg g^{-1} TE, which represented 53.7% of the total quantified. The first extract of carnation exhibited 8.2 mg g^{-1} TE, being the second highest in value, representing 63.3% of the total. Red sweet William (SW_Re), pink orchid (Orc_Pi), or yellow orchid (Orc_Ye), although presenting the lowest values of AOA (1.2–3.8 mg g^{-1} TE), had higher relative amount of AOA in first extract (79.8–86.0%) compared with other flowers (40.4–69.8%). This indicates that for these flowers the AOA was quantified essentially in the first extract, but the final values were still very low.

The AOA obtained by ABTS assay in the first extraction, expressed as milligram per gram TE, and relative percentage of each extract are presented in Figure 7. The first extract of pink rose and carnation contained 5.5 and 5.3 mg g^{-1} TE, respectively. For the analysed flowers, the antioxidant compounds evaluated by this method were preferentially recovered in the first extracts, ranging from 54.2% in case of orange rose to 89.2% in pink orchid.

A factor analysis was undertaken by PCA, because the data were found suitable. The correlation matrix between the variables was analysed and allowed concluding that all values were higher than 0.4. The Kaiser–Meyer–Olkin measure of adequacy of the sample (KMO) and the Bartlett's test were used to verify the intercorrelation between variables (Broen et al. 2015). The value of KMO was acceptable (0.748) and the results of the Bartlett's test indicated adequacy for applying FA because the p-value was significant ($p < 0.0005$), thus leading to the rejection of the null hypothesis that the correlation matrix was equal to the identity matrix. In addition, analysis of the anti-image matrix showed that all values of measure of sampling adequacy (MSA) were over 0.5, confirming that all variables were adequate to include in the analysis (values of MSA for the variables: TPC = 0.741, flavonols = 0.855, anthocyanins = 0.881, DPPH = 0.737, ABTS = 0.657).

The solution obtained by FA with PCA produced only one component, based on the Keiser criterion to consider eigenvalues greater than 1; this solution includes all variables and explains 78% of variance. Because the solution produced only one factor, this could not be rotated. Antioxidant variables have the largest fraction of variance explained by the solution (96.4% for ABTS and 93.9% for DPPH), followed closely by TPC (93.0%), and in the last came anthocyanins and flavonols, but still with high percentages of variance explained (79.1% and 76.4%, respectively). These results confirm that all the measured properties are strongly interconnected.

Cluster analysis was performed for the 11 varieties of flowers, considering the different properties measured (TPC flavonols, anthocyanins, DPPH, and ANTS). The same grouping solution was obtained with methods average linkage between groups and average linkage within group, and so this was considered and presented in Figure 8. The dendogram in Figure 8 shows the two clusters: cluster 1 corresponds to the samples with lower phenolic compounds and AOA (two orchids, two sweet Williams, and lavender), whereas cluster 2 includes the flowers with higher phenolic compounds and AOA (three roses, carnation, Mexican marigold, and blue plumbago). These results are in agreement with previous observations regarding the amounts of phenolic compounds and AOA reported in the analysed flowers (Table 2 and Figures 3–7).

Results of bivariate correlation analysis for Pearson correlation coefficients are presented in Table 3. The weakest correlation found was between TPC and flavonoids ($r = 0.570$), but still it can be considered moderate. The highest value was obtained for the correlation between DPPH and ABTS AOA ($r = 0.966$, correlation significant at 1% level), which is expected, because these two values measure the same property although according to different methods. In addition, very strong correlations were found between TPC and both measurements of AOA ($r = 0.883$ for DPPH and $r = 0.948$ for ABTS), which indicates clearly that the TPC contribute in a high extent for the AOA of the flowers analysed. On the contrary, the correlations between flavonols and anthocyanins are strong, but lower, meaning that these families of compounds also contribute for the AOA of the flowers, although in a lower extent.
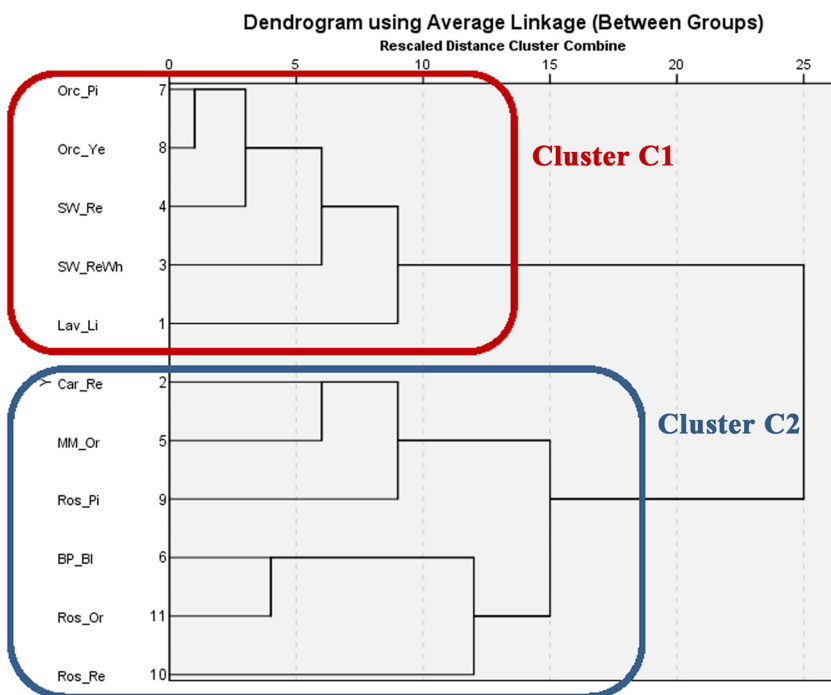
Figure 8: Dendogram obtained through cluster analysis to the flowers.

Table 3: Results of bivariate correlation analysis for Pearson correlation coefficients

Properties	TPC	FLAV	ANT	DPPH	ABTS
TPC	1.000	0.570	0.650[a]	0.883[b]	0.948[b]
FLAV		1.000	0.617[a]	0.614[a]	0.640[a]
ANT			1.000	0.628[a]	0.644[a]
DPPH				1.000	0.966[b]
ABTS					1.000

[a] Correlation significance at 0.05 level. [b] Correlation significance at 0.001 level.

4 Conclusions

In this study, phenolic composition and antioxidant capacity of 11 EF were evaluated. Methanol showed to be an efficient solvent for the extraction of phenolic and antioxidant compounds. This work allowed to obtain new information about the composition of Portuguese EF. The results obtained varied according to the variety analysed. Pink rose, red carnation, and red rose possessed the highest total phenolic contents, TA, and antioxidant power among the flowers analysed in this study. These EF possessed antioxidants as other plants described in literature. As for the content in flavonoids, the richest flowers were red carnation, pink rose, and

Mexican marigold. The first extract accounted for the great majority of TPC, namely flavonoids. These extracts were also accounting for a great part of the anthocyanins or AOA, but to a lesser extent. Cluster analysis allowed classifying the sample into two groups, the first corresponding to flowers with lower phenolic compounds and AOA and the second including the flowers richer in phenolics and antioxidants. Finally, results of bivariate correlation analysis showed particularly strong correlations between TPC and AOA. The obtained results confirm that these flowers could be potential source of natural compounds with antioxidant capacity that could be used as functional foods. This study provided new information on the properties of these flowers grown in Portugal.

Conflicts of interest: The authors declare no conflicts of interest.

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