Trends Phytochem. Res. 3(4) 2019 251-260

Trends in Phytochemical Research (TPR)

Journal Homepage: http://tpr.iau-shahrood.ac.ir

Original Research Article

Health attributes of ten Mediterranean edible flowers: anti-proliferative and enzyme-inhibitory properties

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ABSTRACT

Edible flowers represent an important source of biologically active compounds with positive effects on consumer health. In fact, they contain high quantities of natural antioxidants and have been used since ancient times not only to prepare traditional recipes but also as a remedy against some common illnesses. In the current report, the extracts of ten Mediterranean edible flowers were analyzed to determine their relevant vitamin C, chlorophylls, carotenoids, proanthocyanidins, flavonoids, anthocyanins and total phenolics contents. They were also assayed *in vitro* for inhibitory effects on digestive enzymes relevant to carbohydrates metabolism and for anti-proliferative activity on CaCo2 colon cancer cell line. Accordingly, the extracts inhibited the α-glucosidase activity in a dose-dependent manner. In addition, half-maximal inhibitory concentration (IC_{50}) ranged from 12.5 ± 1.9 to 132 ± 3.2 µg/mL, while for the anti-proliferative activity IC_{50} values varied from 24.26 ± 4.5 to 106.3 ± 5.7 µg/mL. In view of the obtained results, the use of these flowers provides important functional ingredients as well as contributing to the realization of delicate and more appealing flavor recipes.

ARTICLE HISTORY

Received: 03 August 2019
Revised: 29 November 2019
Accepted: 29 November 2019
ePublished: 14 December 2019

KEYWORDS

Anti-proliferative
Anti-α-glucosidase
Edible flowers
Polyphenols

1. Introduction

Chefs utilize edible flowers for the preparation of salads, soups, cakes and drinks (Fernandes et al., 2017), particularly to add a fresh and delicate flavor, rendering their recipes more appetizing or simply to decorate their dishes. Edible flowers have been consumed since ancient times and there is a growing interest for their potential use not only for the aesthetic value added to the recipes but also for their healthy properties. In fact, their intense pigmentation, evolved to attract pollinators (Stintzing and Carle, 2004), corresponds to a significant antioxidant activity that is relevant to human health. The increasing interest in edible flowers has intensified the research on their nutritional value and subsequent biological activities (Loizzo et al., 2016). Scientific research on plants recognized for their medicinal value is a useful strategy for the discovery of novel therapeutic applications. Several studies have revealed that vegetable products might be used in the improvement of some disorders. For example, the active compound of the well-known antidiabetic drug metformin, derives from the *Galega officinalis* plant. Additional examples are the analgesic morphine of *Papaver somniferum*, the anti-inflammatory aspirin of *Salix alba* and the antimalarial drug, the quinine of *Cinchona succirubra*. A large number of new secondary metabolites, belonging to different classes of natural products possessing interesting biological activities, from the anti-proliferative to the anti-inflammatory, to the neuroprotective, have been found in plants from Lamiaceae and Asteraceae families. These families comprise a multitude of widespread flowering plants. Phytochemical research may either validate the ancient folkloric medicinal practices by giving a scientific rationale or even to reveal new uses by assessing their biological activities in both *in vitro* and *in vivo* studies (Mohammadhosseini, 2017; Mohammadhosseini et al., 2017; Venditti et al., 2018). Flowers contain natural antioxidants including

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flavonoids, anthocyanins, and other phenolic compounds, which are responsible for their biological activities as well as their color. Phenolic compounds have been related to the prevention of some chronic diseases, such as diabetes, cardiovascular disease and different types of cancer (Tomé-Carneiro and Visioli, 2016; Ombra et al., 2019). Some substances may act as anti-diabetic agents controlling carbohydrate metabolism by various mechanisms. Compounds that inhibit alpha-amylase and alpha-glucosidase were found to ameliorate diabetes (Adisakwattana, 2017; Ombra et al., 2018). Amylase breaks down large insoluble starch molecules into absorbable molecules. This enzyme is found in pancreatic juice and saliva. On the other hand, α-glucosidase, present in the mucosal brush border of the small intestine, catalyzes the end step of digestion of starch and disaccharides that are abundant in the human diet. α-Glucosidase is a key enzyme for the metabolism of di-saccharides into the diabetogenic mono-saccharides; α-amylase and α-glucosidase inhibitors could be used to reduce the postprandial hyperglycemia levels (Tanaka et al., 2009). To date, several inhibitors of the carbohydrate-hydrolyzing enzymes have been isolated from plants to serve as an alternative drug, with increased potency and fewer adverse effects than existing synthetic drugs, such as acarbose. Moreover, the edible flowers could have an interesting application as natural colorants, representing an alternative to the use of synthetic dyes in foods (Díaz-García et al., 2015). Replacement of artificial dyes with natural coloring agents is one of the crucial interests of the food industries to satisfy actual consumption trends. For instance, some yogurt formulations require specific additives, as represented by dyes and the use of natural colorants, which are generally considered safer than the artificial ones, may be recommended (Pires et al., 2018). Anthocyanins water-soluble pigments extracted from plants, giving the blue, purple and red color of many plant tissues are authorized food colorants. These phenolic compounds are widely found in fruits (especially berries), as well as in flowers and leaves. Several typical recipes of the Mediterranean diet are based on the use of plant species; among these, we have chosen 10 varieties with more common edible flowers. Studies are available in the literature about the composition and bioactivities of edible flowers. However, more reports related to edible flowers usage are needed if they are considered as functional foods. Regarding the selected edible flowers, the phenolic composition has been analyzed (Kee et al., 2013; Miguel et al., 2016), but potential benefits to human health have been studied to a much lesser extent. The main goal of the present study was to evaluate the bioactive potential of ten variously colored edible flowers, namely *Calendula officinalis* L., *Cichorium intybus* L., *Malvus sylvestris* L., *Punica granatum* L., *Tropaeolum majus* L., *Origanum vulgare* L., *Rosmarinus officinalis* L., *Viola tricolor* L., *Cucurbita maxima* L. and *Salvia elegans* Vahl. In particular, the aims of the present study were: (a) to quantize the phytochemical content of eight biochemical parameters involving total phenols, flavonoids, carotenoids, anthocyanins, proanthocyanidins, chlorophyll a, chlorophyll b, and vitamin C; (b) to evaluate their potential enzyme inhibitory activity and (c) to test their *in vitro* anti-proliferative activity.

2. Experimental

2.1. Flower material and preparation of the extracts

Ten commonly consumed flowers of Mediterranean diet were used in the present study: *Calendula officinalis* L.; *Cichorium intybus* L.; *Malvus sylvestris* L.; *Punica granatum* L.; *Tropaeolum majus* L.; *Origanum vulgare* L.; *Rosmarinus officinalis* L.; *Viola tricolor* L.; *Cucurbita maxima* L. and *Salvia elegans* L. see (Fig. 1). The flowers were obtained from a private garden in

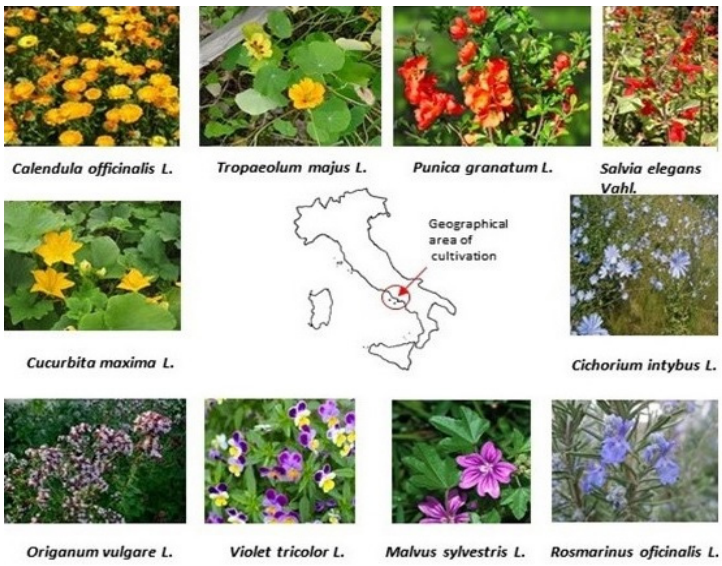

Fig. 1. Photos and scientific names of the plants and the geographical map of the sampling area.

province of Naples (Italy), where the crops were not treated with pesticides. They were hand-picked early in the morning, during spring or summer 2018. Their names and general characteristics are listed in Table 1. Flowers were cleaned by using distilled water and kept at room temperature to drain. Afterwards, they were weighed and incubated with ethanol (1.0 g:10 mL; w:v). Samples were then shaken for 24 h at room temperature (25 °C), centrifuged at 4 °C to recover the supernatants which were used for the *in vitro* assays.

Table 1
Scientific name, Family, common Italian name, color and usage of ten edible flowers.

Scientific Name	Family	Common Italian Name	Color	Edible use
Calendula officinalis L.	Asteraceae	Calendula	yellow	Salad, soup
Cichorium intybus L.	Asteraceae	Cicoria comune	blue	Salad and fries with eggs, soup, and boil
Malvus sylvestris L.	Malvacee	Malva	purple -violet	Salad, decoction
Punica granatum L.	Punicaceae	Melograno	red	Salad, soup
Tropaeolum majus L.	Tropaeolaceae	Nasturzio	yellow	Salad, soup
Origanum vulgare L.	Lamiaceae	Origano	white	Salad, aromatic on tomatoes etc.
Rosmarinus officinalis L.	Lamiaceae	Rosmarino	blue	Aromatic, on roast meat or potatoes etc.
Viola tricolor L.	Violaceae	Violetta	violet	Salad, soup
Cucurbita maxima L.	Cucurbitaceae	Zucca	yellow	Salad and fries
Salvia elegans Vahl	Lamiaceae	Salvia ananas	red	Salad, soup

2.2. Determination of total polyphenol contents

Total phenolic content was determined using the (Singleton and Rossi method 1965), using Folin-Ciocalteau reagent. The absorbance values were determined at 760 nm using a Cary 50 Bio spectrophotometer (Varian, Palo Alto, CA USA). Gallic acid was used as standard reference through a calibration curve. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g of flower.

2.3. Determination of the total flavonoid content

Total flavonoids (TF) were quantified according to the method given by (Zhishen et al. 1999). In this regard, the extract (50 µL) was added to distilled water followed by NaNO2 (5%) and after 5 min by $AlCl_3$ (10.0%). After further 5 min, the reaction mixture was treated with 0.2 mL of NaOH (1.0 mM). Finally, the resulting mixture was diluted to 1 mL with deionized water and the corresponding absorbance was measured at 510 nm (Cary 50Bio Varian, Palo Alto, CA). TF amounts were expressed as mg quercetin equivalents (QEQ)/g ± SD.

2.4. Determination of proanthocyanidin content

The condensed tannins were colorimetrically assayed using the proanthocyanidin assay as explained by (Porter et al. 1985). Briefly, in a test tube, 50 µL condensed tannin samples properly diluted were added to *n*-$C_4H_{10}O$-HCl reagent and to the ferric reagent (2.0% $NH_4Fe(SO_4)_2$ in 2.0 N HCl). The mixture was heated in a boiling water bath at 97 °C for 60 min and allowed to cool to room temperature. The absorbance of the solution was read at 550 nm against a reagent blank. The content of tannins was expressed as A_{550}/mL of sample. Condensed tannins were calculated as µg catechin equivalents/g ± SD by the following formula (Vasic et al., 2012) :

Condensed tannins = (A550 nm x 78.26 x Dilution factor)/(matter) (Eqn.1)

2.5. Determination of anthocyanin content

The total anthocyanin content (TAC) was determined by the pH-differential method (Giusti and Wrolstad, 2001). The colored oxonium form predominates at pH 1.0, whereas the colorless hemiketal form is dominant at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total anthocyanins, even in the presence of other interfering compounds. Samples were diluted to pH 1 (0.025 M KCl) and pH 4.5 (0.4 M CH_3COONa) and then left to equilibrate for 15 minutes. The absorbance of each dilution was finally measured at 510 and 700 nm, against a blank prepared with only distilled water. The samples were quantified in terms of cyanidin-3-glucoside equivalent, used as standard, and calculated using the following formula :

Ant = (A510- A700) pH 1.0 - (A510- A700) pH 4.5 (Eqn. 2)

Where Ant is the anthocyanin content in the sample.

2.6. Determination of ascorbic acid content

The vitamin C content of fresh product was based on the reduction of the dye 2,6-dichlorophenolindophenol

(DCPIP) by the acid solution of ascorbic acid (Davies and Masten, 1991). According to this method, a solution of 0.3 mg/mL DCPIP was included at each sample containing ascorbic acid in 2.5% metaphosphoric acid (HPO_3) and citrate-acetate buffer. In the last step, the absorbance was measured at 520 nm after 45 s.

2.7. Measurement of total carotenoids and chlorophyll A, B

The ethanolic extracts were centrifuged at 10,000 rpm for 15 min at 4 °C (Micro CL21 centrifuge Thermo Scientific). The supernatant was subsequently analyzed for Chlorophyll A (ChA), Chlorophyll B (ChB), and carotenoids content (Cx+c) using a spectrophotometer. Chlorophyll A showed maximum absorbance at 665 nm, chlorophyll b at 649 nm and the amount of these pigments was calculated according to the formulas of (Lichtenthaler and Wellburn 1985). The formulas were :

ChA=13.95 A665 - 6.88 A649 (Eqn. 3)
ChB=24.96 A649 - 7.32 A665 (Eqn. 4)
Cx+c= (1000 A470 –2.05 ChA - 114.8 ChB) /245 (Eqn. 5)

Where the numbers represent specific absorption coefficients, based on the extraction solvent; and the corresponding absorbance values (A_{664}, A_{646} and A_{470}).

2.8. α-Amylase inhibition assay

The α-amylase bioassay method was adopted from Sigma-Aldrich protocol, with some modification (Ombra et al., 2018). The dinitrosalicylic acid (DNSA) color reagent was prepared by diluting 96 mM 3,5-dinitrosalicylic acid in deionized water (20 mL), adding sodium potassium tartrate (5.31 M) in 2 M NaOH (8 mL) and 12 mL of deionized water. α-Amylase from porcine pancreas, (Sigma A 6255, 1151 U/mg of protein, Sigma, Milan, Italy) was dissolved in phosphate buffer (20 mM; pH 6.9), containing NaCl (6.7 mM). The sample (90 µL) was mixed with 10 µL of amylase solution (575U /mL) in phosphate buffer and incubated at 25 °C for 20 min. After pre-incubation, the reaction was started with the addition of 100 µL of starch solution (1.0% w/v) and incubation at 25 °C for 3 min. DNSA reagent (100 µL) was included, incubated at 85 °C for 10 min, then cooled and diluted with distilled water (1.0 mL). Negative controls were realized by replacing extracts with 90 µL of deionized water. Likewise, a blank was prepared by adding the extract to all reaction reagents without enzyme (α-amylase) solution. Absorbance was measured at 540 nm and the value of the blank was subtracted from that of the sample. Acarbose (1.0 mM) was utilized as positive control. The assay was carried out in triplicate, and the calculated percent inhibition was plotted against concentration to calculate the corresponding numerical values of IC_{50}, namely half-maximal inhibitory concentration. The IC_{50} value is defined as the concentration of extract where percent inhibition is equal to 50. In our experiments, the IC_{50} value was the mean value of three independent experiments.

2.9. α-Glucosidase inhibition assay

α-Glucosidase (5.0 mg/mL, Sigma-G5003) and *p*-nitrophenyl-α-D-glucopyranoside (1.0 mM) solutions were prepared in phosphate buffer (20 mM; pH 6.0) (Sharp et al., 2007). The reaction was performed at 37 °C using 10-µL enzyme, 25 µL substrate and 10-µL extract for 7 min, in a total volume of 80-µL. Absorption was read at 400 nm after the addition of 80 µL Na_2CO_3 (0.1 M). Ten µL of aqueous 1.0% dimethyl sulfoxide (DMSO) of acarbose solution was used as control, as well. The test was realized in triplicate, and the respective percent inhibition was plotted against each relevant concentration to obtain IC_{50} estimations.

2.10. Evaluation of anti-proliferative activity

Caco-2 cell line (American Type Culture Collection -ATCC, Rockville, MD) was grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% of heat-inactivated fetal bovine serum (FBS), penicillin ($0.010UL^{-1}$), streptomycin (10 mgL^{-1}), and sodium pyruvate 0.0002 M. Cells were incubated at 37 °C in the presence of humidified atmosphere of CO_2 (5.0%). $CaCo_2$ cell line is one of the most common epithelial colorectal carcinoma cell lines. In reality, the cells originally obtained from a human colon adenocarcinoma are ideal for *in vitro* assays. The extracts were suspended in DMEM medium at various concentrations. Only the concentrations that did not change the culture media conditions were utilized for the assays in terms of pH or as precipitation of components. Cells were seeded in 96-well microplates at a density of 5×103 cells/well and extracts were added after 24 h. The anti-proliferative effect of polyphenolic extracts was carried out against $CaCo_2$ cells using a colorimetric assay, namely 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. It was found out that treatment with the extracts inhibited the cellular growth in a time dependent manner. After 48 hours, cell survival (%) was calculated as the reduction of MTT in formazan at 550 nm. Triton® X-100 (10 µL of 10% solution) was used as a positive control, because in biological research this detergent is used for lysing cells. Untreated cells (vehicle alone) represented the negative control. All the samples were tested in triplicate for each concentration and replicated three times. The absorbance values were transformed into percentages of cell viability using the following formula:

Cell viability = Abs sample/Abs control x 100 (Eqn. 6)

We reported the anti-proliferative activity of the sample with an IC_{50} value accounting for a concentration of polyphenolic extract with 50% of cell growth inhibition, and expressed as µg GAE mL^{-1}.

2.11. Statistical analysis

Results were expressed as mean ± standard deviation of triplicate measurements. Analysis of variance

(ANOVA) was utilized to compare results and significant difference was taken into consideration at $p < 0.05$. The PC software "Excel Statistics" was employed for the calculations. Principal Component Analysis (PCA) was applied to correlate values of biological activities to phenolic composition, using the free software package for statistical computing and graphics R. (https://www.r-project.org/). All IC_{50} values were calculated from the corresponding dose inhibition curve using ED50plus v1.0 online software (Shaji and Varkey, 2013).

3. Results and Discussion

3.1. Biochemical characterization of extracts

Extracts were prepared from fresh flowers of the ten varieties as being listed in Table 1. We chose flowers commonly used to prepare typical recipes of the Mediterranean diet, like salads, soups, fruit salads or only as aromatic in meat or cheese meals. We also selected flowers of various colors from red to violet, as in the rainbow (Fig. 1). As shown in Table 2, the different extracts had a wide range of total polyphenol contents (TP). The highest content was found in *P. granatum* L. (13.669 mg GAE/g), followed by *O. vulgare* L. (4.66 mg GAE/g) and *M. sylvestris* L. (3.59 mg GAE/g). In the extracts of *R. officinalis* L. and *C. officinalis* L., TP values were intermediate. These differences were correlated with genetic factors. Many of the flavonoids that give rise to the color may also provide positive health benefits as antioxidants. This class of polyphenols was present in all samples analyzed, with a very similar trend to that of the polyphenols, as shown in Table 2, with the exception of *P. granatum* extract. The highest quantities of flavonoids were found in the extracts of *O. vulgare* L. (4.481 mgQE/g), as well as in that of *M. sylvestris* L. (3.227 mgQE/g). The extracts obtained from *T. majus* L. and *C. intybus* L. (0.335 and 0.893 mgQE/g, respectively) showed the lowest levels in terms of flavonoid contents. In particular, the presence of anthocyanins contributed towards the red and purple colors of flowers (Mazza, 2007). The results reported in Table 2 also evidenced a higher anthocyanin concentration in the red or purple-violet cultivars than in the white ones. A higher content of anthocyanins was observed in the extracts of *P. granatum* L., while lower amounts were found in those of *C. intybus* L. In *C. officinalis* L., *O. vulgaris* L. and *C. maxima* L. extracts, the anthocyanins were absent (Table 2). The extract of *P. granatum* L. showed the highest value (233.278 µg C3GE/g) of anthocyanins. Moreover, this cultivar proved to be also the richest with regard to total polyphenols among the analyzed samples. It is well-known that carotenoids are valued bioactive compounds contributing to the health properties of several foods (Saini et al., 2015; Langi et al., 2018). Wan et al. (2018) reported that the flowers were also a rich source of carotenoids, the total content of which varied greatly among species. In our set of samples, with regard to the total carotenoid content, *C. officinalis* and *C. maxima* showed the highest values (17.9 µg and 16.3/ µg, respectively), whereas those of *M. sylvestris* and *C. intybus* were lower (1.3 and 0.7 µg/g, respectively) (Table 2). In the yellow group, carotenoids were on average about 4-fold higher than in blue and violet samples (16.13 µg/g with respect to 4.25 µg/g). Proanthocyanidins are oligomeric and polymeric products of the flavonoid biosynthetic pathway (Henry-Kirk et al., 2012). They are present in flowers, fruits or seeds of many plants and their astringency protects the plants from pathogens and predators thus constituting a defense against biotic and abiotic stressors (Rauf et al., 2019). Proanthocyanidins have numerous health beneficial properties (Pajuelo et al., 2012). We measured variable amounts of proanthocyanidins in the selected flowers, which resulted more abundant in S. *elegans*, V. *tricolor* and *P. granatum*, while *O. vulgare* contained the least amount: 1306.241 µgC3GE/g; 761.747 µg C3GE/g; 561.024 µg C3GE/g and 104.757 µg C3GE/g, respectively. Vegetable products are the major source of metabolites such as vitamin C or ascorbic acid. The dietary intake of vitamin C provides protection against a wide range of diseases (Diplock et al., 2007). Based on clinical and epidemiological studies, dietary intake of 100 mg/day of ascorbic acid is recommended, to contribute to a reduced risk of heart diseases, stroke, and cancer. In plant tissues, vitamin C is present in high quantities in almost all subcellular compartments, being responsible for numerous functions in plants (Mellidou et al., 2017). The vitamin C contents of the extracts are shown in Table 2. The highest ascorbic acid content was found in *T. maius* and *C. officinalis* (30.7 and 29.7 mg/100 g, respectively), followed by V. *tricolor* and S. *elegans* (29.3 and 22.4 mg/100 g, respectively). These samples are a valuable natural source of vitamin C, and it has been observed that the content of carotenoids and flavonoids prevents the oxidation of vitamin C, increasing its stability and bioavailability in humans (Mellidou et al., 2017). Chlorophyll and its derivatives have been used in traditional medicine and potentially may act as chemopreventive agent (Levent İnanç, 2011; Mishra et al., 2011). Chlorophylls are most ubiquitous natural pigments and, as other dietary compounds, they possess cellular detoxifying mechanism and antioxidant property that protects against cellular damage. We found a higher content of chlrophyll A, B in *M. sylvestris*, *O. vulgaris* and *S. elegans*, while a minor quantity was present in *C. intybus*, *P. granatum* and *C. maxima* (*Table 2*).

3.2. Anti-proliferative activity

The anti-proliferative activity of the extracts was evaluated on Caco-2 colon carcinoma cells using an MTT colorimetric test. After 48 h of treatment, the cellular growth was significantly inhibited in a dose-dependent manner, and, for each extract, an IC50 value was calculated from the dose-response curves of the cell line. In Table 3, IC_{50} values are reported, indicating the concentration of the extracts (expressed as µg equivalent to gallic acid, GAE, as a standard, of total polyphenols present in the extract/mL) able to reduce cell viability by 50%. The IC_{50} values varied among the different extracts, the highest IC_{50} concentrations

Table 2
Phytochemical contents of edible flower extracts.

Sample	Total Phenols mgGAE/g	Flavonoids (mgQE/g)	Anthocyanins (µgC3GE/g)	Vitamin C (mg/100 g)	Proanthocyanidins (µgCE/g)	Chlorophyll A (µg/g)	Chlorophyll B (µg/g)	Carotenoids (µg/g)
C. officinalis L.	2.321 ± 0.10	1.814 ± 0.12	nd	29.7 ± 0.52	284.057 ± 5.74	32.596 ± 1.02	16.364 ± 0.82	17.9 ± 1.49
C. intybus L.	1.842 ± 0.05	0.893 ± 0.01	4.129 ± 0.28	19.4 ± 0.9	306.290 ± 28.89	6.498 ± 0.48	3.693 ± 0.19	0.7 ± 0.147
M. sylvestris L.	3.591 ± 0.11	3.227 ± 0.09	149.305 ± 5.04	14.4 ± 0.84	293.198 ± 8.52	83.226 ± 4.26	36.728 ± 2.9	1.3 ± 0.22
P. granatum	13.669 ± 3.85	2.859 ± 0.26	233.278 ± 9.15	14.7 ± 1.82	561.024 ± 62.2	0.679 ± 0.07	1.97 ± 0.03	6.445 ± 0.56
T. majus L.	0.689 ± 0.01	0.335 ± 0.01	10.157 ± 1.25	30.7 ± 1.10	150.115 ± 15.21	1.209 ± 0.17	3.247 ± 0.58	14.2 ± 0.31
O. vulgare L.	4.660 ± 0.14	4.481 ± 0.01	nd	21.5 ± 1.01	104.757 ± 11.41	41.186 ± 2.11	21.436 ± 2.19	9.3 ± 0.56
R. officinalis L.	3.074 ± 0.12	2.994 ± 0.16	8.541 ± 1.03	12.8 ± 0.61	366.488 ± 30.1	32.929 ± 0.78	18.509 ± 0.95	9.301 ± 0.73
V. tricolor L.	2.050 ± 0.09	2.042 ± 0.12	71.938 ± 4.83	29.3 ± 0.83	761.747 ± 58.91	17.077 ± 0.04	7.628 ± 1.01	5.7 ± 0.88
C. maxima L.	1.688 ± 0.10	1.877 ± 0.01	nd	18.1 ± 0.69	64.091 ± 7.01	7.701 ± 0.68	3.101 ± 0.33	16.3 ± 0.18
S. elegans Vahl	2.321 ± 0.06	1.499 ± 0.06	67.886 ± 2.28	22.4 ± 1.22	1306.241 ± 50.59	37.71 ± 0.53	39.864 ± 1.14	19.4 ± 1.44

Data represent the mean ± SD (standard deviation) (n = 3); mgGAE/g = mg of gallic acid equivalents/g of fresh flower; mgQE/g = mg of quercetin equivalents/g of fresh flower; µgC3GE/g = µg cyanidin-3-glucoside equivalent /g of fresh flowers; mg/100gr of fresh flowers; µgCE/g = µg Catechin equivalent /g of fresh flowers; µg/g of fresh flowers; nd = not detected.

were measured using the extract of *M. sylvestris*, while the lowest was found with *C. officinalis* extracts. Our results are in agreement with those reported by (Miguel et al. 2016) and (Bekir et al. 2013) on the *in vitro* cytotoxic properties of C. *officinalis* L. and *Punica granatum* L. flowers. In the method of (Mothana et al. 2011), methanolic extracts of *Rosmarinus officinalis* showed higher IC_{50} values for two cell lines, 5637 (IC_{50} = 48.3 ± 5) and MCF-7 (IC_{50} =187.3 ± 0.5), with respect to the IC_{50} value reported in Table 3, with $CaCo_2$ cell line (IC_{50} = 36.42 ± 4.1). These discrepancies are due to the different sensitivity of the cell lines.

3.3. Inhibition of α-amylase and α-glucosidase activities

Table 3
Anti-proliferative (AP) and anti-glucosidase (Aglu) activity of the flower extracts.

Sample	IC_{50} AP ± SD (µg GAE/mL)	IC_{50} Aglu ± SD (µg GAE/mL)
C. officinalis L.	24.26 ± 3.8	32.70 ± 4.5
C. intybus L.	42.75 ± 3.9	100.99 ± 4.4
M. sylvestris L.	106.30 ± 5.4	74.59 ± 6.3
P. granatum L.	85.90 ± 7.2	12.50 ± 5.6
T. majus L.	26.22 ± 9.5	13.99 ± 6.1
O. vulgare L.	50.11 ± 7.2	132.00 ± 15.6
R. officinalis L.	36.42 ± 4.1	47.30 ± 13.1
V. tricolor L.	51.27 ± 7.2	59.69 ± 2.8
C. maxima L.	25.51 ± 5.6	42.49 ± 4.1
S. elegans Vahl	35.80 ± 5.4	53.59 ± 3.6

IC_{50}= Concentration of polyphenolic extract (expressed as µg GAE mL^{-1}) necessary to inhibit 50% of cellular growth or enzyme activity; data are mean values ± standard deviation (SD).

Phenolic compounds have great potential to inhibit enzymes related to carbohydrate metabolism, such as α-amylase and α-glucosidase. Therapies against type-2 diabetes consider the use of drugs as enzyme inhibitors in order to decrease glucose absorption in the gut. Inhibiting enzymes like α-amylase and α-glucosidase, which are involved in carbohydrate digestion, are good approaches for decreasing postprandial hyperglycemia (Tanaka et al., 2009; Thompson and Davis, 2017). For example, flavonoids and other phenolic compounds enclosed in different vegetables or fruits like blueberries, ginger, Olea europea and soybean have been reported to inhibit these enzymes (Ademiluyi and Oboh, 2013). Our samples were weakly active against pancreatic α-amylase, reducing only at high dosage

(100 µg/mL) of about 15-20% of the enzyme activity (data not shown), while showing a significant inhibition against yeast α-glucosidase. Pomegranate was the only sample that released a significant inhibition of amylase, reducing activity to 29.8% at 100 µg GAE/mL compared with control. Our results are in agreement with the study of (Kam et al. 2013), in which the effects of extracts of different pomegranate parts (including flowers) were compared on carbohydrate digestive enzymes, amylase and glucosidase, *in vitro*. The results of the α-glucosidase inhibition assays are shown in Table 3, where we reported the respective IC_{50} values. As shown, all of the extracts possessed inhibitory capacity. Overall, the *T. majus* extract was more efficient than O. *vulgaris* sample. In addition, *P. granatum* cultivar showed the most inhibitory activity since its extracts possessed the lowest IC_{50} concentrations. Ultimately, the analyzed biological activities of the samples are due to the synergic effects exerted by various polyphenolic classes. The varieties with violet flowers (*M. sylvestris* and *V. tricolor*) had lower anti-proliferative and anti-glucosidase power with respect to the red-flowered varieties (*P. granatum* and *S. elegans*), although both groups were characterized by a high concentration of anthocyanins. This suggests that additional phytochemicals in the red cultivars, probably carotenoids could strengthen these biological activities. In fact, the cultivars with red flowers were found to be richer in carotenoids, than the violet cultivars (Table 2). On the other hand, the yellow group characterized by a higher content of carotenoids presented lower IC_{50} values for these biological properties than the other colored groups, thus resulting in the group with a major potentiality. Regarding the correlations between the biochemical parameters and anti-proliferative activity, the correlation test released a negative significant value ($r = -0.70$) only between carotenoids content and IC_{50} value, thus indicating that these compounds are mainly involved in such inhibitory activity.

3.4. Principal component analysis

Principal component analysis (PCA) was applied to characterize the ten varieties of flowers according to their biological activity. The method was successfully used in the treatment of data concerning the study of apples (Xu et al., 2016) and common beans (Ombra et al. 2016). In our study, the ten variables were measured for 10 samples and the subsequent data were analyzed by PCA. The cumulative percentage of the total variance explained by the first two components was 65.02%. A bidimensional plot was designed (Fig. 2). Since pomegranate flowers contained higher phenolic content, it was decided to repeat the PCA study excluding them. The distribution of the varieties along PC1 and PC2 showed that samples could be divided into two main groups: group A, which included yellow varieties positioned on the left half of the biplot; group B, which comprised two blue and one violet varieties near to the center of the bidimensional plot. The remaining samples were more separated from the others and not included in these groups. The varieties of group A differed from the others due to their higher content of carotenoids, which were able to influence the growth of the cancer cells more effectively. The most active varieties were those belonging to group A. The red flowers were only slightly less active, while the extracts of blue-violet varieties, despite a higher content of anthocyanins, resulted less active than the yellow flowers, suggesting that the carotenoids were mostly responsible for such biological properties.

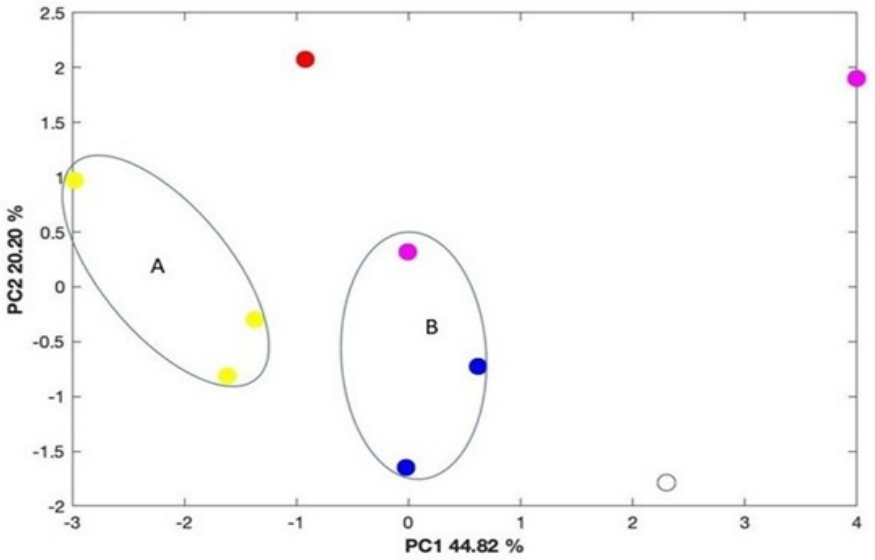

Fig. 2. PCA map showing the relationship among the concentration of total phenolics, flavonoids, proanthocyanidins, anthocyanins, vitamin C, chlorophyll A, B, carotenoids and IC_{50} values of anti-proliferative and anti-glucosidase activities, in nine edible flowers. The circles represent the differently colored flowers.

4. Concluding remarks

In this study, the phytochemical contents of 10 edible flowers as well as the corresponding anti-proliferative and enzyme inhibitory capacities have been explored. The data obtained showed large variability among these ten species, both in the color and in the levels of their main bioactive compounds involving polyphenols, flavonoids, anthocyanins, carotenoids, and vitamin C. According to the results, these edible flowers may be recommended as natural colorants, representing an alternative to the use of synthetic dyes in foods, as well as natural sources of bioactive compounds relevant to human health. The results also highlight the importance of flower color in terms of anthocyanin and carotenoids contents. These pigments are primarily involved in the color-mediated attraction strategy and make the flowers an important resource that should be enhanced from an agronomic and nutritional point of view. The present study increases the interest in these edible flowers as sources of natural agents, with beneficial properties for human health. The findings illustrated above contribute to encourage the consumption of flowers, not merely for enhancing the aesthetic value of food. Finally, further investigation is needed to highlight the mechanism of action underlying the described activities and to find the most bioactive compounds, also to be correlated with *in vivo* studies. Undoubtedly, knowledge of the biological properties of these compounds will enable the realization of new functional foods or new food supplements formulations, thus promoting their utilization in food technology and in the pharmaceutical industry.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

This work was supported by SAFE SMART Italian project "PON4a2. F "Be & Save" and by "iPARK" cod.pog.2015-AMB-0070.

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