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Original research paper

Phytochemical composition, antioxidant, antiglycation and antihyperlipidemic activity of flowering parts from five plant species before and after *in vitro* digestion

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ABSTRACT

This study evaluates the antihyperlipidemic (pancreatic lipase inhibition assay), antiglycation [inhibition of bovine serum albumin (BSA) glycation], and antioxidant activity (ABTS, DPPH and FRAP assays) of ethanolic extracts from flowering parts of five widely distributed plant species in Croatia – *Crocus heuffelianus* Herb. (tepals), *Nicotiana tabacum* L. (petals), *Malva sylvestris* L. (petals), *Calendula officinalis* L. and *Helianthus annuus* L. (both sterile ligulate flowers). An *in vitro*-simulated system of human digestion was employed to assess the bioaccessibility of the selected phenolics, and the stability of the extracts' antioxidant, hypolipidemic and antiglycation potential following each digestion phase. The concentrations of *L*-ascorbic acid, individual flavonoids and phenolic acids were determined using RP-HPLC analysis. Principal component analysis revealed significant differences in the content of bioactive compounds and their biological activity among investigated plant species. All original extracts exhibited high antioxidant activity (> 70 %) in at least one assay, with *N. tabacum* and *H. annuus* demonstrating the strongest antioxidant capacity throughout digestion. *H. annuus* contained the highest levels of total identified phenolic acids (TiPA), total identified phenols (TiP), and total identified compounds (TiC), while *N. tabacum* and *C. heuffelianus* exhibited the highest total flavonoid (TiF) content.

Among individual compounds, protocatechuic acid, quercetin and ferulic acid significantly contributed to antioxidant activity. *N. tabacum* had the strongest antihyperlipidemic potential in the original extracts, as well as in the most digestion phases. Strong BSA glycation inhibition (70–100 %) was observed in all plant extracts across various digestion phases, with the exception of *C. heuffelianus*, which exhibited moderate inhibitory effects. These findings suggest that the analyzed flower-derived plant materials, some of which are often considered agricultural waste, can serve as sustainable and valuable resources of bioactive compounds for functional food, dietary supplements and pharmaceutical applications.

Keywords: Crocus heuffelianus Herb., *Nicotiana tabacum* L., *Malva sylvestris* L., *Calendula officinalis* L., *Helianthus annuus* L., flowering parts, chemical composition, antiglycation activity, antihyperlipidemic activity, antioxidant activity

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INTRODUCTION

Edible flowers have significant potential for enriching the functional food market, offering the dual advantage of enhancing both functional and sensory properties through their nutritive profile, pleasant taste, aroma and vibrant color (1). These bioactive components exhibit diverse health-promoting properties, including antioxidant, anti-inflammatory, antimicrobial, antineoplastic, antihyperlipidemic, antidiabetic and neuroprotective effects, making edible flowers a promising choice for addressing modern-day, chronic conditions like cardiovascular disease, type 2 diabetes, obesity, cancer and neurodegenerative disorders (1, 2).

Historically, marigold (*Calendula officinalis* L., Asteraceae) and common mallow (*Malva sylvestris* L., Malvaceae) flowers have been valued for their nutritional medicinal properties, with documented use dating back to ancient Rome, medieval France and continuing into modern times (1). Common mallow and marigold flower preparations are recognized by the European Medicines Agency (EMA) as traditional herbal medicinal products (3, 4). Marigold flowers are rich in carotenoids and essential oils, offering a slightly sour and pungent flavor, with aromatic and bitter notes, making them suitable for seasoning and coloring dishes (1, 5). They can be used fresh in salads or as dried petal powder in rice, fish, cheese and yogurt, often serving as a substitute for saffron, earning the nickname "poor man’s saffron" (6). Mallow flowers, on the other hand, are commonly added to mixed salads and used for garnishing and decorating meat and fish dishes (5).

De Lima Franzen *et al.* (6) investigated the nutritional properties of sunflower (*Helianthus annuus*, L., Asteraceae) and marigold flowers, finding that both have high water content, low caloric value, and carbohydrate levels of 7.57 % and 5.62 %, resp. Sunflower flowers and marigold petals also demonstrated notable fatty acid content, with marigold being particularly rich in unsaturated fatty acids (59.3 %) (7, 8). Sunflower presented a higher ash content (1.25 %), which refers to the total amount of minerals present in the plant. The researchers concluded that these flowers exhibit chemical properties comparable to conventional vegetables such as broccoli and cauliflower, suggesting their suitability for inclusion in a healthy diet either in raw form or as functional food

ingredients (6). For instance, Bragueto Escher *et al.* (9) fortified organic yogurt with lyophilized marigold extract, significantly enhancing its total polyphenol content as well as its antioxidant and antidiabetic properties. Liang *et al.* (8) demonstrated that sunflower florets could serve as a promising source of dietary fiber, iron and essential amino acids such as valine and leucine, which are beneficial for developing supplements for athletes or prevention of anemia (8, 10). Abundant phenolic content determined by Liang *et al.* (8) and Ye *et al.* (11) suggests that sunflower florets could be considered as a promising resource of natural antioxidants.

Given that different antioxidant capacity assays employ distinct detection mechanisms, and each has its specific applicability, advantages and limitations, multiple *in vitro* methods should always be used to determine the antioxidant activity of a given sample (12). Some of the commonly used assays include DPPH, ABTS and FRAP. The DPPH assay is more suitable for assessing lipophilic antioxidants, while FRAP primarily measures hydrophilic antioxidant activity. In contrast, ABTS is a versatile assay capable of evaluating both hydrophilic and lipophilic antioxidants (13).

As a result of deliberate deflowering process during tobacco (*Nicotiana tabacum* L., Solanaceae) cultivation, substantial quantities of inflorescences are left in the fields (14). Accordingly, Leal *et al*. (15) examined extracts from this pre-harvest tobacco waste using natural deep eutectic solvents (NaDES), finding high total phenolic content and significant antioxidant activity.

To obtain just 1 kilogram of saffron (*Crocus sativus* L., Iridaceae) spice, often referred to as "red gold", an extraordinary amount of over 150,000 flowers is required (16). Since the spice consists solely of dried stigmas, this process generates approximately 350 kilograms of saffron tepals as a by-product, which is typically discarded, leading to significant biomass waste (17). Serrano-Diaz *et al.* (18) and Jadoulai *et al*. (19) analyzed the nutritional properties of saffron tepals and reported high dietary fiber, carbohydrates, protein and ash content, along with a notably low-fat content. Furthermore, investigations into the phytochemical profile of saffron tepals have identified them as the richest source of polyphenolic compounds within the entire saffron flower, including stamens, styles and a whole flower (19, 20). Saffron tepal extracts have demonstrated strong antioxidant, radical scavenging, anti-inflammatory, antispasmodic, and antidiabetic properties (18, 21). To maximize the recovery of polyphenolic compounds from saffron tepals, advanced extraction techniques are being employed (*e.g.*, microwave-, ultrasound- and enzyme-assisted extraction) (22, 23). *Crocus heuffelianus* Herb. was formerly treated as one of the synonyms for *C. vernus* (L.) Hill. ssp. *vernus*, but is now recognized as an independent species (24).

One of the key pathophysiological mechanisms in diabetes involves the non-enzymatic reaction of proteins with sugars, leading to the formation of advanced glycation end products (AGEs). AGEs play a significant role in the development of both microvascular complications, such as retinopathy, cataract formation, peripheral neuropathy and diabetic kidney disease, and macrovascular complications, including coronary heart disease, peripheral arterial disease and stroke. Perhaps the most extensively studied AGE is glycated hemoglobin (HbA1c), a marker used for diabetes diagnosis. Despite its critical role in diabetes management, data from the American Diabetes Association indicate that only 50.5 % of American adults with diabetes achieve the therapeutic target of HbA1c levels below 7 % (25).

Inhibition of pancreatic lipase reduces dietary lipid digestion and absorption, making it an attractive and widely studied target for the development of potential anti-obesity agents (26). Orlistat is currently the only drug with the aforementioned mechanism of action used to treat obesity, however, its clinical use is often connected

with undesirable gastrointestinal side effects, such as diarrhea, flatulence, abdominal pain and oily stools (27). This increases the importance of exploring plant bioactive compounds, such as flavonoids, for their potential to inhibit pancreatic lipase, reduce protein glycation and slow the progression of glycation-related complications (28, 29).

The aim of this study was to quantify individual polyphenolic compounds and L-ascorbic acid (L-AA) in ethanolic extracts prepared from the petals of *Malva sylvestris* L. and *Nicotiana tabacum* L., tepals of *Crocus heuffelianus* Herb. and sterile ligulate flowers of *Calendula officinalis* L. and *Helianthus annuus* L. Additionally, the study sought to investigate the antioxidant, antiglycation and antihyperlipidemic activity of these extracts, both before and after each phase of *in vitro* digestion.

EXPERIMENTAL

Chemicals and apparatus

Enzymes (α-amylase, porcine pepsin, pancreatic lipase and pancreatin) and bile utilized for *in vitro* digestion and antidiabetic activity (α-amylase) were products of Merck KGaA (Germany). Commercial polyphenol standards were produced by Merck KGaA and Extrasynthese (France). All chemicals and reagents were of analytical grade and supplied by Merck KGaA or Kemika (Croatia). Deionized water was used in all experiments and the solvents and chemicals were of analytical or HPLC grade.

RP-HPLC analyses were performed using the Agilent 1100 Series system equipped with a quaternary pump, multiwave UV/Vis detector, autosampler, fraction collector, analytical Zorbax Rx-C18 guard column (4.6 x 12.5 mm, 5 µm particle size) and Poroshell 120 SB-C18 column (4.6 x 75 mm, 2.7 µm particle size) (Agilent Technologies, USA). All absorbance and fluorescence measurements related to antihyperlipidemic, antiglycation and antioxidant potential were performed using Fluostar Optima microplate reader (BMG Labtech GmbH, Germany).

Plant materials

Aerial flowering parts from Heuffel's saffron (*Crocus heuffelianus* Herb., Iridaceae), tobacco (*Nicotiana tabacum* L., Solanaceae), common mallow (*Malva sylvestris* L., Malvaceae), sunflower (*Helianthus annuus* L., Asteraceae) and marigold (*Calendula officinalis* L., Asteraceae) were collected at their full flowering stage in March 2020 (*Crocus heuffelianus*) and July 2020 (other plant species), from three different locations in Croatia, as follows: Heuffel's saffron in the Botanical Garden of the Faculty of Science, University of Zagreb; mallow, marigold and sunflower in Đurđevac area, while tobacco was collected in Pitomača area. The plant material was identified at the Department of Biology (Divison of Botany), Faculty of Science, University of Zagreb, Croatia, where the plant material has been deposited. Tepals (*Crocus heuffelianus*), petals (*Nicotiana tabacum* and *Malva sylvestris*) and sterile ligulate flowers (*Calendula officinalis* and *Helianthus annuus)* were separated from the collected flowers and dried in the dark in a ventilated area at room temperature.

Extract preparation

The extracts at the concentration of 50 mg mL^{-1} were prepared from dry flowering parts using 40 % aq. ethanol (*V/V*) at room temperature on a rotary extraction device for 60 min. The use of 40 % ethanol was specifically chosen to approximate the alcohol concentration found in strong alcoholic beverages, rendering the extracts suitable for consumption. The extracts were then centrifuged for 5 min at 10,000 rpm, and supernatants were stored at –20 °C until analyses. Extractions were performed in triplicate.

Model of human in vitro *digestion*

The *in vitro* model of human digestion was based on the method described by Vujčić Bok *et al*. (30), with minor adjustments. Firstly, 0.15 mL of extract was combined with an equal volume of 20 mmol L^{-1} phosphate buffer (pH 7.0). To initiate the salivary phase of digestion, 5 µL of amylase (0.48 mg mL^{-1} in 20 mmol L^{-1} phosphate buffer, pH 7.0) was added, and the mixture was incubated for 5 minutes at 37 °C in a shaking water bath at 150 rpm. For the gastric digestion phase, 0.2 mL of porcine pepsin solution (3 mg mL^{-1} in 0.1 mol L^{-1} HCl) was added, and acidified with 1 mol L^{-1} HCl (pH 2.0). The samples were then incubated in a shaking water bath at 37 °C for 1 hour at 150 rpm. To simulate the upper intestinal phase, the pH was first adjusted to 5.3 with 5 µL of 1 mol L^{-1} $NaHCO_3$. After the pH adjustment, 0.45 mL of pancreatic juice (containing 2.4 mg mL^{-1} bile acids, 0.2 mg mL^{-1} porcine pancreatic lipase and 0.4 mg mL^{-1} pancreatin, in 20 mmol L^{-1} phosphate buffer, pH 7.0) were added. The total volume of each sample in the intestinal phase was then adjusted to 1 mL using 20 mol L^{-1} phosphate buffer (pH 7.0), and the final pH was brought to 7.0 by adding 1 mol L^{-1} NaOH. These samples were subsequently incubated for 2 hours at 37 °C in a shaking water bath at 150 rpm. After digestion, the final volume of each sample, both pre- and post-digestion, was adjusted to 1 mL with 20 mmol L^{-1} phosphate buffer (pH 7.0). The samples were centrifuged at 15,000 rpm for 5 minutes at 4 °C, and the supernatants were stored at –20 °C until further spectrophotometric and HPLC analyses.

RP-HPLC analysis

For chromatographic identification and quantification of phenolic compounds and *L*-ascorbic acid, the extracts were hydrolyzed with HCl at a final concentration of 1.2 mol L^{-1} for 2 h at 80 °C and 300 rpm in a rotary shaker. Qualitative and quantitative RP-HPLC analyses of plant extracts were performed using the Agilent 1100 Series system. Mobile phase A was 0.2 % aq. acetic acid (*V/V*), and mobile phase B was 0.2 % acetic acid and 80 % methanol (acetic acid:methanol:water; 0.2:80:19.8; *V/V*) and the solvent gradient profile was as reported in Šola *et al*. (31–33). The flow rate was 1 mL min^{-1} and the injected volume of the sample was 25 µL. For quantification, the multiwave UV/Vis detector was set at 220 nm for L-ascorbic acid (L-AA), 254 nm for vanillic acid (VA), *p*-hydroxybenzoic acid (*p*-HBA) and protocatechuic acid, 280 nm for gallic acid (GA), syringic acid (SyrA) and cinnamic acid, 310 nm for caffeic (CA), sinapic (SinA), ferulic (FA) and *p*-coumaric acid (*p*-KA) and 360 nm for quercetin (Q), luteolin (L), kaempferol (K) and isorhamnetin (IzoR).

Phenolic compounds were characterized according to their retention times and UV spectra compared with commercial standards. For the quantitative analyses, calibration curves were obtained by injecting known concentrations (in the range between 1 and 250 µg mL^{-1}) of the combined standard solution in triplicate. The quantification of phenolic compounds was performed by integrating peak areas and referencing them against

calibration curves established using known quantities of available pure standard compounds (Supplementary materials: Figs. S1-5 and Table S1).

Antioxidant activity

The ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] assay was carried out as described by Vujčić *et al*. (34). A volume of 2 µL of the tested plant extract was added to 200 µL of ABTS solution and incubated for 6 min at room temperature. The decrease in absorbance of the reaction mixture was read at 740 nm, and the radical scavenging activity was calculated as percentage of ABTS inhibition.

The radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was performed as described by Radić Brkanac *et al*. (35). The reaction mixture consisted of 10 µL of tested plant extract, or 10 µL of 40 % aq. ethanol (*V*/*V*) for estimating initial absorbance (A_0) and 190 µL of freshly prepared ethanolic DPPH solution (0.1 mmol L^{-1}). The mixture was incubated in the dark for 30 min at room temperature and the decrease in absorbance of the radical solution was measured at 520 nm.

The ferric reducing antioxidant power (FRAP) assay was carried out as described in Vujčić Bok *et al*. (36). The tested plant extracts (10 µL) were mixed with 190 µL of freshly prepared FRAP reagent. Absorbance was measured at 595 nm after 4 min of reaction time and the percentage of ferric tripyridyl triazine (Fe^{3+}-TPTZ) reduction was calculated.

Trolox was used as a positive control for all antioxidant activity methods.

Antihyperlipidemic and antiglycation activity

Inhibition of pancreatic lipase was conducted as described by Spinola *et al.* (37). Twenty µL of 10 mmol L^{-1} *p*-nitrophenyl butyrate (substrate) solution in 96 % ethanol (*V*/*V*) was mixed with 40 µL of tested extract. Subsequently, 40 µL of pancreatic lipase enzyme (2.5 mg mL^{-1} in 0.1 mol L^{-1} phosphate buffer, pH = 8.0) was added and the mixture was homogenized on a vortex mixer. The mixture was incubated for 20 minutes at 37 °C on a shaking water bath and absorbance was read at 405 nm. Solution of pure Orlistat (6 g L^{-1} in ethanol) was used as a positive control. For each sample, a control was prepared in which an equal volume of 0.1 mol L^{-1} phosphate buffer (pH = 8.0) was added instead of the pancreatic lipase enzyme. Pancreatic lipase inhibitory activity was calculated using the Equation *1*:

$$\% \text{ inhibition} = 100 - [(A_t - A_{tb})/(A_c - A_{cb})] \times 100 \qquad (1)$$

where A_t was the absorbance of the test (sample extract with enzyme), A_{tb} was the absorbance of test blank (sample extract without enzyme), A_c was the absorbance of control (with enzyme) and A_{cb} was the absorbance of the control blank (without enzyme).

Inhibition of BSA glycation was performed as described by Spinola *et al.* (38). Volume of 100 µL of BSA solution (10 g L^{-1}) was mixed with 100 µL of fructose solution (0.5 mol L^{-1}) and 40 µL of the tested extract. Incubation was done in incubator shaker for 24 h at 37 °C; after incubation fluorescence was measured (excitation wavelength 405 nm and emission wavelength 460 nm). Catechin solution (6 g L^{-1}) was used as a positive control and enzyme inhibitory activity was calculated.

Statistical analysis

All results were processed using Statistica 13.3 software package (Stat Soft Inc., USA). One-way variance analysis (ANOVA) followed by Duncan's multiple range test was applied for assessment of significant differences between the samples. Principal component analysis (PCA) was employed for visualization of samples grouping. Pearson's correlation coefficients between individual and total compounds, and antioxidant activity, antihyperlipidemic and antiglycation potential were calculated to assess possible correlations between the measured parameters. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

RP-HPLC analysis

Amount of total identified phenolic acids (TiPA), total identified flavonoids (TiF), total identified phenolic compounds (TiP), total identified compounds (TiC = TiP + L-AA) released from saffron tepals, tobacco and mallow petals and sterile flowers of marigold and sunflower before, during and after *in vitro* digestion are presented in Table I.

The highest TiPA, TiP and TiC values were found in sunflower original sample and in almost all *in vitro* digestion samples of sunflower florets compared to other flowering plant samples. In the salivary phase of *in vitro* digestion, amount of 6.73 ± 0.33 μg mL^{-1}, 6.84 ± 0.33 μg mL^{-1} and 6.91 ± 0.33 μg mL^{-1} were detected for TiPA, TiP and TiC for sunflower samples, resp. Amount of L-ascorbic acid and individual phenolics released from selected flowering plants after *in vitro* gastrointestinal digestion are presented in Tables II and III.

In the sunflower samples 11 compounds were detected: gallic acid (GA), protocatechuic acid (PrKa), hydroxybenzoic acid (HBA), vanillic acid (VA), caffeic acid (CA), syringic acid (SyrA), *p*-coumaric acid (*p*-KA), ferulic acid (FA), quercetin and *L*-ascorbic acid in all samples, isorhamnetin in almost all samples, and kaempferol in gastric phase of in vitro digestion (Table II). Liang *et al.* (8) reported also for florets of sunflower that 1,5-di-*O*-caffeoylquinic acid, isoquercitrin and chlorogenic acid are the most abundant phenolic. The main phenolic acid in our sunflower samples was protocatechuic acid (PrKa) and the highest amount (5.42 ± 0.30 μg mL^{-1}) was detected in the salivary phase of *in vitro* digestion.

As Heuffel's saffron was recently recognized as new species, very little phytochemical analysis has been performed so far. After gastric digestion, TiF was the highest in saffron tepals samples (5.79 ± 0.4 μg mL^{-1}). Kaempferol (K) was the main flavonoid in this saffron sample (5.17 ± 0.45 μg mL^{-1}), including the original saffron sample and all *in vitro* digestion samples (Table III). This is in accordance with results from Šola *et al.* (39) where kaempferol was the dominant flavonoid in all saffron tepal extracts. In saffron samples, sinapic acid (SA), FA, *p*-KA and L-AA were also detected.

Tobacco had the highest value of TiF in the initial (extract + phosphate buffer) and salivary phase of *in vitro* digestion and also in original sample in comparison to other plant species. Main compounds in tobacco petals were PrKA (0.02–2.79 μg mL^{-1}), quercetin (Q) (0.61–2.11 μg mL^{-1}), L-AA (0.33–0.89 μg mL^{-1}), K (0.032–0.55

μg mL^{-1}), CA (0.32–0.40 μg mL^{-1}), HBA (0.03–0.04 μg mL^{-1}), FA (0.03–0.04 μg mL^{-1}) and SyrA (0.03–0.04 μg mL^{-1}). Cinammic acid was detected only in tobacco samples after gastric phase of digestion.

In marigold samples, caffeic acid was identified as the dominant phenolic acid. Among the flavonoids, quercetin, isorhamnetin, and kaempferol were detected. These results are in concordance with those reported by Pires *et al*. (2), who identified three caffeic acid derivatives and ten flavonoids, including various glycosides of kaempferol, quercetin, and isorhamnetin.

Antioxidant activity

The free-radical scavenging activities and the ferric ion reduction capacity of the ethanolic extracts of selected flowering parts were assessed using three commonly used tests, ABTS, DPPH and FRAP, followed by spectrophotometric measurements.

In Fig. 1, antioxidant capacity (ABTS, % inhibition; DPPH, % inhibition; FRAP, % reduction Fe^{3+}) of original and digested plant extracts are presented. According to Vujčić *et al.* (34), all original samples exhibited high antioxidant (>70 %) activity with all of the three used methods, with the exception of *M. sylvestris* original sample, which demonstrated moderate activity (58.3 %) in the DPPH assay. The highest value of antioxidant activity measured by DPPH method during digestion was reported in sunflower sample after gastric phase (67.0 %), followed by tobacco sample (53.4 %) for the same phase of digestion. Using the ABTS method, *N. tabacum* exhibited the highest antioxidant activity throughout digestion, with values of 98.8, 94.0, 84.7 and 85.9 % in the initial, salivary, gastric and intestinal phase, resp. Additionally, sunflower samples demonstrated significant antioxidant capacity after the gastric phase (79.5 %). All plant extracts showed high (> 70 %) antioxidant capacity measured by FRAP method after *in vitro* digestion. The highest FRAP values were recorded for tobacco after the initial (89.6 %), salivary (91.3 %), and gastric (95.1 %) phase, while sunflower exhibited similarly high activity after the initial (91.6 %), salivary (89.5 %), and gastric (94.2 %) phase. After the intestinal phase, *N. tabacum* demonstrated the highest antioxidant capacity (90.1 %).

The antioxidant capacity measured using the DPPH method exhibited a strong reduction after intestinal digestion for all tested plant samples. Since the DPPH assay primarily detects lipophilic antioxidants, this decline suggests that lipophilic antioxidant compounds were negatively affected by the digestion process. Still, the ability of the tested plant extracts to inhibit the $ABTS^{\bullet+}$ radical cation and their FRAP antioxidant capacity also decreased following digestion, the reduction was much less pronounced compared to the DPPH method.

Antihyperlipidemic and antiglycation activity

Antihyperlipidemic and antiglycation properties of original samples and predigested extracts measured by the inhibition of pancreatic lipase and BSA glycation are given in Fig. 2.

All original samples showed moderate (35 – 70 %) pancreatic lipase inhibitory activity according to classification used by Rusak *et al.* (40). Saffron and marigold samples exhibited the strongest pancreatic lipase inhibition after the initial phase of digestion, while tobacco and sunflower samples dominated during the salivary phase. Tobacco samples consistently showed high pancreatic lipase inhibitory activity across various digestive phases, particularly in its original sample and during gastric and intestinal digestion. Since pancreatic lipase is

secreted by pancreas into the small intestine (duodenum), the antihyperlipidemic activity observed during the intestinal phase is the most relevant. Flavonoids (Q) and phenolic acids (SyrA, HBA) showed strong correlations with the pancreatic lipase inhibitory activity of the extracts, suggesting that these compounds may play a key role in pancreatic lipase inhibition (see section Pearson's correlations). Hernández-Saavedra *et al*. (41) investigated pancreatic lipase inhibitory activity of *C. officinalis* infusions *in vitro*, reporting that a concentration of approximately 15.0 mg mL^{-1} achieved 50 % inhibition of the reaction. A subsequent *in vivo* study on high-fat-and-fructose-diet-fed rats confirmed statistically significant inhibitory effect on postprandial serum TG and even a significant reduction in body mass. In contrast, Zor *et al*. (42) found that water extracts obtained from aerial parts of *C. officinalis* (0.5–2.0 mg mL^{-1}) exhibited no pancreatic lipase-inhibitory activity *in vitro*. Interestingly, *C. officinalis* and *H. annuus* seed extracts showed pancreatic lipase inhibition *in vitro* (58 and 57 %, resp., of the positive control value). However, a follow-up *in vivo* study on Wistar rats revealed that neither *C. officinalis* nor *H. annuus* seed extracts delayed the postprandial rise in plasma triglycerides (43). In our study, *M. sylvestris* petal extracts showed weak (initial, salivary and gastric phase of *in vitro* digestion) to moderate (original sample and intestinal phase) inhibition of pancreatic lipase. Marrelli *et al.* (44) reported that a 70 % aq. ethanolic (*V*/*V*) leaf extract of *M. sylvestris* exhibited weak pancreatic lipase inhibitory activity, with a concentration required to achieve 50 % inhibition exceeding 2.5 mg mL^{-1}. For illustration, orlistat (positive control) showed IC_{50} of 0.018 ± 0.001 mg mL^{-1}.

According to our results, strong inhibition of BSA glycation (70 – 100 %) was observed in *M. sylvestris*, *H. annuus*, *N. tabacum* and *C. officinalis* in both the original samples and after almost all digestion phases. Sun *et al*. (45) evaluated the AGE inhibitory activity of *H. annuus* sprouts extract, reporting an inhibition rate of 83.3 % at a concentration of 1.0 mg mL^{-1}. For additional context, this was noted alongside the positive control, aminoguanidine solution (1 mmol L^{-1} ≈ 0.07 mg mL^{-1}), which exhibited 80.9 % inhibition in the same study. However, these values serve as illustrative data rather than a direct comparison due to the differing nature of the substances. Likewise, the findings of Ahmad *et al.* (46) align with our results, showing that *C. officinalis* whole plant extracts effectively inhibited BSA glycation. Their evaluation indicated that a concentration of 270 µg mL^{-1} of *C. officinalis* extract achieved 50 % inhibition, while a concentration of 390 µg mL^{-1} resulted in approximately 70 % inhibition. For illustrative purposes, they noted that the IC_{50} of the positive control, aminoguanidine, was 70 µg mL^{-1}.

C. heuffelianus exhibited moderate inhibition during the initial (40.8 %) and salivary (43.4 %) digestion phases, as well as in the original sample (37.6 %). While no prior studies have examined BSA glycation inhibition of *C. heuffelianus*, research on *C. sativus* conducted by Ronsisvalle *et al.* (47) yielded comparable inhibition percentages (30–40 %). According to van der Lugt *et al.* (48), heat-treated food products (*e.g.*, fried foods) represent a major source of pro-inflammatory dietary advanced glycation end products (dAGEs), which can also be endogenously formed during the intestinal digestion of AGE-rich foods. Consequently, the findings from the intestinal digestion phase are particularly relevant. Notably, *N. tabacum* demonstrated the highest statistically significant inhibition of BSA glycation at this stage. While cigarette smoke from cured tobacco contains highly reactive glycation products that can accelerate AGE formation *in vivo*, our findings suggest that *N. tabacum* petal extracts may exert a protective effect by significantly reducing glycation (49). Chemometric analysis revealed that caffeic acid (CA) correlated strongly with BSA glycation inhibition activity of extracts in all phases of *in vitro* digestion (see section

Pearson's correlations). Given the promising antidiabetic potential of these extracts, further investigations could be conducted to assess their inhibitory effects on α-amylase and α-glucosidase, important enzymes involved in glucose metabolism.

Pearson's correlations of bioactive compounds and biological activity

Pearson's correlation coefficients between polyphenolic content, L-ascorbic acid and antioxidant, antihyperlipidemic and antiglycation activity of saffron tepals, tobacco and mallow petals and sterile flowers of marigold and sunflower are presented in Table IV for initial phase (a), intestinal phase (b) and for original samples (c).

Using Evans' (50) interpretation of correlations, a very strong positive correlation (Table IVa) was observed after the initial phase of *in vitro* digestion between TiC and TiP (1.00), TiPA (0.98), PrKA (0.98), CA (0.89), GA (0.84), and FRAP (0.93), as well as a strong positive correlation between TiC and ABTS (0.69) and DPPH (0.71). These findings indicate that the TiC significantly contribute to the antioxidant activity after the initial phase of digestion.

FRAP exhibited very strong correlations with TiP (0.92), TiPA (0.88) and PrKA (0.89), and strong correlations with GA (0.68), CA (0.71), HBA (0.67) and ABTS (0.79). These results suggest that among the individual identified compounds, PrKA was the most responsible for antioxidant activity as measured by the FRAP method, while TiP and TiPA also significantly contributed to the antioxidant (FRAP) activity after the initial phase of *in vitro* digestion. ABTS demonstrated very strong correlations with HBA (0.97), Q (0.88) and FA (0.85), along with a strong correlation with TiC (0.69). This indicates that HBA, Q and FA were the primary contributors to the antioxidant activity measured by the ABTS method. Similarly, DPPH exhibited a very strong correlation with FA (0.89) and strong correlations with TiC (0.71), TiP (0.69), TiPA (0.61), HBA (0.70), CA (0.66), SyrA (0.76) and ABTS (0.80). These results suggest that FA was the most influential compound in antioxidant activity measured by the DPPH method. Overall, the compounds most responsible for the antioxidant activity in the initial phase of digestion were PrKA, Q, FA and HBA, followed by *L*-ascorbic acid, SyrA, CA, *p*-KA and GA. Furthermore, after the initial phase of digestion, a very strong correlation was observed between TiP and TiPA (0.99), PrKA (0.98), CA (0.90) and FRAP (0.92). TiF correlated very strongly with *L*-ascorbic acid (0.93), Q (0.81), and SyrA (0.81), indicating that Q is the dominant flavonoid in the TiF parameter after the initial phase of digestion. Additionally, TiPA correlated very strongly with GA (0.92), PrKA (1.00), CA (0.91), and FRAP (0.88), suggesting that PrKA, GA, and CA were the main phenolic acids contributing to TiPA content. Additionally, a strong positive correlation was found between BSA and CA (0.74), suggesting that CA contributes the most to inhibition of BSA glycation in the initial phase of digestion.

After the intestinal phase of digestion (Table IVb), TiC exhibited very strong or strong positive correlations with TiP (1.00), TiPA (0.99), PrKA (1.00), HBA (0.68), CA (0.79), *p*-KA (0.67), ABTS (0.62), DPPH (0.74), and FRAP (0.82). TiPA correlated very strongly or strongly with PrKA (0.99), HBA (0.62), CA (0.83), *p*-KA (0.72), DPPH (0.69) and FRAP (0.80). These results indicate that PrKA, HBA, CA and *p*-KA significantly contributed to the content of TiC, TiP and TiPA after the intestinal phase of digestion. Furthermore, TiF exhibited very strong or strong positive correlations with K (0.94), SinA (0.87) and HBA (0.64) after the intestinal phase of digestion,

suggesting that K is the dominant flavonoid in TiF after this stage. In terms of antioxidant capacity, Q, GA, PrKA, HBA, CA, SyrA and FA were the most important after the intestinal phase of digestion. Notably, very strong or strong positive correlations were detected for: Q with ABTS (0.64) and DPPH (0.67); GA with DPPH (0.62) and FRAP (0.64); HBA with ABTS (0.72), DPPH (0.85), and FRAP (0.72); CA with FRAP (0.62); SyrA with ABTS (0.62), DPPH (0.71), and FRAP (0.64); FA with ABTS (0.75), DPPH (0.77), and FRAP (0.75). Q and SinA contributed to pancreatic lipase inhibition, as evidenced by their very strong positive correlation (0.83), while GA and CA contributed to BSA glycation inhibition, showing strong positive correlations (0.65 and 0.72, resp.) after the intestinal phase of *in vitro* digestion.

In the original samples (Table IVc), TiC correlated very strongly with TiP (1.00), TiPA (0.96), PrKA (0.96), CA (0.83) and SyrA (0.93), and strongly with GA (0.73), HBA (0.73), *p*-KA (0.67), FA (0.60) and BSA (0.66). TiPA correlated very strongly with GA (0.88), PrKA (0.99), CA (0.88), SyrA (0.81) and *p*-KA (0.83), and showed a strong correlation with DPPH (0.61). These findings suggest that GA, PrKA, HBA, CA, SyrA and *p*-KA significantly contribute to the content of TiC, TiP, and TiPA in original samples. TiF correlated very strongly with Q (0.81), L-AA (0.93) and pancreatic lipase (0.80), indicating that Q was the dominant flavonoid in original samples. Among individual compounds, L-AA and Q showed very strong positive correlations with pancreatic lipase inhibition (0.84 and 0.90, resp.), followed by HBA, FA and SinA, which exhibited strong positive correlations (0.75, 0.62 and 0.76, resp.). Regarding antioxidant activity in original samples, ABTS correlated strongly with K (0.63), FA (0.64) and SinA (0.79); DPPH correlated strongly with GA (0.66) and PrKA (0.70); FRAP correlated strongly with FA (0.64) and ABTS (0.77). Based on these correlation results, K, FA, SinA, GA, and PrKA appear to be the key compounds responsible for the antioxidant activity of the tested original extracts. Additionally, L-AA, Q, HBA and SinA contribute to pancreatic lipase inhibition, while SyrA, CA and FA strongly influence antiglycation activity, as evidenced by their strong positive correlations (0.75, 0.73 and 0.68, resp.).

Principal component analysis (PCA) of bioactive compounds and biological activity

Principal component analysis (PCA) between individual and total compounds and antioxidant, antihyperlipidemic and antidiabetic potential for the initial, intestinal phase and original samples was performed and presented in Fig. 3. This way of visualization effectively highlights the relationship between the phytochemical profile of the plant extracts and their biological activity, while also revealing similarities and differences among the analyzed samples (28, 29, 31, 34, 35, 44).

The first (Factor 1) and the second (Factor 2) principal component (PC) accounted for 43.8 % and 31.2 % of the variance after the initial phase of digestion, resp. (Fig. 3a). Together, the first two PCs represented 75.0 % of the total variability. After the intestinal phase of digestion, the first (Factor 1) and the second (Factor 2) PC accounted for 44.5 % and 29.0 % of the variance, resp. (Fig. 3b.). Together, the first two PCs represented 73.5 % of the total variability. Finally, the first (Factor 1) and the second (Factor 2) PC accounted for 39.2 % and 31.2 % of the variance (Fig. 3c) for original samples, cumulatively explaining 70.3% of the total variability. Across all three phases, a consistent separation of extracts was observed, with saffron and sunflower showing the greatest distance in the PCA plot, while mallow and marigold consistently clustered together, indicating higher similarity

in their phytochemical profiles. Sunflower was strongly associated with polyphenolics (TiPA, TiP, TiC, GA, *p*-KA, CA, PrKA), antioxidant capacity (FRAP, DPPH) and BSA glycation inhibition. Tobacco showed high loadings in TiF, FA, SyrA, HBA, L-AA, Q, pancreatic lipase inhibition and all antioxidant assays results (DPPH, ABTS, FRAP). In contrast, mallow and marigold exhibited less diverse phytochemical profile and showed weaker associations with biological activity. Mallow was primarily associated with IzoR and L, while marigold only with VA. Saffron had consistently strong loadings with SinA and K. These findings underscore the potential of tobacco petals and sunflower sterile ligulate flowers as valuable sources of bioactive compounds, exhibiting significant antioxidant, antiglycation, and pancreatic lipase inhibitory properties, and suggesting their application in health-promoting formulations.

CONCLUSIONS

Based on the results, all original samples can be considered significant sources of antioxidants and moderate sources of antihyperlipidemic compounds. Furthermore, almost all samples exhibited strong antidiabetic activity, with the exception of saffron, which demonstrated moderate antiglycation potential. Among the analyzed plants, sterile sunflower flowers and tobacco petals stood out as the samples with the highest antioxidant capacity both before and after *in vitro* digestion. Throughout nearly all phases of *in vitro* digestion, sunflower exhibited the highest levels of TiPA, TiP and TiC, while tobacco showed the highest TiF values after the initial and salivary phases as well as in the original sample. Saffron, on the other hand, had the highest TiF levels after the gastric and intestinal phases, and the highest TiP and TiC values after the gastric phase. This research contributes to a better understanding of the chemical composition and biopotential of the examined flowering parts during *in vitro* digestion. Our study employs a multi-phase simulated human digestion model which was used for the first time on a flower-derived material from saffron, mallow, marigold, sunflower and tobacco. Our findings demonstrate that extracts prepared from flowering parts of sunflower and tobacco serve as a rich source of phenolic acids and flavonoids and exhibit significant antioxidant and antidiabetic activity. Importantly, their biological activity remains largely preserved throughout *in vitro* digestion, indicating the stability of the bioactive compounds within the gastrointestinal tract. These findings highlight the potential of sunflower and tobacco flower extracts as promising candidates for the development of novel cosmetic formulations and their application in health-promoting products, such as functional food, beverages and dietary supplements. ,

Acronyms, abbreviations, symbols. – ABTS – 2,2 -azinobis(3- ethylbenzothiazoline-6-sulfonic acid), CA – caffeic acid, DPPH – 1,1-diphenyl-2-picrylhydrazyl, FA – ferulic acid, FRAP – ferric reducing/antioxidant power assay, GA – gallic acid, Gl. BSA – glycation of bovine serum albumin, HBA – hydroxybenzoic acid, IzoR – isorhamnetin, K – kaempferol, L – luteolin, L-AA – L-ascorbic acid, LIP – inhibition of pancreatic lipase, *p*-KA – *p*-coumaric acid, PrKA – protocatechuic acid, Q – quercetin, SinA – sinapic acid, SyrA – syringic acid, TiC – total identified compounds, TiF – total flavonoids, TiP – total identified phenols, TiPA – total phenolic acids, VA – vanillic acid.

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Authors contributions. – Conceptualization, V.V.B. and I.Š.; investigation, V.V.B., D.B. I.Š., A.V., original draft preparation, V.V.B. and D.B.; review and editing, V.V.B, D.B., I.Š., G.R. and Ž.M.; funding acquisition, G.R. and Ž.M. All the authors have read and agreed to the published version of the manuscript.

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Table I. Amount of total identified phenolic acids (TiPA), total identified flavonoids (TiF), total identified phenolic compounds (TiP) and total identified compounds (TiC) from selected flowering plants before/after in vitro *gastrointestinal digestion*

	Crocus heuffelianus	*Nicotiana tabacum*	*Malva sylvestris*	*Calendula officinalis*	*Helianthus annuus*
TiPA (μg mL^{-1})					
Initial	0.06 ± 0.00^{e}	2.30 ± 0.02^{b}	0.78 ± 0.06^{c}	0.50 ± 0.04^{d}	5.50 ± 0.31^{a}
Salivary	0.06 ± 0.00^{e}	2.31 ± 0.12^{b}	0.80 ± 0.05^{c}	0.47 ± 0.03^{d}	6.73 ± 0.33^{a}
Gastric	0.04 ± 0.00^{c}	0.51 ± 0.05^{b}	0.47 ± 0.06^{b}	0.15 ± 0.00^{c}	1.85 ± 0.15^{a}
Intestinal	0.06 ± 0.00^{e}	3.43 ± 0.13^{b}	0.87 ± 0.04^{c}	0.63 ± 0.03^{d}	4.98 ± 0.26^{a}
Original sample	0.06 ± 0.00^{d}	2.24 ± 0.48^{b}	0.82 ± 0.04^{c}	$0.54 \pm 0.07^{c,d}$	4.35 ± 0.52^{a}
TiF (μg mL^{-1})					
Initial	0.47 ± 0.02^{c}	1.02 ± 0.04^{a}	0.59 ± 0.04^{b}	0.51 ± 0.04^{c}	0.14 ± 0.02^{d}
Salivary	0.44 ± 0.01^{d}	0.78 ± 0.02^{a}	0.61 ± 0.03^{b}	0.48 ± 0.00^{c}	0.10 ± 0.01^{e}
Gastric	5.79 ± 0.46^{a}	2.72 ± 0.10^{c}	0.87 ± 0.05^{d}	3.16 ± 0.04^{b}	0.36 ± 0.03^{e}
Intestinal	0.55 ± 0.09^{a}	0.18 ± 0.01^{b}	0.03 ± 0.00^{c}	0.18 ± 0.02^{b}	0.01 ± 0.00^{c}
Original sample	0.40 ± 0.01^{c}	1.07 ± 0.10^{a}	0.62 ± 0.06^{b}	0.64 ± 0.02^{b}	0.09 ± 0.06^{d}
TiP (μg mL^{-1})					
Initial	0.52 ± 0.02^{e}	3.33 ± 0.05^{b}	1.38 ± 0.09^{c}	1.00 ± 006^{d}	5.64 ± 0.33^{a}
Salivary	0.50 ± 0.01^{e}	3.09 ± 0.12^{b}	1.42 ± 0.07^{c}	0.95 ± 0.03^{d}	6.84 ± 0.33^{a}
Gastric	5.83 ± 0.46^{a}	3.24 ± 0.13^{b}	1.36 ± 0.11^{d}	3.30 ± 0.03^{b}	2.21 ± 0.18^{c}
Intestinal	0.61 ± 0.09^{d}	3.61 ± 0.13^{b}	0.90 ± 0.04^{c}	$0.81 \pm 0.03^{c,d}$	4.98 ± 0.26^{a}
Original sample	0.47 ± 0.01^{d}	3.31 ± 0.58^{b}	1.46 ± 0.10^{c}	1.19 ± 0.09^{c}	4.43 ± 0.45^{a}
TiC (μg mL^{-1})					
Initial	0.62 ± 0.03^{e}	3.66 ± 0.05^{b}	1.52 ± 0.10^{c}	1.16 ± 0.07^{d}	5.72 ± 0.34^{a}

Salivary	0.58 ± 0.01^{e}	3.42 ± 0.14^{b}	1.56 ± 0.07^{c}	1.09 ± 0.04^{d}	6.91 ± 0.33^{a}
Gastric	5.83 ± 0.46^{a}	3.24 ± 0.13^{b}	1.36 ± 0.11^{d}	3.30 ± 0.03^{b}	2.21 ± 0.18^{c}
Intestinal	1.17 ± 0.09^{d}	4.50 ± 0.15^{b}	1.53 ± 0.02^{c}	1.49 ± 0.03^{c}	5.57 ± 0.25^{a}
Original sample	0.58 ± 0.01^{d}	3.67 ± 0.57^{b}	1.63 ± 0.10^{c}	1.38 ± 0.10^{c}	4.54 ± 0.45^{a}

dm – dry mass basis, TiC = TiP + L-ascorbic acid. Values represent mean ± standard deviation of three biological and three technical replicates (N = 9). Different lower case letters indicate significant difference within each phase separately.

Table II. Content of individual phenolic acid from selected flowering plants before/after in vitro *gastrointestinal digestion*

	Crocus heuffelianus	*Nicotiana tabacum*	*Malva sylvestris*	*Calendula officinalis*	*Helianthus annuus*
Ferulic acid (μg mL^{-1})					
Initial	0.01 ± 0.00^{d}	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}	nd	0.01 ± 0.00^{c}
Salivary	0.01 ± 0.00^{d}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	nd	0.02 ± 0.00^{c}
Gastric	nd	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}	nd	0.02 ± 0.00^{b}
Intestinal	0.01 ± 0.00^{d}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	nd	0.01 ± 0.00^{c}
Original sample	0.01 ± 0.00^{d}	0.03 ± 0.00^{a}	0.02 ± 0.00^{b}	nd	0.02 ± 0.00^{c}
Sinapic acid (μg mL^{-1})					
Initial	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	nd	nd	nd
Salivary	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	nd	nd	nd
Gastric	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	nd	nd	nd
Intestinal	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}	nd	nd	nd
Original sample	0.02 ± 0.00^{a}	0.02 ± 0.00^{b}	nd	nd	nd
Protocatechuic acid (μg mL^{-1})					
Initial	nd	1.68 ± 0.01^{b}	$0.13 \pm 0.03^{c,d}$	0.27 ± 0.02^{c}	4.28 ± 0.27^{a}
Salivary	nd	1.69 ± 0.11^{b}	0.09 ± 0.03^{c}	0.24 ± 0.02^{c}	5.42 ± 0.30^{a}
Gastric	nd	0.02 ± 0.03^{b}	nd	nd	0.87 ± 0.09^{a}
Intestinal	nd	2.79 ± 0.10^{b}	0.15 ± 0.03^{d}	0.40 ± 0.03^{c}	3.83 ± 0.26^{a}
Original sample	nd	1.60 ± 0.47^{b}	0.11 ± 0.01^{c}	0.31 ± 0.06^{c}	2.98 ± 0.40^{a}
Cinammic acid (μg mL^{-1})					
Initial	nd	nd	nd	nd	nd
Salivary	nd	nd	nd	nd	nd

Gastric	nd	0.27 ± 0.09[a]	nd	nd	nd
Intestinal	nd	nd	nd	nd	nd
Original sample	nd	nd	nd	nd	nd
Caffeic acid (μg mL^{-1})					
Initial	nd	0.39 ± 0.01[c]	0.49 ± 0.07[b]	0.16 ± 0.01[d]	0.91 ± 0.05[a]
Salivary	nd	0.40 ± 0.01[c]	0.54 ± 0.05[b]	0.16 ± 0.01[d]	0.93 ± 0.03[a]
Gastric	nd	0.32 ± 0.01[b]	0.35 ± 0.05[b]	0.12 ± 0.00[c]	0.68 ± 0.04[a]
Intestinal	nd	0.36 ± 0.02[c]	0.50 ± 0.04[b]	0.14 ± 0.01[d]	0.86 ± 0.01[a]
Original sample	nd	0.40 ± 0.01[c]	0.56 ± 0.04[b]	0.16 ± 0.01[d]	0.95 ± 0.05[a]
Syringic acid (μg mL^{-1})					
Initial	nd	0.12 ± 0.00[a]	0.07 ± 0.00[b]	0.02 ± 0.00[d]	0.03 ± 0.00[c]
Salivary	nd	0.13 ± 0.01[a]	0.08 ± 0.01[b]	0.02 ± 0.00[d]	0.06 ± 0.00[c]
Gastric	nd	0.09 ± 0.01[a]	0.05 ± 0.01[b]	0.02 ± 0.00[c]	0.06 ± 0.02[b]
Intestinal	nd	0.12 ± 0.02[a]	0.08 ± 0.00[b]	0.02 ± 0.00[d]	0.03 ± 0.00[c]
Original sample	nd	0.14 ± 0.02[a]	0.06 ± 0.01[b]	0.02 ± 0.00[c]	0.11 ± 0.04[a]
p-Coumaric acid (μg mL^{-1})					
Initial	0.03 ± 0.00[c]	0.02 ± 0.00[d]	0.06 ± 0.00[b]	0.01 ± 0.00[c]	0.20 ± 0.00[a]
Salivary	0.03 ± 0.00[c]	0.01 ± 0.00[d]	0.06 ± 0.00[b]	0.01 ± 0.00[d]	0.21 ± 0.01[a]
Gastric	0.03 ± 0.00[c]	0.01 ± 0.00[d]	0.05 ± 0.00[b]	0.00 ± 0.00[d]	0.20 ± 0.01[a]
Intestinal	0.03 ± 0.00[c]	0.01 ± 0.00[d]	0.05 ± 0.00[b]	0.00 ± 0.00[d]	0.19 ± 0.01[a]
Original sample	0.03 ± 0.00[c]	0.02 ± 0.00[c,d]	0.06 ± 0.00[b]	0.01 ± 0.00[d]	0.21 ± 0.02[a]
p-Hydroxybenzoic acid (μg mL^{-1})					
Initial	nd	0.04 ± 0.00[a]	nd	0.00 ± 0.00[c]	0.01 ± 0.00[b]

Salivary	nd	0.03 ± 0.00[a]	nd	0.00 ± 0.00[c]	0.01 ± 0.00[b]
Gastric	nd	0.03 ± 0.00[a]	nd	nd	0.01 ± 0.00[b]
Intestinal	nd	0.03 ± 0.00[a]	nd	0.00 ± 0.00[c]	0.01 ± 0.00[b]
Original sample	nd	0.03 ± 0.00[a]	nd	0.00 ± 0.00[c]	0.01 ± 0.00[b]
Gallic acid (μg mL^{-1})					
Initial	nd	nd	nd	nd	0.03 ± 0.00[a]
Salivary	nd	nd	nd	nd	0.04 ± 0.00[a]
Gastric	nd	nd	nd	nd	0.01 ± 0.00[a]
Intestinal	nd	0.06 ± 0.00[a]	0.06 ± 0.00[a]	nd	0.02 ± 0.00[b]
Original sample	nd	nd	nd	nd	0.03 ± 0.00[a]
Vanillic acid (μg mL^{-1})					
Initial	nd	nd	0.01 ± 0.00[c]	0.04 ± 0.00[a]	0.03 ± 0.00[a]
Salivary	nd	nd	0.01 ± 0.00[c]	0.04 ± 0.00[a]	0.04 ± 0.00[b]
Gastric	nd	nd	0.00 ± 0.00[c]	0.01 ± 0.00[a]	0.02 ± 0.00[b]
Intestinal	nd	nd	0.01 ± 0.00[c]	0.06 ± 0.00[a]	0.03 ± 0.00[b]
Original sample	nd	nd	0.01 ± 0.00[c]	0.04 ± 0.01[a]	0.03 ± 0.00[b]

dm – dry mass basis, nd – not detected. Values represent mean ± standard deviation of three biological and three technical replicates (N = 9). Different lower case letters indicate significant difference within each phase separately.

Table III. Content of L*-ascorbic acid and individual flavonoids from selected flowering plants before/after* in vitro *gastrointestinal digestion*

	Crocus heuffelianus	*Nicotiana tabacum*	*Malva sylvestris*	*Calendula officinalis*	*Helianthus annuus*
L-ascorbic acid (μg mL^{-1})					
Initial	0.10 ± 0.00^{c}	0.34 ± 0.03^{a}	0.14 ± 0.01^{b}	0.16 ± 0.00^{b}	0.08 ± 0.01^{c}
Salivary	0.08 ± 0.00^{c}	0.33 ± 0.02^{a}	0.13 ± 0.01^{b}	0.14 ± 0.01^{b}	0.07 ± 0.00^{c}
Gastric	nd	nd	nd	nd	nd
Intestinal	0.57 ± 0.00^{d}	0.89 ± 0.03^{a}	0.63 ± 0.03^{c}	0.68 ± 0.02^{b}	0.59 ± 0.01^{d}
Original sample	0.11 ± 0.01^{d}	0.36 ± 0.00^{a}	0.17 ± 0.01^{c}	0.19 ± 0.01^{b}	0.11 ± 0.01^{d}
Quercetin (μg mL^{-1})					
Initial	0.07 ± 0.01^{c}	0.82 ± 0.04^{a}	0.01 ± 0.00^{d}	0.18 ± 0.01^{b}	0.09 ± 0.01^{c}
Salivary	0.06 ± 0.00^{c}	0.61 ± 0.02^{a}	0.01 ± 0.00^{d}	0.17 ± 0.01^{b}	0.06 ± 0.00^{c}
Gastric	0.62 ± 0.01^{b}	2.11 ± 0.08^{a}	0.02 ± 0.00^{e}	0.41 ± 0.01^{c}	0.29 ± 0.02^{d}
Intestinal	0.08 ± 0.00^{b}	0.16 ± 0.01^{a}	nd	0.03 ± 0.00^{c}	0.01 ± 0.00^{d}
Original sample	0.06 ± 0.00^{c}	0.85 ± 0.06^{a}	0.01 ± 0.00^{c}	0.21 ± 0.01^{b}	0.06 ± 0.05^{c}
Isorhamnetin (μg mL^{-1})					
Initial	nd	0.03 ± 0.00^{c}	0.56 ± 0.05^{a}	0.32 ± 0.04^{b}	0.05 ± 0.01^{c}
Salivary	nd	$0.03 \pm 0.00^{c,d}$	0.57 ± 0.03^{a}	0.29 ± 0.02^{b}	0.04 ± 0.01^{c}
Gastric	nd	0.06 ± 0.00^{c}	0.81 ± 0.05^{b}	2.71 ± 0.03^{a}	0.00 ± 0.00^{d}
Intestinal	nd	0.00 ± 0.00^{c}	0.03 ± 0.00^{b}	0.16 ± 0.02^{a}	nd
Original sample	nd	0.03 ± 0.00^{c}	0.58 ± 0.06^{a}	0.42 ± 0.03^{b}	0.03 ± 0.02^{c}
Kaempferol (μg mL^{-1})					
Initial	0.40 ± 0.01^{a}	0.18 ± 0.01^{b}	0.02 ± 0.00^{c}	$0.01 \pm 0.00^{c,d}$	nd

Salivary	0.38 ± 0.01[a]	0.14 ± 0.00[b]	0.02 ± 0.00[c]	0.01 ± 0.00[d]	nd
Gastric	5.17 ± 0.45[a]	0.55 ± 0.02[b]	0.04 ± 0.00[c]	0.03 ± 0.00[c]	0.06 ± 0.01[c]
Intestinal	0.47 ± 0.09[a]	0.02 ± 0.00[b]	nd	nd	nd
Original sample	0.34 ± 0.01[a]	0.20 ± 0.03[b]	0.03 ± 0.00[c]	0.02 ± 0.00[c]	nd
Luteoline (µg mL^{-1})					
Initial	nd	nd	0.02 ± 0.00	nd	nd
Salivary	nd	nd	0.02 ± 0.00	nd	nd
Gastric	nd	nd	0.02 ± 0.00	nd	nd
intestinal	nd	nd	nd	nd	nd
Original sample	nd	nd	0.02 ± 0.00	nd	nd

dm – dry mass basis; nd – not detected. Values represent mean ± standard deviation of three biological and three technical replicates (N = 9). Different lower case letters indicate significant difference within each phase separately.

Table IV. Pearson's correlation coefficients between the phytochemical content, antioxidant capacity, antihyperlipidemic and antihyperglycemic activity during simulated in vitro *gastrointestinal digestion: a) initial phase, b) intestinal phase, and c) original samples in 40 % EtOH*

a)	TiC i	TiP i	TiF i	TiP A i	L-AA i	Q i	L i	K i	Izo R i	GA i	PrK A i	HB A i	VA i	CA i	Syr A i	*p*-KA i	FA i	Sin A i	ABTS i	DPPH i	FRAP i	Gl. BSA i	LIP i
TiC i	1.00																						
TiP i	**1.00**	1.00																					
TiF i	–0.22	–0.26	1.00																				
TiPA i	**0.98**	**0.99**	–0.39	1.00																			
L-AA i	0.12	0.07	**0.93**	–0.07	1.00																		
Q i	0.29	0.25	0.81	0.12	**0.96**	1.00																	
L i	–0.27	–0.26	0.08	–0.26	–0.13	–0.37	1.00																
K i	–0.42	–0.42	0.25	–0.44	0.07	0.14	–0.32	1.00															

IzoR i	–0.40	–0.39	0.02	–0.38	–0.15	–0.41	0.85	–0.57	1.00							
GA i	0.84	0.86	–0.71	**0.92**	–0.44	–0.24	–0.25	–0.40	–0.33	1.00						
PrKA i	**0.98**	**0.98**	0.40	**1.00**	–0.07	0.14	–0.35	–0.38	–0.45	**0.93**	1.00					
HBA i	0.50	0.46	0.70	0.34	**0.90**	**0.97**	–0.35	0.06	–0.45	–0.03	0.35	1.00				
VA i	0.31	0.33	–0.63	0.41	–0.41	–0.33	–0.27	–0.70	0.14	0.55	0.42	–0.30	1.00			
CA i	**0.89**	**0.90**	–0.37	**0.91**	–0.12	–0.04	0.16	–0.65	0.02	0.83	0.86	0.19	0.36	1.00		
SyrA i	0.29	0.25	0.81	0.12	**0.88**	0.79	0.25	–0.17	0.10	–0.25	0.08	0.82	–0.44	0.23	1.00	
p-KA i	0.78	0.81	–0.73	0.87	–0.51	–0.36	–0.01	–0.40	–0.18	**0.97**	0.86	–0.13	0.42	0.87	–0.22	1.00

FA i	0.44	0.41	0.72	0.28	0.81	0.78	0.17	–0.05	–0.10	–0.08	0.25	0.85	–0.52	0.35	**0.96**	–0.04	1.00					
SinA i	–0.31	–0.33	0.48	–0.38	0.33	0.38	–0.28	**0.96**	–0.58	–0.45	–0.34	0.31	–0.83	–0.58	0.12	–0.45	0.23	1.00				
ABTS i	0.69	0.65	0.53	0.55	0.77	**0.88**	–0.35	–0.01	–0.51	0.20	0.55	**0.97**	–0.23	0.39	0.76	0.11	0.85	0.23	1.00			
DPPH i	0.71	0.69	0.34	0.61	0.49	0.53	0.17	–0.13	–0.19	0.33	0.57	0.70	–0.38	0.66	0.76	0.39	**0.89**	0.10	0.80	1.00		
FRAP i	**0.93**	**0.92**	–0.02	**0.88**	0.35	0.53	–0.48	–0.41	–0.47	0.68	**0.89**	0.67	0.37	0.71	0.38	0.54	0.47	–0.27	0.79	0.60	1.00	
Gl. BSA i	0.59	0.58	0.04	0.55	0.26	0.20	0.29	**–0.92**	0.42	0.37	0.49	0.31	0.47	0.74	0.51	0.37	0.43	–0.78	0.37	0.46	0.60	1.00
LIP i	–0.60	–0.59	–0.09	–0.55	–0.17	–0.13	–0.55	0.36	–0.19	–0.37	–0.48	–0.32	0.26	–0.77	–0.60	–0.53	–0.70	0.18	–0.45	**–0.89**	–0.37	–0.57	1.00

b)	TiCc	TiPc	TiFc	TiPAc	L-AAc	Qc	Lc	Kc	IzoRc	GAc	PrKAc	HBAc	VAc	CAc	SyrAc	*p*-KAc	FAc	SinAc	ABTSc	DPPHc	FRAPc	Gl. BSAc	LIPc
TiCc	1.00																						
TiPc	**1.00**	1.00																					
TiFc	–0.50	–0.50	1.00																				
TiPAc	**0.99**	**1.00**	–0.58	1.00																			
L-AAc	0.32	0.26	–0.16	0.27	1.00																		
Qc	0.20	0.15	0.47	0.10	0.74	1.00																	
Lc	–0.36	–0.36	–0.42	–0.30	–0.16	–0.46	1.00																
Kc	–0.44	–0.43	**0.94**	–0.50	–0.41	0.28	–0.27	1.00															
IzoRc	–0.46	–0.47	–0.10	–0.43	0.01	–0.34	–0.06	–0.33	1.00														

GA c	0.32	0.29	–0.54	0.33	0.57	0.24	0.58	–0.50	–0.43	1.00							
PrKAc	**1.00**	**1.00**	–0.49	**0.99**	0.31	0.19	–0.41	–0.44	–0.42	0.27	1.00						
HBAc	0.68	0.64	–0.17	0.62	**0.88**	0.78	–0.37	–0.32	–0.32	0.53	0.67	1.00					
VA c	–0.06	–0.04	–0.35	–0.01	–0.18	–0.56	–0.22	–0.47	0.85	–0.51	–0.01	–0.31	1.00				
CA c	0.76	0.78	–0.83	0.83	–0.07	–0.39	0.22	–0.63	–0.37	0.45	0.75	0.20	0.06	1.00			
SyrAc	0.42	0.37	–0.45	0.39	0.84	0.50	0.28	–0.53	–0.31	**0.92**	0.37	0.78	–0.42	0.30	1.00		
p-KAc	0.67	0.71	–0.49	0.72	–0.46	–0.48	–0.04	–0.22	–0.42	–0.01	0.67	–0.08	0.10	0.86	–0.17	1.00	
FA c	0.41	0.38	–0.40	0.40	0.64	0.42	0.42	–0.37	–0.56	**0.97**	0.36	0.66	–0.63	0.40	**0.94**	–0.01	1.00

SinAc	–0.30	–0.30	0.87	–0.38	0.03	0.65	–0.20	0.88	–0.51	–0.10	–0.31	0.09	–0.76	–0.61	–0.06	–0.41	0.07	1.00				
ABTS c	0.62	0.61	0.02	0.57	0.43	0.64	–0.10	0.10	**–0.89**	0.59	0.59	0.72	–0.81	0.33	0.62	0.23	0.75	0.45	1.00			
DPPH c	0.74	0.72	–0.12	0.69	0.58	0.67	–0.18	–0.09	–0.77	0.62	0.71	0.85	–0.66	0.39	0.71	0.22	0.77	0.29	**0.97**	1.00		
FRAP c	0.82	0.81	–0.29	0.80	0.39	0.43	–0.06	–0.18	–0.83	0.64	0.79	0.72	–0.60	0.62	0.64	0.47	0.75	0.13	**0.94**	**0.96**	1.00	
Gl. BSA c	0.44	0.43	**–0.98**	0.51	0.34	–0.33	0.45	**–0.97**	0.15	0.65	0.43	0.28	0.30	0.72	0.59	0.30	0.51	–0.82	0.01	0.16	0.29	1.00	
LIP c	–0.17	–0.20	0.54	–0.25	0.51	0.83	0.03	0.46	–0.48	0.41	–0.20	0.45	–0.84	–0.48	0.49	–0.58	0.54	0.83	0.61	0.53	0.33	–0.39	1.00

c)	TiCm	TiPm	TiFm	TiPAm	L-AAm	Q m	L m	K m	IzoRm	GAm	PrKAm	HBAm	VAm	CAm	SyrAm	*p*-KAm	FAm	SinAm	ABTSm	DPPHm	FRAPm	Gl.BSAm	LIPm
TiC m	1.00																						
TiP m	**1.00**	1.00																					
TiF m	–0.09	–0.15	1.00																				
TiPAm	**0.96**	**0.98**	–0.35	1.00																			
L-AAm	0.29	0.23	**0.93**	0.03	1.00																		
Q m	0.39	0.34	0.81	0.15	**0.95**	1.00																	
L m	–0.24	–0.24	0.08	–0.25	–0.08	–0.36	1.00																
K m	–0.39	–0.40	0.22	–0.43	0.10	0.26	–0.33	1.00															

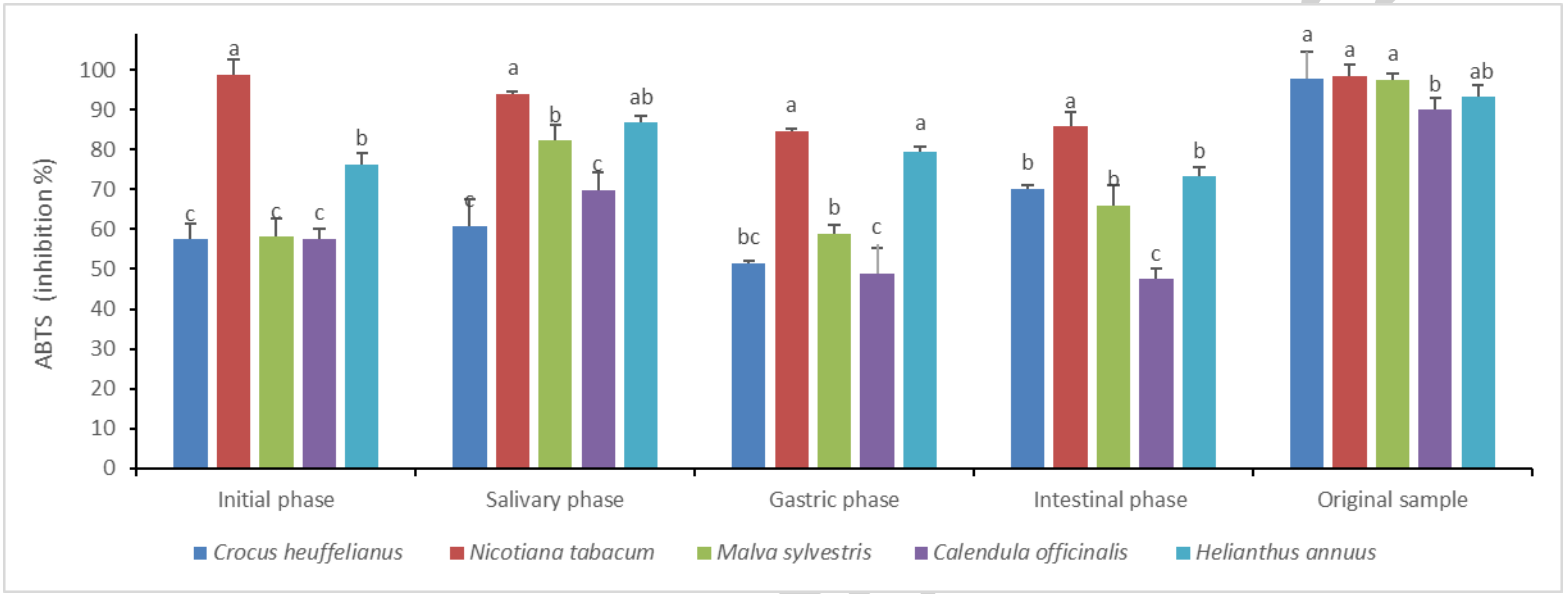
IzoRm	–0.41	–0.41	0.17	–0.43	–0.04	–0.35	0.76	–0.59	1.00							
GA m	0.73	0.77	–0.74	**0.88**	–0.44	–0.29	–0.25	–0.44	–0.38	1.00						
PrKAm	**0.96**	**0.97**	–0.32	**0.99**	0.06	0.22	–0.39	–0.34	–0.53	0.86	1.00					
HBAm	0.73	0.70	0.53	0.55	0.80	**0.91**	–0.41	0.08	–0.50	0.13	0.61	1.00				
VA m	0.17	0.19	–0.40	0.27	–0.31	–0.30	–0.29	–0.72	0.25	0.44	0.27	–0.18	1.00			
CA m	0.83	0.85	–0.39	**0.88**	–0.10	–0.11	0.21	–0.65	–0.02	0.82	0.80	0.28	0.24	1.00		
SyrAm	**0.93**	**0.91**	0.24	0.81	0.57	0.61	–0.07	–0.27	–0.31	0.45	0.80	0.86	–0.10	0.72	1.00	
p-KAm	0.67	0.71	–0.77	0.83	–0.51	–0.41	–0.02	–0.45	–0.26	**0.97**	0.78	0.01	0.30	0.87	0.42	1.00

FA m	0.60	0.56	0.50	0.43	0.68	0.61	0.32	–0.05	–0.09	0.03	0.39	0.71	–0.54	0.48	0.84	0.10	1.00					
SinA m	–0.27	–0.29	0.41	–0.36	0.32	0.44	–0.21	**0.96**	–0.54	–0.49	–0.29	0.25	–0.85	–0.55	–0.06	–0.48	0.22	1.00				
ABTS m	–0.04	–0.06	0.36	–0.13	0.32	0.31	0.32	0.63	–0.26	–0.33	–0.15	0.25	**–0.99**	–0.08	0.23	–0.18	0.64	0.79	1.00			
DPPH m	0.52	0.54	–0.45	0.61	–0.19	0.13	–0.83	0.26	**–0.91**	0.66	0.70	0.38	0.16	0.22	0.29	0.51	–0.12	0.15	–0.13	1.00		
FRAP m	0.47	0.48	–0.11	0.47	0.06	0.18	0.04	0.40	–0.60	0.34	0.46	0.39	–0.69	0.42	0.54	0.44	0.64	0.51	0.77	0.40	1.00	
Gl. BSA m	0.66	0.64	0.26	0.55	0.46	0.29	0.41	–0.75	0.39	0.26	0.47	0.46	0.19	0.73	0.75	0.30	0.68	–0.54	–0.07	–0.29	0.06	1.00
LIP m	0.15	0.10	0.80	–0.07	0.84	**0.90**	–0.22	0.59	–0.41	–0.47	–0.01	0.75	–0.67	–0.29	0.43	–0.52	0.62	0.76	0.65	0.06	0.40	0.03	1.00

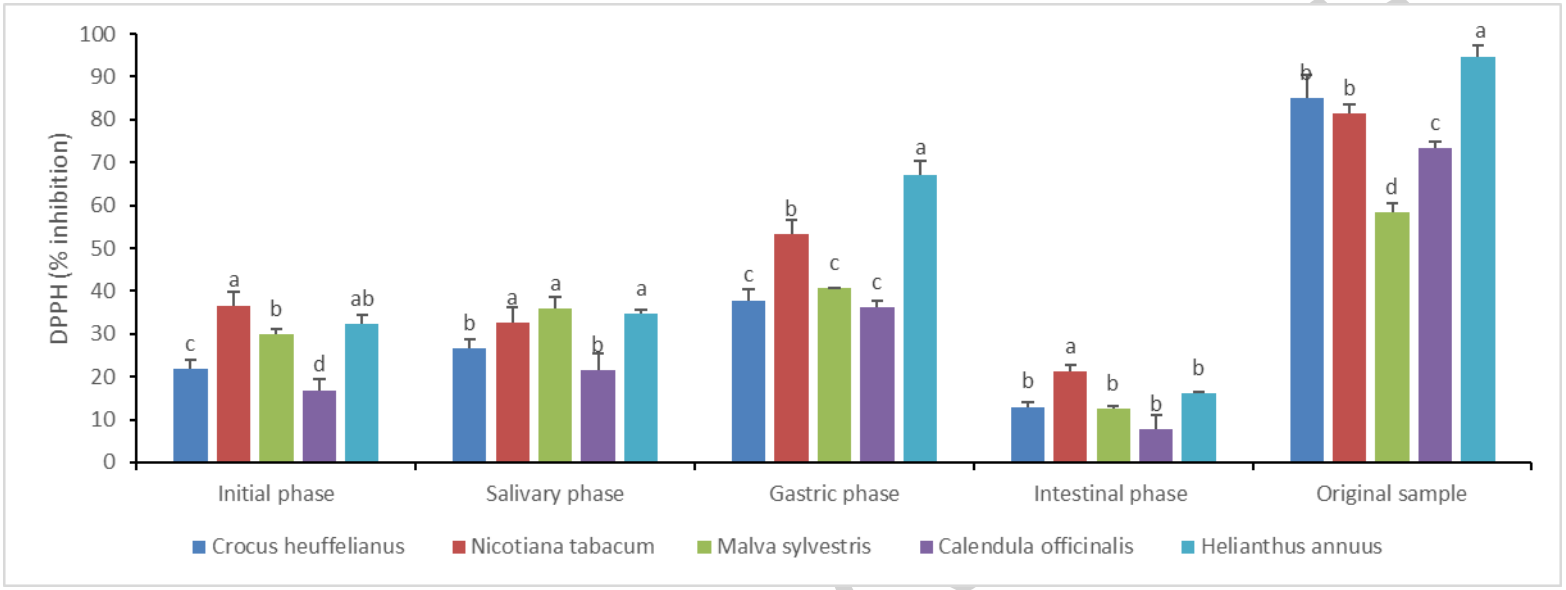
ABTS – 2,2 -azinobis(3- ethylbenzothiazoline-6-sulfonic acid), CA – caffeic acid, DPPH – 1,1-diphenyl-2-picrylhydrazyl, FA – ferulic acid, FRAP – ferric reducing/antioxidant power assay, GA – gallic acid, Gl. BSA – glycation of bovine serum albumin, HBA – hydroxybenzoic acid, IzoR – isorhamnetin, K – kaempferol, L – luteolin, L-AA – L-ascorbic acid, LIP – inhibition of pancreatic lipase, *p*-KA – *p*-coumaric acid, PrKA – protocatechuic acid, Q – quercetin, SinA – sinapic acid, SyrA – syringic acid, TiC – total identified compounds, TiF – total flavonoids, TiP – total identified phenols, TiPA – total phenolic acids, VA – vanillic acid.

Bold values denote significance at $p \leq 0.05$. Phases of *in vitro* digestion are represented by different letters: *i* – initial phase, *c* – intestinal phase, *m* – original samples.

a)



b)



c)

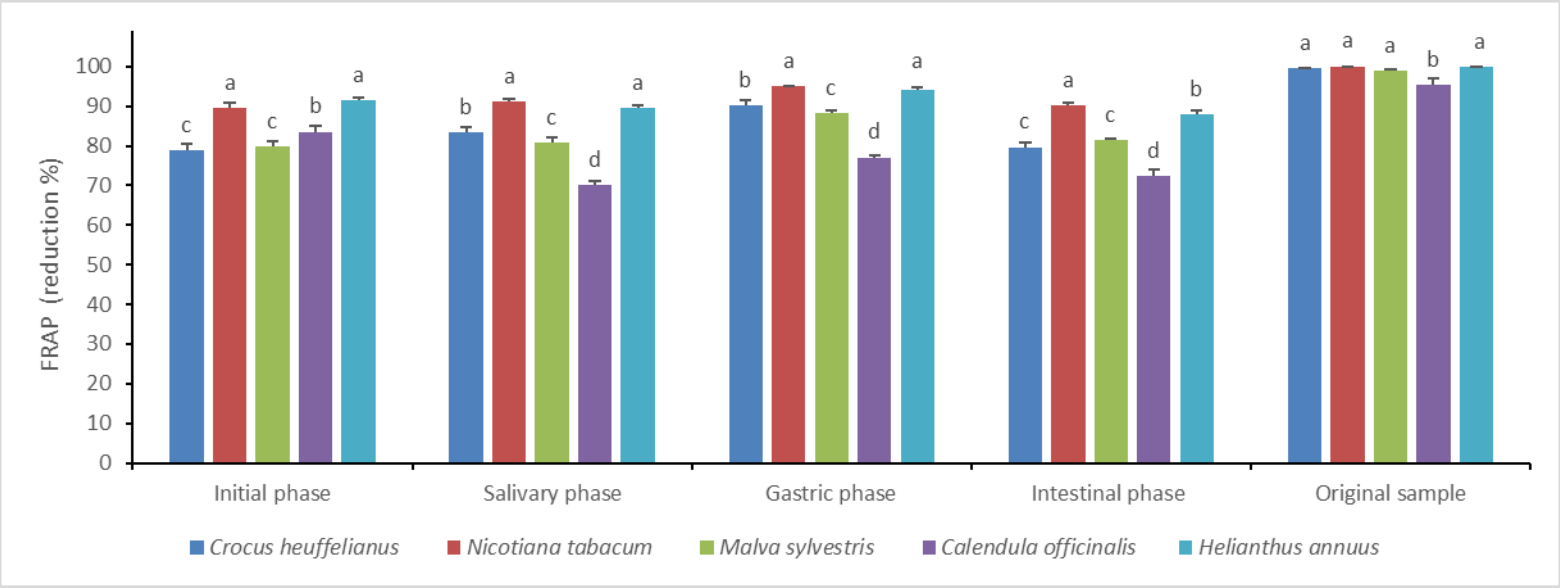
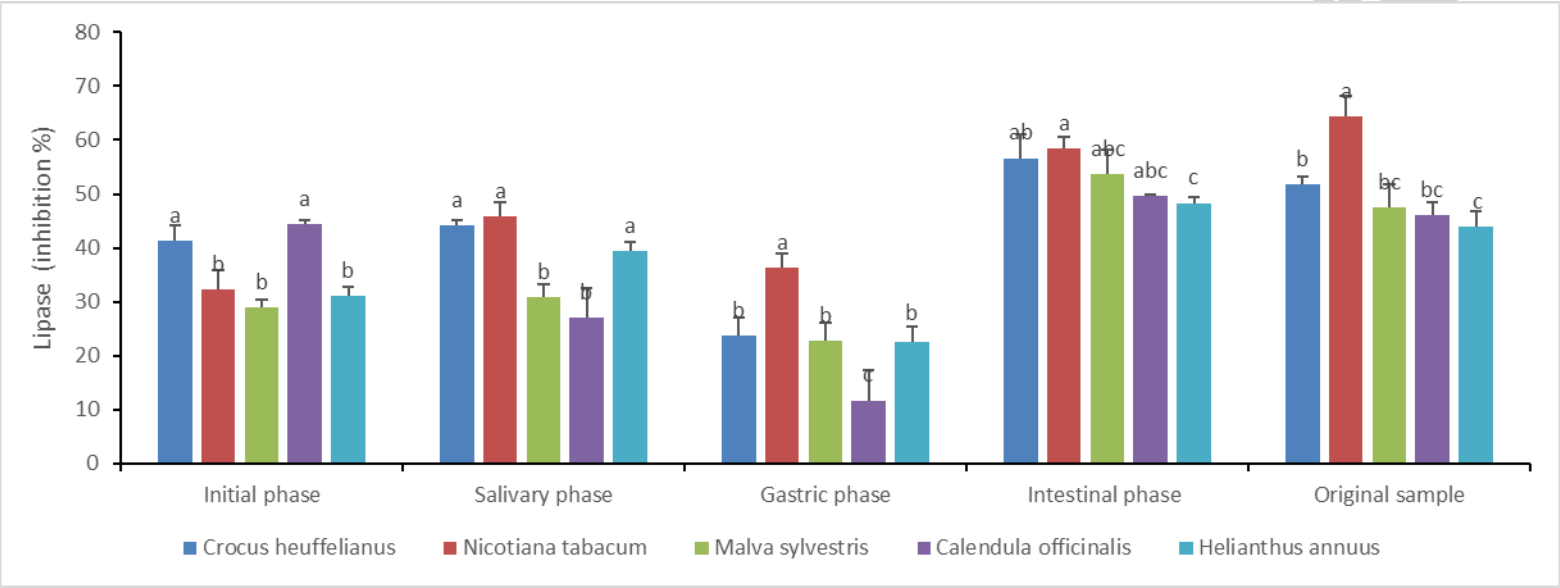


Fig. 1. Antioxidant activity: a) ABTS; b) DPPH and c) FRAP of tested plant extracts. Values represent mean ± SD of 3 replicates. Different letters indicate significant difference at $p < 0.05$.

a)



b)

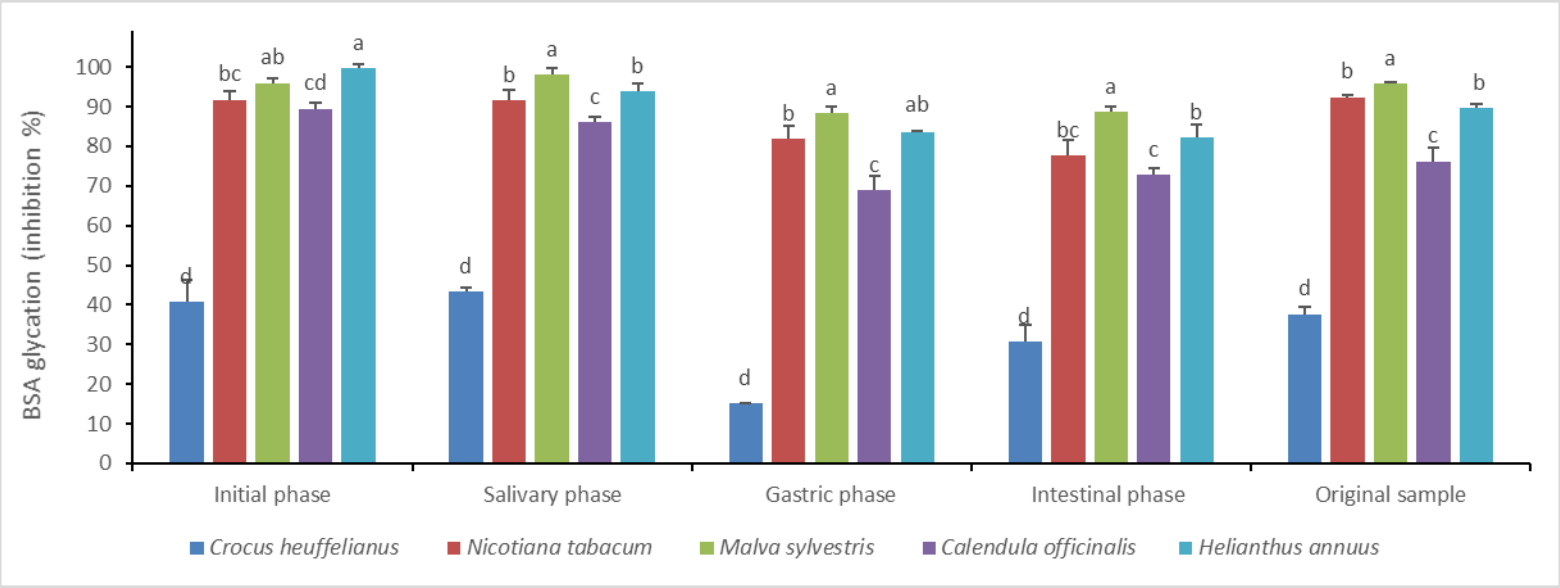
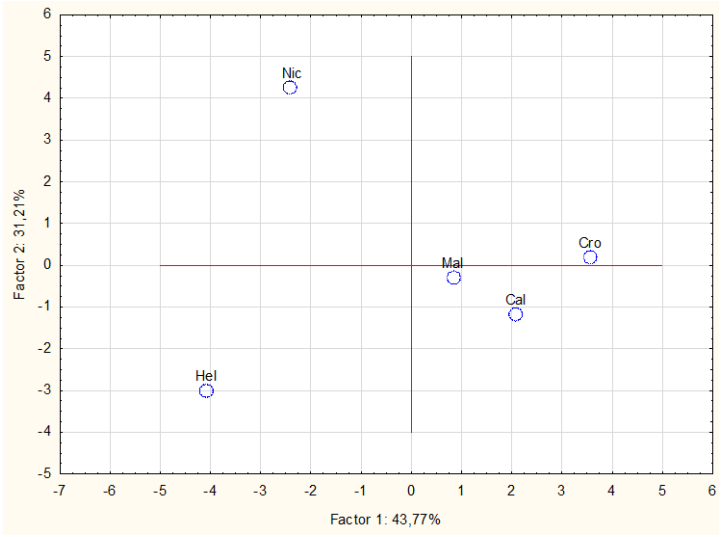
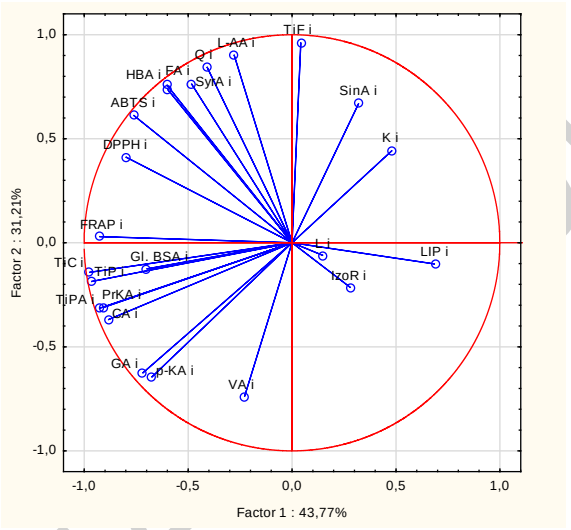


Fig. 2. Antihyperlipidemic and antihyperglycemic activity: a) pancreatic lipase inhibition and b) BSA glycation inhibition of tested plant extracts. Data are presented as mean value ± SD, $N = 3$. Different letters indicate significant differences at $p < 0.05$.

a)



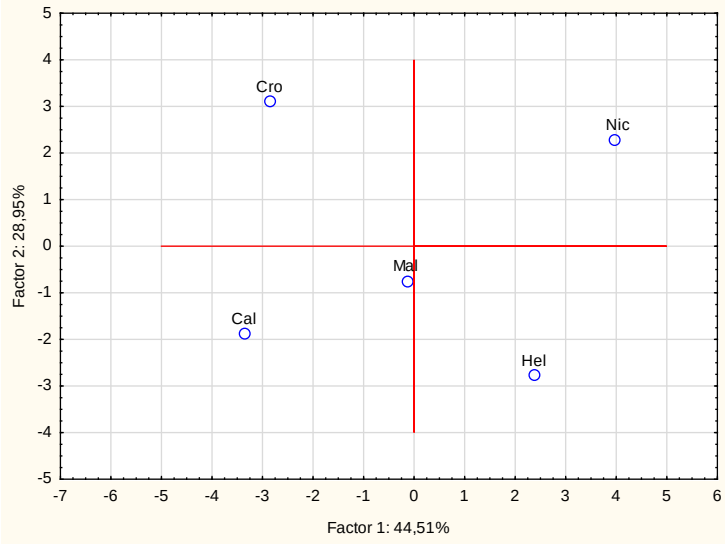
(*i*)



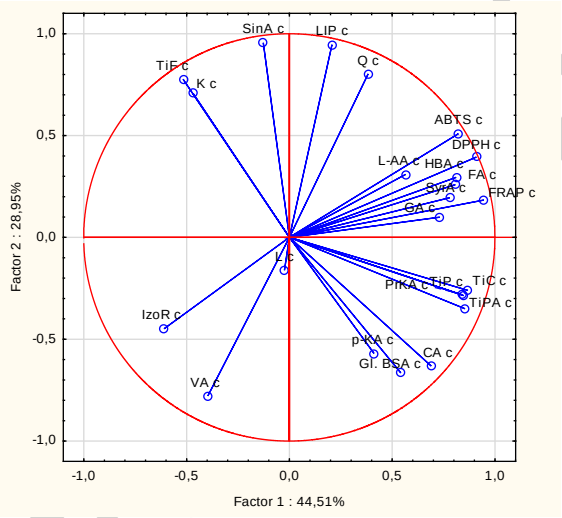
(*ii*)

b)

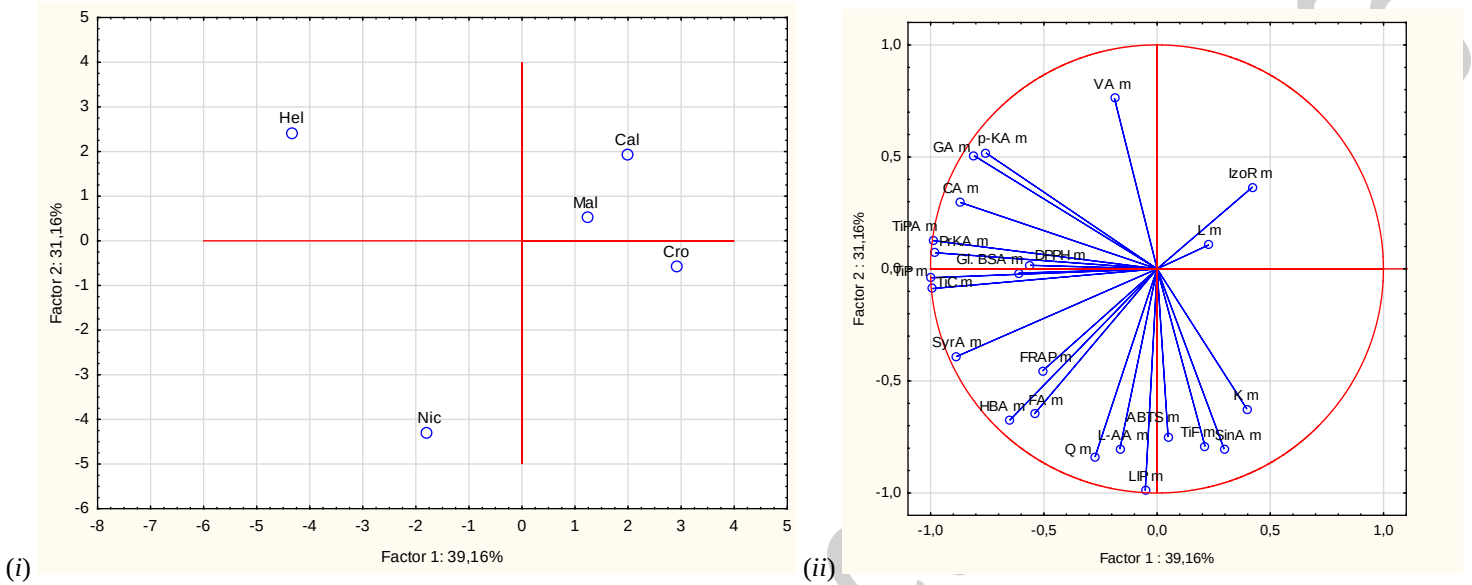
(*i*)



(*ii*)



c)



(*i*) (*ii*)

Fig. 3. Principal component analysis (PCA) diagram of the measured polyphenols, L-ascorbic acid, antioxidant, antihyperlipidemic and antihyperglycemic activity in ethanolic extracts of five plant species during simulated *in vitro* gastrointestinal digestion: a) initial phase, b) intestinal phase, c) original samples: (i) score plot separating samples of tepals (Cro = *Crocus heuffelianus*), petals (Nic = *Nicotiana tabacum*, Mal = *Malva sylvestris*), and sterile ligulate flowers (Cal = *Calendula officinalis*, Hel = *Helianthus annuus*), (ii) loading plot of polyphenols, *L*-ascorbic acid, antioxidant and antidiabetic activity as variables.

ABTS – 2,2 -azinobis(3- ethylbenzothiazoline-6-sulfonic acid), CA – caffeic acid, DPPH – 1,1-diphenyl-2-picrylhydrazyl, FA – ferulic acid, FRAP – ferric reducing/antioxidant power assay, GA – gallic acid, Gl. BSA – glycation of bovine serum albumin, HBA – hydroxybenzoic acid, IzoR – isorhamnetin, K – kaempferol, L – luteolin, L-AA – L-ascorbic acid, LIP – inhibition of pancreatic lipase, *p*-KA – *p*-coumaric acid, PrKA – protocatechuic acid, Q – quercetin, SinA – sinapic acid, SyrA – syringic acid, TiC – total identified compounds, TiF – total flavonoids, TiP – total identified phenols, TiPA – total phenolic acids, VA – vanillic acid.