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Cytotoxic and bioactive properties of different color tulip flowers and degradation kinetic of tulip flower anthocyanins

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ABSTRACT

This study was conducted to determine the potential use of anthocyanin-based extracts (ABEs) of wasted tulip flowers as food/drug colorants. For this aim, wasted tulip flowers were samples and analyzed for their bioactive properties and cytotoxicity. Total phenolic contents of the extracts of the claret red (126.55 mg of gallic acid equivalent (GAE)/g dry extract) and orange–red (113.76 mg GAE/g dry extract) flowers were the higher than those of the other tulip flowers. Total anthocyanin levels of the violet, orange–red, claret red and pink tulip flower extracts were determined as 265.04, 236.49, 839.08 and 404.45 mg pelargonidin 3-glucoside/kg dry extract, respectively and these levels were higher than those of the other flowers. The extracts were more effective for the inhibition of *Listeria monocytogenes*, *Staphylococcus aureus* and *Yersinia enterocolitica* compared to other tested bacteria. Additionally, the cytotoxic effects of five different tulip flower extracts on human breast adenocarcinoma (MCF-7) cell line were investigated. The results showed that the orange red, pink and violet extracts had no cytotoxic activity against MCF-7 cell lines while yellow and claret red extracts appeared to be toxic for the cells. Overall, the extracts of tulip flowers with different colors possess remarkable bioactive and cytotoxic properties.
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1. Introduction

Color is one of the most important food quality characteristics and food manufacturers have been used both natural and synthetic colorants for centuries (Kammerer et al., 2007; Bechtold and Mussak, 2009). However, negative effects of synthetic colorants on health have been well demonstrated, therefore consumers are demanding healthy foods which do not contain those additives. For this reason, natural colorants, especially anthocyanins, have been used extensively in food industry and have drawn the attention of researchers (Silbergeld and Anderson, 1982; Kammerer et al., 2007). Anthocyanins comprise the largest group of water soluble pigments which are responsible for a wide range of colors (Bridle and Timberlake, 1997; Cevallos-Casals and Cisneros-Zevallos, 2004). The color and stability of anthocyanins in food systems can vary depending on several factors such as structure and concentration of the pigment, pH, temperature, light intensity and quality, presence of copigments, metallicions, enzymes, oxygen, ascorbic acid, sugars and their degradation products and sulfur dioxide (Cevallos-Casals and Cisneros-Zevallos, 2004). In addition to their coloring properties, anthocyanins are also attractive for their antioxidant activity which makes them as disease-prevention compounds (Kammerer et al., 2007).

Edible flowers have been used traditionally for their unique taste and colors and they have been more popular by the increase in the number of publications such as edible-flower cookbooks, magazine articles and television programs about them. Edible flowers are used in salads, soups, entrees, desserts, and drinks (Kelley et al., 2003; Mlcek and Rop, 2011). Researchers have been claimed that edible flowers are important sources of antioxidant compounds (Mlcek and Rop, 2011; Tai et al., 2011).

The genus *Tulipa* L. is a member of family *Liliaceae* and represented by 16 taxa (15 species), and two of them are endemic in Turkey (Mlcek and Rop, 2011). Food manufacturers are seeking natural coloring alternatives to synthetic ones due to their possible harmful effects on health and negative public perception. It has been shown that some synthetic colorants provoke hypersensitivity reactions such as urticaria, angioneurotic edema, and asthma (Michaelsson and Juhlin, 1973; Granholt and Thune, 1975; Vidotti et al., 2006).

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Many artificial food colorants also contain toxic chemicals which are responsible for many diseases and behavioral problems such as hyperkinesis and learning disabilities (H-LD) (Feingold, 1975; Ganesan et al., 2011). To overcome these effects, there is an ongoing effort, and plant extracts have been suggested to be used as coloring agents; however, very few of them are approved for use in food industry. Although natural food colorants are more desirable than artificial ones, only limited information has been available in the literature about the toxic effects of natural food dyes on mammals. Therefore, it is important to study the toxicity of the flower extracts that will be used as food coloring, such as tulip flowers based anthocyanin extracts. The aims of the present study were: (i) to determine total phenolic, total anthocyanin, antiradical, antioxidant, antimicrobial and toxicity properties of the tulip flower samples and (ii) to determine thermal degradation kinetics of tulip flower anthocyanin based extracts at 70, 80 and 90 °C.

2. Materials and methods

2.1. Plant material

Tulip (*Tulipa gesneriana* L.) flower samples were obtained from Istanbul Metropolitan Municipality, Tree and Landscape Co. (Istanbul, Turkey). All of the tulip samples can commercially be found reference varieties. Colors of tulip flowers were pink (*T. gesneriana* 'Leo Visser'), orange–red (*T. gesneriana* 'Red Ring Hood'), claret red (*T. gesneriana* 'Ben Van Zanten'), violet (*T. gesneriana* 'Negrita') and yellow (*T. gesneriana* 'Yokohama'). After tulips' sprouts and stem parts were removed, flower petals of the tulips were dried at the room temperature. Dried tulip flower's petals were stored at room temperature in the dark conditions until use.

2.2. Extraction method

Ethanol ($\geqslant$99.8%): distilled water (1:1 v/v) mixture acidified with 0.01% HCl solvent was used for the extraction of anthocyanin pigments from the tulip flowers. Raw materials were allocated to small pieces by a laboratory type grinder. Sample: solvent ratio was adjusted to be 1:19. 200 mL solvent was added in 50 g of sample and homogenized with ultra-turrax (1 min in high speed grinder). After the homogenization, 275 mL of remaining solvent was added to sample and extracted in shaking water-bath (Memmert WB-22, Germany) at 35 °C for 1 h. Following the first extraction, the extract was filtered with filter paper and then 475 mL of solvent was added. Second extraction was carried out under the former conditions. Solvents were collected and filtered using Whatman no: 1 filter paper under vacuum conditions. After that, the residual solvent was evaporated from the extract at 50 °C using a rotary evaporator.

2.3. Determination of total phenolic content

The Folin–Ciocalteu method was used with some modifications to determine total phenolic content of the tulip flower extracts (Singleton and Rossi, 1965). The extracts were diluted in certain concentrations with ethanol: distilled water (1:1). 2400 μL of distilled water, 40 μL of the extract and 200 μL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) were added in each tube, respectively. Then 600 μL saturated Na_2CO_3 (20%) and finally 760 μL of distilled water were added. After 2 h incubation in a dark environment, the absorbances of analytes were measured at 765 nm with a spectrophotometer (Varian Cary 100 Conc. UV–Visible, USA). A standard curve with serial gallic acid solution (0–1 mg/mL) was use for calibration. The results were expressed as mg gallic acid equivalent (mg GAE) per g of dry extract.

2.4. Determination of total anthocyanin content

Total anthocyanin contents of the extracts were determined using the pH-differential method (Fuleki and Francis, 1968). The results were calculated based on pelargonidin-3-glucoside with a molecular weight of 433.2 and molar absorbance of 22400. Total anthocyanin contents were calculated according to the following formula (Wrolstad, 1976):

$$\text{Anthocyanin mg/L} = (\Delta A/\varepsilon \times L)10^3 \ (\text{MW}) \ (\text{DF})$$

ΔA is the difference of absorbance; ε the molar absorbance; L the thickness of cuvette, cm; MW the molecular weight; and DF is the dilution factor.

2.5. Determination of free radical scavenging activity

The scavenging activity of the extracts for the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured as described by Brand-Williams et al. (1995) with some modifications. A series of extract concentration were prepared, and 200 μL of the samples was mixed with 4000 μL of 0.1 mM DPPH (Sigma St. Louis, MO, USA) in methanol. After the incubation for 30 min in ambient and dark conditions, the absorbance of sample was measured at 517 nm using a spectrophotometer. Solvent system was used instead of sample in the control. Methanol was used as blank to zero the absorbance. Antiradical activity (%) of the samples was calculated by the following equation:

$$\%\ \text{Inhibition} = 100 \times ((\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control})$$

The amounts of the extract required for 50% inhibition of DPPH were determined graphically by means of % Inhibition.

2.6. Determination of total antioxidant capacity

The antioxidant activity of the anthocyanin-based extracts (ABEs) was determined by phosphomolybdenum reduction method described by Prieto et al. (1999). In this assay, 400 μL solution of the extracts was added and mixed with 4 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After homogenization with vortex, reaction mixture was incubated in a shaking water-bath (Memmert WB-22, Germany) at 95 °C for 90 min. Then the samples were cooled rapidly at room temperature and the absorbances of the analytes were measured at 695 nm. The antioxidant capacity of the extracts was expressed as the ascorbic acid equivalents in milligrams/g dry extract.

2.7. Determination of antimicrobial activity

The agar well diffusion method was used to test antimicrobial activity of the extracts. Fifteen microorganisms including 13 bacteria and 2 yeast strain were used as test organisms: *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* ATCC 33019, *Bacillus subtilis* ATCC 6633, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 11230, *E. coli* O157:H7 ATCC 33150, *Klebsiella pneumoniae* ATCC 13883, *Listeria monocytogenes* ATCC 7644, *Proteus vulgaris* ATCC 13319, *Pseudomonas aeruginosa* ATCC 17853, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Yersinia enterocolitica* ATCC 1501, *Saccharomyces cerevisiae* BC 5461 and *Candida albicans* ATCC 1223. Bacteria were inoculated in nutrient broth and incubated at 35 °C for 24 h while the yeasts were activated in malt extract broth at 27 °C for 24 h. Then 1% of microorganisms was added in nutrient broth and malt extract broth and re-incubated for 18 h. Final cell concentrations were measured as 10^6–10^7 colony forming unit (CFU)/mL. Microbial cultures (1%) were added to nutrient agar and malt extract agar at 43–45 °C and poured 25 mL of agar into petri dishes. Agars were allowed to be solidified at 4 °C for 1 h. Then four equidistant holes were bored by sterile cork borers (Ø = 4 mm). 50 μL of 1%, 2.5%, 5% or 10% extract solutions in absolute ethanol was added to the holes using a micropipette. Absolute ethanol without the extract was used as a control. Yeasts and bacteria were incubated at 27 °C and 35 °C for 18–24 h, respectively. Following the incubation, formed inhibition zones around the holes were measured and expressed as millimeter (mm) (Sagdic et al., 2003).

2.8. Determination of thermal stability of anthocyanins

The thermal stabilities of tulip flower ABEs were studied at pH 3.5 in sodium citrate buffer solutions at 70 and 80 °C for 420 min and at 90 °C for 180 min. The pH value of the buffer systems was controlled using a calibrated pH meter (WTW, Werkstatten, Germany). For coloring, ABEs containing 4 mg anthocyanin were added into the 100 mL of the buffer, divided into 15 mL portions. Each portion was poured into Pyrex tubes and incubated in a thermostatic water bath (Memmert WB-22, Germany) at following temperature. The samples were removed from the water bath at 30 min intervals and rapidly cooled under running tap water. The anthocyanin contents of the samples were determined according to pH-differential method (Giusti and Wrolstad, 2001).

2.9. Kinetics of anthocyanin degradation during heating

Thermal degradation of the ABEs in the buffer followed first order reaction kinetics. Previous studies have shown that thermal degradation of anthocyanins follows a first-order reaction (Cemeroglu et al., 1994; Dyrby et al., 2001; Wang and Xu, 2007). Kinetic coefficients were calculated by the following equations (Kirca, 2004):

$$-(\mathrm{d}C/\mathrm{d}t) = kC$$

$$\ln(C/C_0) = -kt$$

C_0 is the initial anthocyanin content; C the anthocyanin content after t hour of heating; k the reaction rate constant; and t is the time (hour).

2.9.1. Calculation of reaction rate constant

Applied to the each temperature anthocyanin losses, "y" axis, the times "x" axis, placed in a semi-logarithmic scale graph a linear curve was obtained. The equation of the curve was calculated by applying of linear regression analysis in this curve. The reaction rate constant was calculated using the slope of the curve (Kirca, 2004).

$$k = (\text{slope}) \times 2.303$$

2.9.2. Calculation of activation energy

The Arrhenius model was used to describe the temperature dependency of anthocyanin degradation (Kirca, 2004; Wang and Xu, 2007).

$$k = k_0 \times \exp^{-Ea/RT}$$

Calculations were obtained by taking the logarithm of the above equation and used in the form shown in the following equation:

$$\ln k = [(-E_a/R) \times (1/T)] + \ln k_0$$

k is the reaction rate constant; k_0 the frequency factor; E_a the activation energy (J/mol); R the universal gas constant (8.314 J/mol/K); and T is the temperature (Kelvin, K).

2.9.3. Calculation of half-life periods ($t_{1/2}$)

Half-lives ($t_{1/2}$), the time needed for 50% degradation of anthocyanins, were calculated according to the formula which was given below for first order reaction kinetics (Kirca, 2004).

$$t_{1/2} = -\ln(0.5) \times k^{-1}$$

2.10. Cell culture

Human breast adenocarcinoma (MCF-7) cells were grown to confluence at 37 °C under 5% CO_2 in Dulbecco's Modified Eagle Serum (DMEM) containing 1% penicillin/streptomycin, 10% fetal bovine serum (FBS) and 2 mM L-glutamine.

2.11. Cytotoxicity assay in vitro

Cells in the logarithmic growth phase were washed once with phosphate-buffered saline, trypsinized and resuspended in fresh medium. The cells were seeded in 96-well plates at 5×10^3 cells/well. After 24 h of culture, the medium was removed by aspiration and replaced with 100 μL of fresh medium containing tulip flower extract. The treatments were performed with 500 μg/mL tulip flower extract for 24, 48 and 72 h. After addition of the extracts, cytotoxicity was assayed by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay (Sigma, Aldrich). At least two well columns containing cells without the extract were used as a negative control. MTT solution (10 μL) was added to each well, and the plates were incubated in the dark for 4 h at 37 °C. At the end of the incubation period, the medium was removed and the purple formazan product was dissolved in 50 mL of dimethylsulfoxide. The optical density was measured at 570 nm with 680 nm using a microplate reader (SpectraMax, M5). The optical densities (ODs) of wells containing cells and MTT without the extract were considered as OD control. All the experiments were performed in triplicate (Liu et al., 2005).

2.12. Statistical analysis

All data represent means ± S.E.M. Statistical analysis was performed using analysis of variance followed by the Student's *t*-test.

3. Results

3.1. Total phenolic contents of the flower extracts

Yields of the ABEs obtained from five different tulip flowers were given in Table 1 while Table 2 shows total phenolic (mg GAE/g dry extract), total anthocyanin level (mg pelargonidin 3-glycoside/kg dry extract), antioxidant activity (mg AAE/g dry extract) and antiradical capacity (IC_{50}: μg/mL) of the extracts. Total phenolic content measured by Folin–Ciocalteu method for tulips ranged from 2.55 ± 0.55 to 126.55 ± 0.79 (mg GAE/g dry extract). The claret red sample had the highest value of the total phenolic content which is 126.55 ± 0.79 (mg GAE/g dry extract) and yellow one has the lowest value which is 2.55 ± 0.55 (mg GAE/g dry extract) (Table 2).

Table 1
Yields of ABEs obtained from the tulip flowers at different colors.

Flower petal color	Yield (%)
Claret red	44.64
Orange–red	58.65
Pink	44.70
Violet	50.31
Yellow	43.17

3.2. Total anthocyanin contents of ABEs

Total anthocyanin amounts ranged between 839.08 ± 4.22 and 236.49 ± 3.68 mg pelargonidin 3-glicoside/kg dry extract for claret red and orange–red samples, respectively. Anthocyanin amount of the yellow sample was not detected as expected (Table 2).

3.3. Antioxidant activity and antiradical capacity of ABEs

On the contrary of total phenolic and total anthocyanin amount, orange–red sample had the highest antioxidant activity and antiradical capacity (IC_{50}) values which are 48.69 ± 1.01 mg AAE/g dry extract and 159.82 ± 2.76 μg/mL, respectively (Table 2).

3.4. Antimicrobial activities of ABEs

Antimicrobial activities of ABEs were determined using agar well diffusion method. The antimicrobial activity of the extracts was tested against 15 microorganisms including 13 bacteria and two yeast strains. Antimicrobial activity of ABEs prepared at different concentrations (1%, 2%, 5% and 10%) was indicated in Table 3. The extracts were more efficient against *L. monocytogenes*, *S. aureus* and *Y. enterocolitica* than the other tested bacteria. In general, effects of the extracts on the microorganisms increased at higher concentrations. However, the extracts had no antimicrobial effect against two yeasts tested.

3.5. The heating stabilities of ABEs of tulip flowers

Kinetic parameters for the degradation of tulip anthocyanins in citrate buffers at pH 3.5 during heating can be seen in Table 4. While claret red tulip anthocyanins showed higher temperature stability, violet tulip anthocyanins exhibited the lowest stability. As it can be seen in Table 4, increasing temperature caused decreases in $t_{1/2}$ values. It was determined that degradation kinetics of the ABEs obtained from different colors of tulips followed a first-order reaction at pH 3.5, the typical pH of fruit juices (Fig. 1). These results are in agreement with previous literatures (Kirca et al., 2003, 2007). The heating stability of yellow tulip flowers was not tested since they do not possess anthocyanin. Activation energy values of the tulips were calculated to be 68.69 ± 1.56 kJ/mol for claret red, 76.00 ± 8.29 kJ/mol for orange–red, 70.14 ± 0.00 kJ/mol for both of pink and violet. Statistical analyses revealed that the color of tulip had no significant effect on E_a values of the tulips ($P > 0.05$).

3.6. Toxicity properties of the tulip flower extracts

We studied the cytotoxic effects of tulip flower extracts with five different colors on MCF-7 cell lines using the MTT assay for cell toxicity (Fig. 2). MTT assay was performed in vitro similar to Liu et al. (2005). The cytotoxicity of the plant extracts was determined by comparison of growth of non-treated cells with

Table 2
Bioactive properties of the ABEs obtained from different color tulip flowers.

	Flower petal color				
	Claret red	Orange–red	Pink	Violet	Yellow
TPC	126.55 ± 0.79	113.76 ± 0.27	73.74 ± 1.10	80.52 ± 0.80	2.55 ± 0.55
TA	839.08 ± 4.22	236.49 ± 3.68	404.45 ± 1.99	265.04 ± 3.91	–
AA	31.36 ± 0.91	48.69 ± 1.01	26.15 ± 5.09	28.26 ± 0.27	23.94 ± 0.71
IC_{50}	62.46 ± 3.04	159.82 ± 2.76	122.81 ± 5.54	122.79 ± 1.77	126.45 ± 4.91

TPC: total phenolic content (mg GAE/g dry extract), TA: total anthocyanin content (mg pelargonidin 3-glucoside/kg dry extract), AA: antioxidant activities (mg AAE/g dry extract), IC_{50}: antiradical capacity (μg/mL), –: not detected.

treated ones. Three measurements were performed at time point 24 h, 48 h and 72 h in vitro, after cells were treated with tulip flower extracts. Results showed that plants extracts did not cause any cell death up to 3 days. Orange–red, pink and violet plants did not show any cytotoxicity in MCF-7 cells compared to control. However, yellow and claret red tulip flower extracts decreased cell viability; survival rate of cells reduced up to 90% after 72 h treatments. Additionally, cytotoxicity experiment was performed in the absence of cells in order to show MTT reduction was due to cell respiration and independent of anthocyanin-based extracts (data were not shown).

4. Discussion

Phenolic and anthocyanin contents of the tulips with different colors varied in a wide range and were significantly ($P < 0.05$) different from each other. The highest total phenolic and anthocyanin contents and antiradical capacity were observed in claret red petals. These results are in accordance with the literature. Friedman et al. (2010) found that red petal (San Francisco cultivar) roses had the highest anthocyanin and hydrophilic antioxidant levels among different garden rose cultivars selected as edible flowers while phenolic content of yellow tulip petals were lower than other samples. Kaisoon et al. (2012) reported that *Tagetes erecta* (yellow) flowers contained higher level of phenolic compounds (1107.5 mg/100 g dry weight) than *Cosmos sulphureus* (yellow–orange), *Antigonon leptopus* (pink) and *Bougainvillea glabra* (pink–purple) which are used as flavoring in salads and flower teas in Thailand. Anthocyanin and phenolic contents of the petals of orange *Nasturtium* flowers were 72 mg/100 g FW (Fresh weight) and 406 mg GAE/100 g FW, respectively (Garzón and Wrolstad, 2009) which were incomparable with our results. Total phenolic contents of the extracts of *Chaerophyllum macropodum*, *Sophora viciifolia* and *Michelia champaca* were 30.2 ± 0.4 mg GAE/g, 75.2 ± 4.2 mg GAE/g and 7.75 ± 0.7 mg GAE/g, respectively (Ebrahimabadi et al., 2010; Nagavani and Rao, 2010; Tai et al., 2011). Those values were lower than our data obtained from claret red, orange–red and violet tulip flowers.

In our study, the lowest antiradical capacity (IC_{50}) was detected in orange–red (159.82 ± 2.76 μg/mL) tulip petals while the highest was determined in claret red petals (62.46 ± 3.04 μg/mL). Antiradical capacities of *C. macropodum* and *S. viciifolia* were 167.1 μg/mL and 20.7 μg/mL (Ebrahimabadi et al., 2010; Tai et al., 2011). Antioxidant activities of the tulip flowers ranged from 23.94 AAE/g dry extract to 48.69 ± 1.01 mg AAE/g dry extract (Table 2). Laloo and Sah (2011) reported that antioxidant activities of *Cinnamomum wightii*, *Ochrocarpus longifolius* and *Mesua ferrea* were 205 ± 1.44, 108.33 ± 1.59 and 91.67 ± 2.16 mg AAE/g plant extract, respectively which were all higher than our results.

Ksouri et al. (2009) determined antibacterial and antifungal activity of extracts of *Tamarix gallica* leaves and flowers against the bacteria *Staphylococcus epidermidis*, *S. aureus*, *Micrococcus luteus*, *E. coli* and *P. aeruginosa* and against the fungi *Candida kefyr*, *C. holmii*, *C. albicans*, *C. sake* and *C. glabrata*. Flower extracts were more active against bacteria than the leaf extracts. The most sensitive and resistant bacteria against the extracts were *M. luteus* and *E. coli*, respectively. Organ extracts showed a weakly to moderate activity against the tested *Candida* species. In another study (Koncic et al., 2010), aqueous extracts of *Moltkia petraea* did not show antimicrobial activity against tested fungi (*C. albicans* and *Aspergillus niger*), Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). According to Samec et al. (2010), 66.66 mg/mL of the water extracts of *Teucrium ardiuni* flowers had no antimicrobial activity. Infusions of *T. aurdini* flowers and leaves were also ineffective against *E. coli*, *P. aeruginosa*, *C. albicans* and *A. niger*.

Thermal kinetics of anthocyanins of several plants such as blackberry juice and black carrot have been investigated by some researchers (Kammerer et al., 2004; Wang and Xu, 2007; Harbourne et al., 2008). However, to the best of our knowledge, there has been no report about the heat stability of tulip flower anthocyanins. In the present study, heating stabilities of tulip flower anthocyanins were ordered as claret red > pink ⩾ orange–red > violet. Wang and Xu (2007) determined k values of blackberry juice anthocyanins as 1.32×10^{-3}, 2.47×10^{-3} and 3.94×10^{-3} min^{-1} for 70, 80 and 90 °C, respectively. These results indicate that stability of blackberry anthocyanins was relatively higher than that of tulip anthocyanins. In another research, k values of red cabbage, blackcurrant, elderberry and black grape anthocyanins in pH 3.0 model systems at 80 °C were found to be 0.06×10^{-3}, 1.45×10^{-3}, 3.0×10^{-3} and 5.33×10^{-3} min^{-1}, respectively (Dyrby et al., 2001). In the present study, lower k values indicate that the anthocyanins in all tulip flower samples (claret red, orange red, pink and violet) presented in Table 4 were more stable than black grape anthocyanins. It is well known that as the temperature increases, k value also increases while a decrease occurs in $t_{1/2}$ value (Kirca et al., 2003, 2007; Wang and Xu, 2007; Harbourne et al., 2008). In this study, it can be understood from $t_{1/2}$ values that claret red tulip anthocyanins were more stable than the other tulip anthocyanins.

The dependency of the degradation of tulip anthocyanins on temperature is seen in Table 4. Activation energy (E_a) values of the anthocyanins ranging from 68.69 to 76.00 kJ/mol were consistent with those of elderberry (*Sambucus nigra*), strawberry (*Fragaria x ananassa*) and black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens*) anthocyanins varying from 62.76 to 79.50 kJ/mol (Reyes and Cisneros-Zevallos, 2007). As expected, higher temperature and longer heating periods increased the degradation of anthocyanins in the tulip flowers. Anthocyanin composition, extraction conditions, storage temperature and heating temperature are among the important factors influencing the degradation of anthocyanins (Kirca et al., 2003, 2007; Wang and Xu, 2007; Harbourne et al., 2008; Pliszka et al., 2009).

Cytotoxicity test is a critical evaluation for approving natural colors as food colorings. Choice of the method to test the viability and proliferation of the cells is also crucial for quantitative assessment of cytotoxic activities of the plant extracts. MTT is a

Table 3
Antimicrobial activities of ABEs obtained from different color tulip flowers (inhibition zone diameter, mm).

% Concentration	*Aeromonas hydrophila*	*Bacillus cereus*	*Bacillus subtilis*	*Enterobacter cloacae*	*Escherichia coli*	*Escherichia coli* O157:H7	*Klebsiella pneumoniae*	*Listeria monocytogenes*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Staphylococcus aureus*	*Yersinia enterocolitica*	*Saccharomyces cerevisiae*	*Candida albicans*
Control															
AE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Claret red															
10	11.08 ± 2.06	13.69 ± 1.96	16.17 ± 1.35	19.96 ± 5.54	11.73 ± 1.10	16.35 ± 7.20	–	23.40 ± 4.76	–	12.87 ± 0.88	11.37 ± 2.01	25.64 ± 6.57	27.37 ± 2.54	–	–
5	10.33 ± 0.47	–	8.51 ± 0.47	11.90 ± 0.32	8.21 ± 0.29	11.86 ± 1.96	–	16.49 ± 1.65	–	7.43 ± 0.05	9.31 ± 1.37	21.16 ± 7.07	23.08 ± 3.49	–	–
2	–	–	–	9.49 ± 0.69	–	8.54 ± 0.76	–	12.49 ± 0.95	–	–	–	11.33 ± 0.47	16.63 ± 2.10	–	–
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Orange–red															
10	7.16 ± 0.23	12.32 ± 2.83	9.09 ± 2.45	7.23 ± 0.52	–	9.82 ± 1.43	–	16.20 ± 2.98	–	7.28 ± 0.19	8.95 ± 1.79	14.81 ± 3.12	20.78 ± 2.62	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pink															
10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Violet															
10	–	–	–	10.88 ± 2.57	–	9.57 ± 3.08	–	30.57 ± 2.38	–	–	–	15.99 ± 7.77	13.93 ± 0.93	–	–
5	–	–	–	–	–	–	–	28.43 ± 0.91	–	–	–	10.29 ± 1.71	–	–	–
2	–	–	–	–	–	–	–	18.71 ± 1.71	–	–	–	–	–	–	–
1	–	–	–	–	–	–	–	15.62 ± 0.95	–	–	–	–	–	–	–
Yellow															
10	10.06 ± 2.76	9.61 ± 1.55	–	11.86 ± 2.04	–	13.51 ± 2.20	–	18.51 ± 3.17	–	8.98 ± 2.38	10.90 ± 1.15	19.09 ± 1.91	17.83 ± 0.60	–	–
5	7.99 ± 0.27	–	–	7.38 ± 0.54	–	8.67 ± 1.41	–	–	–	–	9.41 ± 2.20	10.74 ± 0.64	11.22 ± 0.85	–	–
2	–	–	–	–	–	–	–	–	–	–	–	–	9.03 ± 0.24	–	–
1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

AE: Absolute ethanol.
–: not effective.

Table 4
Reaction rate constant (k) and half-life ($t_{1/2}$) values of ABEs obtained from different color tulip flowers at pH 3.5 during heating at 70, 80 and 90 °C.

Sample	Temperature (°C)	$-k \times 10^3$ (min^{-1})	$t_{1/2}$ (h)
Claret red (Ben Van Zanten)	70	$1.38^c \pm 0.00$	$8.40^a \pm 0.00$
	80	$3.11^b \pm 0.16$	$3.75^b \pm 0.21$
	90	$5.19^a \pm 0.16$	$2.25^c \pm 0.07$
Orange–red (Red Ring Hood)	70	$1.50^c \pm 0.16$	$7.79^a \pm 0.83$
	80	$3.68^b \pm 0.00$	$3.12^b \pm 0.03$
	90	$6.45^a \pm 0.33$	$1.81^c \pm 0.12$
Pink (Leo Visser)	70	$1.61^c \pm 0.00$	$7.2^a \pm 0.00$
	80	$3.68^b \pm 0.00$	$3.1^b \pm 0.00$
	90	$6.22^a \pm 0.00$	$1.90^c \pm 0.00$
Violet (Negrita)	70	$1.61^c \pm 0.00$	$7.18^a \pm 0.00$
	80	$4.96^b \pm 0.16$	$2.34^b \pm 0.08$
	90	$6.56^a \pm 0.17$	$1.76^c \pm 0.04$

For each sample and each property; $^{a-c}$: Means in lowercase in the same column compare the temperature and show significant differences at $P < 0.05$.

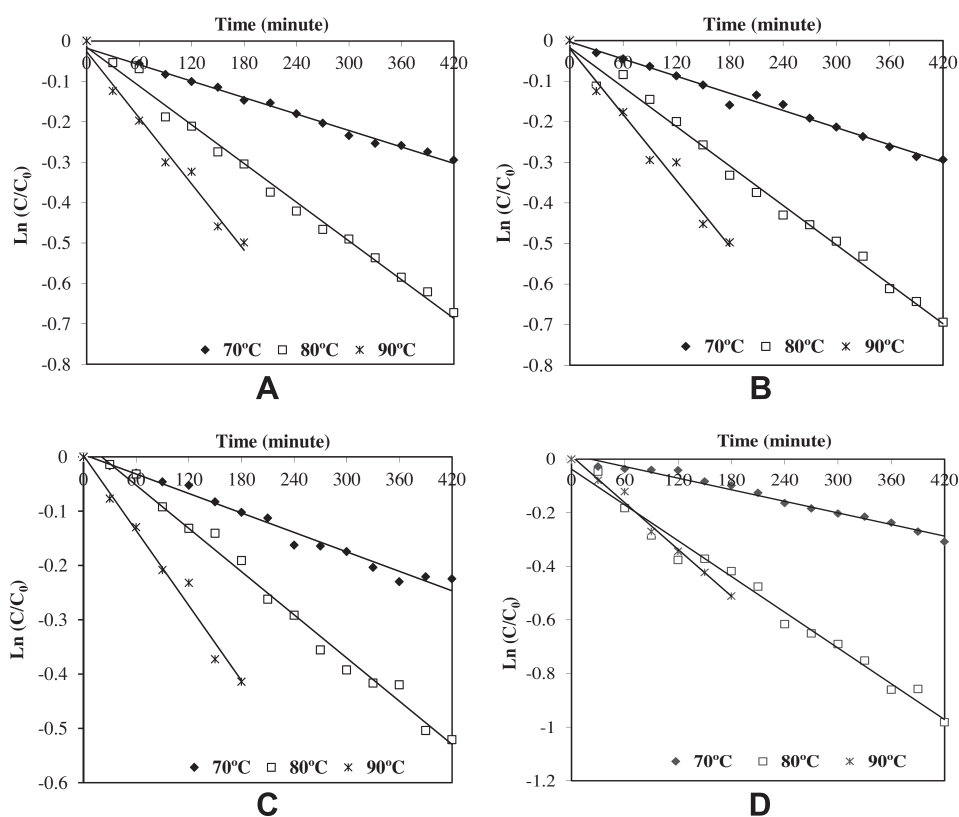

Fig. 1. Degradation of anthocyanins of tulip flower extracts at pH 3.5 buffers. (a) Orange–red (Red Ring Hood), (b) Pink (Leo Visser), (c) claret red (Ben Van Zanten), and (d) violet (Negrita).

tetrazolium salt converted to purple formazan crystals mainly by mitochondrial succinate dehydrogenase (Wang et al., 1996). In this method, color intensity of the formazan dye is correlated to the number of viable cells. Advantages of MTT test include the sensitivity that even very small number of living cells can be detected and that incidence of errors is minimal since it does not require washing step (Ahmadian et al., 2009). This method is more sensitive than qualitative trypan blue which detects only alive cells (Vandeloosdrecht et al., 1994).

In this study, MTT results showed that red, pink and violet tulip extracts had no cytotoxic activity against MCF-7 cell lines. The present study provides the unique information about the nontoxicity of red, pink and violet tulip flower extracts. Therefore, those extracts can be included in the list of natural colorants like annatto, paprika, curcuma and anthocyanin. On the other hand, yellow and claret red tulip extracts showed somewhat toxicity on the cells. Some plant extracts obtained from Northwestern Brazilian plants, extracts of *Carpesium rosulatum* and flowers of *Butea monosperma*, green tea and wheatgrass (*Triticum aestivum*) have been demonstrated to have anti-cancer properties (Schmidt et al., 2005; Choedon et al., 2010; Aydos et al., 2011; Ferreira et al., 2011; Moon and Zee, 2011). Our result can be valuable since yellow and claret red tulip extracts may have antiproliferative potential against MCF-7 cell line. However, further experiments should be conducted to determine the potential toxic effect of yellow and claret red tulip extracts against cancer cells.

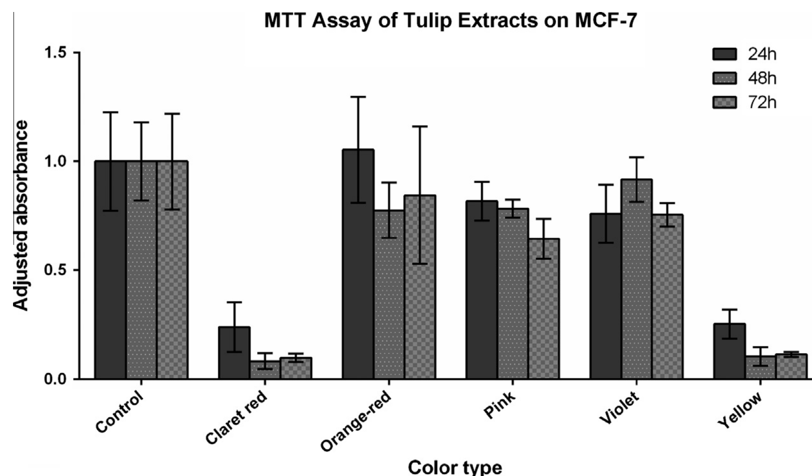

Fig. 2. Comparison of cytotoxic effects of tulip flower extract on MCF-7 cell line. Cells were treated with different tulip extract for 24, 48 and 72 h. Results are mean ± SEM ($n = 3$).

5. Conclusions

Tulip flowers obtained from former planting tulip onion at spring are used as ornamental plants. Agronomically, these flowers are cut and wasted to obtain tulip onion at the end of cultivation. The waste tulip flowers were investigated in terms of their bioactive properties. The results of this study demonstrated that the studied tulip flowers had desirable bioactive characteristics. The toxicity results also revealed that color materials obtained from red, pink and violet tulip flowers can be potentially used as coloring agents to be alternative to synthetic agents in food and pharmacology industries. In the further study, bioactivity and physicochemical properties of food products enriched with anthocyanin extracts obtained from the different color tulip flowers can be investigated.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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