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Carotenoid Assessments and Antioxidant Activities from Flower Petals

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ABSTRACT

The objectives of this study were to evaluate carotenoid pigment profile and the antioxidant activity from 14 commonly grown flowers in Thailand. The result found that orange marigold showed the highest total carotenoid content at as 2,209±75.58 µg/g, followed by deep yellow chrysanthemum at 551.27±47.72 µg/g (P<0.01). The next lower total carotenoid content group was found in yellow silk cotton, yellow trumpetbush, yellow marigold and yellow golden shower with total carotenoid content of 447.42±27.56, 429.46±28.34, 409.85±34.58 and 363.88±12.74 µg/g, respectively. The highest antioxidant activities against DPPH and ABTS radicles were found from orange marigold, which were 32.34±2.16 and 50.08±0.87%, respectively. Deep yellow chrysanthemum, yellow silk cotton, yellow trumpetbush, yellow marigold and yellow golden shower also showed significantly higher antioxidant activities than other flowers (P<0.01). Total carotenoid contents well correlated with antioxidant activities against DPPH and ABTS radicals (r = 0.6924 and r = 0.8270, respectively) at P<0.01. TLC result elucidated that orange marigold, yellow silk cotton and yellow golden shower were a good source of *β*-carotene, while deep yellow chrysanthemum and yellow marigold were a good source of lutein and/or zeaxanthin. The result indicated that flower petals would be useful as natural carotenoid source and provide antioxidants for food industry.

1. Introduction

Carotenoids are pigment, which are commonly found in nature. They exhibit yellow, orange and red color. Their structures consist of 8 isoprene units, connected by covalent bonds (Britton *et al.* 2004). They are categorized into 2 main groups, according to their structures: carotenes and xanthophylls. Carotenes are hydrocarbon, including *β*-carotene, α-carotene and lycopene on the other hand Xanthophylls contain oxygen atoms in their molecules such as lutein, zeaxanthin, astaxanthin and canthaxanthin (Britton *et al.* 2017). Additionally, to advantage as pigment that distribute yellow, orange and red color, they also play important roles regarding health for humans and animals, such as antioxidant activity, enhancement of immunity, contribution to reproduction, prevention of cancer and lifestyle-related diseases, as well as being vitamin A precursor. Although photosynthetic plants, algae and cyanobacteria can synthesize carotenoids, humans and animals are unable to perform carotenoids synthesis de novo. Carotenoids can be obtained from food and modified through metabolic reactions and subsequently incorporate in some specific organs and tissues, such as egg yolk, skin, legs, beak and feather (Zeb and Murkovic 2010; Maoka 2020). As a result of advantages mentioned above, natural carotenoid sources have been studied such as *Chlorella* spp., *Spirulina* spp., carrot, buckthorn berry, linseed, marigold, calendula, basil, and tomato (Karadas *et al.* 2006; Englmaierová *et al.* 2014; Skřivan *et al.* 2015; Kljak *et al.* 2021; Panaite *et al.* 2021).

Flowering plants are one of the interesting pigment sources because of a variety of color in flower petals. Therefore, several research laboratories have explored carotenoid profile in flower petals of monocot and eudicot plants by using common 2 techniques, including thin-layer chromatography; TLC and high performance liquid chromatography; HPLC. In general, the majority carotenoids in flower petals are lutein, *β*-cryptoxanthin, zeaxanthin, lycopene, and *β*-carotene. Interestingly, some flowering plants have modified their carotenoid pathway to synthesis specific carotenoid compositions. Additionally, carotenoid compositions in flower petals are different

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from those found in leaves, resulting to a great diversity of carotenoid composition in flower petals (Ohmiya 2011). Therefore, it could be said that flower petals have a great potential as carotenoid source. However, the study of carotenoids from flower petals is limited in Thailand. Therefore, this research focused on study of carotenoid contents, carotenoid profiles and antioxidant activities from 14 petals of flowers, which have been commonly grown and well adapted to the environment in Thailand. Some of them bloom all year round but some prefer to bloom in specific period. The data obtained from this study would suggest potential carotenoid sources from flower petals that can be used in food and pharmaceutical industry.

2. Materials and Methods

2.1. Sample Preparation

The fully open 14 flowers were collected and dried by a hot air over at 60°C for 24 hours, then grinded into a fine powder and stored in an airtight bag for further study.

2.2. Total Carotenoids Extraction

Carotenoids were extracted and total carotenoids contents were analyzed, followed the methods, explained by (Britton *et al.* 1995a, 1995b) with some modifications. Briefly, 10 ml of acetone and 10 ml of distilled water were used to extract carotenoids from samples, then 20 ml of hexane was added into the mixture. The mixture was gently shaken and set for complete separation. The hexane layer was separated and evaporated by the vacuum evaporator. The absorption at a 450 nm of the pigment extracts were measured by using petroleum ether as solvent. Total carotenoids contents were calculated using the absorption coefficient. ($E^{1\%, 1\ cm}$) as 2,500.

2.3. Carotenoid Profiles

Thin layer chromatography analysis was performed on TLC Aluminum oxide 60 F254, neutral (Merck, Germany). The mobile phase consisted of acetone and petroleum ether, in a ratio of 30:70. Chromatogram was developed at room temperature and under dim light on the distance of 5 cm within 10 min in glass chromatographic chamber, saturated of mobile phase for 30 min. After drying with N_2 gas, plates were analyzed with JustTLC software (version 4.6.1, available at http://www.sweday.com/).

2.4. DPPH Radicle-scavenging Activity

Analysis of DPPH antioxidant properties was partly modified from the method, explained by Brand-Williams *et al.* (1995). Fifty microliter of pigment extract and 0.1 mM 2,2 diphenyl-1-picrylhydrazyl radical solution were mixed and incubated at room temperature for 30 minutes in the dark. After that, measured the absorption values at 517 nm. Calculated the percentage of DPPH antioxidants from the formula.

$$\% \text{ DPPH radical inhibition} = [(A - B) / A] \times 100$$

When A and B were the absorption values of a DPPH solution without pigment extract and the absorption value of a DPPH solution containing pigment extract, respectively. The measurement was repeated 5 times in each extract type.

2.5. ABTS Radicle-scavenging Activity

Analysis of ABTS antioxidant properties was partly modified from the method, explained by Re *et al.* (1999). Prepared a mixed solution between 2.45 mM potassium persulfate and 7 mM ABTS in distilled water, stored in dark for 16-18 hours, diluted the solution to obtain absorption values in the range of 0.8-1.0. Fifty microliter of pigment extract and prepared solution were mixed and incubated at room temperature for 30 minutes in the dark. After that, measured the absorption values at 734 nm. Calculated the percentage of ABTS antioxidants from the formula.

$$\% \text{ ABTS radical inhibition} = [(A - B) / A] \times 100$$

When A and B were the absorption values of an ABTS solution without pigment extract and the absorption value of an ABTS solution containing pigment extract, respectively. The measurement was repeated 5 times in each extract type.

2.6. Statistical Analysis

Statistix version 8.0 was used for statistical analysis. Data were expressed as mean ± standard deviation. Measurement data with normal distribution were analyzed using one-way analysis of variance (ANOVA), followed by Least Significantly Difference (LSD) with a significance level of $\alpha = 0.05$.

3. Results

3.1. Total Carotenoid Contents and Cntioxidant Activities

Fourteen flowers were studied in this study, including rose (*Rosa hybrida*), west Indian jasmine (*Ixora chinensis* Lamk), golden shower (*Cassia fistula* L.), orchid tree (*Bauhinia purpurea* L.), safflower (*Carthamus tinctorius* L.), impala lily (*Adenium obesum* (Fosk.) Roem. and Schult.), bastard teak (*Butea monosperma*), trumpet bush (*Tecoma stans*), marigold (*Tagetes erecta*), bougaville (*Bougainvillea* spp.), globe

amaranth (*Gomphrena globosa* L.), chrysanthemum (*Chrysanthemum morifolium* Ramat), yellow silk cotton (*Cochlospermum regium*), and butterfly pea (*Clitoria ternatea* L.). These flowers showed distinct flower petals in term of color as showed in Figure 1.

The highest total carotenoid content was found from orange marigold which reached 2,209.34±75.58 µg/g and followed by deep yellow chrysanthemum which reached 551.27±47.72 µg/g (Table 1 and Figure 2). Other rather high total carotenoid contents were found from yellow silk cotton, yellow trumpetbush, yellow marigold and yellow golden shower with total carotenoids content of 447.42±27.56, 429.46±28.34, 409.85±34.58, and 363.88±12.74 µg/g, respectively. Moderate low total carotenoid contents, in descending order, were found from yellow chrysanthemum, bougaville with violet, orange and magenta petals as well as west Indian jasmine which were 238.18±31.86, 214.38±10.35, 199.54±15.26, 170.35±9.02, and 113.86±6.68 µg/g, respectively. Flowers with fairly low total carotenoid contents (lower than 80 µg/g) were impala lily, butterfly pea,

Figure 1. Morphology of flower samples (1 = rose, 2 = west Indian jasmine, 3 = golden shower, 4 = orchid tree, 5 = safflower, 6,7 = impala lily, 8 = bastard teak, 9 = trumpetbush, 10-11 marigold, 12-14 = bougaville, 15 = globe amaranth, 16-18 = chrysanthemum, 19 = yellow silk cotton, and 20 = butterfly pea)

magenta chrysanthemum, orchid tree, bastard teak, globe amaranth, safflower, and rose.

The results on antioxidant activities against DPPH and ABTS radicles were also presented in Table 1 and Figure 2. A great diversity among tested flowers in their antioxidant activities was found. Six tested flowers, including orange marigold, deep yellow chrysanthemum, yellow silk cotton, yellow trumpetbush, yellow marigold, and yellow golden shower had significantly higher antioxidant activities than others (P<0.01). Their DPPH-antioxidant activities were as 32.34±2.16, 29.41±0.57, 28.69±1.03, 28.20±0.61, 29.60±0.98, and 20.07±1.11%, respectively. Likewise, high antioxidant activities against ABTS radicles were also found from the same six tested flowers, which were 50.08±0.87, 33.90±0.81, 35.70±1.79, 35.21±1.26, 33.23±0.55, and 28.15±0.15%, respectively. The rest showed some antioxidant activities against DPPH and ABTS radicles, but their antioxidant powers were not as high as those of six flowers. Relationships between total carotenoid content and DPPH and ABTS antioxidant property were analyzed. The significantly positive correlations of total carotenoid content and all antioxidant assay were found at P<0.01 (Table 2). The correlation coefficients between total carotenoid content and DPPH and ABTS antioxidant property were r = 0.6924 and 0.8270, respectively.

3.2. Carotenoid Profiles

The first 6 pigment extracts from flower petals with high total carotenoid content, including orange marigolds, deep yellow chrysanthemum, yellow silk cotton, yellow trumpetbush, yellow marigold and yellow golden shower were subjected to carotenoid profile analysis. Yellow and orange spots were obtained on TLC plates as shown in Figure 3. In order to identify the pigments, JustTLC software, version 4.6.1 was carried out for TLC analysis (Figure 4). Comparing Rf values and color for spots of pigment extracts with those of standard carotenoids, spot 3 (lane 4), spot 17 (lane 6) and spot 22 (lane 9) were identified as β-carotene (R_f = 0.882). Considering spot 25 (lane 5) and spot 21 (lane 8), they were both identified as lutein and/or zeaxanthin with the R_f of 0.266. Nevertheless, spot 23 (lane 4), spot 13, 24, 30 (lane 5), spot 14, 27 (lane 6), spot 19 (lane 7), spot 11 (lane 8), and spot 28, 29 (lane 9) were unknown carotenoids because they showed no coincidence of R_f value with any carotenoid standards.

Table 1. Total carotenoids content and antioxidant activities

Sample		Color of flower petals	Total carotenoids content	Radicle inhibition (%)	
Scientific name	Common name		(µg/gram sample)	DPPH	ABTS
Rosa hybrida	rose	red	5.79±1.32^{k}	15.68±0.68^{cd}	16.36±0.72^{h}
Ixora chinensis Lamk	west Indian jasmine	red	113.86±6.68^{g}	9.12±1.94^{hi}	13.65±1.30^{i}
Cassia fistula L.	golden shower	yellow	363.88±12.74^{d}	20.07±1.11^{c}	28.15±0.15^{d}
Bauhinia purpurea L.	orchid tree	magenta	13.05±1.61^{k}	11.53±2.47^{fgh}	13.20±1.88^{i}
Carthamus tinctorius L.	safflower	red	24.47±1.23^{jk}	13.13±1.18^{cdef}	14.29±0.38^{i}
Adenium obesum (Fosk.) Roem. and Schult.	impala lily	deep magenta	15.37±1.18^{k}	9.16±1.09^{hi}	15.70±0.57^{hi}
Adenium obesum (Fosk.) Roem. and Schult.	impala lily	magenta	75.88±3.63^{gh}	10.40±0.28^{gh}	16.22±0.46^{h}
Butea monosperma	bastard teak	orange	20.37±1.39^{jk}	7.44±1.74^{i}	15.83±0.57^{hi}
Tecoma stans	trumpetbush	yellow	429.46±28.34^{c}	28.20±0.61^{b}	35.21±1.26^{b}
Tagetes erecta	marigold	orange	2,209.34±75.58^{a}	32.34±2.16^{a}	50.08±0.87^{a}
Tagetes erecta	marigold	yellow	409.85±34.58^{c}	29.60±0.98^{b}	33.23±0.55^{c}
Bougainvillea spp.	bougaville	magenta	170.35±9.02^{f}	12.22±0.93^{efg}	18.10±0.47^{g}
Bougainvillea spp.	bougaville	violet	214.38±10.35^{e}	12.22±0.40efg	24.92±0.62^{e}
Bougainvillea spp.	bougaville	orange	199.54±15.26^{ef}	14.16±0.33^{cde}	25.56±1.84^{e}
Gomphrena globosa L.	globe amaranth	violet	15.80±0.84^{jk}	9.30±1.51^{hi}	18.07±0.57^{g}
Chrysanthemum morifolium Ramat.	chrysanthemum	yellow	238.18±31.86^{e}	12.66±0.05^{defg}	16.41±1.01^{h}
Chrysanthemum morifolium Ramat.	chrysanthemum	magenta	56.56±2.15^{hij}	14.51±4.49^{cde}	22.65±0.92^{f}
Chrysanthemum morifolium Ramat.	chrysanthemum	deep yellow	551.27±47.72^{b}	29.41±0.57^{b}	33.90±0.81^{c}
Cochlospermum regium	yellow silk cotton	yellow	447.42±27.56^{c}	28.69±1.03^{b}	35.70±1.79^{b}
Clitoria ternatea L.	butterfly pea	dark blue	62.51±3.96^{hi}	11.53±1.12^{fgh}	11.32±0.19^{j}

Means with different letter within a column of each group are significantly different (P<0.01)

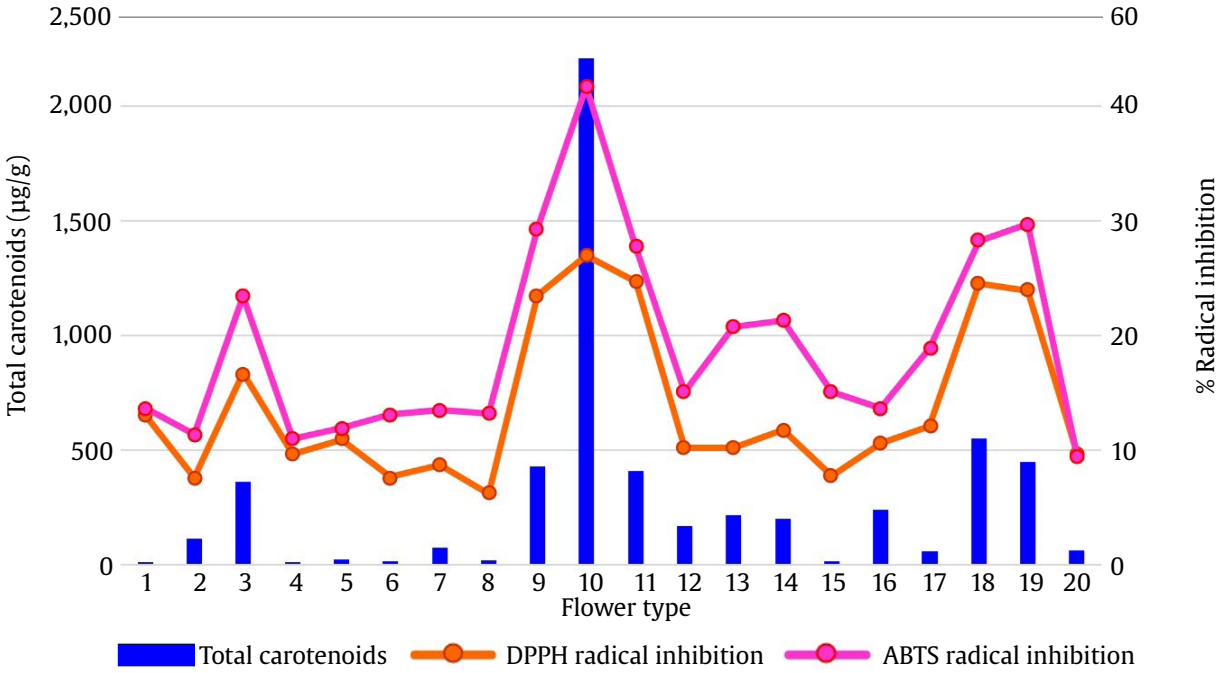

Figure 2. Total carotenoids content and antioxidant activities (1 = rose, 2 = west Indian jasmine, 3 = golden shower, 4 = orchid tree, 5 = safflower, 6,7 = impala lily, 8 = bastard teak, 9 = trumpetbush, 10-11 marigold, 12-14 = bougaville, 15 = globe amaranth, 16-18 = chrysanthemum, 19 = yellow silk cotton, and 20 = butterfly pea)

Table 2. Correlation of total carotenoid content and antioxidant activities

Factor	Total carotenoid content	% DPPH Radical inhibition
% DPPH radical inhibition	0.6924**	
% ABTS radical inhibition	0.8270**	0.8731**

**means significant correlation (P<0.01)

4. Discussion

The results on total carotenoid contents from flower petals coincided with the observation of following studies. Tanoi *et al.* (2006) compared carotenoid pigments among 8 commonly grown garden flowers and concluded that the highest number of total carotenoids was found in the family Compositae, especially in marigold. Supporting results was also provided by Tangmo *et al.* (2019) which showed that yellow silk cotton and golden shower contained higher carotenoid contents in term of β-carotene (28.91±0.43 and 22.95±0.20 µg β-carotene/100 g dried weight, respectively). Notably, high total carotenoid content in this study was obtained by flowers with yellowish flower petals. It seemed that the more yellowish flower petals are, the more total carotenoids obtain. Our findings leaded to the proposed idea that color of flower petals relied on the level of carotenoid content. Our proposed idea well agreed with the research conducted by Wang *et al.* (2018) which compared carotenoid accumulation and investigate its contribution in coloration of *Osmanthus fragrans*. Their research revealed that difference in flower coloration of *O. fragrans* were mainly attributed by the level of carotenoids and color became more deeper with carotenoid accumulation. Not only the level of carotenoid accumulation in flower petals is influent on flower petal color, but the type of carotenoid compound is also a key factor. According to the studies of Moehs *et al.* (2001) and Ohmiya (2011), the xanthophylls which impart pale yellow, deep yellow to orange color to flower petals were lutein, β-cryptoxanthin and zeaxanthin as well as the carotenes such as β-carotene and lycopene were responsible for deep yellow to orange color to flower petals. Though, the petals of some flowers have modified carotenoid biosynthesis pathways to produce specific carotenoid compositions. For example, the petals of *Adonis aestivalis* and *A. annua* accumulate large amount of astaxanthin, resulting in their distinctive blood-red color (Cunningham and Gantt 2005). In addition, the study from Grotewold (2006) explained that there were 3 major classes of pigments that could attribute to flower coloration. One was carotenoids and another were flavonoid and betalain. Therefore, in term of flowers with low total carotenoid contents in this study, the main pigments accumulated in petals could be anthocyanin and betalain. This idea was in

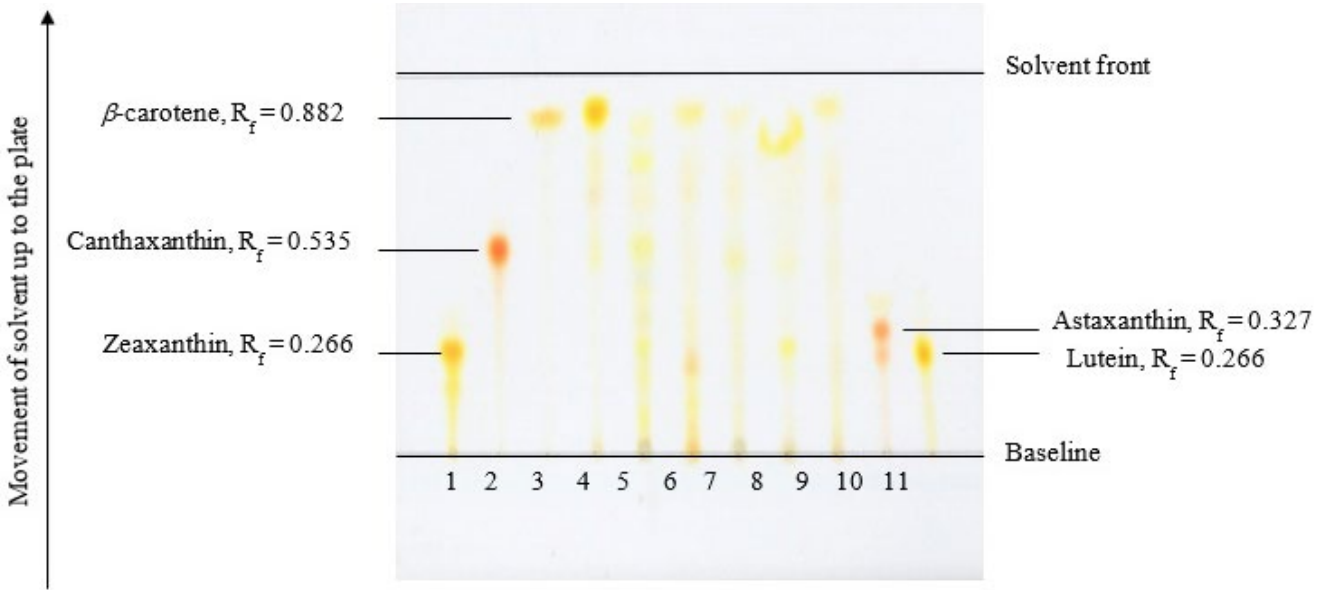

Figure 3. Silica TLC plate on which carotenoids standards and flower petals extracts (1 = zeaxanthin, 2 = canthaxanthin, 3 = β-carotene, 4 = orange marigold, 5 = deep yellow chrysanthemum, 6 = yellow silk cotton, 7 = yellow trumpetbush, 8 = yellow marigold, 9 = yellow golden shower, 10 = astaxanthin, 11 = lutein)

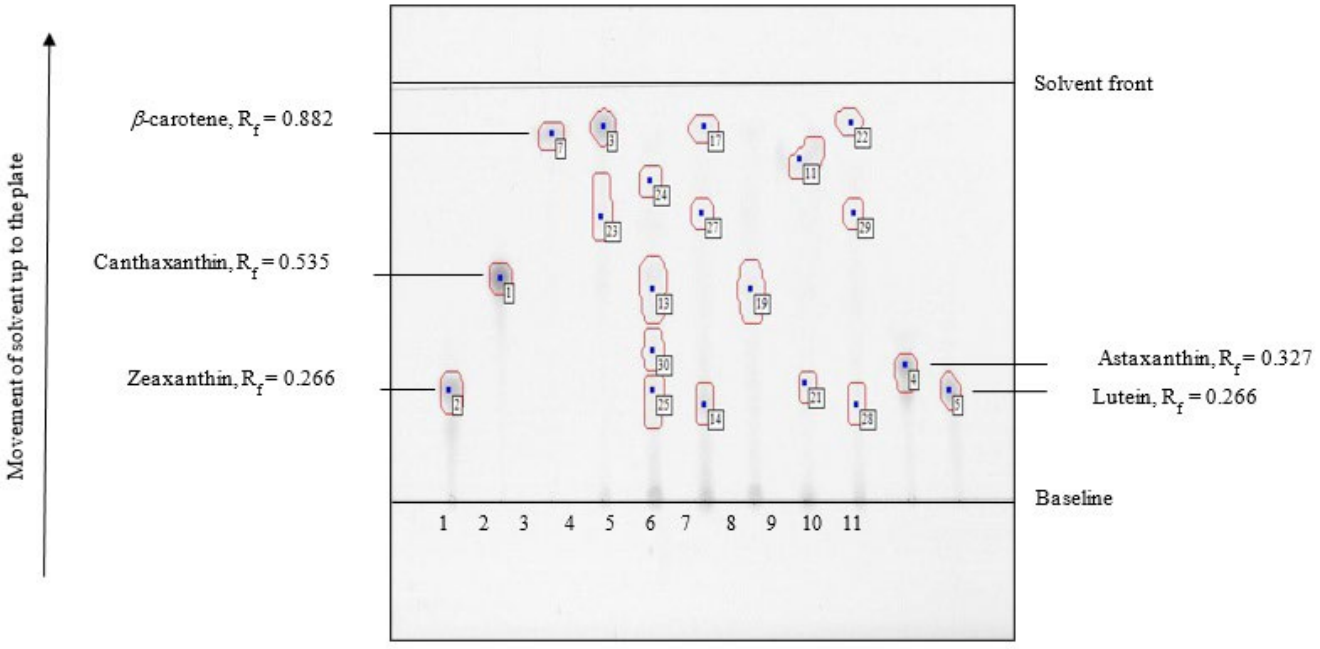

Figure 4. Silica TLC plate on which carotenoids standards and flower petals extracts, analyzed by Just TLC version 4.6.1. (1 = zeaxanthin, 2 = canthaxanthin, 3 = β-carotene, 4 = orange marigold, 5 = deep yellow chrysanthemum, 6 = yellow silk cotton, 7 = yellow trumpetbush, 8 = yellow marigold, 9 = yellow golden shower, 10 = astaxanthin, 11 = lutein)

an agreement with several studies, which explained that anthocyanin was abundant in petals of rose, west Indian jasmine, shoe flower (*Hibiscus rosa-sinensis*) and butterfly pea (Tantituvanont *et al.* 2008; Vankar and Srivastava 2010).

The results of antioxidant activities showed that orange marigold, deep yellow chrysanthemum, yellow silk cotton, yellow trumpetbush, yellow marigold and yellow golden shower were a good source of antioxidants. This finding shared similarity to several studies which previously reported that marigold especially Optiva Orange and Rodeo Gold, yellow silk cotton and golden shower showed high antioxidant activities (Ingkasupart *et al.* 2015; Yaemkong *et al.* 2020). The results implied that total carotenoid contents in flower petals was responsible for their antioxidant activities which was in an agreement to the finding from Benvenuti *et al.* (2016). Their work explained that color of flowers as a result of pigment accumulation in petals not only contribute to increase their appeal towards customers, but also the antioxidant activity. The relationships between total carotenoid content and antioxidant activities were also examined in this study and the positive corrections between total carotenoid content and antioxidant activities against DPPH and ABTS radicles were found. Our finding was supported by the studies from Yaemkong *et al.* (2020) and Ingkasupart *et al.* (2015), which also reported a positive correlation between antioxidant effects and carotenoids. However, the correlation between total carotenoid content and DPPH antioxidant activity in this study was lower than that of total carotenoid content and ABTS antioxidant activity. This might be a result of a structural diversity of antioxidants. Different types of antioxidants might act differently to reduce free radicals, leading to different antioxidant efficiency and correlation (Müller *et al.* 2011). Therefore, it suggested that more than one antioxidant method would rather be used for antioxidant examination.

Carotenoid profile analysis by TLC clearly showed that carotenoid profile of each flower petal was distinct. The β-carotene which is a provitamin A was the dominant pigment in orange marigold, yellow silk cotton and yellow golden shower while lutein and/or zeaxanthin was dominant in deep yellow chrysanthemum and yellow marigold. Our results correlated with the study from Kishimoto *et al.* (2005); Ohmiya (2011); Ingkasupart *et al.* (2015) whose result showed that lutein was the main pigment in yellow chrysanthemum and yellow marigold. Although, in this study lutein and zeaxanthin could not be differentiated because of the same R_f value, to the best of our knowledge, our study firstly identified the carotenoid compositions in yellow silk cotton, yellow golden shower and yellow trumpetbush. Therefore, this new information would be useful for considering these flowers as a new and good β-carotene source. In addition, the method used for extraction and identification of carotenoids in this study was simple, inexpensive and less time consuming, which could be applied for screening carotenoid compositions from many samples in a single run without using sophisticated equipment.

It could be concluded that flower petals possessed different total carotenoid contents and antioxidant activities against DPPH and ABTS. Orange marigold, deep yellow chrysanthemum, yellow silk cotton, yellow trumpetbush, yellow marigold and yellow golden shower provided high total carotenoid content and antioxidant activities. Considering the carotenoid profiles, orange marigolds, yellow silk cotton and yellow golden shower were a good source of β-carotene, while deep yellow chrysanthemum and yellow marigold were a good source of lutein and/or zeaxanthin. Therefor these flower petals would be useful for using as natural carotenoid source as well as beneficial to provide antioxidants for food and pharmaceutical industry.

Conflict of Interest

The authors declare no conflict of interest.

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