

Comparison of antioxidant properties of tamarillo (*Cyphomandra betacea*), cherry tomato (*Solanumly copersicum* var. *cerasiform*) and tomato (*Lyopersicon esulentum*)

Noor Atiqah, A. A. K., Maisarah, A. M. and *Asmah, R.

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, 43400, Selangor, Malaysia

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<u>Abstract</u>

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Keywords

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The emerging studies suggest antioxidant may represent an important role in defence against certain diseases outlined the necessity of determining their contents in tamarillo (Cyphomandra betacea), cherry tomato (Solanum lycopersicum var. cerasiforme), and tomato (Lycopersicon esculentum). This study aims to determine the antioxidant capacity, total phenolic content and total flavonoid content in tamarillo, yellow cherry tomato, red cherry tomato, and tomato in 70% ethanol and water extracts. The ethanol extract showed the highest scavenging activity, ferric reducing activity, phenolic and flavonoid contents, whereas, the water extract showed higher value for antioxidant activity in β -Carotene bleaching assay. Tamarillo showed the highest antioxidant activity ($22.92 \pm 3.60\%$, $28.89 \pm 3.85\%$), scavenging activity ($44.25 \pm 0.82 \mu g/ml$, $47.38 \pm 1.11 \ \mu g/ml$), ferric reducing activity ($12.17 \pm 0.53 \ \mu M$ Fe (II)/g, $3.72 \pm 0.20 \ \mu M$ Fe (II)/g), phenolic content (7.63 \pm 0.37 mg GAE/g edible portion, 1.83 \pm 0.50 mg GAE/g edible portion) and flavonoid content (6.44 ± 0.16 mg CE/g edible portion, 2.22 ± 0.31 mg CE/g edible portion) in ethanol and water extracts respectively. For ethanol extracts a positive correlations existed ($0.66 \le r \ge 0.97$) between ferric reducing activity, antioxidant activity, phenolic content and flavonoid content. While, in water extract correlation test revealed a positive correlations between antioxidant activity, ferric reducing activity and phenolic content (0.645 \leq r \geq 0.706) and between antioxidant activity and flavonoid content (r = 0.820). In conclusion, tamarillo exhibits the highest antioxidant capacity, phenolic content and also flavonoid content.

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Introduction

Oxidative stress is an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, which referred to as antioxidants. These imbalances have leads to the damage of important biomolecules and cells, with potential impact on the whole organism (Durackova, 2010). Antioxidant is a substance that when present at low concentration compare to those of the oxidizable substrate, significantly delays, or inhibits oxidation of that substrate (Niki, 2010).

Antioxidants can be obtained from the diet in the form of vegetables, fruit, green tea and chocolates. Some of the earliest data were collected in animal studies and indicated that antioxidants derived from dietary studies sources may reduce the progression of atherosclerosis, and observational data collected in human suggest that the ingestion of antioxidants is associated with preventing cardiovascular disease (Yoshihara *et al.*, 2010).

Cyphomandra betacea is locally known as "Buah Cinta," "Moginiwang," or "Tamarillo" among local

people in Sabah, Malaysia. Whereas, in Peninsular Malaysia, this fruit is commonly known as "Pokok Tomato" or "Tamarillo". C. betacea can grow naturally in the higher-humidity and low-temperature area. In Malaysia C. betacea is cultivated in Cameron Highland (Peninsular Malaysia), and Kundasang (Sabah). The ripe fruit of C. betacea is usually eaten raw by local community (Ali Hassan and Abu Bakar, 2013). It is an egg-shaped bright red fruit with yelloworange flesh and black seeds that are surrounded by purple gelatine. The red colour is due to pigments called anthocyanins and the yellow-orange colour is due to carotenoids. This fruits are available in both red and yellow varieties. However, the red varieties are more popular and more common (Lister et al., 2005).

Tomato, Lycopersicon esulentum typically grow to 1–3 meters in height, weak stem and perennial in its native habitat. An average common tomato weighs approximately 100 grams. Cherry tomato *Solanum lycopersicum* var. *cerasiform*, have thin skins, high water content and can have variable size (Jones, 2008). Tomato and cherry tomato are believed to have a bright future and recently, there has been renewed attention given to the antioxidant content of tomatoes because many epidemiological studies suggested that regular consumption of fruits and vegetables, including tomatoes, can play an important role in preventing cancer and cardiovascular problems (Giovannucci, 1999; Heber, 2000). Local community usually prepare these fruits fresh, blended with milk or water, cooked in stews and sauces, and incorporated into desserts and salads.

Tamarillo, cherry tomatoand tomato have shown to be very useful plant and it is expectable that the interest of this kind of plant will arise in the forthcoming years. The findings from the study can be used to raise awareness about the advantage of this fruits and its role in assisting people health behaviours. Thus, this study was undertaken to determine the antioxidant capacity, total phenolic content and total flavonoid content of tamarillo (*Cyphomandra betacea*), cherry tomato (*Solanum lycopersicum* var. *cerasiform*) and tomato (*Lycopersicon esulentum*) in ethanol and water extract.

Materials and Methods

Standard and reagents

β-Carotene, linoleic acid, Tween 20, α-tocopherol, 2,2-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acids, sodium acetate trihydrate, acetic acids, 2,4,6tripyridly-1,3,5-triazine (TPTZ), ferric chloride (FeCl₃.6H₂O), ferumsulphate, sodiumcarbonate, gallic acids, sodium nitrate, aluminium trichloride, sodium hydroxide, and (+)- Catechin were purchased from Sigma Chemical Co. (St Louis, Mo, USA). Whereas, absolute ethanol and Folin-Ciocalteu reagent were purchased from Merk (Darmstadt, Germany), while Chloroform and hydrochloric acids were purchased from Fisher Scientific (Loughborough, UK).

Food sampling

Convenience sampling was used to obtain the sample for this study. Freshly harvested Tamarillo (*Cyphomandra betacea*), cherry tomato (*Solanum lycopersicum* var. *cerasiform*) and tomato (*Lycopersicon esulentum*) were purchased from local stall in Cameron Highland and stored at 4°C in Nutrition laboratory, Faculty of medicine and health sciences, UPM.

Sample preparation

Every sample were cleaned and washed to remove any residual compost by using tap water. These samples were cut into pieces and stored at -80°C. Then, the samples were freeze dried to remove the moisture content. After freeze-drying, the dried sample were ground into fine powder by using dry grinder and stored at -20°C prior to analysis.

Sample extraction

Ethanol extraction was prepared according to the method of Andarwulan *et al.* (2010). 2 g of freeze dried sample was extracted by shaking with 100 ml of 70% of ethanol for 1 hour at 50°C using an orbital shaker. Following centrifugation at 1536g for 5 min, aliquots of supernatant were reserved for analysis. For water extraction, sample was prepared according to the method of Reihani and Azhar (2012). By using orbital shaker, 2 g of dried sample was extracted by shaking with 100 ml of deionized water at room temperature for 1 hour. Water extract was obtained by filtering the mixture through Whatman No. 4 filter paper and used for analysis.

Determination of total phenolic content

Total phenolic content was determined according to the method of Singelton and Rossi (1965). Sample solution of 200 μ l was taken into 25 ml volumetric flask, to which 10 ml of water and 1.5 ml of Folin-Ciocalteau reagent was added. The mixture was then kept for 5 min and 4 ml of 20% w/v sodium carbonate solution was added and the volume was made up to 25 ml with distilled water. The mixture was kept for 30 minute until blue colour develops. The samples were then observed at 765 nm. Results were expressed as gallic acid equivalents (mg GAE/g edible portion).

Determination of total flavonoid content

Total flavonoid content was determined according to the method of Marinova *et al.* (2005). An aliquot 1 ml of extracts or standard solution of catechin was added to 10 ml volumetric flask containing 4 ml of distilled water. The flask was then added with 0.3 ml of 5% NaNO₂. After 5 min, 0.3 ml 10% AlCl₃ was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalent (CE)/ g edible portion.

Determination of total antioxidant activity

B-Carotene bleaching assay

The antioxidant activity of sample extracts was assayed based on the β -carotene bleaching method developed by Velioglu *et al.* (1998). Alfa-tocopherol was used as the standard. Beta-carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were transferred into a round bottomed

flask. The mixture was then added to 0.2 ml of sample extract or standard or ethanol (as control). Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary. Following evaporation, 100 ml of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. Then, 5 ml aliquots of the emulsion were pipette into test tubes and immediately placed in a water bath at 50°C. The absorbance was read at 20 min intervals for 2 h at 470 nm. The rate of β -carotene bleaching was calculated using the following formula:

Rate of bleaching (R) = {
$$\ln A_{t=0} / A_{t=1}$$
} X 1/t,

where $A_{t=0}$ is the absorbance of emulsion at 0 min; and $A_{t=t}$ is absorbance at time t (120 min). The calculated average rates were used to determine the antioxidant activity (AA) of the respective samples, and expressed as percentage of inhibition of the rate of β -carotene bleaching using the formula:

% AA =
$$(R_{Control} - R_{Sample} / R_{Control}) \times 100$$

where $R_{Control}$ and R_{Sample} represent the respective average β - carotene bleaching rates for the control and test samples, respectively.

2, 2-diphenyl-2-picrylhydrazyl radical scavenging assay

The scavenging activity was estimated according to the method of Marinova and Batchvarov (2011). 2 ml sample and 2 ml DPPH solution were added in test tube and mixed well. The mixtures were then stored 30 min in dark and the absorbance was determined at 517 nm against diluted blank. The scavenging effect of the DPPH radical is calculated using the following equation:

Scavenging effect (%) = $(A_{C517} - A_{S517} / A_{C517}) / 100$

where A_{C517} is absorbance of control at 517 nm and A_{S517} is absorbance of sample at 517 nm. EC₅₀ value was determined from the plotted graph of scavenging activity against the concentration of sample extract, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

Ferric reducing/antioxidant power (FRAP) assay

FRAP assay was determined based on the reduction of Fe³⁺-TPTZ to a blue colored Fe²⁺ TPTZ (Benzie and Strain, 1996). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6),

10 mM TPTZ and 20 mM FeCl₃.6H₂O in a ratio of 10:1:1, at to 37°C. FRAP reagent (3 ml) was pipetted into test tubes. A total of 100 μ l of sample and 300 μ l of distilled water was then added to the same test tubes, and incubated at 37°C for 4 min. Each sample was run in triplicate. Absorbance was measured at 593 nm. Absorbance values (A_{sample}) were measured after 4 min. Reagent blank reading, of FRAP reagent (A_{reagent blank}), and blank sample reading, using sample and acetate buffer (A_{blank sample}), were taken. The change in absorbance [A_{sample} – (A_{reagent blank}+A_{blank sample})] was calculated. Ascorbic acid was used as positive control. The FeSO₄•7H₂O calibration plot was obtained by plotting the change in absorbance against 200 to 1000 μ M concentrations of FeSO₄•7H₂O.

Statistical analysis

All analysis was performed in triplicate and data were expressed as mean \pm standard deviation. Data were analyzed by using one-way ANOVA using SPSS for windows version 20. One way ANOVA was used to test whether there are significant differences in antioxidant capacity, total phenolic content and total flavonoid content of samples between 70% ethanol extract and water extract. Pearson correlation test was used to assess correlation between antioxidant capacity and total phenolic and total flavonoid contents. A significant difference is considered at the level of p < 0.05.

Result and Discussion

Total phenolic and flavonoid content

The total phenolic content of tamarillo, yellow cherry tomato, red cherry tomato and tomato is shown in Table 1. All ethanol extract showed higher mean total phenolic content compared to water extract. Ethanol extract of tamarillo were significantly higher (p < 0.05) in total phenolic content and followed by yellow cherry tomato, red cherry tomato and tomato. However, analysis of variance showed no significant difference between red cherry tomato and tomato. The total phenolic content of water extracts followed the order of tamarillo > yellow cherry tomato >tomato > red cherry tomato. No significant difference was found between these samples.

It was reported that extract yields and resulting activities of the plant materials are strongly dependent on the nature of extracting solvent, due to the presence of different antioxidant compound of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Aqueous organic of tested plant material exhibiting greater phenolic content due to the fact that phenolics are often

Table 1. Mean of total phenolic content of samples in ethanol extract and water by Folin-Ciocalteu assay

Samples	GAE (mg/g)			
Samples	Ethanol extract	Water extract		
Tamarillo	7.63 ± 0.37d	1.83 ± 0.50a		
Yellow cherry tomato	5.07 ± 0.99c	1.55 ± 0.20a		
Red cherry tomato	4.28 ± 0.51b 1.35 ± 0.09a			
Tomato	4.25 ± 0.13b 1.39 ± 0.10a			
Values are expressed as mean ± standard deviation.				
Means with differe	ent letters were	significantly		
different at the level of	of p < 0.05.			

Table 2. Mean of total flavonoid content by aluminium chloride colorimetric assay in ethanol extract and water extract

Samples	CE (mg/g)			
Samples	Ethanol extract	Water extract		
Tamarillo	6.44 ± 0.16c	2.22 ± 0.31b		
Yellow cherry tomato	1.88 ± 0.26a	1.66 ± 0.02a		
Red cherry tomato	1.71 ± 0.09a	1.64 ± 0.06a		
Tomato	1.66 ± 0.06a	1.66 ± 0.03a		
Values are expressed as mean ± standard deviation.				
Means with differ different at the level		e significantly		

Table 3. Mean antioxidant activity of sample in ethanol extract and water extract calculated on the basis of the rate of β -carotene bleaching at t = 120. AA: Antioxidant

	Antioxidant Capacity		
Samples	Ethanol Extract	Water Extract	
	AA (%)	AA (%)	
Tamarillo	22.92 ± 3.60cd	28.89 ± 3.85d	
Yellow cherry tomato	20.83 ± 3.61abc	17.78 ± 3.85bc	
Red cherry tomato	10.42 ± 3.61ab	15.55 ± 3.85abc	
Tomato	8.33 ± 3.61a	11.11 ± 3.85ab	
Values are expressed as mean ± standard deviation.			
Means with different letters were significantly different			
at the level of $p < 0.0$	5.		

extracted in higher amounts in more polar solvents, and therefore greater reducing power (Bushra *et al.*, 2009).

Table 2 showed ethanol extract of tamarillo were significantly higher (p < 0.05) in total flavonoid content and followed by yellow cherry tomato, red cherry tomato and tomato. Water extract of tamarillo had the highest flavonoid content, followed by yellow cherry tomato, tomato and red cherry tomato. Yellow cherry tomato and tomato had slightly the same mean of total flavonoid content, yet no significant difference existed between these samples. All ethanol extract showed higher mean total flavonoid content compared to water extract.

Flavonoid compounds are considered to be the largest group of naturally occurring phenols. Plant flavonoids and phenols in general, are highly effective free radical scavenging and antioxidants. Polyphenol and flavonoids are used for the prevention and cure of various diseases which are mainly associated with free radicals. It has been reported that compounds such as the flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants (Atanassova *et al.*, 2011).

B-carotene bleaching assay

In β -carotene bleaching assay, the presence of an active antioxidant delays the rate of β -carotene bleaching. Heated at 50°C induced oxidation involves the subtraction of H-atom from an active methylene group of linoleic acids, forming a linolate free radical. The linolate radical then will attack the highly unsaturated β -carotene in an effort to regains its lost H-atom. The presence of a good antioxidant can prevent the attack on β -carotene by neutralizing the linolate radical.

The ethanol extracts of tamarillo showed higher antioxidant activity followed by yellow cherry tomato, red cherry tomato and tomato (Table 3). Tamarillo extracts showed a significant difference (p < 0.05) in antioxidant activity compared to the other extracts except for yellow cherry tomato. With regard to water extracts, tamarillo contained higher value of antioxidant activity, followed by yellow cherry tomato, red cherry tomato and tomato.

There were several factors that may contribute to the antioxidant activity in the samples. As cited in Guil-Guerro and Rebolloso-Fuentes (2012), it has been noted before that together with phenolic compounds, ascorbic acids represent the main watersoluble antioxidant in tomatoes and contributes to the antioxidant activity of the water soluble fraction. In addition Pinela *et al.* (2012) stated that all the differences observed in the antioxidant contents of tomato varieties are related to genotype, but also several factors such as ripening stage, cultivation practices and also climatic environment.

As presented in Table 6, there was a positive correlation existed between antioxidant activity and total phenolic content in ethanol (r = 0.687) and water (r = 0.706) extracts. Besides, a positive correlation also existed between antioxidant activity and total flavonoid content in ethanol (r = 0.656) and also water (r = 0.820) extracts. Based on the correlation, phenolic compounds were the main micro constituents contributing to the antioxidant activity of the samples. It is well known those flavonoids are a subset of phenolic content (Nurul and Asmah, 2012). Thus, this similar correlation with total phenolic content was expected. In addition, water extracts showed higher antioxidant activity compared to ethanol extracts and seems to inhibit the oxidation of β -carotene in a β -carotene-linoleate system better than compound soluble in ethanol.

DPPH radical scavenging assay

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit cellular damage. The DPPH free radical scavenging method is colorimetric assay and can be used to evaluate the radical scavenging capacity of specific compounds or extract. EC_{50} was determined from the plotted graph of scavenging activity against concentration of samples, which is defined as the amount of

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Table 4. Mean scavenging activities (EC_{50}) of samples in ethanol extract and water extract by DPPH radical

Samples	EC ₅₀ (µg/ml)	
	Ethanol extract	Water extract
Tamarillo	44.25 ± 0.82a	47.38 ± 1.11ab
Yellow cherry tomato	46.22 ± 4.51ab	49.01 ± 0.58b
Red cherry tomato	46.47 ± 0.38ab	48.79 ± 1.29ab
Tomato	46.33 ± 2.63ab	47.48 ± 3.48ab
Values are expressed a with different letters we of p < 0.05.		

Table 5. Mean FRAP values (μ M Fe (II)/g) of samples in ethanol extract and water extract by ferric reducing/

antioxi					
Samulas	μM Fe (II)/g		_		
Samples	Ethanol extra	ct Water extrac	t		
Tamarillo	12.17 ± 0.53	e 3.72 ± 0.20b			
Yellow cherry tomato	7.87 ± 0.42d	1 3.32 ± 1.07at)		
Red cherry tomato	7.00 ± 0.40c	d 2.30 ± 0.08a			
Tomato	6.58 ± 0.58c	2.73 ± 1.13at	,		
Values are expressed as mean ± standard deviation. Means					
with different letters	were significan	tly different at tl	he		
level of $p < 0.05$.					
· · ·	0.5	a 1.			
Table 6. r-values of Pearson Correlation test					
Antiox	idant Scav	enging Fe	rric reducing		
activ	rity act	ivity	activity		
Ethanol W	ater Ethanol	Water Eth	anol Water		
	ract extract	extract ext	ract extract		
Total phenolic content 0.687* 0.7	-0.215	0.013 0.9	58** 0.645*		
Total					
flavonoid 0.656* 0.8	20** -0.342	-0.101 0.9	74** 0.515		
content					
**Correlation is significant at the 0.01 level (2-tailed)					

*Correlation is significant at the 0.05 level (2-tailed)

antioxidant necessary to decrease the initial DPPH radical concentration by 50% (Table 4). The lowest EC_{50} indicates the strongest ability of samples to act as DPPH scavengers.

For ethanol extracts, tamarillo had the lowest EC_{50} , meanwhile, yellow cherry tomato, red cherry tomato and tomato had slightly same EC_{50} . However no significant difference existed between these samples. Of the water extracts, tamarillo and tomato had slightly the same EC_{50} which was lower compared to other sample and followed by red cherry tomato and yellow cherry tomato.

Marinova and Batchvarov (2011) stated that there were substantial differences in used solvents, concentration of DPPH working solutions, ratio between volumes of sample/reagent, duration of reaction, wavelength of absorbance measurement, standard solutions and equations for calculation of the result. In addition, determination of the effect of methods conditions by ruggedness testing of methods indicated that the accuracy of the method for determination of free radical scavenging activity is affected by the solvent used (ethanol or methanol) and the sample /reagent DPPH volume ratio.

Whereas, Azeez *et al.* (2012) reported that antioxidant activity decrease due to ripeness. Other reasons for low antiradical efficiency might be that the phenolic compounds are bound to other molecules, such as carbohydrates, which considerably reduce the scavenging activity.

As shown in Table 6, there was no significant

correlation existed between scavenging activity and total phenolic content in ethanol and water extracts. Similarly, there was no significant correlation detected between scavenging activity and total flavonoid content. Therefore, this present study may indicate that scavenging ability on DPPH could not due to polyphenolic compounds found in tamarillo, cherry tomato and tomato extracts.

Ferric Reducing/Antioxidant Power assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe³⁺-TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe²⁺-TPTZ). Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom. As shown in table 5, the ethanol extract of tamarillo exhibited significantly higher (p < 0.05) antioxidant potential on the FRAP assay, and followed by yellow cherry tomato, red cherry tomato and tomato. For water extracts, tamarillo also exhibited the highest antioxidant potential, followed by yellow cherry tomato, tomato and red cherry tomato.

Dragovic-Uzelac *et al.* (2007) stated that higher phenolic content have shown to exert greater reducing power. Therefore, as the reducing power was determined with the Fe^{3+} to Fe^{2+} transformation, the reducing power increased with increasing concentrations of phenolics in the sample extracts.

As stated by Benzie and Strain (1996), the reduction of Fe^{3+} -TPTZ complex to blue coloured of Fe^{2+} -TPTZ occurs at low pH. Jones (2008) stated that the pH value for tomato is between 4.0 and 4.5; the lower the pH the greater the so-called tartness. Whereas Bajaj (1996) stated that tamarillo pulp, which varies in colour from yellow to orange-red, is relatively acidic (pH 3.7-3.8) and has an agreeable aromatic flavour. The highest antioxidant potential of tamarillo could be due to the highly acidic nature of the tamarillo which may influence the pH of the assay medium.

Table 6 showed that there was a positive correlation between ferric reducing activity and total phenolic content in ethanol (r = 0.958) and also water (r = 0.645) extracts. Whereas, a strong significant correlation existed between ferric reducing activity and total flavonoid content in ethanol extract (r = 0.974). However, there was no significant correlation existed between ferric reducing activity and total flavonoid content in water extract.

Water extracts showed no correlation between ferric reducing activity and total flavonoid content possibly because low flavonoid compound extracted in water extracts. The extracting solvent affected the total phenolic content and antioxidant capacity of tamarillo, cherry tomato and tomato extracts. In addition Cheung *et al.* (2003) reported that the amount of phenolic compound in organic extract was higher than in water extracts.

Conclusion

On the basis of the result obtained from the study, ethanolic extracts showed the highest antioxidant activity when determined by the DPPH and FRAP assay, while water extracts showed highest antioxidant activity when evaluated by beta-carotene bleaching assay. Besides, the highest phenolic and flavonoid amount was found in ethanol extracts, it is thus suggested that phenolic and flavonoid compounds present in the sample extracts have strong scavenging ability and ferric reducing power. However, it should be noted that, different solvent and extraction method used and growing condition of the sample material may lead to an overestimation of total phenolic or flavonoid content and antioxidant can exert its effect by different mechanisms and functions. It is interesting to conduct more research and to compare on the polyphenol pattern including flavonols, flavanones and cinnamate derivatives since the biological activities of these polyphenols have become well known in recent years evidencing their beneficial effects on human health.

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