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Fractionation and Identification of Phytochemicals and Antioxidant Activity of Wild Grape (*Ampelocissus martinii* Planch.) Seed Extracts

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A methanolic extract of wild grape (*Ampelocissus martinii* Planch.) seed at green (immature) growth stage was partially purified using silica gel chromatography, before the investigation of total phytochemicals and antioxidant activity of sub-fractions. The ethyl acetate/methanol at 3:1 (v/v) fraction had higher phytochemicals and antioxidant activities than others. The gallic acid, myricetin, and resveratrol were dominant phenolic substances in this fraction. However, ethyl acetate sub-fraction found the highest content of epicatechin and catechin. A correlation test indicated a strong positive trend between antioxidant activity and all tested of the phytochemical contents. The obtained results indicated that the wild grape seed contains compounds with excellent antioxidation capacity. That might be applied as an active ingredient in health-supporting products or active ingredients in cosmetics.

Keywords: Wild grape, Phytochemical, Antioxidant activity, Fractionation.

INTRODUCTION

Recently, the discovery of active ingredients from plants and the study of their biological activities for development as health supplements and cosmetics, as well as their use in traditional medicines have been gradually interested [1,2]. The substances obtained from plants, known as phytochemicals, have also proven their safety without side effects compared with synthetic substances. Many diseases caused by metabolic activities in the body without infection such as cancer, Alzheimer's, diabetes, cataracts, hypertension, chronic inflammatory disease, dermatitis and aging [3]. These diseases called "degenerative diseases" and progress often caused by free radicals. Exactly, protection from damage caused by the free radicals can reduce by supplying electron-donating substances, namely antioxidants, especially phytochemicals [4,5]. They derive from secondary metabolites of plants and include huge groups such as phenolics, flavonoids, quinines, tannins, alkaloids, saponins and phytosterols [6,7]. Various biological activities of these phytochemicals were proposed [8,9]. However, sources are critical factors in obtaining desired phytochemicals.

During the past decade, different types of health supplement products from grape, especially seed extracts, have been produced and commercially advertized [7,10,11]. Many reports confirmed that all parts of grapes composed high phytochemical contents [7]. The authors are interested in phytochemicals found in a native herb, namely wild grape (*Ampelocissus martinii* Planch.). In Thailand, especially the north and northeast have been used the wild grape as an herb. Its stem, fruit, and leaf were similar with a planted grape. Previous study indicated that the fruit extracts of wild grapes have a high content of phytochemicals and biological activities such as antioxidant and antibacterial [12-15]. However, a detailed study to identify the types and content of each phytochemical would be needed to support the development of further applications. Therefore, this work reported the fractionation of the crude extract of wild grape seed using silica gel column chromatography. The obtained fractions were further investigated their total phytochemicals as well as the antioxidant activity. The HPLC was also used for identification of phytochemicals of the fractionated samples.

EXPERIMENTAL

Pure standards of phenolics and flavonoids as well as the reagents for total phytochemicals and antioxidant activity were purchased from Sigma-Aldrich (U.S.A.). All mobile phase was of HPLC grade (Merck, Germany) and used without further purified.

Plant extraction and fractionation: The wild grape (*Ampelocissus martinii* Planch.) fruits at immature growth stage collected in September 2018 from Roi-Et Province, Thailand. The seeds and pulp were separated and the seeds were dried and ground into small pieces. The wild grape seed powder and methanol at 1:10 (w/v) ratio were mixed and stirred for 3 h. The extracted process was performed in triplicate and then pooled, centrifuged before the collection of supernatant. The obtained extract was rotary evaporated to obtain crude extract. A glass column (60 cm × 4.5 cm i.d.) packed with silica gel (60-200 mesh) was prepared and then loaded with the crude extract solution. The mixtures of different polarity solvents used for column elution with a flow rate of 1.0 mL/min. The serial of elution solvent was starting with ethyl acetate/methanol in the following ratios successively: 100:0, 75:25, 50:50, 25:75 and 0:100. The 10 mL of each fraction was collected and read at 280 nm. Sub-fractions were grouped before evaporation by a rotary vacuum evaporator.

Total phenolic content (TPC): The investigation method to determine total phenolic content was adopted as described earlier [16]. The methanolic solution (0.2 mL) was reacted with a mixture solution of Folin-Ciocalteau reagent (1.0 mL) and 7.5% Na_2CO_3 solution (0.8 mL). After standing for 30 min, the mixture was measured absorbance at 765 nm. The TPC were expressed as mg gallic acid equivalent per gram of dry weight (GAE/g DW).

Total flavonoid content (TFC): The investigation method to determine total flavonoid content was adopted as described earlier [17]. The methanolic solution (0.5 mL), distilled water (0.2 mL), and 5% $NaNO_2$ solution (0.1 mL) was firstly mixed and stand for 6 min, before adding 10% $AlCl_3$ solution (0.2 mL). After 5 min, added of 1M NaOH solution (0.5 mL) and left for 15 min. The mixture was measured absorbance at 510 nm. The TFC expressed as mg catechin equivalent per gram of dry weight (CE/g DW).

Total proanthocyanidin content (TPAC): The investigation method to determine total proanthocyanidin content was described earlier [18]. A mixture solution of extract (0.2 mL) and 4% vanillin-ethanol (1.5 mL) were firstly prepared before adding concentrated HCl (0.75 mL). After being left for 15 min, the mixture was measured absorbance at 500 nm. The TPAC expressed as mg catechin equivalent per gram of dry weight (CE/g DW).

Total saponin content (TSC): The investigation method to dertermine total saponin content as described by Hiai *et al.* [19]. Briefly, a mixture of extract and 8% vanillin-ethanol (1:1 ratio) firstly prepared before adding of 72% H_2SO_4 (2.5 mL). After that the mixture solution was then warmed and cooled again until to room temperature before reading absorbance at 560 nm. The TSC was expressed as mg aesin equivalent per gram of dry weight (AES/g DW).

DPPH radical scavenging activity: The extract was determined for DPPH radical scavenging activity following by a previous work [20]. The mixture was measured at the absorbance of 517 nm. The DPPH inhibition (%) of extract was calculated according to eqn 1. Finally, the antioxidant activity expressed as a 50% inhibition (IC_{50}) value.

$$\text{DPPH inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

ABTS radical scavenging activity: The $ABTS^{\bullet+}$ solution and the extract were mixed and left in the place without light for 1 min before measuring absorbance at 734 nm [16]. The percent inhibition of the extract was calculated according to eqn 2 and expressed as IC_{50} value.

$$\text{ABTS inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

Ferric reducing antioxidant power (FRAP): Firstly, the FRAP reagent (acetate buffer (pH 3.6), 20 mM $FeCl_3$, 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl) was firstly prepared. The extract was then mixed before incubation for 15 min at 37 ºC [18]. The mixture was measured absorbance at 593 nm. The result was expressed as mmol ferrous equivalent per gram of dry weight (Fe^{2+}/g DW).

Cupric reducing antioxidant capacity: Firstly, the developing reagent ($CuCl_2$, neocuproine- ethanol and acetate buffer at pH 7.0) was prepared before mixing with the extract. After 30 min of standing, the mixture was measured absorbance at 450 nm and expressed result as mg Trolox equivalent per gram of dry weight (TE/g DW) [21].

HPLC analysis of phenolic content: The separations for phenolics and flavonoids were RP-HPLC using C18 column (4.6 mm × 250 mm, i.d. 5 µm) with LC-20AC pumps (Shimadzu Co., Kyoto, Japan) and a diode array as detector. A 20 µL injection volume was used and the elution was performed by gradient system of deionized water-acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B). A re-equilibration period with 5% solvent B was adjusted between individual runs of 5 min. The flow rate was monitored at 0.8 mL/min. The standard external compounds of hydroxybenzoic acid, stilbene hydroxycinnamic acid and flavonols were recorded at 280, 306, 320 and 360 nm, respectively.

RESULTS AND DISCUSSION

The crude methanolic extract of wild grape seed fractionated by column chromatography (W-SF) was obtained and then analyzed for bioactive compounds and their biological activities. Five sub-fractions divided by eluting solvents; W-SF1, W-SF2, W-SF3, W-SF4 and W-SF5 were grouped regarding the absorbance at 280 nm (Fig. 1). Fractionation yield in the sub-fractions of wild grape seed as shown in Table-1. W-SF2 showed the highest value in wild grape (25%), while the lowest yield was found in W-SF5 (3.07%).

The phytochemical content of TPC, TFC, TPAC, TSC in various sub-fractions found that W-SF2 derived from the crude extracts of wild grape seed had polyphenol content of TPC, TFC and TSC with the highest content (Table-1). However,

TABLE-1
FRACTIONATION YIELDS AND PHYTOCHEMICAL CONTENT IN WILD GRAPE SEED SUB-FRACTIONS

Sub-fraction	Fractionation yield (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)	TPAC (mg CE/g DW)	TSC (mg AES/g DW)
Crude extract	-	417.4 ± 22.5^{b}	384.9 ± 7.0^{a}	324.7 ± 37.0^{c}	687.3 ± 14.3^{de}
W-SF1	18.0 ± 0.2^{c}	211.1 ± 3.15^{d}	288.2 ± 5.8^{f}	369.8 ± 9.7^{a}	725.0 ± 38.6^{cd}
W-SF2	25.0 ± 0.2^{b}	486.7 ± 18.2^{a}	353.5 ± 11.7^{b}	342.0 ± 13.2^{bc}	784.1 ± 27.1^{c}
W-SF3	12.6 ± 0.4^{d}	329.5 ± 28.0^{c}	242.3 ± 10.4^{g}	188.1 ± 1.3^{e}	632.1 ± 18.0^{e}
W-SF4	7.5 ± 0.1^{e}	11.9 ± 0.9^{g}	12.3 ± 0.2^{e}	4.6 ± 0.2^{h}	53.3 ± 4.9^{i}
W-SF5	3.1 ± 0.1^{f}	35.5 ± 1.0^{g}	40.6 ± 1.0^{i}	8.7 ± 0.2^{h}	150.2 ± 9.2^{h}

Results are expressed as mean ± SD of triplicate measurements. Means with different letters in the same column represent significant differences at $p < 0.05$. DW, dry weight of sample; W-SF, wild grape sub-fraction.

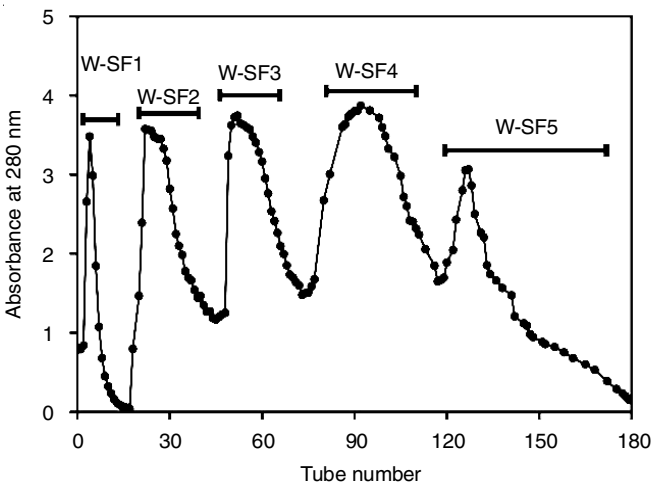

Fig. 1. Column chromatography profile of wild grape seed fractions

observation in different individual phenolics or flavonoids of this sub-fraction is also appeared. The polyphenol contents varied in each sub-fraction, which may have been affected according to the differences of the variable polarity of the mobile phase in the fractionation step [22]. Moreover, species-specific differences in their chemical compositions in the various sub-fractions of wild grape extracts were also considered [23]. The TPAC was found in following as W-SF1 > W-SF2 > W-SF3 > W-SF5 ≈ W-SF4, respectively.

The DPPH$^{\bullet}$ assay has been chosen to evaluate the antioxidant power of the fractionated extracts according to its wildly used [23]. The radical scavenging activities of the fractionated wild grape seeds expressed as IC_{50} values (Table-2). W-SF3 and W-SF2 had the highest efficiency to scavenge DPPH radicals. With ABTS$^{\bullet+}$ scavenging activity assay showed a similar trend as found in the DPPH$^{\bullet}$ scavenging assay, in which W-SF3 had the highest activity. The highest FRAP values found in W-SF2 (666.43 μmol Fe^{2+}/g DW). This assay is also wildly applied for antioxidant evaluation due to it is a simple, rapid and inexpensive method [24]. The cupric reducing antioxidant activity of various sub-fractions ranged from 9.58 to 304.32 mg TE/g DW with the highest value in W-SF2 (304.32 mg TE/g DW). Present findings revealed that the first three sub-fractions of wild grape crude extract (W-SF1, 2 and 3) presented intense antioxidant activity, especially W-SF2. These suggest that W-SF2 had potential antioxidants on free radicals, especially by scavenging and reducing power. The differences antioxidants activities of the various sub-fractions might be caused by variable compounds associated with polyphenols and their chemical structures [2].

Table-3 showed correlations between phytochemical contents and antioxidant activity of each sub-fractions. The correlation coefficient (r) indicated a high positive correlation between the TPC, TFC, TPAC, TSC and antioxidant activities (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP and cupric reducing antioxidant activity assays) ranged from 0.575 to 0.957 ($p < 0.01$). These indicated that all phenolic compounds are essential role contributors to antioxidant activity. These findings are in agreement with previous studies [25-28].

HPLC analysis indicated that the highest content of total phenolic compound found in W-SF1. Among the phenolic compounds, epicatechin was the predominant substance, and the highest content found in W-SF1 (102.25 mg/g DW). Remarkably, W-SF1 exhibited the highest content of catechin and epicatechin (more than 90% of the total phenolic compounds), whereas the antioxidant activity was lower than W-SF2. The results indicate that catechin and epicatechin are not the only

TABLE-2
ANTIOXIDANT ACTIVITIES OF WILD GRAPE SEED EXTRACT AND ITS SUB-FRACTIONS

Sub-fraction	DPPH$^{\bullet}$ assay $IC_{50}{}^{A}$ (μg/mL)	ABTS$^{\bullet+}$ assay IC_{50} (μg/mL)	FRAP assay (μmol Fe^{2+}/g DW)	CUPRAC assay (mg TE/g DW)
Crude extract	39.4 ± 0.4^{h}	6.3 ± 0.1^{k}	1497.7 ± 30.2^{b}	111.6 ± 0.2^{e}
W-SF1	50.5 ± 0.7^{g}	25.37 ± 0.31^{g}	457.47 ± 11.04^{f}	233.61 ± 0.48^{c}
W-SF2	28.4 ± 0.2^{i}	20.99 ± 0.27^{h}	666.43 ± 21.26^{c}	304.32 ± 1.75^{a}
W-SF3	26.1 ± 0.1^{i}	13.97 ± 0.03^{j}	537.30 ± 2.48^{de}	236.39 ± 1.69^{bc}
W-SF4	490.0 ± 10.0^{a}	304.5 ± 1.7^{b}	94.8 ± 1.5^{i}	9.6 ± 0.1^{h}
W-SF5	221.8 ± 3.3^{c}	165.3 ± 2.6^{c}	182.2 ± 7.6^{g}	24.4 ± 0.1^{g}

[A]The concentration of the plant extract that scavenges 50% of free radical. Lower IC_{50} values indicate higher radical scavenging activity. Results are expressed as mean ± SD of triplicate measurements. Means with different letters in the same column represent significant differences at $p <$ 0.05. DW, dry weight of sample.

TABLE-3
CORRELATION COEFFICIENTS (*r*) BETWEEN PHYTOCHEMICAL CONTENTS AND DIFFERENT ANTIOXIDANT ASSAYS OF WILD GRAPE SEED SUB-FRACTIONS

	TPC	TFC	TPAC	TSC	DPPH$^{\bullet}$	ABTS$^{\bullet+}$	FRAP	CUPRAC
TPC	1	.868**	.842**	.703**	.787**	.888**	.836**	.885**
TFC	–	1	.957**	.936**	.701**	.836**	.850**	.815**
TPAC	–	–	1	.855**	.661**	.803**	.809**	.840**
TSC	–	–	–	1	.575**	.736**	.727**	.638**
DPPH$^{\bullet}$	–	–	–	–	1	.820**	.939**	.778**
ABTS$^{\bullet+}$	–	–	–	–	–	1	.796**	.803**
FRAP	–	–	–	–	–	–	1	.871**
CUPRAC	–	–	–	–	–	–	–	1

**Correlation is significant at the 0.01 level.

TABLE-4
COMPOSITION OF INDIVIDUAL PHENOLIC COMPOUNDS (mg/g DW) IN WILD GRAPE SEED SUB-FRACTIONS

Sub-fraction	Gallic acid	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Resveratrol	Catechin
Crude extract	3.06 ± 0.05^{d}	0.23 ± 0.01^{c}	0.06 ± 0.00^{c}	0.33 ± 0.01^{a}	0.48 ± 0.02^{g}	0.75 ± 0.05^{f}
SF 1	5.98 ± 0.32^{b}	0.04 ± 0.00^{i}	0.06 ± 0.01^{c}	0.05 ± 0.00^{ef}	1.05 ± 0.01^{d}	1.83 ± 0.10^{d}
SF 2	10.15 ± 0.13^{a}	0.11 ± 0.02^{g}	0.10 ± 0.01^{b}	0.22 ± 0.00^{b}	8.52 ± 0.04^{a}	0.72 ± 0.09^{f}
SF 3	0.72 ± 0.00^{ij}	0.13 ± 0.00^{f}	0.03 ± 0.00^{g}	0.12 ± 0.02^{d}	3.75 ± 0.15^{b}	1.63 ± 0.07^{e}
SF 4	0.54 ± 0.00^{j}	0.06 ± 0.00^{h}	0.02 ± 0.00^{h}	0.03 ± 0.00^{f}	0.84 ± 0.09^{e}	ND
SF 5	1.58 ± 0.01^{g}	0.14 ± 0.00^{e}	0.04 ± 0.00^{f}	0.08 ± 0.00^{e}	0.46 ± 0.02^{g}	0.02 ± 0.00^{h}

Sub-fraction	Epicatechin	Rutin	Myricetin	Quercetin	Total
Crude extract	60.45 ± 0.60^{b}	0.08 ± 0.00^{b}	0.13 ± 0.00^{c}	0.05 ± 0.00^{b}	65.60 ± 0.52^{c}
SF 1	102.25 ± 0.16^{a}	0.02 ± 0.00^{c}	0.01 ± 0.00^{e}	0.15 ± 0.01^{a}	111.44 ± 0.34^{b}
SF 2	44.78 ± 0.92^{d}	0.14 ± 0.00^{a}	0.39 ± 0.01^{a}	0.01 ± 0.00^{e}	65.18 ± 1.02^{c}
SF 3	0.68 ± 0.05^{h}	0.03 ± 0.00^{b}	0.02 ± 0.00^{d}	ND	7.11 ± 0.24^{f}
SF 4	0.07 ± 0.00^{l}	ND	ND	ND	1.58 ± 0.09^{i}
SF 5	0.01 ± 0.00^{l}	ND	ND	0.01 ± 0.00^{e}	2.43 ± 0.01^{gh}

Results are expressed as mean ± SD of triplicate measurements. Means with different letters in the same column represent significant differences at $p < 0.05$. ND, not detected; DW, dry weight of sample.

substances in sub-fractions that exhibited antioxidant activity. However, flavanol monomers, including catechin, epicatechin, and others, maybe potent anticarcinogens and antiatherogenic agents as evidence by previous work [26]. The most abundant phenolic compounds in W-SF2 were gallic acid, resveratrol and myricetin (10.15, 8.52 and 0.39 mg/g DW) at higher levels than in other sub-fractions. These results might be suggested that gallic acid, resveratrol, and myricetin are the most important antioxidant contributors. The number and position of a hydroxyl group in the phytochemicals structure are also involved in antioxidant activities [29,30]. The results (Table-4) indicated that the fractionation using column chromatography could use to partially purify and concentrate individual phenolic compounds in wild grape seed extract.

Conclusion

Five sub-fractions of wild grape seed extract obtained from silica gel column chromatography, W-SF1 to W-SF5. DPPH, ABTS, FRAP and cupric reducing antioxidant activity assays indicated that the W-SF2 had the highest antioxidant activities as determined, which positively correlated with the TPC, TFC, TPAC and TSC. Based on HPLC analysis, the predominant substances found in W-SF2 were gallic acid, myricetin and resveratrol. Also, besides W-SF1 showed the highest total value of epicatechin and catechin, but the antioxidant activity was lower than in W-SF2. Present findings suggested that W-SF1 was a flavonoid-enriched sub-fraction, while the W-SF2 had the high content of other substances which possessed stronger antioxidant activities. This finding result supported that wild grape seed is an excellent natural source of phenolic compounds with exhibited high antioxidant activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S. Feng, Z. Luo, Y. Zhang, Z. Zhong and B. Lu, *Food Chem.*, **151**, 452 (2014); https://doi.org/10.1016/j.foodchem.2013.11.057
2. H.-I. Jun, B.-T. Kim, G.-S. Song and Y.-S. Kim, *Food Chem.*, **148**, 367 (2014); https://doi.org/10.1016/j.foodchem.2013.10.028
3. L.A. Pham-Huy, H. He and C. Pham-Huy, *Int. J. Biomed. Sci.*, **4**, 89 (2008); https://www.ncbi.nlm.nih.gov/pubmed/23675073

4. A. Apostolou, D. Stagos, E. Galitsiou, A. Spyrou, S. Haroutounian, N. Portesis, I. Trizoglou, A.W. Hayes, A.M. Tsatsakis and D. Kouretas, *Food Chem. Toxicol.*, **61**, 60 (2013); https://doi.org/10.1016/j.fct.2013.01.029
5. P. Pajak, R. Socha, D. Galkowska, J. Roznowski and T. Fortuna, *Food Chem.*, **143**, 300 (2014); https://doi.org/10.1016/j.foodchem.2013.07.064
6. A. King and G. Young, *J. Am. Diet. Assoc.*, **99**, 213 (1999); https://doi.org/10.1016/S0002-8223(99)00051-6
7. K. Farhadi, F. Esmaeilzadeh, M. Hatami, M. Forough and R. Molaie, *Food Chem.*, **199**, 847 (2016); https://doi.org/10.1016/j.foodchem.2015.12.083
8. C. Ignea, C.M. Dorobantu, C.P. Mintoff, N. Branza-Nichita, M.R. Ladomery, P. Kefalas and V.S. Chedea, *Food Chem.*, **141**, 3967 (2013); https://doi.org/10.1016/j.foodchem.2013.06.094
9. D. Sahpazidou, G.D. Geromichalos, D. Stagos, A. Apostolou, S.A. Haroutounian, A.M. Tsatsakis, G.N. Tzanakakis, A.W. Hayes and D. Kouretas, *Toxicol. Lett.*, **230**, 218 (2014); https://doi.org/10.1016/j.toxlet.2014.01.042
10. A.V.S. Perumalla and N.S. Hettiarachchy, *Food Res. Int.*, **44**, 827 (2011); https://doi.org/10.1016/j.foodres.2011.01.022
11. P.R. Poudel, H. Tamura, I. Kataoka and R. Mochioka, *J. Food Compos. Anal.*, **21**, 622 (2008); https://doi.org/10.1016/j.jfca.2008.07.003
12. Y. Boonsod, A. Sangdee and P. Srihanam, *Br. J. Pharm. Res.*, **4**, 23 (2014); https://doi.org/10.9734/bjpr/2014/5626
13. J. Jirum, A. Sangdee and P. Srihanam, *Int. J. Res. Ayurveda Pharm.*, **4**, 337 (2013); https://doi.org/10.7897/2277-4343.04306
14. P. Srihanam and P. Siripipatthana, *Orient. J. Chem.*, **35**, 1690 (2019); https://doi.org/10.13005/ojc/350609
15. W. Simchuer and P. Srihanam, *Orient. J. Chem.*, **34**, 875 (2018); http://doi.org/10.13005/ojc/340235
16. E. Pastrana-Bonilla, C.C. Akoh, S. Sellappan and G. Krewer, *J. Agric. Food Chem.*, **51**, 5497 (2003); https://doi.org/10.1021/jf030113c
17. J. Kubola, S. Siriamornpun and N. Meeso, *Food Chem.*, **126**, 972 (2011); https://doi.org/10.1016/j.foodchem.2010.11.104
18. Y. Li, C. Guo, J. Yang, J. Wei, J. Xu and S. Cheng, *Food Chem.*, **96**, 254 (2006); https://doi.org/10.1016/j.foodchem.2005.02.033
19. S. Hiai, H. Oura and T. Nakajima, *Planta Med.*, **29**, 116 (1976); https://doi.org/10.1055/s-0028-1097639
20. I.S. Choi and E.J. Kwak, *Food Sci. Biotechnol.*, **23**, 1677 (2014); https://doi.org/10.1007/s10068-014-0228-8
21. R. Apak, K. Güçlü, M. Özyürek and S.E. Karademir, *J. Agric. Food Chem.*, **52**, 7970 (2004); https://doi.org/10.1021/jf048741x
22. L. Zahradníková, Š. Schmidt, Z. Sékelyová and S. Sekretár, *Czech J. Food Sci.*, **26**, 58 (2008); https://doi.org/10.17221/1135-CJFS
23. M. Cho, H.-S. Lee, I.-J. Kang, M.-H. Won and S. You, *Food Chem.*, **127**, 999 (2011); https://doi.org/10.1016/j.foodchem.2011.01.072
24. I.F.F. Benzie and J.J. Strain, *Anal. Biochem.*, **239**, 70 (1996); https://doi.org/10.1006/abio.1996.0292
25. N. Cotelle, *Curr. Top. Med. Chem.*, **1**, 569 (2001); https://doi.org/10.2174/1568026013394750
26. R. Guendez, S. Kallithraka, D.P. Makris and P. Kefalas, *Food Chem.*, **89**, 1 (2005); https://doi.org/10.1016/j.foodchem.2004.02.010
27. S.-Y. Kim, S.-M. Jeong, W.-P. Park, K.C. Nam, D.U. Ahn and S.-C. Lee, *Food Chem.*, **97**, 472 (2006); https://doi.org/10.1016/j.foodchem.2005.05.027
28. V. Katalinic, S.S. Mozina, D. Skroza, I. Generalic, H. Abramoviè, M. Miloš, I. Ljubenkov, S. Piskernik, I. Pezo, P. Terpinc and M. Boban, *Food Chem.*, **119**, 715 (2010); https://doi.org/10.1016/j.foodchem.2009.07.019
29. Y.-H. Chen, Z.-S. Yang, C.-C. Wen, Y.-S. Chang, B.-C. Wang, C.-A. Hsiao and T.-L. Shih, *Food Chem.*, **134**, 717 (2012); https://doi.org/10.1016/j.foodchem.2012.02.166
30. M.S. Ola, A.M. Aleisa, S.S. Al-Rejaie, H.M. Abuohashish, M.Y. Parmar, A.S. Alhomida and M.M. Ahmed, *Neurol. Sci.*, **35**, 1003 (2014); https://doi.org/10.1007/s10072-014-1628-5