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ANTIOXIDANT ACTIVITY of DIFFERENT EXTRACTS FROM STALK PART of RHUBARB (*RHEUM RHABARBARUM* L.)

Ravent (*rheum rhabarbarum* l.) Bitkisinin sap kısmının farklı ekstraktlarının antıoksıdan aktivıteleri

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ÖZET

Bu çalışmada, ravent (*Rheum rhabarbarum* L.) saplarının metanol, etil asetat ve heksan ekstraktlarının antioksidan aktiviteleri farklı *in vitro* model sistemleri kullanılarak değerlendirilmiştir. Ravent saplarının polar çözücüdeki ekstraktlarının serbest radikal süpürme aktiviteleri, 2,2'-azino-bis (3-etilbenzotiazolin-6-sülfonik asit), hidroksil ve 1,1-difenil-2-pikrilhidrazil radikalleri kullanılarak ayrı ayrı ölçülmüştür. Her bir ekstraktın demir iyonlarını şelatlama kabiliyetleri ve toplam antioksidan kapasiteleri araştırılmıştır. Buna ek olarak, ravent sap ekstraktlarının toplam fenolik, flavonoid ve flavonol içerikleri belirlenmiştir. Metanol ekstresi, en yüksek 1,1-difenil-2-pikrilhidrazil radikalini temizleme aktivitesini göstermişte olup, ardından etilasetat ve hekzan ekstreleri ve son olarak da bütil hidroksi toluen yer almaktaydı. Etil asetat ekstraktı, en yüksek 2,2'-azino-bis (3-etilbenzotiazolin-6-sülfonik asit) radikal temizleme aktivitesini gösterirken (IC_{50} değeri 59,7±1,1 µg/mL), bunu bütil hidroksi toluen ve metanol ekstraktı takip etmekteydi (sırasıyla 53.1±2.2 ve 47,9±1,3 µg/mL), ancak hidroksil radikalini temizleme aktivitesini en fazla metanol ekstraktı göstermekteydi (IC_{50} değeri 75.1±2.9 µg/mL). Toplam antioksidan aktivite, en fazla metanol ve etilasetat ekstraktlarında bulunmuştur (1 mg ekstrakta sırasıyla 284,1±11,6 ve 237,6±10 µg askorbik asit eşdeğeri). 100 µg/mL konsantrasyonundaki metanol ve etilasetat ekstraktları, demir iyonlarına karşı en yüksek şelatlama aktivitesini göstermişlerdir (sırasıyla % 96,3±2,2 ve % 89,8±1,5). Ravent sap ekstraktları, kullanılan çözücüye ve konsantrasyona bağlı olarak oldukça belirgin bir antioksidan aktivite göstermiştir. Ravent sap ekstraktlarının güçlü bir antioksidan potansiyel göstermesi, *Rheum rhabarbarum* L.'nin ümit verici bir bitki olduğunu düşündürmektedir.

Anahtar Kelimeler:
Rheum rhabarbarum L., ravent, sap, antioksidan aktivite

ABSTRACT

In this study, the antioxidant activities of methanol, ethyl acetate and hexane extracts from stalks of Rhubarb (*Rheum rhabarbarum* L.) were evaluated using different model systems *in vitro*. Free radical scavenging activities were assessed by measuring the scavenging activities of stalks different polar extracts separately using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid, hydroxyl and 1,1-diphenyl-2-picrylhydrazyl radicals. Effect of extracts on ferrous ions chelating ability and total antioxidant capacity were also investigated for each extract. In addition, total phenolic, flavonoid and flavonol content of rhubarb stalks extracts were determined. The stalk methanol extract showed significantly the highest 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, followed by stalks ethylacetate extract, stalks hexane extract and lastly, butylated hydroxytoluene. Ethylacetate extract showed the highest 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity with IC_{50} value of 59.7±1.1 µg/mL, followed by butylated hydroxytoluene and methanol extract with IC_{50} value of 53.1±2.2 and 47.9±1.3 µg/mL, respectively, whereas the highest hydroxyl radical inhibition activity was found for methanol extracts with IC_{50} values of 75.1±2.9 µg/mL. The total antioxidant activity was the highest in methanol and ethylacetate extracts (284.1±11.6 and 237.6±10.0 µg ascorbic acid equivalent for 1 mg extract, respectively). Methanol and ethylacetate extracts at concentration of 100 µg/mL showed the highest chelating activity against ferrous ions (96.3±2.2% and 89.8±1.5%, respectively). All rhubarb stalk extracts showed pronounced antioxidant activities in a dose dependent manner and the effects depend strongly on the solvent used for extraction. The results showed that extracts of stalk of rhubarb exhibit antioxidant potential suggesting that *Rheum rhabarbarum* L. is a promising plant.

Key Words:
Rheum rhabarbarum L., rhubarb, stalk, antioxidant activity

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INTRODUCTION

Herbs, spices and plants have been shown to possess a wide range of pharmacological and therapeutic properties and recently most researches are conducted to isolate and identify the compounds occurring in such plants for their medicinal and biological activities. Many studies have investigated the potential of plant secondary metabolites as antioxidants like phenols, flavonoids, vitamin C and E and tannin, etc against reactive oxygen species (ROS) which are responsible for various diseases including cancer, diabetes mellitus, atherosclerosis, heart diseases, neurodegenerative diseases and many other diseases that induced by free radicals (Broadhurst et. al., 2000).

Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are usually used in oil or in fat rich food to protect against free radicals damage by scavenging reactive oxygen radicals or terminating radical chain reactions by donating hydrogen from their phenolic hydroxyl group, but due to health concerns resulted from the use of synthetic antioxidants much interest is given to the use of natural antioxidants from plants and herbs (Lu and Foo, 2000). It is generally accepted by customers that any medicine derived from plant sources are safer and healthier than their synthetic counterparts. Phenolic compounds derived from plants and herbs are one of the major groups of plant secondary metabolites that act as primary antioxidants and free radical scavengers, and flavonoids, in particular, are the most diverse phenolic compounds and probably the most important natural phenolics that possess a broad spectrum of biological and chemical activities (Oluyemi et. al., 2007).

Rhubarb, the Rheum species, belongs to the Polygonaceae family. There are many kinds of Rheum, some of which, such as *Rheum officinale* B. and *Rheum palmatum* L., are known as medicinal rhubarb, while others, *Rheum rhabarbarum* L. for example, are known as vegetable rhubarb. The vegetable rhubarb, *Rheum rhabarbarum* L., is a wild grown plant of central Asia. The cultivation of rhubarb began more than 2,000 years ago due to its medicinal purposes. Since the eighteen century, rhubarb has been a food crop in North America and is commonly used as an ingredient of jams, pies, and desserts owing to its unique taste (Dregus and Engel, 2003).

Rhubarb (*Rheum rhabarbarum* L.) has been used as a traditional medicine in Far East countries (Matsuda et. al., 2001). The medicinal rhubarb, peeled dried root and rhizome of rhubarb, has been used as a laxative for over a thousand years (Clementi and Misiti, 2010). The medicinal rhubarb has also been well used to treat chronic renal failure in China and Japan for decades (Wang et. al., 2009). Studies have shown the renal protective effect of rhubarb such as reducing glomerulosclerosis and proteinuria, relieving uremic symptoms, lowering serum creatinine, improving hemoglobin level, and adjusting disturbance of lipid metabolism, while the effect on reducing the progression of end stage renal disease and number of deaths is still unclear. The components identified in rhubarb belong to classes including anthraquinones, dianthrones, naphthalins, stilbenes, galloyglucoses, anthocyanins, flavonoids, polyphenols, organic acids and acylglucose derivatives (Agarwal et. al., 2001; Lin et. al., 2006). The anthraquinones in rhubarb such as rhein, emodin, and aloe-emodin were reported to have cathartic, diuretic, antidiarrhea, anti-diabetic, and anti-tumor effects (Clementi and Misiti, 2010).

The antioxidant activity of methanolic extract of *Rheum palmatum* and *Rheum ribes* leaves and stalks has been studied and the isolation of emodin was indicated. In addition, antioxidant activity of *Rheum rhabarbarum* L. leaves has been notified (Matsuda et. al., 2001). However, to date and to our knowledge, antioxidant activity of *Rheum rhabarbarum* L. stalks has not been reported. Therefore, in the present work, the antioxidant properties of different polar extracts from rhubarb stalks with respect to radical scavenging activities, chelating power, total antioxidant activity, phenolics and flavonoids contents were investigated.

MATERIALS and METHODS

Plant Materials

Fresh rhubarb stalks were purchased at Tesco Supermarket in Cracow, Poland and taxonomic determination of rhubarb was confirmed by Assoc. Prof. Dr. Mirosław Krośniak.

Solvents extraction

The ground sun-dried stalks (300g) were extracted separately with hexane, ethyl acetate and methanol using Soxhlet apparatus at 60°C until the refluxed solvent became colorless. The extracts were separately evaporated to dryness at 40°C using a rotary evaporator (Heidolph, Schwabach, Germany). The yields for stalk extracts were 6.6% (w/w) in the hexane extract, 5.3% in the ethyl acetate extract and 4.2% in the methanol extract. All extracts were stored in separate screw cap brown bottles at 4°C before analysis.

Determination of total phenolic compound contents

The total phenolic compounds contents present in stalk extracts were determined according to the method described by Duh and Yen (1997). Catechole was used as the standard for the calibration curve. Aliquot (100 µL) from each extract solution (10 g/L) was transferred into 20 mL volumetric flask and diluted with 10 mL distilled water. Then 1 mL of Folin-Ciocalteau and 2 mL of sodium carbonate solution (10%, w/v) were added to each flask and mixed thoroughly by vortex. The volume is completed

with distilled water and the absorbance was measured at 760 nm against the reagent blank using a UV Visible spectrophotometer (Analyticjena Specord-50, Jena, Germany). The total phenolic compound contents (mg/g) were expressed as catechole equivalent and calculated using the standard curve.

Determination of total flavonoids and flavonols

The total flavonoid and flavonol content of stalk extracts of rhubarb were determined according to the method described by Miliauskas (2004). An aliquot of 1 mL of each plant extract (10 g/L) was mixed with 1 mL of 2 % aluminum trichloride in ethanol. After vortexing the reaction mixture was diluted with 25 mL ethanol and placed in dark place for 40 min at 20°C and the absorbance was measured at 415 nm using spectrophotometer. A mixture of 1 mL from each plant extract and 1 drop of acetic acid diluted with 25 mL ethanol were served as blank and the absorbance was measured separately for each extract as described above. The amount of total flavonoids (mg/g) was calculated as rutin equivalents (RE) and calculated using the standard curve.

Total flavonol content was determined as follows: an aliquot of 1 mL of each plant extract (10 g/L) was taken in a test tube and 2 mL of 2% aluminum trichloride solution in ethanol and 6 mL of 5% sodium acetate solution were added to each tube. After vortexing the reaction mixture, the tubes were allowed to stand for 2.5 hr at 20°C and the absorbance was recorded at 440 nm using spectrophotometer. A mixture of 1 mL from each plant extract and 2 mL of ethanol and 6 mL of sodium acetate solution were served as blank and the absorbance was measured separately for each extract as described above. The amount of total flavonols (mg/g) was calculated as rutin equivalents (RE) and calculated using the standard curve.

1,1-diphenyl-2-picrylhydrazyl free radical-scavenging assay

1,1-diphenyl-2-picrylhydrazyl (DPPH·**)** stable radical was used to determine the free radical scavenging activity of stalk extracts of rhubarb (Hatano et. al.,1988). Sample extracts at various concentrations from 0-50 µL (1000 µg/mL) of methanol, ethyl acetate and hexane extracts was added to 1 mL of methanolic solution of DPPH· ($6x10^{-5}$ M). After vortexing the reaction mixture, the decreases in absorbance of each extract and/or control (BHT) were measured at 517 nm after 30 minutes. The scavenging activity of the extracts was calculated as follows:

$$\text{DPPH.radical scavenging activity (\%)} = \frac{\text{Control radical scavenging - Sample absorbance}}{\text{Control absorbance}} \times 100$$

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical-scavenging activity assay

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\cdot+}$) [2, 2`-azinobis (3-ethylbezthiazoline-6 sulphonic acid] radical-scavenging activity of stalk extracts of rhubarb was assayed by the method of Ozgen et. al. (2006). ABTS was dissolved in 20 mM acetate buffer (pH 4.5) and $ABTS^{\bullet+}$ radical was produced by reacting ABTS solution with potassium per sulfate. Prior to assay The $ABTS^{\bullet+}$ radical solution was diluted with the 20 mM sodium acetate buffer (pH 4.5) to give an absorbance of 0.700±0.01 at 734nm in a 1cm cuvette. The extract of rhubarb stalk was diluted to a concentration that can produce between 20% and 80% inhibition of the blank absorbance. After addition of 2 mL of $ABTS^{\cdot+}$ solution to various concentrations of rhubarb stalk extract, the reaction mixture was vortexed, allowed to stand at room temperature for 20min and the absorbance at 734 nm was recorded. The radical-scavenging activity of extracts was estimated based on percentage of the $ABTS^{\bullet}$ color reduction by calculating the IC_{50} (concentration in µg/mL that cause 50% inhibition of $ABTS^{\bullet+}$ radicals) using a non-linear regression analysis.

Hydroxyl radical scavenging activity assay

The scavenging activity of stalk extract of rhubarb on the hydroxyl radical ($OH^{\bullet}$) was measured using a modified deoxyribose assay (Menaga et. al., 2013). The reaction was generated by Fenton's reaction (from Fe^{2+} - ascorbate - ethylenediaminetetraacetic acid (EDTA) - hydrogen peroxide (H_2O_2) system). The reaction mixture containing 10 µL of $FeCl_3$ (10 mM), 100 µL EDTA (1 mM), 100 µL H_2O_2 (10 mM), and 360 µL of 2-deoxy- D-ribose (10 mM), 0-200 µL (1000 µg/mL)) from rhubarb extract, 330 µL of phosphate buffer (50 mM, pH 7.4). The reaction was started by adding 100 µL (100µM) ascorbic acid. After incubation at 37°C for 1h, aliquot of 1 mL of incubated mixture was mixed with 1mL of 10% trichloroacetic acid (TCA) and 1 mL of 0.5% 2-thiobarbituric acid (TBA) (in 0.025M NaOH containing 0.025% butylated hydroxyl anisole) and the development of pink chromogen was recorded spectrophotometrically at 532 nm against a blank (the same solution but without reagent). $OH^{\bullet}$ scavenging activity of the stalk extract was estimated based on percentage inhibition of deoxyribose degradation from the following formula:

$$\text{OH inhibition (\%)} = \frac{\text{Control absorbance (Sample absorbance Blank absorbance}}{\text{Control absorbance}} \times 100$$

Determination of chelating power activity

Chelating power of stalk extract of rhubarb was determined according to the method described by Kumar et. al. (2008). Briefly: aliquot of 100 µg/mL from each extract was mixed with 0.1 mL of 2 mM

$FeCl_2$ and 0.2 mL of 5 mM ferrozine solutions. The reaction mixture was then incubated at 25ºC for 10 min. The absorbance of the resulting solution was recorded at 562 nm for each extract. The $FeCl_2$ and ferrozine mixture was used as control solution. The percentage inhibition (%) of the ferrous ion in the mixture was calculated by comparing the results of stalk extract of rhubarb with those of the control using the following formula:

$$\text{Chelating activity (\%)} = \frac{[1-(\text{absorbance extract} - \text{Extract blank absorbance})]}{\text{Control absorbance}} \times 100$$

Determination of total antioxidant activity

The total antioxidant activities of stalk extract of rhubarb was determined according to the method described by Umamaheswari and Chatterjee (2008) as follows: an aliquot of 0.1 mL of from each extract was vortexed with 1mL of a reaction solution prepared from 0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The glass tubes were capped and incubated for 1.5h in a water bath at 95ºC, and the tubes were cooled to room temperature. The absorbance of the reactant mixtures was recorded at 695 nm using spectrophotometer against blank. Ascorbic acid was used as standard. The antioxidant activity was expresses as mg equivalents to ascorbic acid.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) by using the SPSS program version 16.0. Tukey Post-hoc tests were performed when significant differences occurred at 5% level. All tests were performed in triplicate for each extracts.

Results

Total phenolic, flavonoid and flavonol contents of extracts

The total phenolics, flavonoids and flavonols of different polar extracts obtained from stalk of rhubarb are shown in Table 1. The content of phenolic compounds varied between 51.6 and 227.1 mg/g. It was the highest in the methanolic extract, followed by the ethyl acetate extract, and finally the hexane extract in decreasing order. The flavonoid content of ethyl acetate extract was significantly the highest (189.0±5.4 mg/g) followed by hexane extract (177.1±3.2 mg/g) and methanolic extract (154.3±6.7 mg/g). The flavonol content varied from 61.7±1.4 mg/g in the methanolic extract to 6.3±0.5 mg/g in the hexane extract.

Radical -scavenging activities on 1,1-diphenyl-2-picrylhydrazyl, hydroxyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid

The DPPH free radical-scavenging activity of stalk extract from rhubarb and the standard antioxidant BHT is shown in Figure 1. Results showed that all rhubarb extracts exhibited stronger DPPH radical-scavenging activity than the standard BHT in a dose dependent manner. The results showed that the methanolic extract exhibited significantly ($p<0.05$) the highest DPPH scavenging activity in a dose dependent manner (IC_{50} value 3.54±0.2 µg/mL). At concentration of 7.5 µg/mL, the inhibition of DPPH radical by methanolic extract was 78.3%, while for ethyl acetate and hexane extracts the inhibition were 78.1% and 14.5%, respectively. The inhibition of DPPH radical by BHT standard at the same concentration was 14.1%. The IC_{50} values of various extracts from rhubarb on DPPH radical scavenging activity were in the following decreasing order; BHT > hexane extract > ethyl acetate extract > methanolic extract (Table 1).

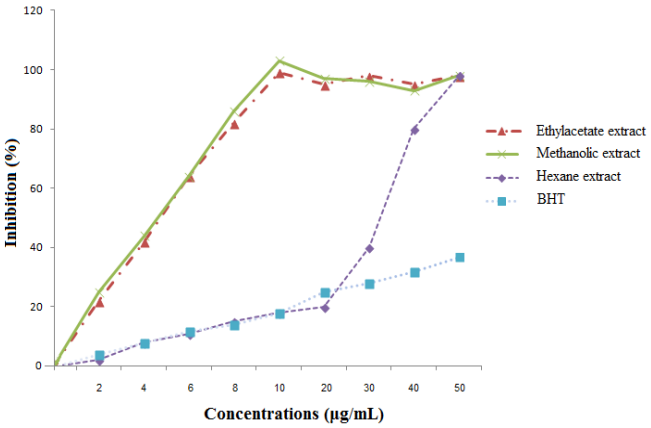

Figure 1. 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activities (% inhibition) for different extracts from rhubarb stalks. (BHT: butylated hydroxytoluene)

The ability of rhubarb extracts to scavenge hydroxyl radical was evaluated using different concentrations from each extract. The IC_{50} values of different rhubarb extracts are shown in Table 1. The methanolic extract showed significantly (IC_{50} values 75.1±2.9 µg/mL) the highest activity in quenching the hydroxyl radicals (diminishing chromogen formation) and in a dose dependent manner.

In ABTS radical scavenging assay, the IC_{50} values for different extracts from rhubarb stalk are shown in Table 1. All the studied stalk extracts showed good ABTS radical scavenging activities and the 50% inhibition of the radical was achieved at concentrations ranged from 46.2-60.9 µg/mL. ABTS radical scavenging activity of ethyl acetate extract was higher than the methanolic extract and BHT ($p<0.05$). However, the activity of hexane extract was not determined because of the turbidity.

Table 1. Average phenolic, flavonoid and flavonol contents (mg/g) and radicals scavenging activities of rhubarb stalk extracts.

	Phenolics (mg/g)	Flavonoids (mg/g)	Flavonols (mg/g)	Total contents IC_{50} (µg/mL) *		
				ABTS·	OH·	DPPH·
Methanol	218.3±8.6[b,c]	154.3±6.7[b,c]	61.7±1.4[b,c]	47.9±1.3[b,d]	75.1±2.9[b,c,d]	3.54±0.2[c,d]
Ethylacetate	153.9±3.5[a,c]	189.0±5.4[a,c]	32.6±3.0[a,c]	59.7±1.1[a,d]	105.7±2.2[a,c,d]	3.7±0.2[c,d]
Hexane	52.8±1.3[a,b]	177.1±3.2[a,b]	6.3±0.5[a,b]	N.D.	93.2±1.8[a,b,d]	7.62±0.4[a,b,d]
BHT	N.D.	N.D.	N.D.	53.1±2.2[a,b]	113.7±4.8[a,b,c]	13.41±0.6[a,b,c]

[a]Values were significantly different from than methanol extract
[b]Values were significantly different from than ethylacetate extract
[c]Values were significantly different from than hexane extract
[d]Values were significantly different from than BHT
(BHT: butylated hydroxytoluene, N.D.: not determined)

Chelating power and total antioxidant activities

Table 2 shows the chelating power and the total antioxidant activities of different extracts from rhubarb at concentration of 100 µg/mL. The methanolic extract of rhubarb stalk exhibited higher chelating activity on ferrous ion (96.3%) than the ethyl acetate and hexane extracts (89.8% and 46.7%, respectively). In addition, methanolic extract of rhubarb had significantly the highest total antioxidant activity (284.1 µg ascorbic acid equivalent/ mg extract) followed by ethyl acetate and hexane extracts (237.6 and 19.8 µg ascorbic acid equivalent/ mg extract, respectively).

Table 2. Average total antioxidant activity and chelating power of various extracts from stalk of rhubarb at concentration of 100 µg/mL.

	Chelating power (Inhibition %)	Total antioxidant activity (µg ascorbic acid equivalent/mg extract)
Methanol	96.3±2.2[b,c]	284.1±11.6[b,c]
Ethylacetate	89.8±1.5[a,c]	237.6±10.0[a,c]
Hexane	46.7±0.9[a,b]	19.8±2.7[a,b]

[a]Values were significantly different from than methanol extract
[b]Values were significantly different from than ethylacetate extract
[c]Values were significantly different from than hexane extract

DISCUSSION

The antioxidant properties of different extracts from rhubarb stalks have been evaluated using different models. It is evident from the results that the phenolic, flavonoid and flavonol contents were found in considerable amount in all extracts from stalks. Many studies have been suggested that the antioxidant properties of plants and herbs are directly related to their contents of phenolic compounds, flavonoids and flavonols which act by donating hydrogen from the phenolic hydroxyl groups (Lu and Foo, 2000; Miliauskas et. al., 2004). Therefore, the relationships between values obtained using different models to evaluate antioxidant activity and the content of phenolic, flavonoid and flavonol compounds were evaluated. With further data analysis a positive correlation between the total phenolic content of extracts and their DPPH radical scavenging activities was found at all concentrations. These results were in agreement with the results of other researchers whom found a linear relationship between phenolic content and antioxidant activity of studied plant extracts (Al-Dabbas et. al., 2010).

Jang et. al. (2018) found that the phenolic compound contents of rhubarb stalk methanolic extract was 42 mg/g. The difference in the results between this study and our study may due to the difference in the standard used to establish the calibration curve to determine phenolic contents and to the method of extraction.

$OH^{\bullet}$ is known to be the most reactive free radical that can cause oxidative damage to DNA, proteins and lipids (Kumar et. al., 2008). The decolorization effect of extracts in this study reflects the potent capacity of extracts to act as antioxidant by donating hydrogen atoms that inactivate this radical. All the stalk extracts showed good hydroxyl radical scavenging activities and the 50% inhibition of the radical was achieved for all solvents. The inhibitory potential of extracts against the hydroxyl radical scavenging activity follows the following decreasing orders; methanolic extract, hexane extract, ethyl acetate extract and BHT. It is obvious that different polar extracts from rhubarb stalks shown to possess a potential free radical scavenging activities against different radicals, suggesting the potential of these extracts in contribution in prevention of free radical mediated diseases.

In our study, ethyl acetate extract of rhubarb stalk exhibited the highest ABTS radical scavenging activity. The ABTS radical assay was mainly evaluated in this study to check the existence of phenolic compounds with high molecular weight. Hagerman et al. (1998) reported that the higher molecular weight of phenolics is the stronger in quenching ABTS radicals.

Transition metals like Fe^{+2} ions have been proposed as catalyst for the formation of radicals that induced damage to living cells. Chelating agents existed in plant extracts have the ability to reduce radical formation and subsequent lipid per oxidation due to their redox potential that stabilize the oxidized form of the metal ions (Elmastas et. al., 2006). The chelating activity of the rhubarb extracts were determined by quantitative measurement of ferrozine complex formation with Fe^{+2} ions. The formation of Fe^{+2} ferrozine complexes were hindered in the presence of extracts, indicating that rhubarb extracts chelate the iron and prevent the completion of the reaction. The 50% chelating activity of the root extract of the same genus *Rheum ribes* L. was reported to be approximately 4-5 µg/mL (Öztürk et. al., 2007), thus *Rheum ribes* L. extract possessed higher chelating activity against ferrous ion than *Rheum rhabarbarum* L.

The total antioxidant activity determination of rhubarb extracts using phosphomolybdenum method is based on the formation of green phosphate/Mo (V) complex resulted from the reduction of Mo (VI) to Mo (V) in acidic medium by the extract. This method is quantitative and the total antioxidant activity is

expressed as microgram ascorbic acid equivalent (Prieto et. al., 1999). The reason of low antioxidant activity in the hexane extract could be due to the presence of prooxidants in stalk which dominate the antioxidant compounds present in the extract.

This study showed that different polar extracts from the stalk of rhubarb exhibited different antioxidant activities according to model system used and these activities are mainly related to their phenolic compounds and flavonoid contents. *Rheum rhabarbarum* L. is considered a potential source of natural antioxidants and can inhibit unwanted oxidation processes. Further study is needed to isolate and characterize the antioxidant compounds from *Rheum rhabarbarum* L.

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