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Antioxidant Activities of Rhubarb Extracts Containing Phenolic Compounds

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페놀화합물이 포함된 대황(Rhubarb)추출물의 항산화성 평가

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국문요약

이 연구에서는 미국산 *R. rhabarbarum* L.과 중국산 *R. palmatum* L.의 줄기 추출물과 그리고 한국산 *R. undulatum* L.의 뿌리 추출물이 천연항산화제로서의 사용 가능성을 평가하였다. 대황의 페놀화합물을 추출하기 위해서 물, 에탄올, 메탄올이 사용되었다. 에탄올이 대황(Rhubarb)에서 페놀화합물을 추출하는데 가장 효과적인 용매였으며, 50% 에탄올은 *R. rhabarbarum* L. 줄기에, 70% 에탄올은 *R. palmatum* L.의 줄기에, 80% 에탄올은 *R. undulatum* L.의 뿌리에서 페놀을 추출하는데 각각 가장 효과적인 용매농도였다. 또한 추출물의 항산화능을 알기 위해 β-carotene bleaching inhibition activity와 DPPH radical scavenging activity를 측정하였으며 합성항산화제인 BHT와 비교하여 측정하였다. β-Carotene bleaching inhibition activity 실험에서 추출물의 농도가 증가하면 대황추출물의 항산화능이 역시 증가하였다. 추출물의 농도가 5 mg/ml와 10 mg/ml 일때 *R. undulatum* L. 뿌리추출물과 BHT의 항산화능은 유의적 차이는 없었다($p \leq 0.05$). 그러나 DPPH radical scavenging activity에서 *R. undulatum* L. 뿌리추출물이 0.4 mg/mL 농도 이상에서는 BHT 보다 높은 항산화능을 나타내었다. 결과적으로 대황(*R. undulatum* L.)의 80% 에탄올 추출물이 천연항산화제로의 사용이 가능하였다. 그러나 합성항산화제인 BHT의 식품에 사용농도를 비교하여 볼 때 대황 추출물의 항산화능이 BHT와 동일하기 위해서는 보다 높은 농도를 필요로 하였다.

Key Words : 항산화제, 대황추출물, phenolic compounds, DPPH radical scavenging activity, β-carotene bleaching inhibition activity

I. INTRODUCTION

Although synthetic antioxidants such as BHT, BHA and TBHQ are commonly used in processed foods, it has been reported that these compounds have some side effects[1]. In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant-rich foods and

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the incidence of number of human diseases[2]. Therefore, natural antioxidants have received considerable interest from the food industry due to the concern over the safety of synthetic antioxidants[3][4]. Tocopherols are the most widely used natural antioxidants found in plant tissue as a blend of α, β, δ, and γ homologues[5]. Although tocopherols have been widely used in food as safe antioxidants, the disadvantages of tocopherols are highly manufacturing costs and low effectiveness in some food products such as cereals and citrus oils[5][6].

During the past several years, many spices have been extensively studied for their natural antioxidant characteristics[7]-[11]. Recently, several antioxidants have been found in other sources such as young green barley leaves, tanshen, *Polygonum hydropiper* leaves, oregano (*Origanum vulgare* L.), eucalyptus leaf waxes, and red turnip (*Brassica campestiris* L.). It has been found that the majority of these natural antioxidants possessed phenolic rings[6][10][12]-[15].

Rhubarb belongs to the family of Polygonaceae (buckwheat family). Rhubarb is a plant name for the many different species of Rheum. Rhubarb has very broad leaves and elongated, often reddish, petioles (leaf stalks). The petioles are edible and often use as food. Rhubarb' s crisp sour stalks can be served as a sauce over ice cream, combined with fresh strawberries, or made into pies, tarts, puddings, bread, jam, jellies and refreshing beverages. The dried roots and rhizome are used in medicinal treatment. Rhubarb has many pharmacological actions, such as purgation, analgesic effects, cures of mental and renal disorders, antibacterial, antioxidative, antitumor and antimutagenecity[16][17].

Recently, much attention has focused on the antioxidant activity of rhubarb. Most of these effects have been attributed to the antioxidative behavior and free radical scavenging property of its content of high polyphenolic compounds[16][17].

The pharmaceutically relevant compounds in rhubarb are anthraquione derivatives including physcion, emodin, rhein, aloe-emodin, chrysophanol and their glucosides. Rhaponticin, a distryrene derivative, only exists in wild rhubarb. Rhubarb remains in use as a laxative, especially as a powder. It produces a variety of secondary phenolic metabolites such as anthraquinones, naphthalenes, stillbenes, chromones, flavonoids, and related compounds[16][17].

Korean rhubarb, the rhizome and roots of *Rheum undulatum* L. is used as a remedy for the blood stagnation syndrome ('Oketsu syndrome' in Japanese traditional medicine) as well as a purgative agent. This rhubarb is considered to have a lesser purgative effect but more potent effect on Oketsu syndrome than other kind of rhubarbs such as *R. palmatum L.*, *R. tanguticum Maxim.*, *R. officinale* Baill., and *R. coreanum* Nakai.[16][17].

Previously, antiallergic and antiinflammatory effects of the hot water extract were reported to be responsible for its anti-Oketsu effect. Recently, the isolation and structure elucidation of antioxidant constituents from the rhizome of *R. undulatum* was reported and the structural requirements for antioxidant activity was elucidated[16][17]. Even though the pharmacological properties in bioactive constituents from rheum spp. were studied intensively, antioxidant properties do not seem to be sufficient.

The aim of this study was the evaluation of antioxidant activities of extracts from stalks in *R. rhabarbarum* L., and *R. palmatum* L., and in roots of *R. undulatum* L. to find new potential sources of natural antioxidant.

II. MATERIALS AND METHOD

1. Materials

1) Plant materials

Fresh *Rheum undulatum* L., Korean rhubarb was purchased, which was cultivated in the year of 2002 in Uisung, Kyongbuk, Korea. The fresh stalks of *R.*

rhabarbarum L. (also known as *R. rhapontiuum* being commonly referred to as wild rhubarb in the U.S.A) and the dried stalks of *R. palmatum L.* (Chinese rhubarb) were provided by 'S' Chemical Company. Then, the fresh rhubarb stalks and roots were deposited in a freezer at -20℃, and then immediately lyophilized in Freeze Dry System(Model 77530-13, Labconco Co., Kansas City, MO, U.S.A.).

2) Preparation of the extract of rhubarbs

The freeze-dried and powdered stalks of *R. rhabarbarum* L., and *R. palmatum* L., and roots of *R. undulatum* L. were extracted by using the method described by Julkunen-Titto[18]and Kahkohen et al.[19]. Each sample (5 g) was defatted with hexane (3 × 24 hr) at room temperature and subsequently extracted with 100 mL of water, aqueous ethanol (50-95%), and 80% aqueous methanol (3 × 24 hr) at room temperature.

The solution was filtered with Whatman No.1 filter paper and centrifuged for 10 min at 1500 × g, which was then evaporated under vacuum to give the clear supernatants to a volume of about 10 mL at 45℃. These concentrated samples were lyophilized to powder and stored in a freezer at -20℃.

3) Reagents

HPLC grade solvents, methanol, chloroform, ethanol, and water were purchased from Duksan Pure Chemical Company (Ansan, Kyoungki, Korea). Sorbic acid was purchased from Junsei Chemical Company (Kyoto, Japan). Folin-Ciocalteu's phenol, gallic acid, sodium carbonate, β-carotene, linoleic acid, polyoxyethylene sorbitan mono palmitate (Tween 40), α, α-diphenyl-β-picrylhydrazyl (DPPH), and butylated hydroxytoluene(BHT) were purchased from Sigma Chemical Co. (St. Louis, MO., U.S.A.).

2. Determination of total phenolic compounds in rhubarb extracts

Content of the total phenolic compounds in rhubarb extracts was determined by the method described by Emmons et al.[20], Ragazzi and Veronese[21], and Yildirim et al.[22].

Accurately 20 mg of gallic acid with 40% ethanol (15 mL) was stirred until no solids presented at room temperature and was filled to full volume up to 25 mL with extraction solvent. Then, this standard solution was diluted to 2, 4, 8, 16, and 32 times. HPLC grade water (60-70 mL) and Folin Ciocalteu's phenol (5 mL) were added into the standard solution (1 mL) at room temperature. Sodium carbonate solution (15 mL) was added into this mixture in 4 min. Subsequently, the mixture was shaken for 2 hr at room temperature and measured absorbance by using an UV/Visible Spectrophotometer at 725 nm (Ultrospec 3000, Pharmacia Biotech, Cambridge, England). The extracts of samples (20 mg) in 40% ethanol (1 mL) were mixed. Each extract of rhubarbs was also evaluated according to the same procedure. Gallic acid equivalents (GAE) were determined from a standard concentration curve and the total phenolic content is expressed as gallic acid equivalents in milligrams per gram as dry material as the following equation;

$$C = (A + 0.0231) / 4.498 \times 10^{-3}, \text{ and}$$

where A is the absorbance at 725 nm, and C is gallic acid equivalent (mg).

3. Antioxidant activity by β-carotene bleaching method

Antioxidant activity of stalks of *R. rhabarbarum* L., stalks of *R. palmatum* L., and roots of *R. undulatum* L. was determined by measuring a coupled autoxidation of β-carotene and linoleic acid as described by Emmons et al.[20], Cruz et al.[23], and Seymour et al.[24]. Antioxidant activity (AOA) is expressed as percent inhibition relative to the absorbance of control after incubation for 1 hr using the following equations:

Antioxidant activity (AOA) = [(DRc - DRs) / DRc] $\times$100(%), and

where AOA is the antioxidant activity, DRc is the degradation rate of the control, $(a/b)/60$ and DRs is the degradation rate of the sample, $(a/b)/60$, and where a is the initial absorbance at time zero, and b is the absorbance at 470 nm after 60 min.

4. α,α-Diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was measured by a Blois method as described by Yildirim et al[22]. This activity is given as percentage of DPPH scavenging as following:

% DPPH scavenging = [(A - E) / A] $\times$ 100, and

where A is absorbance of control, and E is absorbance of extracts of rhubarb.

5. Statistical analysis

The triplicate data were subjected to an analysis of variance using Statistical Analysis System package (Release 8.01, SAS Institute Inc., Cary, NC., U.S.A.). The data was presented as means $\pm$ S.D.. Analyses of variance using the ANOVA were conducted. Differences between the sample means were analyzed by Duncan's multiple range test at $p \leq 0.05$.

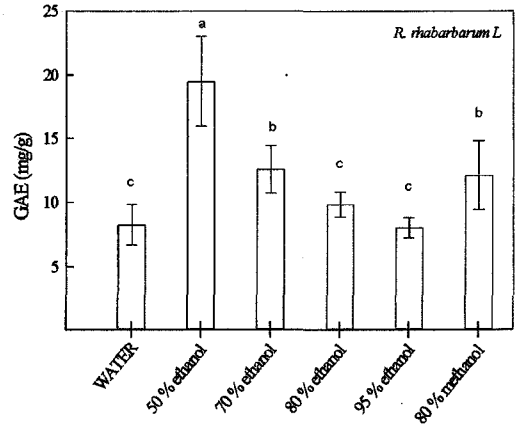
III. RESULTS AND DISCUSSION

1. Evaluation of total phenolic compounds in rhubarb extracts

There are several methods for determination of the total phenolic compounds in plant material[25]. The most often used methods are based on the ability of phenolics to react with oxidizing agents. In this study, the commercially available Folin-Ciocalteu phenol reagent was used, which is unspecific for any phenolics and the color yielded depends on hydroxyl groups and their position in the molecules. But in spite of the fact that phenolic reagent is unspecific, for instance, absence of interfering substances, only relative results may be obtained for the amount of phenolic compounds.

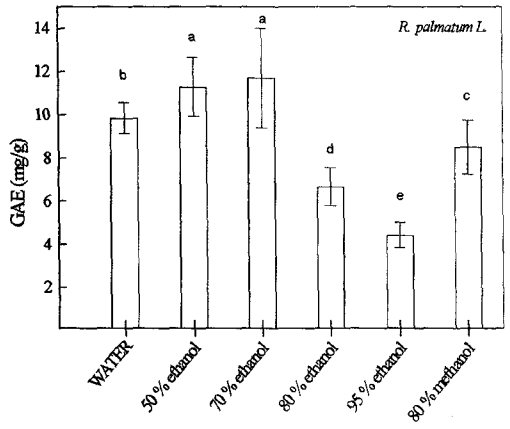
It is obviously difficult to choose suitable standards for the total phenolic determinations in plant extracts due to the chemical heterogeneity of plant products and the unspecificity of phenolic reagents. Thus, it is only possible to get relative equivalents with the standards used. The reactivity of different phenolic standards to Folin-Ciocalteu reagents is reported[18]. Gallic acid showed the most linear relationship between absorptivity and standard concentration with the amounts of phenol reagent and sodium carbonate used. Thus, the total phenolic content is expressed as gallic acid equivalent (GAE) in this study. The result of testing different solvents for the phenolics in rhubarbs was shown in Figures 1-3. The effects depended strongly on the solvent used for the extraction as well as on the extracted residue.

Aqueous methanol (80%) was moderate effective, but it is known to be a rough solvent for certain



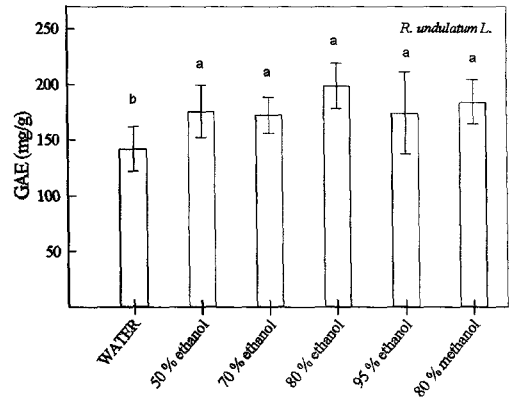
<Figure 1> Effect of solvent extraction on amount of total phenolic compounds in stalks of *R. rhabarbarum* L.

(Means with different letter are significantly different among solvents at $p \leq 0.05$ as determinded by Duncan's multiple range test)



<Figure 2> Effect of solvent extraction on amount of total phenolic compounds in stalks of *R. palmatum L.*

(Means with different letter are significantly different among solvents at $p \leq 0.05$ as determinded by Duncan's multiple range test)



<Figure 3> Effect of solvent extraction on amount of total phenolic compoundsin stalks of *R. undulatum* L.

(Means with different letter are significantly different among solvents at $p \leq 0.05$ as determinded by Duncan's multiple range test)

phenolic compounds, e.g., for their glycosides[18]. In addition, it is not good solvent for food additives. Pure water was a quite acceptable extraction means of the total phenolics in stalks of *R. palmatum* L. (Figure 2), but it has been known to be very deleterious for glycosides[18]. Aqueous ethanol with 50, 70, and 80% concentration were the most effective in stalks of *R. rhabarbarum* L., *R. palmatum* L., and

<Table 1> Comparision of total phenolic compounds in *R. rhabarbarum* L., *R. palmatum* L., and *R. undulatum* L. extracts

Solvents	GAE[1] (mg/g)		
	SRRE[2]	SRPE	RRUE
Water	8.28 ± 1.55^{c}	9.87 ± 0.72^{b}	142.60 ± 19.81^{b}
50% ethanol	19.52 ± 3.54^{a}	11.30 ± 1.36^{a}	176.11 ± 23.4^{a}
70% ethanol	12.60 ± 1.86^{b}	17.72 ± 2.31^{a}	172.83 ± 16.33^{a}
80% ethanol	9.87 ± 0.96^{c}	6.68 ± 0.88^{d}	199.04 ± 20.51^{a}
95% ethanol	8.06 ± 0.77^{c}	4.45 ± 0.58^{e}	174.61 ± 36.80^{a}
80% methanol	12.15 ± 2.71^{b}	8.52 ± 1.26^{c}	184.54 ± 19.98^{a}

[a-e] Means with the different superscripts within each column are significantly different ($p < 0.05$)

[1] Gallic acid equivalent (mg/g)

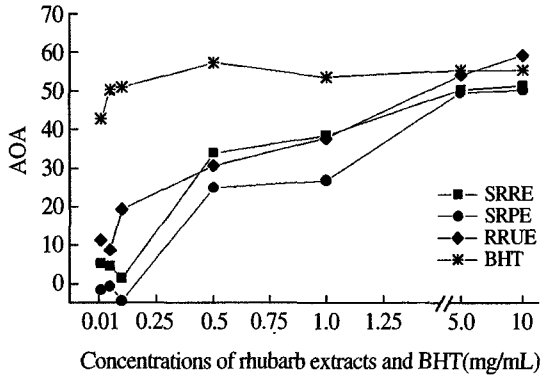
[2] SRRE: stalks of *R. rhabarbarum* L.; SRPE: stalks of *R. palmatum L.*; and RRUE: roots of R. *undulatum* L.

roots of *R. undulatum* L., respectively(Figures 1-3). As shown in Table 1, among all of the extracts, the highest amount was found in the 80% ethanol extract of *R. undulatum* roots, and the lowest amount was measured in the 95% ethanol extract of stalks from R. palmatum stalks. The total amounts of phenolic compounds in roots of *R. undulatum L.* were uncomparatively higher than those of any extracts regardless of all solvents used. Many phenolic compounds are soluble in polar solvents. The choice of solvents depends on the number of hydroxyl groups and sugars in the molecules. For the total phenolic extracts, aqueous ethanol has often been used as one of solvents, at which Yildirim et al.[22] and Mau et al.[26] used.

2. Antioxidant activity of ethanol extracts from rhubarb.

1) β-Carotene bleaching inhibiting activity

β-Carotene bleaching test was selected for determination of antioxidant activity because it is carried out in an emulsion being a situation frequent in food.



<Figure 4> Antioxidant activity of ethanol extract from stalks of *R. rhabarbarum* L. and *R. palmatum* L., roots of *R. undulatum* L., and synthetic antioxidant, BHT by β-carotene bleaching activity.

(SRRE: stalks of *R. rhabarbarum* L.; SRPE: stalks of *R. palmatum* L.; RRUE: roots of *R. undulatum* L.; and BHT: butylene hydroxytoluene)

As shown in Figure 4, antioxidation activities in ethanol extracts of rheum species were concentration-dependent within concentration ranges, while BHT had similar antioxidant activities regardless of concentration.

At the concentration lower than 5 mg/ml, *R. undulatum* root extracts rich in polyphenol compounds (Table 1) had a higher antioxidant activities than those of stalk extracts of *R. rhabarbarum* and *R. palmatum*. As compared with BHT, ethanol extracts of rhubarb showed their antioxidation activities far less than that of BHT.

However, at the concentration higher than 5 mg/mL, all rhubarb extracts showed antioxidant activities comparable to that of butlylated hydroxytoluene (BHT). *R. undulatum* root extracts seemed to give the highest contribution to antioxidation activities, especially at concentration of 10 mg/ml, because they contain high quantities of stilbenes, naphthalene glucosides, torachrysone 8-*O*-glucosides, chromones and flavonoids that show high antioxidant properties[16][17].

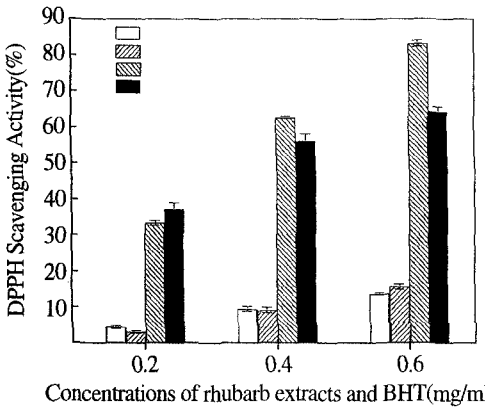
Although the antioxidant activities of extracts increased with increasing amount of extracts, there was no statistically significant difference between 5 mg/mL and 10 mg/mL in root and stalk extract from rheum spp. and BHT ($p \leq 0.05$).

2) DPPH radical scavenging activities of ethanol extracts from rhubarbs

It is generally agreed that the oxidation is initiated by free radical attack; therefore, assays to evaluate the radical scavenging activity are representative of the potential of a compound to retard oxidation. Among the radical scavenging assays, the utilization of DPPH radical was chosen due to its simplicity and worldwide acceptance for comparative purposes.

DPPH is a stable free radical that can accept an electron or hyrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the ethanol solution of DPPH shows a strong absorption band at 517 nm. DPPH radicals react with suitable reducing agents and the electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up[27]. Such reactivity has been widely used to test either the ability to act as free radicals scavengers or the antioxidant activity of plant extracts[28]-[30]. Reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm.

As shown in Figure 5, extracts of rhubarbs at different concentration reduced DPPH radicals significantly. However, DPPH scavenging activities of stalk extracts of R. palmatum and *R. rhabarbarum* were comparatively lower than those of *R. undulatum* and BHT. The percentages of DPPH scavenging activity of ethanol extracts of rhubarb (Figure 5) had also the same result as β-carotene bleaching assay, in which the higher amount phenolic compounds in extracts showed the higher antioxidant activity. Stalk extracts of *R. rhabarbarum* and R. palmatum showed relatively lower DPPH scavenging activities than root extract of *R. undulatum* L. due to the lower amount of phenolic compounds in extracts. In the present study, ethanol extract of roots of *R. undulatum* L. has shown the



<Figure 5> Antioxidant activity of concentrations of ethanol extracts from stalks of *R. rhabarbarum* L., *R. palmatum* L., roots of *R. undulatum* L., and synthetic antioxidant, BHT by DPPH radical scavenging activity.

(Means with different letters (a, b, c) are comparisons among rhubarb extracts and BHT, and significantly different from each other at $p \leq 0.05$ as determinded by Duncan's multiple rang test; Means with different letters (x, y, z) are comparisons amoung concentrations, and significantly Different from each other at $p \leq 0.05$ as determinded by Duncan's multiple rang test; and SRRE: stalks of *R. rhabarbarum* L.; SRPE: stalks of *R. palmatum* L.; RRUE: roots of *R. undulatum* L.; and BHT, butylene hydroxytoluene)

highest DPPH radical scavenging activity.

When antioxidant potentials of rhubarb extracts were also compared with BHT, the higher antioxidant activity was found in ethanol extract of *R. undulatum* L. at the concentration higher than 0.4 mg/ml. It was reported that phenolic compounds could easily donate hydroxyl hydrogen due to resonance stabilization[31]. The high potential of phenolic compounds to scavenge radicals may be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups[32]. By combining this fact with the forementioned obtained results, it could be suggested that the antioxidant activities of ethanol extracts of rhubarbs increase so as to increase their extracted amounts of phenolic compounds.

IV. CONCLUSIONS

The possibility of antioxidant activities from extracts of stalk of *Rheum rhabarbarum* L., stalk of *R. palmatum* L, and root of *R. undulatum* L. were investigated. Solvents such as water, ethanol, and methanol were used to extract phenolic compounds. Ethanol was found the most effective solvent to extract phenolic compounds from rhubarbs, in such that use of 50% ethanol on stalk of *R. rhabarbarum* L., 70% ethanol on stalk of *R. palmatum* L., and 80% ethanol on root of *R. undulatum* L. Both the α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging capacity and the inhibition of the β-carotene bleaching of extracts for antioxidant activity were applied and compared with that of synthetic antioxidant, butylated hydroxytoluene (BHT).

In testing β-carotene bleaching inhibiting assay, roots of *R. undulatum* L. being rich in polyphenolic compounds was shown the higher antioxidant activity than BHT. Although the antioxidant activities of rhubarb extracts showed concentration-dependent, there were no significant differences between ethanol extracts from roots of *R. undulatum* L. and BHT at extract concentrations of 5 mg/ml and/or 10 mg/ml ($p \leq 0.05$). The results of the DPPH radical scavenging activity were shown that roots of *R. undulatum* L. had more significant scavenging activity than BHT at higher than 0.4 mg/ml extract concentration.

In conclusion, extract of *R. undulatum* roots from 80% aqueous ethanol was shown to be a better potential source for natural antioxidant than other rhubarb extracts. However, when considered the real approved concentration of BHT used in the appropriate foods, the higher concentration of ethanol extracts from rhubarb would be used to have the same antioxidant effect as BHT.

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