# Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products

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The antioxidant activities and total phenolics of 28 plant products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, and several fruits, vegetables, and medicinal plants were determined. The total phenolic content, determined according to the Folin−Ciocalteu method, varied from 169 to 10548 mg/100 g of dry product. Antioxidant activity of methanolic extract evaluated according to the $\beta$-carotene bleaching method expressed as AOX ($\Delta$ log $A_{470}$/min), AA (percent inhibition relative to control), ORR (oxidation rate ratio), and AAC (antioxidant activity coefficient) ranged from 0.05, 53.7, 0.009, and 51.7 to 0.26, 99.1, 0.46, and 969.3, respectively. The correlation coefficient between total phenolics and antioxidative activities was statistically significant.

**Keywords:** *Antioxidant activity; phenolics; medicinal plants; oilseeds; buckwheat; vegetables; fruits; wheat products*

## INTRODUCTION

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid (Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Branen, 1975; Ito et al., 1983). Thus, the interest in natural antioxidants has increased considerably (Löliger, 1991). The ability of some phenolic compounds to act as antioxidants has been demonstrated in the literature. Several researchers have investigated the antioxidative activity of flavonoid compounds and have attempted to define the structural characteristics of flavonoids that contribute to their activity (Nieto et al., 1993; Das and Pereira, 1990; Foti et al., 1996). *o*-Dihydroxy groups in the B ring, the presence of a C2−3 double bond in conjunction with 4-oxo in the C ring, and 3- and 5-hydroxy groups and the 4-oxo function in the A and C rings are associated with antioxidant activity. Phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, and *p*-coumaric acids, appear to be more active antioxidants than the hydroxy derivatives of benzoic acid such as *p*-hydroxybenzoic, vanillic, and syringic acids (Dziedzic and Hudson, 1983; Larson, 1988). Burton and Ingold (1981) have shown that α-tocopherol is one of the most active in vitro chain-breaking antioxidants. Carotenoids also have a protective function against oxidative damage, and singlet oxygen is very powerfully quenched by $\beta$-carotene (Foote et al., 1971).

Many of the natural antioxidants, especially flavonoids, exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (Cook and Sammon, 1996). Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property (Cook and Samman, 1996; Huang et al., 1992).

The antioxidant activity of several plant materials has recently been reported (Al-Saikhan et al., 1995; Yen and Duh, 1995; Oomah and Mazza, 1996; Wang et al., 1996; Cao et al., 1996; Amarowicz et al., 1996); however, information on the relationship between antioxidant activity and phenolic content and composition of many food plants is not available. The objective of this study was to determine the contents of total phenolics in several plant products and to explore relationship(s) between phenolic content and antioxidant activity. In addition, the antioxidant activities of alcoholic extracts of herbal products, such as ginseng, echinacea, and sea buckthorn were compared.

## MATERIALS AND METHODS

**Plant Material.** Twenty-eight plant products, buckwheat seeds and hulls, flaxseed and flaxseed gum, sunflower seed and hulls, sea buckthorn fruit, ginseng roots, echinacea roots and flower heads, white and purple flesh potatoes, blueberries, sweet cherries, red onion skin, horseradish roots and oil, and four wheat products, were assayed for antioxidant activity and phenolic content.

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Buckwheat seeds (cv. AC Manisoba) were obtained from the 1995 harvest at Agriculture and Agri-Food Canada Research Centre, Morden, MB. The buckwheat was dehulled as described by Mazza and Campbell (1985) within 1 month after harvest, and hulls and seeds were stored at −25 °C prior to analysis.

The flaxseed were of the cultivar NorMan and Linola 947, grown commercially in 1995 near Winnipeg, MB. The flaxseed gums were extracted with water, precipitated with 95% ethanol as described by Cui et al. (1994), and spray-dried. The four gum samples extracted from NorMan flaxseed, designated Fsg1, -2, -3, and -4, were precipitated with 1, 2, 3, and 4 volumes of 95% ethanol, respectively. The gum extracted from Linola 947 was not precipitated with ethanol.

The sunflower seeds and hulls were from the purple-hulled breeding line CM 160 grown at the Agriculture and Agri-Food Canada Research Centre, Morden, MB (Mazza and Gao 1994).

Fruit of sea buckthorn (*Hippophae rhamnoides* L. cv. Indian-Summer) were from commercial plantings at Indian Head, SK.

The fibroteins were wheat products, rich in fiber and protein, manufactured by Mohawk Oil Ltd. Vancouver, BC.

Flower heads and roots from a 1-year-old crop of echinacea (*Echinacea purpurea*) and horseradish (*Armoracia lapathifolia* Gilib.) roots were from research plots of Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC. Ground roots were used directly for assay or were subjected to steam distillation as described by Mazza (1984). Distillates of fresh roots (90−92% allyl isothiocyanate) were diluted 1/10 times with 80% methanol prior to analysis.

Samples of potatoes (*Solanum tuberosum* L. cv. Russet Burbank) were obtained from a local market in Penticton, BC. Samples of purple flesh potatoes were from a commercial producer in Newfoundland, Canada.

Lowbush blueberries (*Vaccinium angustifolium* Ait. cv. Fandy) were obtained from research plots of Agriculture and Agri-Food Canada, Research Centre, Kentville, NS. Samples were kept at −30 °C until used.

Samples of red onions (*Allium cepa* L. cv. Red Jumbo) were from a commercial farm, near Yakima, WA.

All samples were either ground or homogenized before they were freeze-dried to ensure equal moisture content.

**Extraction.** Ground samples (1 g) were extracted with 80% aqueous methanol (25 mL) on an orbital shaker for 120 min at 70 °C. For the anthocyanin-containing samples—blueberries, cherries, red onion skins, sunflower hulls, and purple potatoes—extraction temperature was 25 °C. The lower extraction temperature for the anthocyanin-containing samples was used to prevent any thermal breakdown of the anthocyanin pigments. The mixture was subsequently filtered (Whatman No. 5) on a Büchner funnel, and the filtrate was assayed for antioxidant activity.

**Determination of Antioxidant Activity.** Antioxidant activity of plant extracts and standards (α-tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, MO) was determined according to the β-carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, 1 mL of β-carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J. T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH Chemicals, Toronto, ON). Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 mL) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autoxidation at 50 °C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (Beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT, BHA, and α-tocopherol in 80% methanol were used as standards, and 80% methanol was used as the control.

Antioxidant activity was calculated in four different ways. In the first, absorbance was plotted against time, as a kinetic curve, and the absolute value of slope was expressed as antioxidant value (AOX). Antioxidant activity (AA) was also calculated as percent inhibition relative to control using the following equation (Al-Saikhan et al., 1995)

$$\mathrm{AA} = \frac{R_{\mathrm{control}} - R_{\mathrm{sample}}}{R_{\mathrm{control}}} \times 100 \qquad (1)$$

where $R_{\mathrm{control}}$ and $R_{\mathrm{sample}}$ were the bleaching rates of β-carotene in reactant mix without antioxidant and with plant extract, respectively.

The third method of expression based on the oxidation rate ratio (ORR) was calculated according to the method of Marinova et al. (1994) using the equation

$$\mathrm{ORR} = R_{\mathrm{sample}}/R_{\mathrm{control}} \qquad (2)$$

where $R_{\mathrm{sample}}$ and $R_{\mathrm{control}}$ are the same as in eq 1.

In the fourth method, the antioxidant activity coefficient (AAC) was calculated as described by Mallet et al. (1994)

$$\mathrm{AAC} = \frac{A_{\mathrm{s}(120)} - A_{\mathrm{c}(120)}}{A_{\mathrm{c}(0)} - A_{\mathrm{c}(120)}} \times 1000 \qquad (3)$$

where $A_{\mathrm{s}(120)}$ was the absorbance of the antioxidant mix at $t$ = 120 min, $A_{\mathrm{c}(120)}$ the absorbance of the control at $t$ = 120 min, and $A_{\mathrm{c}(0)}$ the absorbance of the control at $t$ = 0 min.

**Determination of Total Phenolics.** Total phenolics were determined using Folin−Ciocalteu reagent (Singleton and Rossi, 1965). Two hundred milligrams of sample was extracted for 2 h with 2 mL of 80% methanol containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000*g* for 15 min and the supernatant decanted into 4 mL vials. The pellets were extracted under identical conditions. Supernatants were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin−Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 0.75 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 90 min at 22 °C, absorbance was measured at 725 nm. Results are expressed as ferulic acid equivalents.

## RESULTS AND DISCUSSION

**Antioxidant Activities of Plant Materials.** The antioxidant activities and total phenolics of 28 plant products, including fractions of flaxseeds, wheat, buckwheat, sunflower seeds, and several fruits, vegetables, and medicinal plants, are shown in Table 1. The decrease in absorbance of β-carotene in the presence of different methanolic plant extracts (and well-known antioxidants used as standards) with the oxidation of β-carotene and linoleic acid is shown in Figures 1−4.

Horseradish oil had exceptionally high antioxidant activity, even higher than that of BHA and BHT at 400 mg/L and α-tocopherol at 200 mg/L. The major constituent of horseradish oil is allyl isothiocyanate ($C_2$-$CHCH_2NCS$), which represents 90−92% of the oil. Other components include 2-phenethyl isothiocyanate (3%), vinyl acetonitrile (3%), allyl thiocyanate (2%), and phenyl propionitrile (1%) (Mazza, 1984). To our knowledge there is no known antioxidant mechanism associated with these compounds. However, we suspect that the observed antioxidant activity may result from quenching of singlet oxygen by the isothiocyanates (Hall and Cuppett, 1997).

**Table 1. Antioxidant Activity and Total Phenolics of Methanolic Extracts of Plant Products**

| sample no. | sample name | sample abbrev | AOX[a] (A/h) | AA[b] (%) | ORR[c] | AAC[d] | total phenolics (mg/100 g) |
|---|---|---|---|---|---|---|---|
| 1 | solin gum | Sg | 0.101 | 82.1 | 0.179 | 526.8 | 1422 |
| 2 | flaxgum 1 | Fsg1 | 0.121 | 78.6 | 0.214 | 433.0 | 1354 |
| 3 | solin seed | Ls | 0.217 | 61.6 | 0.384 | 97.7 | 473 |
| 4 | flaxseed | Fs | 0.222 | 60.6 | 0.394 | 51.7 | 509 |
| 5 | flaxseed gum with maltodextrin | FsgM | 0.228 | 59.7 | 0.403 | 183.9 | nd[e] |
| 6 | flaxgum 2 | Fsg2 | 0.235 | 58.5 | 0.415 | 74.7 | 328 |
| 7 | flaxgum 4 | Fsg4 | 0.236 | 58.3 | 0.417 | 195.4 | 338 |
| 8 | flaxgum 3 | Fsg3 | 0.262 | 53.7 | 0.463 | 136.0 | 377 |
| 9 | blueberry | Bl | 0.044 | 92.1 | 0.079 | 796.3 | 4180 |
| 10 | red onion scale | Ros | 0.055 | 90.2 | 0.097 | 743.3 | 10548 |
| 11 | sunflower hull, purple | Sh | 0.063 | 88.9 | 0.111 | 714.5 | 9747 |
| 12 | sweet cherry | Swc | 0.099 | 82.5 | 0.175 | 580.4 | 2098 |
| 13 | potato, purple | Pp | 0.221 | 60.8 | 0.392 | 197.3 | 781 |
| 14 | horseradish oil | Hro | 0.005 | 99.1 | 0.009 | 969.3 | nd |
| 15 | sea buckthorn | Sb | 0.036 | 93.6 | 0.064 | 827.6 | 1112 |
| 16 | echinacea flower heads | Efh | 0.105 | 81.5 | 0.185 | 576.6 | 5467 |
| 17 | echinacea root | Er | 0.126 | 77.7 | 0.223 | 505.7 | 3841 |
| 18 | ginseng root | Gr | 0.175 | 69.1 | 0.309 | 333.3 | 347 |
| 19 | horseradish root | Hr | 0.241 | 57.4 | 0.426 | 134.1 | 481 |
| 20 | buckwheat hulls | Bwth | 0.029 | 94.9 | 0.051 | 827.6 | 3900 |
| 21 | potatoes (R. Burbank) | Pt | 0.113 | 80.0 | 0.200 | 509.6 | 437 |
| 22 | sunflower seed | Ss | 0.153 | 72.9 | 0.271 | 279.7 | 1601 |
| 23 | wheat germ | Wg | 0.199 | 64.9 | 0.351 | 235.6 | 349 |
| 24 | buckwheat seed | Bwt | 0.205 | 63.7 | 0.363 | 124.5 | 726 |
| 25 | fibrotein MK22E3 | Fm22 | 0.028 | 95.1 | 0.049 | 852.5 | nd |
| 26 | fibrotein MK11-DDG | Fm11 | 0.100 | 82.3 | 0.177 | 536.4 | 1241 |
| 27 | fibrotein MK43 | Fm43 | 0.207 | 63.4 | 0.366 | 321.8 | 169 |
| 28 | fibrotein MK37 | Fm37 | 0.249 | 56.0 | 0.440 | 187.7 | 213 |
| | control | Ctrl | 0.565 | 0.0 | 1.000 | 0.0 | |
| | BHT, 50 mg/L | BHT50 | 0.089 | 84.3 | 0.157 | 672.4 | |
| | BHT, 200 mg/L | BHT200 | 0.016 | 97.2 | 0.028 | 911.9 | |
| | α-tocopherol, 50 mg/L | TOC50 | 0.016 | 97.3 | 0.027 | 929.1 | |

[a] AOX, antioxidant value. [b] AA, antioxidant activity. [c] ORR, oxidation rate ratio. [d] AAC, antioxidant activity coefficient. [e] nd, not determined.

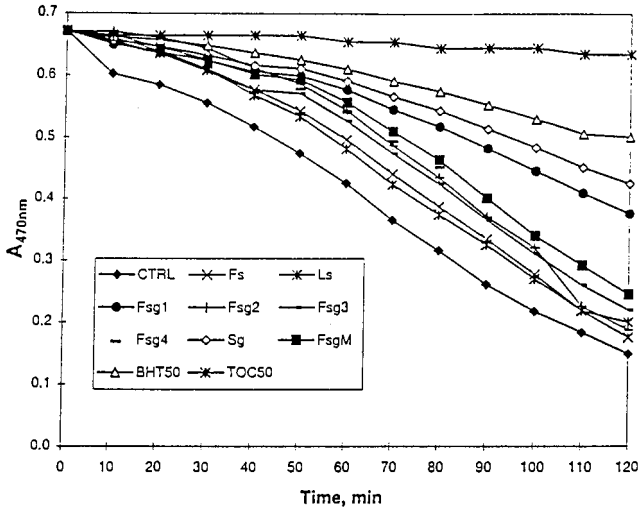


**Figure 1.** Antioxidant activity of methanolic extracts of flaxseed and its products assayed by the $\beta$-carotene bleaching method (BHT α-tocopherol at 50 mg/L concentration was used as a reference).

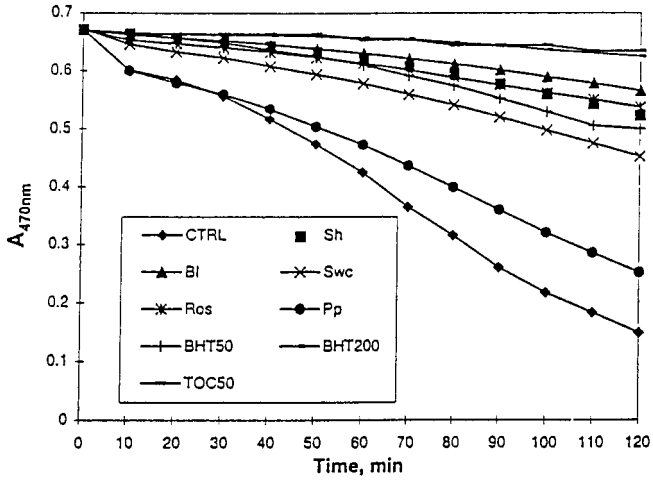


**Figure 2.** Antioxidant activity of anthocyanin-containing extracts assayed by the $\beta$-carotene bleaching method.

The flaxseed gum samples showed considerable differences in antioxidant activity (AA = 53.7−78.6) when it was calculated by the four different methods used in this study (Table 1). Fsg1, which was not precipitated with 95% ethanol, showed strong activity because of its high phenolic content (1354 mg/100 g) (Figure 1). The other three fractions all showed similar activities with AA for Fsg2, -3, and -4 at 53.7, 58.3, and 58.5%, respectively, and a phenolics level ranging from 328 to 377 mg/100 g (Table 1). Flaxseed as a whole showed relatively low antioxidant activity (AA = 60.6%). Similarly, solin gum, which was not precipitated with 95% ethanol, showed high antioxidant activity (AA = 82.1%) because of its high phenolic content (1422 mg/100 g) (Table 1; Figure 1), and solin seed showed lower antioxidant activity (AA = 61.6).

Antioxidant activities of buckwheat seed and hulls were 63.7 and 94.9%, respectively. The significantly high activity of the hulls reflects the higher phenolic content of the hull, 3900 mg of phenolics/100 g in hull versus 726 mg/100 g in seed. Sunflower seed, potato (Russet Burbank), wheat germ, and sunflower seed samples all showed relatively high antioxidant activity; all of these samples also contained above average levels of phenolic compounds.

**Table 2. Relationship between Antioxidant Activities and Total Phenolic Contents of Plant Materials**

| | relationship with total phenolics | $R^2$ |
|---|---|---|
| all plant materials | AOX = −0.00001717[phenolic] + 0.1883 | |
| $n = 28$ | AA = −0.000001660[phenolic] + 0.7016 | 0.4253[b] |
| fibers | AOX = −0.0001260[phenolic] + 0.2854 | |
| $n = 8$ | AA = −0.02220[phenolic] + 49.53 | 0.9632[b] |
| anthocyanin-rich | AOX = −0.00001111[phenolic] + 0.1572 | |
| materials, $n = 5$ | AA = −0.002355[phenolic] + 79.78 | 0.4623 |
| medicinal plant | AOX = −0.00001251[phenolic] + 0.1647 | |
| $n = 6$ | AA = −0.002218[phenolic] + 70.87 | 0.1388 |
| plant fractions | AOX = −0.00004041[phenolic] + 0.1964 | |
| $n = 5$ | AA = −0.007149[phenolic] + 65.25 | 0.6698[a] |
| cereal fractions | AOX = −0.0001200[phenolic] + 0.2505 | |
| $n = 4$ | AA = −0.02130[phenolic] + 55.72 | 0.9051[a] |

[a] $p < 0.01$. [b] $p < 0.001$.

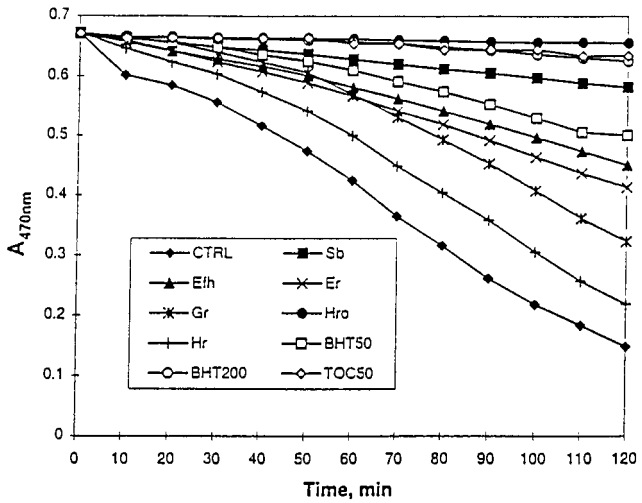


**Figure 3.** Antioxidant activity of medicinal plants assayed by the $\beta$-carotene bleaching method.

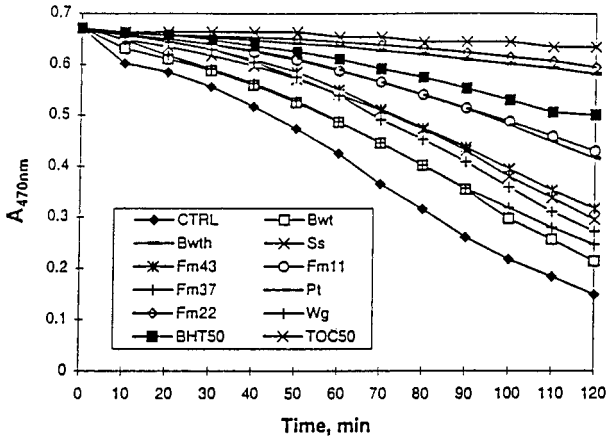


**Figure 4.** Antioxidant activity of buckwheat seed and hulls, sunflower kernel, potatoes, wheat germ, and fibroteins assayed by the $\beta$-carotene bleaching method.

The anthocyanin-rich samples generally showed very strong activities (Figure 2) as well as high phenolic contents (Table 1). The antioxidant activity of sunflower hulls, which contained nearly 10% phenolics of which 2.2% were anthocyanins, showed a very high activity. Potato (Russet Burbank), wheat germ, and sunflower seed samples all had moderate antioxidant activity, consistent with their moderate content of phenolic compounds. The results confirm that anthocyanins possess strong antioxidant activities (Wang et al., 1997; Tsuda et al., 1994a,b). Anthocyanins are probably the largest group of phenolic compounds in the human diet,

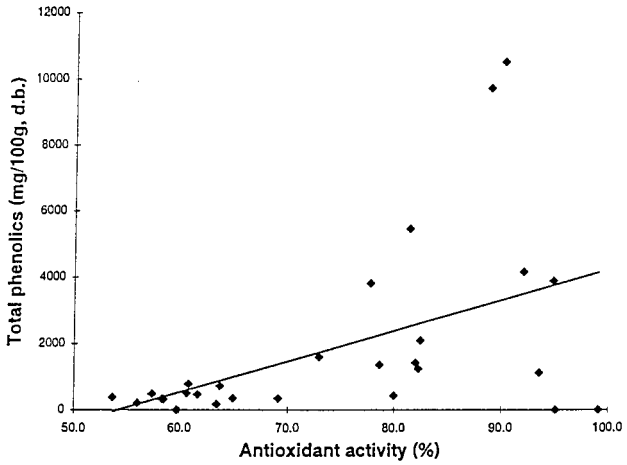


**Figure 5.** Relationship between total phenolic content and antioxidant activity (AOX) of plant materials.

and their strong antioxidant activities suggest their importance in maintaining health.

The medicinal plants showed very strong antioxidant activities (Table 1; Figure 3). Sea buckthorn fruit had the highest activity (AA = 93.6%) among the medicinal plants (Table 1). Other plants were generally less potent, with AA value from 81.5% for echinacea flower head to 57.4% for horseradish root.

Some of the fibrotein samples included in this study showed very strong activity (Fm22), and others showed medium to high activities. The antioxidant activities of these products are probably from the combined action of phenolics and protein in the samples. Cereal protein has been reported to exert strong antioxidant activities (Iwami et al., 1987).

**Relationship between Phenolic Contents and Antioxidant Activity.** The total phenolic content of the plant materials investigated in this study varied from 169 to 10548 mg/100 g of dry product (Table 1). The relationship between total phenolic content and antioxidant activity of plant material is shown in Figure 5 and Table 2. The results indicate that when all plant materials were included in the statistical analysis, there was a positive and highly significant ($p < 0.001$) relationship between total phenolics and antioxidant activity. Statistically significant relationships were also observed between total phenolics and antioxidant activity of flaxseed products ($R^2 = 0.963$; $p < 0.001$) and cereal products ($R^2 = 0.905$; $p < 0.001$). However, the relationship between phenolics and antioxidant activity for the anthocyanin-rich materials and for the medicinal plants was not significant. The lack of a significant correlation between total phenolics and AA of the

anthocyanin-containing plant materials, which included blueberries, cherries, red onion scales, purple sunflower hulls, and purple potatoes, reflects the exceptionally high antioxidant activity of blueberry and red onion scale samples despite their very different contents of total phenolics (Table 1). The composition of the phenolics in red onion scales, primarily flavonoids (Donner et al., 1996), is very different from the phenolics of blueberries, which are primarily anthocyanins (Gao and Mazza, 1995). The lack of correlation between the phenolics and AA of the medicinal plants may be due to the carotenoid-rich sea buckthorn (Li and Wang, 1998). When the results for this fruit were omitted from the regression analysis, the relationship between the phenolic contents and antioxidant activities was significant ($p < 0.05$). This indicates that factors other than total phenolics can play a major role in the antioxidant activity of plant materials such as sea buckthorn.

Further work is in progress in our laboratory to elucidate the identity of compounds responsible for the antioxidant activity.

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