ARTICLES

Antioxidant Activity of Plant Extracts Containing Phenolic Compounds

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The antioxidative activity of a total of 92 phenolic extracts from edible and nonedible plant materials (berries, fruits, vegetables, herbs, cereals, tree materials, plant sprouts, and seeds) was examined by autoxidation of methyl linoleate. The content of total phenolics in the extracts was determined spectrometrically according to the Folin−Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Among edible plant materials, remarkable high antioxidant activity and high total phenolic content (GAE > 20 mg/g) were found in berries, especially aronia and crowberry. Apple extracts (two varieties) showed also strong antioxidant activity even though the total phenolic contents were low (GAE < 12.1 mg/g). Among nonedible plant materials, high activities were found in tree materials, especially in willow bark, spruce needles, pine bark and cork, and birch phloem, and in some medicinal plants including heather, bog-rosemary, willow herb, and meadowsweet. In addition, potato peel and beetroot peel extracts showed strong antioxidant effects. To utilize these significant sources of natural antioxidants, further characterization of the phenolic composition is needed.

Keywords: *Plant extracts; natural antioxidants; total phenolics; antioxidant evaluation*

INTRODUCTION

Phenolic compounds are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects (Löliger, 1991).

Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease. Ingestion of alcohol-free red wine or a phenolic compound mixture extracted from red wine has been shown to improve the antioxidant status of plasma in humans (Serafini et al., 1998; Carbonneau et al., 1998). Consumption of controlled diets high in fruits and vegetables increased significantly the antioxidant capacity of plasma, and the increase could not be explained by the increase in the plasma α-tocopherol or carotenoid concentration (Cao et al., 1998). Moreover, epidemiological studies have found that there is a significant negative association between the intake of fruits and vegetables and heart disease mortality (Hertog et al., 1993, 1995; Knekt et al., 1996).

Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs (Ramarathnam et al., 1995). Flavonoids and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks (Larson, 1988). They are important in the plant for normal growth development and defense against infection and injury. Flavonoids also partly provide plant colors present in flowers, fruits, and leaves. They generally occur as glycosylated derivates in plants, although conjugation with inorganic sulfate or organic acid as well as malonylation are also known (Heldt, 1997). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (Rice-Evans et al., 1995).

Berries and fruits contain a wide range of flavonoids

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and phenolic acids that show antioxidant activity. Main flavonoid subgroups in berries and fruits are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic acids present in berries and fruits are hydroxylated derivates of benzoic acid and cinnamic acid (Macheix et al., 1990). Studies on antioxidative activities of fruit extracts have been focused mainly on grapes, which have been reported to inhibit oxidation of human low-density lipoprotein (LDL) at a level comparable to wine (Meyer et al., 1997). Fresh strawberry extract was reported to have 15 times higher total antioxidant capacity than trolox in an artificial peroxyl radical model system (Wang et al., 1997). Extracts of blackberries, black and red currants, blueberries, and black and red raspberries possessed a remarkably high scavenging activity toward chemically generated superoxide radicals (Constantino et al., 1992). Hydroxycinnamic acids typically present in fruits have been shown to inhibit LDL oxidation in vitro (Meyer et al., 1998). Also phenolic extracts of berries (blackberries, red raspberries, sweet cherries, blueberries, and strawberries) were shown to inhibit human low-density lipoprotein (LDL) and liposome oxidation (Heinonen et al., 1998b).

Studies on phenolic composition and antioxidant action of vegetable extracts have been reported recently. Beans (kidney and pinto), followed by beet, corn, and broccoli, showed highest total phenol content per fresh weight among 22 vegetables analyzed. By using a LDL oxidation method and a combined measure of the quality and quantity of phenol antioxidants present in the vegetables (per fresh weight), kidney and pinto beans were also ranked at the top in respect to the antioxidant ability. Garlic, yellow and red onion, asparagus, snap bean, beet, potato, and broccoli were also evaluated among the 10 most potent vegetables (Vinson et al., 1998). Al-Saikhan et al. (1995) measured antioxidant activity by using β-carotene/linoleic acid as the oxidizing lipid system. Activity of analyzed vegetables decreased in the following order: broccoli > potato > carrot > onion > bell pepper.

Besides flavoring purposes, spices and herbs have been used also for their medical or antiseptic properties. The preservative effect of many spices and herbs suggests the presence of antioxidative and antimicrobial constituents. As early as in 1952 Chipault et al. examined 72 spices, their petroleum ether, and alcohol-soluble fractions and found 32 spices to retard the oxidation of lard. Rosemary and sage were remarkably effective, and oregano, thyme, turmeric, and nutmeg also showed high antioxidant activity in the ground form and as extracts. Several later studies confirmed that many leafy spices, especially those belonging to the Labiatae family such as sage, rosemary, oregano, and thyme, show strong antioxidant activity (Hirasa and Takemasa, 1998). A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts (Nakatani, 1997).

Cereals contain a wide range of phenolic compounds. A significant amount of phenolic acids such as ferulic, caffeic, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, vanillic, and syringic acids is typical to cereals. These compounds occur in the grain primarily in the bound form as conjugates with sugars, fatty acids, or protein (White and Xing, 1997). The phenolic antioxidants in oats have been studied extensively since the 1930s, but less attention has been given to other common cereals. Methanolic extracts of oats have been found to have significant antioxidant effects (Duve and White, 1991; Tian and White, 1994).

A variety of tree materials are known to be remarkable sources of phenolic compounds. Several phenolics have been isolated and identified from conifer needles (Lundgren, 1987; Strack et al., 1989); from bark of birch, spruce, and pine (Pan, 1995); and from white birch and silver birch leaves (Ossipov et al., 1996). Pycnogenol (procyanidin extract of the bark of *Pinus maritima*) is probably the most studied phenolic tree extract. It has been shown to scavenge free radicals, including hydroxyl and superoxide anions (Noda et al., 1997), and it may have beneficial effects in preventing atherosclerosis and other age-related diseases (Fitzpatrick et al., 1998). Very scarce data on the quantity of phenolic compounds and the antioxidant activity of phenolic tree extracts in lipid systems have been reported.

Even though intensive studies on the phenolic constituents in numerous plant sources have been conducted, the composition data are yet insufficient. Phenolic profiles often have been analyzed after hydrolyzation of the glycosidic bonds in order to simplify the identification process; necessary information of the authenthic structure of the compounds is lost. It is known that the degree of glycosylation significantly affects the antioxidant properties of the compound. For example, aglycones of quercetin and myricetin were more active than their glycosides in bulk methyl linoleate (Hopia and Heinonen, 1999). Also the wide variety of oxidation systems and ways to measure activity used in antioxidant evaluations make it difficult to compare results from different studies.

The aim of this study was to screen a large number of plant material extracts of Finnish origin with respect to their total phenolic content and antioxidant activity in order to find new potential sources of natural antioxidants. The study is a part of a larger survey in which other functional properties of these extracts such as their antimicrobial, antiinflammatory, and calcium antagonistic effects are also being evaluated.

MATERIALS AND METHODS

Chemicals. Methyl linoleate (MeLo) was purchased from Nu-Check-Prep, Inc. (Elysian, MN), α-tocopherol was from Sigma (St. Louis, MO), Folin−Ciocalteu's phenol reagent and sodium carbonate were from Merck (Darmstadt, Germany). All organic solvents used were of HPLC grade except methanol (techical quality), which was used as an extraction solvent.

Extracts. The 92 different plant materials were either purchased from a market place or collected from nature. The materials included berries, fruits, vegetables, cereals, herbs, plant sprouts, and seeds as well as tree leaves and bark (Table 1). Berries were extracted with seeds. Apples were cored and onions peeled before extraction. Air-drying was carried out for herbs and medicinal plants (at +24 °C in the dark); other materials were stored in a freezer at −18 °C and lyophilized before analysis. All samples were analyzed within 3 months of collection. Four different extraction methods were used to produce phenolic extracts from different types of plant materials. The methods were selected on the basis of extraction tests performed using different solvents and conditions (Kähkönen et al., unpublished results) and on the basis of literature (Keinänen, 1993; Loponen et al., 1997).

Preparation of Berry and Fruit Extracts (Method A). Grounded lyophilized material (500 mg) was weighed into centrifuge tube, 10 mL of solvent (70% aqueous acetone) was added, and the sample was homogenized (Ultra-Turrax) for 1 min. Tubes were centrifugated (3000*g*, 15 min), and the clear supernatant was collected. The procedure was repeated with

Table 1. Total Phenolics in Plant Extracts and Their Inhibition (% In) of Methyl Linoleate Oxidation

raw materials		drying method[a]	extraction method[b]	dry wt of extract (mg)	total phenolics[c] (mg of GAE/g dw)	% In,[d] 500 ppm	% In,[e] 5000 ppm
		Berries and Fruits					
aronia	*Aronia melanocarpa,* Viking	1	A	67	40.1 ± 0.9	93	94
crowberry	*Empetrum nigrum*	1	A	71	50.8 ± 1.0	98	99
strawberry	*Fragaria ananassa,* Senga Sengana	1	A	42	14.8 ± 0.3	57	21
strawberry	*Fragaria ananassa,* Jonsok	1	A	22	17.7 ± 0.2	52	25
strawberry	*Fragaria ananassa,* Bounty	1	A	24	23.7 ± 0.5	60	22
gooseberry	*Ribes grossularia*	1	A	50	12.4 ± 0.6	93	43
black currant	*Ribes nigrum,* Öjeby	1	A	67	20.3 ± 0.7	83	13
red currant	*Ribes rubrum,* Red Dutch	1	A	48	12.6 ± 0.2	60	11
cloudberry	*Rubus chamaemorus*	1	A	42	16.2 ± 0.1	97	98
raspberry	*Rubus idaeus,* Ottawa	1	A	35	23.9 ± 0.2	88	23
rowanberry	*Sorbus aucuparia*	1	A	35	18.7 ± 0.8	98	88
bilberry	*Vaccinium myrtillus*	1	A	54	29.7 ± 0.9	92	62
cranberry	*Vaccinium oxycoccus*	1	A	59	21.2 ± 0.7	96	87
whortleberry	*Vaccinium uligonosum*	1	A	31	28.7 ± 0.8	95	96
cowberry	*Vaccinium vitis-idaea*	1	A	28	24.9 ± 0.4	91	96
rose, fruit	*Rosa* sp.	1	B	37	12.5 ± 0.1	12	35
apple	*Malus pumila,* Punakaneli	1	A	20	11.9 ± 0.4	88	55
apple	*Malus pumila,* Valkea kuulas	1	A	26	12.1 ± 0.3	95	86
		Vegetables					
onion	*Allium cepa*	2	B	88	2.5 ± 0.1	16	11
red onion	*Allium cepa*	2	B	36	3.0 ± 0.1	12	−1
swede, peel	*Brassica napus rapifera*	1	C	167	1.6 ± 0.0	−1	−1
beetroot, peel	*Beta vulgaris esculenta*	1	C	236	4.3 ± 0.2	98	99
cucumber, leaf	*Cucumber savitus*	1	C	138	3.8 ± 0.1	32	35
carrot, flesh	*Daucus carota*	1	C	281	0.6 ± 0.0	0	10
carrot, leaf	*Daucus carota*	1	C	101	7.4 ± 0.1	34	39
carrot, peel	*Daucus carota*	1	C	210	6.6 ± 0.1	22	52
tomato	*Lycopersicum esculentum*	1	A	21	2.0 ± 0.1	52	9
pea	*Pisum savitum*	1	C	50	0.4 ± 0.0	8	28
pea, legume	*Pisum savitum*	1	C	177	1.6 ± 0.1	24	37
potato, peel	*Solanum tuberosum,* Rosamunda	1	C	67	4.3 ± 0.2	66	86
potato, peel	*Solanum tuberosum,* Matilda	1	C	80	2.5 ± 0.1	53	63
sugar beet, peel	*Beta vulgaris altissima*	1	C	201	4.2 ± 0.2	88	95
		Herbs and Medicinal Plants					
yarrow	*Achillea millefolium*	1	B	54	5.3 ± 0.1	16	17
bog-rosemary	*Andromeda polifolia glaucophylla*	1	B	108	32.8 ± 1.1	97	98
horseradish	*Armoracia rusticana*	1	B	11	0.9 ± 0.0	−3	−24
willow herb	*Epilobium angustifolium*	1	B	178	32.2 ± 0.9	90	88
heather	*Calluna vulgaris*	1	B	110	36.0 ± 1.2	95	93
	Fallopia convolvulus	1	B	58	2.8 ± 0.1	13	2
meadowsweet	*Filipendula ulmaria*	1	B	95	26.8 ± 1.3	95	87
hop	*Humulus lupulus*	1	B	24	23.1 ± 0.8	1	−24
blue lupin	*Lupinus angustifolius*	1	B	183	4.7 ± 0.1	10	6
yellow loosestrife	*Lysimachia vulgaris*	1	B	96	13.5 ± 0.6	44	65
purple loosestrife	*Lythrum salicaria*	1	B	10	42.1 ± 0.9	46	41
camomile, flower	*Matricaria chamomilla*	1	B	14	9.1 ± 0.8	16	20
camomile	*Matricaria chamomilla*	1	B	17	12.7 ± 0.7	8	17
pineapple weed	*Matricaria matricarioides*	1	B	10	4.2 ± 0.9	19	21
lake reed, leaf	*Phragmites australis*	2	C	76	5.7 ± 0.3	46	40
lake reed, stalk	*Phragmites australis*	2	C	43	1.5 ± 0.1	23	7
silverweed	*Potentilla anserina*	1	B	41	2.8 ± 0.2	54	58
damson	*Prunus insititia*	1	B	82	23.0 ± 1.0	48	86
creeping buttercup	*Ranunculus repens*	1	B	97	4.1 ± 0.2	46	81
cloudberry, leaf	*Rubus chamamaemorus*	1	B	21	17.2 ± 0.5	92	97
sorrel	*Rumex acetosa*	1	B	61	3.5 ± 0.1	17	30
goldenrod	*Solidago virgaurea*	1	B	86	8.2 ± 0.1	28	34
field milk thistle	*Sonchus arvensis*	1	B	81	5.6 ± 0.2	4	−6
devil's bit scabious	*Succisa pratensis*	1	B	127	16.4 ± 0.4	−5	10
tansy	*Tanacetum vulgare*	1	B	161	14.2 ± 0.6	44	−12
thyme	*Thymus vulgaris*	1	B	9	17.1 ± 0.2	97	97
alsike clover	*Trifolium hybridum*	1	B	82	5.0 ± 0.1	4	−9
red clover	*Trifolium pratense*	1	B	138	7.8 ± 0.2	19	−1
white clover	*Trifolium repens*	B	1	78	2.9 ± 0.0	10	14
	Tripleurospermun inodorum	1	B	92	3.9 ± 0.1	6	10
bulrush, leaf	*Typha latifolia*	1	C	64	8.2 ± 0.1	35	33
bulrush, stalk	*Typha latifolia*	2	C	59	5.7 ± 0.2	80	90
caraway, seed	*Carum carvi*	1	B	7	6.5 ± 0.1	64	46
flax, seed	*Linum usitatissimum*	1	C	24	0.8 ± 0.0	35	18
rose, seed	*Rosa* sp.	1	B	5	6.0 ± 0.1	71	48

Table 1. (Continued)

raw materials		drying method[a]	extraction method[b]	dry wt of extract (mg)	total phenolics[c] (mg of GAE/g dw)	% In,[d] 500 ppm	% In,[e] 5000 ppm
			Cereals				
oat, grain	*Avena sativa*	1	C	18	0.3 ± 0.0	9	8
oat, chaff	*Avena sativa*	1	C	18	0.7 ± 0.1	3	−5
oat, bran	*Avena sativa*	1	C	14	0.4 ± 0.0	80[f]	−6
oat, flakes	*Avena sativa*	1	C	14	0.3 ± 0.0	9	−2
rye, bran	*Secale cereale*	1	C	48	1.3 ± 0.1	60[f]	8
rye, flour	*Secale cereale*	1	C	36	0.5 ± 0.1	36[f]	16
wheat, bran	*Triticum aestivum*	1	C	33	1.0 ± 0.1	35	6
wheat, grain	*Triticum aestivum*	1	C	19	0.2 ± 0.0	7	2
barley, pearl	*Hordeum sativum*	1	C	8	0.3 ± 0.0	20	6
barley, grain	*Hordeum sativum*	1	C	17	0.4 ± 0.1	13	4
			Tree Materials				
maple, leaf	*Acer platanoides*	1	D	151	31.7 ± 0.2	65	97
white birch, leaf	*Betula pubescens*	1	D	160	38.7 ± 0.5	51	94
silver birch, leaf	*Betula pendula*	1	D	219	38.4 ± 1.0	63	93
silver birch, phloem	*Betula pendula*	1	C	114	85.5 ± 2.1	91	96
silver birch, bark	*Betula pendula*	1	C	7	2.0 ± 0.1	87	18
Scotch pine, needle	*Pinus sylvestris*	1	C	104	17.5 ± 0.2	85	95
Scotch pine, cork	*Pinus sylvestris*	1	C	11	1.1 ± 0.1	93	48
Scotch pine, bark	*Pinus sylvestris*	1	C	132	76.0 ± 2.9	90	97
spruce, needle	*Picea abies*	1	C	111	155.3 ± 6.1	98	98
aspen, leaf	*Populus tremula*	1	D	153	20.2 ± 0.3	68	94
aspen, bark	*Populus tremula*	1	C	115	32.1 ± 0.2	91	94
willow, leaf	*Salix caprea*	1	D	163	37.6 ± 0.1	96	97
willow, bark	*Salix caprea*	1	C	108	75.5 ± 1.5	96	96
silverwillow, leaf	*Salix alba*	1	D	139	27.5 ± 0.3	38	84
silverwillow, bark	*Salix alba*	1	C	113	58.6 ± 0.9	96	98

[a] 1 = air-drying; 2 = lyophilization. [b] Extraction procedures are described in detail in Materials and Methods. A = 70% aqueous acetone, homogenization with Ultra Turrax; B = 80% aqueous methanol, sonication; C = 80% aqueous methanol, Ultra Turrax; D = 70% aqueous acetone, magnetic mixer. [c] Mean of duplicate assays; dw = dry weight of the original sample. [d] Calculated by using the dry weight of the extract. [e] Calculated by using the dry weight of the original sample. [f] Tested at 2000 ppm level.

another 10 mL of solvent. Supernatants were combined and taken to dryness. The solid residue was dissolved in methanol. Solid-phase extraction (SPE) was used to remove sugars in the berry and apple extracts according to the method described before (Heinonen et al., 1998a) in order to avoid interference in the antioxidant test. To determine dry weights, a part of the extract was lyophilized and the solid residue weighed.

Preparation of Plant Extracts (Method B). Grounded dry plant material (500 mg) was weighed into a test tube. A total of 10 mL of 80% aqueous methanol was added, and the suspension was stirred slightly. Tubes were sonicated 5 min and centrifugated for 10 min (1500*g*), and supernatants were collected. Plant materials were re-extracted twice. Combined supernatants were evaporated to a volume of about 1 mL. These concentrated extracts were lyophilized and weighed.

Preparation of Plant Extracts (Method C). Grounded dry plant material (500 mg) was extracted with 2 × 10 mL of 80% aqueous methanol using Ultra Turrax mixer for 1 min. Samples were centrifuged (10 min, 3000*g*), and combined extracts were taken to dryness. The solid residue was dissolved in water and lyophilized, and the residues were weighed.

Preparation of Plant Extracts (Method D). Grounded dry plant material (500 mg) was extracted with 3 × 25 mL of 70% aqueous acetone using magnetic mixer for 45, 45, and 20 min. Extraction was continued as in method C.

Determination of Total Phenolics. The amount of total phenolics in extracts was determined according to the Folin−Ciocalteu procedure (Singleton and Rossi, 1965). Samples (200 μL, two replicates) were introduced into test tubes; 1.0 mL of Folin−Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (Perkin-Elmer λ15 UV−vis spectrophotometer, Norwalk, CT). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

Oxidation of MeLo. Antioxidant testing was carried out by oxidizing MeLo in the presence of the antioxidants. The method was used in a previous antioxidant activity study of berry and fruit wines and liquors (Heinonen et al., 1998a). Extracts were tested at the concentration of 500 ppm according to the dry weight of the extract. As the content of extract varied greatly (from 5 to 281 mg), extracts also were evaluated at a level representing 5000 ppm of original (dry) plant material. Methanolic extracts were added to MeLo (0.2 g), and methanol was evaporated under nitrogen. Oxidation of MeLo was carried out in the dark at 40 °C. Sample aliquots (10 mg) were taken at the starting point and after 72 h of oxidation. Aliquots were dissolved in 5 mL of 2,2,4-trimethylpentane (isooctane), and the conjugated diene absorption at 234 nm was measured (Perkin-Elmer Lambda15 UV−vis spectrophotometer, Norwalk, CT). The amount of conjugated diene hydroperoxides was calculated using molar absorptivity of 26 000 (Fischwick and Swoboda, 1977). The antioxidant activity was expressed as percentage (%) inhibition of formation of MeLo-conjugated diene hydroperoxides after 72 h of oxidation. α-Tocopherol (20 ppm) was used in each experiment as a reference antioxidant. Each oxidation series was repeated once to confirm the results.

RESULTS

Amount of Total Phenolics. The amount of total phenolics varied widely in plant materials and ranged from 0.2 to 155.3 mg GAE/g dry material (Table 1). Among edible materials, low levels were found in cereals (0.2−1.3 mg/g GAE) and vegetables (0.4-6.6 mg/g GAE), whereas berries contained relatively high amounts of phenolics (12.4−50.8 mg/g GAE). Moderate levels were found in herb extracts (9.1−23.1 mg/g GAE). Among nonedible materials, all examined tree materials, excluding pine cork and birch bark, had considerably high contents of phenolic substances (17.5−155.3 mg/g GAE). Significant amounts also were observed in some medicinal plants, for example purple loosestrife (42.1 mg/g GAE), heather (36.0 mg/g GAE), bog-rosemary (32.8 mg/g GAE), and willow herb (32.2 mg/g GAE).

Antioxidant Activities. Antioxidant activities of extracts in MeLo are shown in Table 1. As the yield of

phenolic extract varied significantly (from 5 mg in rose seed to 281 mg in carrot flesh), the percentage inhibition value of a sample at the concentration of 500 ppm of extract is more descriptive in respect to the activity of the extract whereas the 5000 ppm level calculated on the basis of dry weight of the original plant sample gives an estimation of the total content of active extract gained by this extraction method.

At the 500 ppm extract level, berry and tree material extracts showed high antioxidant activities whereas most vegetable and cereal extracts were relatively inactive. The formation of MeLo-conjugated diene hydroperoxides was inhibited over 90% by crowberry, rowanberry, cloudberry, cranberry, whortleberry, aronia, gooseberry, bilberry, and cowberry extracts. Raspberry and black currant were somewhat less active with inhibitions of 88 and 83%, whereas red currant and strawberry were the least active berry extracts in this study, giving inhibitions of 60 and 57%. There were few extracts with significant antioxidant activity among the vegetable subclass. Only beetroot peel extract inhibited hydroperoxide formation by over 90% (98% inhibition). Extract prepared from red colored potato peels of variant Rosamunda gave an inhibition value of 86% whereas extract from variant Matilda with yellow peels reached only 53% inhibition. The antioxidant activities of cereal extracts were very low. Wheat bran extract reached 35% inhibition whereas oat grain, chaff, and flakes; wheat grains; and barley grains and pearls had very weak inhibition effect, under 20%. Oat bran, rye bran, and rye flour were tested at 2000 ppm extract concentration instead of 500 ppm concentration, but despite that the inhibitions of conjugated diene hydroperoxide formation were only 80, 60, and 38%, respectively.

Most of the examined herb and plant sprout extracts showed weak or moderate antioxidant activity. There were, however, some herbs and medicinal plants with considerably strong antioxidant response (over 90% inhibition). The inhibition of MeLo hydroperoxides of these plant extracts decreased in the following order: bog-rosemary = thyme > heather = meadowsweet > cloudberry leaf > willow herb. Seed extracts, namely, caraway, flax, and rose seed extracts, had moderate inhibition effects (from 35 to 71% inhibition). Tree material extracts had overall good antioxidant activities. The inhibition of conjugated diene hydroperoxides of most active tree extracts (>90% inhibition) decreased in following order: spruce needle > willow leaf = willow bark > silverwillow bark > pine cork > silver birch phloem > pine bark.

Somewhat different inhibitions were observed at 5000 ppm of the original sample material level. Among berry extracts there were some materials that showed a remarkably lower activity at this level than at the 500 ppm extract level, for example, bilberry (62%) and raspberry (23% inhibition). Beetroot peel was the only vegetable extract to have high antioxidant activity (99% inhibition) at this level. None of the cereal materials showed significant activity at the 5000 ppm level; inhibition values ranged from −6 to 16%. Herbs also were as inactive as at the 500 ppm level excluding thyme, which showed high activity (97% inhibition). Inhibitions of other plant sprout extracts were approximately the same as at the 500 ppm. The inhibition of MeLo-conjugated diene hydroperoxides of most active plant extracts (>90% inhibition) decreased in following order: bog-rosemary > thyme = cloudberry leaf > heather > bulrush stalk. Caraway, flax, and rose seed extracts had only moderate or weak activity (<48% inhibition). All examined tree extracts, excluding birch bark (18%) and pine cork (48%), had antioxidant activity of over 80%. The inhibition decreased in the following order: silverwillow bark > maple leaf = willow leaf = pine bark > silver birch phloem > willow bark > white birch leaf > aspen leaf > silver birch leaf > silverwillow leaf. The spruce and pine needle extracts were as active as leaf extracts, with 98 and 95% inhibition.

DISCUSSION

No significant correlations could be found between the total phenolic content and antioxidant activity of the plant extracts in any of the studied subgroups. It is known that different phenolic compounds have different responses in the Folin−Ciocalteu method. Similarily the molecular antioxidant response of phenolic compounds in MeLo varies remarkably, depending on their chemical structure (Satue-Gracia et al., 1997). Thus, the antioxidant activity of an extract cannot be predicted on the basis of its total phenolic content.

Berries and Fruits. Overall, berries exhibited high total phenolic contents and high antioxidant activity. Berries with a strong purple color, such as crowberry, aronia, bilberry, and whortleberry, had clearly higher phenolic contents (28.7−50.8 mg/g GAE) than the yellowish rowanberries and cloudberries (18.7 and 16.2 mg/g GAE), but there were no significant differences between the antioxidant activities. The strongly colored berries are rich in anthocyanins (Macheix et al., 1990), and strong antioxidant activities for anthocyanins in different model systems have been reported (Tamura and Yamagami, 1994; Rice-Evans et al., 1995; Satue-Gracia et al., 1997; Wang et al., 1997). In addition, berry wines made of berries with strong color such as bilberries, black currants, cowberries, cranberries, and crowberries showed stronger antioxidative activity in the oxidation of MeLo than those made of berries with light color such as cloudberries, red raspberries, and strawberries (Heinonen et al., 1998a). In an LDL oxidation system, strawberry was found to be a weaker antioxidant than blackberries, red raspberries, sweet cherries, and blueberries (Heinonen et al., 1998b). On the contrary, fresh strawberry extract was reported to have 15 times higher total antioxidant capacity than trolox and greater activity than many fruits, for example plum, orange, red grape, apple, and tomato, in an artificial peroxyl radical model system (Wang et al., 1996). According to Häkkinen et al. (1998), crowberry, bilberry, cranberry, and cowberry are rich in flavonols, mainly quercetin, while strawberry, red raspberry, and cloudberry have an especially high content of ellagic acid, and rowanberry contains high amounts of ferulic acid. In addition, bilberries are also especially rich in hydroxycinnamic acid derivates, which have been shown to exert antioxidant activity (Chen and Ho, 1998; Meyer et al., 1998).

Somewhat surprising is that black currant was not among the most active berries in this study. In the berry wine study mentioned before (Heinonen et al., 1998a), where the MeLo oxidation model similar to the present one was used, black currant was evaluated as one of the most active raw materials. It may be that the wine-making procedure more effectively extracts the active phenolic compounds from the berries as compared to the

solvent extraction used in the present study. Another possible explanation for these different results is that the antioxidant phenolics were concentrated during the wine-making process. Extracts of black currants along with red currants, blackberries, blueberries, and black and red raspberries possessed also a remarkably high scavenging activity toward chemically generated superoxide radicals (Constantino et al., 1992). The total phenol contents of the two apple varieties studied were almost similar (11.9 and 12.1 mg/g GAE) but lower than the contents in berries. However, apples exerted strong antioxidant activities. The very low inhibition values of black currant and raspberry at the concentration of 5000 ppm of original material indicate that the yield of active phenolic compounds in the extraction was low. This inactiviness may result from the high amount of nonactive compounds present such as residual sugars. In fact, the weak antioxidant activity of rose fruit extract, where sugars were not removed, may partly be due to a high concentration of sugars as they increase the dry weight of the extract and thereby affect the antioxidant activities when measured based on the ppm concentrations.

Vegetables. The total phenol contents in the vegetable subgroup were very low as compared to berries, only 0.4−6.6 mg/g GAE. Despite that, three vegetable extracts (beetroot peel, the peel of purple skinned potato variety Rosamunda, and sugar beet peel) showed remarkable antioxidant activity, being superior to other vegetable extracts. The yellow-skinned potato variety Matilda had lower total phenolic content and antioxidant activity than Rosamunda. In a study where several red-fleshed potato varieties and breeding clones were analyzed, the anthocyanin content of 21.7 mg in 100 g of skin (fresh weight) was detected, and the dominant anthocyanin was identified as pelargonidin-3-rutinoside-5-glucoside. The major phenolics in the skin were chlorogenic and *p*-coumaric acids (Rodriguez-Saona et al., 1998). The difference in antioxidant activity between potato varieties may result partly from the presence of anthocyanins, although pelargonin has been reported to show poor antioxidant activity in human LDL and pro-oxidant activity in a lecithin liposome system (Satue-Gracia et al., 1997). Methanolic extracts of purple potatoes as well as other anthocyanin-rich plant products, such as blueberries, sweet cherries, purple sunflower hulls, and red onion scales, showed strong antioxidative activities in a β-carotene bleaching method (Velioglu et al., 1998). In the present study, the antioxidant activity order was approximately similar at both tested concentration levels, which indicates that the yield of the vegetable extract was good. However, while the total phenol content of many vegetable extracts was extremely small, their dry weights were remarkably high, up to 281 mg (originating from 500 mg of original dry sample). To explain this inconsistensy, it would be necessary to study the composition of these extracts to a fuller extent. Sugar removal could have increased the antioxidant activities of some of the vegetable extracts.

Cereals. The concentrations of total phenolic compounds in cereals were even lower than the concentrations in vegetables (0.2-1.3 mg/g GAE). Cereal extracts did not show remarkable antioxidant activity. Oat bran exerted a moderate effect, but the inhibition (as well as inhibitions of rye bran and flour) was measured at a higher level than the other extracts (2000 vs 500 ppm) and would have propably been much lower at the 500 ppm level. In oat flour, *p*-hydroxybenzoic, protocatechuic, vanillic, *trans*-*p*-coumaric, (*p*-hydroxyphenyl)-acetic, syringic, *trans*-sinapic, caffeic, and ferulic acids were found, with ferulic acid being the most abundant (White and Xing, 1997). Ferulic acid is also the dominant form of phenolic acid in rye, wheat, and barley. Ferulic acid showed moderate, caffeic acid showed strong, and *p*-coumaric acid showed neglibigle antioxidant activity in a study by Terao et al. (1993), where the activity was measured by autoxidation of bulk MeLo. In addition, oats contain a series of anionic substituted cinnamic acid conjugates, avenanthramides, which also are potential antioxidants (Dimberg et al., 1993). The processing seems to be important when evaluating the antioxidant activity of cereals as brans were more active than other products from the same cereal species. This is obviously due to the localization of the phenolics in the grain: the outer layers of the grain (husk, pericarp, testa, and aleurone cells) contain the greatest concentrations of total phenolic, whereas their concentrations are considerably lower in the endosperm layers About 80% of the *trans*-ferulic acid of both rye and wheat grain was found in the bran (White and Xing, 1997). The results are also in accordance with the results of Onyeneho and Hettiarachchy (1992), who observed that the freeze-dried fraction from durum wheat (triticum durum) bran exhibited stronger antioxidant activity than extracts from other milling fractions.

Herbs. Thyme possessed strong antioxidant activity in this study as expected on the basis of previous studies (Takácsová et al., 1995; Nakatani, 1997; Hirasa and Takemasa, 1998). Thymol and carvacrol are major aroma components of essential oil of thyme, and both show high antioxidant and antimicrobial activity. Biphenyl compounds, dimerization products of thymol and carvacrol, and a flavonoid (eriodicytol) have also been isolated as efficient antioxidants inhibiting superoxide anion production in the xanthine/xanthine oxidase system and mitochondrial and microsomal lipid peroxidation (Haraguchi et al., 1996). Highly methylated flavonoids with antioxidant activity in linoleic acid oxidation system have been found in the less polar fraction (Miura and Nagatani, 1989). Camomile and hop were practically inactive, although their total phenol contents were rather high (12.7 and 23.1 mg/g GAE). Hop even showed moderate pro-oxidant activity in MeLo. Xanthohumol, a prenylated chalcone, is the principal flavonoid in hop, constituting 80−90% of the total flavonoids. Minor amounts of other prenylchalcones and prenylnaringenins have also been reported (Stevens et al., 1997). Of these constituents, xanthohumol and 6-prenylnaringenin have been found to have antifungal activities (Mizobuchi and Sato, 1984). However, the present results do not suggest that hop phenolics would exert significant antioxidant activity in bulk oil.

Medicinal Plants. Bog-rosemary, heather, meadowsweet, and willow herb, the most active medicinal plants in this study, all accumulate large amounts of phenolic compounds. Quantitative data on the phenolics in these medicinal plants appear to be missing, but the main constituents are suggested to be flavonol aglycones, such as quercetin, myricetin, and kaempferol, and their glycosides. Quercetin, quercetin-monopentosides (quaijaverine and avicularine), and quercetin-dipentoside (polifolioside) were isolated in a methanolic extract

of bog-rosemary (Pachaly and Klein, 1987). Kaempferol, quercetin, myricetin, herbacetin, and isocutellarein and their glycosides were identified in heather. In addition, dihydroflavonols (callunin and taxifolin-3-glucoside) and cyanidin 3-glucoside have been identified in heather flowers (Allais et al., 1991, 1995). Several monoglycosides of kaempferol, quercetin, and myricetin have been isolated from willow herb (Ducrey et al., 1995). It also contains a flavonol, glucuronide, that has very strong antiinflammatory properties (Hiermann et al., 1991). Two of the main polyphenolic constituents of meadowsweet are the tannins tellimagrandin II and rugosin D (Haslam, 1996). Spiraeoside, hyperoside, rutoside, quercetin-3-glucuronide, aviculariside, kaempferol-4′-glucoside, and quercetin have been identified in the flowers and flowering tops (Lamaison et al., 1991).

The same discrepancy as discussed with vegetables, namely, the inconsistency between the total phenol content and the dry weight of extract, can be observed also with some members of this subgroup. For example, the dry weight of blue lupin extract was 183 mg, but the total phenolic concentration was only 4.7 mg/g GAE, which suggests that the extract consists mainly of other than phenolic compounds. Differences in total phenolic content and dry weight ratio do not seem to have significant effect on the activity order of these plant extracts at 5000 ppm.

Tree Materials. Silver birch phloem was one of the strongly active tree materials with high phenolic content. The outer bark extract resulted in a very low amount of total phenolics (2.0 mg/g GAE), but still it inhibited the oxidation of MeLo effectively at the 500 ppm concentration. On the contrary, the antioxidative effect was weak when tested at the concentration of 5000 ppm of the original sample, which indicates that the recovery of phenolic extract was low. Phenols of three different types were found in silver birch inner bark (phloem): arylbutanoid glycosides, benzoic acid derivates, and catechins. Suberin and the triterpenes betulin (10−30% of the outer bark), betulinic aldehyde, and acid, lupeol, erythdiol, oleanic acid, and several triterpene caffeates were identified in outer bark of silver birch (Pan, 1995). The latter compounds have previously been shown to have antioxidant (Takagi and Iida, 1980) and antiinflammatory properties (Otsuka et al., 1981)

Main phenolics in white birch and silver birch leaves have been identified by Ossipov et al. (1996). Total phenolic content in the leaves analyzed by HPLC method was 44.7 mg g^{-1} dry mass in white birch and 27.1 mg g^{-1} dry mass in silver birch. Chlorogenic acid constituted almost 50% of the phenolic content in white birch leaves, but in silver birch leaves chlorogenic acid constituted only 5% of the total phenolic content. (+)-Catechin content was 4% in white birch and 6% in silver birch. Several quercetin, kaempferol, and myricetin glycosides were identified of which quercetin-3-*O*-*β*-D-glucuronopyranoside was most abundant in white birch, and myricetin-3-*O*-α-L-(acetyl)-rhamnopyranoside was most abundant in silver birch.

Several flavonoids have been isolated from the needles of Scotch pine and Norway spruce. The flavan-3-ols (+)-catechin and (+)-gallocatechin have been found in both conifer needles. In addition, dimeric and trimeric proanthocyanidins have been reported in pine needles. Dilignols and their glycosides were found in both pine and spruce needles (Lundgren, 1987; Strack et al., 1989). Several glycosides of quercetin, isorhamnetin, and kaempferol have also been found in both species, but the predominant occurrence of dihydroflavonol glucosides is typical in pine needles, dihydroquercetin 3′-O-glucoside being most abundant (2% of the needle dry weight). The acetophenoneglucosides picein and pungenin and the stilbene glucosides astringin and isorhapontin are predominant in spruce needles (Lundgren, 1987). Also coumarins, hydroxybenzoic acids, and hydroxycinnamic acids have been reported in spruce needles (Strack et al., 1989). Isorhapontin was identified as the most efficient fungistatic compound in phenolic extracts of Norway spruce. Especially high isorhapontin concentrations were found in the outer root bark, stem bark, and fine root extracts (Beyer et al., 1993).

Conclusions. It is obvious that the total phenolic content measured by the Folin−Ciocalteu procedure does not give a full picture of the quantity or quality of the phenolic constituents in the extracts. In addition, there may be some interference rising from other chemical components present in the extract, such as sugars or ascorbic acid (Singleton and Rossi, 1965). Similarly it must be noted that the efficiency of antioxidants depends strongly on the oxidation conditions and lipid substrate, and thus the MeLo bulk oil method used in this screening study gives only one approximation of the possibilities of an extract to act as an antioxidant. Yet the results gained by these methods provide simple data that make it possible to classify extracts in respect to their antioxidant potential. As can be observed also from the present data, antioxidant activity does not necessarily correlate with high amounts of phenolics, and that is why both phenolic content and antioxidant activity information must be discussed when evaluating the antioxidant potential of extracts. On the basis of this study, the most potent Finnish plant sources for natural phenolic antioxidants are berries and apples, certain medicinal plants and vegetable peels, and different tree materials. Further work is under way to confirm the antioxidative effect of these promising plant extracts by using other types of lipid models and to characterize the active phenolic antioxidants, their mechanism of action, and their possible interactive antioxidant effects together with other antioxidants in different lipid environments. In addition, more work on the effect of intraspecies variation, i.e., different cultivars and growing conditions, is in progress.

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