Antioxidant Activities of Selected Oriental Herb Extracts

S.Y. Kim[a], J.H. Kim[a], S.K. Kim[a], M.J. Oh[a] and M.Y. Jung[b,*]
[a]Department of Food Science and Technology, Chungnam National University, Daejon, Republic of Korea and [b]Department of Food Science and Technology, Jeonju Woosuk University, Jeonbuk Province 565-800, Republic of Korea

Antioxidant activities of methanol extracts of 180 Oriental herbs were studied by determining the peroxide values of linoleic acid during storage at 50°C. Among the herb extracts tested, 44 species showed strong antioxidant activities on the oxidation of linoleic acid. The antioxidative effects of these 44 selected herb extracts were studied further in a methyl linoleate system during storage for 35 d. Among the 44 species tested, 11 species had particularly high antioxidative effects. The effects of type of extraction solvent (methanol, petroleum ether and ethyl acetate) on the antioxidant activities of the 11 species were studied. Antioxidant activities of most herb extracts were greatly dependent on the extraction solvent used; however, some of the extracts showed strong antioxidant activities regardless of the solvents used for the extraction. Among the 11 herbs selected, based on the antioxidant activity of their methanol extracts, two (i.e., *Psoralea corylifolia* L. and *Sorphora angustifolia* Sieb. & Zucc.) were selected for further study in lard held at 75°C for 7 d. The methanol extracts of *P. corylifolia* L. and *S. angustifolia* Sieb. & Zucc. greatly decreased the peroxide formation of lard during storage. Treatment with 0.20% methanolic extract of *P. corylifolia* L. exhibited significantly stronger antioxidant effect on the oxidation of lard than treatment with 0.02% butylated hydroxyanisole ($P < 0.05$).

KEY WORDS: Antioxidative activity, herb, lard, methanol extract, natural antioxidants, oxidation, *Psoralea corylifolia* L., *Sorphora angustifolia* Sieb & Zucc., storage.

Fats and oils are easily deteriorated by oxidation. To prevent the oxidation of fats and oils, artificial antioxidants are widely used in lipid-containing foods. Consumers, however, are becoming increasingly conscious of the safety of food additives. Extracts of many plants have been reported to have varying degrees of antioxidant activities in lipids. Chipault *et al.* (1) studied 32 spices for antioxidant activity and reported that rosemary and sage were particularly effective. Bracco *et al.* (2) reported that molecular distillation of rosemary derivatives effectively protected foods against oxidative rancidity. Houlihan *et al.* (3) isolated and identified an antioxidant, rosmariquinone, from the leaves of *Rosmarius officinals* L. Farag *et al.* (4) reported that clove oil showed greater antioxidant activity than thyme oil in cottonseed oil. Economou *et al.* (5) reported that oregano (*Origanum vulgare*) extract was the most effective in stabilizing lard at 75°C, followed by thyme (*Thymus vulgaris*), dittany (*O. dictamnus*), marjoram (*O. majorana*) and lavender (*Levandula vera*) extracts, in decreasing order.

In the Orient, certain herbal plants have long been used for retarding the aging process of humans and for preventing and/or curing certain diseases. Some of the clinical effects of the herbs could be, to some extent, related to their possible antioxidant activities. Several studies have evaluated the antioxidant effects of herbal plant extracts on the oxidative stabilities of fats and oils during storage (6–10,11). Zhang *et al.* (12) isolated and identified the effective antioxidants from the herbal plant *Salvia miltorrhiza* Bung. Even though strong antioxidant activities of some plant extracts have been reported, the need for novel natural antioxidants is obvious, and industry continues to look for natural antioxidants. Thus, the objectives of this research were: (i) to find herb extracts that exhibit strong antioxidant properties; (ii) to study the effects of the type of extraction solvent on the antioxidant activities of selected herbs; and (iii) to evaluate the effects of selected herb extracts on the inhibition of lard oxidation during storage.

*To whom correspondence should be addressed at Department of Food Science and Technology, Jeonju Woosuk University, Hoojeong-Ri, Samrae-Up, Jeonbuk Province 565-800, Republic of Korea.

MATERIALS AND METHODS

Materials. One-hundred eighty different kinds of newly dried herbs were purchased from local Oriental herbal stores in Seoul, Korea. The herbal plants and plant parts used in this research are listed in Table 1. Linoleic acid and methyl linoleate were purchased from Sigma Co. (St. Louis, MO). Lard without any additives was obtained from Donbang Oil Co. (Seoul, Korea).

Extraction. The dried herbs were ground to pass a 60-mesh sieve. The ground herbs were transferred into flasks, flushed with nitrogen and stored in a freezer at −18°C before being used. Fifty mL of methanol, ethyl acetate or petroleum ether was added to 5 g ground herb and shaken for 30 min at room temperature. The sample solution was allowed to stand 24 h at 5°C and then was filtered to obtain particle-free herb extract.

Primary screening of antioxidant activities with methanol extracts of herbs. Fifty μL of methanol extract of herb was added to 100 μL linoleic acid. The prepared samples in test tubes (16 mm × 100 mm) with caps were stored, in triplicate, in a water bath at 50°C for 20 h. Peroxide values (PV) of linoleic acid after 20 h of storage were determined by a colorimetric microassay (13). The PV was expressed as absorbance at 560 nm. The control was prepared by adding 50 μL of methanol to 100 μL of linoleic acid. The level of antioxidant activity of an herb was arbitrarily divided into five categories by calculating the ratio of the PV of the sample containing herb extract (PVs) to the PV of the control (PVc) after 20 h storage at 50°C (++++: PVs/PVc < 0.1, +++: 0.1 < PVs/PVc < 0.2, ++: 0.2 < PVs/PVc < 0.4, +: 0.4 < PVs/PVc < 0.7, −: 0.7 < PVs/PVc). The coefficient of variation of the analysis was 4.23%.

Antioxidant activities of 44 selected herb extracts in methyl linoleate emulsions. After the primary screening, methanol extracts were selected of 44 species that showed strong antioxidant activities. The antioxidative effects of the selected herb extracts were further studied by determining peroxide formation during 35 d of storage at 40°C in the dark. Samples were prepared in 125-mL brown bottles with caps by adding 3 mL of methanol extracts of the herbs into a mixture of 37 mL of 0.1 M phosphate buffer (pH 7.0) and 10 mL ethanol containing 0.1 M methyl linoleate. The control was prepared by adding 3 mL methanol into a mixture of 37 mL of 0.1 M phosphate buffer (pH 7.0) and 10 mL ethanol containing 0.1 M methyl linoleate. Samples in bottles were stored in duplicate at

TABLE 1

Antioxidant Activities of Methanol Extracts of Oriental Herbs on the Oxidation of Linoleic Acid During Storage (at 50°C for 20 h)

Number	Herbs	Plant part[a]	Activity[b,c,d]
1	*Acanthopanax spinosum* Miquel (Acanthopanax Bark)	P	+++ (0.14)
2	*A. spissiliorum* Seemenn (Acanthopanax)	R,Co	+++ (0.16)
3	*Achyranthes japonica* Nakai (Achyranthes Root)	R	− (0.95)
4	*Aconitum cilliare* D.C. (Monkshood)	R	++ (0.23)
5	*A. rocyanum* Raymund (Arbor Monkshood)	R	++++ (0.01)
6	*Acorus calamus* L. var. *angustatum* (Drug Sweetflag)	R,C	− (0.80)
7	*A. gramineus* Solander (Grassleaf Sweetflag)	R,C	− (0.80)
8	*Adenophora verticillate* Fisvher (Ladybell)	R	− (1.02)
9	*A. verticillata* var. *typica* (Upright Ladybell)	R	− (0.75)
10	*Akebia quinata* Decaisne (Fiveleaf Akebia)	St	++++ (0.05)
11	*Alisma plantago L. var. angustifolium* (Arrowhead)	L	− (0.98)
12	*A. plantago* L. var. *parifolium* (Alisma rhizome)	R	− (0.85)
13	*Allium odorum* Regel (Scallion)	C	− (0.93)
14	*A. niponicum* Fr. et Sav. (Wild Rocambole)	C	− (0.78)
15	*Allium tuberosum* Rottler (Tuber Onion)	Wp	− (1.02)
16	*Ampelopsis serjaniaefolia* Bunge (Japanese Ampelopsis)	R	− (1.20)
17	*Anemarrhena asphodeloides* Bunge (Anemarrhena Rhizome)	R,C	++ (0.30)
18	*Angelica anomala* Lallem (Eumenol Angelica)	R	− (0.71)
19	*A. cartilaginomarginata* Nakai (Gristlymargin Agenlica)	R	− (0.89)
20	*A. gigas* Nakai (Gigantic angelica)	R	− (0.85)
21	*A. polymorpha* Max. (Polymorphic Angelica)	R	+++ (0.18)
22	*A. pubescens* Max. (Pubescent Angelica)	R	− (0.86)
23	*Anthriscus sylvestris* Hoffman (Woodland Beakchervil)	R	++++ (0.10)
24	*Aralia canescens* Sieb. et Zucc (Aralia, Fatsia)	Co	++ (0.21)
25	*Arctium lappa* L. (Great Burdock)	R,S	++++ (0.10)
26	*Arisaema japonica* Blume (Diversileaf Jackinthepulpit)	C	− (1.07)
27	*Artemisia capillaris* Thumberg (Capillary Wormwood)	Wp	− (0.94)
28	*A. japonica* Thunb (Japanese Wormwood)	Wp	− (0.88)
29	*A. keiskeana* Miquel (Keiske Wormwood)	S,L	− (0.85)
30	*A. lavandulaefolia* D.C. (Lavenderleaf Wormwood)	Wp	− (0.81)
31	*A. vulgaris* L. var. (Mountain Mugwort)	L	− (0.94)
32	*A. vulgaris* L. var. *indica* (Common Mugwort)	L	− (0.77)
33	*Asarum heterotropoides* Maekawa (Manchurian Wildginger)	R	++++ (0.01)
34	*Asparagus cochinchinensis* (Asparagus Radix)	R	− (1.09)
35	*Astragalus membranaceus* Bunge (Membranous Milkuetch)	R	− (1.11)
36	*Atractyloides japonica* Koizumi (Chinese Atractylodes)	R,C	− (0.80)
37	*A. lyrate* S. & Z. (White Atractylodes)	R,C	++++ (0.10)
38	*A. ovata* Thunb. (Atractylodes Rhizoma)	R,C	++++ (0.10)
39	*Betula japonica* Sieb. (Birch)	S	− (0.91)
40	*B. latifolia* Korn. (Birch)	L	− (0.88)
41	*Biota orientalis* Endlicher (Platycladus Orientalis)	Fr	− (0.95)
42	*Boschniakia glabra* C.A. Mey (Boschniakia)	Wp	++ (0.39)
43	*Brassica alba* L. (White Mustard Seed)	S	++++ (0.01)
44	*Broussonetia kozinoki* Sieb. (Kazinoki Papermulberry)	S	++++ (0.03)
45	*B. papyrifera* Ventenat (Common Papermulberry)	S	+++ (0.18)
46	*Bupleurum falcatum* L. (Throwax)	R	− (1.02)
47	*Cannabis sativus* L. (Hemp Fimble)	St	++++ (0.06)
48	*Carthamus tinctorius* L. (Safflower)	Fl	− (0.80)
49	*Cassia tora* L. (Sickle Senna)	S	− (1.00)
50	*Celosia argenta* L. (Feather Cockscomb)	S	− (0.84)
51	*Chelidoniuim majus* L. var. *Asiaticum* (Celandine)	Wp	++++ (0.01)
52	*Chrysanthemum indicum* L. (Chrysanthemum)	Fl,C	++++ (0.06)
53	*Cimicifuga heracleifolia* Komarov (Largetrifolioliou Bugband)	R,L	++++ (0.00)
54	*Cnidium japonicum* Miq. (Japanese Cnidium)	S	− (1.05)
55	*C. officinale* Makino (Cnidium Rhizome)	At	++++ (0.03)
56	*Cocculus diversifolium* (Snailseed)	R	+ (0.60)
57	*C. trilobus* DC (Japanese Snailseed)	R	− (0.70)
58	*Coix lachryma-jobi* var. Ma-yuen (Jbstears)	S	− (0.89)
59	*Coptis japonica* Makino (Japanese Goldthread)	R,C	++++ (0.00)
60	*Cornus officinale* Nakai (Dogwood)	Fr	− (0.85)
61	*Corydalis ternata* Nakai (Corydalis Tuber)	R	+ (0.61)

(continued)

TABLE 1 *(continued)*

Number	Herbs	Plant part[a]	Activity[b,c,d]
62	*Crataegus curneata* Sieb. et Zucc. (Hawthorn)	Fr	++++ (0.09)
63	*Cuscuta chinensis* Lam (Chinese Dodder)	S	− (1.00)
64	*C. japonica* Choicy (Japanese Dodder)	S	++++ (0.02)
65	*Cynanchum wilfordii* Max. (Wilford Swallowwort)	R	+ (0.70)
66	*Cyperus rotundus* L. (Nutgrass Galingale)	R	− (1.01)
67	*Dendrobium moniliforme* Swartz (Moniliform Dendrobium)	Wp	++ (0.21)
68	*Dianthus chinensis* L. (Rainbow Pink)	S	+++ (0.13)
69	*Dioscorea batatas* Decaisne (Dioscorea Rhizom)	At	− (0.91)
70	*Draba nemorosa* L. var. *hebecarpa* (Woolly Draba)	S	++ (0.38)
71	*Elscholtzia cristata* Willd (Elsholtzia)	Wp	− (0.94)
72	*Epimedium koreanum* Nakai (Koreanum Epimedium)	St,L	++++ (0.01)
73	*Equisetum arvense* L. var. boreale (Field Horetail)	Wp	− (1.00)
74	*E. Hyemale* L. var. *japonica* (Shavegrass)	Wp	+ (0.49)
75	*Erythronium japanicum* Makino (Fawnlily)	R	− (0.81)
76	*Eucommia ulmoides* Oliver (Eucommia)	Co	− (1.02)
77	*Evodia rutaecarpa* Benth. (Evoida Fruit)	S	++++ (0.07)
78	*Foeniculum vulgaris* Miler (Fennel)	Fr,L	− (0.96)
79	*Fritillaria ussuriensis* Max. (Ussuri Fritillary)	R,C	− (1.12)
80	*Garadabthus chinensis* Lemann (Blackberrylily)	R,C	− (0.94)
81	*Gardenia jasminoides* Ellis (Cape Jasmine)	Fr	++ (0.36)
82	*Gastrodia elata* Blume (Tall Gastrodia)	R,C	− (1.00)
83	*Gentiana scabra* Bunge var. *Buergeri* (Rough Gentian)	R	− (0.79)
84	*Ginko biloba* L. (Maiden Hairtree, Ginko)	S	− (0.85)
85	*Glycyrrhiza glabra* L. (Licorice)	R	++++ (0.09)
86	*Gossypium indicum* Lam. (Cotton)	S	++++ (0.01)
87	*Imperata arundiriaca* Cylindrica (Lalang Grass)	R	− (0.91)
88	*Inula britanica* L. var. *chinensis* (British Inula)	Fl,St	++ (0.25)
89	*Inula helenium* L. (Elecampane Inula)	R	+ (0.67)
90	*Juniperus chinensis* L. (Sabina Chinesis)	L	++ (0.36)
91	*Kadsura japonica* Dunal (Japanese Kadsura)	Fr	+ (0.67)
92	*Leonurus sibiricus* L. (Siberian Motherwort)	Wp	− (0.79)
93	*Liriope platyphylla* Wang et Tang (Broadleaf Liriope)	R	− (1.09)
94	*Lithospermum officinale* L. (Common Gromwell)	R	++++ (0.00)
95	*Lonicera japonica* Thumb (Japanese honeysuckle)	Fl,L	+ (0.64)
96	*Lycium chinense* Miller (Chinese Wolfberry)	Fr	− (0.85)
97	*Lycopus coreanus* Levi (Shiny Buleweed)	Wp	− (0.89)
98	*Machilus rimosa* var. *thumbergii* (Machili cortex)	Co	++++ (0.02)
99	*Magnolia kobus* A.P. DE candolle (Kobus Magnolia)	Fl	++++ (0.01)
100	*M. liliflora* Desr. (Lily Magnolia)	Fl	+++ (0.18)
101	*Melandrium firmum* (Sieb. et Zucc) Rohroach (Hard Melandrium)	Wp	+ (0.60)
102	*Maximowiczia chinensis* var. *typica* (Schizandra Fruit)	S	+ (0.67)
103	*M. nigra* (Black Schizandra Fruit)	S	+ (0.65)
104	*Mentha canadensis* Briquet (Mint)	St	− (0.86)
105	*Morus alba* L (White Mulberry)	L	++++ (0.02)
106	*M. bombycis* Koitzumi (Mulberry Bark)	Co	++++ (0.01)
107	*Nelumbo nucifera* Gaertner (Nelumbo Fruit)	Fr	++++ (0.01)
108	*N. nucifera* Gaertner (Nelumbo Semen)	S	++++ (0.08)
109	*Nepeta cataria* L. (Japanese Peppermint)	St	− (0.94)
110	*Nothosmyrnium japonicum* Miquel (Japanese Nothosmyrnium)	R,C	++ (0.20)
111	*Oenanthe stolonifera* D.C. (Waterdropwort)	St	− (0.82)
112	*Ophiopoon jaonicus* Ker. (Dwarf Lilyturf)	R	− (0.92)
113	*Osmunda regalis* Thunberg (Osmund, Royal Fern)	R,C	+ (0.54)
114	*Paeonia albiflora* var. *hirta* Regel (Paeony Root)	R	− (1.09)
115	*Palupara cordata* Busk (Chinese Lizardtail)	Wp	++++ (0.01)
116	*Perilla fritescaus* Brith var. *crispa* (Perilla)	S	− (0.82)
117	*Peucedanum decurcivum* Maxi. (Common Hogfennel)	R	+++ (0.12)
118	*Phelloterus Littoralis* Benthan (Ledebouriella Root)	R	− (0.96)
119	*Phlomis umbrosa* Turz. (Shady Jerusalemsage)	R,C	− (0.89)
120	*Pinellia ternata* Breitenbach (Ternate Pinellia)	R	− (0.89)
121	*Pinus densiflora* Sieb. et Zucc. (Japanese Rad Pine)	Re	− (0.82)
122	*Plantago major* L. var. *asiatica* (Rippleseed Plantin)	Wp	− (0.82)
123	*Platycodon glaucum* Nakai (Balloonflower)	R	− (0.88)
124	*P. grandiflorum* A. De Candolle (Ballonflower)	R	− (1.65)
125	*Polygala japonica* Houtt (Japanese milkwort)	St	+ (0.58)

(continued)

TABLE 1 ***(continued)***

Number	Herbs	Plant part[a]	Activity[b,c,d]
126	*P. tenuifolia* Willdenow (Thinleaf Milkwort)	R	+ (0.46)
127	*P. falcatum* A. Gray (Solomonseal)	R,C	− (1.03)
128	*P. japonica* (Fragrant Solomoneal)	R,C	− (1.08)
129	*Polygonum aviculare* L. (Common Knotgrass)	Wp	++ (0.36)
130	*P. multiflorium* Thunberg (Tuber Fleeceflower)	R	++++ (0.00)
131	*Poncirus trifoliata* Rafiiensque (Trifoliate Orange)	Fr	− (0.90)
132	*Prunus armeniaca* L. var. *ansu* Maxi. (Ansu Apricot)	Fr	− (0.86)
133	*P. buergeriana* Miquel (Apricot)	R	+ (0.54)
134	*P. mume* Sieb. et Zucc. (Mumeplant)	Fl	++++ (0.01)
135	*P. persica* var. vulgaris Persicae Semen)	Fr	− (0.83)
136	*Pseudocydonia sinensis* Schneider. (Floweringquince)	Fr	++++ (0.04)
137	*Psoralea corylifolia* L. (Malaytea Scurfpea)	S	++++ (0.07)
138	*Pueraria hirsuta* Matsum (Pueraria Root)	R	+ (0.51)
139	*Punica granatum* L. (Pomegranate Bark)	Fr	+++ (0.12)
140	*Quercus acutissima* Carcuthers (Sawtooth Oak)	Fr	− (0.83)
141	*Q. acutissima* Carcuthers (Acorn)	Fr	− (0.81)
142	*Raphanus sativus* L. (Garden Radish)	R,S	++++ (0.02)
143	*Rehmannia glutinosa* Libosehitz (Dry Adhesive Rehmannia)	R	− (0.76)
144	*R. glutinosa* Libosehitz (Raw Adhesive Rehmannia)	R	− (0.87)
145	*Rehmannia lutea* Max. (Rehmannia)	Co,L	− (0.95)
146	*Rheum palmatum* L. (Docken)	R,C	++++ (0.00)
147	*R. undelatum* L. (Rhubarb)	R	+++ (0.11)
148	*Ricinus communis* L. (Castor bean)	S	++++ (0.02)
149	*Rubus idaeus* L. (Red Raspberry)	Fr	− (0.93)
150	*Salvia miltiorrhiza Bunge (Dan-Shen)*	R	++ (0.23)
151	*S. officinalis* L. (Salvia)	L	− (0.98)
152	*Sanguisorba officinalis* var. *coreana* Garden Burnet)	St	++++ (0.01)
153	*Schizandra chinensis* var. *glabrata* (Chinese Magnoline)	S	+ (0.60)
154	*Schizonepeta tenuifolia* Briquet (Fineleaf Schizonepeta)	C	− (0.98)
155	*Scrophularia buergeriana* Miquel (Buerger Figwort)	Co,L	− (0.77)
156	*Scutellaria baicalensis* George (Baikal Skullcap)	R	++++ (0.02)
157	*Selinum monniei* L. (Milk Parsely)	Fr	− (1.26)
158	*Siler divaricatum* Bentham et hooker (Saposhinkovia Root)	R	− (0.80)
159	*Smilax china* L. (Chinaroot Greenbrier)	R,C	− (0.95)
160	*S. china* L. (Red Hoelen)	R,C	− (1.21)
161	*S. glabra* Roxb. (Glabrous Greenbrier)	R,C	− (1.03)
162	*Sophora angustifolia* Sieb. & Zucc. (Sophora Root)	R	++++ (0.01)
163	*S. jkaponica* L. (Japanese Burnet)	Fl	++++ (0.01)
164	*Spilodela polyrhiza* L. (Duckweed)	Wp	− (0.92)
165	*Syringa dilata* Nakai (Clove)	Co	++++ (0.01)
166	*Taraxacum piatycaripumh* (Dadelion)	Wp	+ (0.62)
167	*Teucrium japonicum* Houtt (Japanese Germander)	Wp	− (0.80)
168	*Thesium chinese* Turc. (Chinese Bastardtoadflox)	Wp	++++ (0.01)
169	*Torilis anthriscus* Guel (Hedgeparsley)	Fr	− (1.26)
170	*T. scabra* D.C. (Common Hedgeparsley)	Fr	− (1.08)
171	*Tribulus terrestris* L. (Puncturevine Caltrap)	R	+ (0.56)
172	*Trichosanthes kirillowii* Maxim (Mongolian Snakegourd)	R	− (1.01)
173	*T. gradriciana* Miquel (Snakegourd)	R	− (0.86)
174	*Ulmus japonica* Sarg (Elm)	Co	++++ (0.06)
175	*Vaccaria vulgaris* Host (cowherb)	S	+ (0.65)
176	*Vitex rotundifolia L.* (Chastetree)	Fr	++++ (0.06)
177	*Vitis amureniisis* Rupr (Amur Grape)	S	− (0.87)
178	*Xanthium strumarium* L. (Cocklebur)	S	+ (0.68)
179	*Zanthoxylum piperitum* D.C. (Pricklyash)	S	++ (0.35)
180	*Zizphus jujuba* Miller (Jujube)	Fr	− (0.81)

[a]Plant parts used are indicated as follows: At, aerial tuber; C, caulis; Co, cortex; Fl, flower; Fr, fruit; L, leaf; P, pericarp; R, root; Re, resin; S, seed; St, stem; Wp, whole plant.

[b]The level of antioxidant activity of herb was arbitrarily divided into five categories by calculating the ratio of peroxide value (PV) of sample containing herb extracts (PVs) to PV of control (PVc) after 20-h storage at 50°C (++++: PVs/PVc < 0.1; +++: 0.1 < PVs/PVc < 0.1; +++: 0.1 < PVs/PVc < 0.2; ++: 0.2 < PVs/PVc < 0.4; +: 0.4 < PVs/PVc < 0.7; −; 0.7 < PVs/PVc.

[c]PVs/PVc (ratios of PVs to PVc) are shown in parentheses beside the + and − categorization. For the calculation of PVs/PVc, mean values of triplicate measurements were used.

[d]The coefficient of variation of the analysis was 4.23%.

40°C for 35 d in the dark. PVs were determined every 5 d by the colorimetric microassay (13).

Effects of extraction solvents on the antioxidant activities of herb extracts. Eleven kinds of herbs, for which methanol extracts showed the strongest antioxidant activities, were extracted separately with methanol, petroleum ether and ethyl acetate, and the antioxidant activities of these herb extracts were studied. Samples were prepared in 125-mL brown bottles with caps by adding 3 mL of herb extracts into a mixture of 37 mL of 0.1 M phosphate buffer (pH 7.0) and 10 mL ethanol containing 0.1 M methyl linoleate. The control was prepared by adding 3 mL methanol into a mixture of 37 mL of 0.1 M phosphate buffer (pH 7.0) and 10 mL ethanol containing 0.1 M methyl linoleate. The samples in bottles were stored, in duplicate, at 40°C for 35 d in the dark. PV of methyl linoleate were determined by the colorimetric microassay (13).

Application of S. angustifolia *Sieb & Zucc. and* P. corylfolia *L. extracts to lard.* Among the 11 herbs selected based on the antioxidant activity of their methanol extracts, two (i.e., *S. angustifolia* Sieb & Zucc. and *P. corylifolia* L.) were selected for further study in lard because of their easy accessibility, low price and nontoxicity. Methanol extracts of *S. angustifolia* Sieb & Zucc. and *P. corylifolia* L. were evaporated at 40°C in a rotatory vacuum evaporator to remove the methanol. To study the antioxidant activities of these herb extracts on the oxidation of lard, 0, 0.01, 0.02, 0.05, 0.10 and 0.20% (w/w) of the extracts on a dry weight basis were added to lard. Lard without any additives was used as a control. Lard treated with 0.02% BHA was used as a positive control. Fifty grams of the prepared sample was transferred into a 100-mL beaker. Samples were stored, in duplicate, in a forced-draft air-oven at 75°C for 7 d, and the oxidative deterioration of lard was studied by determining PVs according to the American Oil Chemists' Society (AOCS) Official Method Cd 8-53 (14). Because larger samples were used, the AOCS method requiring 3 to 5 g of oil, could be used.

Determination of tocopherols by high-performance liquid chromatography. To determine the tocopherol contents in methanol extracts of *S. angustifolia* Sieb & Zucc. and *P. corylifolia* L., the herb extracts were redissolved in diethyl ether, and the solution was concentrated under vacuum at 40°C. The samples were then transferred to Erlenmeyer flasks. Thirty mL of ethanol, 3 mL of 20 N-KOH solution and 3 mL of pyrogallol/ethanol (1:9, vol/vol) were added to the sample solution. The sample was saponified in a boiling water bath for 30 min. The sample was rapidly cooled in running cold water and then transferred to a separatory funnel. Forty mL of water and 30 mL petroleum ether were added to the separatory funnel, and the sample was shaken vigorously. After separation of the two phases, the petroleum layer was taken. This extraction step was repeated two more times with 30 mL petroleum ether. The sample was then washed with water, and Na_2SO_4 was added to remove any trace of water. The sample was then filtered and evaporated until dry and then redissolved in 1 mL *n*-hexane. Tocopherol contents were determined with a high-performance liquid chromatograph (model 244; Waters Associates, Milford, MA) equipped with an ultraviolet detector. The column used was μBondapak™ NH_2 (10 μm, 300 × 3.9 mm i.d.). The eluting solvent was *n*-hexane/methanol (99.5:0.5, vol/vol) at a flow rate of 1.7 mL/min. Tocopherol components were quantitated at 280 nm.

Statistical analysis. All experiments were carried out in duplicate or in triplicate. Statistical analysis was accomplished with Statistical Analysis System (15) software. Duncan's multiple range test or coefficient of variation of analysis was used to ascertain qualitative or quantitative effects of herb extracts on the oxidation of samples (15,16).

RESULTS AND DISCUSSION

Primary screening of antioxidant activities with methanol extracts of herbs. The effects of methanol extracts of 180 herbs on the oxidation of linoleic acid during 20 h storage at 50°C are shown in Table 1. Among the 180 different kinds of herb extracts tested, eighty-six species showed antioxidative properties in stabilizing linoleic acid during storage (as shown in Table 1). Several species (44 total) showed strong antioxidative effects (++++) on the oxidation of linoleic acid. These results clearly indicated that some of the tested herbs were rich in natural antioxidants, and that the qualities and/or quantities of the antioxidants in methanol extracts of the herbal plants seemed to be very different depending on the kinds of herbs. The antioxidative activities of most of the herb extracts tested in this research have not been reported in previous literature. But the results are similar to previous tests in which some herb extracts are found to be rich in antioxidants. Hirosue *et al.* (6) studied 24 herb extracts for antioxidant activities and found four ethanolic herbal extracts with strong antioxidant activities. Su *et al.* (10) studied the antioxidant activities of methanol extracts of 195 herbal plants and reported that about one-half showed antioxidant activities. Hirosue *et al.* (7) reported that acetone extract of *Glycyrrhiza glabra* L. showed strong antioxidant activity. Our results also showed that the methanol extract of *G. glabra* L. (No. 85) was highly effective in retarding the oxidation of linoleic acid during storage (Table 1). Toda *et al.* (8) reported that 20 species among the methanol extracts of 107 herb extracts tested showed strong antioxidant activities on the oxidation of linoleic acid. Zhang *et al.* (12) reported that the diethyl ether extract of *Salvia miltiorrhiza* Bung had strong antioxidant activity, and that the antioxidants of the herb extract were quinone-type compounds. In the present study, the methanolic extract of *S. miltiorrhiza* Bung (No. 150) showed relatively strong antioxidative activity.

Antioxidant activities of 44 selected herb extracts in methyl linoleate emulsions. Because methanol extracts of 44 species showed strong antioxidant activities on the oxidation of linoleic acid, the antioxidant activities of the methanol extracts of these 44 species were studied further in a methyl linoleate system for an extended storage time. The results for the 44 selected herbs on the oxidation of methyl linoleate during 35-d storage at 40°C are shown in Table 2. The PV of samples were determined by a colorimetric microassay and expressed as absorbance at 560 nm. The control, which contained no herb extract, reached a PV of 1.16 (absorbance at 560 nm) after only 10 d of storage at 40°C. However, all the samples containing methanol extracts of herbs developed PV of less than 0.8 (absorbance at 560 nm) after 10 d of storage at 40°C, indicating strong antioxidant activities. These results

TABLE 2

Effects of Methanol Extracts of Selected Herbs on the Oxidation of Methyl Linoleate During 35-d Dark Storage at 40°C

Herb[a] number	Peroxide value (absorbance at 560 nm)[b-d]						
	5 d	10 d	15 d	20 d	25 d	30 d	35 d
Control	0.34[a]	1.16[a]	—	—	—	—	—
5	0.02[ijk]	0.03[mnopqr]	0.05[pqr]	0.07[stu]	0.07[u]	0.07[x]	0.08[v]
10	0.10[d]	0.79[b]	1.19[a]	—	—	—	—
23	0.01[nopqr]	0.04[m]	0.22[i]	0.40[g]	0.61[g]	0.87[e]	1.13[d]
25	0.12[c]	0.42[d]	0.72[c]	1.12[b]	—	—	—
33	0.01[mnopq]	0.02[tu]	0.03[vw]	0.03[x]	0.03[wx]	0.04[yz]	0.04[wx]
37	0.01[opqrst]	0.03[nopqrst]	0.07[no]	0.07[stu]	0.18[n]	0.46[o]	1.18[b]
38	0.01[nopqr]	0.03[opqrstu]	0.08[mn]	0.15[o]	0.46[h]	0.82[g]	1.25[a]
43	0.01[mnopq]	0.09[j]	0.32[f]	0.70[d]	0.89[c]	1.16[b]	—
44	0.02[klm]	0.03[opqrst]	0.23[hi]	0.80[c]	1.16[b]	—	—
47	0.06[f]	0.08[k]	0.13[l]	0.26[k]	0.35[j]	0.41[p]	0.52[o]
51	0.00[tu]	0.02[rstu]	0.03[vw]	0.03[x]	0.03[wx]	0.03[za]	0.03[wx]
52	0.02[jkl]	0.08[jk]	0.12[m]	0.22[m]	0.31[k]	0.69[i]	0.92[h]
53	0.09[nopqrs]	0.03[opqrstu]	0.04[stu]	0.07[tu]	0.23[l]	0.68[j]	0.79[j]
55	0.02[jkl]	0.06[l]	0.17[j]	0.35[j]	0.77[d]	1.19[a]	—
59	0.00[rstu]	0.02[qrstu]	0.04[stu]	0.09[qr]	0.45[h]	0.57[l]	1.09[e]
62	0.01[lmnop]	0.06[l]	0.06[op]	0.09[r]	0.13[p]	0.32[q]	0.57[n]
64	0.01[lmno]	0.04[mnop]	0.08[m]	0.09[r]	0.10[s]	0.17[u]	0.46[q]
72	0.00[tu]	0.02[tu]	0.03[vw]	0.03[wx]	0.03[x]	0.03[a*]	0.03[x]
77	0.01[qrstu]	0.04[mno]	0.07[no]	0.10[q]	0.15[o]	024[s]	0.30[s]
85	0.03[i]	0.06[l]	0.07[no]	0.07[stu]	0.07[u]	0.07[x]	0.07[v]
86	0.01[opqrstu]	0.03[qprstu]	0.04[tuvw]	0.02[n]	0.35[j]	0.57[l]	0.84[i]
94	0.02[ijk]	0.27[e]	0.57[d]	1.18[a]	—	—	—
98	0.41[g]	0.11[i]	0.12[l]	0.12[p]	0.14[p]	0.16[u]	0.17[t]
99	0.00[stu]	0.04[mno]	0.06[pq]	0.08[s]	0.10[rs]	0.62[k]	0.69[k]
105	0.02[ij]	0.14[g]	0.34[e]	0.49[f]	0.67[e]	0.79[h]	0.99[g]
106	0.01[nopqr]	0.02[u]	0.02[w]	0.05[v]	0.06[v]	0.32[q]	1.06[f]
107	0.02[klmn]	0.04[m]	0.07[no]	0.12[p]	0.21[m]	0.31[q]	0.51[p]
108	0.02[klmn]	0.06[l]	0.07[no]	0.09[qr]	0.11[rs]	0.14[v]	0.64[l]
115	0.02[klmn]	0.03[opqrst]	0.03[uvw]	0.03[wx]	0.03[wx]	0.04[yza*]	0.04[wx]
130	0.26[b]	0.68[c]	1.10[b]	—	—	—	—
134	0.02[jkl]	0.05[l]	0.06[op]	0.07[st]	0.08[tu]	0.08[x]	0.08[v]
136	0.00[rstu]	0.03[mnopqrs]	0.05[pqr]	0.17[n]	0.43[i]	0.98[d]	1.16[c]
137	0.01[qrstu]	0.03[mnopqr]	0.04[tuvw]	0.04[vw]	0.04[w]	0.05[y]	0.05[w]
142	0.01[nopqr]	0.02[u]	0.07[nop]	0.10[q]	0.12[q]	0.21[t]	0.51[p]
146	0.02[ij]	0.13[h]	0.25[g]	0.31[i]	0.35[j]	0.47[n]	0.52[op]
148	0.01[mnopq]	0.04[mn]	0.04[rst]	0.06[u]	0.11[r]	0.29[r]	0.37[r]
152	0.07[e]	0.15[f]	0.25[g]	0.28[j]	0.34[j]	0.48[n]	0.61[m]
156	0.00[u]	0.04[mnopq]	0.05[qrs]	0.07[stu]	0.09[t]	0.08[x]	0.09[v]
162	0.00[u]	0.02[stu]	0.04[tuv]	0.04[vw]	0.04[w]	0.04[yz]	0.10[u]
163	0.02[ij]	0l.09[jk]	0.12[l]	0.23[l]	0.68[e]	1.12[c]	—
165	0.03[h]	0.06[l]	0.08[m]	0.10[q]	0.10[rs]	0.11[w]	0.11[u]
168	0.00[rstu]	0.04[mnopq]	0.15[k]	0.35[h]	0.43[i]	0.54[m]	0.65[l]
174	0.10[d]	0.15[f]	0.23[h]	0.32[i]	0.66[f]	0.84[f]	0.99[g]
176	0.00[rstu]	0.06[l]	0.12[l]	0.65[e]	1.19[a]	—	—

[a]Herb numbers: refer to Table 1 for the scientific and common names of herbs.
[b]Peroxide values were determined by a colorimetric microassay and expressed as absorbances at 560 nm.
[c]Peroxide value of all the samples at 0-d storage was 0.
[d]Means within each column with the same superscript nonitalized letters are not significantly different at $P < 0.05$; a and a* are used as different superscript letters.

were consistent with the results in Table 1. Methanol extracts of 11 of the herbs among the selected 44 herbs showed especially strong antioxidative effects on the oxidation of methyl linoleate during storage. PVs of the treatments of methanol extracts of herbs 5, 33, 51, 72, 85, 115, 134, 137, 156, 162 and 165 were significantly lower than those of other treatments ($P < 0.05$). PVs of treatments of methanolic extracts of the 11 herbs were less than 0.11 (absorbance at 560 nm), even after 35 d of storage at 40°C. These results strongly suggest that methanol extracts of these 11 herbs contain powerful antioxidative components. Phenolic acids and/or flavonoids might be the possible antioxidative components because methanol is a good solvent for extracting these types of components from plant materials. Positive identification, however, is needed to confirm this idea.

Effects of extracting solvents on the antioxidant activities of herb extracts. Figures 1 and 2 show the effects of ethyl acetate and petroleum ether extracts of the 11 selected herbs on the peroxide development of methyl linoleate during storage. These studies were done under the same conditions in Table 2. The antioxidant activities of some herb extracts were greatly dependent on the kinds of solvents used for the extraction. This might be due to

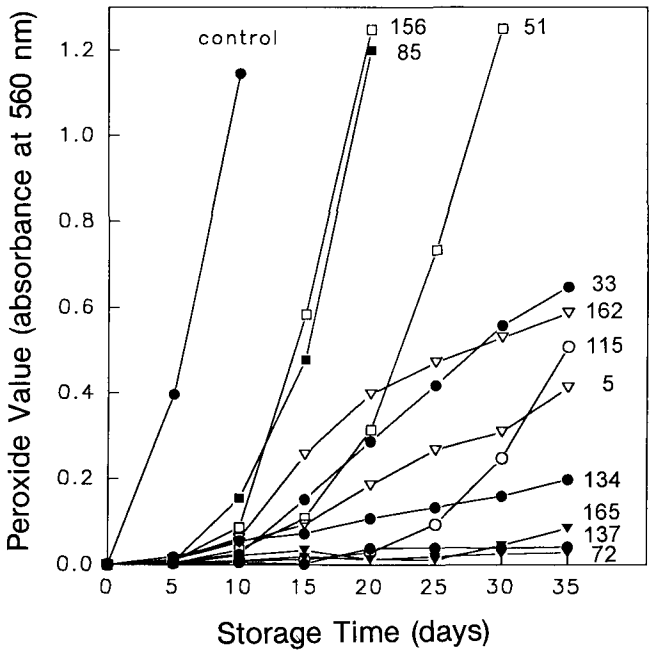

FIG. 1. Effects of ethyl acetate extracts of herbs on the oxidation of methyl linoleate during storage at 40°C. The number beside each line represents herb number. For the scientific and common names of herbs, refer to Table 1.

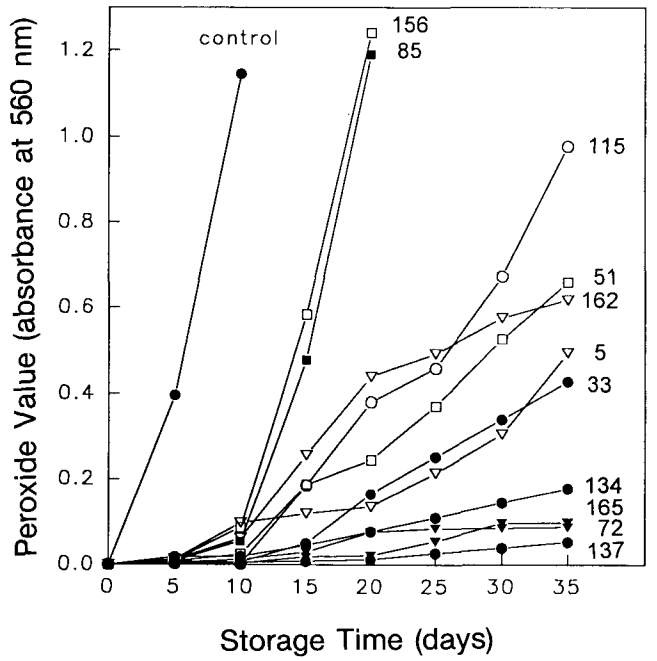

FIG. 2. Effects of petroleum ether extracts of herbs on the oxidation of methyl linoleate during storage at 40°C. The number beside each line represents herb number. For the scientific and common names of herbs, refer to Table 1.

the differences in solubility of antioxidant components of herbs in solvents. For example, the ethyl acetate and petroleum ether extracts of *Scutellaria baicalensis* George (No. 156) and *G. glabra* L. (No. 85) showed weak antioxidant activities, even though methanol extracts of these herbs showed strong antioxidant activities. In this study, the PV of the treatments of methanol, ethyl acetate and petroleum ether extracts of *S. baicalensis* George (No. 156) after 20-d storage at 40°C were 0.07, 1.25 and 1.24 (absorbance at 560 nm), respectively, as shown in Table 2 and in Figures 1 and 2. The PV of the treatments of methanol, ethyl acetate and petroleum ether extracts of *G. glabra* L. (No. 85) after 20-d storage at 40°C were 0.07, 1.20 and 1.19 (absorbance at 560 nm), respectively (Table 2 and Figs. 1 and 2). Therefore, the qualities and/or quantities of antioxidant(s) extracted from herbs depend(s) greatly on the kinds of extraction solvents used. Hirosue *et al.* (6) and Su *et al.* (10) also reported that the antioxidant activities of herbs are greatly dependent on the kind of solvent. In the present study, however, *Epimedium koreanum* Nakai (No. 72), *Prunus mume* Sieb. & Zucc. (No. 134), *P. corylifolia* L. (No. 137) and *Syringa dilata* Nakai (No. 165) extracts showed significantly strong antioxidant activities regardless of the solvents used for the extraction, as shown in Table 2 and Figures 1 and 2 ($P < 0.05$).

Effects of methanol extracts of S. angustifolia *Sieb. & Zucc. and* P. corylifolia *L. on lard oxidation.* Among the 11 herbs selected, based on the antioxidant activity of their methanol extracts, two [i.e. *S. angustifolia* Sieb. & Zucc. (No. 162) and *P. corylifolia* L. (No. 137)] were selected for further study in lard held at 75°C for 7 d. The selection of the two species was based on their easy accessibility, low price and nontoxicity. Figure 3 shows the effects of methanol extract of *S. angustifolia* Sieb. & Zucc. on the peroxide formation of lard during 7-d storage at 75°C. As the storage days increased, PV of lard increased from 0 to 192 meq/kg oil after 7-d storage. As the concentrations of methanol extracts of *S. angustifolia* Sieb. & Zucc. increased from 0 to 0.20%, peroxide formation of lard during storage decreased significantly ($P < 0.05$). The PV of lard containing 0.01, 0.02, 0.05, 0.10 and 0.20% methanol extracts of *S. angustifolia* Sieb. & Zucc. after 7 d of storage at 75°C were 178, 162, 135, 118 and 90 meq/kg oil, respectively. These results clearly showed that the methanol extract of *S. angustifolia* Sieb & Zucc. contained antioxidant activity and that the level of activity was concentration-dependent. However, antioxidant effect of 0.20% of the extract was significantly lower than that of 0.02% butylated hydroxyanisole (BHA) ($P < 0.05$).

Figure 4 shows the effects of adding a methanol extract of *P. corylifolia* L. to lard on the peroxide formation during 7 d of storage at 75°C. As the storage days increased, PVs of the control increased greatly. The addition of *P. corylifolia* L. extract reduced the formation of peroxides during storage. As the concentration of the extracts increased, the inhibitory effects on the peroxide formation increased considerably. After 7-d storage, lard containing no extracts had a PV of 192 meq/kg oil. However, the lard containing 0.01, 0.02, 0.05, 0.10 and 0.20% *P. corylifolia* L. extract after 7-d storage at 75°C had PVs of 166, 117, 83, 32 and 7.5 meq/kg oil, respectively. The treatment containing 0.02% BHA had a PV of 14.6 meq/kg oil after 7-d storage, which was significantly higher than that containing 0.20% *P. corylifolia* L. methanolic extract but lower than that containing 0.10% of the extract ($P < 0.05$). After 6 d of storage, however, the PV of the treatment with 0.10% of the extract was not significantly different from that of the treatment with the 0.02% BHA, and the PV

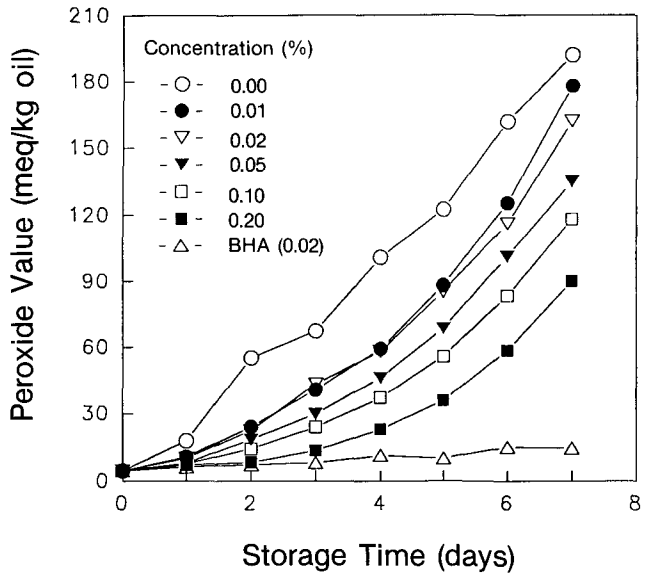

FIG. 3. Antioxidative effects of methanol extract of *Sorphora angustifolia* Sieb. & Zucc. on the oxidation of lard during 7-d storage at 75°C. BHA, butylated hydroxyanisole.

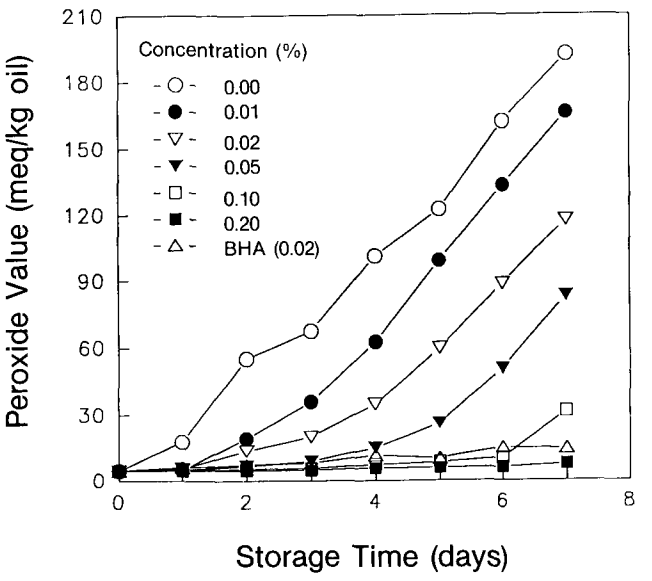

FIG. 4. Antioxidative effects of methanol extract of *Psoralea corylifolia* L. on the oxidation of lard during 7-d storage at 75°C. BHA, butylated hydroxyanisole.

of the treatment with 0.20% of the extract was significantly lower than that with 0.02% BHA ($P < 0.05$). That is, 0.20% methanol extract of *P. corylifolia* L. exhibited stronger antioxidant effects than did 0.02% BHA. The results in this research strongly suggest that the methanol extract of *P. corylifolia* L. can be successfully applied to lard to prevent oxidative deterioration.

Methanol extracts from 100 g dried *P. corylifolia* L. contained higher contents of tocopherol than did *S. angustifolia* Sieb. & Zucc.. The methanol extract from 100 g dried *S. angustifolia* Sieb. & Zucc. contained 18.3 mg total tocopherol with 5.5 mg α-, 1.1 mg β-, 11.0 mg γ- and 0.7 mg δ-tocopherol. The methanol extract from 100 g dried *P. corylifolia* L. contained 67.9 mg total tocopherol with 37.8 mg α-, 16.0 mg γ-, and 13.9 mg δ-tocopherol. No β-tocopherol was detected. The higher contents of tocopherols in the methanol extract of *P. corylifolia* L. might be related, to some extent, to the higher antioxidative activity of the extract. Further research, however, is needed to isolate and identify other possible antioxidative components in the herb extracts. Nevertheless, the data presented provide important information for the discovery of new natural antioxidants from herbs or for new sources of known antioxidants.

REFERENCES

1. Chipault, J.R., G.R. Mizuno, J.M. Hawkins and W.O. Lundberg, *Food Res. 17*:46 (1952).
2. Bracco, U., J. Lölinger and J.L. Viret, *J. Am. Oil Chem. Soc. 58*:686 (1981).
3. Houlihan, C.M., C. Ho and S.S. Chang, *Ibid. 62*:96 (1985).
4. Farag, R.S., A.Z.M.A. Badei and G.S.A. El Baroty, *Ibid. 66*:800 (1989).
5. Economou, K.D., V. Oreopoulou and C.P. Thomopoulos, *Ibid. 68*:109 (1991).
6. Hirosue, T., H. Kawai and Y. Hosogai, *Nippon Shokuhin Kogyo Gakkaishi 25*:691 (1978).
7. Hirosue, T., H. Kawai and Y. Hosoai, *Ibid. 29*:418 (1982).
8. Toda, S., H. Tanizawa, S. Arichi and Y. Takino, *Yakugaku Zassi 104*:394 (1984).
9. Toda, S., S. Arichi, H. Tanizawa and Y. Takino, *Chem. Pharm. Bull. 33*:1270 (1985).
10. Su, J., T. Osawa and M. Namiki, *Agric. Bio. Chem. 50*:199 (1987).
11. Lee, Y.B., Y.S. Kim and C.R. Ashmore, *J. Food Sci. 51*:20 (1986).
12. Zhang, K., Y. Bao, P. Wu, R.T. Rosen and C. Ho, *J. Agric. Food Chem. 38*:1194 (1990).
13. Asakawa, T., and S. Matsushita, *Lipids 15*:965 (1980).
14. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, American Oil Chemists' Society, Champaign, 1989.
15. *SAS User's Guide*, Statistics, SAS Institute, Inc., Cary, 1985.
16. Ott, L., *An Introduction to Statistical Methods and Data Analysis*, 2nd edn., PWS Publishers, Boston, 1984, pp. 342, 376-379.

[Received June 18, 1993; accepted March 28, 1994]