Biological screening of 100 plant extracts for cosmetic use (1) Antioxidative activity and free radical scavenging activity

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Abstract

Methanolic aqueous extracts of 100 plants were screened for antioxidative activity using Fenton's reagent/ethyl linoleate system and free radical scavenging activity using DPPH free radical generating system. The results suggest that at least six plants including Eugenia caryophyllata, Alpinia offiicinarum, Rhus verniciflua, Curcuma longa, Rheum palmatvm and Evodia officinalis may be the potential sources of antioxidant, But only one plant, Cornus officinalis, may be the potential source of free radical scavenger from natural plants.

Key words: Antioxidative activity, Free radical scavenging activity: Plant extracts.

Introduction

Biological activities have been screened for the cosmetic use [1]. Furthermore, plant sources have been evaluated for developing natural antioxidants that may be involved in intiaging and antiwrinkle care [2]. Many endogeneous plant compounds have been reported to retard the oxidation process in their natural environment and in products to which they have been added [3]. Natural antioxidants occur in all higher plants and in all parts of the plant wood, bark, stems, pods, leaves, fruits, roots, flowers, and seeds. These are usually phenolic or polyphenolic compounds. Typical compounds that possess antioxidative activities are including tocopherols, flavonoids, cinnamic acid derivatives, phosphatides and polyfuctional organic acids. Recent studies indicate that the compounds with antioxidative and free radical scavenging activities can inhibit mutagenesis and carcinogenesis in addition to retardation of aging [1-3]. In this study, we have screened the antioxidative and free radical scavenging activities of 100 plant extracts. We first examined the antioxidative activity against lipid peroxidation in Fenton's reagent with ethyl linoleate. Secondly, we also examined the scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical generation.

Materials and Methods

Plant extract

One hundred plants were obtained from the oriental medicinal market located in Chuncheon, South Korea. Each powered plant (100g) was soaked in 300ml of 80% methanolic aqueous solution at room temperature for 7 days. After filteration, the methanolic filtrate was evaporated to dryness under vacuo. These extracts were used for the further biological study including antioxidative and free radical scavenging activities.

Antioxidative activity

A lipid peroxidation system was induced by Fenton's reagent. Each test sample (0.lml) and ethyl linoleate (10μM) were added to incubation medium (4.89ml) containing 2% sodium dodecyl sulfate, 1,μM ferrous chloride and 0.5mM hydrogen peroxide. The known synthetic antioxidants, dibutylated hydroxy tolune (BHT), dl-α-tocopherol and l-ascorbic acid were used as reference compounds. The incubation medium was kept at 55°C for 16 hours. Each reaction mixture (0.2ml) was transfered into a test tube, followed by addition of 4% BHT (50μl) to prevent further oxidation. Antioxidative activity of the sample was measured using thiobarbituric acid (113A) assay according to the method Ohkawa et al [4]. The absorbance was measured at 535nm.

Free radical scavenging activity

Scavenging effect against free radical generation was measured following the procedure of Fugita et al [5]. The sample solution (2ml) was added to 2ml of 60μM 1,1-diphenyl-2-picryl hydrazyl (DPPH) ethanolic solution and kept at room temperature for 30 min. The absorbance was measured at 520nm.

Results and Discussion

Methanolic aqueous extracts of 100 plants were screened for antioxidative activity using Fenton's reagent/ethyl linoleate system and free radical scavenging activity using DPPH free radical generating system. Table 1 represented the antioxidative and free radical scavenging activity of 100 plant extras in the initial screening. In lipid peroxidation assay using TBA method, dozen of the plant extracts including Alpinia officinarum, Brassica alba, Cannabis sativa, Curcuma longa, Eugenfa caryophyllata, Gastrodia ellata, Paeonia suffruitcosa, Plantago asiatica, Rhaphanus sativus, Rheum palmatum, Rhus verniciflua and Trapa bispinosa showed more than 40% inhibition at the concentration of 10 μg/ml or 80% inhibition at the concentration of 1,000μg/rnl. In DPPH free radical assay, several plant extracts including Acorus gramineus, Areca catechu, Chaenomeles speciosa, Citrus aurantium, Citrus unshiu, Cornus officinalis, Eugenia caryophyllata, Evodia officinalis, Gleditsia sinensis, Lindera strychinifolia, Morinda officinalis, Paeonia

suffruiticosa, Polvgala tenuifolia, Prunus armeniaca, Prunus mume, Rhus verniciflua, Schizandra chinensis, Sophora flavescens and Teminalia chebula showed more than 25% inhibition at the concentration of 10μgSml or o0% inhibition at the concentration of 1,000μg/ml.

In order to investigate IC_{50} values of plant extracts showing high biological activities, the experiments of dose-response relationship were performed. Fig. 1 showed the antioxidative acivity of several active plant extracts and reference compounds such as dl-α-tocopherol, l-ascorbic acid and BHT, which gave good dosetresponse relationships. BHT was the most potent inhibitor of TBA-reactive material formation. IC_{50} value of BHT was 1.5μg/ml, while other reference compounds, dl-α-tocopherol and l-ascorbic acid, showed 33.6μg/ml and 219μg/ml, respectively. IC_{50} values of plant extracts selected were 17.2μl/ml for Eugenia caryopyllata, 33.5 μl/ml for Alpinia officinarum, which showed more potent activity than dl-α-tocopherol. And IC_{50} values of other plant extracts were 38.1μg/ml for Rhus verniciflua, 42.8μg/ml for Curcuma longa, 56.2μg/ml for Rheum palmatum, 58.2μg/ml for Evodia officinalis, which showed similar potency with dl-α-tocopherol or more potent activity than l-ascorbic acid.

Naural antioxidants are usually phenolic or polyphenolic compounds and these compounds include tocopherol, flavonoid and cinnamic acid derivatives [6]. It is known that there are two types of antioxidant [7]. The first type of antioxidant inhibits the formation of free radicals which may initiate oxidation. In most case, they are chelators of metal ions such as flavonoids. The second type of antioxidant inhibits the free radical chain propagation reactions. Therefore, some of plant extracts may act at the initiation stage of peroxidation interfering with Fenton's reaction, thus breaking the chain reaction.

Fig.2 showed the free radical scavenging activity of plant extracts and several reference compounds. l-Ascorbic acid was the most potent scavenger. IC_{50} values of l-ascorbic acid, dl-α-tocopherol and BHT were found to be 29.7μg/ml, 33.5 μg/ml and 37.2μg/ml respectively. On the other hand, IC_{50} values of plant extracts tested showed much lower activity than the well-known reference compounds. Most of them showed over l00μg/ml of IC_{50} values except Cornus officinalis showing relative higher activity (50.6μg/ml).

Free radical damage to biosystem is one of the major process that contributes to the degenerative disease like cancer and aging [81. Detailed free radical mechanisms and their quantitative contributions are still not clear. Despite these uncertainties, it is clear that free radical scavenger may inhibit endogeneous, metabolically driven, oxidative DNA damage, as well as mutation and aging by exogeneous agents [9-11].

There are many plants which were reported to show their antioxidative effect. Masaki [12] reported Hamamelis virginiana, Aesclus hippocastanum, Polygonum cuspidatum, Quercus robur, Rosemarinus officinalis, Salvia officinalis and Sanguisorba officinalis have potent activities (IC_{50} <3μg/ml) on the scavenging effect of superoxide anions. Fukuda et al. [13] had screened 64 kinds of plant for their SOD like biological activity, Juglans mandshurica, Ephedra sinica, Phellodendri amurense, Magnolia officinalis,

Rehmannia glutinosa, Scutellaria baicalensis, Cornus officinalis, Citrus unshiu, Perilla frutescens and Paeonia suffruncosa showed more than 40% of inhibitory activity at the concentration of 50μg/ml. Most of these plants have phenolic compounds such as tannins and flavonoids that may contribute to their antioxidatne activity. Yoshikawa [14] reported that Paeonia suffruticosa have a strong scavenging activity against DPPH radicals, and isolated galloyl glucose as active compound.

In this study, we demonstrated that several plant extracts have potent antioxidative activity and/or free radical scavenging activity . However, we do not know what components in the plant extracts show these biological activities. Isolation of the active compounds from each plant extract showing potent biological activities is under investigation.

References

1) Mizuno, M. and Tanaka, T.: Chemistry of phyto-ingredients - Recent advance of crude dmgs research: in The Science of Plants in Cosmetics, Fragrance Press, Tokyo, pp 2 (1986).
2) Wattenberg, L.W.D and Lar4 L.K.T.: Inhibition of chemical carcinogenesis by phenols, coumarins, aromatic isothiocyanates, flavons, and indoles, Plenum Press, New York, pp 1 (1981).
3) Pratt, D.E.: Natural antioxidants from plant material: in Phenolic Compoumds and Their Effects on Health II, ACS Symposium Series 547, American Chemical Society, Washington D.C., pp 54 (1994).
4) Ohkawa, H., Oshini, N., and Yagi, K.: AnaL Chem., 95, 351 (1979).
5) Fugita, Y., Uera, I., Morimoto, Y., Nakajima, M., Hatano, C. and Okuda, T.: Yakugaku Zasshi, 108, 129 (1988).
6) Ames, B.N.: Science, 221, 1256 (1983).
7) Simic, M.G. and Jovanovic, S.V.: In activation of oxygen radicals by dietary phenolic compounds in anticarcinogenesis, in Food Phytochemicals for Cancer Prevention II, ACS Symposium Series 547, American Chemical Society, Wash ington D.C., pp 20 (1994).
8) Davies, KT.A.: Oxidative damage and rep,ur, Pergamon Press, New York (1991).
9) Simic, M G. and Bergtold, D.S.: Mutahon Res., 250, 17 (1991).
10) Hartman, P.E. and Shankel, D.M.: Environ. A~MoL Mutagen., 15, 145 (1990). Il) Wattenberg, L.W.: CancerRes. 52, 2015s (1992).
12) Masaki, H.: Active oxigen scavenging activity in plant extracts, Fragransc J., 8, 64 (1995).
13) Fukuda, T. and Kitada, Y.: Reactive oxygen species-Scavenging effects of clude dmg, Fragransc J., 18, 75 (1995).
14) Yoshikawa, M. and Yamaham, J.: Chem. Pharn. Bull., 40(8), 2248 (1992).

Table 1. Antioxidative and free radical scavenging activities of 100 plant extracts.

Name of plant	Part of plant used	Antioxidative activity(%)		Free radical scavenging activity(%)	
		10μg/ml	1000μg/ml	10μg/ml	1000μg/ml
Acorus gramineus	Rhizoma	3	5	2	54
Agastache rugosa	Leaves	0	57	0	6
Akebia quinata	Stem	0	10	7	11
Alisma orientale	Rhizoma	25	52	0	5
Alpinia officinarum	Rhizoma	0	81	20	24
Amomum cardamomum	Fruit	0	67	0	12
Amomum xanthioides	Seed	0	68	8	4
Anemarrhena asphodeloides	Rhizoma	1	37	6	29
Angelica dahurica	Roots	0	13	14	22
Angelica koreana	Root	6	51	10	17
Angelica tenuissima	Root	11	46	0	5
Anthriscus sylvestris	Root	11	43	1	27
Aralia cordata	Root	16	50	0	2
Areca catechu	Seed	24	73	52	47
Areca catechu	Peel	0	68	9	24
Arisaema heterophyllum	Tuber	10	19	4	7
Asiasarum sieboldii	Root	0	58	1	21
Astragalus membranaceus	Root	0	49	4	17
Atractylodes japonica	Rhizoma	3	48	2	16
Belamcanda chinensis	Rhizoma	0	42	5	46
Brassica alba	Seed	39	89	2	19
Bupleurum falcatum	Root	0	47	4	18
Cannabis sativa	Seed	51	75	9	12
Caragana sinica	Root	6	42	0	14
Chaenomeles speciosa	Fruit	11	41	7	54
Chrysanthemum indicum	Flower	0	48	2	28
Cinnamomum cassia	Bark	20	53	0	22
Citrus aurantium	Fruit	16	59	2	67
Citrus unshiu	Peel	1	30	2	55
Codonopsis pilosula	Root	14	31	2	46

Commiphora molmol	Resin	18	76	2	4
Cornus officinalis	Fruit	24	46	4	69
Coptis japonica	Rhizoma	0	66	2	6
Corydalis ternata	Tuber	27	73	2	20
Curcuma longa	Rhizoma	22	75	16	10
Curcuma longa	Rhizoma	22	81	10	9
Curcuma zedoaria	Rhizoma	0	48	2	14
Cuscuta chinensis	Seed	13	29	3	12
Cyperus rotundus	Rhizoma	0	54	3	13
Dendrobium moniliforme	Leaves	0	72	5	26
Dioscorea batatas	Leaves	7	37	0	11
Dryopteris crassirrhizoma	Rhizoma	11	37	24	48
Equisetum hyemale	Leaves	0	0	3	10
Eucommia ulmoides	Bark	0	34	1	19
Eugenia caryophyllata	Flower	88	84	38	50
Evodia officinalis	Fruit	39	85	26	25
Foeniculum vulgare	Fruit	0	50	0	0
Gardenia jasminoides	Fruit	16	43	0	19
Gastrodia ellata	Rhizoma	42	28	3	33
Gleditsia japonica	Spine	22	63	34	65
Glycyrrhiza glabra	Root	1	73	0	0
Hordeum vulgare	Fruit	0	55	2	35
Kochia scoparia	Fruit	17	70	9	24
Leonurus sibricus	Leaves	0	26	2	9
Liriope platyphylla	Tuber	6	18	2	17
Lindera strychnifolia	Root	19	60	19	62
Lycium chinensis	Fruit	13	31	3	12
Morinda officinalis	Root	14	21	1	66
Morus alba	Stem	0	66	6	16
Myristica fragrans	Seed	11	77	9	15
Nepeta japonica	Leaves	7	67	6	13
Paeonia suffruticosa	Bark	26	81	49	22
Perilla frutescens	Leaves	17	65	18	12
Perilla frutescens	Seed	14	63	7	11
Phellodendron amurense	Bark	11	77	0	9

Phragmites communis	Root	0	22	5	85
Phylostachys nigra	Stem	20	71	5	35
Pinellia ternata	Tuber	0	18	0	8
Plantago asiatica	Seed	44	54	7	8
Platicodon grandiflorum	Roots	0	0	14	37
Polygala tenuifolia	Roots	7	54	15	52
Poncirus trifoliata	Fruit	5	61	5	15
Poria cocos (red)	Hoelen	15	32	1	22
Poria cocos (alba)	Hoelen	5	8	1	20
Prunus armeniaca	Seed	0	26	7	54
Prunus mume	Fruit	0	26	5	89
Prunus persica	Seed	2	0	2	10
Pueraria thunbergiana	Root	0	55	8	25
Pueraria thunbergiana	Flower	10	65	7	15
Rehmannia glutinosa	Root	20	34	1	34
Rhaphanus sativus	Seed	22	83	1	31
Rheum palmatum	Rhizoma	35	80	46	33
Rhus verniciflua	Resin	48	66	56	9
Rubus coreanus	Fruit	0	18	31	32
Sanguisorba officinalis	Root	26	67	41	67
Saussurea lappa	Root	0	9	0	18
Schizandra chinensis	Fruit	0	54	6	88
Sophora flavescens	Root	7	66	3	57
Sophora japonica	Flower	33	65	21	11
Sparganium stoloniferum	Rhizoma	0	11	1	23
Taraxacum platycarpum	Root	2	41	13	22
Trapa bispinosa	Fruit	37	82	33	40
Terminalia chebula	Fruit	11	66	64	69
Torilis japonica	Fruit	0	0	3	6
Trichosanthes kirilowii	Root	5	19	2	14
Trichosanthes kirilowii	Seed	0	39	1	10
Tussilago farfara	Flower	6	37	5	15
Valeriana fauriei	Root	9	59	0	30
Zanthoxylum piperitum	Peel	44	72	18	26
Zingiber officinale	Rhizoma	0	71	23	46

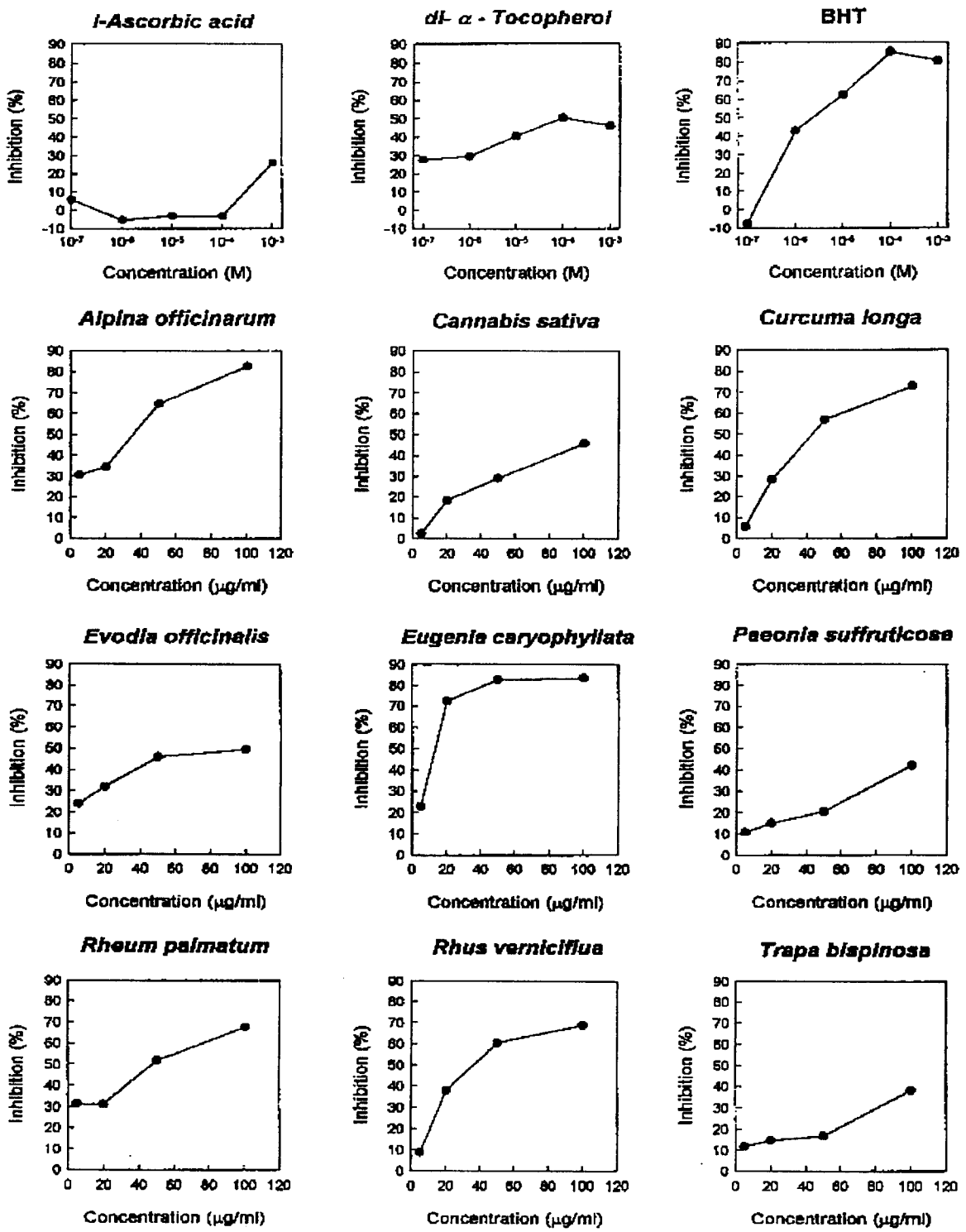

Fig 1. Antioxidative activity of several plant extracts determined by Fenten's reagent/ ethyl linoleate system.

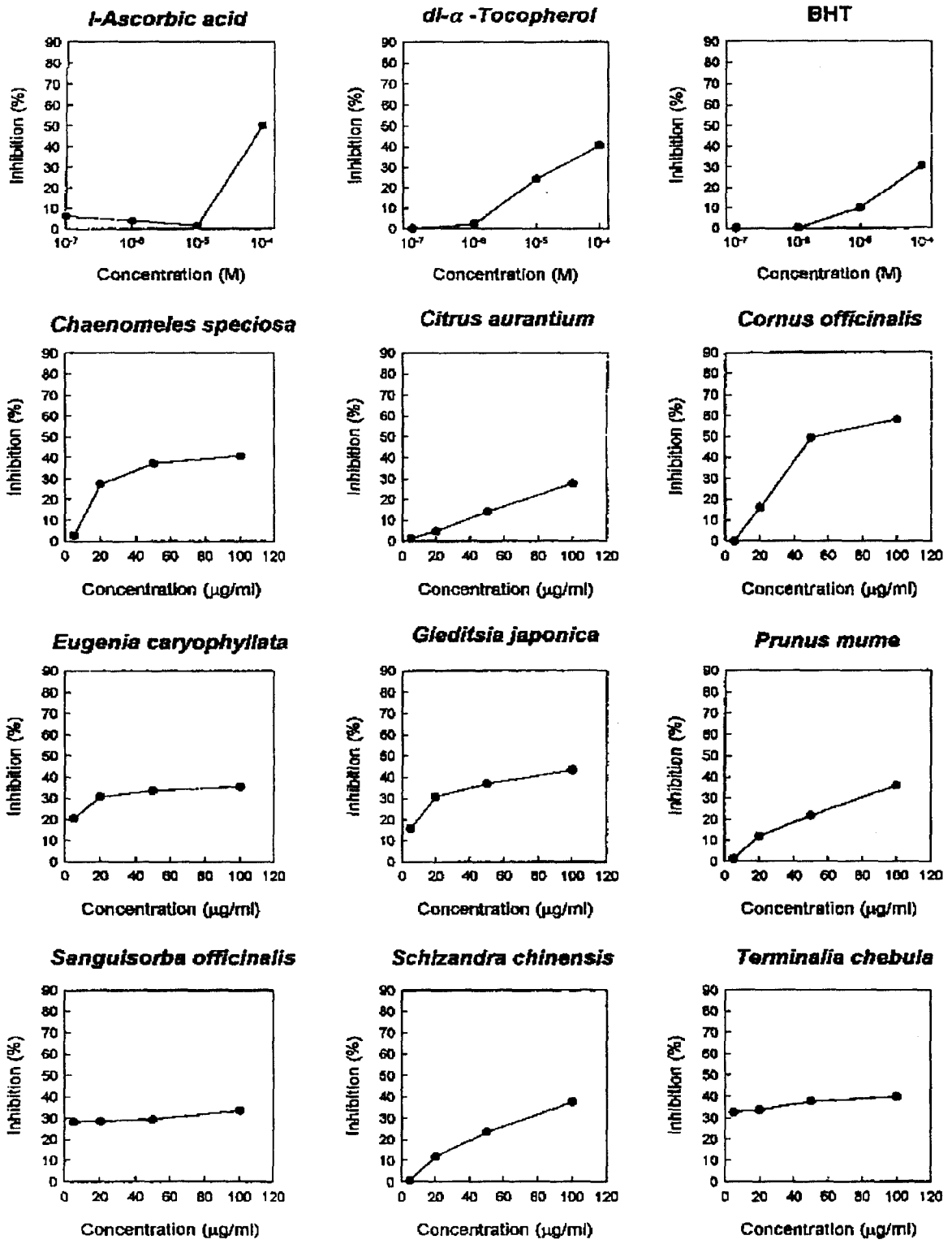

Fig 2. Free radical scavenging activity of several plant extracts determined by DPPH free radical generating system.