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Study on DPPH Free Radical Scavenging and Lipid Peroxidation Inhibitory Activities of Vietnamese Medicinal Plants

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Abstract – Among 90 Vietnamese medicinal plant extracts investigated for their antioxidant activity by DPPH assay at various concentrations from 10 - 100 μg/mL, 67 showed an inhibition rate over 50% at 100 μg/mL; 47 had greater than 50% inhibition at 50 μg/mL; 17 showed over 50% inhibition at 25 μg/mL. 8 extracts which exhibited strong inhibitory activity more than 50% inhibition at 10 μg/mL were further tested for lipid peroxidation inhibition by TBA assay. They displayed activity with IC_{50} values from 30.6 to 158.9 μg/mL. Until now, this is the first report on antioxidant activity of the female flower of *Borassus flabellifer,* and the stem of *Combretum latifolium, Embelia ribes*, *Spatholobus parviflorus*, and *Tetrastigma erubescens.* Fractionations of the EtOAc extract prepared from *S. parviflorus* led to the isolation of protocatechuic acid (**1**), ferulic acid (**2**), epicatechin (**3**), and gallic acid (**4**). These compounds showed significant DPPH inhibitory activity with IC_{50} values from 6.5 to 23.6 μM.
Keywords – Vietnamese medicinal plant, Antioxidant activity, DPPH, Lipid peroxidation, *Spatholobus parviflorus*

Introduction

Although oxygen is necessary for aerobic life, it can also participate in potentially toxic reactions involving oxygen free radicals or reactive oxygen species (ROS). Free radicals are formed in the human body to prevent from virus and bacterial infections. However, they react with macromolecules including protein, lipid, DNA causing serious diseases such as heart disease, macular degeneration, cancer, diabetes, and more. Therefore, antioxidant substances are required for the protection against the oxidizing agents. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant (Nagulendran *et al*., 2007).

Several analytical methods have been developed to determine the antioxidant capacity of natural substances. They can be categorized into two groups: (i) assays for radical-scavenging ability and (ii) assays for lipid oxidation inhibitory effect. However, the total antioxidant activities of plant extracts cannot be evaluated by using one single method, due to the complex composition of phytochemicals as well as of oxidative processes. Therefore, the use of at least two methods should be employed in order to evaluate the total antioxidant activity (B€ohm *et al*., 2001).

Vietnam, a tropical Southeast Asian country, also has a long history of traditional medicine systems. In practical, despite widespread use of wild plants as medicines, the literature contains few reports about antioxidant activity and chemical constituents of the Vietnamese medicinal plants. Therefore, we selected 90 medicinal plants casually according to their therapeutic application in Vietnamese folk medicine to investigate their antioxidant activity by using different antioxidant tests, including DPPH free radical scavenging assay and lipid peroxidation inhibition assay. We hope that the selected plants could be a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated and oxidative stress related degenerative diseases.

Experimental

Plant materials – Vietnamese medicinal plants used in this study were collected at the Seven-Mountain area in An Giang province on August 2009 (Table 1). The plants were identified by Dr. Hoang Viet, Faculty of Biology, University of Science, National University Ho Chi Minh City. The voucher samples are preserved with analytical

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number from 1001 to 1090 at Department of Analytical Chemistry, Faculty of Chemistry, University of Science, National University-Ho Chi Minh City.

Chemical – 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Merck (Darmstadt, Germany). Trolox, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals were of the highest grade available.

Preparation of samples – Each medicinal plant (100 - 213 g) was cut into small pieces and extracted with MeOH (300 mL, reflux, 3 h, × 3). The MeOH solution was evaporated under reduced pressure to give a MeOH extract.

DPPH free radical scavenging assay – The stable free radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Molyneux, 2004). Briefly, a 0.1 mM solution of DPPH in 90% ethanol was prepared and then 1.5 mL of this solution was mixed with 1.5 mL of each sample (crude extract) at concentrations of 100, 50, 25, 10 μg/mL in 90% ethanol. After 30 min incubation in the dark, the decrease in the solution absorbance was measured at 517 nm in a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). DPPH inhibitory activity was expressed as the percentage inhibition (I%) of DPPH in the above assay system, calculated as $(1 - B/A) \times 100$, where A and B are the activities of the DPPH without and with test material. IC_{50} (inhibitory concentration, 50%) values were calculated from the mean values of data from three determinations. Trolox at various concentrations (1, 2.5, 5, 10 μM) was used as a positive control.

Lipid peroxidation inhibition assay *in vitro* – TBA reacts with MDA to form a di-adduct, a red chromogen, which can be detected spectrophotometrically at 532 nm (Singh and Arora, 2007). Normal rats (200 - 250 g) were used for the preparation of brain homogenate. Brain was excised and washed with 0.95 M NaCl solution. Brain homogenate was prepared with homogenizer at −5 ºC with 5 mM phosphate buffered saline (PBS) buffer (1 : 10) for 30 min. The homogenate was centrifuged for 15 min, and clear cell free supernatant was used for the study of *in vitro* lipid peroxidation. 0.1 mL of each extract at different concentrations (10 - 2000 μg/mL) in dimethyl sulfoxide was taken in test tube. 1.4 mL of 50 mM PBS buffer and 0.5 mL of rat brain homogenate were added to the test tubes. After incubation at 37 ºC for 15 min, the reaction was stopped by addition of 1 mL of 10% TCA and 1 mL of 0.8% TBA. The mixture was then heated at 100 ºC for 15 min. The samples were cooled, centrifuged and the absorbance of the supernatants was measured at 532 nm. The percentage inhibition of lipid peroxidation is calculated as $(1 - B/A) \times 100$, where A and B are the activities of the MDA without and with test material. IC_{50} values were calculated from the mean values of data from three determinations. Trolox at various concentrations (10, 50, 100, 500, 1000 μM in 90% ethanol) was used as a standard.

Extraction and isolation of the active compounds from the stem of *Spatholobus parviflorus* – The dried stem (2.3 kg) of *S. parviflorus* was extracted with MeOH (12 l, reflux, 3 h, × 3), to yield a MeOH extract (205.3 g). The MeOH extract was suspended in H_2O and partitioned successively with ether, $CHCl_3$, EtOAc, BuOH to yield ether (8.2 g; IC_{50}, 55.1 μg/mL), $CHCl_3$ (7.5 g; IC_{50}, > 100 μg/mL), EtOAc (15 g; IC_{50}, 4.6 μg/mL), BuOH (12 g; IC_{50}, 8.7 μg/mL) and H_2O (115,2 g; IC_{50}, 13.3 μg/mL) fractions, respectively. The EtOAc fraction (15 g) was applied to silical gel column (3×30 cm) chromatography eluted with MeOH/$CHCl_3$ (0 - 30%) to give eight fractions: fr. 1 (0.5 g; IC_{50}, > 100 μg/mL), fr. 2 (1.3 g; IC_{50}, 97.0 μg/mL), fr. 3 (0.6 g; IC_{50}, 23.7 μg/mL), fr. 4 (0.6 g; IC_{50}, 1.3 μg/mL), fr. 5 (1.0 g; IC_{50}, 13.9 μg/mL), fr. 6 (1.1 g; IC_{50}, 3.6 μg/mL), fr. 7 (2.5 g; IC_{50}, 13.3 μg/mL), and fr. 8 (4.2 g; IC_{50}, 4.2 μg/mL). Fraction 4 was separated by reversed-phase preparative thin-layer chromatography (TLC) with MeOH–H_2O (2 : 8) to give protocatechuic acid (**1**, 15.2 mg) (Zhang *et al*., 2009) and ferulic acid (**2**, 10.0 mg) (Donia *et al*., 2011). Fraction 6 was separated by reversed-phase preparative TLC with MeOH–H_2O (2 : 8) to give epicatechin (**3**, 12.0 mg) (Ban *et al*., 2006) and gallic acid (**4**, 15.0 mg) (Zhang *et al*., 2009). Their structures were identified by spectral analysis and comparison of their data with those in the literature.

Results and Discussion

DPPH free radical scavenging assay – The DPPH analysis is a quick and simple test; it guarantees reliable results and needs only a UV-Vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening (Alvarez-Suarez *et al*., 2009). The 90 MeOH extracts which were prepared from the 90 selected plants were screened for their antioxidant activities by DPPH assay (Table 1). The assay was carried out at different concentrations of extract ranging from 1 - 100 μg/mL (data not shown). Of the extracts assayed, 90 extracts (100%) demonstrated DPPH inhibitory activity at 100 μg/mL, among which 67 (74.5%) showed an inhibition rate greater than 50%. Altogether, 89 extracts (98.9%) were found to be active at a concentration of 50 μg/mL,

Table 1. Vietnamese medicinal plants used in this study, their analytical number (AN), families, part used, local name, therapeutic applications, and IC_{50} values (DPPH assay)

AN	Plant name	Family	Part used	Local name	Therapeutic application	IC_{50} (μg/μl)
1001	*Abutilon indicum* (L.) Sweet.	Malvaceae	Whole plant	Dan xay	Backache, febrifuge, detoxication	>100
1002	*Ageratum conyzoides* L	Asteraceae	Aerial part	Cay cut lon	Inflammation, boil, itch, sinusitis	65.3
1003	*Albizia myriophylla* Benth.	Fabaceae	Bark	Cam thao cay	Detoxication, boil, cough	36.6
1004	*Alstonia scholaris* (L.) R. Br.	Apocynaceae	Bark	Muon cua	Febrifuge, tonic, rheumatism	> 100
1005	*Annona glabra* L.	Annonaceae	Stem	Binh bat nuoc	Diarrhea, dysentery, fever	>100
1006	*Anogeissus acuminata* (Roxb. ex DC.) Guill. et Perr.	Combretaceae	Bark	Ca dam	Hemiplegia, inflammation	9.4
1007	*Antidesma ghaesembilla* Gaertn.	Euphorbiaceae	Root	Choi moi	Cough, digestiver disorder, detoxication	41.7
1008	*Artemisia vulgaris* L.	Asteraceae	Leaf	Ngai cuu	Headache, menstrual disorder, malaria, rheumatism	92.4
1009	*Artocarpus altilis* (Park.) Fosb.	Moraceae	Leaf	Xa ke	Diuretic	28.6
1010	*Biota orientalis* (L.) Endl.	Cupressaceae	Leaf	Trac bach diep	Styptic, hemoptysis, cough, diuretic	> 100
1011	*Blumea balsamifera* (L.) DC.	Asteraceae	Leaf	Dai bi	Influenza, stomachache, angina, metrorrhagia	60.1
1012	*Boehmeria nivea* (L) Gaud.	Urticaceae	Leaf	Gai	Diuretic, fever, influenza, nephritis	>100
1013	*Borassus flabellifer* L.	Arecaceae	Female flower	Thot not	Diuretic, inflammation	4.9
1014	*Caesalpinia sappan* L.	Caesalpiniaceae	Seed	To moc	Rheumatism, inflammation, menstrual disorder	96.1
1015	*Caesalpinia sappan* L.	Caesalpiniaceae	Shell	To moc	Rheumatism, inflammation, menstrual disorder	73.8
1016	*Carica papaya* L.	Papayaceae	Leaf	Du du	Cancer, cough, antibiotic, indigestion	34.7
1017	*Cassia alata* L.	Caesalpiniaceae	Stem	Muong trau	Aperient, ringworm, herpes circine	>100
1018	*Cassytha filiformis* L.	Lauraceae	Whole plant	To hong xanh	Heart disease, nephritis, hepatitis, kidney stone, fever	55.8
1019	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Whole plant	Dua can	Cancer, hypertention	84.2
1020	*Ceiba pentandra* (L.) Gaerth. Var. indica (DC.) Bakh	Bombacaceae	Bark	Gon	Rheumatism, kidney	39.5
1021	*Christia vespertilionis (*L.f) Bakh.f.	Fabaceae	Whole plant	Ngai buom	Diuretic, heart disease	78.8
1022	*Chrysopogon aciculatus* (Retz.) Trin.	Poaceae	Whole plant	Co may	Hepatitis, detoxication, diuretic	51.1
1023	*Circus japonicus.* (DC.) Maxim	Asteraceae	Whole plant	O ro	Kidney, metrorrhagia	35.2
1024	*Citrus deliciusae* Tenore	Rutaceae	Bark	Tran bi	Malaria, aperient, cough	56.9
1025	*Coccinia cordifolia* Cogn.	Cucurbitaceae	Stem	Binh bat day	Diuretic, inflammation, diabetes	> 100
1026	*Coix lachryma- jobi* L.	Poaceae	Seed	Cuom gao	Antiseptic, inflammation, urinary tract infection	>100
1027	*Combretum latifolium* Blume	Combretaceae	Stem	Lang vang	Tonic, backache, kidney, fever , pains, inflammation	5.1
1028	*Costus speciosus* Smith	Costaceae	Bulb	Cat loi	Kidney stone, rheumatism, fever	94.4
1029	*Cycas pectinata* Griff.	Cycadaceae	Seed	Thien tue	Cough	85.1
1030	*Cyperus rotundus* L.	Cyperaceae	Bulb	Co cu	Cancer, anodyne, menstrual disorder	27.8
1031	*Dalbergia cadenatensis* (Denst.) Prain	Fabaceae	Stem	Day co rua	Cough, metrorrhagia, antipyretic, boil	43.6
1032	*Dendrobium crumenatum* Sw.	Orchidaceae	Root	Thach hoc	Kidney, night sweat, pains, anaphrodisia	21.7
1033	*Derris elliptica* Benth.	Fabaceae	Leaf	Day thuoc ca	Worm	17.3
1034	*Desmodium styracifolium* (Osb.) Merr.	Fabaceae	Whole plant	Kim tien thao	Kidney stone, hepatitis, urinary tract infection	58.5
1035	*Dolichandrone spathacea* (L.f) K. Schum.	Bignoniaceae	Whole plant	Quao nuoc	Tonic, asthma, detoxication, hepatitis	37.0

Table 1. continued

AN	Plant name	Family	Part used	Local name	Therapeutic application	IC_{50} (μg/μl)
1036	*Drynaria quercifolia* (L.) J. Smith	Polypodiaceae	Bulb	Rang bay	Kidney, pains, tonic	29.9
1037	*Eclipta alba* Hassk.	Asteraceae	Aerial part	Co muc	Fever, kidney, dysentery	60.1
1038	*Eleusine india* (L.) Gaertn.	Poaceae	Whole plant	Co man trau	Diuretic, febrifuge, fever, hypertension, nephritis, hepatitis	> 100
1039	*Embelia ribes* Burm. f.	Myrsinaceae	Stem	Ngu linh chi	Antibiotic, hypertension, diuretic, inflammation	5.3
1040	*Erythrina orientalis* (L) Murr	Fabaceae	Whole plant	Vong nem	Hypertension, tranquillizer, antiseptic, diuretic	59.5
1041	*Euphorbia thymifoblia* Burm	Euphorbiaceae	Leaf	Co sua la nho	Hypertension, inflammation, diabetes, dysentery, diuretic	8.1
1042	*Excoecaria cochinchinensis* Lour.	Euphorbiaceae	Leaf	La lieu	Boil, itch, dysentery, hypertension, inflammation	6.3
1043	*Fibraurea tinctoria* Lour.	Menispermaceae	Stem	Hoang dang	Inflammation, dysentery, hepatitis, malaria, diarrhea, tonic	80.6
1044	*Ficus sagittata* Vahl *var. sagittata*	Moraceae	Stem	Manh trau	Tonic	36.3
1045	*Gnetum montanum* Mgf.	Gnetaceae	Stem	Gam den	Inflammation, malaria, fever, detoxication	13.9
1046	*Gymnopetalum cochinchinensis* (Lour.) Kurz	Cucurbitaceae	Whole plant	Cut qua	Cough, inflammation	48.4
1047	*Heliotropium indicum* L.	Boraginaceae	Whole plant	Den voi	Inflammation, febrifuge, detoxication, skin-disease	>100
1048	*Homalomena occulta* (Lour.). Schott	Araceae	Root	Thien nien kien	Rheumatism, tonic, pains, stomachache, rheumatism	>100
1049	*Hydnocarpus ilicifolia* King	Kiggelariaceae	Stem	Lo noi	Scabies	23.9
1050	*Hypobathrum racemosum* (Roxb.) Kurz	Rubiaceae	Wood	Nhau rung	Scabies	>100
1051	*Imperata cylindrical* (L.) Beauv.	Poeceae	Root	Co tranh	Febrifuge, asthma, cough, kidney, hypertension	69.6
1052	*Kyllinga monocephala* Rottb.	Cyperaceae	Whole plant	Co bac dau	Febrifuge	32.8
1053	*Lasia spinosa* (L.) Thw.	Araceae	Root	Ray gai	Hepatitis, febrifuge, detoxication, nephritis	> 100
1054	*Leonurus heterophyllus* Sweet	Lamiaceae	Aerial part	Ich mau	Menstrual disorder, inflammation, hypertension	45.7
1055	*Melastoma candidum* D. Don	Melastomataceae	Leaf	Mua	Hepatitis, cough, menstrual disorder	31.8
1056	*Melia azedarach* L.	Meliaceae	Wood	Sau dau	Boil, marsk fever, skin-disease,	42.9
1057	*Momordica charantia* L.	Cucurbitaceae	Fruit	Kho qua	Diabetes, febrifuge, diuretic, aperient, tonic, fever	> 100
1058	*Morinda citrifolia* L.	Rubiaceae	Fruit	Nhau nha	Tonic, fever, hypertension	> 100
1059	*Morinda citrifolia* L.	Rubiaceae	Stem	Nhau nha	Tonic, fever, hypertension	33.1
1060	*Morus alba* L.	Moraceae	Stem	Dau tam	Diuretic, hypertension, cough, hepatitis, pains	22.2
1061	*Oroxylum indicum* (L.), Vent	Bignoniaceae	Fruit	Nuc nac	Febrifuge, detoxication, antiseptic, itch, angina	36.9
1062	*Oroxylum indicum* (L.), Vent	Bignoniaceae	Stem	Nuc nac	Febrifuge, detoxication, antiseptic, itch, angina	18.2
1063	*Orthosiphon aristatus (*Blume) Miq.	Lamiaceae	Whole plant	Rau meo	Diuretic, inflammation, nephritis	33.2
1064	*Pandanus tectorius* Sol.	Pandanaceae	Fruit	Dua gai	Diuretic, inflammation, rheumatism	35.1
1065	*Parameria laevigata* (Juss.) Moldenke	Apocynaceae	Stem	Do trong nam	Hypertension, diuretic, backache, tonic, kidney	45.2
1066	*Passiflora foetida* L.	Passifloraceae	Stem	Nhan long	Tranquillizer, insomnia, neurasthenia, itch	> 100
1067	*Perilla ocymoides* L.	Lamiaceae	Leaf	Tia to	Cough, anodyne, detoxication, headache	33.8
1068	*Phyllanthus urinaria* L.	Euphorbiaceae	Whole plant	Cho de rang cua	Hepatitis, malaria, inflammation	13.8
1069	*Piper lolot* C. DC.	Piperaceae	Leaf	La lot	Rheumatism, diarrhea, headache, inflammation	>100
1070	*Plantago asiatica* L.	Plantaginaceae	Aerial part	Ma de	Inflammation, nephritis, hepatitis, cough	>100

Table 1. continued

AN	Plant name	Family	Part used	Local name	Therapeutic application	IC_{50} (μg/μl)
1071	*Pluchea indica* (L.) Less.	Asteraceae	Whole plant	Tu bi	Cough, influenza, fever, hypertension, rheumatism	35.2
1072	*Pluchea pteropoda* Hemsl.	Asteraceae	Stem	Sai ho	Backache, headache, fever	62.1
1073	*Polyscias fruticosa* (L.) Harms	Araliaceae	Leaf	Dinh lang	Tonic, backache, hemoptysis	> 100
1074	*Pueraria thomsoni* Benth.	Fabaceae	Whole plant	Cat can	Fever, backache, antipyretic, cold	24.9
1075	*Rhinacanthus nasuta* (L.) Kurz	Acanthaceae	Whole plant	Kien co	Ring worm, cough, bronchitis, rheumatism, hypertension	>100
1076	*Sauropus androgynus* (L) Merr.	Euphorbiaceae	Whole plant	Bu ngot	Diuretic, placental retention, fever, rheumatism	54.5
1077	*Schefflera octophylla* (Lour.) Harms	Araliaceae	Whole plant	Chan chim	Rheumatism, tonics, hypertension, febrifuge	80.0
1078	*Seoparia dulcis* L.	Scrophulariaceae	Whole plant	Cam thao dat	Diabetes, fever, cough, detoxication	88.5
1079	*Sesbania javanica* Miquel	Fabaceae	Whole plant	Dien dien	Boil, menstrual disorder	>100
1080	*Smilax glabra* Roxb.	Smilacaceae	Bulb	Tho phuc linh	Inflammation, rheumatism, nephritis, diuretic	30.1
1081	*Solanum nigrum* L.	Solanaceae	Whole plant	Thu lu bao	Diabetes, fever, hepatitis, bronchitis	> 100
1082	*Spatholobus parviflorus* (Roxb. Ex DC.) Kuntze.	Fabaceae	Stem	Huyet rong	Tonic, menstrual disorder, inflammation	5.2
1083	*Stenochlaena palustris* (Burm. f.) Bedd.	Blechnaceae	Stem	Day chay	Fever, febrifuge	24.8
1084	*Streptocaulon juventas* (Lour.) Merr.	Asclepiadaceae	Stem	Ha thu o trang	Stomachache, fever, cough, gonorrhoea	45.5
1085	*Tetrastigma erubescens* Planch.	Vitaceae	Stem	Day rom	Fever, tonic, stomachache, inflammation, hypertension	4.1
1086	*Tetrastigma strumarium* (Planch) Gagnep.	Vitaceae	Stem	Day dac	Headache, fever	35.8
1087	*Uvaria micrantha* (A.DC.) Hook. f. et Thoms.	Annonaceae	Stem	Ky huong	Diuretic, digestive disorder, backache	32.1
1088	*Vitex negundo* L.	Verbenaceae	Whole plant	Ngu trao	Rheumatism, influenza, cold, tonic, fever, pains, hemiplegia	46.6
1089	*Xanthium strumarium* L.	Asteraceae	Fruit	Thuong nhi tu	Cancer, scabies, goiter	31.4
1090	*Zea mays* L.	Poaceae	Beard	Bap	Diuretic, urinary tract infection, kidney, hypertension, hepatitis	> 100

among which 47 (52.8%) showed inhibition of more than 50%. At 25 μg/mL, 85 (94.4%) extracts were active, and 17 (20%) showed an inhibition of over 50%. Of the extracts assayed, 81 (90%) displayed activity at 10 μg/mL, including eight (12.7%) of over 50% inhibition. In total, 67 MeOH extracts showed IC_{50} values below 100 μg/mL, 45 crude extracts with IC_{50} values less than 50 μg/mL, 16 extracts exhibited IC_{50} values below 25 μg/mL, and 8 extracts with IC_{50} values below 10 μg/mL (Table 1). IC_{50} of trolox was 8.7 μM (2.2 μg/mL).

The DPPH scavenging activity of the 8 MeOH extracts in decreasing order was: *Tetrastigma erubescens* > *Borassus flabellifer* > *Combretum latifolium* > *Spatholobus parviflorus* > *Embelia ribes* > *Excoecaria cochinchinensis* > *Euphorbia thymifoblia* > *Anogeissus acuminata*. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. On analyzing the results obtained in DPPH assay, it was noticed that extracts act as good hydrogen donating agent, thereby bleaches the DPPH absorbance.

Lipid peroxidation inhtion assay *in vitro* – Based on the results obtained from the DPPH assay, 8 MeOH extracts which exhibited strong inhibitory activity with IC_{50} values less than 10 μg/mL were tested for lipid peroxidation inhibition by the thiobarbituric acid assay (TBA assay) *in vitro* (Table 2). All the studied extracts showed antioxidant activity on brain rat with their IC_{50} values in the order: *T. erubescens* > *E. ribes* > *A. acuminate* > *E. cochinchinensis* > *E. thymifolia* > *C. latifolium* > *B. flabellifer* > *S. parviflorus*. The eight extracts may inhibit strongly the formation of peroxides and hydroperoxides. IC_{50} of trolox was 24.4 μM (6.1 μg/mL).

The bark of *A. acuminate,* the leaf of *E. cochinchinensis* and *E. thymifolia* are widely used in Vietnam traditional medicine to treat of hypertension, inflammatory diseases. The phytochemical studies on these plant species reported that they contain a number of flavonoids (Do., 2004; Do., 2009). Flavonoids which are antioxidants have been reported to be effective radical scavengers and inhibitors of lipid peroxidation (Pandey *et al*., 2009). Thus, the presence of flavonoids may play an essential role in their antioxidant activity.

Five extracts from the flower of the female flower of *B. flabellifer* and the stem of *C. latifolium, E. ribes* and *S. parviflorus*, and *T. erubescens*, showed strong activity. Although these plants are used in Vietnamese folk medicine to treat of inflammatory diseases this is the first report on their DPPH inhibitory and lipid peroxidation inhibitory activity. Until now, there are no scientific reports on their chemical constituents and biological activities. They could be a potential source of natural antioxidants that could have great importance as therapeutic agents in medicine.

Extraction and isolation of the active compounds from the stem of *Spatholobus parviflorus* – In this study, *S. parviflorus* was selected to isolate and identify its active constituents. The MeOH extract of the stem of *S. parviflorus* was subjected to silica gel column chromatography to give eight fractions. Further separation and purification of the active fractions with reversed-phase preparative TLC led to the isolation of protocatechuic acid (**1**), ferulic acid (**2**), epicatechin (**3**), and gallic acid (**4**) (Fig. 1).

Protocatechuic acid (**1**) (Zhang *et al*., 2009): A white amorphous powder. ^{1}H-NMR (500 MHz- CD_3OD) δ: 7.49 (1H, d, J = 2.0 Hz, H-2), 6.86 (1H, d, J = 8.0 Hz, H-5), 7.43 (1H, dd, J = 8.0 & 2.0 Hz, H-6); ^{13}C-NMR (125 MHz- CD_3OD) δ: 123.3 (C-1), 117.6 (C-2), 145.7 (C-3), 150.7 (C-4), 115.4 (C-5), 123.6 (C-6), 167.7 (-COOH).

Ferulic acid (**2**) (Donia *et al*., 2011): A colorless solid. ^{1}H-NMR (500 MHz- CD_3OD) δ: 7.18 (1H, d, J = 1.5 Hz, H-2), 6.83 (1H, d, J = 8.5 Hz, H-5), 7.07 (1H, dd, J = 8.5 & 1.5 Hz, H-6), 7.62 (1H, d, J = 16 Hz, H-7), 6.33 (1H, d, J = 16 Hz, H-8), 3.80 (3H, s, -OCH_3). ^{13}C-NMR (125 MHz- CD_3OD) δ: 127.8 (C-1), 111.7 (C-2), 149.4 (C-3), 150.5 (C-4), 116.5 (C-5), 124.0 (C-6), 147.0 (C-7), 115.9 (C-8), 171.1 (C-9), 56.8 (-OCH_3).

Epicatechin (**3**) (Ban *et al*., 2006): An orange amorphous powder. ^{1}H NMR ((500 MHz- CD_3OD) δ: d 6.99 (1H, d, J = 1.5 Hz, H-2'), 6.82 (1 H, d, J = 8.0 Hz, H-5'), 6.78 (1 H, dd, J = 8.0 & 1.5 Hz, H-6'), 5.94 (1 H, d, J = 2.5 Hz, H-6), 5.96 (1 H, d, J = 2.5 Hz, H-8), 4.85 (1H, br.s, H-2), 4.20 (1H, s, H-3), 2.88 (1 H, dd, J = 17 & 5.0 Hz, H-4 α), 2.75 (1 H, dd, J = 16.5 & 3.0 Hz, H-4 β); ^{13}C NMR (125

Table 2. Antioxidation activity of 8 selected MeOH extracts using TBA assay

AN	Plant name	IC_{50} (μg/μl)	AN	Plant name	IC_{50} (μg/μl)
1006	*Anogeissus acuminata*	68.3	1041	*Euphorbia thymifoblia*	73.5
1013	*Borassus flabellifer*	99.3	1042	*Excoecaria cochinchinenis*	73.4
1027	*Combretum latifolium*	95.1	1082	*Spatholobus parviflorus*	158.9
1039	*Embelia ribes*	49.5	1085	*Tetrastigma erubescens*	30.6

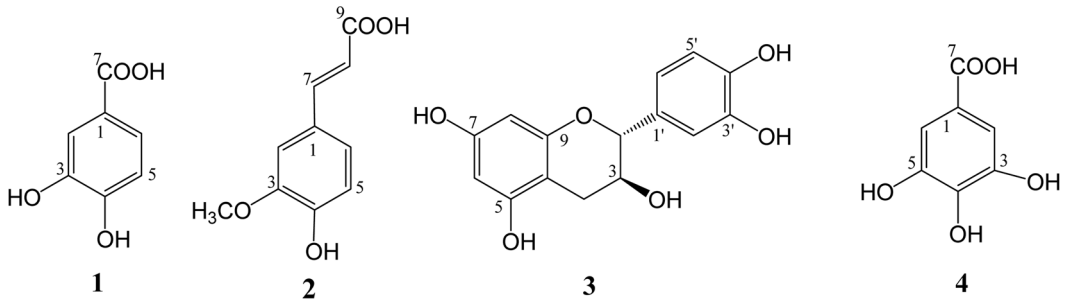

Fig. 1. Structures of the isolated compounds from the stem of *S. parviflorus*.

MHz-CD_3OD) δ: 79.9 (C-2), 67.5 (C-3), 29.2 (C-4), 157.4 (C-5), 95.9 (C-6), 158.0 (C-7), 96.5 (C-8), 157.7 (C-9), 100.1 (C-10), 132.3 (C-1'), 115.4 (C-2'), 145.8 (C-3'), 145.9 (C-4'), 116.0 (C-5'), 119.4 (C-6').

Gallic acid (**4**) (Zhang *et al*., 2009): A white solid. ^{1}H-NMR (500 MHz- CD_3OD) δ: 7.06 (2H, s, H-2,6); ^{13}C-NMR (125 MHz- CD_3OD) δ: 122.1 (C-1), 110.4 (C-2,6), 146.4 (C-3,5), 139.6 (C-4), 170.4 (-COOH).

These compounds were assayed for their DPPH inhibitory activity. IC_{50} values of **1**, **2**, **3** and **4** were 14.1 μM, 23.6 μM, 6.8 μM and 6.5 μM, respectively. Epicatechin which is a flavonol belonging to the group of flavonoids is a strong antioxidant (Yilmaz *et al*., 2004). Besides, protocatechuic, gallic and ferulic acids are polyphenol antioxidants (Karamac *et al*., 2005). Their presence could explain the strong inhibition of lipid peroxidation and DPPH radical-scavenging activity of this plant.

Conclusions

In conclusion, we have carried out a systematic investigation of Vietnamese medicinal plants for DPPH inhibitory activity and lipid peroxidation inhibition. The results indicate a number of medicinal plants that may be useful for the treatment of diseases relating free radical damages, and provide the basis for further investigation on these medicinal plant species to isolate active constituents and drug development. Specifically, this is the first scientific report on antioxidant activity of the female flower of *B. flabellifer,* and the stem of *C. latifolium, E. ribes, S. parviflorus*, and *T. erubescens*.

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