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Evaluation of Antioxidant Polyphenols in Taiwan's Medicinal Plants

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In this paper, 23 forest plants collected from the lowland subtropical rainforest habitat in southern Taiwan were evaluated to determine total polyphenol content and antioxidant activity (by 2,2-diphenyl-1,1-picryl-hydrazyl DPPH free radical scavenging assay against α-tocopherol). The contents of polyphenols in 23 dry plant materials ranged from 2.5 to 164 mg/g in which top 10 is in the order of *Acacia confuse, Macaranga tanarius, Euphoria longana, Mangifera indica, Ficus wightiana, Lantana camara, Pittosporum pentandrum, Leucaena lencocephala, Ficus septica* and *Pittosporum pentandrum*. Except for *Anredera cordifilia, Ophiopogn japonicus* and *Setaria palmifolia*, other plants are able to eliminate significantly DPPH in 0.5 h indicating that the analyzed plants possess free radical scavenging activity. Our research indicates that Taiwan's lowland subtropical rainforest plants have great potential in scavenging free radicals and the plants can be a vital source of antioxidant phytochemicals.

Key Words: Rainforest plants, Total polyphenol, Radical scavenging properties, Taiwan.

INTRODUCTION

Free radicals provoked by various environmental chemicals as well as endogenous metabolism are involved in a number of diseases like tumors, inflammation, shock, atherosclerosis, diabetes, infertility, gastric mucosal injury and ischemia due to the oxidative damage to DNA, lipids and proteins, which can result in failure of cellular functions[1-5]. Polyphenolic compounds containing more than one aromatic hydroxyl group are widely distributed in the plant kingdom. Polyphenols include many classes of compounds such as phenolic acids, anthocyanins and flavonoids[6]. These polyphenols are antioxidants that protect cells against the damaging effects of oxidative stress induced by reactive oxygen species (ROS) like singlet oxygen, superoxide, peroxyl radicals and hydroxyl radicals[7,8].

The primate brain is a large consumer of oxygen and a good substrate for oxidation. Polyunsaturated fatty acids, a major component of cell membranes are highly susceptible to oxidative deterioration of lipids. The free radical theory implicates antioxidant nutrients as being protective against damage to the brain[9]. Consumption

of antioxidants from plant materials that inhibit free radical formation or accelerate their elimination has been associated with a lowered incidence of these diseases as a consequence of alleviating the oxidative stress of free radicals[10,11]. Therefore, dietary antioxidants have recently garnered enormous interest among the general human population. People pay attention now to the potential benefit in consuming more vegetables and fruits since certain vitamins and minerals prevent the formation of free radicals or to scavenge them once they are formed.

Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol group per molecule. The polyphenols have antioxidant characteristics with potential health benefits. According to recent studies, the polyphenol antioxidants are known to reduce the risk of cardiovascular disease and cancer[12]. The antioxidant compounds in a typical diet are mostly derived from plant sources and polyphenolic components of higher plants act as antioxidant or other mechanisms contributing to anti-carcinogenic action[13]. The antioxidants from natural sources are given preference than synthetic sources and the DPPH method has been widely applied for estimating antioxidant activities recently[14]. Research also indicates that the DPPH test is particularly suitable for the evaluation of antioxidant activity of crude extracts[15]. Besides, evaluation of the antioxidant polyphenols from less known ethno-medicinal plants from Asia are urgently needed[16]. In this paper, we present data on the evaluation of 23 plants collected from the lowland subtropical rainforest in southern Taiwan and determined the total polyphenol content and their antioxidant activity by 2,2-diphenyl-1,1-picryl-hydrazyl (DPPH) free radical scavenging assay against α-tocopherol.

EXPERIMENTAL

Collection of plant samples: The Mount Longevity where the plant samples were collected is located in Kaohsiung City adjacent to the Taiwan Strait (22°39' N, 120°15' E) and it has been isolated from other mountains over the last 50 years by to urban development. Its natural forest has been preserved since the beginning of the last century mainly because it was an active military base during the Japanese occupation of Taiwan. The flora of the study area includes 215 species in 73 families and 176 genera. According to the records of the Central Weather Bureau at Kaohsiung, the average annual precipitation between 1997 and 2001 was 2288 mm (SD ± 465), concentrated from June to August. The wet season, with monthly average above 100 mm, was from May to October. The dry season started in November and ended in March with a monthly average rainfall below 50 mm. The average monthly temperature was coldest in January (19.8 ºC) and highest in July (28.8 ºC). The average monthly humidity was above 75 % throughout the year.

Analysis of plant samples: Leaves from selected 23 plant species were collected for laboratory analysis. Plant species identifications were confirmed at the Herbarium of the Biological Sciences Department, National Sun Yat-sen University in Kaohsiung, Taiwan. Vitamin C (ascorbic acid), luminol (5-amino-2,3-dihydro-

1,4-phthalazinedione) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical reagent grade. Polyphenols in dry plant materials were determined spectrophotometrically using the Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction. To 0.2 mL of diluted aqueous acetone sample, 1 mL of Folin-Ciocalteu reagent (Merck, diluted 10 times with water) was added. After that, 0.8 mL of 7.5 % Na_2CO_3 was added and mixed thoroughly. After 0.5 h of standing, the absorbance was measured at 765 nm. The amount of total polyphenols was calculated as a chlorogenic acid equivalent from the calibration curve of chlorogenic acid standard solutions. All measurements were done in triplicate.

DPPH-free radical scavenging activity: The free radical-scavenging activity of polyphenols on the DPPH radical was assessed using modification of previously established methods[17-19]. A stock solution (1 mg/mL) of each extract was prepared and diluted with methanol to various concentrations. An aliquot of 50 µL of each dilution was transferred into a 96-well micro-plate (NUNC, Roskilde, Denmark). A working solution of DPPH (250 mM) in methanol was freshly prepared and then an aliquot of 150 mL was added to each well. After incubation for 0.5 h, the quenching at an absorbance of 490 nm was measured on an ELISA reader (Thermo Labsystems, Cheshire, UK). Each dilution was performed at least in triplicate. Free radical-scavenging activities of test samples and the positive control (vitamin C) were expressed in terms of IC_{50} values, which is the concentration of a sample required to decrease the absorbance at 490 nm by 50 % compared to the control response.

RESULTS AND DISCUSSION

The contents of polyphenols in 23 dry plant materials were estimated and shown in Table-1. The contents of polyphenols in 23 dry plant materials ranged from 2.5 to 164 mg/g in which top ten was in the order of *Acacia confuse, Macaranga tanarius, Euphoria longana, Mangifera indica, Ficus wightiana, Lantana camara, Pittosporum pentandrum, Leucaena lencocephala, Ficus septica* and *Pittosporum pentandrum*. Except for *Anredera cordifilia, Ophiopogn japonicus* and *Setaria palmifolia*, the other plants are able to eliminate significantly DPPH in 0.5 h as shown in Fig. 1 indicating that these plants possess free radical scavenging activity. In order to quantify the antioxidant activity, the IC_{50}, which is the concentration of sample required to decrease the absorbance at 490 nm by 50 %, was calculated and is shown in Table-1. The lower the IC_{50} value, the greater is the free radical-scavenging activity.

Seven plants with potent free radical scavenging activity which efficacy is in the order of *Macaranga tanarius, Acacia confusa, Euphoria longana, Mangifera indica, Lantana camara, Leucaena leucocephala* and *Ficus wightiana* based on the IC_{50} value calculated without extrapolation. By comparing the IC_{50} value of these 23 plants with that of an authentic antioxidant, Vitamin E, the antioxidant activity of all plants was less than that of Vitamin E. The results are due to Vitamin E is pure

TABLE-1
TOTAL POLYPHENOLS AND FREE RADICAL SCAVENGING ACTIVITY FOR 23 PLANT SPECIES FROM TAIWAN

Scientific name of plants	Total polyphenols (mg/g) (Chlorogenic acid equivalent)*	Free radical scavenging activity (μg/mL)**
Euphoria longana	125.9 ± 4.0	65.6 ± 2.9
Broussonetia papyrifera	34.4 ± 0.5	>250 (660.1 ± 50.4)[c]
Leucaena lencocephala	78.1 ± 1.9	169.3 ± 7.0
Passiflora suberosa	38.1 ± 1.3	>250 (674.8 ± 21.3)
Macaranga tanarius	145.2 ± 19.1	41.5 ± 1.3
Panicum repens	20.7 ± 0.4	>250 (715.7 ± 33.8)
Ficus wightiana	78.7 ± 2.6	225.2 ± 7.1
Lantana camara	78.7 ± 1.0	99.5 ± 0.6
Anredera cordifilia	18.0 ± 0.7	>250 (1572.9 ± 192.0)
Pittosporum pentandrum	76.8 ± 1.1	>250 (700.0 ± 18.1)
Synedrella nodiflora	25.2 ± 0.6	>250 (728.6 ± 11.2)
Ophiopogn japonicus	11.4 ± 1.4	>250 (4454.7 ± 217.6)
Ficus septica (leaf)	18.9 ± 0.6	>250 (1139.2 ± 11.3)
Mangifera indica	117.8 ± 3.8	71.5 ± 1.1
Ficus septica (fruit)	40.1 ± 1.6	>250 (415.1 ± 8.7)
Jasminum hemsleyi	19.6 ± 0.6	>250 (555.6 ± 2.2)
Bambusa *oldhami*	22.2 ± 0.8	>250 (1163.8 ± 72.7)
Acacia confusa	164.1 ± 6.8	52.1 ± 0.6
Pittosporum pentandrum	40.9 ± 0.5	>250 (616.0 ± 16.3)
Setaria palmifolia	6.9 ± 1.2	>250 (3361.4 ± 26.9)
Dioscorea doryphora	37.4 ± 1.9	>250 (337.0 ± 7.3)
Dolichos lablab L.	34.6 ± 1.8	>250 (709.6 ± 37.2)
Solanum capsicastrum	31.9 ± 1.9	>250 (884.7 ± 10.0)
Vitamin E (dl-tocopherol)	–	27.3 ± 1.2

*Total polyphenols were expressed as mg chlorogenic acid/g dry plant material.
**The antioxidant activity was evaluated as the concentration of the test sample required to decrease the absorbance at 490 nm by 50 % in comparison to the control response. The data in parenthesis were obtained from concentration-% DPPH remaining profile through extrapolation. Data are presented as the mean ± standard deviation (n= 3).

chemical but plant samples are mixture. In order to estimate correlations of results of polyphenolic contents and free radical scavenging activity, IC_{50} values from the DPPH free radical-scavenging assay were plotted against polyphenolic contents in 23 plant materials. As shown in Fig. 1, a good correlation with square of correlation coefficient (r) of 0.9109 was observed between polyphenolic contents and free radical scavenging activity in 23 plant materials. The results indicated that free radical scavenging potency of these plants is proportional to their polyphenolic contents.

Polyphenols are especially important antioxidants because of their high redox potentials that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers[20]. The analysis of total polyphenols in present study showed that

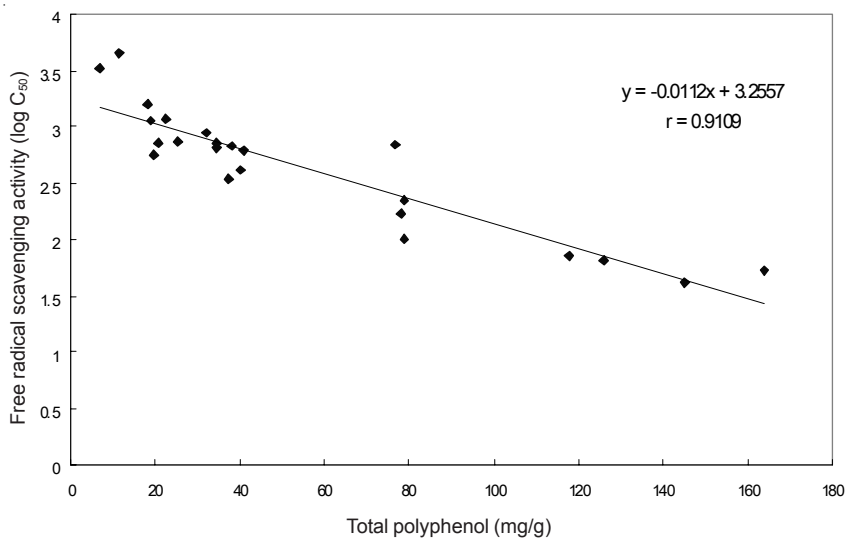

Fig. 1. Correlations of polyphenolic contents and free radical scavenging activity

most of the plants analyzed contained higher amount of polyphenolic contents. The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants[3,11,17,19]. Vascular plants as sources of medicinal compounds continue to play a dominant role in the maintenance of human health since ancient times. Over 50 % of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs of the pharmaceutical industry[19]. Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents[21]. Despite the fact that more research has been focused to derive drugs from medicinal plants to cure wide range of diseases, the antioxidant activities of many traditional medicinal plants have not been systematically studied in South East Asia due to lack of popularity. The compounds that possess antioxidant activity can inhibit mutation and cancer because they scavenge free radicals or induce antioxidant enzymes[22]. Many plant chemicals have been reported to have antioxidant, antiallergic, antiviral, anti-inflammatory, anti-proliferative and anti-carcinogenic properties.

Interest on plant derived food additives has grown enormously in recent years and herbs and spices usually used to flavour dishes are an excellent source of phenolic compounds that have been reported to show good antioxidant activity[13,23]. Plant extracts might substitute synthetic food antioxidants that may influence human health when consumed chronically[24]. Plant derived food additives, especially polyphenolic compounds have been ascribed health promoting properties in terms of preventing cardiovascular diseases[25].

The phenolic compounds are known to have direct antioxidant property due to the presence of hydroxyl groups, which function as hydrogen donor[26,27]. There have been many reports for the increase in antioxidant activity with increased total polyphenol content[28,29]. In present study, there was no increase in antioxidant activity observed with the increase in total polyphenol. A study conducted by Heo *et al.*[30] with enzymatic extracts of some brown seaweeds, some extracts did not possess antioxidant activity, although they contained similar phenolic content like other extracts studied. Similar findings were reported from some tropical plants consumed in South East Asia[31]. The reason for this may be due to differential response of these polyphenols to Folin-Ciocalteu reagent depending on the number of phenolic groups they possess[32].

In conclusion, Taiwan harbors nearly 4000 species of vascular plants with 20 % endemism and many of them have been found to be useful in traditional medicine[33]. The diversity flora therefore offers economic promise in the rapidly emerging biotechnology industry[34]. Therefore the evaluation of the constituents, pharmacological properties, detailed screening of bioactive substances for chemotherapeutic purposes are urgently required. Diet rich in vegetables and antioxidants may contribute to improved cognition and/or memory in the elderly persons. Present study confirms the potential free radical scavenging activities of Taiwan's plants that occur naturally in the lowland subtropical rainforest areas of southern Taiwan. These plants can further be investi-gated for their biological activity against inflammation, cardiovascular diseases, ulcers and cancers influenced by the production of free radicals.

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