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# Antioxidant Properties of Extracts from Medicinal Plants Popularly Used in Taiwan

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**Abstract:** We have examined antioxidant activities of twenty-six medicinal herbal extracts that have been popularly used as folk medicines in Taiwan. The results of scavenging DPPH radical activity show that, among the 26 tested medicinal plants, *Ludwigia octovalvis*, *Vitis thunbergii*, *Rubus parvifolius*, *Lindernia anagallis*, and *Zanthoxylum nitidum* exhibited strong activities and their $IC_{50}$ values for DPPH radicals were 4.6, 24, 27, 36, 50 μg/mL, respectively. As for the superoxide anion scavenging activity ($IC_{50}$, μg/mL), the top five most significant activities were observed in plant extracts of *Ludwigia octovalvis* (26 μg/mL), *Vitis thunbergii* (58 μg/mL), *Prunella vulgaris* (113 μg/mL), *Saurauia oldhamii* (124 μg/mL), and *Rubus parvifolius* (151 μg/mL). The $IC_{50}$ values for DPPH and superoxide anion of catechin (positive control) were 2.5 and 7.2 μg/mL, respectively. It was also observed in the present study that, at 1 mg/mL, *Ludwigia octovalvis* and *Bombax malabaricum* exhibited significant protection on ψx174 supercoiled DNA against strand cleavage induced by UV irradiated $H_2O_2$ with a superior or compatible effect to that of catechin.

**Keywords:** antioxidant activity; medicinal herbs; reactive oxygen species; *Ludwigia octovalvis*.

## 1. Introduction

Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases [1, 2]. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system; however, oxidative stress is generated when equilibrium favors free radical generation as a result of a depletion of antioxidant levels. The oxidation of lipid, DNA, protein, carbohydrate, and other biological molecules by toxic ROS may cause DNA mutation or/and serve to damage target cells or tissues, and this often results in cell senescence and death. Cancer chemoprevention by using antioxidant approaches has been suggested to offer a good potential in providing important fundamental benefits to public health, and is now considered by many clinicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the process of carcinogenesis [3, 4]. Moreover, knowledge and application of such potential antioxidant activities in reducing oxidative stresses *in vivo* has prompted many investi-

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gators to search for potent and cost-effective antioxidants from various plant sources [5-12]. These research activities have contributed to new or renewed public interests worldwide in herbal medicines, health foods, and nutritional supplements.

Botanicals have been used for treatment or prevention of various human diseases throughout history. The cancer chemopreventive activities of naturally occurring phytocompounds is of great interest. Many indigenous herbal plants of regional interest have been used popularly as folk medicines in Taiwan or other Asian countries; however, their bioactivities or pharmacological effects are remained to be elucidated. In this study, we investigated 26 selected, local putative medicinal plants for their potential antioxidant activities using scavenging free radical activity assays. Our results demonstrated that, among the tested plant extracts, *Ludwigia octovalvis* and *Vitis thunbergii* extracts exhibited most potent antioxidant activities are worthy of further investigation for their cancer chemopreventive properties.

## 2. Materials and methods

### 2.1. Chemicals

Chemicals and reagents, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-thiobarbituric acid (TBA), hypoxanthine, xanthine oxidase, nitroblue tetrazolium chloride (NBT), trichloroacetic acid (TCA), catechin, and ψx174 RF1 DNA, were purchased from Sigma Chemical Co. (MO, USA). Agarose was obtained from Bio-Rad (CA, USA). All other chemicals and solvents used in this study were of the reagent grade or HPLC grade.

### 2.2. Plant extracts preparation

Fresh or dried herbal plant materials were purchased from a reputable medicinal plant station in North Taiwan or collected from the Yang-Ming mountain area of Taiwan. Authenticity of the plant species was validated by the specific morphological and anatomical features of the flowers as reported by Li et al. [13]. The leaves of *Ginkgo biloba*, used as a reference plant, were collected in 1999, from the Experimental Forest of National Taiwan University, Taiwan. The air-dried and powdered whole plant materials were extracted twice with methanol. The total crude plant extracts were collected and evaporated to dryness by rotavapor.

### 2.3. Free radical scavenging activity

Scavenging activity of diphenyl-2-picrylhydrazyl (DPPH) radicals of plant extracts or catechin were measured according to the method reported by Chang et al. [14] with minor modifications. Assays were performed in 3 mL reaction mixtures containing 2.0 mL of 0.1 mM DPPH-ethanol solution, 0.9 mL of 50 mM Tris-HCl buffer (pH 7.4), and 0.1 mL of deionized $H_2O$ (as control) or test plant extracts. After 30 min of incubation at room temperature, absorbances of the reaction mixtures at 517 nm were taken. The inhibitory effect of DPPH was calculated according to the following formula:

Inhibition (%) = $[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{control}}] \times 100$

$IC_{50}$ represents the level where 50% of the radicals were scavenged by test samples.

### 2.4. Superoxide scavenging activity

Superoxide anion was generated by xanthine-xanthine oxidase and detected by the nitroblue tetrazolium (NBT) reduction method [15, 16]. Reagents in this study were prepared in 50 mM $KH_2PO_4$-KOH buffer, pH 7.4. The reaction mixture contained 20 μL of 15 mM $Na_2EDTA$ (pH 7.4), 50 μL of 0.6 mM NBT, 30 μL of 3 mM hypoxanthine, 50 μL of xanthine oxidase solution (1 unit in 10 mL buffer) and 150 μL of various concentrations of plant extracts as in-

dicated or 150 μL of $KH_2PO_4$-KOH buffer (as a control). The reaction was initiated by the addition of xanthine oxidase at 25 °C, and the absorbance at 405 nm were recorded every 20 seconds for 5 min using an ELISA reader (Labsystems Multiskan MS, USA). Results were expressed as percentage of inhibition relative to the control, given by [(rate of control – rate of sample reaction) / rate of control] × 100%.

### 2.5. Analysis of hydroxyl radical-induced DNA strand scission

This assay was done according to the method of Wang et al. [17]. The reaction mixture (30 μL) contained 0.3 μg ψx174 RF1 DNA, 30 mM $H_2O_2$, and 10 mM Tris-EDTA buffer (pH 8.0), in the presence or absence of plant extracts. One mg/mL of plant extracts dissolved in 10 μL ethanol were added to the reaction mixture prior to the addition of $H_2O_2$. Hydroxyl radicals were generated by irradiation of the reaction mixtures at a distance of 12 cm with a 12 W UV lamp (254 nm) (Spectroline, Spectronics Co., USA) at room temperature for 10 min. The reaction was terminated by addition of a loading buffer (0.02% bromophenol blue tracking dye and 40% sucrose), and the reaction mixtures were then analyzed by 0.8% submarine agarose gel electrophoresis. The gels were stained with ethidium bromide, destained with water, and photographed on a transilluminator. The inhibitory effect of plant extracts on the formation of open circular form (OC) DNA induced by hydroxyl radicals were calculated using densitometry.

## 3. Results and discussions

Scavenging activity for free radicals of 1.1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. Plant extracts from 26 medicinal plants listed in Table 1 were prepared for investigation of their antioxidant activities. Catechin, a major phenolic constituent of tea soup, was employed as the reference compound in this experiment. Free radical scavenging activity of total crude extracts from the 26 selected medicinal plants was quantitatively determined using a DPPH assay. The dosage of extract is expressed in μg of dry weight of the extract (compound) per mL of the assay mixture. $IC_{50}$ value represents the concentration of test extract or compound where the inhibition of test activity reached 50%. Plant extracts from Ludwigia octovalvis, Vitis thunbergii, Rubus parvifolius, Lindernia anagallii, and Zanthoxylum nitidum, ranked as the top five most active plant extracts among the 26, exhibited strong activity on scavenging DPPH radicals with the determined $IC_{50}$ values 4.6, 24.1, 27.2, 35.6, and 50.2 μg/mL, respectively (Table 2).

As the data shown in Figure 1, the inhibitory effect of the five test plant extracts on DPPH radicals followed dose-dependent manner. Another five plant extracts, i.e., Prunella vulgaris, Bombax malabaricum, Blumea balsamifera, Saurauia oldhamii, and Jassiaea repens, also possessed significant activity and their $IC_{50}$ values were between 68-103 μg/mL (Table 2). Nine other plant extracts were shown $IC_{50}$ below 200 μg/mL (105-187 μg/mL), and little antioxidant activity (>200 μg/mL) was observed for seven medicinal plants (Table 2). The $IC_{50}$ value for DPPH of catechin was 2.5 μg/mL. Ginkgo biloba extract is known to exhibit a high level of antioxidant activity [18], and this in turn has been speculated to contribute to clinical efficacy of this popular medicinal herb. The $IC_{50}$ of Ginkgo biloba leaf extract was estimated to be 930 μg/mL, in a parallel study. Most of the plant extracts investigated in this report exhibited higher scavenging DPPH activity than that of G. biloba.

Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have theirinitial

effects magnified because they produce other kinds of cell-damaging free cals and oxidizing agents, e.g.,radi hydroxyl radicals [1]. The superoxide anion scavenging activities of the 26 plant extracts were also investigated and compared by using a NBT reduction method.

**Table 1.** Twenty-six medicinal plants studied

| Plant names (Family) | Plant names (Family) |
|---|---|
| *Emilia sonchifolia* (Compositae) | *Justicia procumbens* (Acanthaceae) |
| *Adenostemma larenia* (Compositae) | *Saurauia oldhamii* (Actinnidiaceae) |
| *Blumea balsamifera* (Compositae) | *Bombax malabaricum* (Bombacaceae) |
| *Vernonia patula* (Compositae) | *Kyllinga brevifolia* (Cyperaceae) |
| *Ixeris chinensis* (Compositae) | *Cajanus cajan* (Leguminosae) |
| *Tithonia diversifolia* (Compositae) | *Indian pipe* (Orobanchaceae) |
| *Siegesbeckia orientalis* (Compositae) | *Rubus parvifolius* (Rosaceae) |
| *Leucas mollissima* (Labiatae) | *Zanthoxylum nitidum* (Rutaceae) |
| Plectlanthus amboinicus (Labiatae) | *Lindernia anagallis* (Scrophulariaceae) |
| *Prunella vulgaris* (Labiatae) | *Solanum verbascifolium* (Solanaceae) |
| *Sida rhombifolia* (Malvaceae) | *Pteris multifda* (Pteridaceae) |
| *Hibiscus taiwanensis* (Malvaceae) | *Vitis thunbergii* (Vitaceae) |
| *Jassiaea repens* (Onagraceae) | |
| *Ludwigia octovalvis* (Onagraceae) | |

**Table 2**. Free radical (DPPH) scavenging activity of the 26 medicinal plant extracts

| Names | $IC_{50}$ μg/ml | Names | $IC_{50}$ μg/ml |
|---|---|---|---|
| Catechin | 2.5 | | |
| *Ludwigia octovalvis* | 4.6 | *Kyllinga brevifolia* | 139 |
| *Vitis thunbergii* | 24.1 | *Cajanus cajan* | 143 |
| *Rubus parvifolius* | 27.2 | *Solanum verbascifolium* | 149 |
| *Lindernia anagallii* | 35.6 | *Plectlanthus amboinicus* | 155 |
| *Zanthoxylum nitidum* | 50.2 | *Indian pipe* | 164 |
| *Prunella vulgaris* | 68 | *Siegesbeckia orientalis* | 187 |
| *Bombax malabaricum* | 68 | *Vernonia patula* | >200 |
| *Blumea balsamifera* | 74 | *Sida rhombifolia* | >200 |
| *Saurauia oldhamii* | 102 | *Pteris multifda* | >200 |
| *Jassiaea repens* | 103 | *Emilia sonchifolia* | >200 |
| *Ixeris chinensis* | 105 | *Justicia procumbens* | >200 |
| *Adenostemma larenia* | 114 | *Hibiscus taiwanensis* | >200 |
| *Tithonia diversifolia* | 127 | *Leucas mollissima* | >200 |
| | | | |

Each assay was performed in triplicate.
Catechin was used as a reference compound of this experiment.

The six most potent activities ($IC_{50}$, μg/mL) were observed in the plant extracts of Ludwigia octovalvis (25.9 μg/mL), Vitis thunbergii (58.4 μg/mL), Prunella vulgaris (113 μg/mL), Saurauia oldhamii (124 μg/mL), Rubus parvifolius (151 μg/mL), and Jassiaea repens (159 μg/mL) (Table 3). Figure 2 shows that the six plant extracts exhibited dose-dependent inhibition on superoxide anion. The other twenty plant extracts were shown little or no detectable effect on scavenging superoxide anion (data not shown).

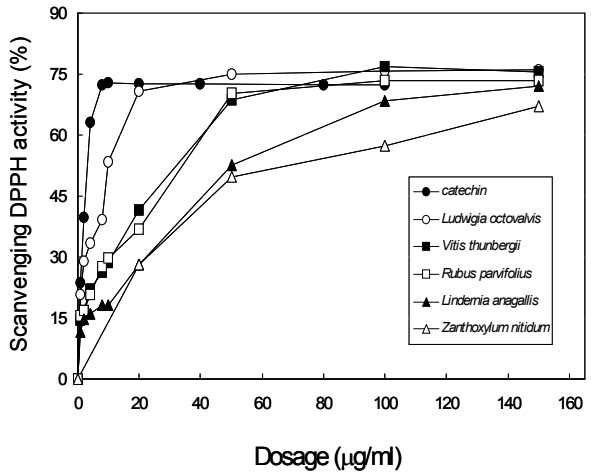


**Figure 1.** Free radical scavenging activities of total crude extract of *Ludwigia octovalvis* (○), *Vitis thunbergii* (■), *Rubus parvifolius* (□), *Lindernia anagallii* (▲), and *Zanthoxylum nitidum* (△) measured using the DPPH assay. Catechin (●) was used as a reference control in this experiment. Each assay was performed in triplicate.

In biochemical systems, superoxide radical can be converted into hydrogen peroxide by the action of superoxide dismutase and the $H_2O_2$ can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions (e.g., iron and copper), or by UV photolysis [1]. Hydroxyl radicals can attack DNA molecules to cause strand scission [1]. The following seven plant species possessed the most potent scavenging DPPH radical activity, i.e., Ludwigia octovalvis, Vitis thunbergii, Rubus parvifolius, Lindernia anagallii, Zanthoxylum nitidum, Prunella vulgaris, and Bombax malabaricum. These were evaluated for their effect on protection of ψx174 RF1 DNA against the cleavage of hydroxyl radicals generated from UV irradiated $H_2O_2$. Supercoiled DNA irradiated with UV (lane II) or treated with $H_2O_2$ alone (lane III) exhibited no DNA cleavage as indicated by the formation of open circular form (OC) DNA, relative to the native untreated DNA (lane I). Treating the native DNA with both $H_2O_2$ and UV caused the supercoiled form of DNA converting to OC DNA (lane IV). The OC DNA content in lane IV was expressed as 1 (100%). At a dose of 1 mg/mL, the magnitude of the inhibition of OC DNA formation by the test plant extracts was in the following increasing order: Ludwigia octovalvis (0.17) > Bombax malabaricum (0.35) > catechin (0.38) > Prunella vulgaris (0.46) > Rubus parvifolius (0.52) = Lindernia anagallii (0.53) > Vitis thunbergii (0.76) > Zanthoxylum nitidum (0.78). Ludwigia octovalvis extract could virtually completely inhibit the DNA damage casused by hydroxyl radical.

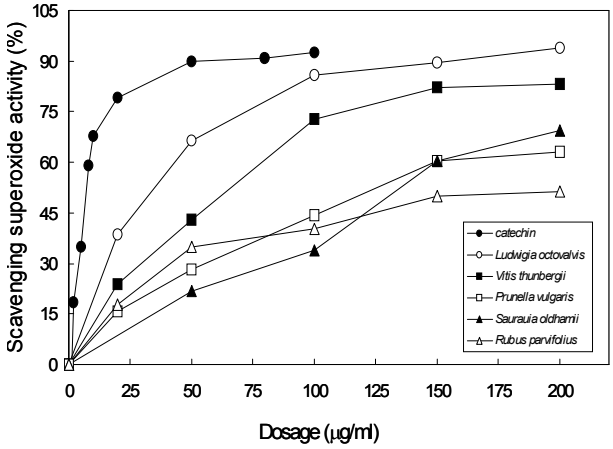


**Figure 2.** Superoxide scavenging activities of total crude extract of *Ludwigia octovalvis* (○), *Vitis thunbergii* (■), *Prunella vulgaris* (□), *Saurauia oldhamii* (▲), and *Rubus parvifolius* (△) measured using the NBT assay. Catechin (●) was used as a reference control in this experiment. Each assay was performed in triplicate.

**Table 3**. Superoxide anion scavenging activity of the 26 medicinal plant extracts

| Names | $IC_{50}$ μg/ml | Names | $IC_{50}$ μg/ml |
|---|---|---|---|
| Catechin | 7.2 | | |
| *Ludwigia octovalvis* | 25.9 | *Emilia sonchifolia* | >200 |
| *Vitis thunbergii* | 58.4 | *Kyllinga brevifolia* | >200 |
| *Prunella vulgaris* | 113 | *Sida rhombifolia* | >200 |
| *Saurauia oldhamii* | 124 | *Solanum verbascifolium* | >200 |
| *Rubus parvifolius* | 151 | *Indian pipe* | >200 |
| *Jassiaea repens* | 159 | *Plectlanthus amboinicus* | >200 |
| *Justicia procumbens* | >200 | *Leucas mollissima* | >200 |
| *Bombax malabaricum* | >200 | *Adenostemma larenia* | >200 |
| *Hibiscus taiwanensis* | >200 | *Blumea balsamifera* | >200 |
| *Cajanus cajan* | >200 | *Vernonia patula* | >200 |
| *Lindernia anagallis* | >200 | *Ixeris chinensis* | >200 |
| *Zanthoxylum nitidum* | >200 | *Tithonia diversifolia* | >200 |
| *Pteris multifda* | >200 | *Siegesbeckia orientalis* | >200 |

Each assay was performed in triplicate.
Catechin was used as a reference compound of this experiment.

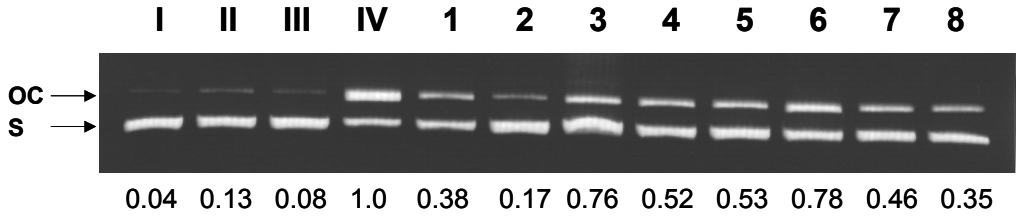


**Figure 3.** Protection effect of plant extracts from *Ludwigia octovalvis*, *Vitis thunbergii*, *Rubus parvifolius*, *Lindernia anagallii, Zanthoxylum nitidum*, *Prunella vulgaris,* and *Bombax malabaricum* on φX174 RF1 DNA strand scission induced by UV photolysis of $H_2O_2$. Results were analyzed by 0.8% agarose gel electrophoresis. Lane I: native supercoiled DNA sample without any treatment; Lane II to IV: DNA samples treated with UV irradiation alone, $H_2O_2$ alone, and with $H_2O_2$ and UV irradiation, respectively. Lanes 1-8: DNA samples treated with $H_2O_2$ + UV irradiation in the presence of 1mg/mL of catechin, *L. octovalvis*, *V. thunbergii*, *R. parvifolius*, *L. anagallii, Z. nitidum*, *P. vulgaris,* and *B. malabaricum*, respectively. Arrows indicate distinct forms of the DNA: OC (open circular) and S (supercoiled). The OC DNA content in lane IV was expressed as 1, and the numbers for OC DNA content indicated in other lanes are the values relative to that of lane IV, calculated using densitometry.

In summary, we observed that some of our selected plant extracts could dose-dependently and significantly inhibit free radical and superoxide anion. In addition, DNA damage by hydroxyl radicals could also be effectively prevented in the presence of specific plant extracts. Very little or undetected antioxidant activities were observed in the plant extracts from Vernonia patula, Sida rhombifolia, Pteris multifda, Emilia sonchifolia, Justicia procumbens, Hibiscus taiwanensis, and Leucas mollissima. Our results in the present report suggest that the plant extracts we have examined such as those from Ludwigia octovalvis and Vitis thunbergii, which exhibited potent antioxidant effect in vitro, can serve as good candidates for further evaluation of their bio-efficacies, active constituents, and molecular and biological mechanisms in vitro as well as in vivo on antioxidation or cancer chemoprevention effects. Possible applications of the selected target plant extracts as food supplement for human health care are also under evaluation.

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