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Studies on antioxidant capacity of *Prunella vulgaris*

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ABSTRACT

Prunella vulgaris is a native traditional anti-bacterial herb for internal and external wounds healing in Taiwan. Results in this study show that the ethanol extract of *Prunella vulgaris* exhibited a strong ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals, the IC_{50} values for DPPH and hydroxyl radicals scavenging of the ethanol crude extract are 76.45 ± 1.54μg/mL and 0.75mg/mL, respecally. On the other hand, the ethanol only showed slightly eliminating activity of ABTS radical cation on TEAC analysis. In summary, these data indicated that *Prunella vulgaris* is a potential ROS scavenger.

Keywords: *Prunella vulgaris*, DPPH, Trolox equivalent antioxidant capacity (TEAC), hydroxyl radical

摘要：

夏枯草台灣民間用來治療外傷的中草藥，其具有抗菌、抗病毒、及增強免疫力等作用，因此其極具開發之潛能。本研究結果顯示夏枯草的乙醇萃取物可以清除 1,1-diphenyl-2-picrylhydrazyl (DPPH) 和氫氧自由基，其 IC_{50} 分別為 76.45 ± 1.54μg/mL 和 0.75mg/mL。在 TEAC 分析方面，夏枯草的乙醇萃取物清除 ABTS 能力與標準品 trolox 相較之下並不佳。總括來看，夏枯草萃出物具有抗氧化能力，值得進一步研究和確認其指標成分。

關鍵字：夏枯草，DPPH, TEAC, 氫氧自由基

INTRODUCTION

Hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\cdot -}$), and hydroxyl radicals ($OH^{\cdot}$), collectively known as the reactive oxygen species (ROS), are the most reactive species derived from the metabolism of oxygen in aerobic systems (Gutteridge, 1994). Classically oxidative stress is described as an imbalance between generation and elimination of ROS. Oxidative stress plays a prominent role in the pathogenesis of many diseases such as respiratory distress syndrome, ischaemia/reperfusion injury, renal failure, rheumatoid arthritis, local or systemic inflammatory disorders, diabetes, atherosclerosis, cancer and neurodegenerative diseases, such as Alzheimer's disease (Aruoma, Kaur & Halliwell, 1991; Emerit, Edeas & Bricaire, 2004). It is well-known that oxidative stress-induced damages disrupt cellular function and membrane integrity, thereby leading to apoptosis (3). As the major component of ROS, H_2O_2 has been extensively used as an inducer of oxidative stress in many *in vitro* models, this effect can be inhibited by the addition of natural antioxidants (Colognato et al., 2006; Heo & Lee, 2005; Pavlica & Gebhardt, 2005).

Dietary intake of naturally occurring antioxidants, which scavenge free radicals, may be effective to prevent or treat atherosclerosis. This is the reason for the current strong interest in natural antioxidants and their roles in human health and nutrition (Enkhmaa et al., 2005; Fuhrman et al., 1997; Koyama et al., 2006; Miura et al., 1995; Yamakoshi, Kataoka, Koga & Ariga, 1999). Self heal has a long history of folk use, especially in the treatment of wounds, ulcers, sores etc. It was also taken internally as a tea in the treatment of fevers, diarrhoea, sore mouth, internal bleeding etc. In Korea it is used to treat oedema, nephritis, scrofula and goitre (Ciraj et al., 2001). The whole plant is alterative, antibacterial, antipyretic, antiseptic, antispasmodic, astringent, carminative, diuretic, febrifuge, hypotensive, stomachic, styptic, tonic, vermifuge and vulnerary (Enkhmaa et al., 2005; Fuhrman et al., 1997; Koyama et al., 2006; Miura et

al., 1995; Yamakoshi, Kataoka, Koga & Ariga, 1999). It has an antibacterial action, inhibiting the growth of *Pseudomonas, Bacillus typhi, E. coli*, Mycobacterium tuberculi etc. It can be used fresh or dried, for drying it is best harvested in mid-summer. The plant is experimentally antibiotic and hypotensive (Aruoma, 1991).

Prunella vulgaris L. (Labiatae), a popular Chinese and Western herbal medicine, has long been associated with anti-viral and anti-bacterial effects. While its anti-viral effects are attributed mainly to the inhibition of virus replication, the biological mechanisms of its anti-bacterial effects remain unknown. As a biological response modifier, the polysaccharides isolated from *P. vulgaris* have been shown to up-regulate the immune responses of monocytes/macrophages (Xuya, 2005).

The aim of this study is to assess the antioxidant activities of *Prunella vulgaris* using DPPH scavenging activity, TEAC and hydroxyl radical scavenging activity as index. Results of the present study facilitate our understanding of the antioxidant effects of *Prunella vulgaris* and its bioactive constituents.

MATERIALS AND METHODS

Plant

Prunella vulgaris L. (Labiatae) was purchased from yi-man traditional Chinese herbal store, Kaohsiung, Taiwan.

Extraction and fractionation

The baked dry *Prunella vulgaris* (50 g) was ground into powder and extracted with ethanol (0.5 L). The combined ethanol extract was lyophilized to yield a dark-brown powder. A small portion of the ethanol extract was redissolved in DMSO prior to use and was denoted as the crude extract.

DPPH scavenging capacities

The crude extract and different layers were evaluated for their activities to scavenge the stable DPPH radical (0.1 mM, Sigma Chemical, St. Louis, MO, USA) according to the method (Dinis, Maderia & Almeida, 1994). The affinity of the test material to quench the DPPH free radical was evaluated according to the equation: scavenging % = $(A_c-A_s)/A_c \times 100\%$. A_s and A_c are absorbance at 517 nm of the reaction mixture with sample and control, respectively. The IC_{50} values were obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to scavenge 50% of DPPH radicals. All experiments were repeated at least three times.

Trolox equivalent antioxidant capacity (TEAC) analysis

The ABTS radical cation was prepared by mixing an ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture has to remain for 12–24 h until the reaction is complete and the absorbance is stable. For measurements the $ABTS^{2+}$ solution was diluted to an absorbance of 0.700- 0.020 at 734 nm. 1 ml of the $ABTS^{2+}$ solution and 100 μl antioxidant solution were mixed for 45 s and the absorbance at 734 nm was recorded after 1 min of incubation. TEAC is defined as the concentration (mg) of Trolox having the antioxidant equivalent to a 1.0 g of the sample under investigation. To calculate the TEAC, the gradient of the plot of the percentage inhibition of absorbance vs. concentration plot for the antioxidant in question is divided by the gradient of the plot for Trolox (Re et al., 1999).

Hydroxyl radical scavenging capacity analysis

The hydroxyl radical scavenging effects of the ethanol extract of *Prunella vulgaris* was measured using a modified luminol-enhanced CL method (Lei et al., 2003). Briefly, the reaction was carried out in a 100 μL mixture containing 5 mM luminol (in PBS), ferrous chloride (40 μM), 1% H2O2, and vehicle, various

concentrations of GT or its major constituents. The hydroxyl-induced luminol CL during the first minute was averaged. The inhibitory efficiency in response to the CL of vehicle control was calculated.

Statistical Analysis

All experiments were repeated at least three times.

RESULTS AND DISCUSSION

50 g dried *Prunella vulgaris* was extracted with 500 mL ethanol at room temperature for 18 hours. The ethanol extract was centrifuged at 8000 rpm for 20 min (Sorval RC-5B, rotor SS-34). The obtained precipitate was lyophilized to yield 1.02g powder.

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging assay and Trolox equivalent antioxidant capacity (TEAC) are two widely used methods to evaluate antioxidant capacity in a short time (Blois, 1958; Re et al., 1999). The ethanol extract of *Prunella vulgaris* also exerted dose-dependent DPPH free radical bleaching activity. The IC_{50} values for DPPH scavenging of the ethanol crude extract is 76.45 ± 1.54μg/mL (Table 1). The Trolox equivalent antioxidant capacity (TEAC) values of the crude extract is 89.86 mg/g. The result of DPPH scavenging activity indicates that the antioxidant compounds may exist in the ethanol extract of *Prunella vulgaris.*

Table 2 shows that the extract inhibited Fenton-mediated hydroxyl radical production. The estimated IC50 values are 0.75 mg/mL. This result indicated that the ethanol extract may have components responsible for the ROS scavenging activity. The traditional function of *Prunella vulgaris* may be therefore attributed in part to its antioxidant activity

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Table 1. The DPPH scavenging potentials of the ethanol extract of *Prunella vulgaris*

Concentration	The percentage of DPPH inhibition (%)
100mg/mL	60.30
50mg/mL	29.99
10mg/mL	6.90
1mg/mL	2.02

Table 2. The hydroxyl radical-elicited chemiluminescence (CL) of the ethanol extract of *Prunella vulgaris*.

Concentration	The percentage of hydroxyl radical-elicited CL (%)
1mg/ml	35.56
0.75mg/ml	56.99
0.5mg/ml	66.15
0.25mg/ml	108.80

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