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Evaluation of Antioxidant Activities of Rice Paddy Herb (*Limnophila aromatica* (Lam.) Merr.)

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Abstract**—**Free radicals are atoms or molecules with unpaired electrons. Many diseases are caused by free radicals. Normally, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Several analytical methods have been used for qualitative and quantitative determination of antioxidants, and each has its own specificity. This project aimed to evaluate antioxidant activity of ethanolic and aqueous extracts from the rice paddy herb (*Limnophila aromatica* (Lam.) Merr.) measured by DPPH and Hydroxyl radical scavenging method. The results showed that averaged antioxidant activity measured in ethanolic extract (µmol Ascorbic acid equivalent/g fresh mass) were 67.09± 4.99 and 15.55±4.82 as determined by DPPH and Hydroxyl radical scavenging activity assays, respectively. Averaged antioxidant activity measured in aqueous extract (µmol Ascorbic acid equivalent/g fresh mass) were 21.08±1.25 and 10.14±3.94 as determined by DPPH and Hydroxyl radical scavenging activity assays respectively.

Keywords**—**Free radical, antioxidant, rice paddy herb, *Limnophila aromatica* (Lam.) Merr.

I. Introduction

PEOPLE have an interest in health and beauty as can be seen by the number of researches of beneficial substances and toxic substances, especially on the research of free radicals and antioxidants. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Some free radicals arise normally during metabolism. Sometimes the body's immune system's cells purposefully create them to neutralize viruses and bacteria. However, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also spawn free radicals. Theses free radicals can get rid of or reduced their toxin with antioxidants. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-"stealing" reaction. The antioxidant nutrients themselves do not become free radicals by donating an electron because they are stable in either form; they act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and disease. There are 2 types of antioxidants: the synthetic and the natural one. The natural antioxidants are believed to be safer than the synthetic. They can be found in Vitamin C, E, and Beta-carotene which can be found in microbes, animals, and plants. Antioxidants do not give any nutritional value due to its composition structure of phenolic compounds such as xanthone and flavonoid. Because plants are the main source of antioxidants, this research investigates the potential of rice paddy herb which is a consumed vegetable. Rice paddy herb contains high amount of antioxidants which play an important role in getting rid of hydroxyl radicals considered to be the free radicals causing several serious diseases such as cancer, heart disease, diabetes, etc. [1]. This project aimed to evaluate antioxidant activity of ethanolic and aqueous extracts from rice paddy herb measured by DPPH and Hydroxyl radical scavenging method.

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II. Materials and Method

A. Plant Material

Limnophila aromatica (Lam.) Merr. was purchased from the Bangkok province of Thailand.

B. Chemicals and Reagents

DPPH (α, α-Diphenyl-β-picrylhydrazyl), $FeSO_4.7H_2O$, 1, 10-phenanthroline and L-ascorbic acid were purchased from Sigma Aldrich Co., Ltd. Ethanol was purchased from Merck Co., Ltd. All other solvents and chemicals were of analytical grade.

C. Preparation of Plant Extracts

The plant samples were ground in a mortar. Slurries were then extracted with distilled water or ethanol then were centrifuged at 13,000g for 10min and the supernatants were collected.

D. Antioxidant Activities

The DPPH radical-scavenging activity of samples was monitored according to the method of Luo et al. [2]. Briefly, a 2.0ml aliquot of test sample (0.2, 0.4, 0.6, 0.8 and 1g/ml) was added 2.0ml of 0.16×10^{-3} mol/l DPPH ethanolic solution. The mixture was vortexed for 1min and then left to stand at room temperature for 30min in the dark, and its absorbance was measured at 517nm. The ability to scavenge DPPH radical was calculated using the following equation:

DPPH radical scavenging activity (%) =
[1 – (A sample – A sample blank)/A control] × 100

where the A control is the absorbance of the control (DPPH solution without sample), the A sample is the absorbance of the test sample (DPPH solution plus test sample), and the A sample blank is the absorbance of the sample only (sample without DPPH solution). L-ascorbic acid was used as positive controls.

The hydroxyl radical-scavenging activity of samples was measured according to the method of the previous report [3]-[5] with some modifications. In this system, hydroxyl radicals were generated by the Fenton reaction. Hydroxyl radicals could oxidize Fe^{2+} into Fe^{3+}, and only Fe^{2+} could be combined with 1, 10-phenanthroline to form a red compound (1,10-phenanthroline-Fe2+) with the maximum absorbance at 536 nm. The concentration of hydroxyl radical was reflected by the degree of de-colorization of the reaction solution. Briefly, 1, 10-phenanthroline solution (1.0ml, 1.865×10^{-3} mol/l), phosphate buffer saline (2.0ml, 0.2 mol/l, pH 7.40), and samples (1.0ml, 0.06, 0.13, 0.25, 0.5 and 1 g/ml) were added into a screw-capped tube orderly and mixed homogeneously. The $FeSO_4.7H_2O$ solution (1.0ml, 1.865×10^{-3} mol/l) was then pipetted into the mixture. The reaction was initiated by adding 1.0 ml H_2O_2 (0.03% v/v). After incubation at 37°C for 60 min in a water bath, the absorbance of reaction mixture was measured at 536 nm against reagent blank. The reaction mixture without any antioxidant was used as the negative control, and without H_2O_2 was used as the blank. The hydroxyl radical scavenging activity was calculated by the following formula:

Hydroxyl radical scavenging activity (%) = [(As-An)/(Ab-An)] × 100

where As, An, and Ab were the absorbance values determined at 536 nm of the sample, the negative control, and the blank after reaction, respectively. L-ascorbic acid was used as positive controls.

II. Results and Discussion

The antioxidant activities of extracts from rice paddy herb on DPPH and hydroxyl radicals, expressed as DPPH radical scavenging activity (%) and hydroxyl radical scavenging activity (%), were showed in Figs. 1-4. The antioxidant activities were found to be dose-dependent.

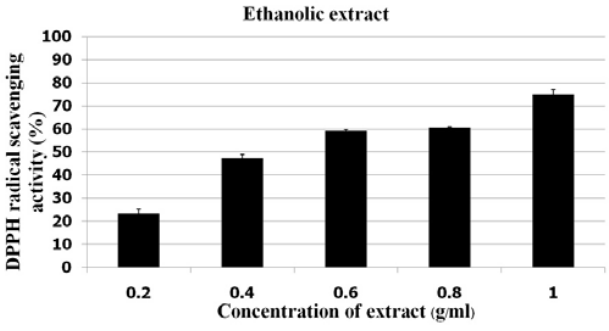

Fig. 1 DPPH radical scavenging activity (%) of ethanolic extracts from rice paddy herb. Data points shown are averages of three measurement ± SE

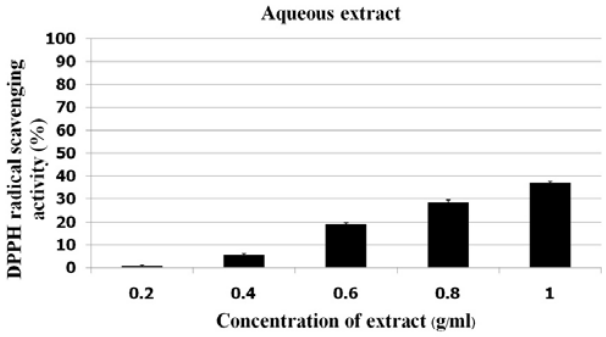

Fig. 2 DPPH radical scavenging activity (%) of aqueous extracts from rice paddy herb. Data points shown are averages of three measurement ± SE

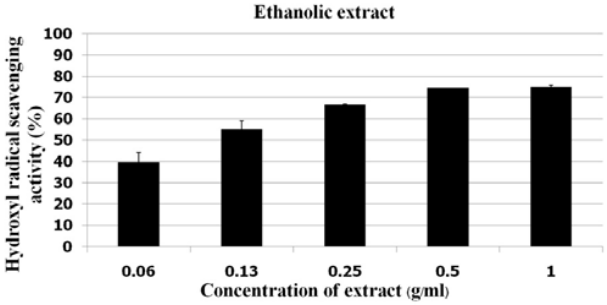

Fig. 3 Hydroxyl radical scavenging activity (%) of ethanolic extracts from rice paddy herb. Data points shown are averages of three measurement ± SE

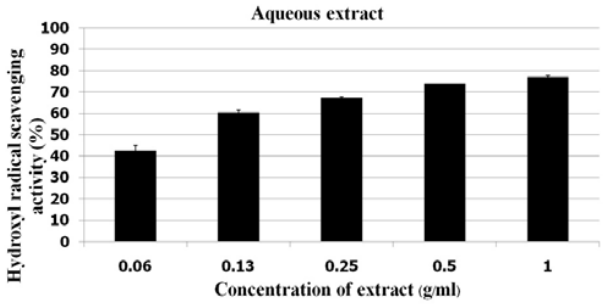

Fig. 4 Hydroxyl radical scavenging activity (%) of aqueous extracts from rice paddy herb. Data points shown are averages of three measurement ± SE.

III. Conclusion

To evaluate antioxidant activity of ethanolic and aqueous extracts from rice paddy herb measured by DPPH and Hydroxyl radical scavenging method, the results show that the averaged antioxidant activity measured in ethanolic extract (µmol Ascorbic acid equivalent/g fresh mass) were 67.09± 4.99 and 15.55±4.82 as determined by DPPH and Hydroxyl radical scavenging activity assays respectively. Averaged antioxidant activity measured in aqueous extract (µmol Ascorbic acid equivalent/g fresh mass) were 21.08±1.25 and 10.14±3.94 as determined by DPPH and Hydroxyl radical scavenging activity assays respectively.

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