Research Article

Free radical scavenging capacity and modulative effect on antioxidant enzyme activity *of Pluchea indica* Less. tea leaf extract

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Abstract

The aim of the present study is to investigate the free radical scavenging and modulative effect on the antioxidant enzyme activity of *Pluchea indica* Less. tea leaf extract. Superoxide and hydroxyl radical scavenging activities were measured. The result showed that the percentage of free radical inhibition markedly increased both on superoxide and hydroxyl radicals in concentration-dependent manners. IC_{50} levels against superoxide and hydroxyl radicals were 265.84 ± 20.55 µg/mL, and 161.91 ± 4.94 µg/mL, respectively. Moreover, the activities of 1 unit/mL of catalase (CAT) and glutathione peroxidase (GPx) were assayed with different concentrations of the extract for 30 minute incubation. The increase of catalase activity in a concentration-dependent profile was observed but it did not alter in case of glutathione peroxidase activity.

Keywords: *Pluchea indica* Less. tea leaf extract, free radical scavenging, antioxidant enzyme

Introduction

Pluchea indica Less. is a widespread medicinal plant that grows naturally in littoral areas of many countries, including Thailand. The leaf of the plant issued to treat dysentery, rheumatism, leucorrhea, bad breath and body odor. The roots are used to treat fever, lumbago, indigestion and headache. [1] A decoction of the leaf has been used to combat fever. A poultice of leaf is applied externally to treat ulcers and soothe sores. [2] *P. indica* leaf is used in the preparation of an herbal tea consumed for promoting good health. [3] A collection of preliminary data from community enterprise in Khlung district, Chanthaburi province, Thailand have found that the local people make liquid soap, spa body scrub powder and facial scrub. *P. indica* has also been taken as herbal tea product (dried leaf), but they are still lack of scientific evidence to support its benefit.

Therefore, the aim of the study was to demonstrate free radical scavenging and modulative effects on the antioxidant enzyme activity of extract obtained from *P. indica* leaf.

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Material and Method

Preparation of extract

P. indica herbal tea (dried leaf) was purchased from community enterprise at Khlung district, Chanthaburi province, Thailand. Then, it was ground to powder using a grinder. Powdered sample (100 g) was added to boiling distilled water (1 L), boiled for 30min. Water extract was filtered by Whatman No.1 with vacuum pump and then centrifuged at 3,000 rpm for 10 min at 25ºC. The remaining residues were re-extracted with boiling distilled water as describe above. The collected filtrate was evaporated by rotary evaporator and dried in a freeze-dryer. The extract was weighed, stored at -20ºC and protected from light until used.

Superoxide radical (O_2^-) scavenging activity

Superoxide radical scavenging activity assay was measured by the pyrogallol autoxidation according to the method of Wang et al [4] with some modifications. 5.6 mL of 50 mM Tris–HCl buffer pH 8.2 and 0.2 mL of samples with different concentrations (31.25, 62.5, 125, 250 and 500 µg/mL) were mixed together. The mixture was then incubated for 10 min in water bath at 25°C. Meanwhile, 0.2 mL of 0.95 mM pyrogallol preheated at 25°C was added immediately. The absorbance of sample and control were determined by UV/visible spectrophotometer at 325 nm. The control reaction mixture did not contain extracted sample. Gallic acid (31.25, 62.5, 125, 250 and 500 µg/mL) was used as a positive control.

Hydroxyl radical (OH°) scavenging activity

The hydroxyl radical scavenging activity assay was carried out by the deoxyribose method. The assay was performed as described by Halliwell, Gutteridge and Aruoma [5] with some minor modifications. All solutions were freshly prepared. The reaction mixture contained 100 µL of 2.8 mM 2-deoxy-D-ribose, 100 µL of 2.8 mM H_2O_2, 100 µL of 25 mM $FeCl_3$, 100 µL of 100 µM EDTA and 500 µL of samples with different concentrations.

The reaction was activated by adding 100 µL of 100 µM ascorbic acid and the reaction mixture was incubated for 1 h at 37°C in an incubator. After that, the color was developed by adding 1 mL of 1% thiobarbituric acid and 1 mL of 2.8% ice-cold trichloroacetic acid and heating in boiling water bath (80°C) for 20 min. The sample was cooled and the absorbance was measured by the microplate reader at 532 nm. The control reaction mixture did not contain the extracted sample. Butylated hydroxytoluene (BHT) was used as the positive control.

Catalase activity assay

Catalase (CAT) activity was determined using the method of Fossati, Prencipe and Berti. [6] The 1x assay buffer 55 µL and sample 20 µL were added to the 1.5 mL microcentrifuge tube. The reaction was started by addition of 25 µL of 200 mM hydrogen peroxide solution mixed by inversion and incubated for 5 min. 900 µL of the stop solution was added and inverted the tube. 10 µL of the solution was then transferred to a new microcentrifuge tube. 1 mL of the color reagent (30 mL of chromogen solution with 30 µL of peroxidase solution) was added. The reaction was mixed and incubated at room temperature for 15 min. The color development was measured spectrophotometrically at 520 nm. One unit of

catalase activity was defined as the amount of enzyme decomposing 1 μmol hydrogen peroxide per minute under assayed conditions.

Glutathione peroxidase activity assay

Glutathione peroxidase (GPx) was determined using the method of glutathione peroxidase assay kit (Sigma-Aldrich). 900 μL of glutathione peroxidase, 50 μL of NADPH assay reagent and 20 μL of sample were added to the cuvette and mixed by inversion. The reaction was started by addition of 10 μL of the 30 mM *t*-butyl hydroperoxide solution. The decrease of NADPH was then monitored by measuring an absorbance at 340 nm. One unit of glutathione peroxidase activity was defined as the amount of enzyme causing the formation of 1 μmol of $NADP^+$ per minute under assayed conditions.

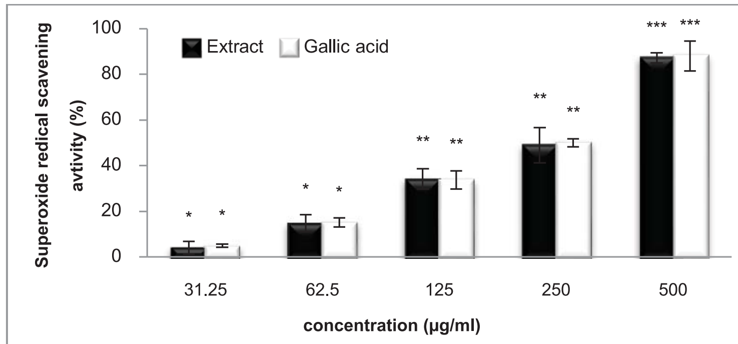
Statistical analysis

All data were expressed as mean values ± standard deviation (n=3). The statistical analysis of data used the one-way ANOVA. When ANOVA results showed statistically significant differences, post hoc testing was performed for intergroup comparisons using Tukey's HSD test. The inhibitory concentration 50% (IC_{50}) was calculated from concentration/effect regression line. The p value <0.05 was considered as statistically significant for the analyzed data. All statistical calculations were computed by the Statistical Package for Social Science (SPSS) version 17.0.

Results

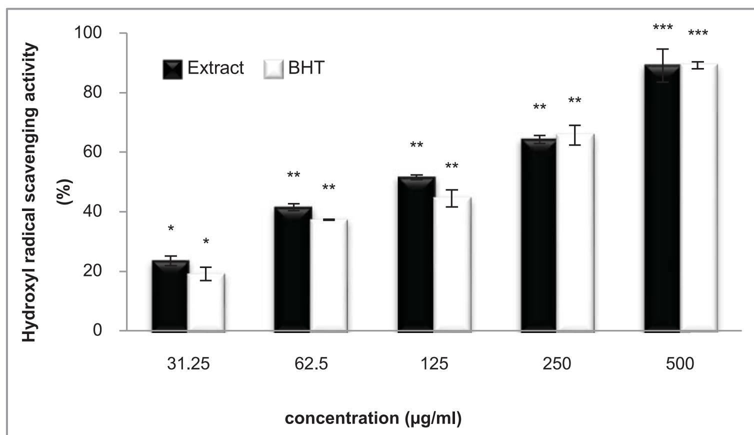
The study was to examine the free radical scavenging and modulative effect on the antioxidant enzyme activity of *Pluchea indica* Less. tea leaf extract *in vitro*. Superoxide and hydroxyl radical scavenging activities were measured. Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in vivo* and can result in the formation of H_2O_2 via dismutation reaction. [4] Superoxide radical plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, or singlet oxygen in living systems. [7] In the experiment, the scavenging activity assay was measured by the pyrogallol autoxidation on superoxide radical as shown in Figure 1. The percentage of free radical inhibition markedly increased by both the extract and gallic acid in concentration-dependent manner ($p<0.05$). There was a slight difference in the percentage of free radical inhibition found between extract and gallic acid treatment groups. The IC_{50} levels of the extract and gallic acid were calculated to be 265.84 ± 20.55 μg/mL, and 262.43 ± 1.51 μg/mL, respectively.

Hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions, such as copper or iron. [4] Hydroxyl radicals, generated by reaction of an iron-EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to form products that, upon heating with thiobarbituric acid at low pH, yield a pink chromogen. [5] The hydroxyl radical scavenging activity assay was measured by the deoxyribose assay. 2-Deoxyribose is attacked by hydroxyl radical to yield a mixture of products. [8] The scavenging ability of the extract on hydroxyl radical inhibition was shown in Figure 2, the percentage of free radical inhibition markedly increased both by the extract and BHT in concentration-dependent manner ($p<0.05$) at various concentrations. The percentage of free radical inhibition by BHT was higher than extract in the higher concentration (from 250 to 500 μg/mL).



*' **' *** $p<0.05$, significant difference in comparison to each concentration for the same sample

Figure 1 Percent of inhibition on superoxide radical scavenging activity of *P. indica* Less. tea extract (black bar) and gallic acid (white bar). Each bar is expressed as mean ± standard deviation from triplicate independent experiments



*, **, *** $p<0.05$, significant difference in comparison to each concentration for the same sample

Figure 2 Percent of inhibition on hydroxyl radical scavenging activity of *P. indica* Less. tea extract (black bar) and BHT (white bar). Each bar is expressed as mean ± standard deviation from triplicate independent experiments

Table 1 The modulative effect on the antioxidant enzyme activity of *P. indica* Less. tea leaf extract

Concentration of extract (µg/mL)	CAT (unit/mL)	GPx (unit/mL)
0	2.75 ± 0.43^{a}	1.77 ± 0.03^{c}
31.25	3.5 ± 0.00^{a}	1.45 ± 0.01^{b}
62.5	4.75 ± 0.43^{b}	1.48 ± 0.03^{b}
125	4.75 ± 0.43^{b}	1.53 ± 0.05^{b}
250	6 ± 0.00^{c}	1.45 ± 0.03^{b}
500	6.5 ± 0.00^{c}	1.25 ± 0.01^{a}

All data are expressed as mean ± standard deviation from triplicates independent experiments

$^{a, b, c}$ $p<0.05$, significant difference in comparison to each concentration for the same enzyme treatment

The IC_{50} levels of the extract and BHT were 161.91 ± 4.94 µg/mL, and 185.26 ± 5.77 µg/mL, respectively. Moreover, the changes of antioxidant enzyme activities such as catalase and glutathione peroxidase were measured. The various methods used to determine antioxidant activity can give more information on the specific free radical scavenging activity. The results of modulative effect on the antioxidant enzyme activity were shown in Table 1. The increase of catalase activity in a concentration-dependent profile was observed significantly ($p<0.05$) whereas it was not significantly altered ($p<0.05$) in the case of glutathione peroxidase activity. The results concerned about the detoxification mechanism of free radical particularly H_2O_2 to H_2O that was non-toxic.

Discussion

The current study is consistent with previous studies of *P. indica* extracts. The methanolic fraction of *P. indica* extracts was found to possess antioxidant activity. [3] In addition, the hot water extract from *P. indica* Less. (HWEP) herbal tea was also found to express the antioxidant. [9] HWEP has potent inhibitory effects against lipopolysaccharide-induced nitric oxide (NO) and prostaglandin E_2 (PGE_2) production in RAW 264.7 macrophages and has potential beneficial effects in preventing various chronic inflammatory diseases, including cancers. [9] Furthermore, phytochemical compounds of *P. indica* have been reported such as kaempferol, myricetin, luteolin, apigenin, tannins, saponins, flavonoids, proanthocyanidins, quercetin and chlorogenic acid. [10-12] Interestingly, quercetin and chlorogenic acid have also been reported to possess antioxidant and anti-inflammatory activities. [12,13]. Moreover, beta-sitosterol and stigmasterol isolated from *P. indica* root methanolic extract was found to be able to neutralize viper and cobra venom and antagonize cobra venom induced lethality, cardiotoxicity, neurotoxicity, and respiratory changes. [14] Further experiments are required to study the modulative effect on the antioxidant enzyme activity of *P. indica* Less. tea leaf extract *in vivo*.

In conclusion, the results of the present study demonstrated that *P. indica* Less. tea leaf extract exhibited a good antioxidant activity significantly ($p<0.05$), and may contain potential beneficial effects to prevent or treat various chronic degenerative diseases. In addition, *P. indica* Less. tea leaf extract may be used as dietary supplements and developed as a prototype herbal medicine in the future.

Acknowledgements

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