Antioxidant and anti-inflammatory activities of hot water extract from *Pluchea indica* Less. herbal tea

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In this study, antioxidant activity of the hot water extract of *Pluchea indica* tea leaves (HWEP) was measured by assays for radical scavenging against 1,1-diphenyl-2-picrylhydrazyl, superoxide and hydroxyl radical, ferric ion reducing power, as well as ferrous ion chelating. Results showed that HWEP exhibited good antioxidant activity in all test systems in a concentration-dependent manner. Furthermore, HWEP has potent inhibitory effects against lipopolysaccharide-induced nitric oxide (NO) and prostaglandin E₂ (PGE₂) production in RAW 264.7 macrophages. The hot water extract of *P. indica* leaves contains a source of antioxidants and inhibitors of NO and PGE₂ production that can be used as dietary supplements containing good health promoting effect.

Key words: Anti-inflammatory activity, antioxidant activity, herbal tea, *Pluchea indica*.

INTRODUCTION

Sources of reactive species (RS) are endogenous and exogenous. Various RS such as superoxide radical, hydroxyl radical, singlet oxygen, hydrogen peroxide, nitric oxide and peroxynitrite, are formed during normal cellular metabolism and in inflammation (Valko et al., 2007; Halliwell and Gutteridge, 2007; Zia-Ul-Haq et al., 2008; 2011a, b, 2012). Exogenous sources of RS include organic solvents, pollutants, pesticides, ionizing radiation and cigarette smoke (Halliwell and Gutteridge, 2007). Under normal conditions, the formation of RS is balanced by the antioxidant defense system within the body that includes antioxidant enzymes and antioxidants. Oxidative stress caused by excessive RS may be harmful to biomolecules (Valko et al., 2007). Several reports have shown that oxidative stress is implicated in several chronic diseases such as atherosclerosis, cancer, diabetes, arthritis, neurodegenerative diseases, inflammatory diseases, and aging (Kaneto et al., 2010; Markesbery, 1997; Valko et al., 2007). Antioxidants can interfere with the oxidative process by acting as electron or hydrogen donors, scavenging RS, and chelation of transition metal ions (Halliwell and Gutteridge, 2007). Additionally, antioxidants, have been shown to reduce inflammation by reducing pro-inflammatory mediators such as nitric oxide (NO) and prostaglandins (Aggarwal and Shishodia, 2006; Lin et al., 2003). Protecting organisms from oxidative damage and inflammation with foods rich in antioxidants is one strategy against chronic diseases, and the properties of some herbal teas have recently been shown to include RS suppression (Joubert et al., 2008; López et al., 2010).

*Pluchea indica* Less. (*Asteraceae*) have been used in folk medicine in South-East Asia, including Thailand. Its leaves are used as a nerve tonic and for treating inflammation and the bark in decoction form, against hemorrhoids. Additionally, *P. indica* leaves are used in the preparation of an herbal tea consumed for promoting...
good health (Office of Mangrove Resources Conservation, 2009; Sen et al., 2002). *P. indica* root extract and methanol leaf extract are reported to possess anti-inflammatory (Choi and Hwang, 2005; Sen et al., 1991; Sen et al., 1993), anti-ulcer (Sen et al., 1993), antioxidant (Choi and Hwang, 2005; Sen et al., 2002), anti-amoebic (Biswas, 2007), and antimicrobial activities (Biswas et al., 2007; Sittiwet, 2009), however, the characteristics of herbal tea are un-described. Further studies would be useful to clarify its health benefits and provide a scientific evidence for its antioxidant and anti-inflammatory activities. Thus, the objective of this study was to determine antioxidant properties of hot water extract of *P. indica* leaves (HWEP). Additionally, the inhibitory effects of *P. indica* extract on NO and prostaglandin E2 (PGE2) production were investigated in LPS-stimulated macrophages.

**MATERIALS AND METHODS**

Commercial *P. indica* herbal tea product (dried leaves) was purchased from community enterprise in Khluang district, Chantaburi province, Thailand, and ground to a fine powder. Powdered sample was added to boiling distilled water (1:5), further heated for 30 min, and then filtered through Whatman No.1 paper and the procedure repeated. The combined filtrate was evaporated at 40°C by rotary evaporator and freeze-dried. Extract yield, 31.6% w/w, was kept at -20°C and protected from light until used.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was performed with minor modification (Nagai et al., 2005). Briefly, 100 µL of 0.2 mM DPPH solution was added to 50 µL of various concentrations of HWEP. The solution was mixed and incubated for 30 min at room temperature and absorbance (517 nm) was measured. Hydroxyl radical scavenger ability was measured with minor modifications (Wang et al., 2008). The reaction mixture (200 µL) contained 67 µL of 1.5 mM FeSO4, 47 µL of 6 mM H2O2, 20 µL of 20 mM sodium salicylate and 66 µL of extract. After incubation (37°C) for 1 h, absorbance (562 nm) was measured. The superoxide radical was generated in vitro by xanthine/xanthine oxidase system and detected indirectly by measuring the rate of reduce XTT production (Rangkadiokl et al., 2007). Reducing power was measured as described by Chan et al. (2007), while chelating effect of ferrous ions was determined as described by Kosem et al. (2007) with some modifications. The extract solution (190 µL) was mixed with 20 µL of 2 mM ferrous chloride. Reaction was initiated by the addition of 5 mM ferrozine (20 µL) before absorbance was measured at 562 nm. The scavenging effects (%) and chelating effects (%) were calculated from the absorbance measurements using equation:

\[
\text{Scavenging effects (\%) or chelating effects (\%) = } \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

The EC50 was obtained by linear regression analysis from the calibration curve between percentage of inhibition and sample concentrations.

Anti-inflammatory effect of HWEP was determined from nitrite and PGE2 levels in cultured media. Nitrite determination was performed using the method described by Srisook et al. (2011) with some modifications. RAW 264.7 macrophage cells were cultured in Dulbecco’s modified eagles’ medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) at 37°C. Cells were incubated with various concentrations of HWEP in the presence and absence of 1 µg/ml LPS for 24 h. Then 100 µL of cell-free conditioned media was incubated with the same volume of Griess reagent for 10 min. Absorbance was measured at 546 nm. In another experiment, concentrations of PGE2 in culture supernatant were determined using ELISA kit according to manufacturer’s instructions (R&D systems, USA). Cell viability was based on the reduction of tetrazolium salt (MTT) into MTT-formazan crystals, mainly by mitochondrial dehydrogenases (Srisook et al., 2011).

**RESULTS AND DISCUSSION**

In this study, several in vitro antioxidant assays were performed, including scavenging of DPPH, superoxide and hydroxyl radicals as well as reducing power. Additionally, metal-chelating activity was also determined by a ferrozine-Fe2+ reaction system. Data on antioxidant activities of HWEP are given in Table 1. The extract showed antioxidant effects in all assays in a concentration-dependent manner. DPPH radical scavenging assay has been widely used to evaluate free radical scavenging activity of natural antioxidants from plant sources. The extract changed the violet color of DPPH radical to yellow of non-radical DPPH-H form. Interestingly, the DPPH radical scavenging activity of HWEP (EC50 value=23.8±1.0 µg/ml) was greater than the positive control BHT (EC50 value=44.4±2.5 µg/ml), whereas slightly less than ascorbic acid (EC50 value=13.0±0.2 µg/ml).

Superoxide and hydroxyl radical, formed in biological system, have been known to be highly reactive with a wide range of molecules found in living cells (Valko et al., 2007; Halliwell and Gutteridge, 2007). The EC50 values of HWEP on superoxide and hydroxyl radical scavenging activity were 446.3±12.1 and 706.3±16.3 µg/ml, respectively. However, superoxide and hydroxyl radical scavenging activity were twenty-fold and five-fold less, respectively, than gallic acid. In biochemical systems, hydrogen peroxide dismutates from superoxide radical, can be converted to hydroxyl radical in the presence of specific transition metal ions such as iron and copper (Halliwell and Gutteridge, 2007; Wang et al., 2008). The metal chelating activity of *P. indica* HWEP interfered with the ferrous-ferrozine complex and varied inversely with extract concentration. Nevertheless, the ferrous ions chelating activity of HWEP was found to be very low relative to the positive control EDTA. Additionally, HWEP reduced Fe3+ to Fe2+ (Table 1).

Furthermore, LPS strongly stimulated the production of NO and PGE2 in RAW 264.7 macrophage cells (Figure 1) but this was decreased by HWEP in a concentration-dependent manner with IC50 values of 314.8±38.9 and 49.1±8.3 µg/ml, respectively. Moreover, HWEP alone at concentrations of 25 to 400 µg/ml showed no cytotoxic effect, indicating that reduction in the level of NO and PGE2 was not due to cell death. Accumulated evidence suggests that chronic inflammation caused by excessive
Table 1. Antioxidant activities (EC\textsubscript{50} values) of hot water extract of \textit{P. indica} leaves\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Samples</th>
<th>EC\textsubscript{50} DPPH (\textmu g/ml)</th>
<th>EC\textsubscript{50} Superoxide (\textmu g/ml)</th>
<th>EC\textsubscript{50} Hydroxyl (\textmu g/ml)</th>
<th>EC\textsubscript{50} Metal chelating (\textmu g/ml)</th>
<th>Reducing power (mg gallic acid equivalents /g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water extract</td>
<td>23.8 ± 1.0</td>
<td>446.3 ± 12.0</td>
<td>706.3 ± 16.3</td>
<td>4.2 ± 0.1</td>
<td>185.2 ± 2.3</td>
</tr>
<tr>
<td>BHT</td>
<td>44.4 ± 2.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>13.0 ± 0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>-</td>
<td>23.1 ± 1.1</td>
<td>145.0 ± 0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EDTA</td>
<td>-</td>
<td>-</td>
<td>0.02 ± 0.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a}All experiments were performed at least three times with triplicate samples. Results are reported as mean ± SD.

Figure 1. Inhibitory effect of hot water extract of \textit{P. indica} leaves (HWEP) on the production of NO (A) and PGE\textsubscript{2} (B) in LPS-stimulated RAW 264.7 macrophages. Cells were co-incubated with the indicated concentrations of HWEP (25 - 400 \textmu g/ml) and LPS (1 \textmu g/ml) for 24 h. The culture supernatants were subsequently isolated and analyzed for nitrite and PGE\textsubscript{2} levels. Each column shows the mean ± SD of three independent experiments with triplicate samples. \#P<0.05, ##P<0.01, ###P<0.001 vs. control and *P<0.05, **P<0.01 and ***P<0.001 vs. LPS alone. (C) Viability of cells was determined using the MTT assay. Each column shows the mean ± SD of four independent experiments with triplicate samples.
cellular secretion of RS promotes tumor initiation (Aggarwal et al., 2006). Antioxidants may be useful in neutralizing RS and prevention of cancer development (Valko et al., 2007). Additionally, prostaglandins including PGE₂ seem to be involved in carcinogenesis (Aggarwal et al., 2006).

Further experiments are required to identify the exact component responsible for the antioxidant activity and anti-inflammatory activities of the hot water extract of *P. indica* tea leaves. However, phytochemical investigations of *P.indica* have reported the presence of eudesmane derivatives, terpene glycosides, benzenoids, phenylpropanoids, lignan glycosides, stigmasteryl glucoside, quercetin and chlorogenic acid (Chakraborty et al., 1994; Mukhopadhyay et al., 1983; Shukri et al., 2011; Uchiyama et al., 1989). Most of plants that exhibit antioxidant activities also possess anti-inflammatory potential (Qayum et al., 2012). Quercetin and chlorogenic acid have also been reported to possess antioxidant and anti-inflammatory activities (Gusdinar et al., 2011; Lin et al., 2003; Shukri et al., 2011; Zhang et al., 2010).

In conclusion, the results of the present study demonstrate that hot water extract of *P. indica* leaves contain anti-oxidative and anti-inflammatory properties, and have potential beneficial effects in preventing various chronic inflammatory diseases, including cancers.

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