ABSTRACT: Several parts of *Lythrum salicaria* were used for this study. Scavenging activities on radicals, inhibitory activity on linoleic acid peroxidation and total phenol contents of extracts from root, flower, and aerial part were evaluated. Flower and root selected from *in vitro* assay were subjected to *in vivo* assay on CCL₄-induced liver injury rat model for two weeks. Carbon tetrachloride intoxication on rats produced large amounts of hepatic lipid peroxidation product, thiobarbituric acid reactive substance (TBARS) compared with normal rats. Treatment with root extract of *L. salicaria* (LSR) showed effective inhibitory activity on lipid peroxidation product. Administration with LSR extract significantly alleviated CCL₄-induced increase in GPT activity which were more effective than silymarin. The results of this study suggest that root and flower of *L. salicaria* have antioxidant and liver protecting activities, and root part is the most effective candidate to develop a new functional material.

Key Words: *Lythrum salicaria*, Antioxidant, Hepatoprotection, Carbon Tetrachloride

INTRODUCTION

Reactive oxygen species (ROS) mostly generated from oxygen used for energy production in living cells evoke hazardous result to human health. The hazardous actions of ROS are performed by various manners including oxidation of fatty acid, mutation through oxidation and bridge bonding of biopolymers such as DNA and protein, and lead to many diseases including cancer and aging (Frei, 1994). Carbon tetrachloride (CCL₄) can be converted into hepatotoxic metabolite, trichloromethyl free radicals (CCL₃ and/or CCL₂OO⁻) by hepatic microsomal cytochrome P450 and can react with sulphhydryl groups in glutathione and protein, and further more lead to membrane lipid peroxidation and finally cell necrosis (Lee and Jeong, 2002). Antioxidants inhibit lipid peroxidation, scavenge free radicals (Bruck *et al.*, 2001), and are proposed as therapeutic agents or drug coadjuvants to ameliorate many diseases such as liver disease (Honeckman, 2003; Paola *et al.*, 2004).

Purple loosestrife or spiked loosestrife named *Lythrum salicaria* is distributed worldwide. The physiological activities such as antioxidant activity on DPPH and linoleic acid, anti-inflammatory and anti-nociceptive effect of the extract prepared from leaves of this plant was reported (Tunalier *et al.*, 2007). Antioxidant activity on superoxide anion and rat liver homogenate (Coban *et al.*, 2003), effect on hyperglycemic mice (Lamela *et al.*, 1986), effect on normal blood glucose rat (Lamela *et al.*, 1985), antimicrobial effect (Rau et al., 2003), antimicrobial compound (Becker *et al.*, 2005), antilisterial activity (Altanlar *et al.*, 2006) were also studied. In Hyangyak-Seangyak Great Encyclopedia (Jung and Shin, 1990), medicinal activity of aerial parts and root of *L. salicaria* have listed about their effect of cleaning blood, blood stopping and effect on ulcer. Compounds such as vitexin, orientin, malvin, cyanidin-3-monogalactoside, ellagic acid and chlorogenic acid exist in flower, and salicarin and tannin exist in aerial parts.
We have conducted evaluation studies on plant resources and selected *L. salicaria*, and tested the plant parts on antioxidant and hepatic protective activities in carbon tetrachloride-intoxicated rats toward the development of a potent antioxidant.

**MATERIALS AND METHODS**

1. Materials and preparation of extracts

   Aerial part, flower and root of *L. salicaria* were collected in the medicinal crop farm of RDA, in 2002 and 2003. For evaluating *in vitro* antioxidant activity, each part of *L. salicaria* was used for preparation of methanol extract, which was made by ASE (accelerated solvent extractor, Dionex, USA), evaporated at 50°C in vacuum evaporator (JPSB1000, Eyela, Japan). For *in vivo* experiment, flower and root of *L. salicaria* were extracted with ethanol in refluxing extraction apparatus at 85°C for 2 hrs twice, and solvent of the extracts were evaporated under vacuum.

2. Chemicals

   Reagents including 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitro blue tetrazodium (NBT), phenazine methosulfate (PMS), β-nicotine amide adenine dinucleotide (NADH), and silymarin were the product of Sigma-Aldrich Co (St. Louis, USA). glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GTP) assay kit was purchased from Asan Pharmaceutical Co. Ltd (Seoul, Korea).

3. Analysis of *in vitro* antioxidant activity and total phenol content of *L. salicaria*

   A reaction mixture composed with 2.97 ml of ethanolic 1.5×10⁻² M DPPH solution and 0.03 ml of sample was incubated at room temperature for 3 min and measured the optical density at 517 nm with UV-spectrophotometer (Cary 300, Varian, Australia) for evaluation of scavenging effect on DPPH radical by the method of Lee *et al.* (2005). For analysis of superoxide anion radical scavenging activity, a mixture of sample (0.5 ml), 0.1 M Tris buffer (pH 8.5, 0.7 ml), 100 μM PMS (0.2 ml), 500 μM NBT (0.2 ml) and 500 μM NADH (0.4 ml) were reacted for 5 min at room temperature and measured the absorbance at 560 nm (Nishikimi *et al.*, 1972). Inhibition effect on linoleic acid peroxidation was tested by the method of Takao *et al.* (1994), which was measured in a mixture composed of 0.03 ml of sample, 0.4 ml of 0.089 M linoleic acid, 0.8 ml of 5×10⁻² M phosphate buffer and 0.77 ml of distilled water. Reaction mixture was incubated at 40°C for 24 hrs. An aliquot (0.1 ml) of the reaction mixture was added with 0.1 ml of 3.94 M ammonium thiocyanate, 0.1 ml of 0.019 M ferrous chloride (in 3.5% HCl) and 2.7 ml of 75% ethanol. The mixture was measured for the absorbance at 500 nm. In above experiments, solvent used for dissolving the sample was used as control, and the result was calculated as IC₅₀ or inhibition rate (%) against the value of control. Total phenol contents of the extracts were obtained by using 50% Folin-Ciocalteau reagent (Kim *et al.*, 1993), and the result was shown as tannic acid equivalent from calculation using the equation, y = 0.0235235x + 0.0646878 (y means optical density and x means tannic acid content), and by multiplying with dilution factor.

4. Animal and treatment

   Flower and root selected from *in vitro* assay were subjected to *in vivo* experiment. Animals for evaluating the activity of two parts of *L. salicaria* were Sprague Dawley male rats which were six week-old age and had about 200 g body weight at the start time of experiment, were divided randomly into six groups (n=8). Rats were acclimated to the environment for one week, used for the experiment in the conditions of 22~24°C for the experiment period (2 weeks), and allowed tap water free. Experiment groups were composed of five groups such as normal, CCL₄ (negative control), CCL₄ plus silymarin (positive control), CCL₄ plus *Lythrum salicaria* flower extract (LSF), and CCL₄ plus *Lythrum salicaria* root extract (LSR) groups. Normal group was administered with basal diet and treated with intra-peritoneal (i.p.) injection of vehicle (olive oil) CCL₄ single treated group was administrated with basal diet and was treated with i.p. injection of 2g/Kg body weight of CCL₄ mixture (1:1 of CCL₄ and olive oil) three times for 2 weeks. CCL₄ plus silymarin group was administrated with 1% silymarin diet and was also treated with CCL₄. CCL₄ plus *L. salicaria* flower extract group was administrated with 1% *L. salicaria* flower extract diet and was injected with CCL₄. CCL₄ plus *L. salicaria* root extract group was administrated with 1% *L. salicaria* root extract and was treated with CCL₄, respectively.
5. Analysis of antioxidant activity and liver protection activity of L. salicaria flower and root extract on CCL₄-induced liver damage of rat

Content of lipid peroxidation product of liver, TBARS, was quantified by the methods of Botsoglou et al. (1994) using standard precursor with tetraethoxypropyne (TEP) which could be converted into MDA. Analysis of Mn-SOD activity in mitochondrial fraction of liver was performed according to the method of Flohe’ (1984) and activities of liver cytosolic GSH-px were analyzed by the method of Flohe’ (1989). Glutathione (GSH) content in liver tissue was analyzed by the method of Ellman (1959) in which a part of liver tissue was homogenized and an aliquot of homogenate was analyzed by the method of Ellman (1959) in which a part of liver tissue was homogenized and an aliquot of homogenate as 400–600 μg of protein was mixed with 2 ml of 4% sulfosalicylic acid, centrifuged 10 min at 3,000 rpm, and then, 0.1 ml of the supernatant was reacted with 2.7 ml of 0.1 mM 5,5’-dithiobis (2-nitrobenzoic acid) in 0.1 M sodium phosphate buffer (pH 8.0) for 5 min at room temperature, was measured on an absorbance at 421 nm and the result was obtained from substitution of the absorbance to glutathione standard curve. Measurements of GOT activity and GTP activity in serum were performed with the GOT and GPT assay kit (Asan Pharmaceutical Co). Content of protein in cytosol fraction was determined by the method of Bradford (1976) using bovine serum albumin as standard compound for calibration curve.

6. Statistical analysis

The results were shown as mean±SD. The significance of data was verified with Duncan’s multiple range test of one way ANOVA test in SAS program (Enterprise Guide 4) at p<0.05. Alphabets displayed on the data means significantly difference between the results.

Table 1. In vitro antioxidant effect and total phenol content of L. salicaria.

<table>
<thead>
<tr>
<th></th>
<th>Flower</th>
<th>Aerial part</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scavenging effect on DPPH radical (IC₅₀, μg/ml)</td>
<td>10.0±0.0ᵇ</td>
<td>18.3±1.1ᵃ</td>
<td>7.7±0.1ᶜ</td>
</tr>
<tr>
<td>Scavenging effect of superoxide radical (%)⁽¹⁾</td>
<td>71.2±0.1ᵇ</td>
<td>55.6±0.4ᵃ</td>
<td>75.0±1.3ᶜ</td>
</tr>
<tr>
<td>Inhibition effect on linoleic acid oxidation (%)⁽²⁾</td>
<td>78.4±2.1ᵃ</td>
<td>82.7±1.4ᵇ</td>
<td>76.2±5.6ᶜ</td>
</tr>
<tr>
<td>Total phenol content (㎎/g)⁽³⁾</td>
<td>1465.4±5.5ᵃ</td>
<td>1125.6±27.3ᵇ</td>
<td>1491.6±8.3ᶜ</td>
</tr>
</tbody>
</table>

Data are mean±SD values (n = 3). Values with different superscripts in the same row are significantly different at P < 0.05.

¹, ² Final concentration was 50 and 10 μg/ml, respectively.
³ Content show as tannic acid equivalent.

RESULTS

1. In vitro antioxidant activity of L. salicaria and total phenol content

In vitro antioxidant effect and total phenol content of L. salicaria extracts were shown in Table 1. In DPPH radical scavenging activity shown as IC₅₀ in root extract of L. salicaria showed 7.7±0.1 μg/ml which was the most effective among the extracts, and flower extract and aerial part extract showed 10.0±0.0 μg/ml and 18.3±1.1 μg/ml, respectively. Root extract was also more effective (75±1.3%) to scavenge superoxide radical at 50 μg/ml than the other parts (55.6±0.4% and 71.2±0.1%). Antioxidant activity of aerial part extract on linoleic acid oxidation tested at final reaction concentration of 10 μg/ml, exhibited the highest value (82.7±1.4%), and those of flower and root extracts showed 78.4±2.1% and 76.2±5.6%, respectively. Contents of total phenol in root, flower, and aerial part extracts were 1491.6±8.3 μg%, 1465.4±5.5 μg% and 1125.6±27.3 μg% as tannic acid equivalent, respectively.

2. Antioxidant activity and liver protection activity of L. salicaria flower and root extract on CCL₄-induced liver damage of rat

Effects of L. salicaria flower and root extract treatment on hepatic lipid peroxidation in short term carbon tetrachloride-intoxicated rats were indicated in Fig. 1. Treatment with CCL₄ increased liver lipid peroxidation to 41.7±3.3 μg/g tissue compared with that (38.4±2.3 μg/g tissue) of normal. Root extract diet (1%) administration significantly decreased liver TBARS (40.3±6.9 μg/g tissue), but 1% flower extract and/or 1% silymarin diet administration increased the product of lipid peroxidation (46.1±3.8 μg/g tissue and 44.0±3.4 μg/g tissue) in CCL₄-intoxicated rats. This data showed that root extract of L. salicaria...
exhibit antioxidant activity on liver lipid peroxidation in CCL4-intoxicated rats. The effects of L. salicaria flower and root extract treatment affected on hepatic Mn-SOD activities in liver intoxication-induced rats (Fig. 2). The activity of hepatic Mn-SOD in CCL4 single treated rats (2.17 ± 0.10 U/㎎ protein) increased, and the activities in L. salicaria flower extract plus carbon tetrachloride treated rats (2.10 ± 0.18 U/㎎ protein), L. salicaria root extract plus CCL4 treated rats (2.01 ± 0.33 U/㎎ protein), and silymarin plus CCL4 treated rats (2.10 ± 0.47 U/㎎ protein) slightly decreased than that in normal rats (1.98 ± 0.19 U/㎎ protein), which were not significantly different among the groups. The serum GOT activity in liver damage induced rats with carbon tetrachloride in short term (Fig. 3) exhibited. GOT activity in CCL4 single treated rats (34.4 ± 8.1 U/L) was very significantly increased compared with that of normal rats (0.1 ± 11.2 U/L). GOT activities in 1% extract of L. salicaria flower extract and L. salicaria root extract diet administered groups were 26.8 ± 14.0 U/L and 11.6 ± 20.3 U/L, respectively, which were reduced values as 22.1% and 66.3% against the activity of CCL4 group. Serum GOT activity of 1% silymarin diet administered group (13.5 ± 15.1 U/L) was reduced to 60.8% against the activity of CCL4 group. As shown in Fig. 4, carbon tetrachloride intoxication in CCL4 single treated rats increased GPT activity (34.4 ± 8.1 U/L) compared with normal group (23.9 ± 2.1 U/L), but the activities in 1% L. salicaria flower extract and 1% L. salicaria root extract diet administered rats were decreased into 25.3 ± 4.0 U/L and 19.6 ± 4.4 U/L, which were significantly lower than that of 1% silymarin diet administrated rats (28.3 ± 4.8 U/L). The effects of root and flower extracts on several hepatic antioxidant enzyme activity and the GSH content in CCL4 treated rat were listed in Table 2. Activity of glutathione peroxidase (GSH-px) in NC rats decreased as 0.36 ± 0.15 U/㎎ protein compared with the activity of normal rats (0.67 ± 0.16 U/㎎ protein). The activities of GSH-px in 1% L. salicaria flower extract and 1% L. salicaria root extract diet fed groups also decreased as 0.28 ± 0.09 U/㎎ protein and 0.27 ± 0.08 U/㎎ protein than those of normal, CCL4 treated group and 1% silymarin diet group. Content of hepatic glutathione in CCL4 single treated, CCL4 plus 1% silymarin diet group, CCL4 plus 1% L. salicaria flower extract treated group and CCL4 plus 1% L. salicaria root extract treated group showed from 1.09 ± 0.06 mg/g tissue.
Table 2. Effect of L. salicaria flower and root extract on the activity of hepatic GSH-px, and the content of GSH on CCL\textsubscript{4}-induced liver damage of rat.

<table>
<thead>
<tr>
<th></th>
<th>GSH-px (Ul/mg protein)</th>
<th>GSH (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.67±0.16\textsuperscript{a}</td>
<td>1.00±0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>CCL\textsubscript{4}</td>
<td>0.36±0.15\textsuperscript{b}</td>
<td>1.15±0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>CCL\textsubscript{4}+1% Sil</td>
<td>0.48±0.08\textsuperscript{b}</td>
<td>1.12±0.09\textsuperscript{a}</td>
</tr>
<tr>
<td>CCL\textsubscript{4}+1% LSF</td>
<td>0.28±0.09\textsuperscript{b}</td>
<td>1.08±0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>CCL\textsubscript{4}+1% LSR</td>
<td>0.27±0.08\textsuperscript{b}</td>
<td>1.09±0.06\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Data are mean±SD values (n = 6). Normal, no CCL\textsubscript{4}-intoxicated and basal diet-administrated group; CCL\textsubscript{4}, CCL\textsubscript{4} (CCL\textsubscript{4}: olive oil = 1:1, 2 g/kg body weight of rat)-intoxicated & basal diet fed group; CCL\textsubscript{4}+Sil, CCL\textsubscript{4}-intoxicated & 1% silymarin-supplemented diet fed group (positive control); CCL\textsubscript{4}+LSF, CCL\textsubscript{4}-intoxicated & 1% L. salicaria flower extract-supplemented diet fed group; CCL\textsubscript{4}+LSR, CCL\textsubscript{4}-intoxicated & 1% L. salicaria root extract-supplemented diet fed group. Values with different superscripts in the same column are significantly different a P < 0.05.

to 0.15±0.05 mg/g tissue, which were not significantly different among the groups.

DISCUSSION

It was suggested that oxidative stress is involved in pathogenic progress of many diseases and the materials having antioxidant activity exhibit ameliorating effect against these (Rodeiro et al., 2008). Many antioxidants originated from plant kingdom have protecting activity on increase of lipid peroxidation products and on depletion of glutathione induced with CCL\textsubscript{4} (MacDonald-Wicks and Garg, 2003; Ha et al., 2005; Kang et al., 2002), and furthermore exhibit attenuating activity on liver disease and/or pathological condition like fibrosis (Pasola et al., 2004; Joy and Kuttan, 1999).

In the study, several plant parts of L. salicaria such as flower, aerial parts and root were preliminarily investigated and compared on their antioxidant activity against free radicals such as DPPH and superoxide and against oxidation of linoleic acid. In these in vitro assay, we obtained the result that flower and root of L. salicaria show more effective radical scavenging activity, and high content in total phenol, but aerial parts of the plant show more effective inhibitory activity on fatty acid peroxidation than other plant parts.

For investigation on liver protectors, chemicals including carbon tetrachloride, and alcohol have been used to induce liver injury in animals (Singh et al., 2008; Rodeiro et al., 2008). Investigations have stated that lipid peroxidation in tissues is increased by trichloromethyl radical (CCL\textsubscript{3} and/or CCL\textsubscript{3}OO\textsuperscript{•}) evoked from CCL\textsubscript{4} by the metabolism of hepatic microsomal P450 (Jeong et al., 2002), and is a crucial step in pathogenesis of free radical-related diseases including hepatic dysfunctions (Singh et al., 2008).

In our study, in vivo assay has been performed with L. salicaria flower and root extract in rats intoxicated with carbon tetrachloride three times for 2 weeks. Supplement with CCL\textsubscript{4} plus 1% L. salicaria root extract extract diet showed significantly protecting activity on hepatic lipid peroxidation (as TBARS) compared with CCL\textsubscript{4} single treatment, CCL\textsubscript{4} plus 1% L. salicaria flower extract diet administration and CCL\textsubscript{4} plus 1% silymarin diet administration.

Antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) constitute a supportive group of antioxidant defense against reactive oxygen species. SOD is the first line of the defense system and carries out the dismutation of superoxide to H\textsubscript{2}O\textsubscript{2} while CAT and GSH-px convert H\textsubscript{2}O\textsubscript{2} to water (Halliwell et al., 1992). Glutathione-S-transferase (GST), a phase enzyme, protects and converts toxic chemicals to less toxic compounds (Wallig et al., 1998), and with GSH-px and GSH work together during decomposition of hydrogen peroxide or other organic hydroperoxides (Meister, 1988).

In present study, Mn-SOD activity was characteristically increased in alcohol supplemented animals (Chen et al., 1992, 1996), was increased in all of the CCL\textsubscript{4} treated rat groups including silymarin plus CCL\textsubscript{4} treated group compared with the normal group, that was not significant. However, rats treated with CCL\textsubscript{4} plus L. salicaria root extract exhibited the most effective data in attenuating the increase of Mn-SOD activity among the CCL\textsubscript{4} treated groups. GSH-px activities were decreased in all of the carbon tetrachloride injected groups except the normal group, which were not significant, too. Although, activity changes in three antioxidant enzymes were not significant among the experiment groups, it is supposed that Mn-SOD plays a defense role against oxidative stress evoked by CCL\textsubscript{4} through formation of large amounts of H\textsubscript{2}O\textsubscript{2}. Increase of SOD activity and concomitant decrease of GSH-px activity were exactly the same features in exhibiting the articles reported by Singh et al. (2008) and Tasaduq et al. (2003).

Supplements with L. salicaria flower and root extract...
decreased serum GOT and GPT activities which increased in CCL₄ singly administered rats compared with normal rats, and *L. salicaria* root extract showed more effective in decreasing GPT activity than silymarin did. These results demonstrate that *L. salicaria* root extract shows potent antioxidant and liver protective activity in CCL₄-induced liver injury. The same result was reported in articles by other researchers (Achliyaa et al., 2004; Wong et al., 2000; Ahmed et al., 2000).

It is supposed that the result which *L. salicaria* root showed inhibitory activity on TBARS production and on elevation of serum GPT activity, but did not on other antioxidant parameters in vivo experiment was caused by short term treatment of carbon tetrachloride on rat.

In conclusion, flower and root of *L. salicaria* showed in vitro antioxidant activities. Furthermore, flower and root selected from in vitro assay showed antioxidant and liver protecting activities on carbon tetrachloride intoxicated liver injury of rats. Compared with other plant parts and silymarin using positive control compound, root part of *L. salicaria* exhibited the most efficient capacity as antioxidant and hepatic protector.

**ACKNOWLEDGMENTS**

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