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Journal of Ethnopharmacology 110 (2007) 539–547
Journal of ETHNO-PHARMACOLOGY
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Antioxidant, anti-inflammatory, anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts

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Received 23 May 2006; received in revised form 15 October 2006; accepted 19 October 2006
Available online 27 November 2006

Abstract

Lythrum salicaria (purple loosestrife) known as "Tıbbi hevhulma" in Turkish is used for its several beneficial health effects against as diarrhea, chronic intestinal catarrh, hemorrhoid and eczema in the form of a decoction or a fluid extract and to treat varicose veins, bleeding of the gums, hemorrhoid and eczema, externally. Dried herbal parts of *Lythrum salicaria* L. (Lythraceae) were sequentially extracted with different solvents such as petroleum ether, ethyl acetate, methanol and 50% aqueous methanol. Water extract of *Lythrum salicaria* was also prepared under reflux. Antioxidant, anti-inflammatory and anti-nociceptive activities of all the extracts were investigated using *in vitro* and *in vivo* methods, respectively. Free radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl, DPPH$^{\bullet}$ assay), iron(III) reductive activity, capacity of the inhibition of linoleic acid peroxidation and MDA formation, anti-nociceptive activity (*p*-benzoquinone-induced abdominal constriction test) and anti-inflammatory activity (carrageenan-induced hind paw edema model) were used for all the extracts. In addition, the content of total phenolics, flavonoids and flavonols in all the extracts were determined with spectrophotometric methods. Results were compared with reference antioxidants *via* ascorbic acid, butylated hydroxytoluene, and gallic acid. Qualitative and quantitative compositions of all the extracts were analysed using a HPLC–PDA system. Polar fractions were found to be rich in flavonoids such as isovitexin and isoorientin.
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Keywords: *Lythrum salicaria*; HPLC; 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$); Linoleic acid peroxidation; Anti-inflammatory activity; Anti-nociceptive activity; Antioxidant activity

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1. Introduction

The genus *Lythrum* (Lythraceae) is spread throughout the world. It is represented by almost 30 species, 9 of which are found in Turkey. Most of them are wetland plants, and about 10 species are found in Europe. *Lythrum salicaria* L. (Syn. *Lythrum tomentosum* DC., *Lythrum cinereum* Gris.) is originally Eurasian, but during the 19th century it was spread *via* the ballast of European ships also into North and South America, as well as Australia. It crowds out native wetland vegetation, such as cattails, grasses, sedges, and rushes. It is known as "Tıbbi hevhulma" in Turkey. Its English name is "blooming sally", "purple willow-herb", "rainbow weed" and "purple loosestrife". It is known in German as "Blutweiderich", in French "Salicaire", and in Swedish "fackelblomster" (Davis, 1982; Baytop, 1999; Rauha, 2001).

Lythrum salicaria (purple loosestrife) was known as medicinal plant already in ancient Greek and Roman times and it has been an important drug for centuries. The whole flowering plant and the flowering branch tips of this plant are used not only in folk medicine but also in pharmaceuticals. *Lythrum salicaria* is used internally for diarrhea, chronic intestinal catarrh, hemorrhoid and eczema in the form of a decoction or a fluid extract. Externally, it is used to treat varicose veins, bleeding of the gums, hemorrhoid and eczema (Baytop, 1999; Mantle et al., 2000; Rauha et al., 2000). This species, as well as other *Lythrum* species, have long been popular with gardeners for their abundant and attractive floral displays and are still sold legally in some places. However, North American horticulturalists have

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doi:10.1016/j.jep.2006.10.024

now abandoned its promotion because of its potential for escape. It has also been utilized as a honey plant by beekeepers. Purple loosestrife seed has occasionally been included in commercial "wildflower" seed mixed (Munger, 2002).

Previously, little work has been done on the phytochemistry of this plant. In these studies, tannins were reported as main compounds in *Lythrum salicaria*. It contains a notable amount of flavonoids represented by anthocyanins and flavon C-glycosides. Vitexin, isovitexin, orientin and isoorientin were reported to occur. In addition, vescalagin, pedunculagin, vanoleic acid dilactone, 1,6-di-*O*-galloylglucose, 1-*O*-galloylglucose and 6-*O*-galloylglucose identified from this plant (Rauha, 2001; Rauha et al., 2001). The phytochemical information of purple loosestrife published in literature was reported by Rauha et al. Furthermore, the phenolic composition of the crude extract of this plant was characterised using HPLC/DAD-UV, LC/APCI-MS and LC/ESI-MS techniques (Rauha et al., 2001). Antioxidant, antimicrobial, and hypoglycemic activities of *Lythrum salicaria* have been reported (Lamela et al., 1986; Kahkönen et al., 1999; Mantle et al., 2000; Çoban et al., 2003). An ethanolic extract of this plant showed concentration-dependent superoxide anion radical scavenging activity and inhibitory effect on lipid peroxidation (Çoban et al., 2003). Aqueous methanolic extract showed a moderate antioxidant activity at autoxidation of methyllinoleate (Kahkönen et al., 1999) and against the ABTS radical (Mantle et al., 2000). The effect of *Lythrum salicaria* on the growth of *Candida albicans*, *Staphyllococcus aureus* and *Escherichia coli* and its antifungal activity has been reported (Rauha et al., 2000). Stems and flowers of *Lythrum salicaria* show hypoglycemic activity and epinephrine-induced hyperglycemia (Lamela et al., 1986).

In this study, different solvent extracts of *Lythrum salicaria* were analysed for their antioxidant, anti-inflammatory, and antinociceptive activities using *in vitro* and *in vivo* methods. In addition, the content of total phenolics, flavonoids and flavonols in all the extracts were determined. Results were compared with synthetic antioxidants, *e.g.* ascorbic acid, butylated hydroxytoluene, and gallic acid. Qualitative and quantitative compositions of all the extracts were analysed using HPLC–PDA system.

2. Materials and methods

2.1. Plant material and reagents

Plant material (*Lythrum salicaria* L. Lythraceae) was collected from Erdek Ocaklar village in Balikesir province in the western part of Turkey. A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy (ESSE 14423). Chromatographic standards were purchased from Extrasynthese, France. Ultra-pure water was used throughout and was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., MA, USA). All remaining reagents were of the highest purity available and obtained from the Sigma Chemical Company (St. Louis, MO, USA). Isoorientin and isovitexin were provided by one of us (I. Calis).

2.2. Preparation of extracts

Air-dried aerial parts (80.12 g) were powdered and sequentially extracted with petroleum ether, ethyl acetate, methanol, and 50% aqueous methanol using a Soxhlet apparatus for 8 h each. The extracts were filtered and evaporated to dryness *in vacuo* at 40 °C. The herb of *Lythrum salicaria* (96.26 g) was extracted with water under reflux for 8 h. The water phase was filtered and freeze-dried. All the extracts were stored at −20 °C. Immediately before analysis, an aliquot of each extract was dissolved and filtered through a 0.45 μm membrane (Whatman, UK) and used in all the experiments.

2.3. Total phenolics, flavonoids and flavonols

Total phenols were estimated as gallic acid equivalents (GAE), expressed as $mg_{gallic\ acid}\ g^{-1}_{extract}$ (Singleton et al., 1999). To *ca.* 6.0 mL H_2O, 100 μL sample was transferred in a 10.0 mL volumetric flask, to which was subsequently added 500 μL undiluted Folin-Ciocalteu reagent. After 1 min, 1.5 mL 20% (w/v) Na_2CO_3 was added and the volume was made up to 10.0 mL with H_2O. After 2 h incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses.

Total flavonoids were estimated as rutin equivalents (GAE), expressed as $mg_{rutin\ acid}\ g^{-1}_{extract}$ (Miliauskas et al., 2004). One milliliter of plant extract in methanol (10 g L^{-1}) was mixed with 1 mL aluminium trichloride in ethanol (20 g L^{-1}) and diluted with ethanol to 25 mL. The absorption at 415 nm was read after 40 min at 20 °C. Blank samples were prepared from 1 mL plant extract and one drop acetic acid, and diluted to 25 mL. The rutin calibration curve was prepared in ethanolic solutions with same procedure. All determinations were carried out in quadruplicate and the mean values were used.

Total flavonols were estimated as rutin equivalents (GAE), expressed as $mg_{rutin\ acid}\ g^{-1}_{extract}$ (Miliauskas et al., 2004). The rutin calibration curve was prepared by mixing 2 mL of 0.5–0.015 mg mL^{-1} rutin ethanolic solutions with 2 mL (20 g L^{-1}) aluminium trichloride and 6 mL (50 g L^{-1}) sodium acetate. The absoption at 440 nm was read after 2.5 h at 20 °C. The same procedure was carried out with 2 mL of plant extract (10 g L^{-1}) instead of rutin solution. All determinations were carried out in quadruplicate and the mean values were used.

2.4. Qualitative–quantitative chromatographic analysis

The liquid chromatographic apparatus (Shimadzu LC 10Avp, Ant Ltd., Istanbul, Turkey) consisted of an in-line degasser, pump and controller coupled to a SPD-M10Avp photo diode array detector equipped with an automatic injector interfaced to Class VP chromatography manager software (Shimadzu, Ant Ltd., Istanbul, Turkey). Separations were performed on a 250 mm × 4.6 mm i.d., 5 μm particle size, reverse-phase Discovery-C18 analytical column (Supelco, PA, USA) operating at room temperature (22 °C) at a flow rate of 1 mL min^{-1}. Detection was carried out with a sensitivity of 0.1 a.u.f.s between

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the wavelengths of 200–550 nm. Elution was affected using a non-linear gradient of the solvent mixtures aqueous 5% HCOOH (solvent A) and acetonitrile (solvent B). The composition of B was held in 5% for 5 min, increased to 15% in 15 min, increased to 85% in 10 min and held for 5 min, then returned to initial conditions in 3 min and held for 7 min. A 10 min equilibrium time was allowed between injections. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and UV spectra with an in-house PDA-library.

All extracts and standards were dissolved in 70% aqueous acetonitrile at a concentration of 1 and 10 mg mL^{-1}, respectively. The concentration used for the calibration of reference compounds was 0.00–0.10 mg mL^{-1}. All the standard and sample solutions were injected triplicate.

2.5. Iron(III) to iron(II) reduction activity

The ability of the extracts to reduce iron(III) was assessed by the method of Oyaizu (1986). One milliliter of each extract dissolved in H_2O, was mixed with 2.5 mL 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) potassium hexacyanoferrate solution. After 30 min incubation at 50 °C, 2.5 mL 10% (w/v) trichloroacetic acid (TCA) was added and the mixture was centrifuged for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL H_2O and 0.5 mL 0.1% (w/v) $FeCl_3$ and the absorbance was recorded at 700 nm. The reductive activities of the extracts are expressed as ascorbic acid equivalents (AscAE) in mmol ascorbic acid/g sample (Dorman et al., 2003). The bigger the AscAE value, the greater the reducing power of the sample. The data are presented as the average value of quadruplicate analyses.

2.6. 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

The ability of the extracts to scavenge $DPPH^{\bullet}$ radicals was determined by the method of Gyamfi et al. (1999). A 50 μL aliquot of each extract, in 50 mM Tris–HCl buffer (pH 7.4), was mixed with 450 μL of Tris–HCl buffer and 1.0 mL of 0.1 mM $DPPH^{\bullet}$ in MeOH. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using Eq. (1). Estimated IC_{50} values are presented as the average of quadruplicate analyses.

$$\text{percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

2.7. Determination of inhibition of linoleic acid peroxidation

2.7.1. Iron(II) thiocyanate method

The ability of the extracts to inhibit the linoleic acid peroxidation was determined by the method of Llorach et al. (2002). All reagents were prepared fresh. Reaction mixture (2.525 mL) in screw cap bottle was included linoleic acid (2.55) (0.25 mL) in ethanol, 50 mM sodium phosphate buffer, pH 7 (1 mL), ethanol (0.25 mL), distilled water (0.9 mL), sample solution (0.1 mL), and 1.8 mM AAPH (25 mL) for acceleration. This mixture was mixed vigorously and placed in oven at 50 °C for 10 h incubation. Thirty microliters of reaction mixture was take in every 2 h into the 2910 μL ethanol, and added 30 mL of ammonium thiocyanate (3.86 M) solution. Thirty microliters of iron(II) solution was added and mixed vigorously then the absorbance at 500 nm was read after 3 min. Blank solution was included all reagents except sample. Ascorbic acid, BHT and gallic acid were used as positive controls. The average of quadruplicate analyses was given as results.

2.7.2. Measurement of MDA value (TBA method)

The amount of malondialdehyde (MDA) formed in reaction mixture used in the above method was determined by thiobarbituric acid (TBA) reagent. One milliliter of reaction mixture, 1 mL trichloroacetic acid (TCA, 2.8%) and 1 mL TBA (1%) were mixed vigorously, and then placed in water bath at 90 °C for 20 min. After incubation, reaction was stopped in ice-bath for 10 min. Two millilitres of *n*-butanol was added into the reaction mixture and mixed vigorously. The butanol phase was separated after centrifuging at 3000 rpm for 5 min. The absorbance of *n*-butanol phase was measured at 532 nm using *n*-butanol as blank. The average of quadruplicate analyses was given as results.

2.8. Pharmacological procedures

2.8.1. Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for 2 days for acclimatization to animal room conditions were maintained on standard pellet diet and water *ad libitum*. The food was withdrawn on the day before the experiment, but free access of water was allowed. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

2.8.2. Preparation of test samples for bioassay

All the materials were given orally to test animals in 200 mg kg^{-1} doses after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg kg^{-1}) or acetyl salicylic acid (ASA) (200 mg kg^{-1}) in 0.5% CMC was used as reference drug.

2.8.3. Anti-nociceptive activity

p-Benzoquinone-induced abdominal constriction test was performed on mice for the determination of anti-nociceptive activity (Okun et al., 1963). According to the method; 60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml 10 g^{-1} body weight of

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2.5% (w/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent average of the total number of writhes observed. The anti-nociceptive activity was expressed as percentage change from writhing controls. Aspirin (ASA) at 100 and 200 mg kg^{-1} doses was used as the reference drug in this test.

2.8.4. Anti-inflammatory activity

Carrageenan-induced hind paw edema model was used with modifications in measuring periods for determination of anti-inflammatory activity (Kasahara et al., 1985). The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Sixty minutes after the oral administration of test sample or dosing vehicle, each mice was injected with freshly prepared (0.5 mg 25 μL^{-1}) suspension of carrageenan (Sigma, St. Louis, MO, USA) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μL saline solutions were injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg kg^{-1}) was used as the reference drug.

2.8.5. Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded, if happens, for each group at the end of observation period.

2.8.6. Gastric-ulcerogenic effect

After the anti-nociceptive activity experiment, mice were killed under deep ether anesthesia and stomachs were removed. Then, the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleedings.

2.9. Statistical analysis

Data are presented as mean values ± standard error. All statistical analyses were carried out using SPSS 10.0.1. (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was performed by ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $p < 0.05$. IC_{50} values were estimated using a non-linear regression algorithm. Data obtained from animal experiments were expressed as mean standard error (±S.E.M.). Statistical differences between the treatments and the control were evaluated by ANOVA and Students–Newman–Keuls post hoc tests. $p < 0.05$ was considered to be significant ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

3. Results

3.1. Extract yields, total phenol, total flavonoids, total flavonols and compositional analysis

Lythrum salicaria was sequentially extracted with petroleum ether, ethyl acetate, methanol and 50% aqueous methanol and extracted with water under reflux, as well. The data of yields, total phenols, total flavonoids and flavonols content of all the extracts are shown in Table 1. The amount of extractable components from *Lythrum salicaria* ranged from 10.32 mg/g (ethyl acetate extract) to 261.35 mg/g (water extract). According to Table 1 presented data, the amounts of total phenol contents were increased by polarity, but total flavonoid and flavonol contents did not change linearly. On the other hand, all the fractions were found to be rich in flavonoids except for the

Table 1
Extract yield, total phenols, flavonoids and flavonols, and HPLC qualitative and quantitative data for *Lythrum salicaria*

Sample	Yield[a]	Spectrophotometric results			HPLC results[b]			
		Total phenols[c]	Total flavonoids[d]	Total flavonols[e]	Isoorientin	Isovitexin	Unidentified flavonoids[f]	∑
PE	11.73	20.03 ± 0.11	nd[g]	nd	nd	nd	nd	–
E	10.32	35.74 ± 0.24	49.64 ± 0.42	8.43 ± 0.11	1.32 ± 0.02	0.67 ± 0.01	4.07 ± 0.05	6.05 ± 0.02
M	81.16	191.35 ± 0.45	37.57 ± 0.26	18.56 ± 0.07	7.60 ± 0.05	3.71 ± 0.03	11.22 ± 0.07	22.53 ± 0.04
MS	119.97	525.76 ± 0.86	37.34 ± 0.09	0.30 ± 0.01	6.18 ± 0.01	1.94 ± 0.00	11.71 ± 0.06	19.82 ± 0.03
∑	223.18	772.88 ± 0.55	124.55 ± 0.38	27.29 ± 0.12	15.10 ± 0.03	6.32 ± 0.02	27.00 ± 0.06	48.40 ± 0.03
S	261.35	305.22 ± 0.32	27.64 ± 0.59	1.12 ± 0.03	1.11 ± 0.01	0.23 ± 0.01	11.54 ± 0.10	12.88 ± 0.05

PE, petroleum ether extract; E, ethyl acetate extract; M, methanolic extract; MS, aqueous methanolic extract; S, water extract.
[a] Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material.
[b] Values (mg/g) are expressed as means ± standard error.
[c] Total phenols expressed as gallic acid equivalents: milligrams of gallic acid per gram (dry weight) of extract.
[d] Total flavonoids expressed as rutin equivalents: milligrams of rutin per gram (dry weight) of extract.
[e] Total flavonols expressed as rutin equivalents: milligrams of rutin per gram (dry weight) of extract.
[f] Quantified using isoorientin.
[g] nd, not detected.

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petroleum ether fraction. The order of increasing phenolic content, expressed as milligrams of gallic acid per gram of extract was 50% aqueous methanol > water > methanol > ethyl acetate > petroleum ether fractions (Table 1).

The qualitative and quantitative analyses of the extracts carried out using an HPLC coupled with a PDA detector are presented in Table 1, with selected chromatograms shown in Fig. 1. The components isoorientin and isovitexin were identified by comparing retention times and UV spectra of authentic standards, while quantitative data were calculated from their calibration curves. Unidentified flavonoids were calculated at 360 nm using the calibration curve of isoorientin. Values (mg/g) are expressed as means ± standard error. Isoorientin and isovitexin were found as the main identified components in both methanolic and aqueous methanolic extracts while they were present in lesser amount in the others. The total amounts of isoorientin and isovitexin within the fractions of *Lythrum salicaria* were obtained 15.10 ± 0.03 and 6.32 ± 0.02 mg g^{-1}, respectively (Table 1).

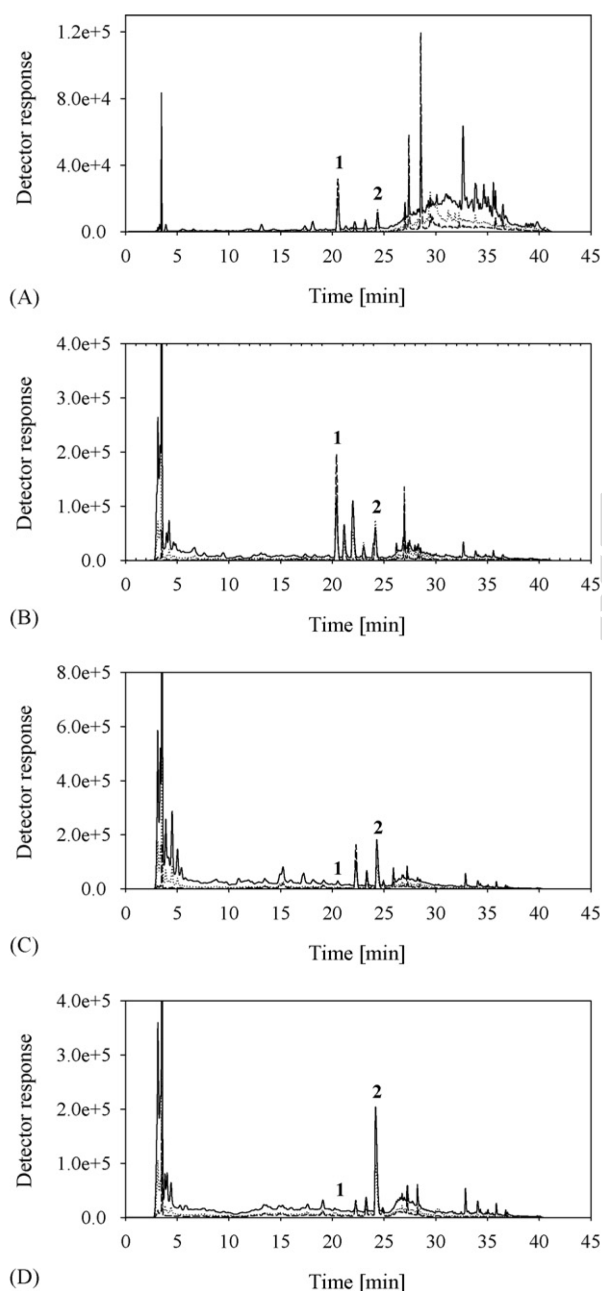

Fig. 1. HPLC–PDA analysis of *Lythrum salicaria* with responses at 280, 330 and 360 nm overlaid. (A) Ethyl acetate extract; (B) methanolic extract; (C) aqueous methanolic extract; (D) water extract; **1**, isoorientin; **2**, isovitexin.

There was a moderate association between total phenol content and DPPH$^{\bullet}$ radical scavenging ($r^2 = 0.7750$; $p = 0.225$) and MDA values ($r^2 = 0.6306$; $p = 0.369$). It is known that the position of the number of hydroxyl groups in flavonoids determines their antioxidant properties; in general, these properties depend on the ability to donate hydrogen or electron to a free radical (Miliauskas et al., 2004). Total flavonol contents ranged from 1.12 mg/g (water extract) to 18.56 mg/g (methanol extract). There was a strong correlation between the total flavonol contents and the inhibition of formation of MDA in both concentrations ($r^2 = 0.8849$; $p = 0.115$ and $r^2 = 0.9311$; $p = 0.069$, respectively).

3.2. *Iron(III) to iron(II) reduction activity*

Different studies have indicated that the antioxidant effect is related to the development of reductones (Yen and Duh, 1993). Reductones are reported to be terminators of free radical chain reactions (Gordon, 1990); thus, the antioxidant activity of an extract may be related to its reductive activity. As seen in Table 2, all the extracts possessed the ability to reduce iron(III) and did so in a linear concentration-dependent fashion. The aqueous methanolic extract was the best iron(III) reducer and its activity was similar to that of BHT. The water extract had a significant ($p < 0.05$) activity but lower than that of the aqueous methanolic extract.

3.3. *1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity*

Free radicals involved in the process of lipid peroxidation are considered to play a cardinal role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others, and are implicated in the aging process. Therefore, the extracts were assessed against DPPH radical serving as the oxidizing substrate, which can be reduced by an antioxidant compound to its hydrazine derivative *via* hydrogen donation, and as the reaction indicator molecule (Dorman et al., 2003).

All the extracts were capable of scavenging DPPH$^{\bullet}$ radicals at pH 7.4 in a dose-dependent fashion except for the petroleum ether extract, which was not effective to DPPH$^{\bullet}$ radicals. From the estimated IC_{50} values, that aqueous methanolic extract was the most potent scavenger followed by methanolic extract > ethyl acetate extract (Table 2). Activity of the methanol extract activity was significantly similar to that of BHT. The water extract had significant activity but was lower than that of the aqueous methanolic extract. The results of the DPPH$^{\bullet}$ radical scavenging assay reveal that these extract (except for the petroleum ether extract), especially aqueous methanolic and water extracts, are capable of scavenging free radicals in solution at pH 7.4 and may

Table 2
Antioxidant properties of the extracts of *Lythrum salicaria*

Sample[a]	AscAE[b] (mmol/g)	IC_{50}[c] (mg/mL)	Inhibition (%)			
			Fe-thiocyanate[d]		TBA[e]	
			0.25	1	0.25	1
AscA	5.7 ± 0.2[f]	0.1 ± 0.0	40.4 ± 0.4	92.3 ± 0.5	21.2 ± 0.6	83.7 ± 1.9
BHT	5.2 ± 0.0	0.1 ± 0.0	95.1 ± 0.4	93.6 ± 0.8	85.4 ± 0.5	96.2 ± 0.5
GA	13.7 ± 0.3	0.0 ± 0.0	71.0 ± 0.6	59.6 ± 0.7	69.5 ± 0.9	70.9 ± 0.8
A	0.7 ± 0.0	n.d.	6.0 ± 0.8	−15.6 ± 0.9[g]	18.4 ± 0.6	−29.5 ± 0.9[g]
B	0.5 ± 0.0	2.7 ± 0.1	93.8 ± 0.9	88.2 ± 0.2	88.5 ± 0.4	75.7 ± 0.2
C	1.5 ± 0.0	0.3 ± 0.0	49.1 ± 1.4	77.0 ± 1.9	64.7 ± 0.4	70.7 ± 0.6
D	5.2 ± 0.0	0.1 ± 0.0	85.7 ± 1.2	75.7 ± 0.5	86.0 ± 0.6	85.1 ± 1.4
E	3.1 ± 0.0	0.1 ± 0.0	84.0 ± 0.3	85.8 ± 0.6	92.8 ± 0.8	89.7 ± 1.2

[a] AscA, ascorbic acid; BHT, butylated hydroxytoluene; GA; gallic acid; A, petroleum ether extract; B, ethyl acetate extract; C, methanol extract; D, aqueous methanol extract; E, water extract.
[b] Iron(III) reduction.
[c] DPPH radical scavenging.
[d] Inhibition of linoleic acid peroxidation.
[e] Inhibition of malondialdehyde formation.
[f] Values (mg/mL) are expressed as mean ± standard error.
[g] Pro-oxidant activity.

prevent initiation of free radical-mediated chain reactions by preventing the abstraction of hydrogen from susceptible polyunsaturated fatty acids.

3.4. Determination of inhibition of linoleic acid peroxidation

3.4.1. Iron(II) thiocyanate method

In this method, two concentrations of all the extracts were used (0.25 and 1%). All extracts, except for the petroleum ether extract, showed a high capacity to inhibit linoleic acid peroxidation when the FTC assay was performed (Table 2). The ethyl acetate extract was found to be the most active extract. Its activity was the same as that of BHT at 0.25% concentration and similar to BHT and ascorbic acid at 1% concentration. The activity decreased in the following order was ethylacetate > aqueous methanol > water > methanol extracts at 0.25% concentration and ethylacetate > water > methanol > aqueous methanol at 1% concentration. Under this assay condition, ethylacetate, aqueous methanol, water extracts were able to prevent peroxidation 100% through 10 h of the assay period. But methanol extract and ascorbic acid were able to prevent peroxidation 50% through 10 h at 0.25% concentration. All the extracts were able to prevent peroxidation 100% through 10 h of assay at 1% concentration.

3.4.2. Measurement of MDA value (TBA method)

All the extracts, except for petroleum ether, showed strong activity at iron(III) to iron(II) reduction (Table 2). Two concentrations of the water extract and 0.25% concentration of ethyl acetate extract were the most potent extracts. They were statistically ($p < 0.05$) significant similar to the activity of 0.25% concentration of BHT. The order of decreasing is as follows: aqueous methanolic extracts > ethyl acetate (1% concentration of the extract) > methanolic extracts. The activity of these extracts were statistically similar and the activity of aqueous methanolic extract was not statistically ($p < 0.05$) different from that of 1% BHTs. But petroleum ether extract showed similar results with the control at two concentrations.

3.5. Anti-nociceptive and anti-inflammatory activities

Lythrum salicaria has been evaluated for its *in vivo* anti-inflammatory and anti-nociceptive activities. Inhibitory effects of the extracts prepared from *Lythrum salicaria* on *p*-benzoquinone-induced writhing for the assessment of anti-nociceptive activity and carrageenan-induced hind paw edema model, a widely used screening protocol for anti-inflammatory activity to test the non-steroidal anti-inflammatory drugs, were examined in mice.

The dose-dependent swelling thickness and inhibition effects of *Lythrum salicaria* extracts are presented in Table 3. Ten milligrams per kilogram of Indomethacin ($10\,mg\,kg^{-1}$) was used as positive control in this assay. As shown in Table 3, methanol extract showed an inhibitory activity ($p < 0.05$) at $200\,mg\,kg^{-1}$ dose after 270 and 360 min (28.9 and 35.5%, respectively). Other extracts from *Lythrum salicaria* were found inactive against the positive control.

The analgesic activities of the samples were studied by using a *p*-benzoquinone-induced writhing model in mice. The number of writhes, inhibition ratio and the ratio of ulceration by the extracts are given in Table 4. According to Table 4, methanol extract was found to have inhibitory activity ($p < 0.05$) at 100 and 200 mg/kg dose (26.9 and 30.1%, respectively). None of the extracts caused any gastric damage whereas ASA was found to have high ulceration ratio (5/6).

4. Discussion

Lythrum salicaria has been traditionally used for many purposes such as antihemorrhagic, cicatrizant, moderating men-

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Table 3
Effects of the *Lythrum salicaria* extracts against carrageenan-induced paw edema in mice

Samples	Swelling thickness ($\times 10^{-2}$ mm) ± S.E.M. (inhibition, %)			
	90 min	180 min	270 min	360 min
Control	44.8 ± 3.37	50.0 ± 3.37	56.0 ± 3.65	62.5 ± 4.68
Indomethacin (10 mg/kg)	34.5 ± 2.99 (22.9)*	31.0 ± 2.02 (38.0)**	33.2 ± 2.15 (40.7)***	34.6 ± 2.38 (44.6)***

Samples	Swelling thickness ($\times 10^{-2}$ mm) ± S.E.M. (inhibition, %)							
	90 min		180 min		270 min		360 min	
	100[a]	200[a]	100[a]	200[a]	100[a]	200[a]	100[a]	200[a]
A	47.2 ± 3.29	44.7 ± 3.55	51.2 ± 4.01	49.3 ± 3.68 (1.4)	58.9 ± 4.12	53.5 ± 3.39 (4.5)	65.9 ± 4.76	61.0 ± 3.07 (2.4)
B	43.3 ± 3.15 (3.3)	41.0 ± 2.59 (8.5)	45.8 ± 3.02 (8.4)	43.5 ± 2.73 (13.0)	48.9 ± 3.14 (12.7)	45.7 ± 3.59 (18.4)	54.3 ± 3.25 (13.1)	49.7 ± 3.23 (20.5)
C	42.3 ± 3.85 (5.5)	41.2 ± 3.90 (8.0)	41.1 ± 3.26 (17.8)	38.8 ± 3.77 (22.4)	44.2 ± 3.88 (21.1)	39.8 ± 3.82 (28.9)*	45.9 ± 4.15 (26.6)	40.3 ± 4.01 (35.5)*
D	47.3 ± 3.02	45.2 ± 3.83	51.2 ± 3.65	47.8 ± 3.89 (4.4)	56.8 ± 3.89	51.3 ± 3.88 (8.4)	63.3 ± 3.92	54.2 ± 3.85 (13.3)
E	51.7 ± 4.89	50.7 ± 4.77	50.3 ± 4.15	55.5 ± 4.70	57.9 ± 5.02	60.5 ± 4.29	65.8 ± 5.11	66.7 ± 3.86

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ significant from control; S.E.M., mean standard error; A, petroleum ether extract; B, ethyl acetate extract; C, methanol extract; D, aqueous methanol extract; E, water extract.
[a] Dose (mg/kg).

strual flow, antidiarrhoeal, typhoid, and astringent. Phenolic compounds, especially, tannins and flavonoids were found to be responsible for these actions (Rauha et al., 2000; Rauha et al., 2001; Çoban et al., 2003). *Lythrum salicaria* has shown inhibitory activity against *Staphylococcus aureus* and *Candida albicans*. The flower and stem extracts of *Lythrum salicaria* were reported to decrease serum glucose levels in hyperglycemic rats. *Lythrum salicaria* was shown to exhibit antiparasitic activity against schistosomiasis in tropical and subtropical countries (Rauha, 2000; Becker et al., 2005).

Relatively, little work has been done on the phytochemistry of *Lythrum salicaria* in recent years. Tannins, flavone-C-glycosides and anthocyanins have been identified in *Lythrum salicaria* extracts. Isoorientin, isovitexin and their derivatives have been reported as its main flavonoids (Rauha et al., 2000; Rauha, 2001).

Flavonoids have certain health effects and their antioxidant, radical scavenging, anti-mutagenic and anti-carcinogenic properties are well known (Middleton et al., 2000; Rauha, 2001; Havsteen, 2002). Their beneficial effects on coronary heart disease have also been studied. Few studies exist on the structure activity relationship of *Lythrum salicaria* flavonoids. In this study, flavonoids of *Lythrum salicaria* and some of their biological activities were investigated. Polar extracts of *Lythrum salicaria* were found to be more active in both *in vitro* antioxidant and *in vivo* pharmacological activity tests. These fractions were also found to be the richest in flavonoids content. Flavonoids are good free radical scavengers that donate hydrogen. Free radical scavenging and lipid peroxidation activities of flavonoids are also well known (Rauha, 2001; Havsteen, 2002). These activities seem to be directly related to the number of hydroxyl groups at ring B (Pelzer et al., 1998). Methanolic and aqueous methanolic fractions and water extract scavenged the DPPH$^\bullet$ free radical in physiological pH while ethyl acetate fraction of *Lythrum salicaria* was found to be the most active in inhibiting linoleic acid peroxidation and in MDA formation tests.

The therapeutic applications of flavonoids on inflammation have previously been reported (Middleton et al., 2000; Havsteen, 2002). Inflammation is important in many serious diseases, including cancer, Alzheimers and AIDS. Therefore, intake of flavonoids is very important in the management of these diseases. In addition, flavonoids are known to prevent the synthesis of prostaglandins. Biochemical investigations on the mechanism of action of flavonoids have shown that these compounds can inhibit a wide variety of enzymes. Linoleic acid and arachidonic acid are indigenous compounds of the cell membrane with a task to protect the cell. The release of arachidonic acid is closely related to the cyclooxygenase (CO) and 5-lipoxygenase (LO) enzyme systems. The ability of flavonoids to inhibit both CO and LO pathways of the arachidonate metabolism have been suggested to contribute to anti-inflammatory action (Middleton et al., 2000). Therefore, the protection of membrane lipids is important in inducing both anti-inflammatory and antioxidant activities.

Rauha (2001) reported the amount of flavonoids in *Lythrum salicaria* to be high. The results of this study agreed with the previous works regarding the high content of flavonoids (Table 1).

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Table 4
The effects of *Lythrum salicaria* extracts against *p*-benzoquinone-induced writhings in mice[a]

Test samples	Number of writhings ± S.E.M.		Inhibitory ratio (%)		Ratio of ulceration	
Control	50.5 ± 4.15				0/6	
	100[b]	200[b]	100[b]	200[b]	100[b]	200[b]
ASA	24.8 ± 1.96	21.8 ± 1.74	50.9^{***}	57.0^{***}	4/6	5/6
A	48.2 ± 3.56	44.0 ± 3.33	4.6	12.9	0/6	0/6
B	41.7 ± 3.98	38.0 ± 3.53	17.4	24.8	0/6	0/6
C	36.9 ± 3.01	35.3 ± 2.68	26.9^{*}	30.1^{**}	0/6	0/6
D	44.8 ± 3.69	41.5 ± 3.13	11.3	17.8	0/6	0/6
E	48.9 ± 4.11	44.3 ± 4.62	3.2	12.3	0/6	0/6

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ significant from control; S.E.M., mean standard error; A, petroleum ether extract; B, ethyl acetate extract; C, methanol extract; D, aqueous methanol extract; E, water extract.
[a] Number of rats whose stomachs were completely prevented from bleeding.
[b] Dose (mg/kg).

The flavonoid rich polar extracts were found to be active in both antioxidant and anti-inflammatory activities. These extracts contain isoorientin and isovitexin as flavonoid-C-glycosides. Methanolic and aqueous methanolic and water extracts were found to be equally rich in flavonoids (Table 1). The methanolic extract in which isoorientin and isovitexin occurred together showed the highest anti-inflammatory activity, while the extracts that contained the highest amount of isovitexin and lesser amount of isoorientin exhibited the highest antioxidant activity. Little work has been reported on the antioxidant and anti-inflammatory activities of pure isovitexin and isoorientin (Ko et al., 1998; Arimoto et al., 2000; Küpeli, 2004; Küpeli et al., 2004). These studies confirm their antioxidant and/or anti-inflammatory activities.

These preliminary results thus confirm the reported antioxidant and anti-inflammatory uses of *Lythrum salicaria*.

References

Arimoto, T., Ichinose, T., Yashikawa, T., Shibamoto, T., 2000. Effect of the natural antioxidant 2″-*O*-glycosylisovitexin on superoxide and hydroxyl radical generation. Food and Chemical Toxicology 38, 849–852.

Baytop, T., 1999. Therapy with Plants in Turkey. Istanbul University Publications, Istanbul, p. 374 (No. 3255).

Becker, H., Scher, J.M., Speakman, J.B., Zapp, J., 2005. Bioactivity guided isolation of antimicrobial compounds from *Lythrum salicaria*. Fitoterapia 76, 580–584.

Çoban, T., Çitoğlu, G.S., Sever, B., Işan, M., 2003. Antioxidant activities of plants used in traditional medicine in Turkey. Pharmaceutical Biology 41, 608–613.

Davis, P.H., 1982. Flora of Turkey and the East Aegean Islands, vol. 4. University Press, Edinburgh, pp. 174–176.

Dorman, H.J.D., Koşar, M., Kahlos, K., Holm, Y., Hiltunen, R., 2003. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. Journal of Agricultural and Food Chemistry 51, 4563–4569.

Gordon, M.H., 1990. The mechanism of antioxidant action in vitro. In: Hudson, B.J.F. (Ed.), Food Antioxidants. London, UK, Elsevier, pp. 1–18.

Gyamfi, M.A., Yonamine, M., Aniya, Y., 1999. Free-radical scavenging action of medicinal herbs from Ghana *Thonningia sanguinea* on experimentally induced liver injuries. Genetic Pharmacy 32, 661–667.

Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics 96, 67–202.

Kahkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural Food Chemistry 47, 3954–3962.

Kasahara, Y., Hikino, H., Tsurufiji, S., Watanabe, M., Ohuchi, K., 1985. Anti-inflammatory actions of ephedrines in acute inflammations. Planta Medica 51, 325–331.

Ko, F.N., Chu, C.C., Lin, C.N., Chang, C.C., Teng, C.M., 1998. Isoorientine-6″-*O*-glucoside, a water-soluble antioxidant isolated from *Gentiana arisanensis*. Biochimica et Biophysica Acta 1389, 81–90.

Küpeli, E., 2004. Studies on the antirhomatizmal effect of *Clematis vitalba* L. PhD Thesis, Pharmacognosy Department, Pharmacy Faculty, Gazi University.

Küpeli, E., Aslan, M., Gürbüz, I., Yesilada, E., 2004. Evaluation of *in vivo* biological activity profile of isoorientin. Zeitschrift für Naturforschung C 59, 787–790.

Lamela, M., Cadavid, I., Calleja, J.M., 1986. Effects of *Lythrum salicaria* extracts on hyperglycemic rats and mice. Journal of Ethnopharmacology 15, 153–160.

Llorach, R., Espin, J.C., Tomas-Barberan, F.A., Ferreres, F., 2002. Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. Journal of Agricultural and Food Chemistry 50, 3458–3464.

Mantle, D., Eddeb, F., Pickering, A.T., 2000. Comparison of relative antioxidant activities of British medicinal plant species in vitro. Journal of Ethnopharmacology 72, 47–51.

Middleton, E., Kandaswami, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacological Reviews 52, 673–751.

Miliauskas, Venskutonis, P.R., Van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 85, 231–237.

Munger, G.T., 2002. Fire Effects Information System, http://www.fs.fed.us/database/feis (October 3, 2005).

Okun, R., Liddon, S.C., Lasagnal, L., 1963. The effect of aggregation, electric shock and adrenergic blocking drugs on inhibition of the “writhing syndrome”. Journal of Pharmacology and Experimental Therapeutics 139, 107–109.

Oyaizu, M., 1986. Studies on products of browning reaction: antioxidative activity of products of browning reaction. Japan Journal of Nutrition 44, 307–315.

Pelzer, L.E., Guardia, T., Juarez, A.O., Guerreiro, E., 1998. Acute and chronic anti-inflammatory effects of plant flavonoids. Il Farmaco 53, 421–424.

Rauha, J.P., 2001. The research for biological activity in Finnish plant extracts containing phenolic compounds, PhD Thesis, Pharmacognosy Department, Pharmacy Faculty of Science, University of Helsinki, pp. 31–45.

Rauha, J.P., Remes, S., Heinonen, M., Hopia, A.I., Kahkönen, M., Kujala, T.S., Pihlaja, K., Vuorela, H.J., Vuorela, P., 2000. Antimicrobial effects of Finnish

plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology 56, 3–12.
Rauha, J.P., Wolfender, J.L., Salminen, J.P., Pihlaja, K., Hostettmann, K., Vuorela, H., 2001. Characterization of the polyphenolic composition of purple loosestrife (*Lythrum salicaria*). Zeitschrift für Naturforschung C 56, 13–20.
Singleton, V.L., Orthofer, R., Lamuela-Ravento, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer, L. (Ed.), Methods in Enzymology, vol. 299. Academic Press, San Diego, CA, pp. 152–315.
Yen, G.C., Duh, P.D., 1993. Antioxidative properties of methanolic extracts from peanut hulls. Journal of the American Oil Chemical Society 70, 383–386.

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