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Isolation of active constituents from cherry laurel (*Laurocerasus officinalis* Roem.) leaves through bioassay-guided procedures

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ABSTRACT

Ethnopharmacological relevance: The fresh leaves of *Laurocerasus officinalis* Roem. (Rosaceae) are externally used against pain and feverish symptoms in Turkish folk medicine.
Aim of the study: Effects of the extracts, fractions and isolated compounds from the leaves of *L. officinalis* were investigated using *in vivo* models of inflammation and pain in mice.
Methods: The crude ethanolic extract from the leaves of plant was sequentially fractionated into five subextracts; explicitly, *n*-hexane, chloroform, ethyl acetate (EtOAc), *n*-butanol, and remaining water extracts. Further studies were carried out on the most active EtOAc subextract was further subjected to fractionation through column chromatography. For the anti-inflammatory activity, carrageenan-induced hind paw edema and acetic acid-induced increase in capillary permeability models, and for the antinociceptive activity *p*-benzoquinone-induced writhing test in mice were employed.
Results: Ethanolic extract of the leaves was shown to possess significant inhibitory activity in the assay methods without inducing any gastric damage. Through bioassay-guided fractionation and isolation procedures three phenolic compounds, 2-*O*-β-D-glucopyranosyl-2-hydroxyphenyl-acetic acid (**1**), kaempferol-3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**) and (+)-catechin (**3**) were isolated from the active fraction and their structures were elucidated by spectral techniques (1D and 2D NMR, ESIMS).
Conclusion: The experimental data verified that *Laurocerasus officinalis* leaves displayed remarkable anti-inflammatory and antinociceptive activity.

1. Introduction

Cherry laurel (*Laurocerasus officinalis* Roem. syn: *Prunus laurocerasus* L.) belongs to the Rosaceae family, locally called "Taflan" or "Karayemis", is an evergreen shrub or small tree of up to 6 m in height characteristic in the northern coastal area of Turkey by the Black Sea (Ayaz et al., 1995). The cherry laurel fruit is a popular food in the eastern Black Sea region, where mostly consumed as fresh fruits in local markets, but also as dried, pickled or processed into pekmez (concentrated juice), jam, marmalade or as fresh fruit juice (Ayaz, 1997). Besides its consumption as food, both fruit and seed of cherry laurel have been utilized as traditional remedy in Turkey for the treatment of digestive system complaints, including stomach ulcer, bronchitis (seeds), eczema, hemorrhoids and as diuretic (fruits) (Ayaz, 1997). It is also used externally for its analgesic (Alpınar and Yazıcıoglu, 1991; Ayaz et al., 1998) and antipuriginous effects (Alpınar and Yazıcıoglu, 1991; Çubukçu, 1989).

In Turkish folk medicine, fresh leaves of the plant are employed against pain and feverish symptoms, externally. Fresh broad leaves are applied on the forehead, after being wilted over open fire (Yesilada et al., 1999). In some other sources also several traditional utilizations of the leaves have been reported, such as against asthma, to alleviate cough, for indigestion and dyspepsia (Grieve, 1994). In a previous preliminary screening study ethanolic extract of the leaves exerted significant anti-inflammatory and antinociceptive activity, while aqueous extract was inactive (Erdemoglu et al., 2003).

The cherry laurel and its cultivars have been studied for fatty acids compositions in their seeds (Ayaz et al., 1995), phenolic acids, fatty acids and sugar contents (Ayaz et al., 1997a) as well as volatile constituents in the leaves and fruits (Mchedlidze and Kharebava, 1988), and benzyl-β-primveroside in the green fruits (Weinges et al., 1991). The ripe fruit of the plant was reported to contain high levels of fructose and glucose as sugars, mainly vanillic acid as a phenolic acid, and linoleic acid as an unsaturated fatty acid (Ayaz et al., 1997b).

The aim of the present study was to investigate the *in vivo* anti-inflammatory and antinociceptive effects of *Laurocerasus officinalis*

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leaves and to isolate the active constituent(s) through bioassay-guided fractionation techniques and to elucidate the structure(s) for the verification of the folkloric information.

2. Material and methods

2.1. Plant material

Laurocerasus officinalis Roemer (Rosaceae) leaves were collected from Trabzon, Akçaabat, Darıca and was identified by Prof. Dr. M. Vural from the Department of Botany, Faculty of Science, Gazi University. Material was dried under shade and course powdered before extraction. Voucher specimen is deposited in the Herbarium of Faculty of Pharmacy, Gazi University (GUE-2789).

2.2. Extraction and fractionation

2.2.1. Preparation of extracts for preliminary activity testing

Two types of extracts were prepared for the preliminary activity assessment of the leaves following the traditional utilization way. First a portion of the powdered leaf (10 g) was extracted twice by continuous stirring for 24 h with distilled H_2O (50 ml) at room temperature. Aqueous extract was then lyophilized to yield "H_2O extract" (3.81 g). Another portion of the powdered leaf (10 g) was extracted twice with 96° EtOH (50 ml) at room temperature for 24 h and evaporated to dryness under reduced pressure not exceeding 40 °C to yield 'EtOH extract' (3.93 g).

2.2.2. Preparation and fractionation of the [EtOH-extract]

One thousand five hundred grams of powdered leaf was extracted in EtOH 96% (15 l) by intermittent stirring in a water bath adjusted at 40 °C for 4 days and the extract was filtered through a filter paper and the filtrate was then evaporated to dryness under reduced pressure. The residual plant was processed several times in a similar way to remove the EtOH-extractable components. All extractives were combined to give EtOH extract (yield: 378.37 g). The EtOH extract was then dissolved in 500 ml of EtOH (90% in H_2O) and extracted with *n*-hexane (10 × 500 ml) in a separatory funnel. Combined hexane extract was evaporated under reduced pressure to yield 'Hexane Fr.' (77.81 g). Then EtOH was removed from the remaining extract and diluted with distilled H_2O to 400 ml and further fractionated by successive solvent extractions with chloroform (5 × 750 ml), ethyl acetate (5 × 750 ml) and *n*-butanol saturated with H_2O (4 × 750 ml). Each extract as well as remaining aqueous phase after solvent extractions was evaporated to dryness under reduced pressure to yield "$CHCl_3$ Fr." (105.33 g), "EtOAc Fr." (25.16 g), "*n*-BuOH Fr." (55.84 g) and "R-H_2O Fr." (80.25 g), respectively.

2.2.3. Chromatographic separation and isolation of active constituents

Five grams of EtOAc Fr. was subjected to column chromatographic separation on Silicagel (Kieselgel 230–400 mesh, Merck Art. No. 1.09385) using $CHCl_3$, $CHCl_3$:MeOH (95:5) → $CHCl_3$:MeOH:H_2O (70:30:3) as solvent systems and eluents were combined as follows: Fr.1 (0.39 g), Fr.2–3 (0.48 g), Fr.4–7 (0.63 g), Fr.8–17 (1.89 g), Fr.18–20 (0.93 g), and Fr.21–35 (0.68 g).

Fr. 8–17 (670 mg) was separated by C_{18}-MPLC (130 g, 5–60% MeOH/H_2O to afford 2-*O*-β-D-Glucopyranosyl-2-hydroxyphenyl-acetic acid (**1**) (20 mg), and subfraction (50 mg) containing crude **3**, which was further purified by SiO_2 column chromatography (9 g) (mobil phase $CHCl_3$–MeOH–H_2O 90:10:1 to 70:30:3) to yield (+)-catechin (**3**) (3 mg). Fr. 18–20 (230 mg) was similarly applied to C_{18}-MPLC (43 g, 15–50% MeOH/H_2O) and yielded a fraction (15 mg), which includes impure **2**. The final purification of kaempferol 3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**) (3 mg) was achieved by Sephadex LH-20 (20 g) column chromatography using MeOH as eluent.

2.2.4. Structure elucidation of the compounds *1*–*3*

The chemical structures of the isolates were identified as 2-*O*-β-D-Glucopyranosyl-2-hydroxyphenyl-acetic acid (**1**) (D'Abrosco et al., 2001), kaempferol 3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**) (Kapusta et al., 2007) and (+)-catechin (**3**) (Markham and Geiger, 1994) by comparison of their spectroscopic data (1D and 2D NMR, ESIMS) with those previously published in the literature (Fig. 1).

2.3. Biological activity tests

2.3.1. Test 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 and were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but free access to water was allowed. A minimum of six animals was used in each group. The study was permitted by the Institutional Animal Ethics Committee (Gazi University Ethical Council Project Number: G.U.ET-05.004) and was performed following the international rules considering the animal experiments and biodiversity rights.

2.3.2. Preparation of test samples for bioassay

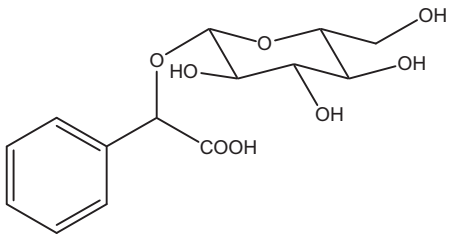
Test samples were given orally to test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetylsalicylic acid (ASA) (100 and 200 mg/kg) in 0.5% CMC was used as reference drug.

2.3.3. Antinociceptive activity

2.3.3.1. p-Benzoquinone-induced abdominal constriction test in mice. *p*-Benzoquinone-induced abdominal constriction test (Okun et al., 1963) was performed on mice for the determination of antinociceptive activity. According to the method evaluated, 60 min after the oral administration of a test sample, the mice were intraperitonally injected with 0.1 ml/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ) solution in distilled water. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for the observation and the total number of the abdominal contractions (writhing movements) was counted for the following 15 min, starting 5 min after the PBQ injection. The data represent the average of the total number of writhes observed. Antinociceptive activity was then expressed as the percentage change from writhing controls. Acetylsalicylic acid (ASA) at 100 and 200 mg/kg doses was used as the reference drug in this test.

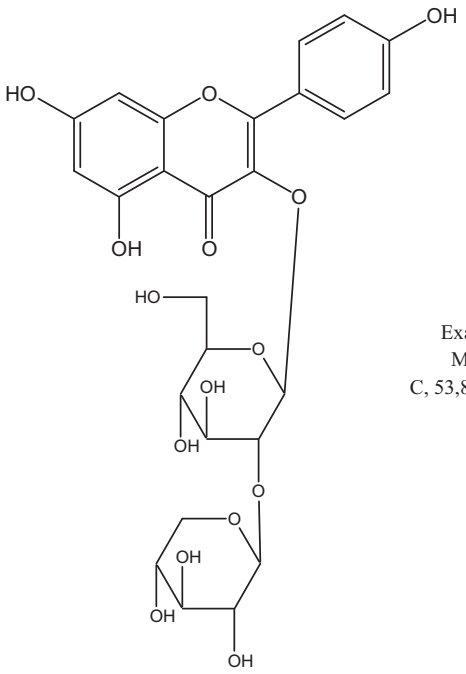
2.3.4. Anti-inflammatory activity

2.3.4.1. Carrageenan-induced hind paw edema. Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity (Yesilada and Küpeli, 2002). 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 those of a control group and analyzed by using statistical methods. 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared



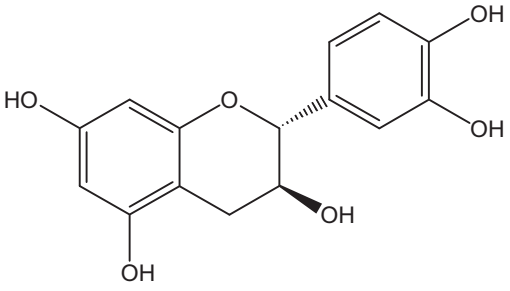
$C_{14}H_{18}O_8$
Exact Mass: 314,10
Mol. Wt.: 314,29
C, 53,50; H, 5,77; O, 40,73

2-O-β-D-Glucopyranosyl-2-hyroxyphenyl-acetic acid **(1)**



$C_{26}H_{28}O_{15}$
Exact Mass: 580,14
Mol. Wt.: 580,49
C, 53,80; H, 4,86; O, 41,34

Kaempferol-3-O-β-D-xylopyranosyl-(1→2)-O-β-D-glucopyranoside **(2)**



$C_{15}H_{14}O_6$
Exact Mass: 290.08
Mol. Wt: 290,13

(+)-Catechin **(3)**

Fig. 1. Chemical structures of isolated compounds **1**–**3**.

suspension of carrageenan (0.5 mg/25 μl) 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 then 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 those of a control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

2.3.4.2. Acetic acid-induced increase in capillary permeability. Effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method (Whittle, 1964) with some modifications (Yesilada et al., 1988). Each test sample was administered orally to a group of 10 mice in 0.2 ml/20 g body weight. Thirty minutes after the administration, tail of each mouse was injected with 0.1 ml of 4% Evans blue in saline solution (i.v.) and waited for 10 min. Then, 0.4 ml of 0.5% (v/v) AcOH was injected i.p. After 20 min incubation, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 ml volumetric flasks through glass wool. Each flask was made up to 10 ml with distilled water, 0.1 ml of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer; Beckman, Fullerton, CA, USA). A mixture of distilled water and 0.5% CMC was given orally to control animals, and they were treated in the same manner as described above.

2.3.5. Toxicity evaluation

2.3.5.1. 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.

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

2.3.6. Statistical analysis of data

Data obtained from animal experiments were expressed as the mean standard error (±SEM). Statistical differences between the treated and the control groups 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. Result and discussion

In Turkish folk medicine, the fresh leaves of *Laurocerasus officinalis* are externally used to reduce pain and fever. Since fresh leaves are directly applied on the skin surface of forehead, after being wilted over a fire (Yesilada et al., 1999), this application might be an indication for the nonpolar character of active ingredient(s). For this reason two types of extracts (aqueous and ethanolic) were prepared and submitted to preliminary anti-inflammatory and antinociceptive activity tests in mice.

As shown in Tables 1 and 2, aqueous extract of the leaves did not show any remarkable anti-inflammatory effect in carrageenan-induced hind paw edema, while a weak antinociceptive effect against *p*-benzoquinone induced writhing reflex models. However EtOH extract was significantly active in both assays without inducing any gastric toxicity. These results also confirmed our previous findings (Erdemoglu et al., 2003). Hence, EtOH extract was

Table 1
Effects of the extracts and fractions of *Laurocerasus officinalis* leaves on carrageenan-induced hind paw edema in mice.

Material	Dose mg/kg	Swelling thickness ($\times 10^{-2}$ mm) ± SEM (%inhibition)			
		90 min	180 min	270 min	360 min
Control		49.8 ± 4.94	57.2 ± 4.83	63.5 ± 5.04	68 ± 3.95
Aqueous extract	500	47.5 ± 4.47 (4.6)	51.7 ± 3.82 (9.6)	57.3 ± 4.14 (9.8)	58 ± 3.22 (14.7)
Ethanol extract	500	37.6 ± 3.69 (24.5)	43 ± 3.42 (24.8)	47.2 ± 5.30 **(25.6)***	47.5 ± 4.1 **(30.1)****
Indomethacin	10	30.5 ± 3.11 **(38.8)****	35.4 ± 2.76 **(38.1)****	35.8 ± 3.11 **(43.6)*****	40.6 ± 3.20 **(40.3)*****
Control		44.0 ± 4.32	48.5 ± 4.09	52.7 ± 4.31	58.2 ± 4.05
n-Hexane Fr.	205.64	42.2 ± 5.38 (4.1)	46.8 ± 5.13 (3.5)	49.5 ± 5.61 (6.1)	52.5 ± 6.19 (9.8)
Chloroform Fr.	278.38	43.0 ± 3.16 (2.3)	45.3 ± 4.17 (6.6)	49.7 ± 4.49 (5.7)	53.8 ± 4.60 (7.7)
Ethyl acetate Fr.	66.50	34.8 ± 2.69 (20.9)	38.2 ± 2.56 (21.2)	39.5 ± 2.79 **(25.0)***	39.8 ± 3.52 **(31.6)****
n-Butanol Fr.	147.58	41.2 ± 3.74 (6.4)	44.0 ± 4.20 (9.3)	47.2 ± 4.11 (10.4)	49.8 ± 4.65 (14.4)
Remaining aqueous Fr.	217.88	42.3 ± 3.23 (3.9)	47.0 ± 3.54 (3.1)	50.3 ± 3.89 (4.6)	51.7 ± 3.53 (11.2)

SEM, standard error mean.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

employed for the following bioassay-guided fractionation and isolation procedures using the same assay systems.

The EtOH extract was subjected to successive organic solvent extractions in increasing polarity as the first step of fractionation. Each solvent extract was then administered to experimental animals in doses proportioned to their ratio in the original EtOH extract. Among the fractions obtained EtOAc Fr. was significantly active, while the other fractions were found to be inactive in carrageenan-induced edema and p-benzoquinone induced writhing reflex models (Tables 1 and 2). Therefore further fractionation and bioassay procedures were carried out on EtOAc Fr.

EtOAc Fr. was further subjected to chromatographic separation on silica gel column and six subfractions were obtained. The anti-inflammatory effect of these subfractions was investigated using two *in vivo* test models; carrageenan-induced paw edema model (Table 3) and inhibition of increased capillary permeability induced by acetic acid injection into peritonal cavity (Table 4). A very similar activity pattern was observed in both *in vivo* models. Subfraction [Fr.8–17] exerted 21.7–33.6% and 30.9% inhibition, while subfraction [Fr.18–20] exerted 17.4–25.9% and 21.9% inhibition, respectively.

The antinociceptive activity of the subfractions was also investigated using *p*-benzoquinone induced writhing reflex model in mice. Same subfractions [Fr.8–17 and Fr.18–20] demonstrated significant antinociceptive activity, which rise up to 37.4% and 29.9% inhibition without inducing any gastric toxicity, respectively (Table 5).

These results revealed that subfractions [Fr.8–17] and with a lesser potency [Fr.18–20] have significant anti-inflammatory and antinociceptive activities. On the other hand, none of the test samples showed any sign of gross toxicity during the observational period of 48 h. These active subfractions were further subjected to separation by C_{18}-MPLC. Subfraction [Fr.8–17] afforded 2-*O*-β-D-glucopyranosyl-2-hydroxyphenyl-acetic acid (**1**) and (+)-catechin (**3**), while subfraction [Fr.18–20] gave kaempferol 3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**) as the main constituents. Due to insufficient amount of these compounds they were not submitted individually for further bioassay testing.

Previously, in a phytochemical study two kaempferol glycosides from the chloroform fraction of the hydroalcoholic extract of *Laurocerasus officinalis* were isolated and their structures were elucidated as kaempferol 3-*O*-(6-*O*-α-D-xylofuranosyl-β-D-galactopyranoside) and kaempferol 3-*O*-(6-*O*-β-D-glucofuranosyl-β-D-galactopyranoside) (Tsiklauri et al., 1979). However, they were not investigated for any activity.

Various phenolic compounds including phenolic acids and flavonoids, both glycosides and aglycones, were previously reported having potent anti-inflammatory and antinociceptive activities (Küpeli and Yesilada, 2007). Among the flavonoids, quercetin derivatives are the most widespread plant metabolites and they are reported to possess anti-inflammatory and antinociceptive activity. In our previous studies, along with quercetin derivatives we have also isolated several kaempferol derivatives as the active anti-inflammatory and antinociceptive components from traditional remedies. For example, three anti-inflammatory flavonoids isolated from *Cistus laurifolius* L., 3-*O*-methylquercetin, 3,7-*O*-dimethylquercetin and 3,7-*O*-dimethylkaempferol, were found to be effective in the both earlier and delayed stages,

Table 2
Effects of the extracts and fractions of *Laurocerasus officinalis* leaves against *p*-benzoquinone-induced writhings in mice.

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration[a]
Control		46.8 ± 2.79		0/6
Aqueous extract	500	37.3 ± 1.87	**20.3***	0/6
Ethanol extract	500	19.2 ± 1.76	**58.9*****	0/6
Acetylsalicylic acid	100	22.6 ± 1.77	51.7***	1/6
Control		47.3 ± 3.31		0/6
n-Hexane Fr.	205.64	41.3 ± 3.02	12.7	0/6
Chloroform Fr.	278.38	41.2 ± 2.37	12.9	0/6
Ethyl acetate Fr.	66.50	27.3 ± 2.29	**42.3*****	0/6
n-Butanol Fr.	147.58	40.8 ± 3.19	13.7	0/6
Remaining aqueous Fr.	217.88	46.7 ± 2.47	–	0/6
Acetylsalicylic acid	200	22.3 ± 2.28	**52.9*****	4/6

SEM, standard error mean.
[a] Number of stomachs in experimental animals induced gastric lesions.
* $p < 0.05$.
*** $p < 0.001$.

Table 3
Effect of the subfractions from the ethyl acetate fraction by silicagel column chromatography against carrageenan-induced hind paw edema in mice.

Material	Dose (mg/kg)	Swelling thickness ($\times 10^{-2}$ mm) ± SEM (%inhibition)			
		90 min	180 min	270 min	360 min
Control		49.4 ± 3.32	54.2 ± 4.11	59.9 ± 4.21	64.5 ± 4.45
Fr. 1	10.4	54.2 ± 4.01	57.9 ± 4.15	60.5 ± 4.13	65.2 ± 3.98
Fr. 2–3	12.6	47.2 ± 3.11 (4.5)	49.4 ± 3.92(8.9)	55.7 ± 4.02 (7.0)	59.7 ± 4.92 (7.4)
Fr. 4–7	16.7	40.5 ± 3.13 (18.0)	44.8 ± 3.74 (17.3)	49.9 ± 3.26 (16.7)	51.1 ± 3.79 (20.8)
Fr. 8–17	50.3	38.7 ± 2.96 (21.7)	39.2 ± 2.34 **(27.7)***	39.8 ± 3.05 **(33.6)***	43.2 ± 3.15 **(33.0)****
Fr. 18–20	24.8	40.8 ± 3.01 (17.4)	44.5 ± 3.56 (17.9)	47.5 ± 3.18 (20.7)	47.8 ± 3.02 **(25.9)***
Fr. 21–35	18.3	50.2 ± 3.18	57.8 ± 3.82	61.2 ± 3.95	65.7 ± 3.26
Indomethacin	10	33.1 ± 2.98 **(32.9)****	37.6 ± 2.65 **(30.6)****	39.3 ± 2.26 **(34.4)****	36.1 ± 3.04 **(44.0)*****

SEM, standard error mean.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 4
Effects of the subfractions from the ethyl acetate fraction by silicagel column chromatography on increased vascular permeability induced by acetic acid in mice.

Material	Dose (mg/kg)	Evans blue concentration (μg/ml) ± SEM	Inhibition (%)
Control		11.7356 ± 3.07	
Fr. 1	10.4	13.4598 ± 2.99	–
Fr. 2–3	12.6	11.8923 ± 3.24	–
Fr. 4–7	16.7	10.0966 ± 2.90	13.9
Fr. 8–17	50.3	8.1107 ± 1.93	**30.9****
Fr. 18–20	24.8	9.1572 ± 2.41	21.9
Fr. 21–35	18.3	9.9482 ± 2.08	15.2
Indomethacin	10	5.0843 ± 1.01	**56.7*****

S.E.M., standard error of the mean
** $p < 0.01$.
*** $p < 0.001$ significant from the control.

in fact somewhat more active in the delayed stage of inflammatory response (Küpeli and Yesilada, 2007). Two flavonoid glycosides were isolated from *Tilia argentea* leaves, kaempferol-3,7-*O*-α-dirhamnoside and quercetin-3,7-*O*-α-dirhamnoside, exerted potent anti-inflammatory and antinociceptive activity (Toker et al., 2004). Two kaempferol glycosides (tiliroside and astragalin) were also reported to possess potent *in vitro* inhibitory effect on TNF-α, an inflammatory cytokine, production (Matsuda et al., 2002). Palanichamy and Nagarajan (1990) also reported the antinociceptive activity of kaempferol 3-*O*-sophoroside from *Cassia alata* leaves. However, no experimental report has been found on the anti-inflammatory and antinociceptive activity of kaempferol-3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**).

Another component isolated as the anti-inflammatory and antinociceptive principle in the present study was (+)-catechin (**3**). This compound was also isolated as an anti-inflammatory and antinociceptive constituent from several other plants such as from the EtOAc subextract of *Arceuthobium oxycedri* (D.C.) M. Bieb. (Loranthaceae) (Küpeli et al., 2010). Nardi et al. (2003) also isolated this compound as an active anti-inflammatory ingredient from the EtOAc fraction of *Croton celtidifolius* against carrageenan-induced edema model. Dias et al. (2007) also reported that a catechin derivative (4′-methyl epigallocatechin) from the EtOAc extract of *Maytenus rigida* stem bark exerted antinociceptive activity in the tail-flick test. They further suggested that this compound may have an opioid-like activity since naloxone (a non-selective opioid antagonist) reversed the antinociceptive effect observed.

In a reference survey 2-*O*-β-D-glucopyranosyl-2-hydroxyphenyl-acetic acid (**1**) was first time isolated from *Sambucus nigra* (D'Abrosco et al., 2001), but not any biological activity has been reported for this phenolic glucoside so far. Therefore, this is the first report for this compound to possess anti-inflammatory and antinociceptive activity. Interestingly, hydroxyphenylacetic acids are also reported as one of the metabolites of flavonoids by the microbiota in human colon (Aura et al., 2002).

In conclusion, results of the present study support the folkloric use of the leaves in Turkish folk medicine against headache and feverish symptoms (Yesilada et al., 1999). Through bioassay-guided

Table 5
Effect of the subfractions from the ethyl acetate fraction by silicagel column chromatography against *p*-benzoquinone-induced writhings in mice.

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration[a]
Control		56.1 ± 2.03		0/6
Fr. 1	10.4	51.5 ± 2.72	8.2	0/6
Fr. 2–3	12.6	49.3 ± 2.11	12.1	0/6
Fr. 4–7	16.7	47.4 ± 2.15	15.5	0/6
Fr. 8–17	50.3	35.1 ± 1.57	**37.4*****	0/6
Fr. 18–20	24.8	27.1 ± 1.42	**29.9****	0/6
Fr. 21–35	18.3	58.3 ± 2.86	–	0/6
Acetylsalicylic acid	200	25.1 ± 1.13	**55.3*****	4/6

SEM, standard error mean.
[a] Number of stomachs in experimental animals induced gastric lesions.
** $p < 0.01$.
*** $p < 0.001$.

fractionation procedures three phenolic compounds, 2-*O*-β-D-glucopyranosyl-2-hydroxyphenylacetic acid (**1**), kaempferol-3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**), (+)-catechin (**3**), were isolated as the potent antinociceptive and anti-inflammatory principles. To the best of our knowledge, this is the first report describing the anti-inflammatory and antinociceptive effects of *Laurocerasus officinalis* leaves hitherto. However, the precise mechanism underlying the anti-inflammatory and antinociceptive effects of the isolated compounds from the plant has still to be determined.

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