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Bioactivity guided evaluation of anti-inflammatory and antinociceptive activities of *Arceuthobium oxycedri* (D.C.) M. Bieb.

Esra Küpeli Akkol[a], Ilkay Orhan[a], Murat Kartal[b], Erdem Yeşilada[c,*]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey
[b] Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey
[c] Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, 34755 Istanbul, Turkey

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ABSTRACT

Ethnopharmacological relevance: Arceuthobium oxycedri (D.C.) M. Bieb. (Loranthaceae) or dwarf mistletoe is a rare semi-parasitic plant that lives on the branches of *Juniperus oxycedrus* as the host plant. In Turkish folk medicine, the plant is prescribed as a panacea for every kind of diseases, including, against infectious and inflammatory disorders of upper respiratory system and gastro-intestinal complaints or as a hypotensive remedy.
Aim of the study: In the current study, *in vivo* anti-inflammatory and antinociceptive effects of *Arceuthobium oxycedri* have been investigated.
Material and methods: The crude ethanolic extract of the whole 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 subextract, *i.e.* the EtOAc subextract, was further subjected to fractionation through successive column chromatographic applications on Silica gel 60, Sephadex LH-20 and LiChropep RP-18. For the activity assessment, each extract or fraction was submitted to bioassay systems; carrageenan-induced hind paw edema model for anti-inflammatory activity and *p*-benzoquinone induced abdominal contraction test for antinociceptive activity assessment.
Results: Among the extracts obtained, the ethanolic extract, EtOAc and *n*-butanol subextracts showed significant inhibitory activity in the bioassay systems. From the EtOAc subextract, a major component was isolated and its structure was determined as (+)-catechin by means of spectral techniques.
Conclusion: Present study confirms the claimed utilization of the plant against inflammatory complaints in Turkish folk medicine.

1. Introduction

Arceuthobium genus or dwarf mistletoe, as they are commonly known, is of biological interest, because they are one of the most evolutionarily specialized genera of Loranthaceae. They show a rare distribution, approximately 8–15 species, which are grown in central Spain, across southern Europe, North Africa, the Near East, the Himalayas and western China. Like other members of the family, *Arceuthobium* species are semi-parasitic in nature, particularly live on *Juniperus* species and some other hosts of the family Cupressaceae (Sarangzai, 2004). A literature survey revealed that there have been very little phytochemical studies on *Arceuthobium* species. In 1979, Crawford and Hawksworth (1979) investigated flavonoid composition in 36 taxa of the genus *Arceuthobium* including *Arceuthobium oxycedri* and found out that all contained flavonol 3-O-glycosides; in particular quercetin and myricetin derivatives. In another study, three species of *Arceuthobium* were analyzed for their triacylglyceride fatty acids (Tocher et al., 1982). However, there has been no report on the biological activity profile of this genus so far.

Arceuthobium oxycedri (D.C.) M. Bieb. is a rare species living on the branches of *Juniperus oxycedrus* as the host plant. Possibly due to their rare availability, it is prescribed as a panacea for every kind of diseases in Turkish folk medicine. In particular, against infectious and inflammatory disorders of upper respiratory system (*i.e.*, common colds, bronchitis, flu, cough, etc.) and gastro-intestinal

Abbreviations: ASA, acetylsalicylic acid (Aspirin); BuOH, butanol; $CHCl_3$, chloroform; CMC, carboxymethyl cellulose; COX-2, cyclooxygenase-2; EtOAC, ethyl acetate; EtOH, ethanol; FT-NMR, Fourier transform-nuclear magnetic resonance; H_2O, water; iNOS, inducible nitric oxide synthase; LC–MS, liquid chromatography–mass spectrometry; MCP-1, monocyte chemotatctic protein-1; MPLC, medium pressure liquid chromatography; PBQ, *p*-benzoquinone; PDG, prodelphinidin B-4 3′-O-gallate; PMA, phorbol 12-myristate 13-acetate; RAW254, murine macrophage cell line; SEM, mean standard error; SFr, subfraction; TLC, thin layer chromatography.

* Corresponding author. Tel.: +90 216 5780000x3021; fax: +90 216 5780068.
E-mail address: yesilada@yeditepe.edu.tr (E. Yeşilada).

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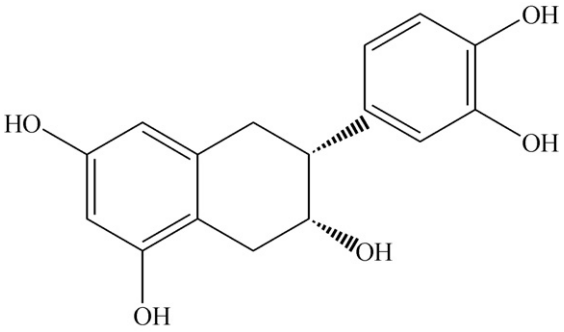

Fig. 1. Structure of (+)-catechin.

complaints (gastric pain, hemorrhoids) or as a hypotensive remedy (Yesilada et al., 1999; Gürhan and Ezer, 2004). Other species of the plant have also traditional uses in some other parts of the world to treat similar or closely related disorders. For instance, a record in the book of Hawksworth and Wien (1996) mentioned that a decoction of *A Arceuthobium occidentale* was used to treat stomach ache by the California Indians, while Indians in Butte County, California used an undetermined species of dwarf mistletoe for treating hemorrhage of the lungs and mouth, tuberculosis, emaciation, stomach ache, cough, colds, and rheumatism. An infusion of another species, *Arceuthobium vaginatum* has been suggested for the treatment of cough and diabetes in Mexico (Hawksworth and Wien, 1996).

Various other mistletoes from the Loranthaceae family, particularly *Viscum album* and its subspecies, have been studied for their various biological activities, but the dwarf mistletoes have received relatively little attention in this regard (Yesilada et al., 1998; Deliorman-Orhan et al., 2006). Concerning the above-mentioned traditional uses, the current study was undertaken to assess anti-inflammatory and antinociceptive effects of *Arceuthobium oxycedri* in order to verify the traditional utilization of the plant in Turkish folk medicine. For this purpose, the crude ethanolic extract, subextracts, and fractions obtained from the plant was submitted to bioassay-guided processes by using *in vivo* carrageenan-induced hind paw edema model for anti-inflammatory activity and *p*-benzoquinone induced abdominal contraction test for antinociceptive activity assessment.

2. Materials and methods

2.1. Plant material

The plant material was collected from Kuzey district of Kastamonu province, Turkey, in May 2004. Authenticated voucher specimen was preserved in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey (GUE 2322).

2.2. Extraction of the material for preliminary activity assessment

Plant material was dried under shade and powdered to a fine grade by using a laboratory scale mill. The extracts were prepared as given below.

Ethanol (EtOH) extract: Dried plant material (10 g) was extracted with 96% EtOH at room temperature two times (×200 mL). The combined ethanol layers were evaporated to dryness *in vacuo* to give crude EtOH extract (yield: 14.9%).

Aqueous (H_2O) extract: Dried plant material (10 g) was extracted with distilled water at room temperature two times (×200 mL). The combined aqueous layers were lyophilized to give the crude H_2O extract (yield: 10.8%).

2.3. Subextracts by successive solvent extraction of the EtOH extract

Dried and powdered whole plant material of *Arceuthobium oxycedri* (620 g) was extracted with 96% ethanol (3 × 2 l) at room temperature. Following filtration, the combined ethanol extract was evaporated to dryness *in vacuo* to give a crude extract (75.15 g, 12.12%, *w*/*w*).

The crude EtOH extract (51.15 g) dissolved in water/methanol (9:1) (1000 ml) was extracted with *n*-hexane (500 ml, Merck Co.) in a separatory funnel. The *n*-hexane phase was collected and evaporated under reduced pressure until dryness affording the "*n*-Hexane extract" (8.73 g, 17.1%). Methanol was removed from the remaining hydroalcoholic extract and diluted with sufficient amount of distilled water and was then successively extracted with chloroform (400 ml, Merck Co.), ethyl acetate (1200 ml, Merck Co.), and *n*-butanol saturated with distilled water (400 ml, Merck Co.). Each solvent extracts were evaporated to dryness under reduced pressure to yield "$CHCl_3$ extract" (4.26 g, 8.3%), "EtOAc extract" (5.42 g, 10.6%), and "*n*-BuOH extract" (10.73 g, 20.9%). Finally, the remaining aqueous extract was evaporated to dryness under reduced pressure to give "R-H_2O extract" (19.98 g, 39.1%).

2.4. Fractionation of the EtOAc extract through column chromatography

The EtOAc extract (4.58 g) of *Arceuthobium oxycedri* was first submitted to silica gel 60 (Merck, Germany) column chromatography and eluted with the solvent systems comprising of dichloromethane: methanol (100:2, 100:4, 100:7, 100:10, 100:20), and then dichloromethane:methanol:water (100:30:1, 85:30:1, 85:35:1, 80:40:1, 75:40:2, 75:40:5, 70:40:7, 70:50:10, 60:50:10) mixtures which gave 28 fractions in total. Following TLC monitoring using chloroform:methanol (3:1), similar fractions were combined together and 8 major fractions (SFr.1–2, SFr.3–10, SFr.11–12, SFr.13, SFr.14–15, SFr.16–18, SFr.19–23, and SFr.24–28) were obtained.

Among the fractions from silica gel column chromatography, SFr.11–12 (1.63 g) was then further fractionated on Sephadex LH-20 (Pharmacia, Sweden) column chromatography. SFr.11–12 (1.36 g) was applied to column and eluted with methanol to give 14 fractions in total, which were then combined into five subfractions: SeFr.A (15.2 mg), SeFr.B (29.0 mg), SeFr.C (51.1 mg), SeFr.D (17.3 mg), SeFr.E (21.1 mg) depending on their TLC profiles.

2.5. Isolation of the (+)-catechin (Fig. 1) by bioactivity guided fractionation

In TLC profiling of the active fraction coded SeFr.C, a yellowish-colored major spot was observed which was not observed in the other subfractions. Therefore SeFr.C was applied to medium pressure liquid chromatography (MPLC) in a Master Flex L/S Digital Economy Drive MPLC pump equipped with Spectra/Chrom 1.5 c × 25 c LC Column using Foxy 200 Fraction Collector. The column packing material was LiChroprep RP-18 (25-40 UM) (Merck, Germany). Methanol:water (50:50) mixture was used as the initial elution solvent system and then the ratio of methanol was gradually increased which afforded 22 subfractions. A yellow-colored precipitate was observed in the sequential subfractions and they were combined and encoded as LiFr.14–17. This subfraction was then evaporated until dryness and the residue was washed with methanol to give a pure compound on TLC analysis. Spectral analysis for the isolated compound was achieved by using Varian Mercury 400, 400 MHz High Performance Digital FT-NMR Spectrometer for ^1H and ^{13}C NMR spectra in $CDCl_3$, while mass spectrum was taken in LC–MS Waters 2695 Alliance Micromass ZQ apparatus.

Table 1
Effect of H_2O and EtOH extracts and subextracts from EtOH extract of *Arceuthobium oxycedri* against *p*-benzoquinone-induced writhings in mice.

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration
Control		53.8 ± 5.8		0/6
H_2O extract	250	46.9 ± 3.7	12.8	0/6
	500	35.4 ± 4.3	**34.2***	0/6
EtOH extract	250	38.4 ± 3.1	**28.6***	0/6
	500	32.3 ± 3.3	**39.9****	0/6
Hexane extract	170.7	56.1 ± 5.7	–	0/6
$CHCl_3$ extract	83.3	43.5 ± 4.6	19.1	0/6
EtOAc extract	105.9	36.3 ± 3.6	**32.5****	0/6
n-BuOH extract	209.8	404 ± 3.8	**24.9***	0/6
R-H_2O extract	390.6	54.5 ± 3.9	–	0/6
ASA	100	27.5 ± 3.5	**48.8****	3/6

Bold characters refer the percent inhibitory rates of significantly active values.
* $p < 0.05$.
** $p < 0.01$.

Table 2
Effect of fractions from EtOAc extract by silica gel column chromatography against *p*-benzoquinone-induced writhings in mice.

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration
Control		47.6 ± 4.2		0/6
SFr. 1–2	3.17	59.5 ± 3.1	–	0/6
SFr. 3–10	14.36	38.9 ± 2.9	18.3	0/6
SFr. 11–12	75.42	33.8 ± 2.9	**28.9****	0/6
SFr. 13	19.86	40.6 ± 2.1	14.7	0/6
SFr. 14–15	52.19	51.4 ± 4.2	–	0/6
SFr. 16–18	14.17	50.2 ± 3.9	–	0/6
SFr. 19–23	15.56	49.1 ± 4.0	–	0/6
SFr. 24–28	14.69	47.9 ± 2.8	–	0/6
ASA	100	26.3 ± 2.1	**44.7*****	4/6

Bold characters refer the percent inhibitory rates of significantly active values.
** $p < 0.01$.
*** $p < 0.001$.

Table 3
Effect of H_2O and EtOH extracts and subextracts from EtOH extract of *Arceuthobium oxycedri* 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		45.0 ± 6.0	50.2 ± 5.7	57.7 ± 6.1	64.0 ± 5.7
H_2O extract	250	50.8 ± 5.0	54.8 ± 5.0	58.3 ± 5.0	61.0 ± 4.7 (4.7)
	500	35.5. ± 4.7 (21.1)	38.2 ± 4.4 (23.9)	42.2 ± 3.7 (**26.9**)*	44.5 ± 4.1 (**30.5**)*
EtOH extract	250	39.5 ± 5.3 (12.2)	40.2 ± 5.9 (19.9)	45.5 ± 6.8 (21.2)	48.7 ± 7.0 (23.9)
	500	31.4 ± 3.1 (**30.2**)*	35.7 ± 3.2 (**28.9**)*	39.5 ± 3.8 (**31.5**)*	42.1 ± 3.4 (**34.2**)*
Hexane extract	170.7	47.4 ± 7.6	54.1 ± 6.8	61.4 ± 6.5	63.4 ± 5.5
$CHCl_3$ extract	83.3	43.2 ± 9.4 (4.0)	45.3 ± 8.5 (9.8)	50.0 ± 8.1 (13.3)	54.7 ± 7.2 (14.5)
EtOAc extract	105.96	36.9 ± 5.5 (18.0)	37.1 ± 4.0 (26.1)	42.1 ± 4.1 (**27.0**)*	43.8 ± 2.3 (**31.6**)**
n-BuOH extract	209.8	39.8 ± 6.2 (11.6)	43.2 ± 5.8 (13.9)	44.9 ± 6.3 (22.1)	47.6 ± 6.2 (**25.6**)*
R-H_2O extract	390.6	48.9 ± 7.1	53.5 ± 7.4	58.2 ± 7.7	63.0 ± 6.6
Indomethacin	10	29.5 ± 3.4 (**34.4**)*	30.3 ± 3.3 (**39.6**)**	35.0 ± 2.7 (**39.3**)***	38.3 ± 4.0 (**40.2**)***

SEM: standard error mean.
Bold characters refer the percent inhibitory rates of significantly active values.
* $p < 0.05$
** $p < 0.01$.
*** $p < 0.001$.

Table 4
Effect of fractions from EtOAc extract by Silica gel 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		39.7 ± 3.1	43.7 ± 3.9	48.8 ± 4.0	51.4 ± 3.9
SFr. 1–2	3.17	41.2 ± 2.7	45.9 ± 3.1	50.5 ± 3.2	54.9 ± 3.0
SFr. 3–10	14.36	38.9 ± 2.9 (2.0)	39.5 ± 3.2 (9.6)	42.5 ± 3.1 (12.9)	47.8 ± 2.9 (7.0)
SFr. 11–12	75.42	34.4 ± 3.0 (13.4)	36.2 ± 2.9 (17.2)	36.8 ± 2.6 (**25.6**)*	35.5 ± 2.2 (**30.9**)**
SFr. 13	19.86	37.9 ± 3.0 (4.5)	38.9 ± 3.0 (10.9)	41.9 ± 2.9 (14.1)	45.5 ± 2.4 (11.5)
SFr. 14–15	52.19	41.5 ± 2.1	44.7 ± 2.5	47.8 ± 3.0 (2.0)	49.8 ± 3.1 (3.1)
SFr. 16–18	14.17	40.2 ± 3.1	44.8 ± 3.2	49.8 ± 2.8	50.4 ± 3.1 (1.9)
SFr. 19–23	15.56	43.6 ± 4.2	47.1 ± 4.3	49.1 ± 3.9	525 ± 3.1
SFr. 24–28	14.69	45.7 ± 3.2	48.9 ± 3.0	51.6 ± 2.9	54.5 ± 2.9
Indomethacin	10	31.1 ± 2.0 (**21.7**)*	31.4 ± 2.4 (**28.1**)*	30.5 ± 2.2 (**37.5**)***	30.1 ± 2.6 (**41.4**)***

SEM: standard error mean.
Bold characters refer the percent inhibitory rates of significantly active values.
* $p < 0.05$
** $p < 0.01$.
*** $p < 0.001$.

Table 5
Effect of subfractions obtained from SFr. 11–12 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		43.5 ± 4.2	49.8 ± 5.1	53.2 ± 4.7	58.3 ± 3.9
SeFr.A	16.86	51.4 ± 6.3	55.9 ± 5.7	59.2 ± 5.4	62.1 ± 4.8
SeFr.B	32.16	40.8 ± 3.4 (6.2)	43.2 ± 3.1 (13.3)	46.9 ± 2.9 (11.8)	49.7 ± 3.3 (14.8)
SeFr.C	56.68	38.1 ± 2.5 (12.4)	39.9 ± 2.9 (19.9)	37.9 ± 2.5 (**28.8**)**	41.1 ± 2.3 (**29.5**)**
SeFr.D	19.19	45.9 ± 5.0	52.6 ± 4.3	57.3 ± 3.6	59.2 ± 4.9
SeFr.E	23.40	49.3 ± 5.2	53.6 ± 4.8	57.8 ± 5.1	61.2 ± 6.4
Indomethacin	10	33.6 ± 2.1 (**22.8**)*	35.5 ± 2.4 (**28.7**)**	34.8 ± 2.2 (**34.6**)**	36.1 ± 2.5 (**38.1**)***

SEM: standard error mean.
Bold characters refer the percent inhibitory rates of significantly active values.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

2.6. Pharmacological procedures

2.6.1. 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 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 prior to the experiment, but was allowed free access of water. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested European ethical guidelines for the care of laboratory animals.

2.6.2. Preparation of test samples and dose estimation for bioassays

Since no ethnomedical data was available on the amount for treatment in traditional medicine, all extracts in the preliminary activity testing were administered in 250 and 500 mg/kg doses after suspending in 1% Tween 80 in distilled H_2O. 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. However, the dose of the subextracts and fractions obtained by chromatographical processing were estimated based on their ratio in the corresponding subextract or fraction. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (100 mg/kg and 200 mg/kg) in 1% carboxymethyl cellulose (CMC) was used as the reference drugs.

2.6.3. Antinociceptive activity

2.6.3.1. p-Benzoquinone-induced abdominal constriction test in mice. In accordance with the method of Okun et al. (1963), 60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (*v*/*v*) *p*-benzoquinone (PBQ; Merck) solution in distilled water. 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 antinociceptive activity was expressed as percentage change from writhing controls. Aspirin (ASA) at 100 mg/kg was used as the reference drug.

2.6.4. Anti-inflammatory activity

2.6.4.1. Carrageenan-induced hind paw edema. As described previously (Yesilada and Küpeli, 2002), 60 min after the oral administration of test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of carrageenan (0.5 mg/25 μl) (Sigma, St. Louis, MO, USA) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As to the control, 25 μl saline solution was 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) was used as a reference drug.

2.6.5. 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.

2.6.6. Gastric toxicity

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

Table 6
Effect of subfractions obtained from SFr. 11–12 against *p*-benzoquinone-induced writhings in mice.

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration
Control		54.7 ± 5.1		0/6
SeFr.A	16.86	53.1 ± 5.7	2.9	0/6
SeFr.B	32.16	49.9 ± 3.1	8.8	0/6
SeFr.C	56.68	40.1 ± 2.4	**26.7****	0/6
SeFr.D	19.19	56.2 ± 4.8	–	0/6
SeFr.E	23.40	59.3 ± 5.1	–	0/6
ASA	100	22.5 ± 2.6	**58.9*****	5/6

SEM: standard error mean.
Bold characters refer the percent inhibitory rates of significantly active values.
** $p < 0.01$.
*** $p < 0.001$.

2.6.7. Statistical analysis

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Student's–Newman–Keuls post hoc tests. $p < 0.05$ was considered to be significant [$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$].

3. Results and discussion

In the current study, anti-inflammatory and antinociceptive activities of *Arceuthobium oxycedri* were investigated in mice in order to verify the claimed conventional uses of the plant in Turkish folk medicine. For the assessment of anti-inflammatory activity carrageenan-induced hind paw edema model and for the antinociceptive activity *p*-benzoquinone-induced abdominal contraction test were used. Furthermore, gastric toxicity of the extracts and fractions was also evaluated to reveal possible ulcerogenic risks.

In the preliminary activity assessment, the H_2O and EtOH extracts prepared from the whole plant were tested in the bioassay systems. Both were shown active against the test systems dose-dependently, however, the activity of the EtOH extract was higher. Therefore, following studies were carried out using this extract. Among the subextracts obtained through successive solvent extractions, the EtOAc extract was found to be the most active in both experimental models at 105.96 mg/kg dose providing 32.5% inhibition in writhing and between 18.0 and 31.6% inhibition in edema induced by agents (Tables 1 and 3). Afterward, this extract was fractionated through successive column chromatography techniques and the fractions were also submitted to the same bioassay systems (Tables 2–6). Successive bioassay-guided fractionation procedures have yielded a major component from the active subfraction. The structure of this component was elucidated to be (+)-catechin by comparison of the one- and two-dimensional NMR data with those of the previously reported (Cren-Olive et al., 2002). On the other hand, none of the test samples showed any sign of gross toxicity during the observational period of 24 h.

On the other hand, catechin, the major component that we elucidated in the active fraction of *A Arceuthobium oxycedri*, has been studied extensively for a number of biological activities in different experimental models (Gabor, 1986). Nardi et al. (2003) isolated catechin as one of the active anti-inflammatory ingredient from the ethyl acetate fraction of *Croton celtidifolius* against carrageenan-induced paw edema model, whereas this compound did not show any superoxide scavenger effect. Previously Dias et al. (2007) also reported that a catechin derivative (4′-methyl epigallocatechin) isolated from the methanol and EtOAc extracts of *Maytenus rigida* stem bark exerted antinociceptive activity in the tail-flick test at 75 mg/kg oral dose. They further suggested that 4′-methyl epigallocatechin may have an opioid-like activity since the non-selective opioid antagonist naloxone (3 mg/kg; *i.p.*) reversed the antinociceptive effect observed.

In a recent report (Hou et al., 2007), prodelphinidin B-4 3′-O-gallate (PDG), consisting of (+)-gallocatechin and (−)-epigallocatechin 3-O-gallate moieties in one molecule, was investigated for its anti-inflammatory activity on cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in murine macrophage RAW264 cells. The results showed that PDG inhibited successfully both COX-2 and iNOS in dose-dependent manner.

As well-known; catechins constitute approximately 25% of the dry weight of fresh tea leaves made from *Camellia sinensis*, including white tea, green tea, black tea, and Oolong tea. Anti-inflammatory activity of the hot water extract of Indian black tea was also examined in a number of experimental models and found to have inhibitory profile against carrageenan-, histamine-, serotonin- and prostaglandin-induced paw inflammations (Chaudhuri et al., 2005). In the same study, the extract remarkably reduced the development of granulation tissue suggesting that it was effective in the proliferative stage of inflammation. Catechin and epicatechin are epimers, with (−)-epicatechin and (+)-catechin being the most common optical isomers found in nature. Epigallocatechin and gallocatechin contain an additional phenolic hydroxyl group when compared to epicatechin and catechin, respectively. (−)-Epigallocatechin-3-gallate, a major green tea catechin, was reported to inhibit phorbol 12-myristate 13-acetate (PMA)-induced monocyte chemotactic protein-1 (MCP-1) which is the most important chemokine regulating migration and infiltration of monocytes and macrophages (Hong et al., 2007). In accordance with this article, anti-inflammatory effect of epigallocatechin gallate was studied on isolated peripheral blood monocytes and apoptosis possibility was evaluated. The results indicated that epigallocatechin gallate induced apoptosis of monocytes (Kawai et al., 2005).

In conclusion, the present study has proven the traditional utilization of *Arceuthobium oxycedri* in inflammatory disorders and (+)-catechin was isolated and structure was elucidated as one of the major components of the active fraction which might be responsible from the anti-inflammatory and antinociceptive activities of the plant. To the best of our knowledge, this is the first report describing the anti-inflammatory and antinociceptive effects of *Arceuthobium oxycedri* hitherto. However, the precise mechanism underlying the anti-inflammatory and antinociceptive effects of (+)-catechin and involvement of other components in the plant has still to be determined.

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