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RESEARCH ARTICLE

Antioxidant, anti-inflammatory, and antinociceptive activities of Turkish medicinal plants

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Abstract

Hypericum orientale L. (Hypericaceae), *Helichrysum plicatum* Dc. subsp. *plicatum* (Asteraceae), *Centaurea drabifolia* Sm. subsp. *drabifolia* (Asteraceae), *Centaurea drabifolia* Sm. subsp. *detonsa* (Bornm.) Wagenitz (Asteraceae), *Achillea wilhelmsii* C. Koch (Asteraceae), and *Rubus canescens* Dc. var. *canescens* (Rosaceae) are used for the treatment of hemorrhoids, abdominal pains, and wound healing in traditional Turkish medicine. In order to assess these uses, methanol extracts prepared from their aerial parts were investigated for antioxidant, anti-inflammatory, and antinociceptive activities. All extracts demonstrated scavenging properties against superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in a non-cellular system, and toward 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. They also inhibited Cu^{2+}-induced low-density lipoprotein (LDL) peroxidation. Among the tested plants, *R. canescens* var. *canescens*, *H. orientale*, and *H. plicatum* subsp. *plicatum* were the most effective on ROS in a non-cellular system. Another goal in this work was to test *in vivo* anti-inflammatory and antinociceptive activities of some of these plants not previously studied. The methanol extracts of *C. drabifolia* subsp. *drabifolia*, *H. orientale*, and *C. drabifolia* subsp. *detonsa* were shown to possess significant inhibitory activity in mice against carrageenan-induced hind paw edema and in *p*-benzoquinone-induced writhings.

Keywords: Hypericum orientale; Helichrysum plicatum; Centaurea drabifolia; Achillea wilhelmsii; Rubus canescens; *antioxidant activity; anti-inflammatory and antinociceptive activities*

Introduction

There is a growing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders such as chronic inflammatory diseases (Candan et al., 2003). Inflammatory diseases are among the most common ailments treated through traditional remedies. It is therefore crucial to assess the potential of herbal remedies to discover novel bioactive compounds that might serve as leads to develop effective drugs. The present article concerns a preliminary screening in ongoing research on plants used in Turkish traditional medicine for their antioxidant activity and treatment of inflammatory diseases. The list of plants selected for the present study is given in Table 1, with vernacular names and their recorded use in Turkish traditional medicine (Robson, 1967; Davis & Meikle, 1972; Davis & Kupicha, 1975; Huber-Morath, 1975; Wagenitz, 1975). Phytochemical studies on these plants have revealed the presence of phenolic and terpenic compounds such as flavonoids, anthocyanins, lignans, and sesquiterpene lactones (Wada & Ou, 2002; Cakir et al., 2003; Kumarasamy et al., 2003;

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Table 1. Traditional use of plant materials in Turkish folk medicine.

Plant name	Family	Local name	Part used	Traditional use[a]
Hypericum orientale	Hypericaceae	Sarı çiçek	Flowers	Stomach diseases, sedative[b]
Helichrysum plicatum subsp. *plicatum*	Asteraceae	Gündöndü	Aerial parts	Abdominal pain
Centaurea drabifolia subsp. *detonsa*	Asteraceae	Basurotu	Aerial parts	(Internally) hemorrhoids
Centaurea drabifolia subsp. *drabifolia*	Asteraceae	Basurotu	Aerial parts	(Externally) hemorrhoids
Achillea wilhelmsii	Asteraceae	Ayvadene	Aerial parts Inflorescence	Diarrhea, abdominal pain Toothache, headache, common cold, cough, sore throat, hypertension
Rubus canescens var. *canescens*	Rosaceae	Böğürtlen	Root	Diuretic, hemorrhoids

Suzgec et al., 2005; Tuberoso et al., 2005; Karamenderes et al., 2007). The functions of such compounds in plants, i.e. anti-inflammatory, antitumor, antiexudative, antiulcer, analgesic, antipyretic, and immunostimulant activities, have been known for many years, while new activities are continually being discovered (Hall et al., 1979; Arif et al., 2004; Standen & Myers, 2004; Tatli & Akdemir, 2006; Shiow et al., 2008).

As the antioxidant, anti-inflammatory, and antinociceptive activities of these plants have not been studied so far, except for *H. plicatum* subsp. *plicatum* (Tepe et al., 2005; Kupeli et al., 2006) and *A. wilhelmsii* (Nickavar et al., 2006; Kupeli et al., 2007), the present work was undertaken to search for these activities in methanol extracts so as to validate their use in traditional medicine.

Materials and methods

Plant materials

Hypericum orientale: A5 Amasya, Merzifon, 3 km northwest of Yukaribuk Village, 1500–1600 m, 07.07.2003 (HUEF 03031).

Helichrysum plicatum subsp. *plicatum*: B3 Afyon, Suhut, Ortapinar Village, Sahbendi location, 1750 m, 22.07.2004 (HUEF 04122).

Centaurea drabifolia subsp. *detonsa*: B3 Afyon, Suhut, Balcikhisar Town, Andiz Mountain, 1300 m, 07.07.2003 (HUEF 03073).

Centaurea drabifolia subsp. *drabifolia*: B3 Afyon, Suhut, Karacaoren Town, Ucler location, 1450 m, 22.06.2004 (HUEF 04076).

Achillea wilhelmsii: B3 Afyon, Suhut, Atlihisar Town, 1100 m, 07.07.2003 (HUEF 03071).

Rubus canescens var. *canescens*: B3 Afyon, Suhut, Kocyatagi Village, Tastepe location, 1200 m, 07.07.2003 (HUEF 03078).

Voucher specimens were deposited in the Herbarium of Hacettepe University (HUEF). Plant specimens were authenticated by Ahmet Sezgin and Oyku Arisan.

Preparation of extracts

Each plant material was dried in the shade and powdered to a fine grain using a laboratory mill. Dried aerial parts of each plant material (7.5 g) were extracted twice with methanol (100 mL each) by macerating at room temperature. The combined extracts were evaporated to dryness *in vacuo* to give methanol extracts with the following yields: *H. orientale*: 2.5 g (33%), *H. plicatum* subsp. *plicatum*: 0.9 g (12%), *C. drabifolia* subsp. *detonsa*: 0.8 g (11%), *C. drabifolia* subsp. *drabifolia*: 0.7 g (9%), *A. wilhelmsii*: 1.1 g (15%), *R. canescens* var. *canescens*: 1.2 g (16%).

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 were left for 2 days to acclimatize to the room conditions (temperature 23 ± 2°C and lighting 12:12 h light–dark cycle) and were maintained on a standard pellet diet with water *ad libitum*. 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. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

Pharmacological studies

Reduction of DPPH radical by bioautographic assay
Methanol extracts were chromatographed on silica gel thin layer chromatography (TLC) plates sprayed with 0.2% DPPH (1,1-diphenyl-2-picrylhydrazyl; Sigma) solution. Extracts showing a yellow-on-purple spot were regarded as antioxidants (Takao et al., 1994).

Reduction of DPPH radical by spectrophotometric assay
The radical scavenging activity of the methanol extracts (100 μL) at varying concentrations (from 5 to 100 mg/L) was assessed spectrophotometrically on DPPH radical. The absorbance of the remaining DPPH was measured at 520 nm, after 30 min incubation. Percentage inhibition of DPPH free radical was calculated (Hatano et al., 1989). The percent radical scavenging activity was plotted against sample concentration, and a logarithmic regression curve was drawn to calculate the IC_{50} value (n = 3). Chlorogenic acid was used as the reference compound.

Scavenging activity of methanol extracts versus superoxide anion (O2•−) in cell-free system
Scavenging effects of the methanol extracts were established on an acellular generation of $O_2^{\bullet-}$ using the hypoxanthine–xanthine oxidase system (Aruoma & Halliwell, 1989). This system produces $O_2^{\bullet-}$ and the reaction is based on cytochrome C reduction by $O_2^{\bullet-}$ unscavenged by the studied extracts. Reduced cytochrome C was quantified spectrophotometrically at 550 nm. The amount of $O_2^{\bullet-}$ was calculated using the Beer–Lambert law with the extinction coefficient $\varepsilon_{550} = 2.1 \times 102\ mol^{-1}\,cm^{-1}$. The percentage of $O_2^{\bullet-}$ production inhibited by the extracts was calculated. Results are expressed as IC_{50} values (n = 6, mean ± SD). Chlorogenic acid was used as the reference compound.

Scavenging activity of methanol extracts versus hydrogen peroxide (H2O2) in cell-free system
H_2O_2 was determined through the peroxide-dependent oxidation of phenol red (Pick & Keisari, 1980). Phenol red oxidation by H_2O_2 leads to a color change in H_2O_2 basic medium (addition of NaOH) and is detected spectrometrically at 610 nm. The percentage of H_2O_2 inhibited by each extract was calculated. Results are expressed as IC_{50} values (n = 6, mean ± SD). Chlorogenic acid was used as the reference compound.

LDL oxidation inhibition test
Human low-density lipoproteins (LDLs) were isolated as previously described (Seidel et al., 2000). Ethanol solutions of the tested extracts were added to the LDL solution to give final concentrations of 10–100 mg/L, in a total concentration of 1% (v/v) (Esterbauer et al., 1993). LDL oxidation was induced and measured spectrophotometrically according to a method previously described (Seidel et al., 2000).

Data analysis
Results are expressed as mean value ± standard deviation. Activities expressed as IC_{50} were calculated using a computer program (Boniface et al., 1972). Comparison of data between more than two groups was done by analysis of variance (ANOVA). Significant differences were then subjected to *post hoc* analysis with the Sheffe test. A value of $p < 0.05$ was accepted as statistically significant.

Anti-inflammatory activity

The carrageenan-induced hind paw edema model was used to determine anti-inflammatory activity (Yesilada & Kupeli, 2002). Paw edema was measured every 90 min during the 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calliper (Ozaki Co.). Mean values of treated groups were compared with those of a control group and analyzed statistically. Indomethacin (10 mg/kg) was used as the reference drug.

Antinociceptive activity

The *p*-benzoquinone (PBQ)-induced abdominal constriction test (Okun et al., 1963) was performed on mice to determine antinociceptive activity. The mice were kept separate for observation, and the total number of abdominal contractions (writhing movements) was counted over 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 compared to writhing controls. Acetylsalicylic acid at 100 mg/kg doses was used as the reference drug in this test.

Acute toxicity

Animals used in the carrageenan-induced paw edema experiment were observed over 48 h; morbidity or mortality was recorded if necessary at the end of the observation period.

Gastric-ulcerogenic effect

After the antinociceptive activity experiment, mice were killed under deep ether anesthesia and the stomach of each mouse was removed. The abdomen of each mouse was then opened through the greater curvature and examined under a dissecting microscope for lesions or bleeding.

Data analysis

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

Results and discussion

The aerial parts of six plant species (Table 1) were evaluated for their *in vitro* antioxidant and their *in vivo* anti-inflammatory and antinociceptive activities. Their methanol extracts were examined using TLC autographic and solution-based spectrophotometric assays with the DPPH radical (Table 2). All extracts showing a strong yellow-on-purple spot were considered as antioxidants in the TLC autographic method because of their rich phenolic and terpenic contents, such as flavonoids, anthocyanins, tannins, and sesquiterpene lactones. In the spectrophotometric assay, antioxidants reacted with the stable free radical DPPH (which gives high absorbance at 520 nm), and resulted in the production of colorless 1,1-diphenyl-2-picrylhydrazine. The six tested extracts showed a dose-dependent reduction in DPPH, corresponding to

Table 2. Antioxidant activities of methanolic plant extracts.

	IC_{50} (mg/L)			
Plant name	DPPH	$O_2^{\bullet-}$	H_2O_2	LDL
Hypericum orientale	17.0 ± 1.5	295.1 ± 18.5	309.5 ± 26.9	17.3 ± 2.1
Helichrysium plicatum subsp. *plicatum*	39.0 ± 4.8	305.2 ± 15.9	301.6 ± 32.6	36.5 ± 4.6
Centaurea drabifolia subsp. *detonsa*	59.0 ± 5.2	430.6 ± 25.2	583.6 ± 41.9	54.4 ± 5.0
Centaurea drabifolia subsp. *drabifolia*	80.0 ± 6.1	290.2 ± 15.5	310.5 ± 18.9	71.0 ± 5.8
Achillea wilhelmsii	46.0 ± 4.0	435.2 ± 32.0	>2000	34.2 ± 3.6
Rubus canescens var. *canescens*	10.0 ± 1.1	283.8 ± 12.0	1740 ± 120	13.1 ± 2.1
Chlorogenic acid[a]	7.4 ± 0.3	57.9 ± 1.9	85.0 ± 8.2	9.2 ± 0.8

[a]Reference compound.

Table 3. Effects of the extracts on carrageenan-induced hind paw edema in mice.

		Swelling thickness ($\times 10^{-2}$mm) ± SEM (% inhibition)			
Material	Dose (mg/kg)	90 min	180 min	270 min	360 min
Control		42.7 ± 5.3	51.2 ± 4.9	56.9 ± 5.1	61.1 ± 5.4
Hypericum orientale	100	36.0 ± 3.1(15.7)	41.5 ± 3.4(18.9)	47.2 ± 3.9(17.0)	48.4 ± 3.8(20.8)
Rubus canescens var. *canescens*	100	45.8 ± 4.2	54.9 ± 4.5	59.5 ± 3.7	64.8 ± 5.0
Centaurea drabifolia subsp. *drabifolia*	100	36.6 ± 4.4(14.2)	39.5 ± 4.0(22.9)	45.3 ± 3.4(20.4)	46.1 ± 3.1(24.6)*
Centaurea drabifolia subsp. *detonsa*	100	37.9 ± 2.8(11.3)	43.5 ± 3.9(15.0)	49.4 ± 4.0(13.2)	49.4 ± 3.5(19.1)
Indomethacin[a]	10	28.1 ± 2.4(34.1)*	31.3 ± 2.7(38.9)**	36.4 ± 3.2(36.0)**	35.1 ± 2.9(42.5)***

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; SEM, standard error of the mean.
[a]Reference compound.

Table 4. Effects of the extracts against *p*-benzoquinone-induced writhings in mice.

Material	Dose (mg/kg)	Number of writhings ± SEM	Inhibitory ratio (%)	Ratio of ulceration[a]
Control		42.0 ± 4.7		0/6
Hypericum orientale	100	36.7 ± 4.1	12.6	0/6
Rubus cenescens var. *canescens*	100	48.1 ± 5.3	—	0/6
Centaurea drabifolia subsp. *drabifolia*	100	35.2 ± 3.6	16.2	0/6
Centaurea drabifolia subsp. *detonsa*	100	37.2 ± 3.2	11.5	0/6
Acetylsalicylic acid[b]	100	22.2 ± 2.5	47.2***	5/6

$^{***}p < 0.001$; SEM, standard error of the mean.
[a]Number of stomachs in experimental animals with induced gastric lesions.
[b]Reference compound.

the quenching intensity of the DPPH radical, with ED_{50} ranging from 10 to 80 mg/L, *R. canescens* and *H. orientale* extracts being the most active (Table 2). The scavenging effects of the methanol extracts were also studied in a cell-free system versus $O_2^{\bullet-}$ and H_2O_2. As shown in Table 2, the extracts had strong antioxidant and scavenging activities in a dose-dependent manner, ED_{50} varying from 283 to 435 mg/L and from 301 to 583 mg/L versus $O_2^{\bullet-}$ and H_2O_2, respectively, except for *A. wilhelmsii* and *R. canescens* var. *canescens* extracts which were not active versus H_2O_2 (ED_{50} > 1.7 g/L). One of the aims of this study was also to test the protective effect of six natural extracts against Cu^{2+}-induced LDL oxidation. All of them inhibited Cu^{2+}-induced *in vitro* LDL oxidation in a dose-dependent manner. Results of the assay (Table 2) show ED_{50} ranging from 13 to 71 mg/L for the six extracts, *R. canescens* and *H. orientale* extracts being the most antioxidant. Four methanolic plant extracts were also studied for the first time for their anti-inflammatory and antinociceptive activities in mice (100 mg/kg). For three of the four extracts, results of these assays (Tables 3 and 4) showed an inhibition percentage ranging from 19.1 to 24.6% on carrageenan-induced hind paw edema and 11.5 to 16.2% on PBQ-induced abdominal constriction. The C*entaurea drabifolia* subsp. *drabifolia* extract was the most active in the two systems without causing any gastric damage. No acute toxicity was observed with any of the four extracts.

In conclusion, all of the extracts were shown to possess dose-dependent antioxidant activity. Whereas the antioxidant activities of *H. plicatum* subsp. *plicatum* and *A. wilhelmsii* were previously reported by Nickavar et al. (2006) and Tepe et al. (2005), respectively, the information given here can be considered as the first on those of *H. orientale*, *C. drabifolia* subsp. *drabifolia*, *C. drabifolia* subsp. *detonsa*, and *R. canescens* var. *canescens*. The methanol extract of *C. drabifolia* subsp. *drabifolia* exhibited the most active anti-inflammatory activity against carrageenan-induced hind paw edema in mice without inducing any gastric damage, as well as antinociceptive activity against PBQ-induced writhings in mice. To the best of our knowledge, the present study is the first to report anti-inflammatory and antinociceptive activities in *H. orientale*, *C. drabifolia* subsp. *drabifolia*, and *C. drabifolia* subsp. *detonsa*, which grow in Turkey. Further studies are under way to define their active constituents.

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***Declaration of interest*:** The authors report no conflicts of interest.

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