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Anti-inflammatory activity of a HPLC-fingerprinted aqueous infusion of aerial part of *Bidens tripartita* L.

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ABSTRACT

The anti-inflammatory potential of three doses of an aqueous infusion of aerial parts *Bidens tripartita* L. against carrageenan-induced acute paw edema in rats was investigated. A phytochemical study and qualitative-quantitative analyses revealed the presence of flavonoids, tannins, polysaccharides, phenols, amino acids, ascorbic acid, organic acids and polyacetylenes. Infusion doses of 20 ml/kg body wt. exhibited significant anti-inflammatory activity in rats, as compared with indomethacin. In addition, the infusion showed analgesic properties in a hot-plate test and antipyretic properties in carrageenan-induced local hyperthermia, both in rats. The effects were dose-dependent. Our results provide evidence for the potential usefulness of *B. tripartita* infusion in the treatment of inflammatory disorders.

Introduction

Bidens tripartita L. (family Asteraceae) is an annual plant, 30–100 cm in height with yellow flowers (common names include threelobe beggarticks, water agrimony and Burr marigold). In Russian traditional medicine, infusions of *B. tripartita* are widely used in the treatment of catarrhal rhinitis, angina, acute respiratory infection, and as an anti-inflammatory in colitis, gout, and rachitis (Sokolov 2000). Infusion of *B. tripartita* is used in traditional Chinese medicine to treat chronic dysentery (Zhang 1989). It is also used in oriental medicine as a diaphoretic and diuretic in nephrolithiasis, and as a bath for children, mainly at diathesis (antiallergic action) (Sezik et al. 2004).

An extract of *B. tripartita* demonstrated a high level of activity in the inhibition of cancer L1210 cells (mouse leukemia) and against thrombin (Goun et al. 2002). *B. tripartita* extract inhibited hemolysis induced by psoralen and by UV-A irradiation (Bezdetnaya et al. 1992). Extracts from the aerial parts of *B. tripartita*, using solvents of different polarity, have shown antiradical activity against the DPPH radical. Evaluation of the antioxidant activity showed it to be due, in large part, to the presence of flavonoids (Wolniak et al. 2007).

It has been reported that *B. tripartita* contains a significant amount of phenolic compounds, flavonoids, chalcones and aurones (Christensen et al. 1990; Mikayelyan et al. 2008), coumarins (Mikayelyan et al. 2008), tannins (Mojab et al. 2003), phenolic acids (Mikayelyan et al. 2008), and carbohydrates, including neutral and acidic polysaccharides and pectin (Isakova et al. 1986; Olennikov and Tankhaeva 2006). Phytochemical studies showed the presence of small amounts of vitamin C, carotenoids and a volatile oil (Tomczykowa et al. 2005). The green parts of *B. tripartita* contain polyacetylenic compounds, linoleic acid and ocimene, whereas thiophene, traces of cosmene and eugenol were identified in the flowers (Christensen et al. 1990).

Models induced by pro-inflammatory agents such as carrageenan, dextrane, formaldehyde, serotonin, histamine and bradykinin in rat paws are employed to investigate the effects of drugs on the acute phase of inflammation (Campos et al. 1995). Carrageenan is perhaps the most commonly used and well studied of these phlogistics (Leme et al. 1973; Shikov et al. 2008) producing a maximal edema in 3 h. The objective of this work was to evaluate, for the first time, the anti-inflammatory activity of infusions of aerial parts of *B. tripartita* in carrageenan-induced acute paw edema in rats.

Materials and methods

Plant material

The herbs of *B. tripartita* were collected in August 2007 from the plantation of MTT Agrifood Research Finland (Mikkeli,

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Finland) and from Begunitsy farm (Leningrad Region, Russia). The samples were dried at 40 °C in an air-forced dryer, ground to a powder in an excelsior mill and stored in closed vessels. The plants were identified by Dr. Vera Kosman, and voucher specimens (BiR0807 and BiF0807) have been deposited in the herbarium of the St. Petersburg Institute of Pharmacy (St. Petersburg, Russia).

Extraction

The dried samples of *B. tripartita* were extracted using water in accordance with State Pharmacopoeia of USSR, XI. Briefly, 10 g of dried herb of each sample was extracted with 200.0 ml of water in a boiling water bath for 15 min, infused for 45 min at ambient temperature, filtered, and then made to volume in a 200-ml volumetric flask. The final ratio of plant to water was 1:20 w/v.

Phytochemical screening

Phytochemical investigation of *B. tripartita* was carried out using standard methodology (Ladygina et al. 1983; State Pharmacopoeia of USSR, XI) to identify and quantify the major chemical constituents.

Flavonoids. The total flavonoids content was estimated as rutin equivalents in mg rutin/g dry weight. About 0.5 g of the plant material was extracted with 70 ml of ethanol for 30 min in a boiling waterbath and filtered through Whatman No. 1 filter paper. A 1.0-ml aliquot of each extract was mixed with 5 ml of 10% solution of aluminium chloride in ethanol and 1 drop of acetic acid, and then made to volume with ethanol in a 25-ml volumetric flask. Blanks were prepared as described above except aluminium chloride was replaced by ethanol. The absorption at 410 nm was measured after 30 min and compared to a rutin calibration curve.

Coumarins. The total coumarins content was estimated as coumarin equivalents in mg coumarin/g dry weight. 1.0 g of plant material was extracted with 15 ml of ethanol for 10 min in a boiling water bath, and immediately filtered through Whatman No. 1 filter paper. Phenolic compounds were precipitated by addition of 5 ml of 10% (w/v) lead acetate. After filtration, coumarins were extracted sequentially with 20 ml, and 2×10 ml of chloroform. The resulting chloroform extracts were combined and evaporated *in vacuo*. The residue was dissolved in ethanol in a 25-ml volumetric flask. The absorption at 310 nm was measured and compared to a coumarin calibration curve.

Tannins. The total tannins content was estimated as tannin equivalents in mg tannin/g dry weight. Briefly, 2.0 g of plant material was extracted with 250 ml of hot water for 30 min by refluxing. A 25 ml sample was mixed with 500 ml of water and 25 ml of indigo sulfuric acid solution, then titrated using 0.02 M $KMnO_4$.

Total phenolics. The total phenolics content of the extracts was estimated as gallic acid equivalents, expressed as mg of gallic acid/g (dry wt) of extract, according to the Folin-Ciocalteu reagent method (Singleton et al. 1999). Briefly, 1.0 g of the plant material was extracted with 100 ml of water for 60 min at 40 °C and filtered through Whatman No. 1 filter paper; 0.25 ml was transferred to a 25.0 ml volumetric flask containing 6 ml of water, to which was added 1.25 ml of undiluted Folin-Ciocalteu reagent. After 1 min, 3.75 ml of 20% (w/v) aqueous Na_2CO_3 was added, and the volume was made to 25.0 ml with water. The controls contained all of the reaction reagents but not the extract. After incubation for 2 h at 25 °C, the absorbance at 760 nm was measured and compared to a gallic acid calibration curve.

Polysaccharides. A 10.0 g sample of plant material was refluxed with 100 ml of water for 30 min and immediately filtered and this treatment was repeated four times. The resulting extracts were combined, evaporated *in vacuo* to a volume of ~10 ml and 45 ml of ethanol was added. After 30 min, the precipitate was centrifuged, washed with 50 ml of ethanol, manually pressed and lyophilized. The polysaccharides content was determined gravimetrically in mg/g dry wt.

Amino acids. The total amino acids content was estimated as arginine equivalents in mg arginine/g raw plant material (dry wt.). About 1.0 g of plant material was extracted with 10 ml of ethanol for 10 min in a boiling water bath, and immediately filtered. A 1.0-ml aliquot was mixed with 20 mg of ninhydrin and 200 mg of sodium acetate and heated for 10 min in a boiling water-bath, cooled and made to volume with water in a 50-ml volumetric flask. The absorption at 565 nm was measured and compared to an L-arginine calibration curve.

Chromatographic fingerprint analyses. The liquid chromatographic apparatus Shimadzu (Kyoto, Japan) comprising two LC20AD pumps, a DGU-20 A3 degasser, and an SPD-M20 A diode-array detector. Separation was achieved on a 4.6 mm i.d. × 150 mm, 5 μm particle, Luna C18 column (Phenomenex, USA) with a SecurityGuard pre-column (2 mm) containing the same adsorbent. Elution of tested compounds was effected using the solvent mixtures of 0.03% aqueous trifluoroacetic acid (v/v) (solvent A) and acetonitrile (solvent B) as follows: linear gradient from 10% B to 30% B in 30 min and then returned to initial conditions in 7 min. A 5-min equilibrium time was allowed between injections. The flow rate was 1.0 ml/min. Detection was carried out with a sensitivity of 0.1 aufs. between the wavelengths of 200 and 800 nm. A Shimadzu LC Solution data-analysis system was used. Sample volume was 20 μl and each sample was analyzed in triplicate. The components were identified by comparison of their retention times to those of authentic standards under identical analysis conditions and the UV spectra with our in-house PDA library.

Ascorbic acid. The content of ascorbic acid was expressed as mg of ascorbic acid/100 g (dry wt.). Briefly, 1.0 g of plant material was extracted with 15 ml of water at room temperature for 10 min, filtered and analyzed by HPLC on the same equipment. Isocratic elution was performed with 0.03% aqueous trifluoroacetic acid–methanol 95:5 (v/v) as mobile phase; the flow rate was 1.0 ml/min. The detection wavelength was 240 nm. Sample volume was 20 μl and each sample was analyzed in triplicate.

Polyacetylenes. Thin-layer chromatography (TLC) was carried out in Kieselgel 60 F 254 on glass plates (Merck, Darmstadt, Germany). The solvent system *n*-hexane /ethyl acetate/acetic acid (85:25:5, by vol.) was used as the mobile phase for the separation and identification of compounds. The components were visualized by UV irradiation at 320 nm by direct densitometric scanning of the developed plate (Camag TLC scanner 3 under software control of WinCats v. 1.3.2, Muttenz, Switzerland).

Chromatographic Standards preparation

Stock solutions of the standards were prepared in water and 95% (v/v) aqueous ethanol to final concentrations of 1 and 10 mg/ml, respectively. The concentration used for the calibration of reference compounds was 0.01-0.10 mg/ml. All standard and sample solutions were injected in triplicate.

Chemicals

The used reference compounds (±)-catechin (purity ≥ 95%), chlorogenic acid (purity ≥ 95%), caffeic acid (purity ≥ 98%), chicoric acid (purity ≥ 95%), luteolin (purity ≥ 98%), rosmarinic acid (purity ≥ 97%) were from Fluka Sigma–Aldrich (Seelze, Germany), luteolin-7-O-glucoside (purity ≥ 95%) was from Vilar-impex

(Moscow, Russia), and L-Ascorbic acid was from DSM Nutritional Products, Kaiseraugst, Switzerland. Acetonitrile (HPLC grade) was from Vekton (St.-Petersburg, Russia); methanol (HPLC grade) was from Merck (Darmstadt, Germany). Trifluoroacetic acid was obtained from Sigma-Aldrich (Seelze, Germany). Before use, the solvents were filtrated through a 0.45 mm Millipore membrane (Millipore, Saint-Quentin, Yvelines, France) after sonication for 15 min. Carrageenan was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Indomethacin dissolved in starch slime (5 mg/kg, Sopharma, Bulgaria) was used for the purpose of comparison. All other reagents and solvents were of analytical grade. Ultrapure water was prepared using a Milli-Q system (Millipore, MA, USA).

Animals

Female Wistar rats (Rappolovo, St. Petersburg, Russia) weighing 180–220 g each were used for the experiments. The animals were kept singly in standard cages and maintained under standard laboratory conditions (temperature 19–25 °C, relative humidity 50–70%, 12 h light/12 h dark cycle) with access to solid pellet diet and water *ad libitum* throughout the study except during the experiment. The experimental procedures were performed in accordance with the directive No. 267 from 19.06.2003 (About the statement of regulation of laboratory practice of the Ministry of Health of the Russian Federation).

Biological tests

Doses and treatments of animals

The rats were divided randomly into eight groups of 6 rats/group to minimize any inter-group differences in body weight. Group one represented the control group that received physiological solution orally. Group 2 received indomethacin at a dose of 5 mg/kg body wt. in starch slime in a volume of 5 ml/ rat by gastric gavage. Groups R-1, R-2, R-3, F-1, F-2 and F-3 received *B. tripartita* infusions orally at a high (20 ml/kg body wt.), middle (10 ml/kg body wt.), and low (4 ml/kg body wt.) doses, respectively. The test substances were given orally at 12 h and at 2 h before, and immediately after, injection of carrageenan.

Carrageenan-induced edema in rats

As a model for examining the anti-inflammatory activity, we used carrageenan-induced edema of rat's paw as described by Winter et al. (1962). Edema was induced by injection of 0.05 ml of carrageenan solution (0.5% (w/v) in normal saline) in the planter aponeurosis of the right hind paw. The edema volume was determined using the oncometric method (Shikov et al. 2008) at 3.5 h after the injection of carrageenan. The ratio of the anti-inflammatory effect of preparations was calculated by the following equation:

$$\text{Inhibition of edema rate } (\%) = 100 \times (1 - PV_{tr}/PV_c),$$

where PV_{tr} represents the percentage difference in paw volume after the compounds were administered to the rats, and PV_c represents the percentage difference of the volume in the control group.

Hot-plate test

To measure the analgesic property of the extract, rats were subjected to a hot-plate test (Turner 1965) using a Termoplate (LenChrom, St.-Petersburg, Russia). The temperature of the hot-plate was maintained at 65 ± 0.5 °C. The response time on the hot-plate was recorded at 3 h after the injection of carrageenan.

Carrageenan-induced local hyperthermia

A method for determining the surface temperature of the inflamed rat's paw was used. The antipyretic effect on decreasing temperature of the rat's paw skin in the center of an inflammation was evaluated. The temperature was measured with an electrothermometer (DT-635, A&D Medical Ltd., Japan) at 1 h, at 2 h and at 3 h after the injection of carrageenan.

Statistical analysis

Data were analyzed using Statistica version 6.0. The results are presented as mean ± SEM. Differences among groups were analyzed by ANOVA for unequal variance and those at $p < 0.05$ were accepted as statistically significant.

Results

The phytochemical profiles of *B. tripartita* samples are shown in Table 1. The Finnish sample was rich in flavonoids, tannins, polysaccharides and ascorbic acid. The Russian sample contains more coumarins and amino acids. The data from the qualitative-quantitative analysis of the extracts made using high performance liquid chromatography are presented in Table 1, while the chromatograms with detector responses at 280 nm are presented in Fig. 1. The components (±)-catechin, chlorogenic acid, caffeic acid, luteolin-7-O-glucoside, chicoric acid, rosmarinic acid and luteolin were identified by comparisons to the retention time and UV spectra of authentic standards analyzed under identical analytical conditions, while the quantitative data were calculated from their respective calibration curves. A number of components within the samples could only be tentatively identified by chemical class from their chromatographic behavior and UV spectra. Accordingly, all the extracts contained hydroxycinnamic acids (peaks 4, 9), glycoside of luteolin (peak 6) and polyacetylenes (peaks 7, 11, 13) (Fig. 1). The presence of polyacetylenes was also confirmed by TLC, in agreement with literature data (Brandão et al. 1997).

The effect of *B. tripartita* infusions on inhibition of the acute paw edema in rats evoked by carrageenan injection into the subplantar tissues of the right hind paws is presented in Table 2. The paw volumes from animals treated with all doses of *B.*

Table 1
Extract yield, phytochemical composition (qualitative-quantitative HPLC data) of *Bidens tripartita*.

Constituent	RUS	FIN
Extract yield	280.0	240.0
Flavonoids	14.0 ± 1.0	33.3 ± 0.4
Coumarins	1.5 ± 0.1	0.7 ± 0.1
Tannins	9.5 ± 0.5	17.8 ± 0.8
Total phenolics	18.3 ± 0.6	25.0 ± 1.0
Polysaccharides	97.4 ± 0.6	128.0 ± 5.0
Amino acids	51.0 ± 2.1	37.0 ± 4.0
Ascorbic acid	0.025 ± 0.001	0.400 ± 0.081
(±)-Catechin	1.76 ± 0.08	Traces
Chlorogenic acid	4.10 ± 0.15	6.29 ± 0.20
Caffeic acid	0.12 ± 0.01	0.36 ± 0.02
Luteolin-7-O-glucoside	0.41 ± 0.02	0.74 ± 0.04
Chicoric acid	0.65 ± 0.03	0.57 ± 0.03
Rosmarinic acid	0.91 ± 0.03	0.95 ± 0.04
Luteolin	0.32 ± 0.01	0.70 ± 0.03

The data (mg/g (dry wt.) herbs) are presented as mean ± SEM ($n = 3$).
RUS – Sample of *Bidens tripartite* collected in Russia; FIN – Sample of *Bidens tripartite* collected in Finland.

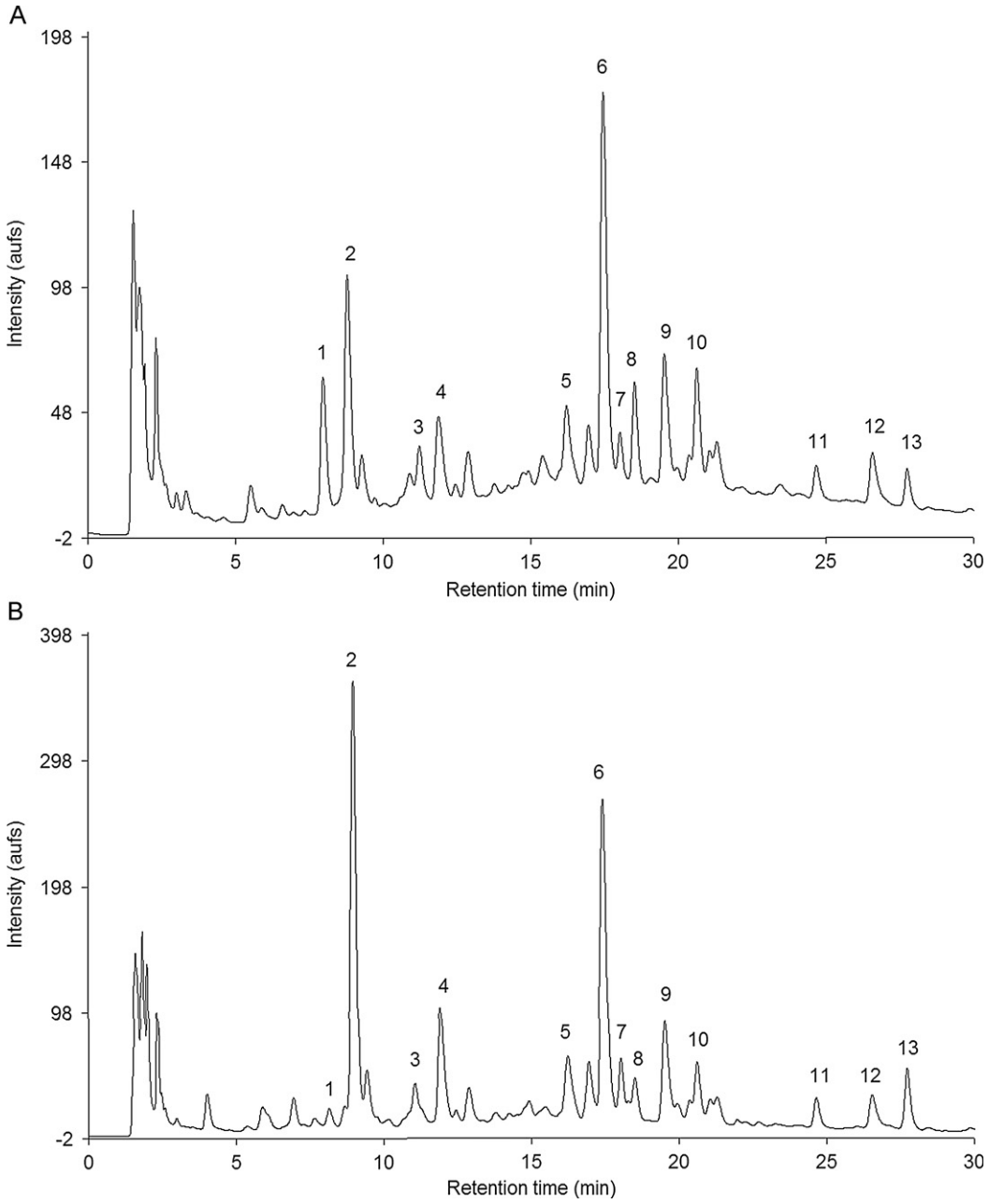

Fig. 1. HPLC chromatograms of aqueous infusions of *B. tripartita* samples-Russian (A), and Finnish (B), with detector responses at 280 nm. Peaks: (1) (±)-catechin, (2) chlorogenic acid, (3) caffeic acid, (5) luteolin-7-O-glucoside, (8) chicoric acid, (10) rosmarinic acid, (12) luteolin, (4,9) hydroxycinnamic acids, (6) glycoside of luteolin, and (7, 11, 13) polyacetylenes.

Table 2
Effect of *Bidens tripartita* infusion on carrageenan-induced rat paw-edema.

Group	Dose	Inhibition of edema rate, % (mean ± SEM)
Control	–	0
Indomethacin	5 mg/kg	53.1 ± 5.1
R-1	20 ml/kg	51.1 ± 4.9
R-2	10 ml/kg	28.2 ± 2.5
R-3	4 ml/kg	4.7 ± 0.9
F-1	20 ml/kg	41.4 ± 3.1
F-2	10 ml/kg	20.1 ± 2.0
F-3	4 ml/kg	3.0 ± 0.8

Groups R-1, R-2, R-3 received infusions of *Bidens tripartita* collected in Russia.
Groups F-1, F-2, F-3 received infusions of *Bidens tripartita* collected in Finland.

tripartita after induction of paw edema showed a marked decrease in comparison with the data of non-treated animals at 3 h. Even middle doses of infusions (10 ml/kg body wt.) were effective. Indomethacine at a dose of 5 mg/kg body wt. showed similar effect to *B. tripartita* infusion of Russian origin at a dose of 20 ml/kg body wt. The Finnish samples displayed slightly less effect. The effect of *B. tripartita* infusion on hot-plate response in rats is reported in Table 3. The response time of rats receiving an infusion of R-1 at 3 h after injection of carrageenan was increased 1.3-fold compared to the control group. The antipyretic effect of *B. tripartita* infusion is reported in Table 4. Our results show that administration of the infusion practically normalizes the temperature of the rat paw at 1 h after injection of carrageenan; i.e., it shows rapid action. These results indicate that the

Table 3
Analgesic effect of *Bidens tripartita* infusion assessed using the 65 ºC thermal-induced hot-plate test.

Group	Dose*	Hot-plate latency, s × 10 (mean ± SEM)
Control	–	42.3 ± 4.1
Indomethacin	5 mg/kg	47.1 ± 6.3
R-1	20 ml/kg	55.9 ± 8.0**
R-2	10 ml/kg	45.9 ± 6.0
R-3	4 ml/kg	42.9 ± 4.2
F-1	20 ml/kg	47.7 ± 4.0
F-2	10 ml/kg	43.9 ± 4.5
F-3	4 ml/kg	40.9 ± 4.0

Groups R-1, R-2, R-3 received infusions of *Bidens tripartita* collected in Russia.
Groups F-1, F-2, F-3 received infusions of *Bidens tripartita* collected in Finland.

* mg/kg or ml/kg body wt.
** Data differ significantly ($p < 0.05$) as compared to control group.

Table 4
Antipyretic effect of *Bidens tripartita* infusion assessed using adjuvant-inflamed rat's paw.

Group	Dose*	Mean temperature (°C) of rat's paw skin after injection of carrageenan (mean ± SEM)		
		1 h	2 h	3 h
Control	–	30.9 ± 0.8	30.3 ± 0.7	30.0 ± 0.5
Indomethacin	5 mg/kg	28.2 ± 0.4**	26.9 ± 0.3**	27.2 ± 0.7**
R-1	20 ml/kg	29.6 ± 0.5	31.9 ± 0.5	31.5 ± 1.1
R-2	10 ml/kg	30.6 ± 0.7	30.9 ± 0.5	31.3 ± 1.0
R-3	4 ml/kg	30.5 ± 0.6	31.0 ± 0.7	30.9 ± 0.9
F-1	20 ml/kg	30.8 ± 0.9	31.3 ± 0.7	31.0 ± 1.1
F-2	10 ml/kg	29.7 ± 0.6	30.3 ± 0.7	30.5 ± 1.0
F-3	4 ml/kg	30.5 ± 0.9	31.1 ± 0.7	30.7 ± 0.9

Groups R-1, R-2, R-3 received infusions of *Bidens tripartita* collected in Russia.
Groups F-1, F-2, F-3 received infusions of *Bidens tripartita* collected in Finland.

* mg/kg or ml/kg body wt.
** Data differ significantly ($p < 0.05$) as compared to control group.

anti-inflammatory hot-plate response and antipyretic effects of *B. tripartita* infusion in rats were dose-dependent.

Discussion

The oral administration of an infusion of *B. tripartita* is commonly used in folk medicine for the treatment of catarrhal and infectious diseases. We obtained an aqueous infusion and studied its anti-inflammatory, analgesic and antipyretic properties in rats.

It was established that the major groups of components of *B. tripartita* are flavonoids, polysaccharides and ascorbic acid for the Finnish samples, and coumarins and amino acids for the Russian samples (Table 1).

There are two main groups of active constituents of *B. tripartite*, flavonoids and polyacetylenes, which reduce inflammation. The flavonoids have long been recognized to possess anti-inflammatory activity (Middleton et al. 2000). Analyses of various species of *Bidens* have been conducted in several countries. Although there is some variation in the level of activity of the different species of *Bidens*, probably due to different levels of active constituents, the general properties appear similar.

The anti-inflammatory effect of aqueous extracts of *Bidens pilosa* L. and *Bidens chilensis* DC. against rat's paw edema induced by carrageenan, and chronic arthritis induced by complete Freund's adjuvant, was studied in rats by Chih et al. (1995). The results indicated that rat's paw edema induced by carrageenan was decreased significantly by treatment with aqueous extracts (150 or 300 mg/kg body wt). of *Bidens* sp. The effect of *B. pilosa* was the most potent of the three Taiwanese varieties tested. The extract (500 mg/kg body wt.) of *B. pilosa* decreased significantly the rat's paw edema induced by Freund's adjuvant.

Carrageenan-induced rat's paw inflammation has been accepted us a useful phlogistic tool for investigating systemic anti-inflammatory agents. The infusions showed inhibitory activity in carrageenan-induced rat's paw inflammation over a period of 3 h. *B. tripartita* infusions showed an analgesic effect in the procedure used. The antipyretic effect of the aqueous infusions was significantly less prolonged for a dose of 20 ml/kg body wt. in comparison to indomethacine at a dose of 5 mg/kg body wt.

The polyacetylenes can also manifest an anti-inflammatory action, probably mediated by a mechanism different from that of the flavonoids. It was indicated earlier that the polyacetylene isolated from *B. pilosa* may be involved in the immunosuppressive effect of the crude extract (Pereira et al. 1999). The same polyacetylene has been isolated from *B. camphylotheca* (Bauer et al. 1992), a species used traditionally in Hawaiian folk medicine. The polyacetylenes isolated from *B. camphylotheca* inhibited prostaglandin biosynthesis (Redl et al. 1994). Taken together, these data indicate a potent immunosuppressive action of components present in *B. pilosa*, suggesting a promising application as an anti-inflammatory drug.

B. tripartia also contains coumarins, triterpenes and essential oils, which may contribute to the observed therapeutic action of the herb. It is likely that coumarins, which are found in significant quantities in *B. tripartita* (Table 1), also contribute to the anti-inflammatory action. It was found recently that coumarins might affect the formation and scavenging of reactive oxygen species (ROS), and influence processes involving free radical-mediated injury. Coumarin can reduce tissue edema and inflammation. Moreover, coumarin and its 7-hydroxy-derivative inhibit prostaglandin biosynthesis, which involves fatty acid hydroperoxy intermediates. Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognized as inhibitors of the lipoxygenase and cycloxygenase enzymic systems, and of neutrophil-dependent superoxide anion generation (Fylaktakidou et al. 2004). Carrageenan stimulates the release of several inflammatory mediators such as histamine, serotonin, bradykinin and prostaglandins (Lino et al. 1997). Non-steroidal anti-inflammatory drugs (NSAID) block the synthesis of prostaglandins by inhibiting cyclooxygenase (COX). COX and 5-lipoxygenase (5-LOX) catalyze peroxidation of arachidonic acid, and polyphenols like coumarins and flavonoids might be expected to interfere with this process (Hoult et al. 1994b). Some coumarins have been shown to inhibit the generation of leukotriene B4 (a 5-LO product) (Hoult et al. 1994a). In other experiments, coumarin and umbelliferone were found to have a mechanism of action similar to that of NSAID in a carrageenan-induced inflammation, and the effect lasted for at least 3 h, which is the time for the maximum effect of carrageenan (Lino et al. 1997).

Synergy effects of the mixture of bioactive constituents and their byproducts contained in plant extracts are claimed to be responsible for the improved effectiveness of many extracts (Wagner and Ulrich-Merzenich 2009). We may assume that the anti-inflammatory activity of *B. tripartia* infusions is due to the synergistic effect of polyphenols, polyacetylenes, coumarins, triterpenes and other active compounds.

In conclusion, this study demonstrated the anti-inflammatory activity of an aqueous infusion of aerial parts of *B. tripartita*. To the best of our knowledge, this is the first report on the anti-inflammatory property of *B. tripartita in vivo* and our findings justify the traditional use of this plant against inflammatory

diseases. However, it will be important to determine the specific compound(s) responsible for the anti-inflammatory activity, as well as to establish the mechanism of action of the extract.

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