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Antinociceptive and anti-inflammatory effects of the essential oil from *Eremanthus erythropappus* leaves

Orlando V. Sousa, Marcelo S. Silvério, Glauciemar Del-Vechio-Vieira, Filipe C. Matheus, Célia H. Yamamoto and Maria S. Alves

Abstract

The chemical composition of the essential oil from air-dried leaves of *Eremanthus erythropappus* was studied. The main compounds were β-pinene (23.24%), β-caryophyllene (22.92%), β-myrcene (10.03%) and germacrene D (9.40%). The essential oil had an LD50 of 2.90 g kg^{-1} in mice. Doses of 200 and 400 mg kg^{-1} inhibited 10.69% and 27.06% of acetic-acid-induced writhing in mice, respectively. In the formalin-induced nociception test in mice, the essential oil inhibited the first phase of paw licking by 29.13% (400 mg kg^{-1}) and the second phase by 32.74% (200 mg kg^{-1}) and 37.55% (400 mg kg^{-1}). In the hot-plate test in mice, doses of 200 mg kg^{-1} and 400 mg kg^{-1} significantly increased the reaction time after 30, 60 and 90 min of treatment. Doses of 200 and 400 mg kg^{-1} inhibited carrageenan-induced paw oedema in rats by 15.18% and 36.61%, respectively. Doses of 200 and 400 mg kg^{-1} administered 4 h before intrapleural injection of carrageenan significantly reduced exudate volume (by 20.20% and 48.70%, respectively) and leucocyte mobilization (by 5.88% and 17.29%, respectively). These results demonstrate that *E. erythropappus* has analgesic and anti-inflammatory properties, supporting the use of this plant in folk medicine.

Introduction

Essential oils are lipophilic molecules responsible for flavour and fragrance and are obtained by steam distillation (Burt 2004; Pichersky et al 2006). Most of them are mixtures of terpene and sesquiterpene hydrocarbons, phenylpropanoids and benzenoid oxygenated derivatives (Pichersky et al 2006). Recently, some studies have demonstrated the antinociceptive and anti-inflammatory properties of essential oils (Öztür & Özbek 2005; Lino et al 2005; Iscan et al 2006; Vendruscolo et al 2006; Passos et al 2007). These properties have been attributed to the chemical composition, such as terpinen-4-ol (Hart et al 2000), β-caryophyllene (Passos et al 2007) and linalool and linalyl acetate (Peana et al 2002).

Eremanthus erythropappus (DC) McLeisch (Asteraceae) (*Vanillosmopsis erythropappa* Schultz-Bip), known commonly as candeia, is used in folk medicine as an antiphlogistic and antimicrobial agent (Sousa et al 2003). The essential oil obtained from the wood of *E. erythropappus* contains α-bisabolol, costunolide and eremanthine (Lopes et al 1991; Braun et al 2003), compounds with the cyclocostunolide and eremanthine skeleton (Lima et al 1985; Lopes et al 1991), vanillosmin (Corbrella et al 1974), 15-deoxygoyazenzolide (Vichnewski et al 1976) and lychnopholide (Vichnewski et al 1989). The α-bisabolol, present in other members of Asteraceae such as *Chamomilla recutita*, exhibits anti-inflammatory properties (Jakovlev et al 1979) and has been widely used in pharmaceutical and cosmetic products (Sousa et al 2003).

Despite the exploration and trade of products containing essential oil from the wood of *E. erythropappus*, as well as chemical and biological studies, no published reports of the pharmacological and toxicological effects of the essential oil from the leaves of this species have been described. Therefore, in the present study we investigated the anti-inflammatory and analgesic effects of the essential oil obtained from *E. erythropappus* leaves, using a variety of different experimental models. We also established the chemical composition and acute toxicity of the essential oil studied.

Departamento Farmacêutico, Faculdade de Farmácia e Bioquímica, Universidade Federal de Juiz de Fora, 36016-330, Juiz de Fora, MG, Brazil

Orlando V. Sousa, Marcelo S. Silvério, Glauciemar Del-Vechio-Vieira, Filipe C. Matheus, Célia H. Yamamoto

Departamento de Análises Clínicas, Faculdade de Farmácia e Bioquímica, Universidade Federal de Juiz de Fora, 36016-330, Juiz de Fora, MG, Brazil

Maria S. Alves

Correspondence: O. V. Sousa, Departamento Farmacêutico, Faculdade de Farmácia e Bioquímica, Universidade Federal de Juiz de Fora, 36016-330, Juiz de Fora, MG, Brazil. E-mail: orlando.sousa@ufjf.edu.br

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Materials and Methods

Plant material

The plant material used in this study was collected in Juiz de Fora, State of Minas Gerais, Brazil in July 2005. The species was identified by Dr Fátima Regina Gonçalves Salimena Pires and a voucher specimen (CESJ number 25363) was deposited in the Herbarium of the Universidade Federal de Juiz de Fora, Brazil.

Essential oil extraction

The air-dried leaves (1.2 kg) of *E. erythropappus* were hydrodistilled in a Clevenger-type apparatus. After 2 h of distillation, the essential oil was removed from the surface of the water and dried over anhydrous sodium sulfate for analysis.

For evaluation of analgesic and anti-inflammatory activities as well as acute toxicity, each 500 mg of the essential oil was solubilized with 100 μL DMSO and diluted in saline.

Identification of the components of the essential oil

The volatile components were analysed by GC/MS in a gas chromatograph (Hewlett-Packard 6890, Fremont, CA, USA) coupled to a mass spectrometer (MS HP5972, Hewlett-Packard) using the following conditions: ZB-5MS column (30 m × 0.25 mm × 0.25 μm film; Phenomenex, Torrance, CA, USA); helium (1 mL min^{-1}); programmed temperature 60–240°C (3°C min^{-1}); injector temperature (260°C) and interface (200°C); ionization energy, 70 eV; split ratio 1/10; scan range, 30–300 amu; scan time, 1 s. Compound identification was based on a comparison of retention indices (determined relative to the retention times of a series of n-alkanes), mass spectra and the NIST spectrometer data bank, together with comparison with literature data (Adams 1995).

Chemicals

Drugs and reagents used in this study (and their sources) were as follows: acetic acid (Vetec Química Farm Ltda, Rio de Janeiro, Brazil), formaldehyde and acetylsalicylic acid (Reagen Quimibrás Ind. Química S.A., Rio de Janeiro, Brazil), morphine hydrochloride (Merck Inc., Whitehouse Station, NJ, USA), naloxone, indometacin and carrageenan (Sigma Chemical Co, St Louis, MO, USA).

Animals

Male Wistar rats weighing 180–240 g and male Swiss albino mice weighing 25–30 g were used in the experiments. The animals were provided by the Central Biotery of the Universidade Federal de Juiz de Fora. The animals were divided into groups and kept in plastic cages at room temperature (25 ± 4°C), with free access to Purina rations and water. Animal care and the experimental protocol followed the principles and guidelines suggested by the Brazilian College of Animal Experimentation (COBEA) and were approved by the local ethical committee.

Acute toxicity

Groups of ten mice received oral doses of 0.5, 1, 1.5, 2 and 3 g kg^{-1} of essential oil from *E. erythropappus*, while the control group received the vehicle (saline). The groups were observed for 48 h and mortality at end of this period was recorded for each group (Dietrich 1983). The LD50 (50% lethal dose) was determined by probit test using a log plot of percentage death versus dose (Litchfield & Wilcoxon 1949). The determination of LD50 served to define the doses used in experiments of analgesic and anti-inflammatory activities.

Acetic acid-induced writhing response in mice

Analgesic activity was evaluated using the test of abdominal writhing induced by acetic acid in mice (Collier et al 1968). Animals were divided into groups of eight mice. Control mice received an i.p. injection of acetic acid 0.6% (0.25 mL) and 10 min later the writhes were counted over a period of 20 min. One group of mice received oral acetylsalicylic acid (200 mg kg^{-1}) as a reference compound, and the other three groups received oral doses of the essential oil at doses of 100, 200 and 400 mg kg^{-1}, 1 h before the acetic acid injection.

Formalin-induced nociception in mice

Mice received subplantar injections of 20 μL 2.5% formalin (in 0.9% saline) and the duration of paw licking was determined over 0–5 min (first phase) and 15–30 min (second phase) after formalin injection (Hunskaar & Hole 1987). Animals (n = 8) were pretreated with essential oil (100, 200 or 400 mg kg^{-1}; 0.1 mL per 10 g body weight, administered orally) or the reference compound, subcutaneous morphine (5 mg kg^{-1}), 1 h before administration of formalin. Control animals were treated with sterile saline (10 mL kg^{-1}).

Hot-plate latency assay in mice

Animals were placed on a hot-plate (Model LE 7406, Letica Scientific Instruments, Barcelona, Spain) heated at 55 ± 1°C (Eddy & Leimbach 1953). Three groups of mice (n = 8) were treated orally with essential oil (100, 200 or 400 mg kg^{-1}; 0.1 mL per 10 g body weight); the control group received sterile saline (10 mL kg^{-1}). Measurements were performed at time 0 and 30, 60 and 90 min after drug administration, with a cut-off time of 40 s to avoid lesions to the animals' paws. The effect of pretreatment with naloxone (1 mg kg^{-1} subcutaneously) on the analgesia produced by the essential oil (400 mg kg^{-1}) was determined in a separate group of animals. Morphine (5 mg kg^{-1} subcutaneously)

in the absence and presence of naloxone treatment was used as a reference.

Carrageenan-induced oedema in rats

Anti-inflammatory activity was assessed on the basis of inhibition of paw oedema induced by the injection of 0.1 mL 2% carrageenan (an oedematogenic agent) into the subplantar region of the right hind paw of the rat (Winter et al 1962). Male Wistar rats were divided into groups of six animals which received oral doses of essential oil (100, 200 and 400 mg kg^{-1}; 0.1 mL per 10 g body weight), saline or indometacin (10 mg kg^{-1}) 1 h before the injection of carrageenan. Paw volume was measured 4 h after administration of carrageenan using a plethysmometer (model LE 7500, Letica Scientific Instruments).

Carrageenan-induced pleurisy in rats

Pleurisy was induced in male Wistar rats by intrapleural administration of 0.5 mL 2% carrageenan suspension in saline solution between the third and fifth ribs on the right side of the mediastinum (Vinegar et al 1973). Essential oil (100, 200 and 400 mg kg^{-1}) was given 60 min before injection of the irritant. Animals were killed 4 h after carrageenan injection, and the skin and pectoral muscles were retracted. A longitudinal incision was made between the third and fifth ribs on each side of the mediastinum. The exudate was collected and transferred to a 15 mL conical centrifuge tube and the total volume determined. A 50 μL aliquot of the exudate was used to determine the total leucocyte count in a Neubauer chamber.

Statistical analysis

Data are expressed as mean ± s.e.m. Statistical significance was analysed by one-way analysis of variance followed by the Student–Newman–Keuls test. P values below 0.05 were considered significant.

Results

Chemical composition

The yield of essential oil from the air-dried leaves of *E. erythropappus* was 0.3%. The chromatogram data and the chemical composition are presented in Table 1. Table 1 shows the 30 compounds identified (99.79% of the total oil). The main compounds were β-pinene (23.24%), β-caryophyllene (22.92%), β-myrcene (10.03%) and germacrene D (9.40%). Bicyclogermacrene, α-copaene, α-pinene and δ-cadinene were also present in significant amounts. The essential oil consisted of monoterpenes (39.12%) and sesquiterpenes (60.20%).

Acute toxicity

The essential oil from *E. erythropappus* was toxic for mice, with an LD50 of 2.90 g kg^{-1} (95% confidence intervals 1.78–4.73). This value was important to define the doses for pharmacological activities.

Table 1 Chemical composition of the essential oil from *E. erythropappus* leaves

Constituents	RT	RI	%	Method of identification
α-pinene	5.49	1002	3.80	RI, GC-MS
β-pinene	6.61	1041	23.24	RI, GC-MS
β-myrcene	7.07	1049	10.03	RI, GC-MS
limonene	8.76	1073	0.42	RI, GC-MS
β-phellandrene	8.84	1075	0.44	RI, GC-MS
cis-ocimene	9.13	1078	0.27	RI, GC-MS
trans-ocimene	9.44	1084	0.81	RI, GC-MS
terpinen-4-ol	15.39	1316	0.30	RI, GC-MS
δ-elemene	21.70	1470	0.38	RI, GC-MS
α-copaene	23.09	1506	4.43	RI, GC-MS
β-bourbonene	23.69	1522	0.35	RI, GC-MS
β-elemene	23.83	1526	2.28	RI, GC-MS
α-gurjunene	24.53	1543	0.47	RI, GC-MS
β-caryophyllene	24.66	1547	22.92	RI, GC-MS
α-humulene	26.34	1590	2.61	RI, GC-MS
cis-muuroladiene	27.10	1609	0.20	RI, GC-MS
γ-muurolene	27.13	1611	0.24	RI, GC-MS
germacrene-D	27.24	1614	9.40	RI, GC-MS
valencene	27.79	1628	0.30	RI, GC-MS
bicyclogermacrene	27.92	1633	5.04	RI, GC-MS
α-muurolene	28.23	1640	0.73	RI, GC-MS
γ-cadinene	28.71	1654	0.60	RI, GC-MS
δ-cadinene	28.82	1656	3.42	RI, GC-MS
spathulenol	31.33	1727	0.47	RI, GC-MS
caryophyllene oxide	31.41	1729	1.58	RI, GC-MS
di-*epi*-cubenol	31.73	1768	0.28	RI, GC-MS
cubenol	32.29	1781	0.55	RI, GC-MS
cadinol	33.64	1797	1.48	RI, GC-MS
muurolol	33.80	1801	0.46	RI, GC-MS
α-cadinol	34.15	1812	1.29	RI, GC-MS

RT, retention time; RI, retention index; GC-MS, gas chromatography-mass spectrometry.

Writhing response induced by acetic acid in mice

At 400 mg kg^{-1} the essential oil caused 27.06% inhibition of acetic-acid-induced abdominal writhing compared with controls (number of constrictions: 53.90 ± 3.11 vs 73.90 ± 3.29; $P < 0.05$) (Table 2).

Table 2 Effects of the essential oil from *E. erythropappus* leaves on acetic acid-induced writhing in mice

Group	Dose (mg kg^{-1})	Number of writhes	Inhibition (%)
Control	Saline	73.90 ± 3.29	–
Essential oil	100	74.50 ± 2.13	–
	200	66.00 ± 1.73*	10.69
	400	53.90 ± 3.11‡	27.06
AA	200	19.75 ± 1.31‡	73.27

AA, acetylsalicylic acid (positive control).
Data are mean ± s.e.m. of eight mice. *$P < 0.05$, ‡$P < 0.001$ vs control group.

Effects on formalin-induced nociception in mice

Essential oil at a dose of 400 mg kg^{-1} inhibited the first phase of paw licking (Table 3). The second phase was inhibited at doses of 200 mg kg^{-1} and 400 mg kg^{-1}. As expected, morphine (reference drug) significantly inhibited both phases of formalin-induced paw licking.

Effects on hot-plate latency assay in mice

The effect of the essential oil in the hot-plate assay varied according to the doses and observation time (Table 4). No significant antinociceptive effects were observed at time 0. The reaction time was increased significantly at 30, 60 and 90 min in animals that received 200 or 400 mg kg^{-1} of the essential oil.

Hot-plate testing was also performed in the presence of naloxone, an opioid antagonist. Naloxone reduced the morphine-induced antinociceptive effect but did not affect the antinociceptive effect of the essential oil (Table 4).

Effects on carrageenan-induced oedema in rats

The anti-inflammatory effect of the essential oil is shown in Table 5. The 200 mg kg^{-1} dose inhibited oedema by 15.18% compared with controls, indicating that the essential oil has anti-oedematogenic properties.

Effects on carrageenan-induced pleurisy in rats

The effects of the essential oil on carrageenan-induced pleurisy were determined by measuring the volume and leucocyte counts of pleural exudates. Essential oil (200 and 400 mg kg^{-1}) administered 4 h before intrapleural injection of carrageenan significantly reduced exudate volume (Table 6) and leucocyte mobilization (Table 7).

Discussion

The essential oil obtained from *E. erythropappus* leaves comprised monoterpenes (39.12%) and sesquiterpenes (60.20%); β-Pinene (23.24%) and β-caryophyllene (22.92%) were identified as main constituents, as has been reported for *V. pohlii* (Andrade et al 2004). However, such constituents were not identified in essential oil obtained from the stem wood of *E. erythropappus* (Braun et al 2003).

Our experimental results show that the essential oil from *E. erythropappus* leaves possesses antinociceptive and anti-inflammatory activities. The essential oil inhibited abdominal writhing induced by acetic acid in mice. These data support the hypothesis that essential oil inhibits prostaglandin synthesis, given that the nociceptive mechanism of abdominal writhing induced by acetic acid metabolites involves the cyclooxygenase pathway and

Table 3 Effects of the essential oil from *E. erythropappus* leaves on formalin-induced nociception in mice

Groups	Dose (mg kg^{-1})	Duration of paw licking (s)			
		First phase		Second phase	
		Number	Inhibition (%)	Number	Inhibition (%)
Control	Saline	90.70 ± 4.45	–	95.60 ± 4.68	–
Essential oil	100	95.00 ± 3.90	–	94.70 ± 4.61	–
	200	91.40 ± 3.63	–	64.30 ± 3.56‡	32.74
	400	65.70 ± 2.98‡	29.13	59.70 ± 2.87‡	37.55
Morphine	5	13.70 ± 1.89‡	85.22	16.80 ± 1.90‡	85.56

First phase = 0–5 min after formalin injection; second phase = 15–30 min.
Data are mean ± s.e.m. of eight mice. ‡$P < 0.001$ vs control group.

Table 4 Effects of the essential oil (EO) from *E. erythropappus* leaves on the reaction time (s) of mice exposed to the hot-plate test

Group	Dose (mg kg^{-1})	Time after drug administration			
		0 min	30 min	60 min	90 min
Control	Saline	6.25 ± 0.53	6.87 ± 0.67	6.50 ± 0.42	6.87 ± 0.74
EO	100	6.75 ± 0.53	7.37 ± 0.68	7.50 ± 0.71	7.62 ± 0.50
	200	6.50 ± 0.57	9.25 ± 0.56*	10.12 ± 0.44‡	9.25 ± 0.73*
	400	6.87 ± 0.44	13.62 ± 0.86‡	14.12 ± 0.51‡	12.25 ± 0.73‡
Morphine	5	6.25 ± 0.59	18.25 ± 0.70‡	21.37 ± 0.84‡	24.37 ± 0.73‡
Naloxone + morphine	1 + 5	6.62 ± 0.65	12.00 ± 0.71‡	11.12 ± 0.55‡	10.25 ± 0.59†
Naloxone + essential oil	1 + 400	6.50 ± 0.68	12.75 ± 0.70‡	13.00 ± 0.71‡	11.87 ± 0.64‡

Data are mean ± s.e.m. of eight mice. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ vs control group.

Table 5 Effects of the essential oil from *E. erythropappus* leaves on carrageenan-induced paw oedema in rats

Group	Dose (mg kg^{-1})	Volume of hind paw (mL)	Inhibition (%)
Control	Saline	1.12 ± 0.06	–
Essential oil	100	1.15 ± 0.05	–
	200	0.95 ± 0.05*	15.18
	400	0.71 ± 0.04‡	36.61
Indometacin	10	0.43 ± 0.04‡	61.61

Data are mean ± s.e.m. of six rats. *$P < 0.05$, ‡$P < 0.001$ vs control group.

Table 6 Effects of the essential oil from *E. erythropappus* leaves on number of leucocytes in carrageenan-induced pleurisy in rats

Group	Doses (mg kg^{-1})	N° leucocytes (× 10^3 cells mm^{-3})	Inhibition (%)
Control	Saline	23.27 ± 0.32	–
Essential oil	100	23.24 ± 0.30	–
	200	21.90 ± 0.29*	5.88
	400	19.25 ± 0.36†	17.29
Indometacin	10	13.55 ± 0.24‡	41.78

Data are mean ± s.e.m. of six rats. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ vs control group.

Table 7 Effects of the essential oil from *E. erythropappus* leaves on pleural exudation induced by carrageenan in rats

Group	Doses (mg kg^{-1})	Exudate volume (mL)	Inhibition (%)
Control	Saline	2.16 ± 0.11	–
Essential oil	100	2.05 ± 0.09	–
	200	1.76 ± 0.11*	20.20
	400	0.81 ± 0.05‡	48.70
Indometacin	10	0.50 ± 0.05‡	71.50

Data are mean ± s.e.m. of six rats. *$P < 0.05$, ‡$P < 0.001$ vs control group.

prostaglandin biosynthesis (Duarte et al 1992). In addition, the essential oil of *E. erythropappus* affected both phases of paw licking, suggesting both peripheral and central actions (Hunskaar & Hole 1987). The central action was confirmed in the hot-plate test (200 and 400 mg kg^{-1}), showing maximal effect after 60 min. Our results indicate that the analgesia induced by this oil is not dependent on the opioid system, since previous treatment with naloxone did not reverse the effect.

The anti-inflammatory effect was confirmed in carrageenan-induced paw oedema in rats, an animal model widely employed for the screening of anti-inflammatory compounds. The inflammatory response induced by carrageenan is characterized by the formation of marked oedema resulting from the release of several mediators such as histamine, serotonin and bradykinin; this is subsequently sustained by release of prostaglandins produced by inducible isoforms of cyclooxygenase (COX-2) (Di Rosa et al 1971; Seibert et al 1994; Nantel et al 1999). In the present study, oral treatment with the essential oil of *E. erythropappus* markedly inhibited carrageenan-induced paw oedema in rats. This treatment consistently attenuated the paw oedema induced by carrageenan, as well as by several inflammatory mediators known to participate in the carrageenan response, such as bradykinin, histamine, substance P and platelet-activating factor (Stochla & Maslinski 1982; Hwang et al 1986; De Campos et al 1994; Gilligan et al 1994). This evidence suggests that the anti-inflammatory actions of the essential oil of *E. erythropappus* are related to the inhibition of one or more intracellular signalling pathways involved in the effects of these mediators.

The inflammation model of carrageenan-induced pleurisy was used to gain further insights into the anti-inflammatory effects of the essential oil from *E. erythropappus* (Vinegar et al 1973; Ammendola et al 1975; Compasso et al 1975). Carrageenan-induced pleurisy has been used to investigate the mechanisms involved in acute inflammation and also to assess the effectiveness of anti-inflammatory drugs (Vinegar et al 1973; Miyasaka & Mikami 1982). As expected, in our experiments intrapleural injection of carrageenan caused a marked accumulation of pleural exudate, followed by intense migration of inflammatory cells into the pleural cavity. Treatment of rats with the essential oil (200 and 400 mg kg^{-1}) significantly reduced the volume of pleural exudate accumulated in response to carrageenan injection and also inhibited the migration of leucocytes.

Analgesic and anti-inflammatory activities found in this model have also been reported for essential oils from *Achillea schischkii* and *A. aleppica* (Iscan et al 2006). Similar components detected in our experiments could be responsible for these properties, for example β-caryophyllene, α-humulene (Passos et al 2007) and α-pinene (Orhan et al 2006). α-Pinene and β-pinene, which were found in appreciable amounts in the essential oil of this study, have been reported to be responsible for the anti-inflammatory activity of the oil from *Bupleurum fruticosum* (Lorente et al 1989). One of the major components of this oil, β-myrcene, has been shown to exhibit analgesic activity (Lorenzetti et al 1991). The others important components of this oil, germacrene-D and bicyclogermacrene, found in the essential oil from *Cesearia sylvestris*, could be responsible for the analgesic and anti-inflammatory activities of this oil (Esteves et al 2005). Limonene, although a minor constituents of the oil under study, is known to have anti-inflammatory properties in species of *Protium* (Siani et al 1999). However, it should be considered that minor and major components, as well as possible interactions between the substances, could contribute to the studied pharmacological properties.

Conclusion

The essential oil from *E. erythropappus* leaves is a potential candidate for use as an analgesic and anti-inflammatory agent in new drugs for the therapy of pain and inflammatory diseases. In addition, the results may justify the use of *E. erythropappus* in traditional medicine. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

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