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Anti-inflammatory effects of essential oils from *Mangifera indica*

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ABSTRACT. *Mangifera indica* is widely found in Brazil, and its leaves are used as an anti-inflammatory agent in folk medicine. The aim of this study is to perform composition analysis of essential oils from the *M. indica* varieties, espada (EOMIL1) and coração de boi (EOMIL2), and confirm their anti-inflammatory properties. Twenty-three volatile compounds were identified via gas chromatography-mass spectrometry (GC-MS) in two essential oils from the leaves. Paw edema and myeloperoxidase (MPO) activity were evaluated using the carrageenan-induced paw model, while leukocyte migration was analyzed using the pleurisy model. At oral doses of 100 and 300 mg/kg, the essential oils significantly reduced edema formation and the increase in MPO activity induced by carrageenan in rat paws. For a dose of 300 mg/kg EOMIL1, 62 ± 8% inhibition of edema was observed, while

EOMIL2 led to 51 ± 7% inhibition of edema. At a dose of 100 mg/kg, the inhibition was 54 ± 9% for EOMIL1 and 37 ± 7% for EOMIL2. EOMIL1 and EOMIL2 significantly reduced MPO activity at doses of 100 mg/kg (47 ± 5 and 23 ± 8%, respectively) and 300 mg/kg (50 ± 9 and 31 ± 7%, respectively). In the pleurisy model, inhibitions were also observed for EOMIL1 and EOMIL2 in the leukocyte migration test. The results of the present study show that essential oils from *M. indica* differ in chemical composition and anti-inflammatory activity in rats.

Key words: Espada; Coração de boi; Paw edema; Pleurisy; Rats

INTRODUCTION

The volatile oils from plants are known for their biological properties, especially antibacterial, antifungal, and antioxidant effects (Deans and Waterman, 1993). In addition, there has been an increased interest in the use of new volatile oils in the pharmaceutical, food, and cosmetic industries.

The species *Mangifera indica* L., which belongs to the Anacardiaceae family, is one of the 40 species of the *Mangifera* genus that can be found in the tropical and subtropical regions of Southeast Asia, Africa, and Latin America (Nikhal and Mahajan, 2010). The leaves of *M. indica* have been used in the Indian systems of medicine to treat diseases such as asthma, dysentery, cough, leucorrhea, jaundice, pain, and malaria (Basha et al., 2011). Studies have been conducted on aqueous extracts of the bark of a selected variety of *M. indica,* which have been used in the pharmaceutical formulation named Vimang®. The volatile constituents of the fruits of *M. indica* present considerable variation in their chemical composition, and they have been extensively investigated (Dzamic et al., 2010). The variability of the volatile constituents can be influenced by factors, such as stage of development, variety, and method of extraction (Pino et al., 2005).

The volatile compounds in *M. indica* are normally obtained via hydrodistillation and analyzed through gas chromatography-mass spectrometry (GC-MS) (Pino et al., 2005; Moreno et al., 2010; Dzamic et al., 2010). In the present study, the anti-inflammatory effects and chemical composition of essential oils from the leaves of *M. indica* were evaluated.

MATERIAL AND METHODS

Collection and material selection

The leaves of *M. indica* varieties espada and coração de boi were collected between November 2011 and May 2012 from two trees in the town of Campo Grande, Mato Grosso do Sul State, Brazil. The varieties espada (collected in location on the geographical coordinates 20°30'7"S and 54°37'17"W) and coracão de boi (20°30'13"S and 54°37'14"W) were identified by Dr. Ronaldo Posella Zaccaro (Centro Universitário Moura Lacerda, Ribeirão Preto, SP, Brazil).

Extraction

After collection, the samples were immediately subjected to the extraction of essential

oils. Oils from 200 g of fresh leaves were extracted via hydrodistillation in a modified Clevenger apparatus with 3 L water for 4 h. The oil was collected in a glass vial with a small amount of water and stocked in a freezer for use in chromatographic analysis. Consecutive extractions from 200 g leaves were performed to obtain the amount of oil sufficient for testing the anti-inflammatory activity.

Chromatographic analysis

The analyses of oil were performed with Varian GC-MS system comprising a CP-3900 gas chromatograph (Walnut Creek, CA, USA) and an ion-trap mass spectrometer (Saturn 2100-T/MS/MS). Chromatographic separation was performed in a silica capillary column with 5%-Phenyl-Arylene-95%-Dimethylpolysiloxane (ZB-5 MS) as the stationary phase. The column was 30 cm in length with an inner diameter of 0.25 mm and film thickness of 0.25 μm. The initial temperature of the furnace was 50°C, followed by heating from 50° to 250°C at 3°C/min. One microliter hexane solution was injected in split mode (1:20). The temperatures of the manifold, GC-MS interface, and ion trap were 50°, 250°, and 200°C, respectively. The MS scan parameters included electron-impact ionization voltage of 70 eV, mass range of 40-450 m/z, and scan interval of 0.5 s. Saturn GC/MS Workstation Version 5.52 software was used for instrument control and data treatment. The retention indices (RI) were calculated using the retention times of linear alkanes (C_9-C_{22}) and retention data of the essential oil components. The volatile compounds were identified by comparing their RI with those of the compounds described by Adams (2007) and their mass spectra with the data in NIST 2.1 and Saturn Libraries.

Animals

The experiments were conducted with male Wistar rats (150-200 g) and Swiss mice (20-30 g), housed (5 animals per cage) at 22° ± 2°C under a 12 h light/12 h dark cycle (lights on at 06:00). The rats were provided *ad libitum* access to food and water. One hour after the experiments, the animals were divided into groups (N = 6) and acclimatized to laboratory conditions. The experiments were carried out according to the protocol of the Institutional Ethics Committee and in accordance with the guidelines for the care of laboratory animals and ethical guidelines (Zimmermann, 1983).

Drugs

The following substances were used: lambda carrageenan type IV (Sigma-Aldrich Co., St. Louis, USA), dexamethasone (Sigma Co., St. Louis, USA), and essential oils from *M. indica* varieties 1 (EOMIL1) and 2 (EOMIL2). All drugs used in this study were dissolved in saline, except EOMIL1 and EOMIL2, which were dissolved in Tween 80 plus saline. The final concentration of Tween 80 (Sigma-Aldrich Co., St. Louis, MO, USA) did not exceed 10%, and it did not induce any pharmacological effect.

Measurement of paw edema induced by carrageenan

The test animals received 100 μL subcutaneous injection of saline solution (0.9%)

containing carrageenan (300 µg) into the right paw. The contralateral paw received 100 µL subcutaneous injection of saline (0.9%). The rats were pretreated (1 h before carrageenan) through the oral route [peroral (*po*)] with vehicle (solution of saline 0.9% with 10% Tween 80, control group), EOMIL1 and EOMIL2 at doses 100 and 300 mg/kg, and dexamethasone (1.0 mg/kg, subcutaneous route, positive control). The thickness of the rat paw (Sharma et al., 2004) was measured using a digital micrometer before induction of edema and 2 h after the injection of inflammatory agent.

Analysis of myeloperoxidase activity

To verify whether EOMIL1 and EOMIL2 can interfere with neutrophil migration induced by carrageenan, myeloperoxidase (MPO) activity was measured in the skin of the rat paw. Six hours after the carrageenan injection, the rats were euthanized in order to measure the MPO activity (De Young et al., 1989). A homogenate was prepared from each paw skin by homogenization of tissues in 5% (w/v) 80 mM phosphate buffer, pH 5.4, containing 0.5% hexadecyltrimethylammonium bromide. Next, it was centrifuged at 11.500 g for 20 min at 4°C. The supernatant of each sample (30 µL) was mixed with 100 µL 80 mM phosphate buffer, 85 µL 0.22 M phosphate buffer, and 15 µL 0.017% hydrogen peroxide on a 96-well plate. The reaction was triggered by the addition of 20 µL 3,3,3-tetramethylbenzidine (dissolved in N,N-dimethylformamide). The plate was kept at 37°C for 3 min, after which the reaction was stopped by adding 30 µL 1.46 M sodium acetate (pH 3.0. The enzymatic activity was determined by measuring the optical density at 630 nm, and it was expressed as mean optical density per mg of protein. All reagents were purchased from Sigma-Aldrich Co., St. Louis, USA.

Pleural cell migration and protein exudation

The mice were divided into six groups. The first group (naive group) received vehicle alone, without the carrageenan pleural injection. All others group received carrageenan 1 h after oral/subcutaneous treatment. The groups were formed as follows: the control group received vehicle alone; group 2 received 100 mg/kg EOMIL1; group 3: 300 mg/kg of EOMIL1; group 4: 100 mg/kg of EOMIL2; group 5: 300 mg/kg of EOMIL2; and group 6: 1.0 mg/kg dexamethasone (subcutaneously, positive control). Pleurisy was induced through an intrapleural injection of 100 µL 1% carrageenan as previously described (Velo et al., 1973). Carrageenan was diluted in saline buffer. Briefly, an adapted needle was inserted into the right side of the thoracic cavity of animals to enable intrapleural administration of carrageenan. The naive mice received an equal volume (100 µL) of sterile, pyrogen-free saline. After 4 h, the animals were killed, and the pleural cavity was washed with 1 mL phosphate-buffered saline. The exudate volume was measured. An aliquot of 20 µL exudate was diluted with Turck solution (1:20) and used for total leukocyte count in a Neubauer chamber. Protein exudation was evaluated using a Bradford assay kit (Bioagency, São Paulo, Brazil). The total cell count was measured under a light microscope, and the results were reported as the number of cells per mL of pleural fluid.

Statistical analysis

The results are reported as means ± SE (standard error of the mean) of experiments.

The statistical significance among the groups was assessed via one-way analysis of variance according to the Student-Newman-Keuls test. Comparisons between the control and vehicle groups were analyzed using the Student *t*-test. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The percentage yields (w/w) of essential oils obtained through the hydrodistillation of *M. indica* leaves were 0.01% for both varieties. In the two essential oils obtained from the two *M. indica* varieties, 23 volatile compounds were tentatively identified and characterized as monoterpenes and sesquiterpenes. The retention times in GC-MS were determined from three independent experiments showing a coefficient of variation less than 2%.

A comparison of the two essential oils revealed qualitative and quantitative differences in composition (Table 1). In the essential oil obtained from the variety espada, the major compounds were sesquiterpenes, such as β-selinene (34.90%), cyperene (22.40%), (E)-caryophyllene (16.39%), α-humulene (10.84%), terpinolene (2.31%), and α-selinene (2.31%). In the essential oil obtained from the variety coração de boi, the major compounds were cyperene (32.62%), (E)-caryophyllene (26.91%), α-humulene (17.12%), terpinolene (2.32%), β-selinene (5.70%), and myrcene (2.80%). These compounds were also reported to be the most important constituents in the leaves of the variety coquinho analyzed by GC-MS (Gebara et al., 2011).

Table 1. Percentage composition of volatile compounds of the leaves of *Mangifera indica*.

Compounds	KI[a]	KI[b]	Relative composition (%)	
			Espada	Coração de boi
α-thujene	931	931	0.11	1.71
Sabinense	974	976	-	0.13
myrcene	990	991	-	2.80
3-Hexen-1-olacetate	1006	1004	-	0.71
γ-carene	1011	1011	0.51	-
o-cimene	1020	1022	1.00	0.11
Limonene	1026	1031	0.10	1.41
β-phellandrene	1029	1031	0.12	2.70
Cisocimene	1035	1034	-	0.70
Terpinolene	1083	1086	2.32	-
p-cimenene	1089	1089	0.11	-
p-cimen-8-ol	1187	1183	0.11	-
δ-elemene	1332	1339	0.12	0.10
Ciclosativene	1366	1368	-	0.10
Isoledene	1370	1373	0.12	0.10
β-elemene	1385	1391	1.43	0.51
Cyperene	1402	1398	22.40	32.62
α-gurjunene	1407	1409	0.52	0.72
(E)-Caryophyllene	1414	1418	16.39	26.91
α-humulene	1450	1454	10.84	17.12
Allo-aromadendrene	1454	1461	1.73	2.03
Drima-7.9(11)diene	1465	1469	2.11	-
γ-gurjunene	1467	1473	0.71	1.71
γ-murolene	1476	1477	0.71	0.40
β-Selinene	1485	1485	34.90	5.70
Viridiflorene	1492	1493	-	1.10
α-selinene	1494	1494	2.31	-
γ-cadinene	1514	1513	1.21	0.50
caryophylleno oxide	1576	1581	0.80	0.30

KI[a]: Retention index calculated. KI[b]: Retention index literature from Adams, 2007. (-): not identified. Espada = essential oil of mango variety espada. Coração de boi = essential oil of mango variety coração de boi.

Some studies show that the main compounds of the essential oils obtained from mango leaves in countries, such as Brazil, Nigeria, China, and Colombia, are sesquiterpenes [e.g., (E)-caryophyllene, caryophyllene oxide, δ-carene, α-gurjunene, β-selinene, and humulene epoxide] and monoterpenes (e.g., β-pinene, α-pinene, limonene, myrcene, cis- and trans ocimene) (Pino et al., 20005; Moreno et al., 2010; Dzamic et al., 2010). These results corroborate with the data on the composition of *M. indica* leaves in the espada and coração de boi varieties.

Although this plant has been used in the treatment of various inflammatory diseases, the pharmacological importance of these essential oils has not yet been scientifically demonstrated.

Several pharmaceutical products currently used to treat inflammation are not completely efficient in chronic diseases, and they produce several side effects. Therefore, it is necessary to develop more effective and less toxic agents (Hoff Brait et al., 2015). The identification of compounds capable of interacting with molecular targets responsible for the amplification of inflammatory processes is a very interesting field of research.

The results depicted in Figure 1A (100 mg/kg) and Figure 1B (300 mg/kg) show that EOMIL1 or EOMIL2 significantly reduced edema formation at oral doses of 100 and 300 mg/kg. In the carrageenan-induced edema model, at a dose of 100 mg/kg, 54 ± 9% inhibition of edema for EOMIL1 and 37 ± 7% inhibition for EOMIL2 were observed. However, for the positive control in paw edema model, dexamethasone (1 mg/kg, subcutaneous route) showed 75 ± 4% inhibition of edema (Figure 1A).

At 300 mg/kg, maximal inhibition, with 62 ± 8% inhibition of edema, was observed for EOMIL1, while the inhibition for EOMIL2 was 51 ± 7% (Figure 1B).

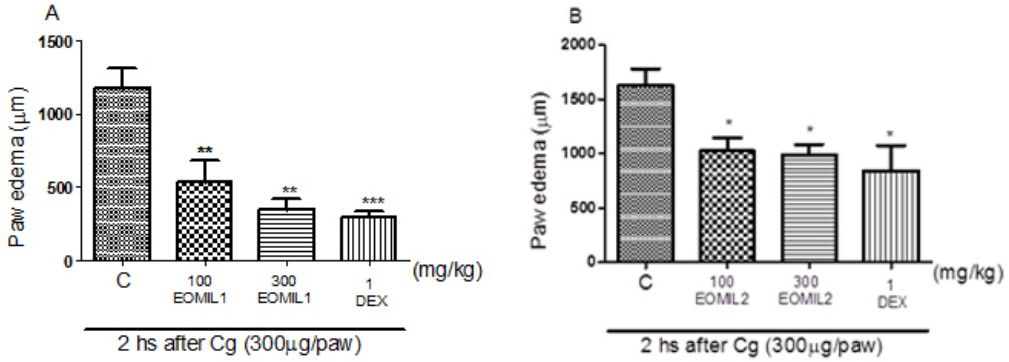

Figure 1. Effect of essential oil of *Mangifera indica* 1 (EOMIL1) and 2 (EOMIL2) at doses of 100 (**A**) and 300 (**B**) mg/kg administered orally on carrageenan-induced paw edema in rats. The column represent the two doses inhibition of edema at the time of 120 min after carrageenan injection, which mean of 6 animals and vertical lines show the SE. Asterisks denote the significance levels when compared with control values (carrageenan): *P < 0.05, **P < 0.01 and ***P < 0.001.

In Figure 2A (100 mg/kg) and 2B (300 mg/kg), it is shown that oral treatment with essential oils from *M. indica* varieties significantly reduced the increase in MPO activity. EOMIL1 and EOMIL2 significantly reduced the MPO activity at doses of 100 mg/kg (47 ± 5 and 23 ± 8%, respectively) and 300 mg/kg (50 ± 9 and 31 ± 7%, respectively). For dexamethasone, the inhibition of MPO activity was found to be 83 ± 5% (Figure 2A and B).

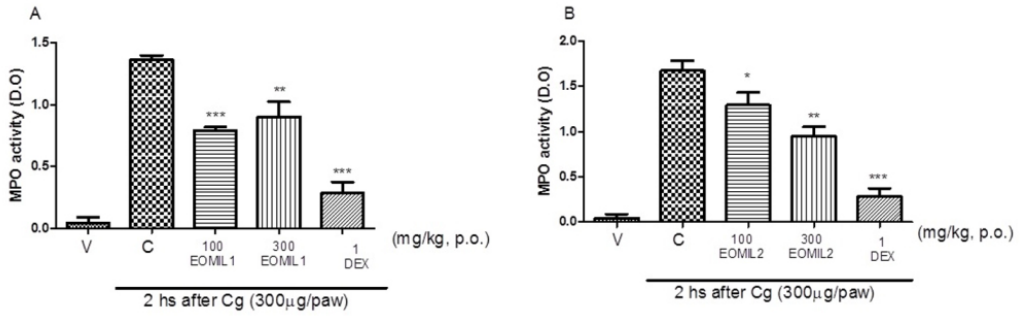

Figure 2. Effect of essential oil of *Mangifera indica* 1 (EOMIL1) and 2 (EOMIL2) at doses of 100 (**A**) and 300 (**B**) mg/kg administered orally on carrageenan-induced increase in MPO activity in rats. The column represent the two doses inhibition of edema at the time of 360 min after carrageenan injection, which mean of 6 animals and vertical lines show the SEM. The comparison of with control versus vehicle group were analyzed by the *t*-test and the symbol # denoted the statistical differences. Differences between EOMIL1,d EOMIL2 and DEXA treated groups versus control were analyzed by analysis of variance (one-way ANOVA) followed by the Newman-Keuls test and asterisks denote statistic values: *P < 0.05, **P < 0.01 and ***P < 0.001.

In Figure 3A and B, it is shown that the oral administration of EOMIL1 and EOMIL2 decreased cell migration. Leukocyte migration was assessed in the pleurisy model, and inhibitions of 39 ± 6 and 72 ± 7% were observed at 100 and 300 mg/kg of EOMIL1, respectively, while inhibitions of 91 ± 1 and 64 ± 2% were observed at 100 and 300 mg/kg of EOMIL2, respectively. However, in the evaluation of plasma extravasation, only EOMIL2 at 100 mg/kg caused significant reduction (Figure 3).

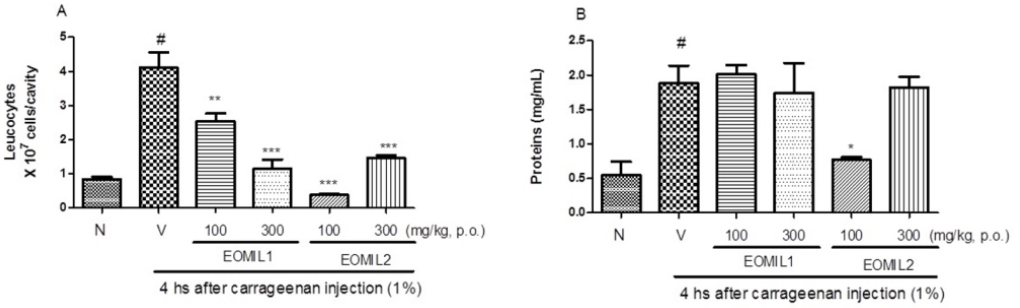

Figure 3. Effects of essential oil of *Mangifera indica* 1 (EOMIL1) and 2 (EOMIL2) at dose of 100 (**A**) and 300 (**B**) mg/kg on total leukocytes (**A**) and protein extravasation (**B**) induced by carrageenan in the pleural cavity of mice. Animal received the oral treatment with EOMIL1 or EOMIL2 (100 or 300 mg/kg), or vehicle, and after 1 h they received an intrapleural injection of Cg (100 µL of a 1% solution/cavity). Control animals received only the vehicles. Animals were killed after Cg injection. The bars express the mean ± SEM of 6 animals. The comparison of with Naive versus vehicle treated (control) group were analyzed by test t and the symbol # denoted the statistical differences. Statistical differences between vehicle (V, Control group) versus treated group were denote by asterisks: **P < 0.01 and ***P < 0.001, one-way ANOVA followed by Student-Newman-Keuls.

These results suggest that the *M. indica* varieties espada and coração-de-boi show anti-edematogenic action against the inflammatory agent carrageenan, in addition to reducing MPO activity in the experimental model of inflammation. However, more in-depth studies are

required to identify the underlying mechanisms of the bioactive compounds and understand their action. These results may be correlated with the presence of major compounds, such as caryophyllene and α-humulene. Studies evaluating the anti-inflammatory properties of α-humulene and caryophyllene, isolated from *Cordia verbenacea* essential oil, showed that oral treatment with these compounds exerted inhibitory effects in different inflammatory experimental models in mice and rats. This indicates that these compounds represent important tools for the management or treatment or both of inflammatory diseases (Fernandes et al., 2007).The synergistic effect of chemical compounds of the essential oils should also be taken into consideration for the anti-inflammatory effects.

Although the mechanism of action of terpenes is not fully understood, it is thought to involve membrane disruption by lipophilic compounds (Cowan, 1999). The results of this study may justify the use of *M. indica* leaves as an anti-inflammatory agent in folk medicine.

Conflicts of interest

The authors declare no conflict of interest.

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