Gastroprotective effect of the essential oil of Myrcia loranthifolia (Myrtaceae) on acute ethanol-induced gastric lesions in mice and possible mechanisms of action

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Research Article

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Abstract

In this work, we investigated the chemical composition and gastroprotective potential of the essential oil from leaves of *Myrcia loranthifolia* collected in Atlantic Forest and Seasonally Dry Forest in Brazil (= Caatinga). The essential oil was extracted by hydrodistillation and characterized GC–MS. Gastroprotection was evaluated in an absolute ethanol-induced gastric ulcer model in mice. To elucidate the gastroprotective mechanisms, the roles of prostaglandins, K_{ATP} channels, and calcium channels were investigated. The essential oil extracted from the leaves of *M. loranthifolia*, collected in the Atlantic Forest, was found to contain (*E*)–caryophyllene (47.54%), *α*–humulene (9.22%), and germacrene D (8.94%) as the primary constituents. In turn, the oil from samples of the Caatinga presented (*E*)–caryophyllene (17.68%), *trans*–calamenene (12.44%), germacrene D (10.38%), *α*–humulene (10.19%), and bicyclogermacrene (9.11%) as predominant constituents. *M. loranthifolia* essential oil at doses of 50, 100, and 200 mg/kg significantly reduced the severity of gastric lesions, but differences were found in the gastroprotective potential according to the geographical origin of the oil. Our findings suggest that the essential oil extracted from *M. loranthifolia* leaves may offer partial protection to the gastric mucosa through the activation of prostaglandins.

Introduction

Gastritis is an inflammatory disease that can lead to morbidity or even mortality when untreated (Klopell et al. 2007; Laine et al. 2008; Carlotto et al. 2019). According to Fahmy et al. (2020), about 4.6 million people are diagnosed with gastric ulcers annually, making gastritis a global public health problem.

The development of ulcers results from an imbalance between the body's natural protective factors (e.g., mucosal layer, mucosal blood flow, cell regeneration, prostaglandins, and epidermal growth factors) and aggressive agents of the stomach, such as *Helicobacter pylori* infection, acid-pepsin secretion, excess HCl, among others (Lu and Graham 2006; Klein et al. 2010). The available treatments, which include antacids, prostaglandins, muscarinic antagonists, histamine-2 receptor antagonists (H2RAs), and proton pump inhibitors, cause adverse effects and may even induce the development of gastric cancer (Babu et al. 2009; Lakshmi et al. 2010; Gupta et al. 2021). For this reason, there is a constant search for more effective and safer therapies with fewer side effects.

Myrtaceae is one of the most economically important botanical families, being the eighth richest family in the Northeast region of Brazil (Sobral and Proença 2006). Myrtaceae species are rich in essential oils and some of them have shown gastroprotective potential (Santin et al. 2011; Batiha et al. 2020; de Paulo Farias et al. 2020; Cilingir-Kaya and Gürler 2021; Hobani et al. 2022; Mansour et al. 2022; Neves et al. 2022). Among its genera, *Myrcia* DC., a heterotypic synonym of *Calyptranthes* Sw., has been reported to occur in various phytogeographic domains, including the Brazilian Atlantic Forest and the Brazilian Dry Forest (Santos et al. 2023). Some species of this genus have already had the chemical composition of their essential oils and their bioactive potential described (de Cerqueira et al. 2007; dos Santos et al. 2014; Ferreira et al. 2021; de Moraes et al. 2022).

Our study focuses on *Myrcia loranthifolia* (DC.) G.P.Burton & E.Lucas [synonym of *Calyptranthes dardanoi* Mattos]. Due to the evidenced potential of the essential oils of representatives of Myrtaceae for the treatment of gastric ulcer, our hypothesis is that the geographic distribution of *M. loranthifolia* plants influences the content and composition of their essential oil, which could have implications for their potential gastroprotective effects, an area of investigation that has not been explored previously.

Material and methods

Plant material

Myrcia loranthifolia was collected at Usina São José (7°49'55.8" S 35°00'21.2" O), an area of Atlantic Forest located in the municipality of Igarassu, state of Pernambuco (Northeastern Brazil). Other specimens were collected in the Vale do Catimbau National Park (8°35'05.6"S, 37°14'34.1"W), an area of Caatinga in the municipality of Buíque, also in the state of Pernambuco. Representatives of the species were deposited in the Dárdano de Andrade Lima Herbarium – IPA (Agronomic Institute of Pernambuco) under numbers 93554 and 92921, respectively.

Extraction of essential oil

After collection, the leaves were dried at room temperature and the oil was extracted by hydrodistillation in a Clevenger apparatus using 100 g of leaves and 500 mL of distilled water. The extractive procedure lasted four hours (Pereira et al. 2011). The resulting oil was weighed and stored at -4°C in amber flasks. The yield of the essential oil was expressed as percentage (%) and calculated as the volume (mL) of oil obtained per dry mass (g) of leaves (Santos et al. 2014).

Identification and quantification of oil constituents

For the qualitative analysis, 1 mg of the essential oil of plants from each locality was diluted in 1 mL of *n*-hexane and 1 µL of the solution was injected in a split mode of 1:50 into a gas chromatograph coupled to an Agilent 5975C GC–MS series mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent J&W HP–5 nonpolar column (60 m × 0.25 mm x 0.25 µm). The initial oven temperature was set at 40°C for 2 min, increased 4°C/min up to 230°C, and held at this temperature for 5 min. The detector was set to scan the range m/z 35–350 at a rate of 1.0 scans/s. Spectra were obtained in EI mode at 70 eV. The identification of the oil components followed da Silva Barbosa et al. (2020). Quantitative analyses were performed by gas chromatography with flame ionization detection (GC–FID) on a Thermo Trace GC (Thermo Scientific, Waltham, MA, USA). A VB–5 nonpolar column (60 m × 0.25 mm x 0.25 µm, ValcoBond®, Valco Instruments Company Inc., Houston, TX, USA). The temperature programming was as described above. The injector and detector temperatures were set at 250°C and 280°C, respectively (da Silva Barbosa et al. 2020).

Animals

Male Swiss mice (25–30 g) obtained from the vivarium of the Keizo Asami Immunopathology Laboratory - LIKA of the Federal University of Pernambuco (Recife, Pernambuco, Brazil) were used in the experiments. Mice were maintained at 22 ± 2 ºC under a 12-hour light/dark cycle and had free access to standard granulated diet and water. Before experimentation, the animals were deprived of food for 18 hours, but had free access to water.

Ethanol-induced gastric lesions

Gastric mucosal hemorrhagic lesions in mice were induced by intragastric instillation of absolute ethanol (Robert et al. 1979). Animals were pretreated with vehicle (saline, 0.1 mL/100 g, p.o.), omeprazole (40 mg/kg, p.o.), and *M. loranthifolia* essential oils (200, 100 and 50 mg/kg, p.o.). After 60 min of pretreatment, 0.5 mL/200 g of absolute ethanol was administered orally. One hour after ethanol administration, the animals were euthanized, the stomachs removed and opened along the smaller curvature for analysis of gastric lesions. The lesions areas of each stomach were calculated using the ImageJ® program Version 1.8 (National Institutes of Health, USA), added and expressed as percentage (%) of the total gastric area.

Ethanol-induced gastric lesions in mice pretreated with indomethacin, glibenclamide and nifedipine

In order to elucidate the possible pharmacological mechanisms involved in the gastroprotective effect of the essential oils, groups of mice (n = 6) were treated with indomethacin, a prostaglandin blocker (20 mg/kg, i.p.), glibenclamide, a K_{ATP} channel blocker (5 mg/kg, i.p.), and nifedipine, a calcium channel blocker (5 mg/kg, i.p.), 30 min prior to administration of a single dose of the essential oils at 200 mg/kg, p.o. One hour later, each animal received orally 0.5 mL/200 g ethanol. After ethanol administration, the animals were euthanized, and the stomachs removed for analysis of gastric lesions (Morimoto et al. 1991).

Statistical analysis

The results for the bioassays were expressed as mean ± standard deviation (n = 6). Differences between treatments were analyzed using analysis of variance (ANOVA) followed by the Tukey's test ($P < 0.05$). These statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). We also performed a PCA (Principal Component Analysis) to better describe how the oil composition varies across each location. The PCA was performed using the XLSTAT software, trial version 2023.1.4 (Addinsoft, Paris, France).

Results

Chemical composition of the essential oil

The yield of the essential oil from *M. loranthifolia* leaves collected in Brazilian Atlantic Forest (BAF) and Brazilian Dry Forest (BDF) was 0.23% and 0.33%, respectively. We identified 25 compounds in the

essential oil of *M. loranthifolia* from BAF and 37 in the essential oil of plants from BDF, totaling 95.15% and 93.26% of the oil composition identified, respectively (Table 1).

Table 1
Chemical composition of the essential oil from the leaves of *Myrcia loranthifolia* growing in the Brazilian Atlantic Forest (BAF) and Brazilian Dry Forest (BDF).

Locality/Composition (%)			**Retention index (RI)**	
Constituents	BAF	BDF	Calculated[a]	Reported[b]
α–Pinene	1.21 ± 0.04	1.38 ± 0.03	932	930
β–Pinene	0.20 ± 0.01	0.39 ± 0.07	974	972
Myrcene	-	0.07 ± 0.04	988	988
ρ–Cymene	0.08 ± 0.01	0.07 ± 0.04	1020	1021
Limonene	-	0.58 ± 0.07	1024	1025
1,8–Cineole	0.69 ± 0.01	1.36 ± 0.14	1026	1027
γ–Terpinene	-	0.05 ± 0.02	1054	1056
δ–Elemene	0.13 ± 0.01	0.73 ± 0.05	1335	1335
α–Cubebene	0.99 ± 0.02	1.58 ± 0.01	1348	1347
α–Ylangene	-	0.2 ± 0.14	1373	1369
α–Copaene	2.63 ± 0.11	2.91 ± 0.11	1374	1373
β–Bourbonene	2.25 ± 0.05	1.37 ± 0.11	1387	1382
β–Elemene	5.46 ± 0.24	4.92 ± 0.5	1389	1390
(*E*)–Caryophyllene	**47.54 ± 0.37**	**17.68 ± 0.57**	1417	1421
β–Copaene	0.82 ± 0.01	1.11 ± 0.62	1430	1427
Aromadendrene	0.15 ± 0.06	0.81 ± 0.43	1439	1436
α–Humulene	**9.22 ± 0.18**	**10.19 ± 0.47**	1452	1452
Allo–aromadendrene	0.23 ± 0.03	1.82 ± 0.28	1458	1459
trans–Cadina-1(6),4–diene	-	0.7 ± 0.7	1475	1472
γ–Muurolene	-	0.91 ± 0.91	1478	1475
Germacrene D	**8.94 ± 1.61**	**10.38 ± 0.23**	1481	1480
β–selinene	-	1.86 ± 0.87	1489	1485

[a] Mean value of the Retention Index, [b] Retention Index obtained from Adams (2009), % = area of the compound in relation to the total area of the chromatogram. Bold numbers represent the major constituents of the oil. The values are means of three determinations ± standard deviation. - = compound not detected (concentration < 0.01%).

Locality/Composition (%)			Retention index (RI)	
trans–Muurola–4(14),5–diene	-	0.7 ± 0.14	1493	1490
Bicyclogermacrene	3.40 ± 0.84	**9.11 ± 0.73**	1500	1494
α–Muurolene	-	0.58 ± 0.02	1500	1498
Germacrene A	0.93 ± 0.31	2.00 ± 0.05	1508	1503
γ–Cadinene	0.55 ± 0.05	0.53 ± 0.04	1513	1512
trans–calamenene	-	**12.44 ± 1.46**	1521	1523
δ–Cadinene	3.79 ± 0.01	1.75 ± 0.27	1522	1531
trans–Cadina–1,4–diene	0.21 ± 0.06	-	1533	1530
α–Cadinene	0.15 ± 0.01	0.19 ± 0.05	1537	1535
α–Calacorene	0.07 ± 0.01	0.12 ± 0.03	1544	1541
Germacrene B	-	0.42 ± 0.25	1559	1555
Spathulenol	-	2.29 ± 0.42	1577	1577
Caryophyllene oxide	2.88 ± 0.13	-	1582	1582
Viridiflorol	-	0.27 ± 0.06	1592	1590
Ledol	-	0.56 ± 0.04	1602	1601
Humulene epoxide II	-	0.48 ± 0.12	1608	1608
α–Muurolol	1.42 ± 0.45	-	1644	1644
α–Cadinol	1.21 ± 0.25	0.75 ± 0.29	1652	1653
Total	95.15%	93.26		
Monoterpene hydrocarbons	1.49	2.54		
Oxygenated monoterpenoids	0.69	1.36		
Sesquiterpene hydrocarbons	87.46	85.01		
Oxygenated sesquiterpenoids	5.51	4.35		
Others	4.85	6.74		

[a] Mean value of the Retention Index, [b] Retention Index obtained from Adams (2009), % = area of the compound in relation to the total area of the chromatogram. Bold numbers represent the major constituents of the oil. The values are means of three determinations ± standard deviation. - = compound not detected (concentration < 0.01%).

The essential oil extracted from *M. loranthifolia* leaves collected from BAF was found to be primarily composed of (*E*)–caryophyllene (47.54%), *α*–humulene (9.22%), and germacrene D (8.94%). In contrast, the essential oil extracted from *M. loranthifolia* leaves collected from BDF had (*E*) –caryophyllene (17.68%), *trans*–calamenene (12.44%), germacrene D (10.38%), *α*–humulene (10.19%), and bicyclogermacrene (9.11%) as the main constituents. Sesquiterpene hydrocarbons were identified as the main class of compounds in the essential oil of *M. loranthifolia* collected from both BAF (87.46%) and BDF (85.01%), as shown in Table 1. The chromatograms are displayed as supplementary information.

Figure 1 displays the outcome of the PCA analysis, illustrating the relationship between the essential oil composition and each respective locality. The PCA analysis revealed that 99.9% of the data variance was explained by two main components, PC1 and PC2, which accounted for 87.6% and 12.3% of the variance, respectively. According to the rotated matrix component (Varimax method), the PC1 axis had (*E*)-caryophyllene (7.00), germacrene D (2.07), *α*-humulene (2.06), *trans*-calamenene (1.60), and bicyclogermacrene (1.32) as the main variables and their corresponding factor scores. The PC2 axis showed significant influence from *trans*-calamenene (-2.05), (*E*)-caryophyllene (1.45), bicyclogermacrene (-1.13), germacrene D (-0.84), and *α*-humulene (-0.78). According to the PCA analysis, the quantitative distribution of the essential oil is highly influenced by local climatic factors and/or genetic components of the plants.

Ethanol-induced gastric lesions

The effect of *M. loranthifolia* essential oil from BAF and BDF orally administered on absolute ethanol-induced gastric lesions is shown in Fig. 2. Animals that received absolute ethanol presented lesions compromising 88% of the total gastric area. Ethanol-induced gastric damage in the groups of animals pretreated with 50, 100 and 200 mg/kg of *M. loranthifolia* essential oil from BAF was significantly reduced by 42, 60 and 77%, respectively. The gastric lesion area in the group treated with *M. loranthifolia* essential oil from BDF showed a slightly lower but still significant reduction of 20.7, 39.9 and 57.1%, respectively. The treatment with 200 mg/kg of *M. loranthifolia* essential oil from BAF showed similar results to treatment with the drug omeprazole at 50 mg/kg (Fig. 2).

The antiulcerogenic effect of *M. loranthifolia* essential oil in the ethanol-induced ulcer model was visualized through macroscopic analyses. The essential oil reduced inflammatory cell infiltration in a dose-dependent manner. Our findings showed that the administration of 200 mg/kg of *M. loranthifolia* essential oil from BAF and BDF significantly improved the structure of gastric tissue, especially in the case of the essential oil from BAF (Fig. 3).

Ethanol-induced gastric lesions in mice pretreated with indomethacin, glibenclamide and nifedipine

Figure 4 illustrates the gastroprotective potential of *M. loranthifolia* essential oil when animals were pre-treated orally with indomethacin, glibenclamide and nifedipine. Treatment with indomethacin (20 mg/kg)

significantly blocked the gastroprotective effect of the essential oil of *M. loranthifolia* plants from BAF and BDF, indicating the possible role of prostaglandins in gastroprotection. On the other hand, pretreatment with glibenclamide and nifedipine at 5 mg/kg did not influence the gastroprotection produced by *M. loranthifolia* essential oil, suggesting that K_{ATP} channels and calcium channels, respectively, play no role in the gastroprotection induced by the essential oil of the species. The effects of the analyzed blockers on the gastroprotective potential of *M. loranthifolia* essential oil can be observed in the photomicrographs shown in Fig. 5.

Discussion

This study aimed to investigate the chemical composition of the essential oils of *M. loranthifolia* and their potential gastroprotective effects against acute ethanol-induced gastric lesions in mice. Furthermore, we investigated the potential mechanisms of action of the essential oils.

According to the literature, various species of *Myrcia* have been found to contain different major constituents in their essential oils, such as (*E*)–nerolidol (92.21%) in *M. multiflora* (Ferreira et al. 2021), *α*–bisabolol (83.8%) in *M. fallax* (Henriques et al. 1997), (*Z*)–*α*–bisabolene (79.65%), *trans*-nerolidol (67.81%), and (*E*)–caryophyllene (45.8%) in *M. splendens* (Nakamura et al. 2010; Scalvenzi et al. 2017; Jerônimo et al. 2021), *α*–pinene (67.81%) in *M. myrtifolia* (de Cerqueira et al. 2007), caryophyllene oxide (22.2%) in *M. pubiflora* (Andrade et al. 2012), (2*E*, 6*E*)–methyl farnesoate (48.30%), spatulenol (40.7%), and *γ*–muurolene (40.16%) in *M. tomentosa* (Borges et al. 2013; Franco et al. 2021), myrcene (48.1%) and germacrene D in *M. cuprea* (Zoghbi et al. 2003), nerolic acid (35.6%) in *M. lundiana* (Alves et al. 2016), (*E*)–caryophyllene (45.0%), spatulenol (40.2%), and *cis*–calamenene (30.1%) in *M. sylvatica* (Zogbi et al. 2003; Rosa et al. 2016), and *trans*–calameneno (29,3%) in *M. obtecta* (Stefanello et al. 2010).

Our findings show that (*E*)-caryophyllene predominated among the identified constituents in the essential oil of *M. loranthifolia*. However, depending on the geographical origin of the plants, quantitative differences were found, especially in relation to the content of (*E*)-caryophyllene. The PCA clearly showed that the quantitative composition of the oil differed between localities. Consequently, pharmacological findings also differed according to the origin of the essential oil from *M. loranthifolia*. Our initial hypothesis, that the plant origin influences the chemical composition of the oil, which in turn affects its pharmacological activity, was therefore corroborated in this study.

In comparison, the content of (*E*)–caryophyllene in *M. loranthifolia* samples from BAF was 47.5%, which is higher than the content reported by Jerônimo et al. (2021) for *M. splendens* and by Rosa et al. (2016) for *M. sylvatica*. The high content of this sesquiterpene in the essential oil of *M. loranthifolia* makes this species a potential source of bioactive compounds still pharmacologically under-exploited. The essential oil content of *M. loranthifolia* (ranging from 0.23 to 0.33%) falls within the range previously reported for other species of *Myrcia* (see references above).

According to the literature, *β*–caryophyllene has important pharmacological activities, including anticancer, cardioprotective, hepatoprotective, nephroprotective, antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, neuroprotective, and, the target of the present study, gastroprotective activity (Diniz et al. 2013; Cho et al. 2015; Fidyt et al. 2016; Sharma et al. 2016; Francomano et al. 2019; Li et al. 2020; Hashiesh et al. 2021). *β*–Caryophyllene is also a selective CB2 receptor agonist with antinociceptive potential, but without the psychotic effects produced by substances that activate CB1 receptors (Gertsch et al. 2008; Klauke et al. 2014; Jha et al. 2021). Thus, species rich in this sesquiterpene, such as *M. loranthifolia*, are very interesting for pharmacological tests related to the phytocannabinoid system.

trans–Calamenene was the second major constituent identified in the essential oil of *M. loranthifolia* from BDF, not being detected in the samples collected from BAF, whose oil demonstrated greater gastroprotective activity. According to the literature consulted, no gastroprotective activity has been described for *trans*-calamenene. Therefore, we believe that the gastroprotective activity of the essential oil of *M. loranthifolia* from BAF may also be due to the presence of *β*–caryophyllene. Similarly, we did not find studies regarding the gastroprotective activity of germacrene D, whose occurrence in the oil of *M. loranthifolia* ranged from 8 to 10%.

On the other hand, *α*–humulene, also known as *α*–caryophyllene, has demonstrated protective efficacy against HCl/ethanol-induced gastritis through various mechanisms, including histamine, NF–κB, and mucus regulation (Yeo et al. 2021). *α*-Humulene is commonly found together with *β*–caryophyllene in essential oils from various plant sources (Di Sotto et al. 2020). In the present study, *α*–humulene and *β*–caryophyllene were identified together in the essential oil of *M. loranthifolia* from both locations, corroborating Di Sotto et al. (2020). The gastroprotective effect of *M. loranthifolia* essential oil may be due to the joint action of these two constituents.

Our findings showed that the gastroprotective action of the essential oil from *M. loranthifolia* was significantly reduced with the administration of indomethacin, a non-selective cyclooxygenase inhibitor, indicating the important role of endogenous prostaglandins in the mechanism of gastroprotection (Peskar et al. 2002). On the other hand, no reversal of the gastroprotective effect of *M. loranthifolia* essential oil was seen with the administration of glibenclamide and nifedipine. According to some authors, K_{ATP} and calcium channels play an important role in protecting the gastric mucosa (Ulak et al. 1991; Peskar et al. 2002). Therefore, the gastroprotective effect of *M. loranthifolia* essential oil observed in this study probably does not depend on these pathways. On the other hand, the treatment with indomethacin effectively inhibited the gastroprotective effect of the essential oil of *M. loranthifolia*, indicating a potential role of prostaglandins in gastroprotection.

Conclusion

Samples of *M. loranthifolia* leaves collected in two Brazilian phytogeographic domains had different essential oil content and peculiar chemical composition. Our findings showed different gastroprotective

activity of *M. loranthifolia* essential oil from the two collection areas, which points to the influence of environmental and biotic factors in the biosynthesis and composition of the oil. The essential oil of *M. loranthifolia* is an interesting source of (*E*)–caryophyllene, notably when obtained from individuals of Brazilian Atlantic Forest, becoming promising for pharmacological purposes. The gastroprotection of *M. loranthifolia* essential oil in the ethanol-induced ulcer model with mice may involve the activation of prostaglandins with participation of (*E*)–caryophyllene and *α*–humulene. Further research on species that are rich in these compounds is necessary to gain a better understanding of their potential for gastroprotection.

Declarations

Supplementary Information The online version contains supplementary material available at

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Author contributions RHGS: Term, Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft. JRSO: Formal analysis, Investigation. JCROFA: Formal analysis, Investigation. VLML: Validation, Formal analysis, Investigation, Resources, Data Curation. DMAFN: Validation, Formal analysis, Investigation, Resources, Data Curation. AFMO: Term, Conceptualization, Resources, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

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Data availability All data generated or analyzed during this study are included in this article.

Conflict of interest The authors declare no conflict of interest.

Ethical approval All animal care and experimental procedures were conducted in accordance with the ethical standards established by the National Guidelines for the Use of Experimental Animals of Brazil and the protocols were approved by the Committee for the Ethical Use of Animals of the Federal University of Pernambuco (number 102/2021 CEUA-UFPE).

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Figures

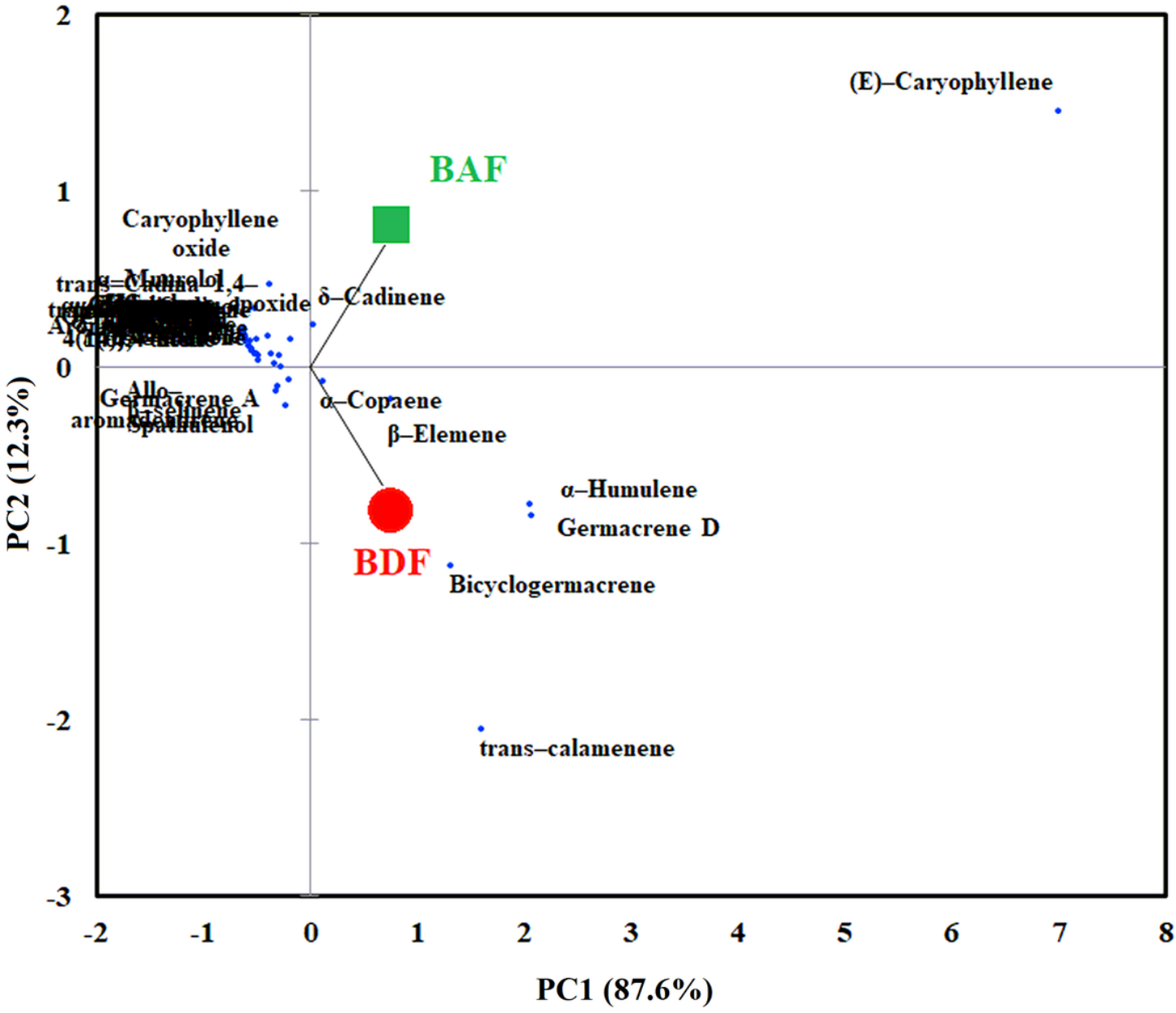

Figure 1

Biplot (score and loading plots) resulting from the principal component analysis (PCA) of the essential oil composition of *Myrcia loranthifolia* leaves collected from the Brazilian Atlantic Forest (BAF) and the Brazilian Dry Forest (BDF). The PCA was performed based on the analysis of the GC–MS profile, which is detailed in Table 1.

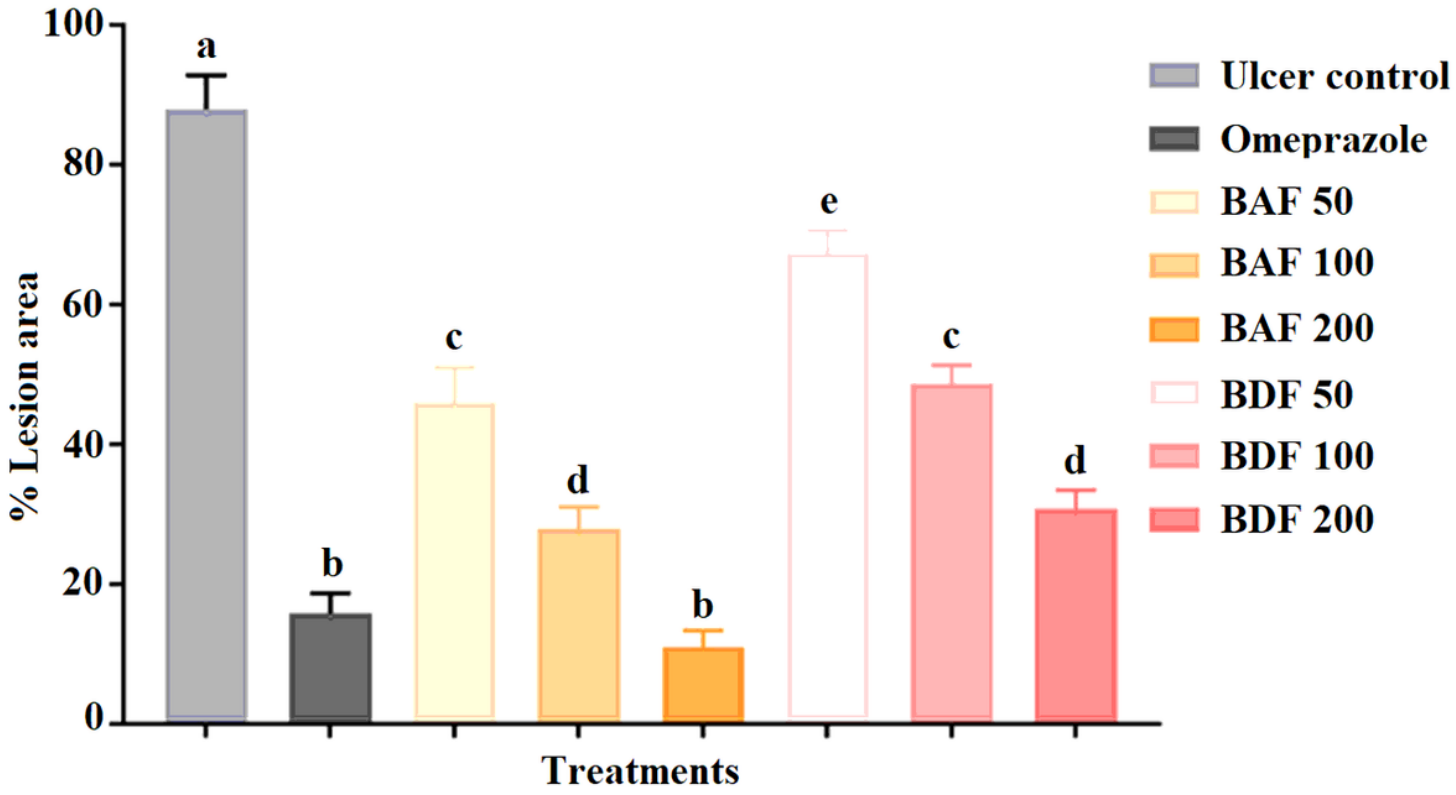

Figure 2

Gastroprotective effect of *Myrcia loranthifolia* essential oil at 50, 100 and 200 mg/kg on absolute ethanol-induced lesions in mice. The results are means ± standard deviation (n = 6). Bars that have the same letter do not significantly differ by Tukey's test ($P < 0.05$). BAF and BDF = Essential oil from Brazilian Atlantic Forest and Brazilian Dry Forest, respectively.

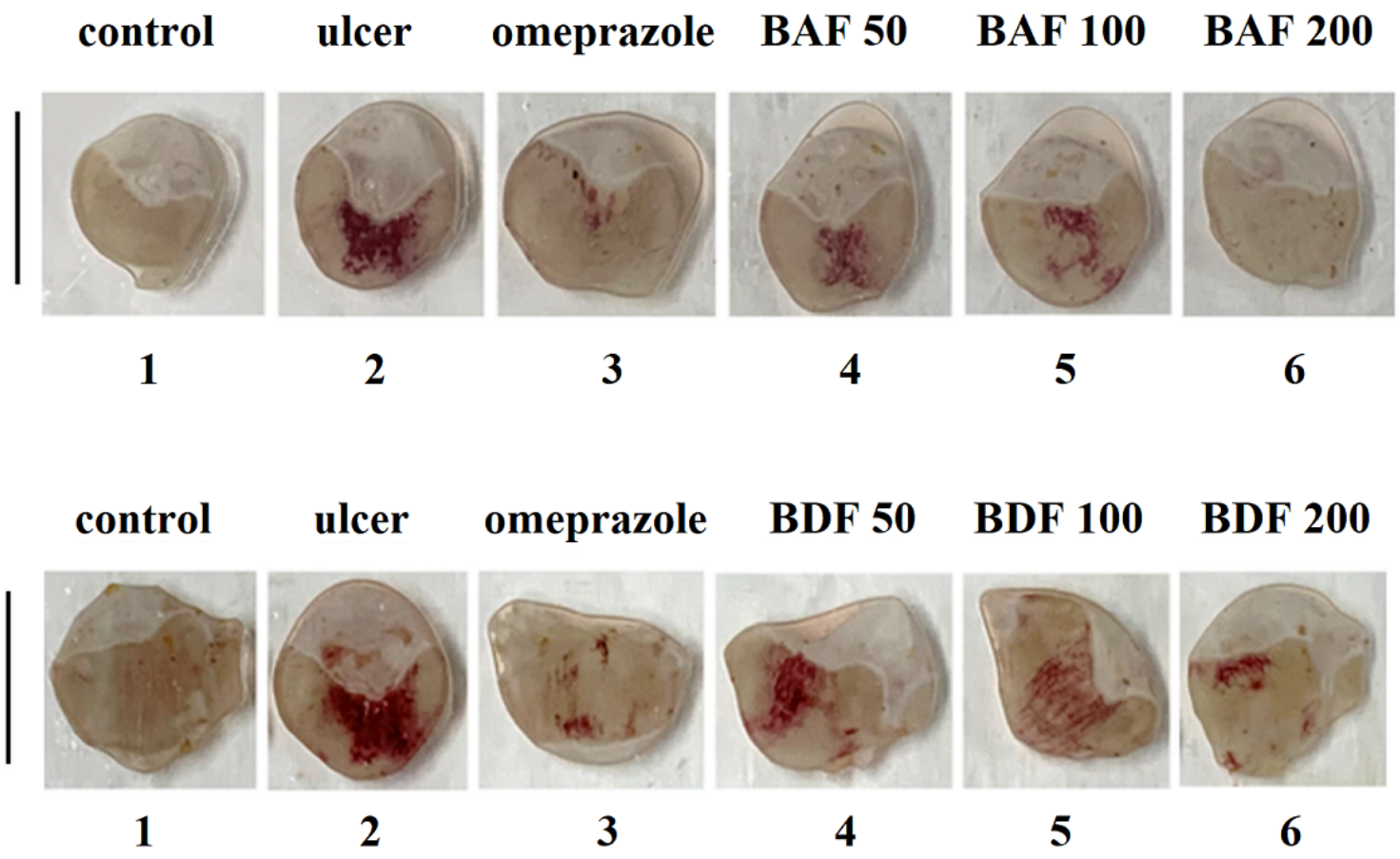

Figure 3

Photomicrographs showing the gastric mucosal surface of mice treated with *Myrcia loranthifolia* essential oil: 1 – stomach of control animal, 2 – stomach of animal treated orally with 0.5 mL/200 g of absolute ethanol, 3 – stomach of animal treated orally with 50 mg/kg of omeprazole, 4–6) stomachs of animals treated orally with 50, 100 and 200 mg/kg of essential oil. Reddened areas indicate tissue injury. BAF and BDF = Essential oil from Brazilian Atlantic Forest and Brazilian Dry Forest, respectively. Bar = 3 cm.

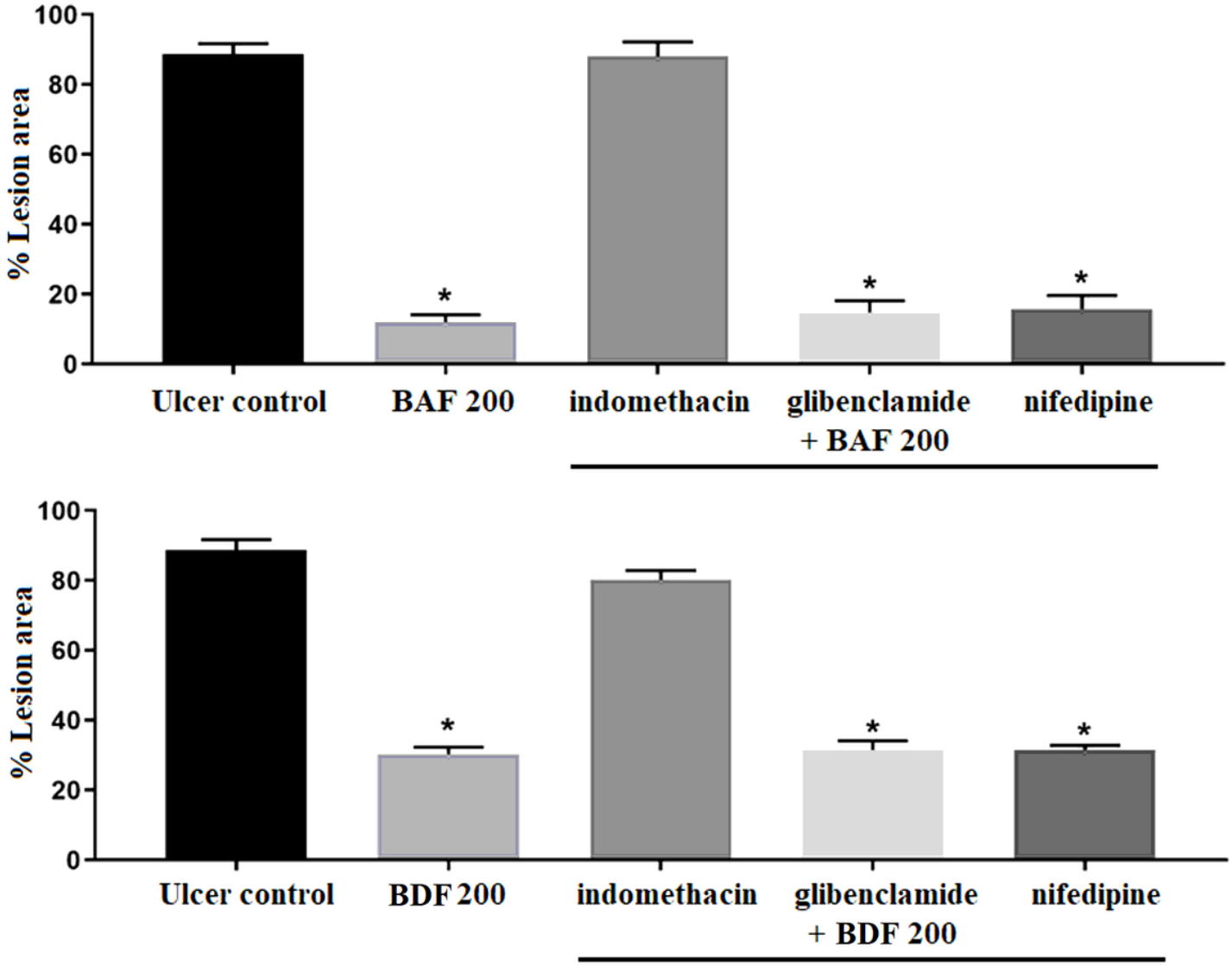

Figure 4

Effect of oral pretreatment with indomethacin (20 mg/kg), gliblenclamide (5 mg/kg) and nifedipine (5 mg/kg) on the gastroprotective potential of *Myrcia loranthifolia* essential oil at 200 mg/kg against ethanol-induced gastric damage in mice. The results are means ± standard deviation (n = 6). * Significant difference relative to the control by Tukey's test ($P < 0.05$). BAF and BDF = Essential oil from Brazilian Atlantic Forest and Brazilian Dry Forest, respectively.

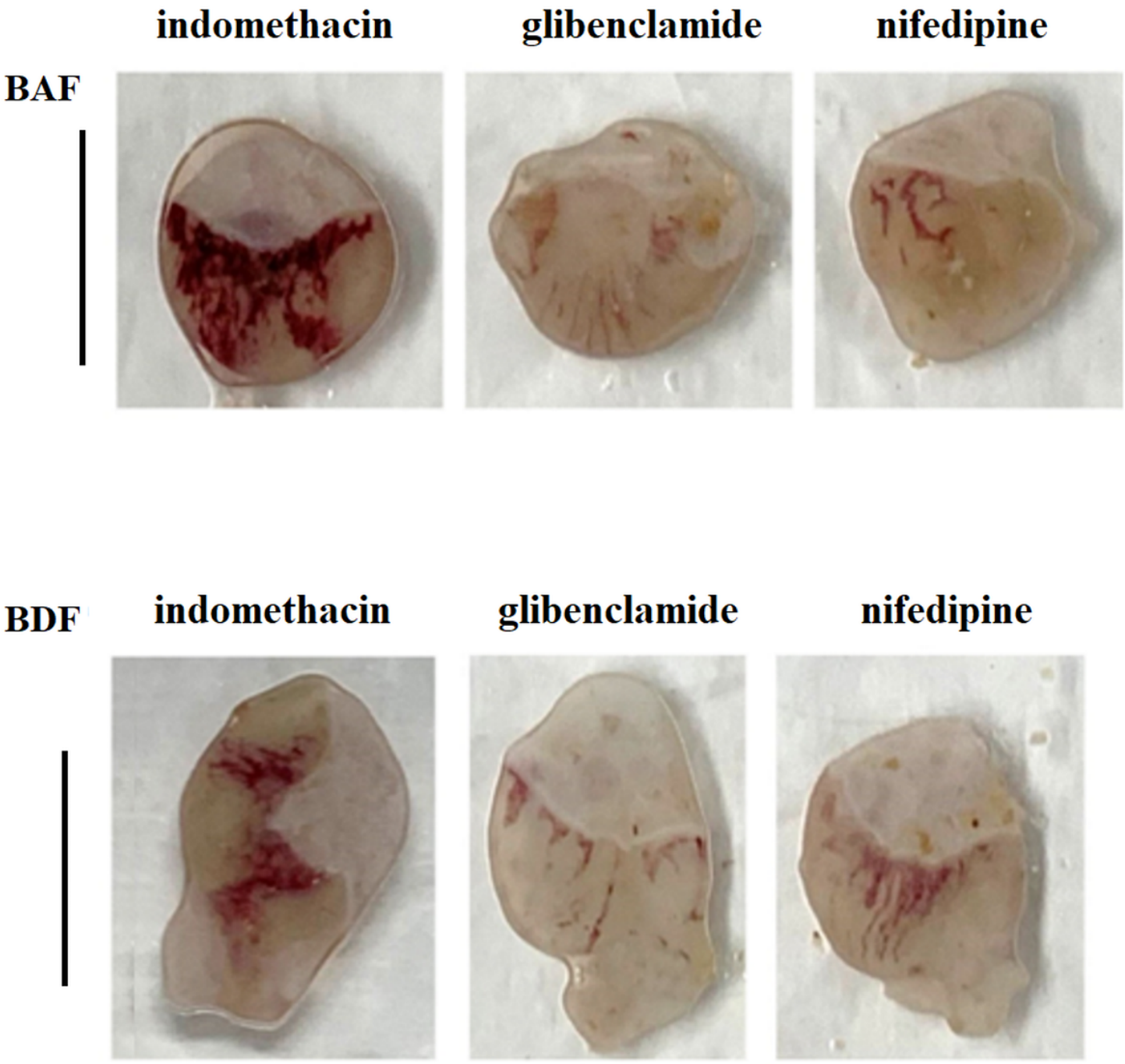

Figure 5

Photomicrographs of the stomachs of animals treated orally with indomethacin (20 mg/kg), gliblenclamide (5 mg/kg) and nifedipine (5 mg/kg) prior to administration of *Myrcia loranthifolia* essential oil (200 mg/kg) in the ethanol-induced gastric damage model in mice. Reddened areas indicate tissue injury. BAF and BDF = Essential oil from Brazilian Atlantic Forest and Brazilian Dry Forest, respectively. Bar = 4 cm.

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