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ORIGINAL ARTICLE

Chemical Profile and Anxiolytic- and Anticonvulsant-Like Effects of *Miconia albicans* (Sw.) Triana (*Melastomataceae*) Leaves in Adult Zebrafish

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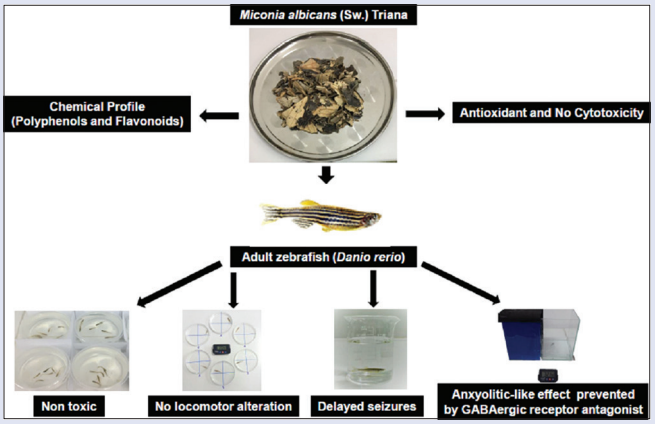
ABSTRACT

Background: *Miconia albicans* is a vegetable widely used in folk medicine as an alternative for the treatment of pain and inflammation. This study aimed to evaluate the chemical profile and anxiolytic- and anticonvulsant-like effects of the aqueous extract *M. albicans* leaves (CAVEL) in adult zebrafish. **Materials and Methods:** The antioxidant activity, chemical prospecting, and toxicity tests were performed. Then, the anxiolytic- and anticonvulsant-like effects were investigated in adult zebrafish. **Results:** It was detected the presence of flavonoids, xanthones, besides alkaloids, flavonoids, and polyphenols in CAVEL. Eighteen polyphenols and nine flavonoids were identified in CAVEL. CAVEL showed antioxidant activity and no cytotoxic potential. The effects of CAVEL in adult zebrafish were dependent on the route of administration: CAVEL (*Per os* [*p.o.*] and intraperitoneal [*i.p.*]) did not alter the locomotion of the animals, presented anxiolytic-like effect (*p.o.* and *i.p.*) and delayed anticonvulsant-like effect (*p.o.*). The anxiolytic-like effect was prevented by GABAergic receptor antagonist. *M. albicans* has pharmacological potential for the treatment of anxiety and anticonvulsant and these results support studies of isolation and characterization of bioactive principles. **Conclusion:** *M. albicans* has pharmacological potential for the treatment of anxiety and anticonvulsant and these results support studies of isolation and characterization of bioactive principles.

Key words: Adult zebrafish, anticonvulsant, anxiolytic-like, flavonoids, *Miconia albicans,* polyphenols

SUMMARY

- The aqueous extract from *Miconia albicans* leaves (CAVEL) is rich in polyphenols and flavonoids. CAVEL administered by gavage, but not by intraperitoneal route, prevented seizures in adult zebrafish. The anxiolytic-like effect of CAVEL was prevented by the GABAergic receptor antagonist, flumazenil. CAVEL has no cytotoxic potential.



Abbreviations used: CAVEL: Aqueous extract *Miconia albicans* leaves; CCD: Thin layer chromatography; CEUA-UECE: Ethics Committee on Animal Research of the Ceará State University; CSM: Classes of Special Metabolites; Cypro: Cyproheptadine; Dic: Dichloromethane; DMEM: Dulbecco's Modified Eagle Medium; DMSO: Dimethyl sulfoxide; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DZP: diazepam; EAG: Equivalent to gallic acid; FC: Flavonoids content; FMZ: Flumazenil; GABA: Gamma aminobutyric acid; GAE: Gallic acid equivalents; Gran: Granisetron; HEX: Hexane; i.p.: Intraperitoneal; PC: Phenolic content; Piz: Pizotifen; *p.o.: Per os;* PTZ: Pentylenetetrazole; QuerE: Quercetin equivalents; RFU: Relative fluorescence units, UPLC-ESI-QTOF-MS/MS: Ultra-efficient liquid chromatography coupled to mass spectrometry.

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INTRODUCTION

Miconia albicans (Sw.) Triana (*Melastomataceae*), popularly known as "canela de velho", is a shrub that can reach tree size and occurs from the south of Mexico and the Antilles to Paraguay and Paraná.[1] It is widely used in folk medicine as an alternative for the treatment of pain and inflammation.[2] Its use is also indicted against digestive disorders.[3]

Tomé *et al.*[4] reported the presence of coumarins, triterpenes, tannins, flavonoid, and saponins in the powder of *M. albicans* leaves. Vasconcelos *et al.*[3] demonstrated the peripheral antinociceptive effect of extracts of the leaves of *M. albicans* and that this action must be related to the presence of triterpene acids and β-sitosterol. Celotto *et al.*[5] presented the antimicrobial effect of extracts of this species. Vasconcelos *et al.*[6] demonstrated *in vivo* analgesic and anti-inflammatory activities of ursolic acid and oleanoic acid isolated by *M. albicans*. The most recent study with this plant concluded that *M. albicans* has antidiabetic properties with high potential as a source of inhibitors of PTP1B.[7]

Since 2014, thousands of chikungunya cases have been recorded in Brazil and more than two million cases worldwide.[8] The disease is characterized by fever, asthenia, arthralgia, myalgia, headache and rash[9] and there was increased use of *M. albicans* due to its analgesic and anti-inflammatory properties. However, there are few studies on the pharmacological properties of the plant and hence, the present study was undertaken to investigate the pharmacological potential of *M. albicans* in adult zebrafish.

MATERIALS AND METHODS

Drugs and reagents

The following drugs and reagents were used in the study: ethanol (96%; Ciclo Farma). Hexane (Hex), Dichloromethane (Dic), Folin–Ciocalteu reagent, and formaldehyde were purchased from Dinâmica. Ferric chloride, sodium chloride, and dimethyl sulfoxide were purchased from Synth. Saline solution (0.9%; Arboreto). Flumazenil (FMZ; Sandoz®). Granisetron hydrochloride (Corepharma LLC, Middlesex Corporation, England). Pizotifen maleate (Novartis Pharmaceutical cooperation, Basel, Switzerland). Cyproheptadine (Actavis UK Ltd., Devon Quercetin, UK). Aluminum chloride ($AlCl_3$), Diazepam (DZP), Gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Pentylenetetrazole (PTZ) were purchased from Sigma Aldrich (Brazil).

Botanical material and preparation of extracts

Commercial samples of the dried leaves of *M. albicans* (Sw.) Triana were acquired (Chás da Amazônia, Lot: 138) for the accomplishment of this study. Tea was obtained from infusion of 70 g of dry material and ground into 1 L of boiling mineral water (10 min). After cooling (25°C ± 2°C), the tea was filtered and placed in a sterile container and later lyophilized to obtain the aqueous extract of the canela de velho (CAVEL).

To obtain the organic extracts, the methodology described by Matos[10] was used. For each extract 70 g of leaf samples of *M. albicans* and 3 L of solvents of different polarities were used: Hex and Dic. The extracts (aqueous and organic) were packed in aseptic vials in the refrigerator (5°C) until use.

Analysis of antioxidant activity

Solutions of DPPH (3.9 mL, 6.5×10^{-5} M)[11] were added to the solutions containing the samples (10–10,000 μg/mL). The tests were performed in triplicate. The absorbance values were measured in spectrophotometer at 515 nm. The antioxidant capacity was compared with quercetin. EC_{50} was calculated to determine the antioxidant potential.

Chemical prospecting

Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical screening to detect the main classes of secondary metabolites through chemical reactions that result in changes in color and/or formation of precipitates which are specific to each class of substances.[10]

Estimation of total phenolic content

Folin–Ciocalteu reagent[12] was used with a gallic acid standard curve (1–500 μg/mL). The value obtained from the equation was $C = 0.0009$ A, where C is the concentration of gallic acid, A is the absorbance (750 nm) and the correlation coefficient $R = 0.9916$. The total phenolic content was expressed in mg gallic acid equivalents per 100 g of extract. Pearson's correlation (r) was used to assess the relationship between total phenolic contents and antioxidant activities.

Estimation of total flavonoids content

The total flavonoids content of the extracts was analyzed utilizing $AlCl_3$ method.[13] The calibration curve was prepared with quercetin (500–31.25 μg/mL in ethanol at 80% [v/v]). Samples of 0.5 mL of each extract (1 mg/mL) or standard solutions were mixed with ethanol (95%; 1.5 mL; v/v), 10% $AlCl_3$ (0.1 mL; w/v), 1 mol/L sodium acetate (0.1 mL) and distilled water (2.8 mL). In the blank test, the volume of $AlCl_3$ was substituted by distilled water. The incubation occurred at room temperature for 30 min and the absorbance reading was measured in a spectrophotometer (415 nm). The measures were performed in triplicate and the average value was expressed in μg of quercetin equivalents per milligrams of extract (μg/mg).

Chemical characterization by ultra-efficient liquid chromatography coupled to mass spectrometry

CAVEL was the extract with potential antioxidant potential (see Results) and therefore was chosen for complementary phytochemical analysis. CAVEL (25 mg) was cleaned up by solid phase extraction using reverse phase cartridge (Supelco® C_{18}-500 mg/6 mL). Initially the adsorbent was activated with methanol (5 mL; HPLC-LiChrosolv®) followed by packing with deionized water (5 mL; Milli-Q®). CAVEL was solubilized in 0.3 mL of methanol and applied to the cartridge. For the removal of interferents, the cartridge was eluted with 5 mL of water. Finally, the cartridge was eluted with 5 mL of methanol: water (9: 1, v/v) to recover the analytes of interest (6.9 mg). This fraction was solubilized in 1 mL of methanol and filtered on a 0.22 μm PTFE membrane (Analytical®).

The chemical characterization was performed using an Acquity ultra-performance liquid chromatography (UPLC) chromatograph coupled to a Xevo quadrupole and time-of-flight mass spectrometers (UPLC-Q-TOF, Waters®). The chromatographic separation was achieved on a Waters Acquity UPLC BEH column (ID 150 mm × 2.1 mm, 1.7 μm diameter) at 40°C. The sample (5.0 μL) was eluted with a mobile phase composed of water (A) and acetonitrile (B), both acidified with 0.1% formic acid, with the following elution gradient: 2%–95% of B (0–15 min), 100% of B (15.1–17 min) and equilibrating with 2% of B (17.1–19.1 min) at a flow rate of 0.4 mL/min. The ionization was performed using an electrospray ionization source in a negative mode at 120°C with a desolvation gas flow rate of 500 L/h at 350°C, extraction cone of 0.5 V and capillary voltage of 2.6 kV. Leucine enkephalin was used as the lock mass. The acquisition mode was MSE. He UPLC-Q-TOF system was managed by MassLynx 4.1 software (Waters®). CAVEL constituents were tentatively

characterized using the molecular formula provided by MassLynx 4.1 software based on their accurate masses (error < 10 ppm), MS fragmentation pattern and previous reports of its occurrence in the same species, genus, family or botanical order. Furthermore, the compounds were assigned by comparison with authentic standards (when available) and public MS database (ChemSpider, PubChem and MassBank and KNapsack).

Infrared (Fourier-transform infrared) spectroscopy

CAVEL was subjected to infrared (IR) spectroscopic study using Shimadzu (Model IR-tracer 100, Japan) spectrophotometer by employing standard potassium bromide (KBr) pellet technique. Sample was ground with KBr in the ration of 1:100. Mixture was placed in the mold and pressed. The pellet was scanned over the wavelength ranged from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans per min.[14]

In vitro toxicity

Non-specific toxicity to Artemia salina L.

Extracts concentrations (100, 500 and 1000 μg/mL) were tested in triplicate. The surviving nauplii were counted after 24 h and the LC_{50} was determined using Probit analysis with 95% confidence intervals.[15] The toxicity potential of the samples was classified into: a) Non-toxic (LC_{50} >1000 μg/mL); b) Toxic (LC_{50} ≤1000 μg/mL).

Cytoxicity to HEK-293 cells

The extract of *M. albicans* with the highest antioxidant potential (CAVEL; see Results) was subjected to the test of specific cytotoxicity to HEK-293 cells (human embryonic kidney cells). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM-Gibco®-Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Subsequently, the cell line was grown at 37°C in a humidified atmosphere containing 5% CO_2.[16]

The log-growing cells were seeded in 96-well plates at the density of 20,000 cells per well and incubated for 24 h at 37°C, with 5% CO_2 and 95% humidity before application of CAVEL (0–1500 μg/mL). After application of CAVEL, the plates were incubated for 72 h at 37°C, with 5% CO_2 atmosphere and 9 5% humidity. 4 h before the end of the incubation period, 10 μL/well of Alamar blue solution (0.312 mg/mL) was added. At the end of the 72 h, fluorescence was measured by ELISA reader (BioTek Synergy HT) using excitation wavelength at 530–560 nm and emission at 590 nm. The following formula, considering relative fluorescence units (RFUs), was used to calculate the cell viability (%): Viability (%) = $(RFU_{samples}/RFU_{control}) \times 100$.

Nonlinear regression was used to obtain the inhibition concentration required to produce a 50% reduction in cell viability (half maximal inhibitory concentration [IC_{50}]). The cytotoxicity potential of the sample was classified into (a) Toxic (IC_{50} ≤30 μg/mL), (b) Non-toxic (IC_{50} >30 μg/mL).[17]

Animals

Adult wild zebrafish (*Danio rerio*), male and female (n = 6/group), short-fin phenotype, 60–90 days, of similar size (3.5 ± 0.5 cm) and weight (0.4 ± 0.1 g) were obtained from Agroquímica: Comércio de Produtos Veterinários LTDA, a supplier located in Fortaleza (Ceará, Brazil). The group of 50 fish were acclimated for 24 h in a 10-L glass tank (30 cm × 15 cm × 20 cm) containing dechlorinated tap water (ProtecPlus®) and air pump with submerged filter at 25°C and pH 7.0, under near-normal circadian rhythm (14:10 h of light/dark cycle). The fish received food (Alcon Gold Spirulina Flakes®) *ad libitum* 24 h prior to the experiments. After the experiments, the animals were euthanized by immersion in icy water (2°C–4°C) for 10 min until loss of opercular movements.[18] All experimental procedures were approved by the Ethics Committee on Animal Research of the Ceará State University (CEUA-UECE; #7210149/2016).

General protocol

On the day of the experiments, the fish were randomly selected, anesthetized with cold water (12°C–15°C) until they were motionless, with all reflexes and muscle control temporarily impaired, transferred to a moist sponge, treated with test samples or controls or drugs, orally (*p.o.*)[19] or intraperitoneally (*i.p.*)[20] or intramuscular (*i.m.*) as described by Magalhães *et al.*[21] Subsequently, they were individually placed in glass beakers (250 ml) containing 150 mL of aquarium water for resting. For the oral treatments an automatic pipette (20 μL) was used. For the *i.p.*, *i.m.*, an insulin syringe (0.5 mL; UltraFine® BD) was used with a 30-G gauge needle.[21]

Behavioral activities

CAVEL was chosen to continue the studies, since it was the extract that presented the most prominent antioxidant activity (see Results). The animals' behavior was recorded by calibrated and blinded analyzers.

Locomotor behavior (open-field test)

The animals (n = 6/group) were pretreated (20 μL-*p.o.* or -*i.p.*) with CAVEL (3 or 10 or 30 mg/mL) or vehicle (distilled water; 20 μL; *p.o* or *i.p.*) and submitted to the open field test[22] to evaluate whether there was an alteration in fish motor coordination, either through sedation and/or muscle relaxation. A naive group (n = 6) was included. The number of line crosses was recorded during 0–5 min.

Light and dark test

In this study, we used the same methodology previously described by Ferreira *et al.*[23] adapted from Gebauer *et al.*[24] Adult zebrafish (n = 6/group) were treated as previously described in open-field test and 1 h later were placed in the bright half of a glass tank (30 cm × 15 cm × 20 cm) containing drug-free dechlorinated tap water. The anxiolytic effect was quantified only in terms of percentage of permanence in the bright zone during 5 min. The water level in the tank was reduced to 3 cm to increase anxiety, inducing the fish to remain in the dark zone.

To evaluate the involvement of 5-HT and GABA,[23] other groups of animals (n = 6/each) received (20 μL; *p.o.*) pizotifen (0.8 mg/mL, 5-HT_1, 5-HT_{2A} 5-HT_{2C} antagonist), cyproheptadine (0.8 mg/mL, 5-HT antagonist), granisetron (0.5 mg/mL; 5-TH_3 antagonist) or flumazenil (0.1 mg/mL, GABA antagonist) 30 min prior to administration of CAVEL (3 mg/mL; *p.o.*) or DZP (0.2 mg/mL; *p.o.*). Naive groups (n = 6/each) were included.

Pentylenetetrazol-induced seizures

Adult zebrafish were individually exposed to 7.5 mM PTZ.[25] Animals (n = 6/group) were treated as previously described in open-field test and 1 h later were individually immersed in 250 mL beakers containing PTZ. The seizure-like behavior was classified according to each stage: stage I – dramatically increased swimming activity, Stage II – whirlpool swimming behavior and Stage III – clonus-like seizures followed by loss of posture, when the animal falls to one side and remains immobile for 1–3 s.

Statistical analysis

The results were expressed as mean values ± standard error of the mean for each group of 6 animals. After confirming the normality of distribution and data homogeneity, the differences between the groups were submitted to one-way analysis of variance (ANOVA), followed by Tukey's test. All analyses were performed with the software GraphPad Prism v. 6.01 (GraphPad Software, San Diego, California USA). The level of statistical significance was set at 5% ($P < 0.05$).

RESULTS

Yields and antioxidant activity

CAVEL was the extract with the highest extraction yield (7.53%) resulting in 5.27 g [Table 1]. The antioxidant activity test by thin layer chromatography identified the presence of antioxidant phenolic compounds presenting free radical reducing potential, as well as chelating ions in CAVEL and hex extract. They had radical elimination activity against DPPH.

Phytochemical prospecting

All extracts tested have phenolic compounds and alkaloids. Dic extract and CAVEL also demonstrated the presence of flavonoids of the flavone type, flavonols, flavanones, as well as xanthones. Triterpenoids were detected in hex extract [Table 2].

Total phenolic content varied significantly between the extracts. However, CAVEL presented the highest phenol content (318.520 ± 0.002 mg equivalent to gallic acid/g extract) [Table 1]. The total flavonoid concentration varied significantly, with the Dic extract having the highest concentration (244.03 ± 0.40 EQuer/g extract) [Table 1].

Eighteen polyphenols were identified in CAVEL, including nine hydrolysable tannins and nine flavonoids, of which eight are glycosylated or galloylated derivatives of quercetin [Table 3 and Figure 1].

The results of FTIR analysis suggest the presence of alkaloids, flavonoids and polyphenols with the following chemical groups of alkanes, aromatics, hydroxyls, carbonyls, aldehydes, amines, nitriles, amides and ethers [Figure 2].

Table 1: Yields, total phenolic, and total flavonoids from extracts of *Miconia albicans*

Extract	Solvent (L)	Weight of plant material (g)	Yield		PC (mg EAG/g of dry extract)	FC (mg EQuer/g of dry extract)
			(g)	(%)		
Hex	3	70	1.2053	1.72	66.670^{a}±0.005	54.440^{a}±0.027
Dic	3	70	1.9286	2.75	71.600^{b}±0.001	244.030^{b}±0.140
CAVEL	1	70	5.2700	7.53	318.520^{c}±0.002	0.610^{c}±0.003

EAG: Equivalent to gallic acid; EQuer: Equivalent to quercetin. Different letters in the column indicate that there was a significant difference among the contents of total phenols or flavonoids. PC: Phenolic content; FC: Flavonoids content; Hex: Hexane; Dic: Dichloromethane; CAVEL: Aqueous extract, obtained from lyophilized tea

Table 2: Preliminary phytochemical analysis of extracts from the dried leaves of *Miconia albicans*

Extract	CSM									
	CSM1	CSM2	CSM3	CSM4	CSM5	CSM6	CSM7	CSM8	CSM9	CSM10
Hex	+	-	-	-	-	-	-	-	+	+
Dic	+	-	+	-	-	+	-	+	-	+
CAVEL	+	-	+	-	-	+	-	-	-	+

CSM1: Phenols; CSM2: Tannins; CSM3: Flavonoids, flavones, flavonols and xanthones; CSM4: Flavonoids (anthocyanins and anthocyanidins); CSM5: Flavonoids (leucoanthocyanidins, catechins); CSM6: Flavanoids (flavanones); CSM7: flavonoids (flavanonols); CSM8: Steroids; CSM9: Triterpenoids; CSM10: Alkaloids; +: Present; -: Absent; Hex: Hexane; Dic: Dichloromethane; CAVEL: Aqueous extract, obtained from lyophilized tea; CSM: Classes of special metabolites

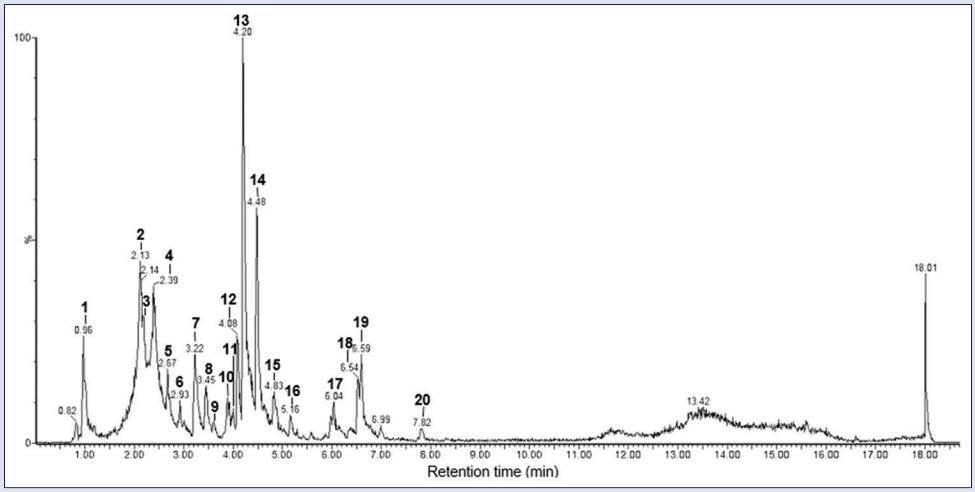

Figure 1: Chromatogram of aqueous extract *Miconia albicans* leaves obtained in ultra-efficient liquid chromatography coupled to mass spectrometry

Table 3: Phytochemical compounds found in the aqueous extract of *Miconia albicans* leaves by using ultra-performance liquid chromatography-ESI-QTOF-mass spectrometry/mass spectrometry

Peak (*n*)	Rt (min)	30	[M-H]- calculated	Product ions (MS/MS)	Empirical formula	Ppm (error)	Putative name
1	0.96	1085.0758	1085.0744	933.0493; 915.0388; 871.0775; 631.0751; 300.9967; 169.0061	$C_{48}H_{30}O_{30}$	1.3	Galloyl-Castagalin/ Vescalagin derivative I[a]
2	2.13	933.0441	933.0423	915.0426; 871.0403; 631.0535; 300.9923	$C_{44}H_{22}O_{24}$	1.9	Ellagitannin derivative[a]
3	2.17	1085.0748	1085.0744	933.0434; 915.0386; 871.0485; 783.0674; 300.9940; 169.0085	$C_{48}H_{30}O_{30}$	0.4	Galloyl-Castagalin/ Vescalagin derivative II[a]
4	2.39	1085.0752	1085.0744	933.0510; 915.0522; 871.0625; 783.0683; 631.0510; 300.9933; 169.0109	$C_{48}H_{30}O_{30}$	0.7	Galloyl-Castagalin/ Vescalagin derivative III[a]
5	2.67	933.0640	933.0634	915.0485; 871.0702; 783.0635; 631.0735; 300.9943	$C_{41}H_{26}O_{26}$	0.6	Castalagin/ Vescalagin isomer I[a]
6	2.93	933.0649	933.0634	915.0547; 871.0047; 783.0749; 631.0519; 300.9905	$C_{41}H_{26}O_{26}$	1.6	Castalagin/ Vescalagin isomer II[a]
7	3.22	467.1203	467.1190	387.1659; 300.9993;169.0125	$C_{21}H_{24}O_{12}$	2.8	Unknown
8	3.45	305.0664	305.0661	275.0143; 125.0180	$C_{15}H_{14}O_{7}$	1.0	Epigallocatechin*[,b]
9	3.61	935.0817	935.0791	783.0544; 633.0924; 300.9952; 169.0101	$C_{41}H_{28}O_{26}$	2.6	Casuarictin[a]
10	3.88	615.0988	615.0986	463.0908; 301.0071; 169.0168	$C_{28}H_{24}O_{16}$	0.3	Quercetin-galloyl-hexoside[c]
11	3.97	761.1551	761.1565	615.1005; 609.1479; 300.9983; 169.0064	$C_{34}H_{34}O_{20}$	−1.8	Quercetin-galloyl-rutinoside[c]
12	4.08	463.0851	463.0877	301.0196; 300.0233; 151.0142	$C_{21}H_{20}O_{12}$	−5.6	Hyperoside[c]
13	4.20	609.1452	609.1456	301.0234; 300.019; 179.0003	$C_{27}H_{30}O_{16}$	0.7	Rutin*[,c]
14	4.48	609.1461	609.1456	447.0869; 301.0273; 300.0234; 151.0117	$C_{27}H_{30}O_{16}$	0.8	Quercetin rhamnosyl-hexoside derivative I[c]
15	4.83	609.1606	609.1608	447.0978; 301.0171; 293.1381	$C_{31}H_{30}O_{13}$	−0.3	Quercetin rhamnosyl-hexoside derivative II[c]
16	5.16	483.1859	483.1886	447.0914; 343.0542; 328.0214; 301.0190; 300.0255; 151.0323	$C_{23}H_{32}O_{11}$	−1.4	Quercetin rhamnoside derivative[c]
17	6.04	711.3922	711.3897	503.3332; 301.0193; 169.0217	$C_{37}H_{60}O_{13}$	−4.8	Galloyl-quercetin derivative[c]
18	6.54	817.3941	817.3917	655.3634; 503.3026; 331.2388; 169.0321	$C_{32}H_{66}O_{23}$	2.9	Galloyl-hexoside derivative I[a]
19	6.59	817.3953	817.3917	655.3369; 503.3491; 331.2460; 169.0293	$C_{32}H_{66}O_{23}$	4.4	Galloyl-hexoside derivative II[a]
20	7.82	503.3216	503.3220	329.2219; 201.1106; 171.0967	$C_{26}H_{48}O_{9}$	−0.8	Unknown -

*Authentic analytical standard; [a]Hydrolysable tannin; [b]Flavonoid; [c]Glycosidic flavonoids

Table 4: Effect of CAVEL on locomotor behavior in adult zebrafish

Groups	Concentration (mg/mL)	Open field (*n*° of crossings/5 min)	
		p.o.	i.p.
Control	-	97.67±11.70	50.83±5.87
CAVEL	3	75.00±14.92	45.00±7.29
	10	75.50±19.42	41.33±15.56
	30	63.67±7.62	24.17±5.21
Naive	-	120.50±13.26	80.67±7.68

The results are expressed as mean values±SEM. (*n*=6/group). ANOVA followed by the Tukey test. ANOVA: Analysis of variance; p.o.: *Per os*; i.p: Intraperitoneal; SEM: Standard error of mean; CAVEL: Aqueous extract *Miconia albicans* leaves

In vitro and *in vivo* toxicity

None of the samples promoted mortality of nauplii (data not shown). CAVEL was not considered to be toxic (IC_{50} of 1.264 µg/mL) against HEK-293 cells.

Behavioral activities

Locomotor behavior (open-field test)

CAVEL did not alter the locomotor activity of the animals [Table 4].

Light and dark test

CAVEL (5 or 10 or 30 mg/mL; *p.o.* and *i.p.*) promoted an anxiolytic effect like DZP [Figure 3] and this effect was not prevented by

pretreatment with 5-HT antagonists (pizotifen, cyproheptadine, and granisetron) [Figure 4]. The GABA antagonist (flumazenil) prevented the anxiolytic-like effect of CAVEL (3 mg/mL; *p.o.*) [Figure 5].

Pentylenetetrazol-induced seizures

CAVEL (30 mg/mL; *p.o.*) delayed the onset of Stages I ($P < 0.01$ vs. control) and II ($P < 0.05$ vs. control). In contrast, the lowest concentration (3 mg/mL; *p.o.*) anticipated the onset of Stage III ($P < 0.05$ vs. control). Animals pretreated intraperitoneally reached Stage III without significant differences in time of onset in the seizure stages [Table 5].

The results are expressed as mean values ± SEM (n = 6/group). Stage I – dramatically increased swimming activity, Stage II – whirlpool swimming behavior and Stage III – clonus-like seizures followed by loss of posture, when the animal falls to one side and remains immobile for 1–3 s. ANOVA followed by the Tukey test (*$P < 0.05$ and ***$P < 0.001$ vs. Control [vehicle]). *p.o.*– *per os*; i. p.– intraperitoneal.

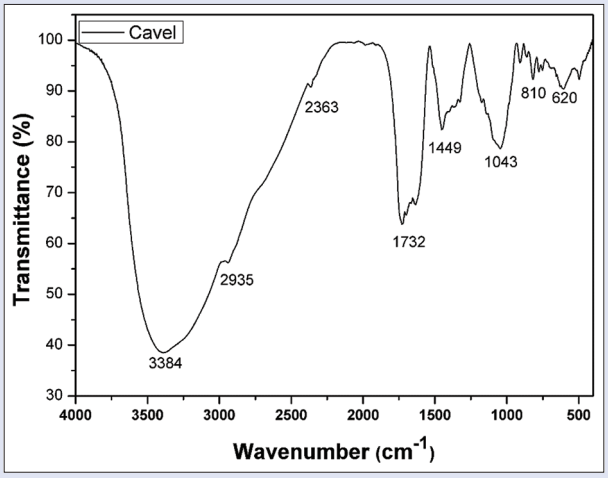

Figure 2: Fourier-transform infrared spectra (400 to 4000 cm^{-1}) of aqueous extract *Miconia albicans* leaves in water extracts

DISCUSSION

The present research is the first to report the assessment of *M. albicans* capacity to alter the behavior in experimental animal models.

A bioguided assay detected the presence of phenolic compounds in the extracts of *M. albicans*. CAVEL (aqueous extract from Canela de Velho) presented the highest antioxidant action against DPPH and this action maybe due the high content of phenolic compounds.[26]

Pieroni *et al.*[27] detected antioxidant substances such as flavonoids and phenols in the methanolic extract of *M. albicans*. Castrillón and Bedoya[28] prove that the tannins are present in several plants of this group and affirm the presence of flavonoids, triterpenoids and steroids in *M. albicans*.

The absence of toxicity points to the potential phytotherapeutic use of CAVEL as samples non-toxic to *Artemia salina* and HEK-293 cells will not be toxic to humans.[15] Scalco and Munhoz[29] also did not find cytotoxicity for methanolic extract of the whole *M. albicans* plant.

The open-field test revealed alteration of the locomotor system of the animals receiving CAVEL by intraperitoneal route. This can be justified

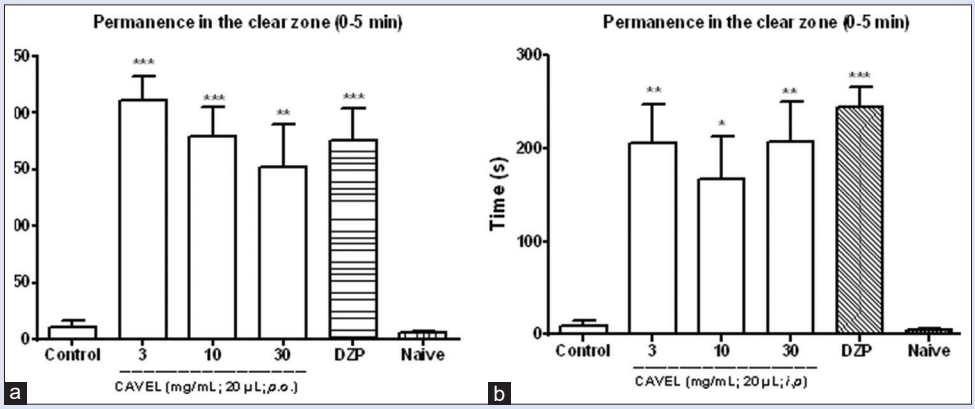

Figure 3: Anxiolytic-like effect of aqueous extract *Miconia albicans* leaves in adult zebrafish on light and dark test. (a) – gavage (*p.o.)* and (b) – *i.p*. The results are expressed as mean values ± SEM (n = 6/group). Analysis of variance followed by the Tukey test ([*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ vs. control (vehicle) or naïve]). DZP - Diazepam (5.0 mg/mL; *p.o.* - A or 2.5 mg/mL); *i.p.* – B. i.p.: intraperitoneal; *p.o.: per os*; SEM: Standard error of mean

Table 5: Effect of aqueous extract *Miconia albicans* leaves on pentylenetetrazole-induced seizure in adult zebrafish

Groups	Concentration (mg/mL)	Time (s)					
		Stage I		Stage II		Stage III	
		p.o.	i.p.	p.o.	i.p.	p.o.	i.p.
Control	-	49.83±22.58	91.50±16.83	86.17±16.90	104.20±8.48	131.70±18.40	119.30±9.49
CAVEL	3	59.50±2.09	93.17±12.45	60.50±2.09	94.17±12.45	68.33±6.50*	147.50±20.54
	10	64.33±5.39	125.30±9.47	65.33±5.39	126.30±9.47	83.67±7.44	135.00±13.58
	30	136.70±10.91***	103.00±9.62	137.70±10.91*	104.00±9.61	167.00±12.61	110.00±8.57

The results are expressed as mean values±SEM. (n=6/group). Stage I: Dramatically increased swimming activity; Stage II: Whirlpool swimming behavior; Stage III: Clonus-like seizures followed by loss of posture, when the animal falls to one side and remains immobile for 1–3 s. ANOVA followed by the Tukey test (*P<0.05 and ***P<0.001 vs. control [vehicle]). ANOVA: Analysis of variance; p.o.: *Per os*; i.p.: Intraperitoneal; CAVEL: Aqueous extract *Miconia albicans* leaves

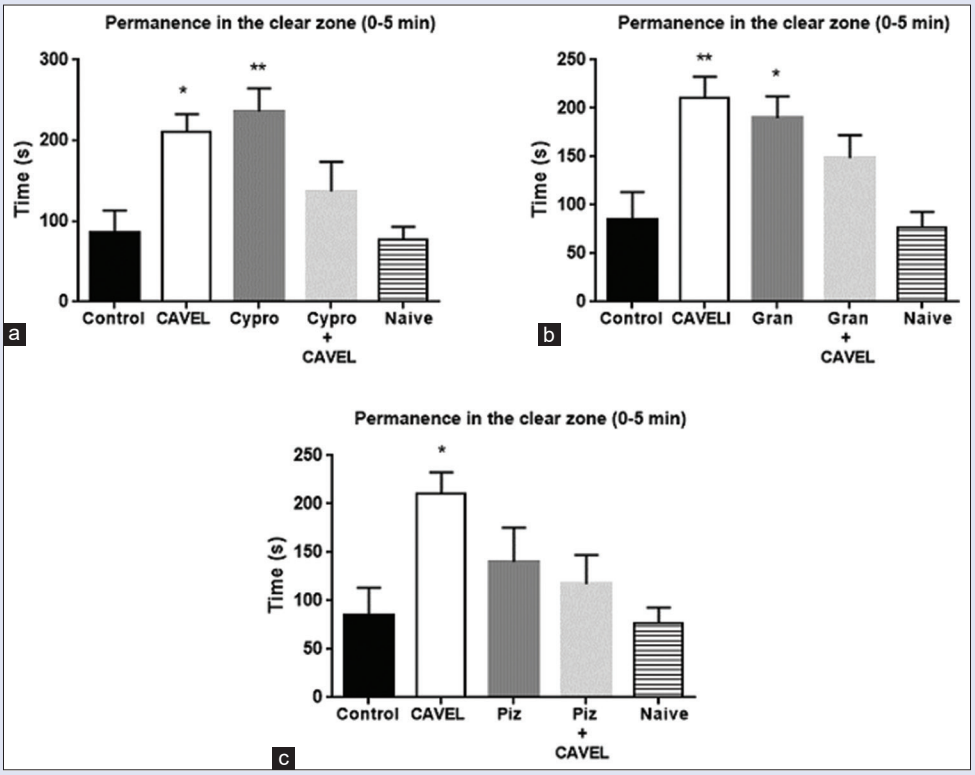

Figure 4: Evaluation of the interference of the cyproheptadine (a), granisetron (b) and pizotifen (c) in the anxiolytic-like effect of aqueous extract *Miconia albicans* leaves (3 mg/ml; 20 µL; *p.o.*) on the light and dark test. The results are expressed as mean values ± SEM (n = 6/group). Analysis of variance followed by the Tukey test ([*P < 0.05 and **P < 0.01 vs. control (vehicle)]). Cypro – cyproheptadine (0.8 mg/mL; 20 µL; *p.o.*); Gran – granisetron (0.5 mg/mL; 20 µL; *p.o.*); Piz – pizotifen (0.8 mg/mL; 20 µL; *p.o.*); SEM: Standard error of mean; *p.o.: per os*

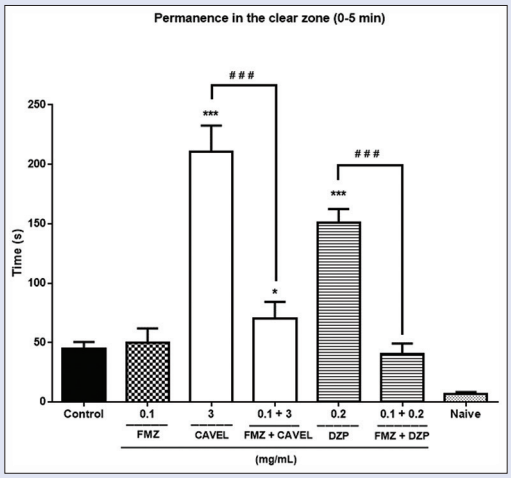

Figure 5: Participation of the GABAergic system in the anxiolytic-simile effect of aqueous extract *Miconia albicans* leaves (3 mg/mL; 20 µL; *p.o.*) on the light and dark test. The results are expressed as mean values ± S.E.M. (n = 6/group). Analysis of variance followed by the Tukey test ([*P < 0.05 and ***P < 0.001 vs. control (vehicle)]. DZP – Diazepam (20 µL; *i.p.*); FMZ – Flumazenil (20 µL; *i.p.*); *p.o.: per os.* ###P< 0.001 *vs. CAVEL or DZP.*

by the fact that the routes of administration determine the time of action, intensity and duration of the drugs in general.[30]

CAVEL (30 mg/mL; *p.o.*) increased the latency time for the onset of PTZ-induced seizures, suggesting an antiseizure action of the drug. This effect may be related to the antioxidant potential of CAVEL, since oxidative stress seems to be involved in the initiation and progression of epileptogenesis.[31]

CAVEL increased the time the animals stayed in the light zone of the aquarium, suggesting an anxiolytic-like action.[23,24,32] From this result, a possible mechanism of anxiolytic action of CAVEL was evaluated. Pretreatment with GABA antagonist, but not with 5-HT antagonists, prevented the CAVEL anxiolytic-like effect, indicating that this pathway is involved in the anxiolytic-like effect of the extract.

Flavonoids, one of the constituents found in *M. albicans*, present as strong neuroprotective agents, acting in several pathological conditions related to oxidative stress, such as anxiety and stress.[33] In addition, research has shown that flavones, another polyphenol present in *M. albicans*, can act as positive modulators of GABA receptors, the main inhibit neurotransmitter channels that articulate the synapses in the central nervous system.

CONCLUSION

M. albicans has pharmacological potential for the treatment of anxiety and seizures and these results support studies of isolation and characterization of bioactive principles.

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Conflicts of interest

There are no conflicts of interest.

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