

Antitumor Properties of the Leaf Essential Oil of *Zornia brasiliensis*

Authors

Emmanuel V. Costa^{1,2}, Leociley R. A. Menezes³, Suellen L. A. Rocha⁴, Ingrid R. S. Baliza⁴, Rosane B. Dias⁴, Clarissa A. Gurgel Rocha^{4,5}, Milena B. P. Soares^{4,6}, Daniel P. Bezerra⁴

Affiliations

The affiliations are listed at the end of the article

Key words

- *Zornia brasiliensis*
- Fabaceae
- essential oil
- cytotoxicity
- B16-F10

Abstract

Zornia brasiliensis, popularly known as “urinária”, “urinana”, and “carrapicho”, is a medicinal plant used in Brazilian northeast folk medicine as a diuretic and against venereal diseases. The aim of this study was to investigate the chemical composition and antitumor potential of the leaf essential oil of *Z. brasiliensis*. The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus and analyzed by GC-MS and GC-FID. Its composition was characterized by the presence of *trans*-nerolidol, germacrene D, *trans*-caryophyllene, α -humulene, and farnesene as major constituents. *In vitro* cytotoxicity of the essential oil and some of its major constituents (*trans*-nerolidol, *trans*-caryophyllene, and α -humulene)

was evaluated for tumor cell lines from different histotypes using the Alamar blue assay. The essential oil, but not the constituents tested, presented promising cytotoxicity. Furthermore, mice inoculated with B16-F10 mouse melanoma were used to confirm its *in vivo* effectiveness. An *in vivo* antitumor study showed tumor growth inhibition rates of 1.68–38.61% (50 and 100 mg/kg, respectively). In conclusion, the leaf essential oil of *Z. brasiliensis* presents *trans*-nerolidol, germacrene D, *trans*-caryophyllene, α -humulene, and farnesene as major constituents and is able to inhibit cell proliferation in cultures as well as in tumor growth in mice.

Supporting information available online at <http://www.thieme-connect.de/products>

Introduction

The genus *Zornia* belongs to the Fabaceae family and is found in tropical and subtropical regions of the world, with distribution predominantly in America and Africa [1,2]. Numerous biological properties have been reported for the plants belonging to this genus, including molluscicidal [3], spasmolytic [4], antibacterial [5], cytotoxic [6,7], and antinociceptive activities [8].

Zornia brasiliensis Vogel, popularly known as “urinária”, “urinana”, and “carrapicho”, is a medicinal plant used in Brazilian northeast folk medicine as a diuretic and against venereal diseases. Usually, decoctions of the whole plant are taken as water until the symptoms disappear [9]. On the other hand, only two scientific articles on the effects of this plant were found. In the first, the methanol extract of the aerial parts of *Z. cf. brasiliensis* showed radical scavenging, antioxidant, and cytotoxic activities [6]. In the second, the antinociceptive effect of 7-methoxyflavone, an isolated compound from *Z. cf. brasiliensis*, was demonstrated

in murine models [8]. The aim of this study was to investigate the chemical composition and antitumor potential of the leaf essential oil of *Z. brasiliensis*, which are reported here for the first time.

Results and Discussion

Hydrodistillation of the leaves of *Z. brasiliensis* resulted in 0.3 ± 0.0% of essential oil (light yellow) in relation to the dry weight of plant material. The oil obtained was clear with a good smell. GC-FID and GC-MS analyses allowed for the identification of 13 compounds in the essential oil and revealed *trans*-nerolidol (48.0%), germacrene D (13.9%), α -humulene (9.3%), *trans*-caryophyllene (8.4%), and (*Z,E*)- α -farnesene (7.3%) as major constituents (● **Table 1**). The essential oil was dominated by sesquiterpenes with 98.0%.

According to literature data, works that reported the chemical composition of the essential oil of *Zornia* species are very scarce, and only one work was found that reported the chemical composition

received Sept. 18, 2014
revised January 14, 2015
accepted February 16, 2015

Bibliography

DOI <http://dx.doi.org/10.1055/s-0035-1545842>
Published online
Planta Med © Georg Thieme
Verlag KG Stuttgart · New York ·
ISSN 0032-0943

Correspondence

Prof. Dr. Daniel P. Bezerra
Oswaldo Cruz Foundation
(Fiocruz)
Gonçalo Moniz Research Center
Rua Waldemar Falcão, 121,
Candeal
40296–710 Salvador, Bahia
Brazil
Phone: + 55 71 31 762272
danielpbezerra@gmail.com

Table 1 Chemical composition of the leaf essential oil of *Zornia brasiliensis*.

Compound	RI ^a	RI ^b	Peak area %
1 Benzaldehyde	963	952	0.4 ± 0.3
2 α -Copaene	1374	1374	0.6 ± 0.0
3 β -Bourbonene	1381	1387	1.2 ± 0.0
4 β -Elemene	1388	1389	1.6 ± 0.1
5 <i>trans</i> -Caryophyllene	1418	1417	8.4 ± 0.2
6 α -Humulene	1455	1452	9.3 ± 0.2
7 Germacrene D	1481	1484	13.9 ± 0.4
8 (<i>Z,E</i>)- α -Farnesene	1491	1494 ^c	7.3 ± 0.5
9 (<i>E,E</i>)- α -Farnesene	1495	1505	2.0 ± 0.3
10 β -Bisabolene	1508	1505	1.5 ± 0.1
11 δ -Cadinene	1518	1522	1.6 ± 0.0
12 (<i>E</i>)- γ -Bisabolene	1541	1544 ^d	2.5 ± 0.1
13 <i>trans</i> -Nerolidol	1565	1561	48.0 ± 1.1
Aromatic compound			0.4 ± 0.3
Sesquiterpene			98.0 ± 0.2
Total identified			98.4 ± 0.2

Data are expressed as mean \pm SD of three analyses; RI (retention indices): ^a calculated on an RT^x-SMS column according to Van Den Dool and Kratz [21], based on a homologous series of normal alkanes; ^b according to Adams [22]; ^c according to Seo et al. [18]; and ^d according to Dugo et al. [19]

tion of the essential oil from the whole plant *Z. diphylla* (L.) Pers [10]. In this specie, 37 compounds comprising 96.3% of the oil were identified by GC-MS. Monoterpenoids predominated the essential oil (83.9%), and the major constituent was sabinene (43.1%) followed by terpinene 4-ol (13.2%), γ -terpinene (7.3%), β -pinene (4.7%), and δ -2-carene (3.7%) [10]. The chemical composition was very different from the one described in our work. However, it is important to say that in this work the authors used the whole plant, while in our work we used only the leaves. On the other hand, most of the major compounds identified in the essential oil of *Z. brasiliensis*, such as *trans*-nerolidol, α -humulene, and *trans*-caryophyllene, were found as minor constituents in *Z. diphylla*. This work contributes to the chemical knowledge of the species belonging to the genus *Zornia*, in particular to the chemical composition of their essential oil, which is little explored.

The cytotoxicity of the essential oil and some of its major constituents (*trans*-Nerolidol, *trans*-caryophyllene, and α -humulene)

was evaluated for tumor cell lines from different histotypes using the Alamar blue assay. **Table 2** shows the obtained IC₅₀ values. The IC₅₀ values ranged from 1.05 to 9.58 μ g/mL on HepG2 and HL-60 cell lines for the essential oil, from 17.58 to > 25 μ g/mL on K562 and B16-F10 cell lines for *trans*-nerolidol, from 12.82 to > 25 μ g/mL on HepG2 and B16-F10 cell lines for *trans*-caryophyllene, from 4.37 to > 25 μ g/mL on K562 and B16-F10 cell lines for α -humulene, respectively. 5-Fluorouracil (5-FU), used as the positive control, showed IC₅₀ values from 0.04 to 0.87 μ g/mL for the HepG2 and B16-F10 cell lines, respectively. According to our pre-clinical anticancer drug screening program, only those extracts/oils presenting IC₅₀ values below 30 μ g/mL and pure compounds presenting IC₅₀ values below 4 μ g/mL in tumor cell line assays are considered promising [11–14]. Thus, the essential oil obtained from *Z. brasiliensis* presented promising results. Interestingly, the essential oil was not cytotoxic to non-tumor cells (PBMCs). On the other hand, this effect seems to be associated to a mixture of its major and minor constituents, since the pure compounds present only weak cytotoxicity (IC₅₀ > 4 μ g/mL for all tumor cell lines tested).

As cited above, the methanol extract of the aerial parts of *Z. cf. brasiliensis* has been reported to have a cytotoxic effect. The cytotoxic property was determined through the brine shrimp lethality test, and the lethal concentration was 7.2 μ g/mL [6]. Arunkumar et al. [7] isolated a steroid positive compound from *Z. diphylla* that showed promising *in vitro* and *in vivo* anticancer activity to Dalton's lymphoma ascites cells and was devoid of any toxicity to mice in a short-term toxicity evaluation, indicating that this genus is a source of cytotoxic compounds.

The cytotoxic activity of some major constituents present in the leaf essential oil of *Z. brasiliensis* has been previously assessed, but they showed IC₅₀ values > 4 μ g/mL [13–19]. Herein, we confirmed that *trans*-caryophyllene, α -humulene, and *trans*-nerolidol presented only weak anticancer potential.

To investigate whether *Z. brasiliensis* essential oil has *in vivo* anti-tumor activity, C57BL/6 mice were subcutaneously inoculated with B16-F10 melanoma and treated by the intraperitoneal route once a day for nine consecutive days with the essential oil. The essential oil was also able to inhibit tumor growth in mice.

Fig. 1 shows the obtained inhibition. On the 16th day, the average tumor weight of the control mice was 3.87 \pm 0.20 g. In the

Table 2 *In vitro* cytotoxic activity of the leaf essential oil of *Z. brasiliensis* and some of its major constituents.

Cell lines	IC ₅₀ (μ g/mL) ^a				
	Essential oil	<i>trans</i> -Nerolidol	<i>trans</i> -Caryophyllene	α -Humulene	5-FU ^b
Tumor cells ^c					
B16-F10	4.93 1.83–13.28	> 25	> 25	> 25	0.87 0.41–1.83
HepG2	1.05 0.16–7.00	> 25	12.82 11.03–14.89	17.46 12.71–23.97	0.04 0.01–1.42
HL-60	9.58 6.23–14.72	21.99 13.74–35.18	13.67 8.37–22.34	14.32 8.07–25.42	0.18 0.01–3.76
K562	7.26 3.00–17.57	17.58 12.78–24.20	20.63 8.80–38.39	4.37 3.90–4.90	0.32 0.24–0.43
Non-tumor cells ^d					
PBMCs	> 25	> 25	> 25	> 25	14.85 9.03–24.41

^a Data are presented as IC₅₀ values, in μ g/mL, and 95% confidence interval obtained by nonlinear regression from three independent experiments performed in duplicate, measured by the Alamar blue assay after 72 h incubation; ^b 5-FU was used as the positive control; ^c Tumor cells: B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), HL-60 (human promyelocytic leukemia), and K562 (human chronic myelocytic leukemia); ^d Non-tumor cells: PBMCs (human peripheral blood mononuclear cells activated with concanavalin A – human lymphoblast)

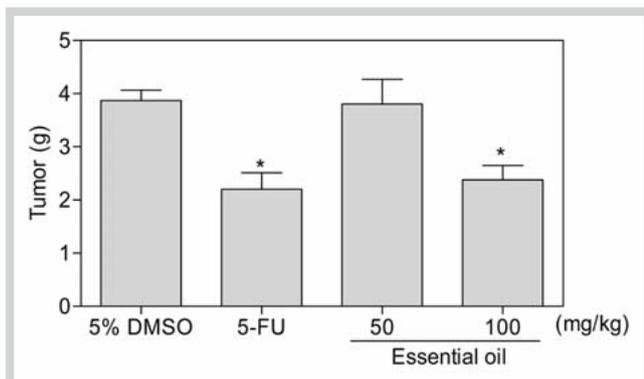


Fig. 1 *In vivo* antitumor effect of the leaf essential oil of *Z. brasiliensis*. Mice were injected with B16-F10 melanoma (2.0×10^6 cells/animal, s.c.). The animals were treated by intraperitoneal administration for nine consecutive days, starting one day after tumor implantation. 5-FU (25 mg/kg) was used as a positive control. The negative control was treated with the vehicle used for diluting the tested substance (5% DMSO). Data are presented as mean \pm SEM of nine animals. * $P < 0.05$ compared to the 5% DMSO group by ANOVA followed by Student-Newman-Keuls.

presence of the essential oil (50 and 100 mg/kg), the average tumor weights were 3.80 ± 0.47 and 2.38 ± 0.27 g, respectively. Tumor growth inhibition rates were 1.68–38.61%. 5-FU (25 mg/kg), used as the positive control, reduced the tumor weight by 43.11%.

Tumors obtained from all experimental groups showed typical tumoral proliferation consisting of round and oval cells exhibiting large nuclei, irregular or multiple nucleoli, and frequent abnormal mitoses. Some of these neoplastic cells also presented intracellular melanin pigment. The stroma was scarce but displayed many areas of congested vessels and inflammation.

Systemic toxicological parameters were also examined in essential oil-treated mice. No significant changes in body and organ (liver, kidney, spleen, lungs, and heart) weight were seen in the essential oil-treated groups ($p > 0.05$, **Table 1S**, Supporting Information). Alkaline phosphatase, gamma-glutamyl transpeptidase, alanine aminotransferase, albumin, globulin, and total protein were measured to investigate liver function alterations, and urea and uric acid were measured to assess the renal parameters. Amylase was measured to investigate the pancreatic function, and creatine kinase was measured as a myocardial marker. Cholesterol and glucose were measured to investigate alterations on blood lipids and sugar, respectively. Moreover, the total count of erythrocytes and leukocytes as well as a differential count of leukocytes, such as neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were measured as a hematologic parameter. No statistically significant changes on peripheral blood biochemistry and hematologic parameters were seen in the essential oil-treated groups ($p > 0.05$, **Tables 2S** and **3S**, Supporting Information, respectively).

Histopathological analysis of the organs (liver, kidneys, spleen, lungs, and heart) showed that in the livers, hydropic changes, focal areas of ischemic necrosis, and vascular congestion were observed in all experimental groups but more intensely in 5-FU-treated mice. Essential oil-treated animals also exhibited chronic inflammation and focal hemorrhage. In the kidneys, hydropic change and vascular congestion were also observed in the groups, but more intensely in the essential oil-treated mice. In the spleens, hemosiderosis found in all the groups could be re-

lated with the hemocaterese function of this organ. Atelectasis, moderate emphysema, and focal hemorrhage were observed in the lungs of animals treated with the essential oil and 5-FU. In addition, inflammation, metastatic emboli, and nodules were also observed in the lungs of the essential oil-treated mice. In respect to this, B16-F10 cells present a higher metastatic potential [20]. Concerning the histopathological features mentioned above, it is important to note that reversible changes (e.g., hydropic change) are an acute cellular response to nonlethal injuries, and the cells are able to return to the homeostatic state when the stimulus ends. In addition, inflammation could be related with tissue necrosis, stressful conditions, and even circulating and metastasizing tumor cells. No histopathological alterations were observed in hearts for all treated groups.

In conclusion, the leaf essential oil of *Z. brasiliensis* presents *trans*-nerolidol, germacrene D, *trans*-caryophyllene, α -humulene, and farnesene as major constituents and is able to inhibit cell proliferation in cultures as well as in tumor growth in mice.

Materials and Methods



Cells

Tumor cells lines B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), K562 (human chronic myelocytic leukemia), and HL-60 (human promyelocytic leukemia) were donated by Hospital A. C. Camargo, São Paulo, SP, Brazil. Cells were maintained in RPMI-1640 (Gibco-BRL) medium supplemented with 10% FBS (Cultilab), 2 mM L-glutamine (Vetec Química Fina), and 50 μ g/mL gentamycin (Novafarma). Adherent cells were harvested by treatment with 0.25% trypsin EDTA solution (Gibco-BRL). All cell lines were cultured in cell culture flasks at 37 °C in 5% CO₂ and subcultured every 3–4 days to maintain exponential growth. All experiments were conducted with cells in the exponential growth phase. All cell lines were tested for mycoplasma with a Lookout[®] Mycoplasma qPCR detection kit (Sigma-Aldrich) and found to be free from contamination.

Heparinized blood (from healthy, 20- to 35-year-old, nonsmoker donors who had not taken any drugs at least 15 days prior to sampling) was collected, and peripheral blood mononuclear cells (PBMCs) were isolated by a standard protocol by Ficoll (GE Ficoll-Paque Plus) density gradient centrifugation. PBMCs were washed and resuspended at a concentration of 0.3×10^6 cells/mL in RPMI-1640 medium supplemented with 20% FBS, 2 mM glutamine, and 50 μ g/mL gentamycin at 37 °C with 5% CO₂. In addition, *concanavalin A* (ConA, Sigma-Aldrich) was used as a mitogen to trigger cell division in T-lymphocytes. ConA (10 μ g/mL) was added at the beginning of culture and, after 24 h, cells were treated with the test drugs. The Research Ethics Committee of the Oswaldo Cruz Foundation (Salvador, Bahia, Brazil) approved the experimental protocol (No. 031 019/2013). All participants signed their written informed consent to participate in the study.

For all experiments, cell viability was performed by the Trypan blue exclusion assay. Over 90% of the cells were viable at the beginning of the culture.

Animals

A total of 36 specific-pathogen-free C57BL/6 mice (males, 25–30 g) was obtained and maintained at the animal facilities at the Gonçalo Moniz Research Center-FIOCRUZ (Salvador, Bahia, Brazil). Animals were housed in cages with free access to food and water, and kept under a 12:12-h light-dark cycle (lights on at

6:00 a.m.). The animals were treated according to the ethical principles for animal experimentation of SBCAL (Brazilian Association of Laboratory Animal Science), Brazil. The local Animal Ethics Committee approved the experimental protocol (number 01/2013).

Botanical material

The leaves of *Z. brasiliensis* were collected in July 2012 at the 'Poço do Cajueiro', city of Poço Redondo (coordinates: S 09°47'44.6" and W 37°40'35.3") in Sergipe State, Brazil. The identity of the plant was confirmed by Dr. Ana Paula do Nascimento Prata of the Department of Biology from Sergipe Federal University (UFS), Brazil, and a voucher specimen (24663) was deposited in the Herbarium of UFS (ASE/UFS). The authors have authorization from the Chico Mendes Institute for Biodiversity Conservation from Brazilian Ministry of the Environment for plant collection (approval No. 25637-1). This work was performed according to the special authorization for access to genetic resources in Brazil # 010344/2014-4, issued by CNPq/MCTI.

Hydrodistillation of the essential oil

The essential oil from dried leaves (for 24 h) of *Z. brasiliensis* (200 g) was obtained by hydrodistillation for 3 h using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and the percentage content was calculated on the basis of the dry weight of plant material. It was then stored in a freezer until analysis. The hydrodistillation was performed in triplicate.

GC-FID and GC-MS analyses of the essential oil

GC-FID and GC-MS analyses were performed on a Shimadzu GC-2010 Plus GCMS-QP2010 Ultra GC-FID, equipped with a Shimadzu AOC-20i auto-injector. The separation of the compounds was achieved on an RTx[®]-5MS fused capillary chromatography column (30 m × 0.25 mm × 0.25 μm film thickness) coated with 5%-diphenyl-95%-dimethylpolysiloxane. Helium was the carrier gas at a 1.2 mL/min flow rate. The column temperature program was 40 °C/4 min at a rate of 4 °C/min to 240 °C, then at 10 °C/min to 280 °C, and at 280 °C/2 min. The injector and detector temperatures were 250 °C and 280 °C, respectively. Samples (10 mg/mL in CH₂Cl₂) were injected with a 1 : 30 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C₈-C₁₈). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without an FID response factor correction. MS were taken at 70 eV with scan intervals of 0.5 s and fragments from 40–550 Da. Essential oil components were identified by comparing the retention times of the GC peaks with standard compounds run under identical conditions, the retention indices [21], and MS [22] with those in the literature, and the MS with those stored in the NIST and Wiley libraries.

Pure compounds

trans-Nerolidol (purity ≥ 85%), *trans*-caryophyllene (purity ≥ 98.5%, sum of enantiomers) and α-humulene (purity ≥ 96%) were obtained from Sigma-Aldrich. The chemical structures are presented in **Fig. 2**.

In vitro cytotoxic activity assay

Cell growth was quantified by the Alamar blue assay, as previously described [23]. For all experiments, cells were seeded in 96-well plates (0.7 × 10⁵ cells/mL for adherent cells or 0.3 × 10⁶

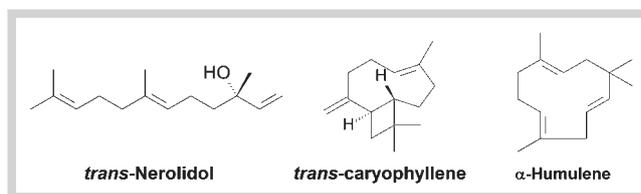


Fig. 2 Chemical structures of *trans*-nerolidol, *trans*-caryophyllene, and α-humulene.

cells/mL for suspended cells in 100 μL of medium). After 24 h, the essential oil and some of its major constituents (0.19–25 μg/mL) dissolved in DMSO (Sigma-Aldrich) were added to each well and incubated for 72 h. The compound 5-FU (purity > 99%; Sigma-Aldrich) was used as a positive control. The negative control received the vehicle used for diluting the tested oil (0.5% DMSO). Four (for cell lines) or 24 (for PBMCs) h before the end of the incubation, 20 μL of stock solution (0.312 mg/mL) of Alamar blue (resazurin, Sigma-Aldrich) were added to each well. The absorbance was measured using a SpectraMax 190 multiplate reader, and the drug effect was quantified as the percentage of control absorbance at 570 and 600 nm. The absorbance of Alamar blue in culture medium was measured at a higher wavelength and a lower wavelength. The absorbance of the medium was also measured at the higher and lower wavelengths. The absorbance of the medium alone was subtracted from the absorbance of medium plus Alamar blue at the higher wavelength. This value was called AO_{HW}. The absorbance of the medium alone was subtracted from the absorbance of the medium plus Alamar blue at the lower wavelength. This value was called AO_{LW}. A correction factor R_0 was calculated from AO_{HW} and AO_{LW}, where $R_0 = AO_{LW}/AO_{HW}$. The percent of Alamar blue reduced was then expressed as follows: % reduced = $A_{LW} - (A_{HW} \times R_0) \times 100$.

In vivo antitumor activity assay

The *in vivo* antitumor effect was evaluated in C57BL/6 mice inoculated with B16-F10 melanoma. Tumor cells (2 × 10⁶ cells per 500 μL) were implanted subcutaneously into the left hind groin of the mice. The essential oil was dissolved in 5% DMSO and given to mice intraperitoneally once a day for nine consecutive days. At the beginning of the experiment, the mice were divided into four groups of nine animals as follows: group 1: animals treated by injection of vehicle 5% DMSO; group 2: animals treated by injection of 5-FU (25 mg/kg); group 3: animals treated by injection of the essential oil (50 mg/kg); group 4: animals treated by injection of the essential oil (100 mg/kg). The treatments were started one day after tumor injection. On day 16, peripheral blood samples of the mice were collected from the retro-orbital plexus under phenobarbital (Aventis Pharma Ltda) for biochemical and hematological analyses, as described below. Then, the animals were euthanized by cervical dislocation, and the tumors were excised and weighed. The drug effects were expressed as the percent inhibition of the control.

Systemic toxicological evaluation

The mice were weighed at the beginning and at the end of the experiment. In addition, the animals were observed for signs of abnormalities throughout the study. Livers, kidneys, spleens, hearts, and lungs were removed, weighed, and observed for any signs of gross lesions or color changes and hemorrhage.

Biochemical analyses of serum samples were performed using a Vet-16 rotor and quantified by the Analyst benchtop clinical chemistry system (Hemagen Diagnostics, Inc.). The biochemical parameters measured were alkaline phosphatase, gamma-glutamyl transpeptidase, alanine aminotransferase, albumin, globulin, total protein, urea, uric acid, amylase, creatine kinase, cholesterol, and glucose.

Hematological analyses were performed by light microscopy. Hematological parameters, including total count of erythrocytes and leukocytes as well as a differential count of leukocytes, such as neutrophil, lymphocyte, monocyte, eosinophil and basophil, were measured.

After a gross macroscopic examination, tumors, livers, kidneys, spleens, hearts, and lungs were fixed in 4% formalin buffer and embedded in paraffin. Tissue sections were stained for hematoxylin and eosin chromogens, and the analysis was performed under light microscopy by a pathologist.

Statistical analyses

Data are presented as mean \pm SEM/SD or half maximal inhibitory concentration (IC₅₀) values and their 95% confidence intervals obtained by nonlinear regression. The differences between experimental groups were compared by ANOVA (analyses of variance) followed by Student-Newman-Keuls ($p < 0.05$). All statistical analyses were performed using the GraphPad program (Intuitive Software for Science).

Supporting information

Systemic toxicological parameters (body and organ weights, peripheral blood biochemistry, and hematologic parameters) are available as Supporting Information.

Acknowledgements

This work was financially supported by the Brazilian agencies CAPES, CNPq, FAPESB, and FAPITEC/SE. The authors are grateful to Marlene da Silva Cerqueira for technical assistance, professor Dr. Ana Paula do Nascimento Prata of the DBI/UFS for the botanical identification, and the histotechnology platform of FIOCRUZ-Bahia for performing the histological techniques.

Conflict of Interest

The authors have declared that there is no conflict of interest.

Affiliations

¹ Department of Chemistry, Federal University of Amazonas, Manaus, Amazonas, Brazil

² Department of Chemistry, Federal University of Sergipe, Itabaiana, Sergipe, Brazil

³ Department of Chemistry, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil

⁴ Gonçalo Moniz Research Center, Oswaldo Cruz Foundation (Fiocruz), Salvador, Bahia, Brazil

⁵ Department of Propedeutics, Federal University of Bahia, Salvador, Bahia, Brazil

⁶ Center of Biotechnology and Cell Therapy, Hospital São Rafael, Salvador, Bahia, Brazil

References

- Mohlenbrock RJ. A monograph of the leguminous genus *Zornia*. *Webbia* 1961; 16: 1–141
- McIvor JG, Bray RA. Genetic resources of forage plants. Melbourne: CSIRO; 1983

- Kloos H, Thiongo FW, Ouma JH, Butterworth AE. Preliminary evaluation of some wild and cultivated plants for snail control in Machakos District, Kenya. *J Trop Med Hyg* 1987; 90: 197–204
- Rojas A, Bah M, Rojas JI, Serrano V, Pacheco S. Spasmolytic activity of some plants used by the Otomi Indians of Quéretaro (México) for the treatment of gastrointestinal disorders. *Phytomedicine* 1999; 6: 367–371
- Obi CL, Ramalivhana J, Samie A, Igumbor EO. Prevalence, pathogenesis, antibiotic susceptibility profiles, and *in-vitro* activity of selected medicinal plants against *Aeromonas* isolates from stool samples of patients in the Venda region of South Africa. *J Health Popul Nutr* 2007; 25: 428–435
- David JP, Meira M, David JM, Brandão HN, Branco A, de Fátima Agra M, Barbosa MR, de Queiroz LP, Giuliotti AM. Radical scavenging, antioxidant and cytotoxic activity of Brazilian Caatinga plants. *Fitoterapia* 2007; 78: 215–218
- Arunkumar R, Nair SA, Subramoniam A. Induction of cell-specific apoptosis and protection of mice from cancer challenge by a steroid positive compound from *Zornia diphylla* (L.) Pers. *J Pharmacol Pharmacother* 2012; 3: 233–241
- Silva AD, Cavalcante-Silva LH, da Matta CB, Silva Dde F, Araújo MV, Tavares JF, da Silva MS, Alexandre-Moreira MS. Antinociceptive effect of 7-methoxyflavone isolated from *Zornia brasiliensis*. *Nat Prod Res* 2013; 27: 1695–1699
- Agra MF, Silva KN, Basilio IJLD, Freitas PF, Barbosa-Filho JM. Survey of medicinal plants used in the region Northeast of Brazil. *Braz J Pharmacogn* 2008; 18: 472–508
- Arunkumar R, Nair SA, Rameshkumar KB, Subramoniam A. The essential oil constituents of *Zornia diphylla* (L.) Pers, and anti-inflammatory and antimicrobial activities of the oil. *Rec Nat Prod* 2014; 8: 385–393
- Britto AC, Oliveira AC, Henriques RM, Cardoso GM, Bomfim DS, Carvalho AA, Moraes MO, Pessoa C, Pinheiro ML, Costa EV, Bezerra DP. *In vitro* and *in vivo* antitumor effects of the essential oil from the leaves of *Gutteria friesiana*. *Planta Med* 2012; 78: 409–414
- Ferraz RP, Cardoso GM, da Silva TB, Fontes JE, Prata AP, Carvalho AA, Moraes MO, Pessoa C, Costa EV, Bezerra DP. Antitumor properties of the leaf essential oil of *Xylopia frutescens* Aubl. (Annonaceae). *Food Chem* 2013; 141: 196–200
- Ribeiro SS, Jesus AM, Anjos CS, Silva TB, Santos AD, Jesus JR, Andrade MS, Sampaio TS, Gomes WF, Alves PB, Carvalho AA, Pessoa C, de Moraes MO, Pinheiro ML, Prata AP, Blank AF, Silva-Mann R, Moraes VR, Costa EV, Nogueira PC, Bezerra DP. Evaluation of the cytotoxic activity of some Brazilian medicinal plants. *Planta Med* 2012; 78: 1601–1606
- Fontes JEN, Ferraz RP, Britto AC, Carvalho AA, Moraes MO, Pessoa C, Costa EV, Bezerra DP. Antitumor effect of the essential oil from leaves of *Gutteria pogonopus* (Annonaceae). *Chem Biodivers* 2013; 10: 722–729
- Tundis R, Loizzo MR, Bonesi M, Menichini F, Dodaro D, Passalacqua NG, Statti G, Menichini F. *In vitro* cytotoxic effects of *Senecio stabianus* L. (Asteraceae) on human cancer cell lines. *Nat Prod Res* 2009; 23: 1707–1718
- Silva EB, Matsuo AL, Figueiredo CR, Chaves MH, Sartorelli P, Lago JH. Chemical constituents and cytotoxic evaluation of essential oils from leaves of *Porcelia macrocarpa* (Annonaceae). *Nat Prod Commun* 2013; 8: 277–279
- Gautam N, Mantha AK, Mittal S. Essential oils and their constituents as anticancer agents: a mechanistic view. *Biomed Res Int* 2014; 2014: 154106
- Seo WH, Baek HH. Identification of characteristic aroma-active compounds from water dropwort (*Oenanthe javanica* DC.). *J Agric Food Chem* 2005; 53: 6766–6770
- Dugo P, Mondello L, Zappia G, Bonaccorsi I, Cotroneo A, Russo MT. The composition of the volatile fraction and the enantiomeric distribution of five volatile components of faustrime oil (*Monocitrus australatica* x *Fortunella* sp. x *Citrus aurantifolia*). *J Essent Oil Res* 2004; 16: 328–333
- Fidler I. Selection of successive tumor lines for metastasis. *Nat New Biol* 1973; 242: 148–149
- Van Den Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr* 1963; 11: 463–471
- Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Illinois: Allured Publishing Corporation; 2007
- Ahmed SA, Gogal RM, Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes an alternative to [³H] thymidine incorporation assay. *J Immunol Methods* 1994; 170: 211–224