Research, Society and Development, v. 9, n. 9, e286997072, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i9.7072

Avaliação da atividade tripanocida e citotóxica *in vitro* dos óleos essenciais de plantas nativas do cerrado brasileiro

In vitro trypanocidal and cytotoxic activity of essential oils from native plants of the brazilian cerrado

Evaluación in vitro de la actividad tripanocida y citotóxica de aceites esenciales de plantas nativas del cerrado brasileño

Recebido: 28/07/2020 | Revisado: 04/08/2020 | Aceito: 11/08/2020 | Publicado: 17/08/2020

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Resumo

Uma das principais doenças tropicais negligenciadas é a doença de Chagas (DC), causada pelo *Trypanosoma cruzi* (Chagas, 1909). O tratamento da DC é feito com os antiparasitários nifurtimox e benznidazol que possuem diversos efeitos colaterais. O mercado de produtos naturais vem crescendo a cada ano e a utilização de plantas tem-se destacado como alternativa para desenvolvimento de medicamentos para cura dessa doença. Neste contexto, o presente trabalho descreve o estudo químico dos óleos essenciais das folhas de *Zanthoxylum riedelianum* (Rutaceae), *Zanthoxylum rhoifolium* (Rutaceae) e K*ielmeyera coriacea* (Calophyllaceae) e dos frutos da *Xilopia aromática* (Annonaceae) e *Zanthoxylum rhoifolium* (Rutaceae), bem como avalia a capacidade tripanocida e os efeitos citotóxicos dos mesmos. As análises químicas dos óleos foram realizadas por cromatografia gasosa acoplada a um espectrômetro de massa. Os ensaios biológicos foram realizados sobre formas tripomastigota de *Trypanosoma cruzi*, e a avaliação da atividade citotóxica foi realizada em células da linhagem $LLCMK_2$. Os óleos essenciais com maior atividade tripanocida foram os das folhas de *K. coriacea* (IC_{50} = 6,4 µg.mL^{-1}) e dos frutos de *X. aromatica* (IC_{50} = 6,4 µg.mL^{-1}), seguidos pelo óleo essencial obtido dos frutos de *Z. rhoifolium* (IC_{50} = 8,1 µg.mL^{-1}). Os dois últimos óleos essenciais apresentaram os melhores índices de seletividade (SI). Além disso, o sabineno pode ser responsável por essas propriedades, uma vez que é o principal composto presente nesses óleos essenciais. A análise citotóxica indicou que todos os óleos essenciais avaliados foram tóxicos para as células $LLCMK_2$ em concentrações maiores que 100 µg.mL^{-1} e, portanto, são excelentes candidatos ao desenvolvimento de novos fármacos antiparasitários.

Palavras-chave: Doença de Chagas; *Trypanosoma cruzi*; Plantas do cerrado brasileiro; Óleo essencial; Fármacos antiparasitários.

Abstract

Chagas disease (CD) is a major neglected tropical disease that is caused by *Trypanosoma cruzi* (Chagas, 1909). CD is treated with the antiparasitic drugs nifurtimox and benznidazole, which have several side effects. The market for natural products has grown and the use of plants has emerged as an alternative for the development of novel drugs to cure this disease. In this context, this study reports the chemical analysis of the essential oils from the leaves of *Zanthoxylum riedelianum* (Rutaceae), *Zanthoxylum rhoifolium* (Rutaceae) and K*ielmeyera coriacea* (Calophyllaceae) and the fruits of *Xilopia aromática* (Annonaceae) and *Zanthoxylum rhoifolium* (Rutaceae), as well as the trypanocidal and cytotoxic activities of these. The chemical analysis of the oils was performed by gas chromatography coupled to mass spectrometry. The trypanocidal assay was performed on trypomastigote forms of *Trypanosoma cruzi*, and the evaluation of the cytotoxic activity was performed on the $LLCMK_2$ cell line. The essential oils with stronger trypanocidal activity were those from the leaves of *K. coriacea* (IC_{50} = 6.4 µg.mL^{-1}) and fruits of *X. aromatica* (IC_{50} = 6.4 µg.mL^{-1}), followed by those from the fruits of *Z. rhoifolium* (IC_{50} = 8.1 µg.mL^{-1}). The latter two essentials oils showed a better selectivity index (SI). Additionally, sabinene may be responsible for these properties since it is the major compound present in this essentials oils. The cytotoxic analysis indicated that all of the essential oils evaluated were toxic to the $LLCMK_2$ cells at concentrations higher than 100 µg.mL^{-1} and therefore they are excellent candidates for the development of novel antiparasitic drugs.

Keywords: Chagas disease; *Trypanosoma cruzi*; Brazilian Cerrado plants; Essential oil; Antiparasitic drugs.

Resumen

Una de las principales enfermedades tropicales desatendidas es la enfermedad de Chagas (EC), causada por *Trypanosoma cruzi* (Chagas, 1909). El tratamiento de la EC se realiza con medicamentos antiparasitarios nifurtimox y benznidazol que tienen varios efectos secundarios. El mercado de productos naturales ha estado creciendo cada año y el uso de plantas se ha destacado como una alternativa para el desarrollo de medicamentos para curar esta enfermedad. En este contexto, el presente trabajo describe el estudio químico de los aceites esenciales de las hojas de *Zanthoxylum riedelianum* (Rutaceae), *Zanthoxylum rhoifolium* (Rutaceae) y K*ielmeyera coriacea* (Calophyllaceae) y de los frutos de la *Xilopia aromática* (Annonaceae) e *Zanthoxylum rhoifolium* (Rutaceae), así como evalúa su capacidad tripanocida y sus efectos citotóxicos. Los análisis químicos de los aceites se realizaron por

cromatografía de gases acoplada a un espectrómetro de masas. Se realizaron ensayos biológicos en formas de tripomastigote de *Trypanosoma cruzi*, y la evaluación de la actividad citotóxica se realizó en células de la cepa $LLCMK_2$. Los aceites esenciales con la mayor actividad tripanocida fueron los de las hojas de *K. coriacea* (IC_{50} = 6.4 µg.mL^{-1}) y de los frutos de *X. aromatica* (IC_{50} = 6.4 µg.mL^{-1}), seguidos del aceite esencial obtenido de los frutos de *Z. rhoifolium* (IC_{50} = 8.1 µg.mL^{-1}). Los últimos aceites esenciales mostraron el mejor índice de selectividad (SI). Además, el sabinene puede ser responsable de estas propiedades, ya que es el principal compuesto presente en estos aceites esenciales. El análisis citotóxico indicó que todos los aceites esenciales evaluados eran tóxicos para las células $LLCMK_2$ en concentraciones superiores a 100 µg.mL^{-1} y, por lo tanto, son excelentes candidatos para el desarrollo de nuevos fármacos antiparasitarios.

Palabras clave: Enfermedad de Chagas; *Trypanosoma cruzi*; Plantas del cerrado brasileño; Aceite esencial; Drogas antiparasitarias.

1. Introduction

One of the major neglected tropical diseases is Chagas disease (CD), which was discovered and described by Carlos Ribeiro Justiniano das Chagas (Carlos Chagas) in 1909. Human CD is caused by *Trypanosoma cruzi* Chagas, a flagellated protozoan that requires two hosts to complete its life cycle (Brasil, 2019; Rey, 2013). It is estimated that eight to ten million people have acquired CD, mainly in Latin America, with an annual increase of more than 40,000 new cases and resulting about 12,500 annual deaths since 2006 (WHO, 2020).

In Brazil, the region of high prevalence of human CD covers an area of three million square kilometers from the state of Maranhão to Rio Grande do Sul. This area comprises approximately 2,450 municipalities, with more than 28 million people at risk of contamination (Cimerman & Cimerman, 2010)

CD is treated with the antiparasitic drugs nifurtimox and benznidazole, with cure rates of 60%–80% in the acute phase of the disease. In Brazil, only benznidazole has been approved by the Brazilian Health Surveillance Agency (Agência Nacional de Vigilância e Inspeção Sanitária – ANVISA) because nifurtimox has several side effects (Dias et al., 2016). However, drug therapy against CD is partially effective despite the efforts of the scientific community. Several drugs have been tested without positive results for the permanent cure of patients, owing to the natural resistance of some parasite populations, among other factors (Neves, 2011).

The market for natural products such as extracts, isolated compounds, and essential oils from plants has increased annually, and the use of plants is an alternative for the development of novel drugs for the treatment of various diseases, including CD, in view of the great potential of these compounds (de Melo et al., 2017; Lee et al., 2020; Moraes et al., 2020). The *in vitro* activity of the essential oil of fruits of *Piper aduncum* L. (Piperaceae) and *Piper hispidinervum* C. DC. (Piperaceae) on trypomastigotes and amastigotes of *Trypanosomacruzi* cultured on a $LLCMK_2$ cell culture line was shown to be effective (L. H. V Silva, 2015). A study conducted by Carneiro et al. (2017) with essential oil from flowers of *Eugenia klotzschiana* Berg. (Myrtaceae) showed potential trypanocidal activity against trypomastigotes.

The Cerrado (Brazilian savannah) is a Brazilian natural heritage owing to its diversity and endemism of biological species, and it is an important source of novel natural substances and essential oils with different biological properties (Estevam et al., 2016).

The species *Zanthoxylum riedelianum* Engl. (Rutaceae), *Zanthoxylum rhoifolium* Lam. (Rutaceae), *Kielmeyera coriacea* Mart. & Zucc. (Calophyllaceae), and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) are present in the Brazilian Cerrado (Lorenzi, 2014), and all of these species have been shown to exhibit antiparasitic activity (Facundo et al., 2003; Pereira et al., 2010; Sobral et al., 2009). In this context, this study aimed to analyze the chemical composition of the essential oils obtained from the leaves of *Zanthoxylum riedelianum*, *Zanthoxylum rhoifolium* and *Kielmeyera coriacea* and the fruits of *Xylopia aromatica* and *Zanthoxylum rhoifolium* as well as to evaluate their trypanocidal and cytotoxic activities.

2. Methods

2.1 Collection of plant material

All species were collected in the period between October 2013 and June 2014 in riparian areas in a rural area of Iporá (Goiás State, Brazil) and were identified by Dr. Vania Sardinha dos Santos Diniz, professor at the Goiano Federal Institute (Instituto Federal Goiano), Iporá campus. All of the collected plant material was transported fresh to the Organic Chemistry Laboratory of the Federal Institute of Goiás, Iporá campus, where the procedures of raw material preparation and essential oil extraction were performed.

2.2 Extraction of essential oils

To obtain the essential oils the steam distillation technique was used. For this, 300 g of each fresh plant sample, composed of leaves of *Zanthoxylum riedelianum*, *Zanthoxylum rhoifolium* and *Kielmeyera coriacea*, and fruits of *Xylopia aromatica* and *Zanthoxylum rhoifolium* was ground and transferred to a 3,000 mL round bottom flask containing 1,500 mL of water; the flask was placed on a heating mantle and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The hydrolate was extracted three times using 30 mL of dichloromethane solvent per extraction, and the organic phase was separated with a separating funnel. Subsequently, the solution was dried with anhydrous sodium sulfate for the complete removal of water and it was filtered. The solvent was removed under vacuum using a rotary evaporator. The essential oils were transferred to previously clean and sterilized glass bottles wrapped in aluminum foil and was stored in the freezer to prevent degradation by light and temperature until analysis. The percent yield was calculated by correlating the essential oil mass obtained and the mass of fresh plant material used in the extraction.

2.3 Chemical analysis of the essential oil

The chemical constituents of the essential oils were analyzed in the Organic Chemistry Laboratory of the Federal University of São Carlos (Universidade Federal de São Carlos–UFSCar) using a Shimadzu QP 5000 GC gas chromatograph equipped with a fused-silica capillary column (OPTIMA®- 5 0.25 µm; 30 m x 0.25 mm) and an electron impact (EI) ionization detector (70 eV). Briefly, a 1.0 µL aliquot of the solution containing 10 µL essential oil in 1 mL of acetone was injected. An initial temperature of 50 °C was maintained for 4.5 min. The temperature was programmed to increase 8 °C/min to 190 °C, and the solution was maintained at this temperature for 1 min. Next, the temperature was programmed to increase 15 °C/min to 250 °C, and then, the solution was maintained at this temperature for 1 min. The run time was 28 min. The other parameters were as follows: injector temperature of 250 °C, interface temperature of 280 °C, injection pressure of 80 kPa, Np Splitless mode of 30 s, scan range of the mass spectrometer of 43–450 m/z, start time of 5 min, solvent cut time of 3 min and flow rate of 1.4 mL/min.

The constituents of the essential oils were identified using the Kovats index. A standard mixture of n-alkanes (C_9–C_{26}) was used to evaluate the performance of the GC-MS system and to calculate the Kovats index of each compound in the sample. A 1.0 µL aliquot of each alkane was injected using the same experimental conditions as the study samples, and the corresponding retention times were used as an external reference standard to calculate the

Kovats index. To assist in the identification and characterization of the compounds, the values obtained were compared with those from the literature for the same column (DB-5) used for the samples.

2.4 Evaluation of *in vitro* trypanocidal activity

The assays on trypomastigotes were performed in the Parasitology Laboratory of the University of Franca (Universidade de Franca - UNIFRAN), state of São Paulo, using parasites cultured in the $LLCMK_2$ cell line derived from monkey kidney fibroblasts. Cells were cultured in RPMI medium supplemented with 2 x 10^{-6} mol/L of L-glutamine, 10^{-5} mol/L of $NaHCO_3$, 100 U/mL of penicillin, 100 mg/L of streptomycin, and 10% inactivated fetal bovine serum in culture bottles at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. The trypomastigotes were obtained in the parasitemia peak from the blood of animals infected by cardiac puncture and were added to the cell culture. After seven days of cultivation, the culture supernatant was collected and centrifuged to obtain the free parasite forms used in the assays.

Approximately $1x10^6$ trypomastigotes were added to a 96-well microtiter plate, followed by the addition of the essential oils at concentrations of 200, 100, 50, 25, and 12.5 $\mu g.mL^{-1}$. After a 24 h incubation, the biological activity was evaluated by direct quantitation of live parasites in a Neubauer chamber through the observation of the flagellar motility of the protozoa.

Samples containing benznidazole and 25% dimethyl sulfoxide (DMSO) were used as positive controls, and samples containing 0.5% DMSO were used as negative controls. All assays were performed in triplicate in three independent experiments.

The results were evaluated using GraphPad Prism software version 5.0 (GraphPad Software, San Diego, CA, USA) to obtain the IC_{50} values. The percentage of lysis achieved by different concentrations of each essential oil was calculated relative to the negative control.

2.5 Evaluation of cytotoxic activity

Fibroblasts of the $LLCMK_2$ cell line were cultured in RPMI 1640 medium supplemented with 100 mL of 10% inactivated fetal bovine serum containing 5 mL of antibiotic, in culture bottles at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. On the day of the experiment, an aliquot of the cell culture was transferred to a sterile Falcon tube using a pipette tip. Subsequently, the Falcon tube was centrifuged at 4 °C at 1,500 rpm

for 15 minutes. After centrifugation, the supernatant was collected by inversion, and 1 mL of complete RPMI was transferred to the tube to adjust the cell count to 10^6.

Approximately $1x10^6$ cells were added to a 96-well microtiter plate, followed by the addition of the essential oil at concentrations of 400, 200, 100, 50, 25, 12.5, and 6.25 $\mu g.mL^{-1}$. DMSO samples at 25% and 0.5% were used as positive and negative controls, respectively. After this procedure, the plate was incubated in a CO_2 incubator for 24 h. After the incubation period, 5 mg/mL of MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well, and the plate was incubated for an additional 4 h.

At the end of this period, the plate was read in an ELISA reader using MTT as the colorimetric technique. The assays were performed in triplicate in three independent experiments. The 50% cytotoxic concentration (CC_{50}) was calculated using regression analysis. The selectivity index (SI) was calculated using the CC_{50} to IC_{50} ratio.

2.6 Ethical Standards

All experiments that involved the use of laboratory animals were performed in accordance with the ethical standards of the Oswaldo Cruz Foundation and were approved by the Research Ethics Committee University of Franca 010/145 (Comitê de Ética da Universidade de Franca 010/145).

3. Results and Discussion

The main compound found in the essential oil of the fruits of *Xylopia aromatica* was sabinene (69.72%). The compounds found in the essential oil of the fruits of *Zanthoxylum rhoifolium* were sabinene (55.94%) and D-germacrene (17.12%), and the leaves contained β-elemene (31.26), D-germacrene (18.16%), α-farnesene (15.05%), and β-caryophyllene (12.09%). The leaves of *Zanthoxylum riedelianum* contained bicyclo [5.3.0] decane-2-methylene-5-(1-methylvinyl)-8-methyl (24.81%) and α-bisabolene epoxide (16.18%). The leaves of *Kielmeyera coriacea* contained n-tetradecane (13.46%) and 1,6-cyclodecadiene (12.08%). The results obtained by the chemical analysis of the evaluated essential oils are shown in Table 1.

Table 1: Qualitative and quantitative composition (%) of the main compounds present in the essential oils of *X. aromatica*, *Z. rhoifolium*, *Z. riedelianum* and *K. coriacea* as analyzed by GC/MS.

Constituents	***IK Cal.**	****IK Lit.**	***X. aromatica* Fruit**	***Z. rhoifolium* Fruit**	***Z. rhoifolium* Leaf**	***Z. riedelianum* Leaf**	***K. coriacea* Leaf**
4,7,7-trimethylbicyclo [4.1.0] heptane-3-ol	1329.2	NF	-	-	0.32	-	-
1R-**α**-pinene	NI	NF	6.85	-	-	-	-
α-thujene	930	931	5.12	-	-	-	-
α-pinene	935	933	3.39	-	-	-	-
β-myrcene	947	991	3.69	8.03	-	-	-
cis-β-ocimene	962	1040	0.22	-	-		-
α-phellandrene	964	1005	2.91	-	-	-	-
Limonene	987	1031	7.09	-	-	-	-
Sabinene	989	976	69.72	55.94	-	-	-
Isolendene	1282.0	NF	-	-	-	-	2.75
Caryophyllene	1328.8	NF	-	-	-	-	10.04
2-undecanone	1336.2	NF	-	1.95	-	-	-
bicyclo [5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-	1338.6	1432	-	-	-	24.81	-
δ elemene	1337.9	1335	-	2.33	7.63	-	-
1H-cyclopropene-azulene, decahydro-1,1,7-trimethyl	1348.1	NF	-	-	-	-	3.75
Azulene	1354.4	NF	-	-	-	-	2.38
Geranyl acetate	1362.3	1379	-	3.39	0.75	-	-
Cis α-bisabolene	1366.0	NF	-	-	-	-	3.49
1H-cyclopentanol	1370.0	NF	-	-	-	-	1.09
α-cubebene	1378.9	1387	-	3.69	0.44	-	-
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-dimethyl	1382.0	NF	-	-	-	-	4.30
2,4-diisopropenyl-1-methyl-1-vinylcyclohexane	1382.6	NF	-	0.22	2.08	-	-
β-bourbonene	1386.1	1387	-	-	-	-	2.29
β-elemene	1390.0	1389	-	3.96	31.26	-	-
1,6-cyclodecadiene	1390.1	NF	-	-	-	-	12.08
Naphthalene,1,2,4a,5,8,8a-hexahydro-4,7-dimethyl	1394.1	NF	-	-	-	-	2.44
n-tetradecane	1399.4	1340	-	-	-	-	13.46
(+)-Cycloisosativene	1404.6	NF	-	-	-	-	11.55
β-caryophyllene	1419.6	1408	-	1.73	12.09	-	-
δ-cadinene	1419.9	1439	-	-	0.71	2.49	-
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl	1424.7	NF	-	-	-	-	11.72
β-cedrene	1430.0	1419	-	-	6.69	-	3.76
cis-nerolidol	1430.8	1478	-	-	0.28	2.12	-
Seichelene	1444.1	1446	-	-	1.91	-	-
α-muurolene	1444.5	NF	-	-	-	-	2.24
Spathulenol	1445.0	NF	-	-	-	0.35	-
Cyclohexene	1447.4	NF	-	0.45	-	-	-
β-farnesene	1448.5	1454		-	0.42	-	2.75
E-caryophyllene	1452.5	1464	-	-	3.63	-	-

Caryophyllene oxide	1453.0	NF	-	-	-	4.10	-
7,11-dimethyl-3-methylene-1,6,10-dodecatriene	1456.8	NF	-	2.56	-	-	-
Tau-cadinol	1462.0	1475	-	-	0.82	-	1.08
1,1,7-trimethyl-4-methylenedecahydro-1H-cyclopropane azulene	1473.6	NF	-	-	0.56	-	-
D-germacrene	1479.1	1484	-	17.12	18.16	1.90	-
Eudesmol	1479.3	1484	6.52				
Isopulegol	1485.4	NF	-	-	-	1.08	-
Cedrene	1489.6	NF		-	0.36	-	
Bicyclogermacrene	1493.8	1500		-	4.57	3.68	-
α-bisabolene epoxide	1494.4	1500	-	-	-	16.18	-
α-farnesene E	1502.5	NF	-	-	15.05	0.32	-
5-azulene methanol	1507.2	NF	-	-	-	-	1.20
D-cadinol	1508.8	NF	-	-	-	0.25	-
Bicyclo [4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl	NI 1516.9	NF	-	-	-	0.95	-
α-muurolol	1517.5	1522	-	-	-	1.40	-
β-bisabolene	1523.9	1509	-	1.25	-	-	
Cubenol	1535.0	1537	-	-	-	-	1.39
3-hexene-1-ol benzoate	1545.8	NF		-	0.16		-
Nerolidol E	1549.0	1561	-	-	0.55	-	-
1-naphthalenol	1550.9	NF	-	-	-	-	3.19
Viridiflorol	1577.1	1592	-	-	0.12	0.39	-
1-hexanol, 2-(hydroxymethyl)-	1577.7	NF	-	-	-	0.55	-
1,2-15,16-diepoxyhexadecane	1584.7	1600	-	-	0.25	-	-
1,11-undecanediol	1585.1	1592	-	-	-	0.65	-
Palmitaldehyde	1593.2	1619	-	-	-	0.20	-
2-isopropenyl-5-methyl-6-heptene-1-ol	1596.2	NF	-	-	0.13		-
α-linolenic acid	1596.6	NF				0.15	
2-nitro-2-heptene-1-ol	1602.6	NF	-	-	0.13	-	-
cis-Z-α-bisaboleneepoxide	1613.3	NF	-	-	0.52	-	-
Tau-muurolol	1628.9	1644	-	-	0.68	-	3.54
5,6-dimethyldecane	1629.3	1644	-	-	-	0.51	-
α-cadinol	1641.1	1652	-	-	2.09	8.26	-
Hexadecanal	1837.0	NF	-	-	0.24		-
Phytol	1999.6	1942	-	-	1.26	1.58	-
2,4-dimethyl-1-heptanol	2024.9	NF	-	-	0.17	-	-

* = Retention index calculated using the equation of Van den Dool and Kratz.

** = Retention index from the literature (Adams, 2007) based on the equation of Van den Dool and Kratz.

NI = Not identified.

NF = Not found.

Fonte: Elaboração dos autores.

All of the tested essential oils showed inhibitory activity, causing the loss of viability of trypomastigotes at all concentrations tested (Table 2). The essential oils that exhibited

stronger trypanocidal activity were those extracted from the leaves of *K. coriacea* (IC_{50} = 6.4 µg.mL^{-1}) and the fruits of both *X. aromatica* (IC_{50} = 6.4 µg.mL^{-1}) and *Z. rhoifolium* (IC_{50} = 8.1 µg.mL^{-1}). The essential oils from the leaves of *Z. riedelianum* (IC_{50} = 13.1 µg.mL^{-1}) and *Z. rhoifolium* (IC_{50} = 29.5 µg.mL^{-1}) showed excellent activity, although their inhibitory concentration values were higher than those of the benznidazole positive control (IC_{50} = 9.8 µg.mL^{-1}). The cytotoxicity analysis indicated that all of these essential oils were toxic to the cells at concentrations higher than 100 µg.mL^{-1}. This indicates that all the essential oils evaluated have low cytotoxicity. The selectivity index (SI), calculated by the ratio between the CC_{50} values of the host cells ($LLCMK_2$cell line) and the IC_{50} values for the parasites, indicates that the oils of the fruits of *X. aromatica* and *Z. rhoifolium* are excellent candidates for the development of novel antiparasitic drugs.

Table 2: Trypanocidal and cytotoxic activities* and selectivity index of the essential oils of *Kielmeyera coriacea*, *Xylopia aromatica*, *Zanthoxylum rhoifolium* and *Zanthoxylum riedelianum*.

Essential oils	**Trypomastigotes IC_{50} (µg.mL^{-1})**	***$LLCMK_2$ fibroblasts CC_{50} (µg.mL^{-1})**	**Selectivity Index (SI)**
K. coriacea (leaves)	6.4	262.1	40.9
X. aromatic (fruits)	6.4	392.6	61.3
Z. rhoifolium (fruits)	8.1	365.0	45.1
Z. rhoifolium (leaves)	29.5	215.9	7.3
Z. riedelianum (leaves)	13.1	116.3	8.9

* Cytotoxic activity

Fonte: Elaboração dos autores.

Previous studies have reported that the major compound of the essential oil of *Piper cubebais* sabinene (19.99%), which is also one of the main compounds present in the oil of the fruits of *X. aromatica* (69.72%) and *Z. rhoifolium* (55.94%). The results of the *in vitro* trypanocidal assay against trypomastigote forms cultured in the $LLCMK_2$ cell line using the essential oil of *Piper cubeba* were positive, showing an inhibitory activity with IC_{50} values of 45.5 µg.mL^{-1} (Esperandim et al., 2013).

Borges et al. (2012) reported that the LC_{50}/24h values of essential oils from *Lippia sidoides*, *Ocimum gratissimum*, *Chenopodium ambrosioides*, and *Lippia origanoides* from the Brazilian northeast region were 10.3, 11.5, 28.1, and 39.7 µg.mL^{-1}, respectively, for

trypomastigotes. All of the essential oils they studied were toxic for mammalian cells at concentrations greater than 100 µg.mL^{-1} and, therefore, they were considered non-cytotoxic. The CC_{50} values for the essential oils of *Vitexagnus-castus*, *Chenopodium ambrosioides*, *Lippia sidoides*, *L. origanoides*, *Justicia peitorais*, and *Ocimum gratissimum* were 617.9, 275.6, 192.7, 175.7, 176.9, and 180.4 µg.mL^{-1}, respectively.

A study of the essential oils of oregano, thyme, and thymol (the main constituent of thyme) at different concentrations showed remarkable trypanocidal activity against epimastigote and trypomastigote forms. The essential oil of oregano inhibited the growth of epimastigotes with an IC_{50}/24 h of 175 µg.mL^{-1} and induced trypomastigote lysis at 115 µg.mL^{-1}. The essential oil from thyme exhibited an IC_{50}/24 h of 77 µg.mL^{-1} for epimastigotes and of 38 µg.mL^{-1} for trypomastigotes, whereas thymoloil had an IC_{50}/24h of 62 µg.mL^{-1} for epimastigotes and of 53 µg.mL^{-1} for trypomastigotes. The data indicate that the essential oils of oregano and thyme are effective against *T. cruzi*, with greater activity of thyme oil; therefore, thymol may be the main compound responsible for the trypanocidal activity. Thyme oil did not exhibit cytotoxic activity against mouse macrophages at concentrations that were at least 1.5–2.0 times higher than the IC_{50} values, which corresponded to 38 µg.mL^{-1} for bloodstream trypomastigotes (Santoro et al., 2007).

The essential oils of the leaves of *Xylopia frutescens* and two specimens of *X. laevigata* (XLMC and XLSI) were evaluated for their trypanocidal activity against the Y strain of *T. cruzi* and showed IC_{50} values lower than 30 µg.mL^{-1} and 15 µg.mL^{-1} against epimastigotes and trypomastigotes, respectively. These essential oils also decreased the number of macrophages infected with *T. cruzi in vitro* and amastigote forms at non-cytotoxic concentrations for macrophages (Da Silva et al., 2013).

4. Conclusions

This study reports high trypanocidal activity associated with the essential oils of the leaves of *Zanthoxylum riedelianum*, *Zanthoxylum rhoifolium* and *Kielmeyera coriacea* and fruits of *Xylopia aromatica* and *Zanthoxylum rhoifolium*; furthermore, these oils exhibited low toxicity to mammalian cells, making these oils promising antiparasitic drug candidates. Considering their trypanocidal activity, the essential oils that were most effective and worthy of further investigation are the fruit of *X. aromatica* and *Z. rhoifolium*. In addition to *in vivo* biological assays, additional studies are required to elucidate the mechanisms of death of the parasites induced by the most promising essential oils and to identify the intracellular targets

of the oils.

5. Acknowledgements

To the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), Goiás State *Research Foundation (Fundação de Amparo à Pesquisa do Estado de Goiás* – FAPEG) and Coordination for the Improvement of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES) for financial support.

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