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# Chemical Composition and Anti-*Candida* and Anti-*Trypanosoma cruzi* Activities of Essential Oils from the Rhizomes and Leaves of Brazilian Species of *Renealmia* L. fil.

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**Abstract:** This work aimed to determine the chemical composition of essential oils from rhizomes and leaves of *Renealmia chrysotricha* Petersen, *R. breviscapa* Poepp. & Endl. and *R. nicolaioides* Loes., and to evaluate the biological activities of these oils on three *Candida* species and the parasite *Trypanosoma cruzi*. The rhizomes and leaves were collected in the Atlantic and Amazon rainforests. Essential oils were isolated and characterized by gas chromatography. *Beta*-Caryophyllene was found to be the most predominant compound in the essential oils of rhizomes and leaves of *R. breviscapa*, and the rhizomes of *R. nicolaioides*, whereas (*E*)-nerolidol was the most abundant compound in the oils of leaves of *R. nicolaioides*. In *R. chrysotricha*,  $\alpha$ -terpineol, coronarin-E and 1,8-cineole were found to be the most predominant compounds in the essential oils of rhizomes, whereas *cis*-3-hexenol was predominant in the leaves. The tested oils did not inhibit *C. albicans* growth at 1000 µg/mL, whereas leaf oils from *R. chrysotricha* and *R. nicolaioides* inhibited the growth of *C. buinensis* and *C. tropicalis* by about 50%. Essential oils from the rhizomes and leaves of *R. chrysotricha* exhibited efficient antiparasitic activity against *Trypanosoma cruzi*. Damage to *T. cruzi* epimastigotes was confirmed by LM and TEM.

**Keywords:** Zingiberaceae; rhizomes; leaves; terpenoids; anticandidal activity; antiparasitic activity. © 2018 ACG Publications. All rights reserved.

# 1. Introduction

Fungal and protozoan diseases are widely disseminated and very common among tropical countries. These diseases are difficult to treat because the pathogens can develop resistance to

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medications or because of side effects of treatments [1], and so natural products derived from plants have been used as a source of replacement drugs [2].

Plants of the family Zingiberaceae are well known for producing essential oils (EOs) that are used to prevent and control several diseases, according to descriptive and biological studies [3-9].

The activity of essential oils from the rhizomes of nine species of Zingiberaceae (Zingiber officinale Rosc., Z. cassumunar Roxb., Z. zerumbet Smith, Curcuma aeruginosa Roxb., C. mangga Valeton and van Zyp, C. xanthorrhiza Roxb., Kaempferia galanga Linn., Alpinia galanga Swartz and Boesenbergia pandurata (Roxb.) Schlecht) against five dermatophytic fungi, three filamentous fungi and five yeast strains, including Candida albicans and C. tropicalis, obtained from the Institute for Medical Research Kuala Lumpur, was evaluated [3]. The essential oils from Zingiber officinale and Z. cassumunar showed the greatest activity against species of Candida, forming an inhibition zone of 11.7-15.7 mm. The rhizome essential oil of Zingiber nimmonii was found to inhibit the fungi Candida glabrata, C. albicans and Aspergillus niger, with the inhibition zones reaching 9-12 mm in diameter [4].

The essential oils from different parts (leaf blade, leaf sheath and rhizome) of *Aframomum* sceptrum (Oliv. & T. Hanb) K. Schum. did not inhibit the growth of *C. albicans* [5], whereas rhizome oil of *Curcuma longa* L. exhibited moderate activity (MIC =  $0.625 \ \mu g/mL$ ) against 11 *Candida* albicans strains and one *C. glabrata* strain isolated from human clinical material [6].

The antifungal and antiparasitic activity of rhizome essential oil from *Aframomum spectrum* (Oliv. and D. Hanb) K. Schum., as well as that of its major compounds ( $\beta$ -pinene and caryophyllene oxide) [7]. The authors concluded that the oil exhibited moderate activity against *C. albicans*, and high activity against *Trypanosoma brucei brucei*.

The activity of rhizome and leaf essential oils from *Hedychium coronarium* J. König., as well as the activity of their major compounds against the procyclic *Trypanosoma brucei*. Caryophyllene oxide exhibited the greatest activity, with its lethal concentration for 50% of parasites varying between 24.53 and 65.77  $\mu$ g/mL for a variety of strains [8].

*Renealmia* L. fil. (Zingiberaceae) comprises 75 herbaceous and rhizomatous species from the Neotropical region and Africa [10]. A review by Negrelle [11] addressed botanical, ecological, pharmacological and agronomic aspects of the species of *Renealmia*, and emphasized that few of the species have had their chemical composition evaluated. In Brazil, this genus is represented by 21 species distributed in all phytogeographic domains and in all regions of the country, 14 of which are found in the Northern Region [12]. Recent studies with essential oil-producing species of the Amazon region have been performed with the purpose of evaluating biological activities, such as antimicrobial, antioxidant and cytotoxic behaviors [13,14].

A previous study by Luz et al. [15] elucidated the composition of leaf oil of *R. floribunda* K. Schum., and found  $\beta$ -pinene to be the predominant compound. For *Renelamia alpinia* (Rottb.) Maas,  $\beta$ -caryophyllene, allo-aromadendrene and  $\beta$ -pinene were detected in stems, leaves and fruits, respectively [16]. For leaves of *R. thyrsoidea* (Ruiz &Pav.), terpinolene and  $\alpha$ -phellandrene were identified as major, and were found to exhibit antimicrobial activity against gram-negative bacteria, and antioxidant activity via the ABTS method [17].

The present work aimed to determine the composition of essential oils from leaves and rhizomes of *Renealmia chrysotricha* Petersen, *R. breviscapa* Poepp. & Endl. and *R. nicolaioides* Loes., and to evaluate the biological activities of these compounds on different yeasts and the parasite *Trypanosoma cruzi*.

## 2. Materials and Methods

# 2.1. Plant Materials

Rhizomes and leaves of *R. chrysotricha* were collected in the Parque Nacional de Itatiaia in May of 2015, while those for *R. breviscapa* and *R. nicolaioides* were collected at the Fazenda Experimental Catuaba  $(10^{\circ}4'40"S \times 67^{\circ}37'35"W)$  and in the Reserva Florestal Humaitá  $(9^{\circ}45'17"S \times 67^{\circ}40'15"W)$  located in Rio Branco, state of Acre, in June of 2015. The material was identified and deposited in the Herbarium of the Universidade Federal Rural do Rio de Janeiro (RBR), voucher 33416 for *R.* 

*chrysotricha*, and in the Herbarium of the Universidade Federal do Acre (UFACPZ), vouchers 6645 and 6646 for *R. breviscapa* and *R. nicolaioides*, respectively.

# 2.2 Extraction of Essential Oils

Fresh rhizomes (115 g  $\pm$  10) and leaves (420 g  $\pm$  20) of *R. chrysotricha, R. breviscapa* and *R. nicolaioides* were hydrodistilled using a modified Clevenger Extractor [18]. The extraction was carried out for 2 h. The obtained oil layers were separated and dried over anhydrous sodium sulphate, stored in hermetically sealed glass containers and kept under refrigeration at 4 °C before analysis. The yield of the essential oil was calculated and expressed as weight of oil per weight of fresh rhizomes.

The oils obtained from rhizomes and leaves were analyzed by analytical gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The GC-FID analyses were performed with a Hewlett-Packard 5890 SERIES II chromatograph equipped with a flame ionization detector and a fused silica capillary column (Factor Four-VF-5ms - 30 m x 0.25 mm i.d. x 0.25 mm film thickness). The analyses were performed first between the programmed temperatures of 60-260 °C (increments of 3 °C/min), and subsequently from 10°C/min to 290 °C, using helium as carrier gas at a flow rate of 1 mL/min, with an injector temperature of 220 °C, interface at 310 °C, detector temperature of 280 °C, and the injection of 1.0  $\mu$ L of essential oil diluted in dichloromethane.

The GC-MS analyses were carried out with a Shimadzu GC-EM, GC-17A/QP2010 Plus, with ion source at 220 °C and electron ionization mode at 70 eV. The chromatographic conditions were identical to those described above. The analyses were performed at a scanning speed of 0.5 scan/s with a m/z 40–550 scanning layer. The relative amount (%) of each oil component was expressed as a percentage of signal area in relation to the total signal area of the oil.

The components of the essential oils were characterized by comparing their spectra with a database, with literature records and by calculating the Kovat's retention index [19-21].

## 2.3 Anti-Candida Activity

The yeasts *Candida albicans* (CE022), *Candida buinensis* (3982) and *Candida tropicalis* (CE017) were cultivated in Sabouraud medium [peptone 10 g/L, D-(+) glucose 20 g/L, agar-agar 17 g/L; Merck S/A], maintained at 23 °C and stored at the Laboratório de Fisiologia e Bioquímica de Microrganismos, in the Centro de Biociências e Biotecnologia, of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, Rio de Janeiro, Brazil.

*Yeast cells (C. albicans, C. buinensis* and *C. tropicalis inoculates)* were removed from the tubes containing Sabouraud agar and transferred to Petri dishes containing the same agar. The cells were maintained at 30 °C for two days. After this period, the cells were used in the assay by removing colonies with an inoculation loop, adding them to 10 mL of culture medium (Sabouraud broth) and quantifying them using a Neubauer chamber (same as used for quantifying fungal spores) under a Zeiss Axiovision 4 light microscope.

After quantification, the cells of the yeasts *C. albicans*, *C. buinensis* and *C. tropicalis*  $(1 \times 10^4 \text{ cells m/L})$  were incubated in 200 µL of Sabouraud containing a stock solution of the essential oil samples was prepared in 10% DMSO at a concentration of 1000 µg m/L of essential oil from rhizomes or leaves of *R. chrysotricha*, *R. breviscapa* or *R. nicolaioides*.

The assay was performed in 96-well culture plates and incubated at 30 °C for 24 h. To observe inhibition of fungal growth, optical density was calculated from readings with a microplate reader (EZ Read 400, Biochrom), at 620 nm every 6 h. Negative controls without addition of essential oil samples were also determined. The entire assay was done in triplicate and under aseptic conditions in a laminar flow hood following an adaptation of the methodology of [22].

# 2.4 Antiparasitic Activity

Epimastigotes of *Trypanosoma cruzi* (DM28 strain) were cultivated in 5 mL of liver infusion tryptose (LIT) medium supplemented with 10% FCS (fetal bovine serum, Gibco) and 4% of hemin at 28 °C. Every five days, 1 mL of parasites, still at exponential population growth, were placed in a

fresh tube, in which fresh culture medium was added to reach 5 mL. Much research has been performed using the epimastigote of *T. cruzi* because it is easily cultivated *in vitro* and, consequently, widely used in preliminary experimental studies.

For cytotoxicity testing of *R. chrysotricha* essential oil on epimastigotes, different volumes of stock solution were incubated in a 96-well plate to give final concentrations of 25, 100, 400 and 800  $\mu$ g/mL, with the final concentration of DMSO being less than 1.5% at all concentrations. In addition, 100  $\mu$ L of parasites, at a density of 1.3 x 10<sup>6</sup> cells/mL, were added per well and the volume was adjusted to 200  $\mu$ L using culture medium. The plate containing the experiment was incubated at 28 °C for 24 and 48 h. Negative controls without addition of essential oil samples were also determined. Quantification of parasites was done in a Neubauer chamber.

For morphological analysis, epimastigotes of *T. cruzi* were centrifuged at 1700 rpm for 10 min, and washed with PBS (pH 7.2) at room temperature. The parasites were fixed in a solution of 4% paraformaldehyde diluted in PBS, and stained with Giemsa (10% v/v) for 2 h at room temperature. Aliquots of 100  $\mu$ L were spread on microscope slides, dried at 37 °C and examined using a photo-equipped Zeiss Axioplan light microscope. The images were obtained using the software Axiovision (ZEISS).

For analysis of ultrastructure, the samples treated with 100 µg/mL of aqueous extract for 24 h were centrifuged for 10 min at 1700 RPM, washed with PBS for 10 more min and re-centrifuged at the same RPM. Fixation was performed using 4% formaldehyde, 1% glutaraldehyde, 0.2 M sodium cacodylate buffer, 1.5 mL distilled water and 5% sucrose for 1 h at room temperature. The samples were then centrifuged again for 10 min at 1700 RPM and washed with a 0.1 M solution of sodium cacodylate buffer. The resulting pellet was post-fixed with 2% osmium tetroxide and 0.8% potassium ferrocyanide for 1.5 h at ambient temperature and protected from light. The post-fixed samples were washed in sodium cacodylate buffer, centrifuged twice and dehydrated in an increasing series of acetone. After dehydration, the samples were incubated in a solution of 100% acetone-epon resin in proportions of 2:1, 1:1, 1:2 and pure epon resin, for 6 h each step. The material was then embedded in epon (Epoxy Resin) and polymerized in a kiln at 60 °C for 48 h. Ultrathin sections were obtained using an ultramicrotome (Reichert Ultracuts Leica Instruments®) and contrasted with 5% aqueous uranyl acetate for 20 min in the dark and lead citrate for 5 min. The material was observed and photographed with a Zeiss TEM 900 transmission electron microscope operating at an accelerating voltage of 80 kV.

#### 3. Results and Discussion

## 3.1. Essential Oil Composition

The essential oil yields for *R. chrysotricha*, *R. breviscapa* and *R. nicolaioides* were 0.024%, 0.012% and 0.018% for rhizomes, and 0.0075%, 0.008% and 0.017% for the leaves, respectively. Tables 1 and 2 show the oil constituents.

Nineteen constituents were identified in the rhizome essential oil of *R. chrysotricha*, which corresponded to 92.98% of all the oil, with the major constituents being  $\alpha$ -terpineol, coronarin E and 1,8-cineole (Table 1). The monoterpene  $\alpha$ -terpineol has been referred to as both antinociceptive [26] and bactericidal [27]. The compound 1,8-cineole has been found to be profuse in rhizome essential oils of species of Zingiberaceae [8,28], and exhibits anti-inflammatory and antinociceptive activities [29]. The labdane diterpene coronarin E, also classified as a furanolabdanoid, was first isolated from the rhizome of *Hedychium coronarium* Roscoe [30], and later from *H. gardnerianum* Roscoe, *H. ellipticum* Buch.-Ham. ex Sm., *H. longicornutum* Bak., and *H. spicatum* [31-34]. This compound was also isolated from other genera of the same family, including *Aframomum, Alpinia, Amomum* and *Curcuma* [34-37]. This diterpene exhibited moderate activity against *Mycobacterim tuberculosis*, as well as cytotoxic activity [32,33].

KI <sup>a</sup>	KI <sup>b</sup>	Compound <sup>c</sup>	Co	Composition %		
		-	Rc	Rb	Rn	
		Oxygenated monoterpenes				
1036	1026-1032 <sup>b-d,g</sup>	1.8-cineole	15.87			
1073	1067-1075 <sup>b-d</sup>	cis-linalool oxide		_	$\overline{0}.20$	
1074	1066-1070 <sup>b,g</sup>	cis-sabinene hydrate	0.67	_		
1090	1084 <sup>c.d</sup>	trans-linalool oxide		_	$\overline{0.23}$	
1096	1095-1101 <sup>b-d,h,g</sup>	linalool	$\bar{1.49}$	_	1.71	
1103	1098 <sup>c,g</sup>	<i>trans</i> -sabinene hydrate	0.85	_		
1124	1118 <sup>c,d</sup>	exo-fenchol	0.08	_	$\overline{0.80}$	
1129	1136 <sup>c</sup>	menth-2-en-1-ol. trans-p-	0.41	_		
1146	$1140^{b,d}$	<i>trans</i> -pinocarveol	1.08	_	$\bar{1.48}$	
1161	1144 <sup>b,d</sup>	<i>cis</i> -verbenol		_	1.01	
1168	1145-1149 <sup>b-d</sup>	camphene hydrate	-	-	0.32	
1168	1160-1161 <sup>b-d</sup>	pinocarvone	$\overline{0}$ 38	-	0.52	
1178	1165-1166 <sup>b-d</sup>	borneol	4 27	-	$\frac{1}{1}47$	
1189	1174-1178 <sup>a-d</sup>	terpinen-4-ol	9.94	_	1.17	
1201	$1186_{-}1100^{a-d.g}$	a-ternine ol	26.14	$\frac{1}{1}$ 10	14 15	
1201	110/ 1105a,c,d	myrtenol	20.14	1.10	0.32	
1214	1207b.d	trang piporital	$\overline{0}$ 25	-	0.32	
1210	1207 1282 1286 <sup>b-d</sup>	isobornyl acetato	0.23	-	$\overline{0}$ 20	
1200	1203-1200	tugung sahinyi acetate	-	-	0.29	
1299	1209 1246 1250a-d	trans-sability acetate	0 45	-	0.05	
1350	1346-1350" "	$\alpha$ -terpinyl acetate	0.45	-	-	
1425	1411 1400a-dfh	Hydrogenated sesquiterpenes	2 00	(2.20	22 70	
1425	$1411-1420^{a-d.f.h}$	$\beta$ -caryophyllene	2.99	62.38	22.78	
1454	1447-1454 <sup>a-u.i.ii</sup>	α-Humulene	0.36	9.56	4.44	
1461	1457	sesquisabinene	0.23	_	<u> </u>	
1479	1460 <sup>c</sup>	dehydro-aromadendrene	_	_	1.29	
1523	1511 <sup>c-a</sup>	∂-amorphene	_	_	0.62	
		Oxygenated sesquiterpenes				
1564	1561-1564 <sup>a-d</sup>	(E)-nerolidol	0.19	3.27	11.06	
1591	1581-1589 <sup>a-d,t,g</sup>	caryophyllene oxide	2.23	9.27	9.90	
1604	1600°	guaiol	_	0.36	0.71	
1619	1601-1608 <sup>c,f,g</sup>	humulene epoxide II	_	1.43	1.67	
1642	1639°	carophylla-4(12).8(13)-dien-5β-ol	_	0.23	0.49	
1650	1640-1643 <sup>a-d</sup>	epi-α-muurolol	_	0.63	0.89	
1664	1642°	selina-3.11-dien-6-α-ol	_	0.57	0.89	
		Carboxylic acid				
1965	1968 <sup>b</sup>	<i>n</i> -hexadecanoid acid	_	0.40	1.61	
		Oxygenated Diterpenes	_			
2027	2026 <sup>c</sup>	(E - E)-geranyl linalool		0.36	0.53	
2146	2136 <sup>e</sup>	coronarin E <sup>#</sup>	$\overline{2}5.1$			
		Oxygenated monoterpenes	61.88	1.10	24.45	
		Hydrogenated sesquiterpenes	3.58	71.94	29.13	
		Oxygenated sesquiterpenes	2.42	15.76	25.61	
		Carboxylic acid		0.40	1.61	
		Oxygenated Diterpenes	$\overline{2}5.1$	0.36	0.53	
		Total	92.98	89.56	81.33	

**Table 1.** Essential oil constituents of rhizomes of *Renealmia* species

**<sup>a</sup>KI**= Kovats indices.

<sup>b</sup>**KI** = Kovats indices of literature. a) [16], b) [19], c) [20], d) NIST08= database, e) [21], f) [22], g [23], h [24].

<sup>c</sup>Compounds listed in order of elution from a VF-5ms column.

**Rc**= *Renealmia chrysotricha*, **Rb**= *R. breviscapa*, **Rn**= *R. nicolaioides*.

# Identify confirmed on the basis of mass spectra: m/z (relative intensity) 284(50.  $M^+$ ). 160(20). 147(100). 137(30). 95(50). 81(80). 69(40). 55(70). 41(40).

Via Kib Compounds Composition 9/							
KI"	KI"	Compound	Composition Re Rh		211 70 Rn		
		Oxygenated hydrocarbons	m	110	AU		
858	850-857 <sup>b-d</sup>	cis-3-hexenol	57.28	15.05	9.45		
866	859 <sup>c,d</sup>	cis-2-hexenol			0.19		
869	863-870 <sup>b-d</sup>	<i>n</i> -hexanol	0.99	_	0.75		
		Hydrogenated hydrocarbons		_			
936	932-940 <sup>a-c,f-h</sup>	$\alpha$ -pinene			0.09		
983	974-982 <sup>a-d,f,h</sup>	<i>B</i> -pinene	-	-	0.16		
991	988-992 <sup>a-d,g</sup>	Mvrcene	-	-	0.10		
1032	1024-1030 <sup>a-d</sup>	Limonene	-	-	0.26		
1002	1021 1000	Oxygenated monoterpenes	-	-	0.20		
1036	1026-1032 <sup>b-d,g</sup>	1 8-cineole	0.33		0.11		
1074	d	5isopropyl2methylbicyclo[3,1,0]hexan-2-ol	0.55	-	0.11		
1103		Linalool	0.10	111	$\bar{0}$ 60		
1105	1099-1101 1098¢,g	trans-sabinene hydrate	$\overline{0}$ 51	1.11	0.00		
1125	1114c,d	endo-fenchol	0.01	-	0 97		
1123	1124 <sup>b,d</sup>	a-campholenal	-	-	0.27		
1147	1127 1140 <sup>b,d</sup>	trans-pinocarveol	$\frac{1}{1}$ 44	-	0.40		
1151	$1140_{11}/5^{a,d}$	trans-verbenol	0.44	-	-		
1162	1140-1145 1145 1140 <sup>b-d</sup>	comphene hydrote	0.44	_	1 33		
1162	1145-1149 1160 1161b-d	Pineservene	0.28	_	1.55		
1170	1165 1160b-d.g	Pinocal	0.28	_	$\frac{1}{2}$ 10		
11/0	1103-1109 ~	borneon terminen 4 el	0.57	$\overline{0}$ 74	2.19		
1107	11/4-11/8 <sup>a d</sup>	terpinen-4-01	3.24	0.74	0.82		
1193	11/9-1184° "	<i>p</i> -cymen-8-ol	4 12	0.43	11.02		
1201	1186-1190 <sup>a d,g</sup>		4.12	3.37	11.92		
1205	1194-1195 <sup>a,c,u</sup>	Myrtenol	0.21	_	<u> </u>		
1226	1215-1217 <sup>b-d</sup>	trans-carveol	0.16	_	0.53		
1229	1204-1206 <sup>b-d</sup>	cis-verbenone	0.16	_	_		
1051	1015 1051hd	Hydrogenated sesquiterpenes			0.65		
1351	1345-1351 <sup>b-d</sup>	a-cubebene	_		0.65		
1379	1369-13/9 <sup>a-d,1-h</sup>	α-copaene	_	0.89	3.31		
1389	1384-1387/ <sup>a-d</sup>	$\beta$ -bourbonene	_	_	0.57		
1393	1400°	Sibirene	_	_	0.89		
1413	1408-1409 <sup>6,d</sup>	α-gurjunene	-	-	0.86		
1426	1417-1420 <sup>a-u</sup>	$\beta$ -caryophyllene	6.85	28.25	3.40		
1436	1430-1433 <sup>6,c,g</sup>	$\beta$ - copaene	-	-	1,06		
1455	1447-1454 <sup>a-d,1,n</sup>	α-Humulene	0.84	_	-		
1462	1454-1456 <sup>b-d</sup>	$(E)$ - $\beta$ -farnesene	0.56		<del>.</del>		
1467	1451-1462 <sup>a,c,t-h</sup>	allo-aromadendrene	_	1.39	1.24		
1478	1471°	dauca-5,8-diene	_	_	0.35		
1482	1474°	α-neocallitropsene	_	-	0.93		
1488	1473-1484 <sup>a-c,g,h</sup>	germacrene D	_	3.89	10.33		
1499	1493°	trans-muurola-4(14),5-diene	_	_	0.75		
1504	1494-1500 <sup>b,c,g</sup>	Bicyclogermacrene	_	6.90	7.45		
1520	1511 <sup>c-d</sup>	$\delta$ -amorphene	_	_	0.17		
1524	1521-1523 <sup>a,b,d,g</sup>	$\delta$ -cadinene	_	1.06	4.04		
1530	1533°	trans-cadina-1,4-diene	_	_	0.85		
		Oxygenated sesquiterpenes					
1567	1561-1564 <sup>a-d</sup>	(E)-nerolidol	0.60	2.28	21.03		
1589	1572-1583 <sup>a-d,g,h</sup>	Spathulenol	_	2.34	_		
1591	1581-1583 <sup>a-d,g</sup>	caryophyllene oxide	4.92	3.38	_		
1597	1590-1597 <sup>a-c,g</sup>	Viridiflorol	_	2.75	0.98		
1606	1595°	cubeban-11-ol	_		0.85		
1616	1602 <sup>c,d</sup>	Ledol	_	_	0.61		
1618	1600°	Guaiol	_	$\overline{1.87}$			
1632	1631°	(E)-sesquilavandulol	_	0.90	_		

 Table 2. Essential oil constituents of leaves of *Renealmia* species

Table 2 Continued							
1635	1607°	5-epi-7-epi-α-eudesmol	_	_	0.35		
1638	1625-1630 <sup>a-c</sup>	1-epi-cubenol			0.69		
1645	1639°	allo-aromadendrene epoxide		1.74	_		
1650	1639°	carophylla-4(12),8(13)-dien-5β-ol	2.56		_		
1654	1640-1643 <sup>a-d</sup>	epi-a-muurolol		_	0.17		
1657	1645°	Cubenol	_	_	0.13		
1654	1649-1654 <sup>a,b,h</sup>	$\alpha$ -cadinol		0.58	1.42		
1667	1649 <sup>c,d</sup>	$\beta$ -eusdemol	2.34	_	_		
1683	1674°	$(Z)$ - $\alpha$ -santalol	_	1.07	_		
1697	1685°	germacra-4(15),5,10(14)-trien-1-α-ol	_	0.53	_		
1752	1740°	mint sulfide	_	4.15	_		
		Carboxylic acid					
1977	1968 <sup>b</sup>	<i>n</i> -hexadecanoid acid	_	3.31	_		
		Oxygenated hydrocarbons	58.27	15.05	10.39		
		Hydrogenated hydrocarbons	_	_	0.61		
		Oxygenated monoterpenes	11.26	5.65	18.87		
		Hydrogenated sesquiterpenes	8.25	42.38	36.85		
		Oxygenated sesquiterpenes	10.42	21.59	26.23		
		Carboxylic acid	_	3,31	_		
		Total	88.20	87.98	92.95		

<sup>a</sup>**KI**= Kovats indices.

<sup>b</sup>**KI** = Kovats indices of literature. a) [16], b) [19], c) [20], d) NIST08= database, e) [21], f) [22], g) [23], h [24].

<sup>c</sup>Compounds listed in order of elution from a VF-5ms column.

 $\mathbf{Rc}$ = Renealmia chrysotricha,  $\mathbf{Rb}$ = R. breviscapa,  $\mathbf{Rn}$ = R. nicolaioides.

Twelve and 26 components were detected in the rhizome essential oils of *R. breviscapa* and *R. nicolaioides*, respectively, with  $\beta$ -caryophyllene being the major constituent of both (Table 1).

Among the 20 components identified in the essential oil of leaves of *R. chrysotricha*, *cis*-3-hexenol was the most prominent compound (Table 2). In the leaves of *R. breviscapa*, 23 compounds were identified, with  $\beta$ -caryophyllene being the major constituent, as was also found for *R. alpinia* [16].

The essential oil of *R. nicolaioides* revealed 42 constituents, with (*E*)-nerolidol being predominant (Table 2). Studies have shown that the sesquiterpene (*E*)-nerolidol causes bacteria to be more susceptible to antibiotics [36] and inhibits growth of species of *Leishmania* [39].

The major constituents of the leaf oils of the studied species differed from those previously reported for *R. alpinia*, *R. floribunda* and *R. thyrsoidea* [15-17].

# 3.2. Anti-Candida Activity

The antimicrobial activities of the essential oils studied in this work were evaluated by exposing the yeasts *C. albicans, C. buinensis* and *C. tropicalis* to 1000  $\mu$ g/mL essential oil, as shown in Figure 1.

The results show that all tested oils, whether from rhizomes or leaves, failed to inhibit the growth of *C. albicans* cells (Figure 1A). A similar result was found for essential oils from the rhizome, leaf blade and leaf sheath of *Aframomum spectrum* [5].

The oil from leaves of *R. chrysotricha* exhibited greater activity, inhibiting 56% of the growth of *C. buinensis* at 1000  $\mu$ g/mL (Figure 1B), whereas leaf oil from *R. nicolaioides* exhibited an effect on *C. tropicalis*, also at 1000  $\mu$ g/mL (Figure 1C). Similar results were reported for essential oil from the rhizome of *Curcuma longa* L. (Zingiberaceae) against *C. tropicalis* [6].

Oxygenated compounds were the major constituents of essential oils from leaves (91,3% and 59.7%, respectively) and rhizomes (96.15% and 62.2%, respectively), of R. chrysotricha and R. nicolaioides, whereas in R. breviscapa hydrogenated compounds were found in greater amounts in rhizomes (80.33%), and in the same proportion of oxygenated compounds in leaves (48.17%).Some studies have shown that oxygenated compounds exhibit higher antimicrobial activity than





**Figure 1.** Activity of the essential oils of rhizomes and leaves at a concentration of 1000  $\mu$ g/mL for: A. *Candida albicans*. B. *Candida buinensis*. C. *Candida tropicalis*. Rb = *Renealmia breviscapa*, Rc = *Renealmia chrysotricha* and Rn = *Renealmia nicolaioides*.

In our study, essentials oils of *R. breviscapa* exhibited a greater percentage of  $\beta$ -caryophyllene and  $\alpha$ -caryophyllene in rhizomes (71.94%) and no activity against *Candida albicans*, which is in disagreement with the findings of Sabulal et al. [4], who observed caryophyllene-rich rhizome oil (oil dilution - 1:2 in DMSO), which showed significant activity against this yeast.

The rhizome oil of *R. chrysotricha* exhibited slight fungicidal activity, but marked antiparasitic activity. Similar results were recorded after treatment with rhizome oil of *Aframomum sceptrum* K. Schum. (Zingiberaceae), for which the minimum inhibitory concentration (MIC) was 0.625 µg/mL against *Candida albicans* (the reference drug exhibited MIC = 0.064 µg/mL), and the minimum lethal concentration (MLC) was 1.51 µg/mL against *Trypanosoma brucei* (the reference drug exhibited MLC = 7.4 µg/mL) [7].

# 3.3. Antiparasitic Activity

The essential oil from the rhizome of *R. chrysotricha*, at 25  $\mu$ g/mL, reduced the number of parasites by 50% and 61%, after 24 and 48 h, respectively. Treatment with 100  $\mu$ g/mL reduced the population of parasites by 56% after 24 h, with all parasites being eliminated within 48 h (Figure 2A).

The leaf essential oil of *R. chrysotricha* was found to reduce the population of parasites by 28-59% in concentrations of 25, 100, 400 and 800  $\mu$ g/mL, after 24 h; and by 26-53% in concentrations of 25, 100 and 400  $\mu$ g/mL, with total death of the parasites at 800  $\mu$ g/mL, after 48 h (Figure 2B).



**Figure 2.** Antiparasitic activity of essential oils of *Renealmia chrysotricha* at the concentrations of 25, 100, 400 and 800  $\mu$ g/mL after 24 and 48 h. A- rhizome. B- leaves.

Our results using essential oils clearly show their potential lethality and ability to alter the morphology of *T. cruzi* epimastigotes. Both light and transmission electron microscopy revealed that untreated epimastigotes had a typical elongate shape, one flagellum and no visible alterations (Figure 3A and 4A). However, in the presence of essential oils from leaves (100  $\mu$ g/mL) and rhizomes (25  $\mu$ g/mL) for 48 h, the epimastigotes exhibited a rounded body, changes to flagellum morphology (Figure 3B and 3C), cytoplasmic vacuolization and mitochondrial swelling (Fig. 4B and 4C).



**Figure 3.** *Trypanosoma cruzi* cells incubated for 48 h, visualized by light microscopy. A. Control. B. Treatment – leaf essential oil of *Renealmia chrysotricha* 100  $\mu$ g/mL. C. Treatment - rhizome essential oil of *R. chrysotricha* 25  $\mu$ g/mL. Bars = 20  $\mu$ m



**Figure 4.** *Trypanosoma cruzi* cells incubated for 48 h, and visualized by transmission electron microscopy. A. Non-treated parasites showing typical morphology. B and C. Parasites treated with *Renealmia chrysotricha* essential oil. B. 100  $\mu$ g/mL leaf essential oil. C. 25  $\mu$ g/mL rhizome essential oil. Kinetoplast (K), flagelar pocket (FP), nucleus (N) and mitochondria (arrows).

The remarkable anti-*Trypanosoma cruzi* activity of the essential oil from the rhizome of *R*. *chrysotricha* may be related to the presence of the labdane diterpenoid coronarin E, as one of the major components of that oil. Some studies have demonstrated potent activity by labdane diterpenoids against T. cruzi [42,43].

Changes to the morphology of species of *Trypanosoma* have been reported in other works after incubation of the parasite, whether in the presence of essential oil from species of Lamiaceae and Fabaceae [44,45]. Changes in ultrastructure were observed when the control was compared to treatments, similar to the results reported by Menna-Barreto et al. [45], suggesting that *T. cruzi* was undergoing an autophagic pathway [46].

In the present study, the oils tested against *T. cruzi* epimastigotes decreased the amount of parasites by 50% at 25  $\mu$ g/mL, as reported for essential oils from *Hedychium coronarium* J. Koenig (Zingiberaceae) against the procyclic form of *Trypanosoma brucei* [8].

Essential oils from leaves were found to contain more substances than rhizome oils, probably due to the greater exposure of leaves to seasonal and climatic variation. The yield, recovery and quality of essential oil are influenced by photosynthesis, photoperiodic modulation, seasonal and climate variation, nutritional relationships, plant growth regulators and some abiotic stresses (light, moisture, salinity and temperature) [47,48].

Essential oils are composed of a variety of different compounds, with terpenes being the most representative. Antifungal and antiparasitic activities shown by some of the evaluated oils may be due to either synergism between their components or the action of predominant compounds. The most significant results against the *Candida* species studied were for the essential oil of *R. chrysotricha* leaves against *C. buinenses*, and for *R. nicolaioides* leaf oil against *C. tropicalis*. The significant activity of the rhizome oil of *R. chrysotricha* against *T. cruzi* suggests that the plant is a potential new source of agents to help the control of trypanosomiasis.

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# References

- L.C. Albernaz; J.E. Paula; G.A.S. Romero; M.R.R. Silva; P. Grellier; L. Mambu and L.S. Espindola (2010). Investigation of plant extracts in traditional medicine of the Brazilian Cerrado against protozoans and yeasts, *J. Ethnopharmacol.* 131, 116-121.
- [2] J.S. Raut and S.M. Karuppayil (2014). A status review on the medicinal properties of essential oils, *Ind. Crops. Prod.* **62**, 250-264.
- [3] I. Jantan; M. Salleh; M. Yassin; C.B. Chin; L.L. Chen; N.L. Sim; C. Roxb and K. Linn (2003). Antifungal activity of the essential oils of nine Zingiberaceae species, *Pharm. Biol.* 41, 392–397.
- [4] B. Sabulal; M.J.A.J. Dan; R. Kurup; N.S. Pradeep; R.K. Valsamma and V. George (2006). Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: chemical characterization and antimicrobial activity, *Phytochemistry* 67, 2469–2473.
- [5] E. Adjalian; F.T.D. Bothon; B. Yehouenou; J. Noudogbessi; G. Figueiredo; F. Avlessi and D.C.K. Sohounhloue (2014). GC/MS and GC/FID analysis and evaluation of antimicrobial performance of *Aframomum sceptrum* essential oils of Benin, *World J. Pharm.* Sci. 2(8), 787-792.
- [6] D.I. Ribeiro; M.S. Alves; M. Graciela; I. Faria; T. Inez; E. Svidzinski; I.A. Nascimento; F. Borges; P. Ferreira and G.A. Ferreira (2010). Determinação da atividade antifúngica dos óleos essenciais de *Curcuma longa L.* (Zingiberaceae) e de *Achillea millefolium* (Asteraceae) cultivadas no Noroeste do Paraná, *Arq. Ciênc. Saúde UNIPAR* 14, 103–109.
- [7] Z. Cheikh-Ali; M. Adiko; S. Bouttier; C. Bories; T. Okpekon; E. Poupon and P. Champy (2011). Composition, and antimicrobial and remarkable antiprotozoal activities of the essential oil of rhizomes of *Aframomum sceptrum* K. Schum. (Zingiberaceae), *Chem. Biodivers.* 8, 658–667.
- [8] D.F. Rodrigues; A.M.A. Velásquez; C. Cavaleiro; L. Salgueiro; G.Z. Martins; N.O. Magalhães; M.B.G. Martins; R.M.B. Cicarelli and R.R.D. Moreira (2013). Chemical composition and trypanocidal activity of the essential oils from *Hedychium coronarium* J. Koenig (Zingiberaceae), *ISRN Infect. Dis.* 1–6.
- [9] I. Gómez-Betancur; D. Benjumea; J.E. Gómez; N. Mejía and J.F. León (2018). Antinociceptive activity of essential oils from wild growing and micropropagated plants of *Renealmia alpinia* (Rottb.) Maas., *Rec. Nat. Prod. in press*, 1-8.
- [10] P.J.M. Maas (1977). *Renealmia* (Zingiberaceae-Zingiberoideae), Costoideae (Zingiberaceae). Flora Neotropica, New York, The New York Botanical Garden.
- [11] R.R.B. Negrelle (2015). Renealmia L.F.: Aspectos botânicos, ecológicos, farmacológicos e agronômicos, Rev. Bras. Plantas Med. 17, 274–290.
- [12] P.J.M. Maas and H. Maas (2018). Zingiberaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Available at <u>http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB110707</u>.
- [13] L. Scalvenzi; A. Grandini; A. Spagnoletti; M. Tacchini; D. Neill; J.L. Ballesteros; G. Sacchetti and A. Guerrini (2017). *Myrcia splendes* (Sw.) DC. (syn. M. fallax (Rich.) DC.) (Myrtaceae) essential oil from Amazonian Ecuador: A chemical characterization and bioactivity profile, *Molecules* 22, 1-12.
- [14] J.K.R. Silva; E.H.A. Andrade; L.H. Barreto; N.C.F. Silva; A.F. Ribeiro; R.C. Montenegro and J.G.S. Maia (2017). Chemical composition of four essential oils of *Eugenia* from the Brazilian Amazon and their cytotoxic and antioxidant activity, *Medicines* 4(51), 1-10.
- [15] A.I.R. Luz; M.G.B. Zoghbi; L.S. Ramos; J.G.S. Maia and M.L. Silva (1984). Essential oils of some amazonian Zingiberaceae, Br. Reports 47, 907–908.
- [16] J.G.S. Maia; E.H.A. Andrade; L.M.M. Carreira and M.H.L. Silva (2007). Essential oil composition of *Renealmia alpinia* (Rottb.) Maas., J. Essent. Oil Bear Plants 10, 10–14.
- [17] P.F. Noriega; E.A. Paredes; T.D. Mosquera; E.E. Diaz; A. Lueckhoff; J.E. Basantes and A.L. Trujillo (2016). Chemical composition antimicrobial and free radical scavenging activity of essential oil from leaves of *Renealmia thyrsoidea* (Ruiz Pav.) Poepp. Endl., *J. Med. Plants Res.* 10, 553–558.
- [18] O. Gottlieb and M. Taveira-Magalhães (1960). Modified distillation trap, *Chem-anal.* 49, 114–115.

- [19] V.I. Babushok; P.J. Linstrom and I.G. Zenkevich (2011). Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **40**(4), 1-47.
- [20] R.P. Adams (2007). Identification of essential oil components by gas chromatography/mass spectrometry, 4rd ed.; Allured Publ. Corp.; Carol Stream.
- [21] N.R. Andriamaharavo (2014). Retention Data. NIST Mass Spectrometry Data Center.
- [22] A.L. Corrêa; H.S. França; L.A.C. Tietbolhl; B.N. Luna; M.G. Santos; M.F. Freitas; A.P. Oliveira and L. Rocha (2017). Volatile constituents of three myrsine L. species from Brazil, *Rec. Nat. Prod.* 11, 82-87.
- [23] A. Hajdari; B. Mustafa; A. Kaçiku; X. Malu; B. Lukas; A. Ibraliu; G. Stefkov and J. Novak (2016). Chemical composition of essential oil, total phenolics, total flavonoids and antioxidant activity of methanolic extracts of *Satureja montana* L., *Rec. Nat. Prod.* 10, 750-760.
- [24] V. Morocho; M.L. Toro; L. Cartuche; D. Guaya; E. Valarezo; O. Malagón and J. Ramírez (2017). Chemical composition and antimicrobial activity of essential oil of *Lepechinia radula* Benth Epling, *Rec. Nat. Prod.* 11, 57-62.
- [25] W.F. Broekaert; F.R.G. Terras; B.P.A. Cammue and J. Vanderleyden (1990). An automated quantitative assay for fungal growth inhibition, *Fems. Microbiol. Lett.* **69**, 55–60.
- [26] L.J. Quintans-Júnior; M.G.B. Oliveira; M.F. Santana; M.T. Santana; A.G. Guimarães; J.S. Siqueira; D.P. De Souza and R.N. Almeida (2011). α-Terpineol reduces nociceptive behavior in mice, *Pharm. Biol.* 49, 583–586.
- [27] S.O. Oyedemi; A.I. Okoh; L.V. Mabinya; G. Pirochenva and A.J. Afolayan (2009). The proposed mechanism of bactericidal action of eugenol, α-terpineol and γ-terpinene against *Listeria* monocytogenes, Streptococcus pyogenes, Proteus vulgaris and Escherichia coli, African J. Biotechnol. 8, 1280–1286.
- [28] I. Lechat-Vahirua; P. François; C. Menut; G. Lamaty and J.M. Bessiere (1993). Aromatic plants of French Polynesia. I. Constituents of the essential oils of rhizomes of three Zingiberaceae: Zingiber zerumbet Smith, Hedychium coronarium Koenig and Etlingera cevuga Smith, J. Essent. Oil Res. 5, 55– 59.
- [29] F.A. Santos and V.S.N. Rao (2000). Antiinflammatory and antinociceptive effects of 1, 8-cineole a terpenoid oxide present in many plant essential oils, *Phytother. Res.* **14**, 240–244.
- [30] H. Itokawa; H. Morita; K. Takeya and M. Motidome (1988). Diterpenes from rhizomes of *Hedychium* coronarium, Chem. Pharm. Bull. **36**, 2682-2684.
- [31] P.P. Reddy; R.R. Rao; J. Shashidhar; B.S. Sastry; J.M. Rao and K.S. Babu (2009). Phytochemical investigation of labdane diterpenes from the rhizomes of *Hedychium spicatum* and their cytotoxic activity, *Bioorganic Med. Chem. Lett.* **19**, 6078-6081.
- [32] I. Kumrit; A. Suksamrarn; P. Meepawpan; S. Songsri and N. Nuntawong (2010). Labdane-type diterpenes from *Hedychium gardnerianum* with potent cytotoxicity against human small cell lung cancer cells, *Phytother. Res.* 24, 1009-1013.
- [33] S. Songsri and N. Nuntawong (2016). Cytotoxic labdane diterpenes from *Hedychium ellipticum* Buch.-Ham. ex Sm, *Molecules* 21, 749.
- [34] H.M. Sirat; D. Masri and A.A. Rahman (1994). The distribution of labdane diterpenes in the Zingiberaceae of Malaysia, *Phytochemistry* **36**(6), 699-701.
- [35] S.V. Sob; P. Tane; B.T. Ngadjui; J.D. Connoly and D. Ma . (2007). Trypanocidal labdane diterpenoids from the seeds of *Aframonum aulacocarpos* (Zingiberaceae), *Tetrahedron* **63**, 8993-8998.
- [36] R. Chokchaisiri; N. Chaneiam; S. Svasti; S. Fucharoen; J. Vadolas and A. Suksamrarn (2010). Labdane diterpenes from the aerial parts of *Curcuma comosa* enhance fetal hemoglobin production in an erythroid cell line, *J. Nat. Prod.* 73, 724-728.
- [37] W. Chate and N. Nuntawong (2015). Diterpenes and kavalactone from the rhizomes of *Amomum uliginosum* J. Koenig, *Biochem. Syst. Ecol.* **63**, 34-37.
- [38] B.F. Brehm-Stecher and E.A. Johnson (2003) Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone, *Antimicrob. Agents Chemother.* **47**, 3357–3360.
- [39] D.C. Arruda; F.L.D. Alexandri; A.M. Katzin and S.R.B. Uliana (2005). Antileishmanial Activity of the Terpene Nerolidol, *Antimicrob. Agents Chemother.* 49, 1679–1687.
- [40] F.C. Klopell; M. Lemos; J.P.B. Sousa; E. Comunello; E.L. Maistro; J.K. Bastos and S.F. De Andrade (2007). Nerolidol, an antiulcer constituent from the essential oil of *Baccharis dracunculifolia* DC (Asteraceae), *Zeitschrift fur Naturforsch. - Sect. C. J. Biosci.* 62, 537–542.
- [41] M. Soković; P.D. Marin; D. Brkić and L.J.L.D. Griensven (2007). Chemical composition and antibacterial activity of essential oils of ten aromatic plants against human pathogenic bacteria, *Glob. Sci. Books* 1, 220–226.
- [42] J. Varela; M.L. Lavaggi; M. Cabrera; A. Rodríguez; P. Miño; X. Chiriboga; H. Cerecetto and M. González (2012). Bioactive-guided identification of labdane diterpenoids from aerial parts of

Aristeguietia glutinosa as anti-Trypanosoma cruzi agents, Nat. Prod. Commun. 7(9), 1139-1142.

- [43] J. Varela; E. Serna; S. Torres; G. Yaluff; N.I.V. Bilbao; P. Miño; X. Chiriboga; H. Cerecetto and M. González (2014). In vivo anti-*Trypanosoma cruzi* activity of hydro-ethanolic extract and isolated active principles from *Aristeguietia glutinosa* and mechanism of action studies, *Molecules* 19, 8488-8502.
- [44] G.F. Santoro; M.G. Cardoso; L.G.L. Guimarães; A.P.S.P. Salgado; R.F.S. Menna-Barreto and M.J. Soares (2007). Effect of oregano (*Origanum vulgare L.*) and thyme (*Thymus vulgaris L.*) essential oils on *Trypanosoma cruzi* (Protozoa: Kinetoplastida) growth and ultrastructure, *Parasitol. Res.* 100, 783–790.
- [45] R.F.S. Menna-Barreto; G.A.T. Laranja; M.C.C. Silva; M.G.P. Coelho; M.C. Paes; M.M. Oliveira and S.L. De Castro (2008). Anti-Trypanosoma cruzi activity of *Pterodon pubescens* seed oil: Geranyl geraniol as the major bioactive component, *Parasitol. Res.* 103, 111–117.
- [46] M.V. Braga and W. Souza (2006). Effects of protein kinase and phosphatidylinositol-3 kinase inhibitors on growth and ultrastructure of *Trypanosoma cruzi*, *Fems. Microbiol. Lett.* **256**, 209–216.
- [47] N.S. Sangwan; A.H.A. Farooqi; F. Shabih and R.S. Sangwan (2001). Regulation of essential oil production in plants, *Plant Growth Regulation* **34**, 3–21.
- [48] H.R.P. Lima; M.A.C. Kaplan and A.V.M. Cruz (2003). Influência dos fatores abióticos na produção e variabilidade de terpenoides em plantas, *Floresta e Ambiente* **10(2)**, 71–77.

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