ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 13:3 (2019) 268-280

records of natural products

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.

Kathlyn V. Gevú[1], Helena R.P. Lima[2*], Ilzenayde A. Neves[3], Érica O. Mello[4], Gabriel B. Taveira[4], Laís P. Carvalho[1], Mário G. Carvalho[3], Valdirene M. Gomes[4], Edésio J.T. Melo[1] and Maura Da Cunha[1]

[1]*Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brazil*

[2]*Laboratório de Anatomia Vegetal, Departamento de Botânica, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil*

[3]*Laboratório de Química de Produtos Naturais, Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil*

[4]*Laboratório de Fisiologia e Bioquímica de Microrganismos, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brazil*

(Received August 04, 2018; Revised October 16, 2018; Accepted October 17, 2018)

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*, α-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.

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

* Corresponding author: E-Mail: limarural63@gmail.com

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products © May-June 2019 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.105.18.08.125

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 μg/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 (*β*-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 μg/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 *β*-pinene to be the predominant compound. For *Renelamia alpinia* (Rottb.) Maas, *β*-caryophyllene, allo-aromadendrene and β-pinene were detected in stems, leaves and fruits, respectively [16]. For leaves of *R. thyrsoidea* (Ruiz &Pav.), terpinolene and *α*-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°4′40"S×67°37′35"W)and in the Reserva Florestal Humaitá (9°45′17"S×67°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 ± 10) and leaves (420 g ± 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 μL 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* i*noculates)* 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* (1×10^4 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 µg/mL, with the final concentration of DMSO being less than 1.5% at all concentrations. In addition, 100 µL of parasites, at a density of 1.3 x 10^6 cells/mL, were added per well and the volume was adjusted to 200 µL 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 µL 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 α-terpineol, coronarin E and 1,8-cineole (Table 1). The monoterpene α-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].

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

KI[a]	KI[b]	Compound[c]	Composition %		
			Rc	**Rb**	**Rn**
		Oxygenated monoterpenes			
1036	1026-1032[b-d,g]	1.8-cineole	15.87	_	_
1073	1067-1075 [b-d]	*cis*-linalool oxide	_	_	0.20
1074	1066-1070[b,g]	*cis*-sabinene hydrate	0.67	_	_
1090	1084[c,d]	*trans*-linalool oxide	_	_	0.23
1096	1095-1101 [b-d,h,g]	linalool	1.49	_	1.71
1103	1098[c,g]	*trans*-sabinene hydrate	0.85	_	_
1124	1118[c,d]	exo-fenchol	0.08	_	0.80
1129	1136[c]	menth-2-en-1-ol. trans-p-	0.41	_	_
1146	1140[b,d]	*trans*-pinocarveol	1.08	_	1.48
1161	1144[b,d]	*cis*-verbenol	_	_	1.01
1168	1145-1149[b-d]	camphene hydrate	_	_	0.32
1168	1160-1161[b-d]	pinocarvone	0.38	_	_
1178	1165-1166[b-d]	borneol	4.27	_	1.47
1189	1174-1178[a-d]	terpinen-4-ol	9.94	_	1.82
1201	1186-1190[a-d.g]	*α*-terpineol	26.14	1.10	14.15
1214	1194-1195[a,c,d]	myrtenol	_	_	0.32
1216	1207[b,d]	*trans*-piperitol	0.25	_	_
1288	1283-1286[b-d]	isobornyl acetate	_	_	0.29
1299	1289[c]	*trans*-sabinyl acetate	_	_	0.65
1350	1346-1350[a-d]	*α*-terpinyl acetate	0.45	_	_
		Hydrogenated sesquiterpenes			
1425	1411-1420[a-d.f.h]	*β*-caryophyllene	2.99	62.38	22.78
1454	1447-1454[a-d.f.h]	*α*-Humulene	0.36	9.56	4.44
1461	1457[c]	sesquisabinene	0.23	_	_
1479	1460[c]	dehydro-aromadendrene	_	_	1.29
1523	1511[c-d]	*δ*-amorphene	_	_	0.62
		Oxygenated sesquiterpenes			
1564	1561-1564[a-d]	(*E*)-nerolidol	0.19	3.27	11.06
1591	1581-1589[a-d,f,g]	caryophyllene oxide	2.23	9.27	9.90
1604	1600[c]	guaiol	_	0.36	0.71
1619	1601-1608[c,f,g]	humulene epoxide II	_	1.43	1.67
1642	1639[c]	carophylla-4(12).8(13)-dien-5β-ol	_	0.23	0.49
1650	1640-1643[a-d]	epi-α-muurolol	_	0.63	0.89
1664	1642[c]	selina-3.11-dien-6-α-ol	_	0.57	0.89
		Carboxylic acid			
1965	1968[b]	*n*-hexadecanoid acid	_	0.40	1.61
		Oxygenated Diterpenes			
2027	2026[c]	(*E*-*E*)-geranyl linalool	_	0.36	0.53
2146	2136[e]	coronarin E[#]	25.1	_	_
		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	25.1	0.36	0.53
		Total	**92.98**	**89.56**	**81.33**

[a]**KI**= Kovats indices.

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

[c]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. $M^{+\cdot}$). 160(20). 147(100). 137(30). 95(50). 81(80). 69(40). 55(70). 41(40).

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

KI[a]	KI[b]	Compound[c]	Composition %		
			Rc	Rb	Rn
		Oxygenated hydrocarbons			
858	850-857[b-d]	*cis*-3-hexenol	57.28	15.05	9.45
866	859[c,d]	*cis*-2-hexenol	_	_	0.19
869	863-870[b-d]	*n*-hexanol	0.99	_	0.75
		Hydrogenated hydrocarbons			
936	932-940[a-c,f-h]	*α*-pinene	_	_	0.09
983	974-982[a-d,f,h]	*β*-pinene	_	_	0.16
991	988-992[a-d,g]	Myrcene	_	_	0.10
1032	1024-1030[a-d]	Limonene	_	_	0.26
		Oxygenated monoterpenes			
1036	1026-1032[b-d,g]	1,8-cineole	0.33	_	0.11
1074	_[d]	5isopropyl2methylbicyclo[3.1.0]hexan-2-ol	0.16	_	_
1103	1095-1101[b,d,g,h]	Linalool	_	1.11	0.60
1106	1098[c,g]	*trans*-sabinene hydrate	0.51	_	_
1125	1114[c,d]	endo-fenchol	_	_	0.97
1132	1124[b,d]	*α*-campholenal	_	_	0.40
1147	1140[b,d]	*trans*-pinocarveol	1.44	_	_
1151	1140-1145[a,d]	*trans*-verbenol	0.44	_	_
1162	1145-1149[b-d]	camphene hydrate	_	_	1.33
1168	1160-1161[b-d]	Pinocarvone	0.28	_	_
1178	1165-1169[b-d,g]	Borneol	0.37	_	2.19
1187	1174-1178[a-d]	terpinen-4-ol	3.24	0.74	0.82
1193	1179-1184[b-d]	*p*-cymen-8-ol	_	0.43	_
1201	1186-1190[a-d,g]	α-terpineol	4.12	3.37	11.92
1205	1194-1195[a,c,d]	Myrtenol	0.21	_	_
1226	1215-1217[b-d]	*trans*-carveol	_	_	0.53
1229	1204-1206[b-d]	*cis*-verbenone	0.16	_	_
		Hydrogenated sesquiterpenes			
1351	1345-1351[b-d]	*α*-cubebene	_	_	0.65
1379	1369-1379[a-d,f-h]	*α*-copaene	_	0.89	3.31
1389	1384-1387[a-d]	*β*-bourbonene	_	_	0.57
1393	1400[c]	Sibirene	_	_	0.89
1413	1408-1409[b,d]	*α*-gurjunene	_	_	0.86
1426	1417-1420[a-d]	*β*-caryophyllene	6.85	28.25	3.40
1436	1430-1433[b,c,g]	*β*- copaene	_	_	1,06
1455	1447-1454[a-d,f,h]	*α*-Humulene	0.84	_	_
1462	1454-1456[b-d]	(*E*)-β-farnesene	0.56	_	_
1467	1451-1462[a,c,f-h]	allo-aromadendrene	_	1.39	1.24
1478	1471[c]	dauca-5,8-diene	_	_	0.35
1482	1474[c]	*α*-neocallitropsene	_	_	0.93
1488	1473-1484[a-c,g,h]	germacrene D	_	3.89	10.33
1499	1493[c]	*trans*-muurola-4(14),5-diene	_	_	0.75
1504	1494-1500[b,c,g]	Bicyclogermacrene	_	6.90	7.45
1520	1511[c-d]	*δ*-amorphene	_	_	0.17
1524	1521-1523[a,b,d,g]	*δ*-cadinene	_	1.06	4.04
1530	1533[c]	*trans*-cadina-1,4-diene	_	_	0.85
		Oxygenated sesquiterpenes			
1567	1561-1564[a-d]	(*E*)-nerolidol	0.60	2.28	21.03
1589	1572-1583[a-d,g,h]	Spathulenol	_	2.34	_
1591	1581-1583[a-d,g]	caryophyllene oxide	4.92	3.38	_
1597	1590-1597[a-c,g]	Viridiflorol	_	2.75	0.98
1606	1595[c]	cubeban-11-ol	_	_	0.85
1616	1602[c,d]	Ledol	_	_	0.61
1618	1600[c]	Guaiol	_	1.87	_
1632	1631[c]	(*E*)-sesquilavandulol	_	0.90	_

Table 2 Continued..

1635	1607[c]	5-epi-7-epi-α-eudesmol	_	_	0.35
1638	1625-1630[a-c]	1-epi-cubenol	_	_	0.69
1645	1639[c]	allo-aromadendrene epoxide	_	1.74	_
1650	1639[c]	carophylla-4(12),8(13)-dien-5β-ol	2.56	_	_
1654	1640-1643[a-d]	epi-α-muurolol	_	_	0.17
1657	1645[c]	Cubenol	_	_	0.13
1654	1649-1654[a,b,h]	*α*-cadinol	_	0.58	1.42
1667	1649[c,d]	*β*-eusdemol	2.34	_	_
1683	1674[c]	(*Z*)-α-santalol	_	1.07	_
1697	1685[c]	germacra-4(15),5,10(14)-trien-1-α-ol	_	0.53	_
1752	1740[c]	mint sulfide	_	4.15	_
		Carboxylic acid			
1977	1968[b]	*n*-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**

[a]**KI**= Kovats indices.
[b]**KI** = Kovats indices of literature. a) [16], b) [19], c) [20], d) NIST08= database, e) [21], f) [22], g) [23], h [24].
[c]Compounds listed in order of elution from a VF-5ms column.
Rc= *Renealmia chrysotricha*, **Rb**= *R. breviscapa*, **Rn**= *R. nicolaioides*.

Twelve and 26 components were detected in the rhizome essential oils of *R. breviscapa* and *R. nicolaioides*, respectively, with *β*-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 *β*-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 µg/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 µg/mL (Figure 1B), whereas leaf oil from *R. nicolaioides* exhibited an effect on *C. tropicalis*, also at 1000 µg/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 hydrogenated compounds because of their ability to promote the formation of hydrogen bonds and the solubility of the oil in water [40,41], which corroborate our results.

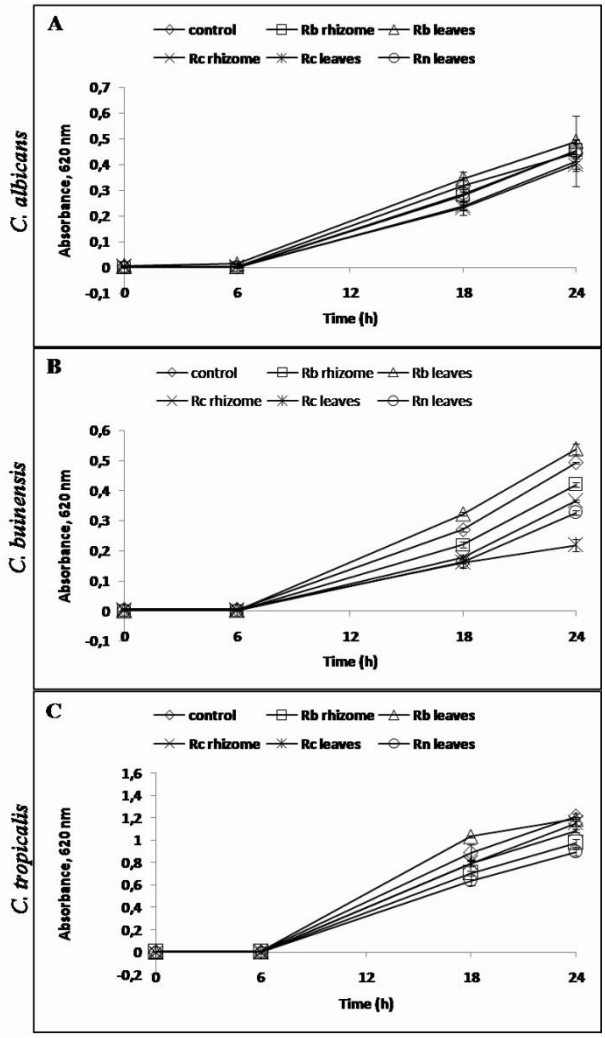

Figure 1. Activity of the essential oils of rhizomes and leaves at a concentration of 1000 µg/mL for: A. *Candida albicans*. B. *Candida buinensis*. C. *Candida tropicalis*. Rb = *Renealmia breviscapa*, Rc = *Renealmia chrysotricha* and Rn = *Renealmia nicolaioides*.

In our study, essentials oils of *R. breviscapa* exhibited a greater percentage of *β*-caryophyllene and α-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 µg/mL, reduced the number of parasites by 50% and 61%, after 24 and 48 h, respectively. Treatment with 100 µg/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 µg/mL, after 24 h; and by 26-53% in concentrations of 25, 100 and 400 µg/mL, with total death of the parasites at 800 µg/mL, after 48 h (Figure 2B).

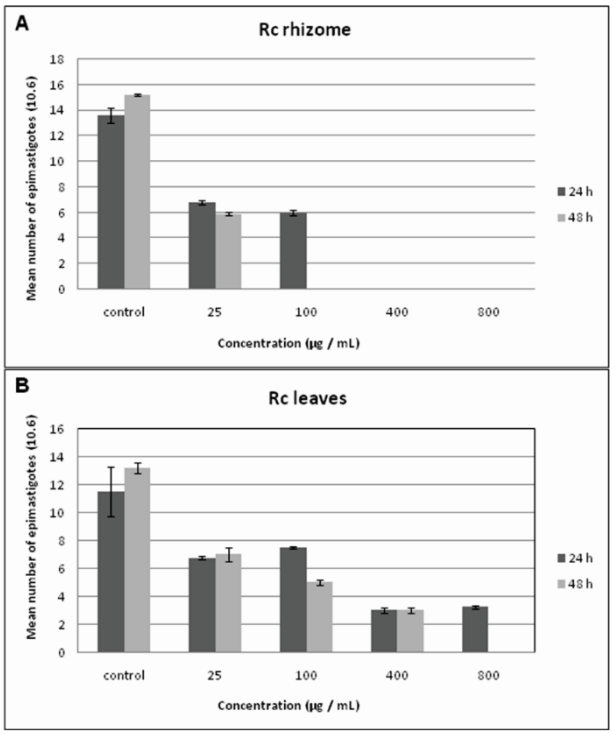

Figure 2. Antiparasitic activity of essential oils of *Renealmia chrysotricha* at the concentrations of 25, 100, 400 and 800 µg/mL after 24 and 48 h. A- rhizome. B- leaves.

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 µg/mL) and rhizomes (25 µg/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).

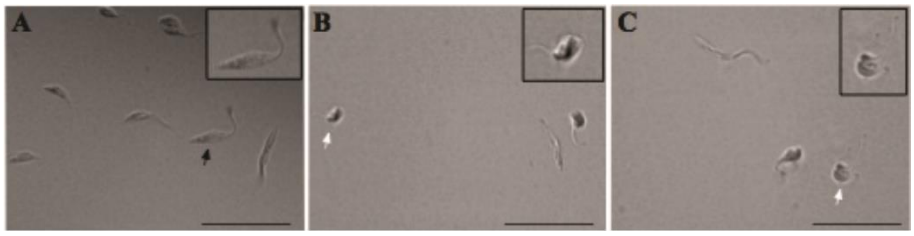

Figure 3. *Trypanosoma cruzi* cells incubated for 48 h, visualized by light microscopy. A. Control. B. Treatment – leaf essential oil of *Renealmia chrysotricha* 100 µg/mL. C. Treatment - rhizome essential oil of *R. chrysotricha* 25 µg/mL. Bars = 20 µm

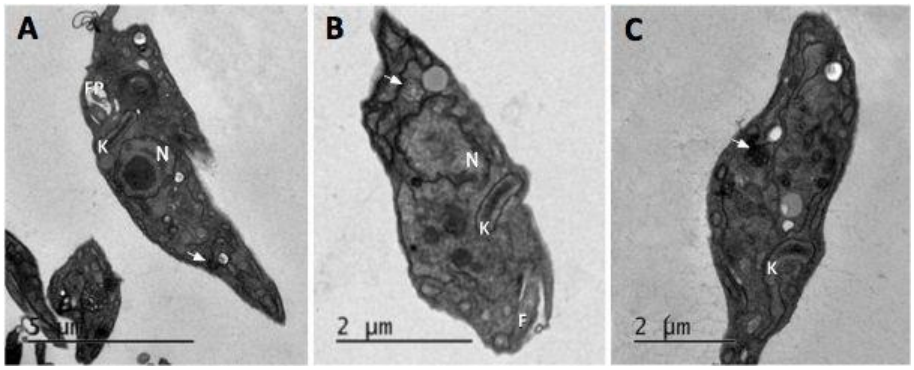

Figure 4. *Trypanosoma cruzi* cells incubated for 48 h, and visualized by transmission electron microscopy. A. Non-treated parasites showing typical morphology. B and C. Parasites treated with *Renealmia chrysotricha* essential oil. B. 100 µg/mL leaf essential oil. C. 25 µg/mL rhizome essential oil. 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 µg/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.

Acknowledgments

The first author would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a scholarship. The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ) for their financial support, and Herison Medeiros and staff of Herbarium of the Universidade Federal do Acre (UFACPZ) for the logistical and technical support. This study was part of the first author's PhD thesis to the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF).

ORCID

Kathlyn V. Gevú: 0000-0002-3372-2144
Helena R.P. Lima: 0000-0002-7982-4146
Ilzenayde A. Neves: 0000-0002-5224-9583
Érica O. Mello: 0000-0002-1965-4560
Gabriel B. Taveira: 0000-0002-1733-3034
Laís P. Carvalho: 0000-0002-3857-5462
Mário G. Carvalho: 0000-0001-5805-734X
Valdirene M. Gomes: 0000-0003-4145-428X
Edésio J.T. Melo: 0000-0003-4210-7749
Maura Da Cunha: 0000-0003-1078-3742

References

[1] 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 http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB110707.

[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 *Aframomum 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.