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Research Article

Chemical Composition and Trypanocidal Activity of the Essential Oils from *Hedychium coronarium* J. Koenig (Zingiberaceae)

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The composition of the essential oils (EO) from leaves and rhizomes of *Hedychium coronarium* was analyzed both by gas chromatography and gas chromatography-mass spectroscopy. Thirty and thirty-nine compounds were identified, respectively, in the oils from leaves and rhizomes, representing 88% and 86.1% of the whole compositions. Caryophyllene oxide is the major component in rhizomes while 1,8-cineole predominates in leaves oil. Essential oils and major components were tested for trypanocidal activity using procyclic forms of *Trypanosoma brucei* (427 and 29-13 strains). The cytotoxicity index (CI_{50}), using the MTT colorimetric method, showed that essential oils and 1,8-cineole were inactive (>100 $\mu g \cdot mL^{-1}$. Nevertheless, caryophyllene oxide revealed a remarkable activity against both *T. brucei* strains (CI_{50} = 65.77 $\mu g \cdot mL^{-1}$ and 24.53 $\mu g \cdot mL^{-1}$, resp.), and the synergism between caryophyllene oxide plus pentamidine (1 : 1, v/v) highly increased the trypanocidal activity (<1.0 $\mu g \cdot mL^{-1}$).

1. Introduction

As part of our ongoing research, exploring the potential of the Brazilian plant biodiversity as a source of bioactive compounds, we looked now for the trypanocidal proprieties of the essential oils isolated from *Hedychium coronarium* J. Koenig (Zingiberaceae). *H. coronarium* is a monocotyledon from the tropical Asia, well adapted in South-America, especially in Brazil, where it is popularly named "lírio-do-brejo" or "gengibre-branco" [1–3]. It is an invasive weed used by locals for healing bruise injuries, infections, sore throats, rheumatism, diabetes, headaches, and severe pain [1–3].

Human African trypanosomiasis (HAT) or sleeping sickness is caused by the parasite *Trypanosoma brucei,* a flagellate protozoan transmitted by flies of *Glossina* genus, known as *tsetse* flies. Trypanosome has two hosts—the insect vector and mammalian host [4]. Due to the great differences among the hosts, the parasite undergoes complex changes during the life cycle that facilitate its survival inside the insect gut or inside the mammalian bloodstream [5]. It also features a unique

Table 1: Composition of the volatile oils of leaves and rhizomes of *Hedychium coronarium* from Brazil.

RI[a]	RI[b]	Compound	Percent in samples (%)	
			Leaves	Rhizome
921	n.d.	α-Thujene	—	0.1
928	1025	α-Pinene	0.2	1.9
941	1072	Camphene	—	0.3
963	1124	Sabinene	t	0.2
968	1115	β-Pinene	1.1	11.7
980	1159	Myrcene	—	0.4
997	1167	α-Phellandrene	—	0.1
1005	1150	δ-3-Carene	—	0.2
1008	n.d.	α-Terpinene	t	—
1012	1272	*p*-Cymene	0.2	1.4
1019	1204	Limonene*	t	2.4
1020	1212	1,8-Cineole*	0.7	31.7
1025	n.d.	*Z*-Ocimene	—	0.3
1046	1246	γ-Terpinene	—	0.5
1051	1457	*E*-Sabinene hydrate	—	0.5
1077	1284	Terpinolene	—	0.3
1082	1542	*Z*-Sabinene hydrate	0.2	0.4
1084	1539	Linalool	—	0.7
1105	n.d.	Fenchol	—	0.2
1106	1488	α-Campholenal	0.3	0.2
1109	n.d.	*Z*-*p*-Menth-2-en-1-ol	—	0.2
1119	1514	Camphor	—	0.1
1120	n.d.	Nopinone	t	—
1122	1645	*E*-Pinocarveol	1.3	0.4
1123	1645	*Z*-Verbenol	—	0.3
1129	1668	*E*-Verbenol	0.4	—
1136	1564	Pinocarvone	1.3	0.4
1145	1691	Borneol	t	3.1
1160	1594	Terpinene-4-ol	1.0	6.8
1164	1622	Myrtenal	1.4	0.1
1171	1687	α-Terpineol	0.6	12.0
1178	1780	Myrtenol	1.4	—
1186	n.d.	*E*-Piperitol	—	0.4
1191	n.d.	*E*-Carveol	—	0.2
1329	1688	α-Terpinyl acetate	—	1.2
1403	1840	α-Ionone	0.2	—
1407	1591	*E*-Caryophyllene	12.1	1.3
1440	1661	α-Humulene	0.8	0.2
1446	1659	*E*-β-Farnesene	t	0.2
1460	1927	β-Ionone	0.6	—
1469	n.d.	β-Selinene	—	0.2
1502	1752	γ-Cadinene	—	0.4
1559	1971	Caryophyllene oxide	43.9	1.1
1569	2078	Caryophylla-2(12),6(13)-dien-5-one	0.4	—
1582	2026	Humulene epoxide II	2.8	0.2
1607	2277	Caryophylla-2(12),6(13)-dien-5-beta-ol	1.8	—
1609	2283	Caryophylla-2(12),6(13)-dien-5-alpha-ol	7.7	—
1631	2270	Caryophylla-2(12),6-dien-5 alpha-ol	2.0	—
1645	2360	Caryophylla-2(12),6-dien-5 beta-ol	5.6	—
2091	n.d.	Coronarin E#	—	14.1
		Monoterpene hydrocarbons	**1.5**	**19.5**
		Oxygen containing monoterpenes	**8.6**	**58.8**
		Sesquiterpene hydrocarbons	**12.9**	**2.4**
		Oxygen containing sesquiterpenes	**64.2**	**1.3**
		Other compounds	**0.8**	**14.1**
		Total identified	**88.0**	**86.1**

Compounds listed in the order of their elution on the SPB-1 column; t: traces; n.d.: not determined. [a]Experimental retention indices on the SPB-1 column relative to C8–C24 *n*-alkanes. [b]Experimental retention indices on the Supelcowax-10 column relative to C8 to C24 *n*-alkanes. *Quantification based on peak areas from Supelcowax-10 chromatogram. #Identity proposed on the basis of mass spectra: *m*/*z* (relative intensity) 284 [M^+] (78), 147 (100), 81 (45), 55 (39), 41 (34), 91 (34), 95 (33), 137 (31), 69 (25), 77 (24), 131 (23), 148 (23), 117 (22), 129 (22), 79 (21), 115 (21).

and notable variable surface glycoprotein (VSG) coat that protects the parasite from the host's immune system. These characteristics contribute to the extraordinary resistance of the trypanosome which contributes to the difficult or ineffective therapies of trypanosomiasis [6].

There are several therapeutic approaches for the disease, however, not entirely efficient, causing severe adverse effects and leading to resistance. Patients usually need to be examined in subsequent years after treatments to certify that parasites were deleted and no resistant strains overcame [7]. The treatment with pentamidine is assumed to be the most effective in the acute infection but it can cause allergic and toxic side effects, most commonly affecting pancreas, with serious hypoglycemia, which, in part, depends on the daily and/or cumulative dose. In cases of brain impairment caused by the parasite, the condition may be irreversible [8]. Thus, there is an urgent need for the development of new effective and safe therapies for trypanosomiasis.

Aiming at developing of novel drugs, natural products emerge as good alternatives for the search of hits and new leading compounds. The ethanolic extract of *Hedychium coronarium* from the Yanesha (Peru) was proved to be active (IC_{50} < 10 μg/mL) on *Leishmania amazonensis* amastigote stages [9]. Essential oils and essential oil constituents have been studied, in the last years, for their activity on several pathogenic protozoan as *Giardia* sp. [10–12], *Leishmania* sp. [13, 14], and *Trypanosoma* sp. [15–20]. Mechanisms for such activities were not definitively elucidated. However, the impairment of membranes and other protozoa structures and the interference in the redox balance, targeting crucial metabolic pathways and leading to autophagic processes,

were suggested as consistent mechanisms to explain the activity of essential oils and some of their constituents.

The chemical composition of the essential oils from leaves and rhizomes of *H. coronarium* was established and the trypanocidal effects of the oils and their major components, were tested on procyclic forms of *Trypanosoma brucei* (427 and 29-13 strains). As far as we know, this is the first study dealing with the activity of the essential oils of *H. coronarium* and its constituents against *T. brucei*.

2. Experimental Section

2.1. Plant Material. Leaves and rhizomes of *H. coronarium* were collected in the Ecological Station "Juréia-Itatins" by Dr. Maria Bernadette G. Martins on March 15, 2011, and the specimens were identified by Dr. Vinicius Castro Souza. Voucher specimens were deposited in the Herbarium of the College of Agriculture, with ESA/USP having registeration number 93272 ESA.

2.2. Preparation of Essential Oils. Leaves and rhizomes were submitted to gentle air drying at room temperature and then processed independently, leaves were coarsely divided, and rhizomes were cut and crushed. Essential oils were then prepared by hydrodistillation of plant material for 4 hours, using a Clevenger apparatus. Oils were stored at the dark at 4°C until used.

Caryophyllene oxide and 1.8-cineole were acquired from Sigma-Aldrich.

2.3. Essential Oil Analysis. Compositions of essential oils were accessed by means of gas chromatography (GC) and gas chromatography-mass spectroscopy (GC/MS). Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detection (FID) systems. The Graphpak divider (Agilent Technologies, part no. 5021-7148) was used for simultaneous sampling to two Supelco (Supelco, Bellefonte, PA, USA) fused silica capillary columns with different stationary phases: SPB-1 (polydimethylsiloxane 30 m × 0.20 mm id, film thickness 0.20 μm) and Supelcowax-10 (polyethyleneglycol 30 m × 0.20 mm id, film thickness 0.20 μm). Oven temperature program is 70–220°C (3°C·min^{-1}), 220°C (15 min), with injector temperature: 250°C, carrier gas: helium, adjusted to a linear velocity of 30 cm·s^{-1}; splitting ratio 1 : 40; detectors temperature: 250°C. GC-MS was carried out in a Hewlett-Packard 6890 gas chromatograph fitted with an HP1-fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., 0.25 μm film thickness) interfaced with a Hewlett-Packard 5973 mass selective detector (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters are described above, with interface temperature: 250°C, MS source temperature: 230°C, MS quadrupole temperature: 150°C, ionization energy: 70 eV, ionization current: 60 mA, scan range: 35–350 units, and scans·s^{-1}: 4.51.

Essential oil components were identified by their retention indices on both SPB-1 and Supelcowax-10 columns and from their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of C8–C23 of *n*-alkanes, were compared with those of reference samples included in the Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra database. Acquired mass spectra were compared with reference spectra from laboratory database, Wiley/NIST library [21], and literature data [22, 23]. Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

2.4. Trypanocidal Activity. Procyclic forms of *T. brucei* strains—427 [24] and 29-13 [25]—were grown at 28°C in SDM-79 medium [26] containing 10% fetal bovine serum (Gibco), penicillin (Sigma-Aldrich), and streptomycin (Sigma-Aldrich). Procyclic forms cultures were carried out to obtain the exponential growth phase (1×10^6 parasites/mL). Log phase procyclic parasites were frozen at −80°C with 10% glycerol for storage.

2.5. MTT Colorimetric Assay of T. brucei Strains. Procyclic forms of *T. brucei* (427 and 29-13 strains) were incubated for 24 hours with the essential oils or with pure compounds for determination of cytotoxicity index (CI_{50}) accessed by the MTT colorimetric assay. This assay is based on the determination of the ability of living cells to reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to the corresponding formazan. Assays were performed in 96-well plates, according to the methodology described by Mosmann (1983) [27] and modified by Cotinguiba and colaborators (2009) [28]. Each test was made in triplicate. The essential oils and isolated compounds were solubilized in DMSO (dimethyl sulfoxide) (Synth), and at the time of use dilutions were made in different concentrations using SDM-79 medium; the final concentration of the solutions reached 3% DMSO, which does not affect the viability of the parasites. Pentamidine and SDM-79 medium were used as positive and negative controls, respectively.

3. Results and Discussion

The compositions of the essential oils from leaves and from rhizomes of *H. coronarium* are described in Table 1, where compounds are listed in the order of their retention on the SBP-1 column (Figures 1 and 2).

Thirty-nine components were identified in the oil from rhizomes, representing 86.1% of the whole composition. Monoterpene hydrocarbons and oxygen containing monoterpenes prevail in the rhizomes oil, with 1,8-cineole (31.7%), α-terpineol (12.0%), β-pinene (11.0%), and terpinen-4-ol (6.8%) as the major constituents. The occurrence of these compounds was previously described [29–31]. Additionally, the labdane diterpenoid coronarin E was also found at the concentration of 14.1%. The occurrence of diterpenoids in the composition of the rhizomes oil of *H. coronarium* was never reported.

In the leaves oil, thirty components were identified, representing 88% of the whole composition. The oil is mainly

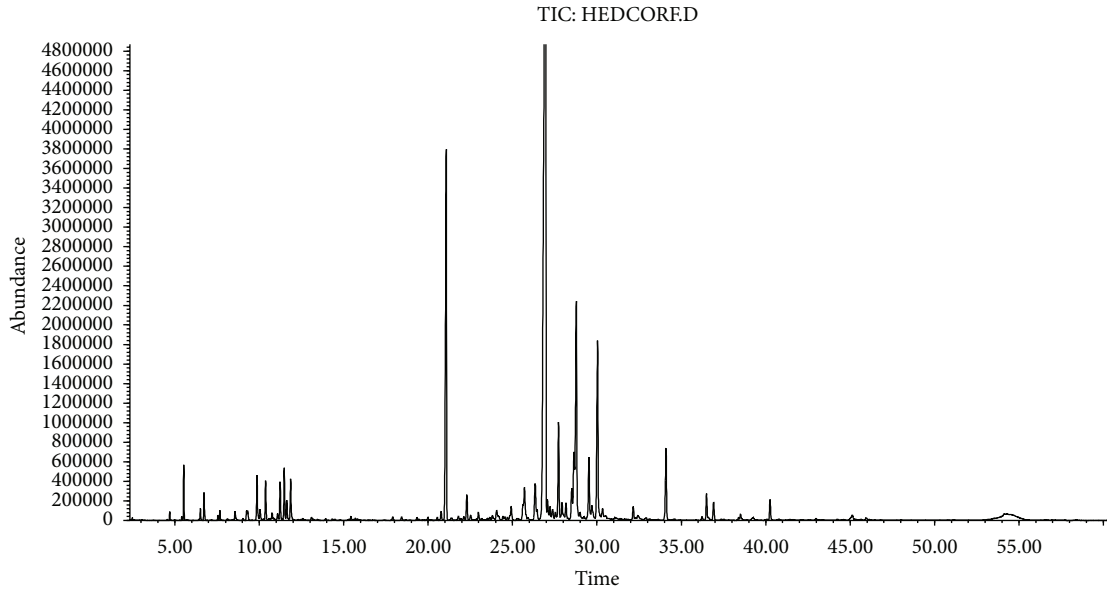

Figure 1: Chromatogram of *Hedychium coronarium* leaves essential oil (SBP-1 column).

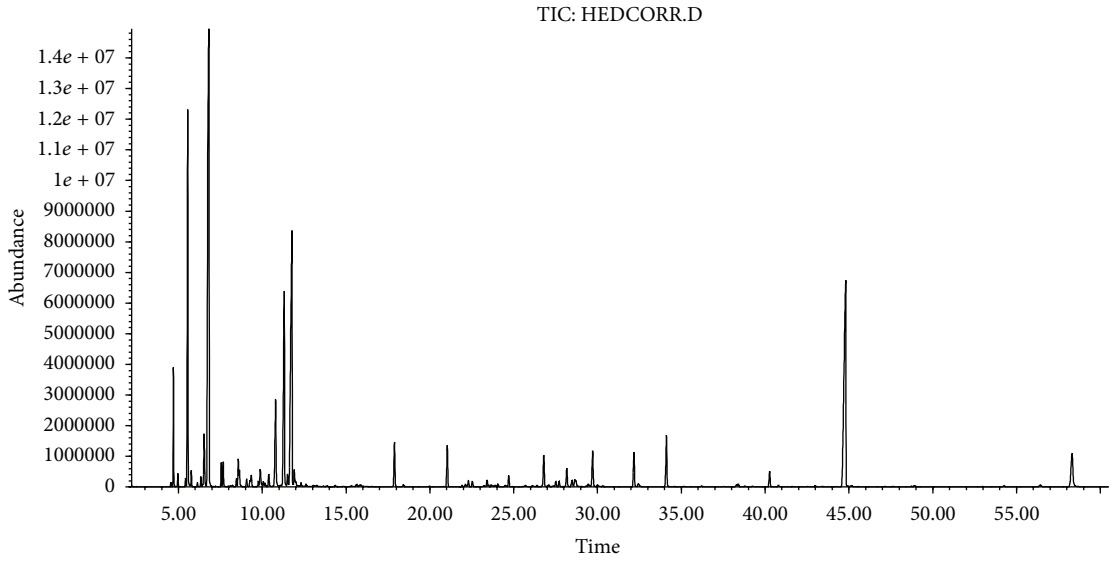

Figure 2: Chromatogram of *Hedychium coronarium* rhizomes essential oil (SBP-1 column).

composed by sesquiterpenoids, particularly caryophyllene derivatives (73.5%), with caryophyllene oxide (43.9%), *E*-caryophyllene (12.1%), caryophylladienol I (7.7%), caryophyllenol II (5.6%), caryophyllenol I (2.0%), and caryophylladienol II (1.8%) as the most representative. This composition is different from those reported by Ali et al. (2002) [30] and Dos Santos et al. (2010) [29]. Ali et al. (2002) describe plants growing in Fiji islands composed by monoterpenes (β-pinene, 53.6%) and sesquiterpenes (β-caryophyllene, 17.7%); Dos Santos et al. (2010) report β-caryophyllene (43.0%), caryophyllene oxide (12.1%), and α-humulene (2.2%) as the major constituents of an oil from plants growing in Brazil.

Table 2 summarizes the results of the trypanocidal effects of the essential oils and pure compounds.

Cytotoxicity index (CI_{50}) values of the crude essential oils (>100 $\mu g \cdot mL^{-1}$), as well as of 1,8-cineole, the major constituent of the rhizomes oil, indicated no trypanocidal activity (CI_{50} value of pentamidine, used as positive control, was estimated as 2.19 $\mu g \cdot mL^{-1}$). Nevertheless, caryophyllene oxide, the major constituent of the essential oil from leaves, revealed a remarkable activity against both *T. brucei* strains, 427 (CI_{50} = 65.77 $\mu g \cdot mL^{-1}$) and 29-13 (CI_{50} = 24.53 $\mu g \cdot mL^{-1}$). The mixture caryophyllene oxide, pentamidine (1 : 1, v/v), revealed a stronger activity (CI_{50} = <1.0 $\mu g \cdot mL^{-1}$), similar

Table 2: *In vitro* trypanocidal activity of essential oils and major components of *Hedychium coronarium* against *Trypanosoma brucei* procyclic forms.

Compounds	*T. brucei* (427 strain) $\mu g \cdot mL^{-1}$	*T. brucei* (29-13 strain) $\mu g \cdot mL^{-1}$
Essential oil from leaves	>100	>100
Essential oil from rhizomes	>100	>100
1,8-Cineol	>100	>100
Caryophyllene oxide	65.77	24.53
Caryophyllene oxide + pentamidine	<1.0	<1.0
Pentamidine	2.19	2.19

to both *Trypanosoma* strains, indicating a probable synergic effect. Caryophyllene oxide was previously recognised as one of the key ingredients for the activity of the hexane extract of *Serjania yucatanensis* against trypomastigotes of *Trypanosoma cruzi* [32]. Furthermore, cytotoxic effects of caryophyllene oxide on several cellular models due to the inhibition of the mitochondrial electron transport chain [33] and apoptosis induction [34] can also explain our results.

4. Conclusions

The compositions of the essential oils from leaves and from rhizomes of *H. coronarium* from Brazil were established. Despite the lack or the feeble trypanocidal activity of these essential oils, the major constituent of the leaves oil, caryophyllene oxide, revealed remarkable potential trypanocidal activity. This activity was strain dependent since it is higher on *T. brucei* 29-13. Interestingly, a potential synergism between caryophyllene oxide and pentamidine was demonstrated in this study. This can be an issue for further studies using natural compounds as an alternative treatment of chronic parasite diseases.

Conflict of Interests

The authors declare no conflict of interests.

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