



BIOLOGICAL SCIENCES

Antiplatelet and antibacterial activities of Essential Oils obtained from rhizomes and leaves of *Hedychium coronarium* J. Koenig

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Abstract: *Hedychium coronarium* J. Koenig, belonging to Zingiberaceae family, is a perennial herb with fleshy aromatic rhizomes. There are no information about the antiplatelet properties of essential oils (EOs) from rhizomes (HCR) and leaves (HCL) of this herb, additionally, there are reports about the antibacterial activity of the Zingiberaceae species, however, no studies have been carried out in the Colombian Amazon Region. The EOs were characterized by GC-MS, the antiaggregant activity was assessed by ADP and Collagen as platelet agonist and the antibacterial activity against *E. faecalis* and *S. aureus* were evidenced by the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A high content of oxygenated monoterpenes were found in HCL essential oil (EO) and 20 compounds were identified in HCR EO. The HCL EO showed antiaggregant activity when collagen was used and HCR EO showed a concentration-dependent activity against ADP and collagen, meanwhile only the HCR EO showed antibacterial activity against *E. faecalis* and *S. aureus*.

Key words: *Hedychium coronarium* J. Koenig, antiaggregant property, antibacterial, essential oil.

INTRODUCTION

Herbs and spices have been widely used for generations as food ingredients and for treating ailments; nowadays, scientific information demonstrate that these natural products contain essential oils (EOs) with different properties used in the prevention and treatment of diseases. Continual research motivated by the increasing incidence of cardiovascular and infectious diseases have recognized in different herbal species, an important source of compounds with antiplatelet and antibacterial properties (Tognolini et al. 2006, El Haouari & Rosado 2016, Vieitez et al. 2018).

Hedychium coronarium J. Koenig, belonging to Zingiberaceae family, is a perennial

herb growing to a height of 0.5 - 1.5 m tall, with fleshy aromatic rhizomes. It is used worldwide, as food flavoring and seasoning (Prakash et al. 2010), but also in traditional medicine for treatment of diabetes, diphtheria, fever, headache, rheumatism and cancer (Bhandary et al. 1995, Matsuda et al. 2002, Oh et al. 2006, Kunnumakkara et al. 2008); its EOs has antioxidant, antimicrobial, anti-inflammatory and mosquito larvicidal properties (Prakash et al. 2010, Noriega et al. 2019, Lu et al. 2009, Ho 2011, Phukerd & Soonwera 2013, Suksathan et al. 2013, Zhao et al. 2017).

Although there are reports about the antibacterial activity of the Zingiberaceae species (El Haouari & Rosado 2016, Jantan et al. 2008), no reports have been carried out in the

Colombian Amazon Region and no information is available about the antiplatelet properties of EOs of *H. coronarium* J. Koenig.

Given the importance of the prevention and treatment of cardiovascular diseases and in order to expand the knowledge about the antibacterial activity of *H. coronarium* J. Koenig, the aim of this study was to evaluate the antiplatelet and antibacterial activities of EOs obtained from leaves and rhizomes of this plant.

MATERIAL AND METHODS

Plant Material and essential oils (EOs)

Extraction

The plant material (leaves and rhizomes) of *Hedychium coronarium* J. Koenig (HCL and HCR) were collected in May 2012 (wet season, winter), folio number 5-2018, from the research center MACAGUAL of the Universidad de la Amazonia (Colombia) with coordinates, North: 01° 29' 51.1" West: 75° 39' 35.5", at 254 m above sea level and classified taxonomically. A voucher specimen of plant (Nº 9001) was deposited in the herbarium of the Universidad de la Amazonia (Colombia). Dried material of HCL and HCR (200 g) were subjected to hydro-distillation for 6 h, using a Clevenger apparatus to afford EOs yields of 0.36% (v/w) for HCL and 0.07% for HCR.

Moisture traces were removed by drying the EOs over anhydrous sodium sulfate (Na_2SO_4), the EOs were collected in glass vials and kept at 4 °C until GC-MS analysis.

GC-MS analysis

The analysis of the EOs obtained from HCL and HCR were performed on an GC-MS (HP 5890-II GC/MS system), equipped with a detector HP 5972 (MSD), scanned in the 40-350 m/z range, with an optimum value of the capillary voltage of 70 eV. Compounds were separated on a DB-5MS capillary column (60 m x 0.25 mm x 0.25

µm). The column temperature was set at 50 °C (2 min) with an increase of 8 °C min⁻¹ until 325 °C (50 min), the carrier gas was helium at a flow of 1 mL min⁻¹.

The identities of the oil components were identified based on comparison of the linear Retention Index (RI) calculated relative to a series of *n*-alkanes (C7-C40; Sigma-Aldrich), confirmed by comparison with published RI values (Adams 2007), and via mass spectral comparison using the mass spectral library software (NIST 08 2008).

Platelet Aggregation Assay

Blood samples were obtained from volunteers donors who signed an informed consent approved by the scientific ethics committee of the Universidad de Talca (Folio number 5-2018). The samples were collected by phlebotomy in 3.2% citrate tubes, mixed and allowed to stand for 5 min. The samples were then centrifuged at 1000 rpm for 10 min to obtain a supernatant rich in platelets, which were adjusted to 2×10^5 platelets/µL with platelet-depleted plasma, using a hematological counter.

The platelet aggregation was performed *in vitro* using a lumi-aggregometer (Mekhfi et al 2004). For this, 480 µL of platelets (2×10^5 platelets/µL) were put in an aggregometer tube and pre-incubated with 20 µL of the respective EOs (1, 0.5, 0.25 and 0.1 mg/mL), solubilized in dimethylsulfoxide (DMSO), after 5 min of incubation, 20 µL of ADP 4 µM or collagen 1 µg/mL as agonist were added to initiate the platelet aggregation. The aggregation process was followed for 6 min, and the platelet aggregation were determined (maximal amplitude (%)) by the software AGGRO/LINK (Chrono-Log, Havertown, PA, USA). All the measurements were performed in triplicate; DMSO (1%) was used as negative control (100% aggregation).

Antibacterial Activity

Strains used were *Enterococcus faecalis* (ATCC 51299) and *Staphylococcus aureus* (ATCC 25923). Prior the assay, they were seeded in Brain heart infusion (BHI) medium, incubated at 37 °C and spiked for 24 h for their metabolic activation.

Antibacterial Screening

As screening the agar diffusion method was used to evaluate the antibacterial activity of the EOs (HCR and HCL). In 100 mm diameter plates with Mueller-Hinton agar were seeded with a 0.5 McFarland standard of each bacterium under study (per plate). Subsequently, 6 mm diameter paper discs impregnated with 15 µL of each of the EOs (HCR and HCL) were deposited on the Mueller-Hinton agar plates with the microorganisms. The agar plates were incubated at 37 °C for 24 h and the inhibition diameters were measured in millimeters and as a reference antibiotic was used chloramphenicol (CAF) at a concentration of 30 µg/Sensi-Disc.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) by 96-well plate microdilution

The MIC and MBC were determined by the microdilution test according to the recommendations of the National Committee of Clinical Laboratory Standards (NCCLS, 1999) (CLSI 1999). The assay was carried out in Mueller-Hinton broth, and the HCR and HCL EOs were dissolved in 1% DMSO. The concentrations tested for each essential oil (EO) ranged from 0.02 to 48 mg/mL, bacterial suspensions were obtained by incubation at 37 °C for 12 h and for the assay were adjusted to 2×10^5 CFU/mL. The microplates were incubated at 37 °C for 24 h and the MIC was defined as the lowest concentration of the EO in which the microorganism does not show visible growth, determined by the absence of turbidity

in the microwell. To determine the MBC, those microwells without turbidity were subcultured by seeding 10 µL of the content in a plate free of microorganisms with Mueller-Hinton agar and incubated at 37 °C for 24 h. The MBC was defined as the concentration of the EO that eliminated the 99.99% of the microorganism tested. The antibiotic of reference (CAF) was used at a concentration of 0.195 to 100 µg/mL.

Statistical Analysis

The results were analyzed statistically, comparing the results of each EO under study with the negative control (DMSO 1%). The results were expressed as mean \pm SE, the statistical analysis *t-test* was used with the software SPSS 15.0 (StatisticalProduct and ServiceSolutions). *p*-value < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Chemical Composition of HCL and HCR essential oils

From the HCL EO were identified 19 compounds (92.9%), with a high content of oxygenated monoterpenes (50.33%), it was also found a 23.35 and 19.20% corresponding to monoterpene hydrocarbons and sesquiterpene hydrocarbons, respectively. A total of twelve compounds constitute the oxygenated monoterpenes group, being the 1,8-cineole (22.68%) and β -terpineol (7.90%) the major components, additionally, in decreasing order were found pinocarvone (4.45%), myrtenol (2.00%), cis-carveol (2.05%) and perillyl alcohol (2.79%), whereas the monoterpene hydrocarbons content comprise in decreasing order: β -pinene (14.78%), α -pinene (7.10%) and γ -terpinene (1.47%). Moreover, the presence of β -caryophyllene (12.95%), α -humulene (2.87%), β -cis-farnesene (1.90%) and β -guaiene (1.48%) were present in the 19.2% of the sesquiterpene

hydrocarbons (Table I). An EO extracted from HCL cultivated in Choco, Colombia, showed a similar composition to that found in this study, being the 1,8-cineole (31.5 %), β -pinene (13.7 %), terpinen-4-ol (6.0 %) and α -pinene (3.8 %), the major constituents (Caballero-Gallardo et al. 2014).

From the HCR EO were identified 20 compounds (89.90%), composed of a high content of monoterpene hydrocarbons (52.41%), followed by 4 oxygenated monoterpenes (18-75%) and 5 sesquiterpenes (8.91%). A total of five compounds constitute the monoterpene hydrocarbons group, being the tricyclene (33.46%) and α -pinene (13.11%) the major components. The oxygenated monoterpenes, camphor (6.20%), pinocarvone (5.39%), linalool (4.72%), the sesquiterpene caryophyllene (3.59%) and the oxygenated sesquiterpene caryophyllene oxide (5.87%) are also important constituents of this EO.

In *H. coronarium*, tricyclene has been reported as constituent (2.7%) of fresh leaves EO, from Choco, Colombia (Caballero-Gallardo et al. 2014) and in lower percentage (0.04%) in HCR EO from Eastern India (Ray et al. 2017). There are not reports about occurrence of this metabolite as major compound in HCR EO. However, metabolites as α -pinene and linalool have also been found as important constituents of EO from HCR growing in Eastern India (Ray et al. 2017, Parida et al. 2015). Furthermore, cadinol, caryophyllene oxide, nerolidol, caryophyllene, pinocarvone, camphor, geraniol, γ -terpinene, cis- β -terpineol, γ -gurjunene have been found in lower quantities (< 1 and < 0.1 %) (Ray et al. 2017), leaving in evidence that the different components varies greatly with the crop, climate and geographical position.

Inhibition of platelet aggregation

The EO obtained from HCL showed no antiplatelet activity against the aggregation induced by ADP 4 μ M ($p > 0.05$), nevertheless, when collagen 1 μ g/mL was used as agonist, an antiaggregant activity was observed at a concentration of 0.5 mg/mL ($p < 0.05$) and 1 mg/mL ($p < 0.001$), with a percentage of inhibition of the platelet aggregation of $30.8 \pm 1.2\%$ and $71.7 \pm 2.2\%$, respectively.

For its part, the EO extracted from HCR showed an increase in the inhibition of the platelet aggregation (using ADP 4 μ M as agonist) as the concentration of the EO increased, with a percentage of inhibition of the platelet aggregation of $18.3 \pm 0.9\%$ at a concentration of 0.25 mg/mL, until a $85.1 \pm 1.8\%$ of inhibition of the platelet aggregation at a concentration of 1 mg/mL (Table II). A similar and more intense effect was observed when collagen was used as agonist with a percentage of inhibition of the platelet aggregation of $48.6 \pm 1.5\%$ at a concentration of 0.25 mg/mL, until $97.2 \pm 2.1\%$ of inhibition of platelet aggregation at a concentration of 1 mg/mL.

The presence of platelets within the bloodstream is crucial for the regulation of the clotting process. The HCL and HCR EOs extracted from *H. coronarium* presented different activities against the platelet aggregation induced by ADP. The ADP is considered a mild platelet agonist and is involved in the activation and platelet aggregation amplification (Murugappa & Kunapuli 2006). The HCR was the only EO that showed antiplatelet activity against the aggregation induced by ADP, among the different compounds identify in this EO highlight the presence of α -pinene which was the second most abundant with a percentage of 13.11% of the total compounds found in the HCR EO, which is almost twice the amount presented in the HCL EO, the presence of this compound could be the

Table I. Chemical constituents of EOs of *Hedychium coronarium* J. Koenig leaves (HCL) and rhizomes (HCR). Retention index (RI) calculated relative to a series of n-alkanes.

Compound	RI calc.	RI Lit. ^a	% in HCL essential oil	% in HCR essential oil
Tricyclene	916	921	-	33.46
α -pinene	939	932	7.12	13.11
β -pinene	978	974	14.78	-
1,8-cineole	1033	1026	22.68	-
δ -Terpinene	1051	1048	-	2.32
γ -terpinene	1056	1054	1.47	1.45
Linalool	1102	1095	-	4.72
<i>trans</i> -pinocarveol	1133	1135	1.39	-
Camphor	1136	1141	-	6.20
β -terpineol	1145	1140	7.90	0.93
Pinocarpone	1157	1160	4.45	5.39
terpinen-4-ol	1172	1174	1.63	-
α -terpineol	1187	1186	1.48	-
Myrtenol	1189	1194	2.00	-
Verbenone	1206	1204	1.78	-
<i>cis</i> -(-)-carveol	1223	1226	2.05	-
Carvone	1229	1239	1.07	-
Geraniol	1256	1252	1.11	2.07
Isocarveol	1280	1294	2.79	-
Carvacrol	1295	1298	-	1.51
β -panasinsene	1413	1381	-	1.42
<i>trans</i> - β -caryophyllene	1417	1417	12.95	-
Caryophyllene	1423	1418	-	3.59
β - <i>cis</i> -farnesene	1459	1440	1.90	1.06
α -humulene	1461	1452	2.87	-
γ -gurjunene	1476	1475	-	1.74
β -selinene	1491	1490	-	1.10
<i>cis</i> - β -guaiene	1494	1502	1.48	-
Nerolidol	1546	1540	-	1.08
Elemol	1552	1548	-	1.09
Caryophyllene oxide	1572	1582	-	5.87
Apiole	1681	1677	-	1.85

^aretention index (RI) calculated relative to a series of n-alkanes.

Table II. Percent inhibition of platelet aggregation of HCL and HCR EOs.

	Collagen 1 µg/mL					IC ₅₀ mg/mL
	Inhibition ratio (%)					
	DMSO 1%	0.1 mg/mL	0.25 mg/mL	0.5 mg/mL	1 mg/mL	
HCL	0.2 ± 0.1	0.5 ± 0.3	0.8 ± 0.5	30.8 ± 1.2 *	71.7 ± 2.2 **	0.75
HCR	0.3 ± 0.2	1.2 ± 0.8	48.6 ± 1.5 *	85.0 ± 1.4 **	97.2 ± 2.1 **	0.38
	ADP 4 µM					IC ₅₀ mg/mL
	Inhibition ratio (%)					
	DMSO 1%	0.1 mg/mL	0.25 mg/mL	0.5 mg/mL	1 mg/mL	
HCL	0.3 ± 0.1	0.6 ± 0.3	0.9 ± 0.4	1.2 ± 0.8	1.9 ± 0.9 *	> 1
HCR	0.3 ± 0.2	0.5 ± 0.3	18.3 ± 0.9 *	43.2 ± 1.8 **	85.1 ± 1.8 **	0.61

n = 6, * *p* < 0.05 and ** *p* < 0.001 respect to the negative control (DMSO 1%).

responsible of the antiplatelet activity, since Yang et al. (2011), described an important antiplatelet activity of the (+)- α -pinene purified from *Angelica sinensis* against the aggregation induced by ADP with an IC₅₀ of 8.23 µg/mL. Collagen, for its part, is important for platelet adhesion and activation, 2 major platelet receptors have been identified; the receptor GPIa/IIa ($\alpha_2\beta_1$ integrin) that contributes to platelet adhesion and GPVI, which is responsible for intracellular signaling and platelet activation (Zhou & Schmaier 2005). Both EOs (HCL and HCR), showed an antiplatelet activity against the aggregation induced by collagen being the HCR EO the most active, this antiplatelet activity could be due to the presence of α -pinene found in the HCL which is also one of the most abundant in the HCR EO. As in case of the agonist ADP, this compound could prevent the release of calcium from the dense granules which is one of the main events in the process of platelet activation and aggregation induced by agonist such as ADP and collagen (Berna-Erro et al. 2016, Ware et al. 1987), moreover, in the HCR highlighted the presence of carvacrol, which despite being in low concentration, may exert an effect enhancing the antiplatelet effect of the HCR EO against the agonist collagen.

Carvacrol is one of the main substances of EO from the herb *Origanum vulgare* and has been described many activities as antimicrobial (Friedman et al. 2002), antioxidant (Alma et al. 2003) and anticarcinogenic (Friedman 2014), also the antiplatelet activity has been described; at this respect, Son et al. 2005, showed that carvacrol, extracted from *T. dolabrata* var. *hondai* presented an inhibition of platelet aggregation (IC₅₀, µM) induced by collagen of 12.6 ± 1.3 µM (Son et al. 2005). At the same time, Karkabounas et al. (2006), reported the anticarcinogenic and antiplatelet activity of carvacrol with a dependent dose-effect over the platelet aggregation by different agonists.

Antibacterial Activity

For both EOs tested, only the HCR showed antibacterial activity against *E. faecalis* ATCC 51299 and *S. aureus* ATCC 25923 with an inhibitory halo of the agar diffusion method of 10 mm and 14 mm, respectively (Table III). The MIC value of the HCR EO against *E. faecalis* was 3 mg/mL; meanwhile the MBC obtained was 24 mg/mL. Similarly, when the same EO was evaluated against *S. aureus*, the MIC value was 12 mg/mL and the MBC was 24 mg/mL (Table III). Regarding to the reference

Table III. Antibacterial activity of the HCR EO against *E. faecalis* and *S. aureus*.

	<i>Enterococcus faecalis</i> ATCC 51299	Chloramphenicol	<i>Staphylococcus aureus</i> ATCC 25923	Chloramphenicol
Inhibition halo (mm)	10	11	14	19
MIC (mg/mL)	3	3.1E-03	12	3.1E-03
MBC (mg/mL)	24	5.0E-02	24	2.5E-02

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal concentration.

to the reference antibiotic, the diameter of the inhibitory halo in the agar diffusion method was for *E. faecalis* and *S. aureus* of 11 mm and 19 mm, respectively. The MIC values of the antibiotic for both microorganisms were 3.125 µg/mL and the MBC value for *E. faecalis* was 50 µg/mL and 25 µg/mL for *S. aureus* (Table III).

Both EOs (HCL and HCR) were investigated about their antibacterial activity, nevertheless, only the HCR EO showed an inhibition halo against *E. faecalis* and *S. aureus*, with MIC and MBC in the order of the mg/mL (Table III); as known the biological activity of an EO could be due to the presence of a single active constituent or more probably, due to the synergistic interactions of several compounds present in it (Tajkarimi et al. 2010). Thus, the antibacterial activity of HCR EO could be attributed to their main compounds such as α -pinene, pinocarvone, tricyclene, camphor and caryophyllene oxide, or synergistic effects between major and minor components. At this regard, Memariani et al. (2017) showed the antibacterial activity of the EO of *Pistacia atlantica* Desf. against the Gram-negative bacteria *Helicobacter pylori*; the main constituent of this essential oil was α -pinene (93.17%) which was indicated as the responsible of the antibacterial activity.

In this study, tricyclene was the major component of the HCR EO (33.46%), compound that was not found in HCL EO. Several EOs containing tricyclene as the major constituent has been described with antibacterial activity, among them, EOs from *Azorella spinosa*

(10.81- 16.99% of tricyclene) (Jara-Bermeo et al. 2016), *Cordia cylindrostachya* (Ruiz & Pav.) Roem. & Schult. (*Boraginaceae*) also showed high content of tricyclene (Oza MJ & Kulkarni YA 2017) and the EO of *Cordia verbenacea* D.C. (*Boraginaceae*), that grows in Mérida-Venezuela with 23.9% of tricyclene (Meccia et al. 2009); all these EOs showed regular or good antibacterial activity against different bacteria, which may suggest the important antibacterial activity of tricyclene.

CONCLUSION

The EO obtained from leaves of *H. coronarium* J. Koenig showed a predominance of oxygenated monoterpenes, followed by monoterpene hydrocarbons and sesquiterpene hydrocarbons, for its part, in the HCR EO were identified 20 compounds with a different predominance, being the monoterpene hydrocarbons the largest number of identified compounds, followed by oxygenated monoterpenes and sesquiterpenes.

When ADP and Collagen were used as agonist of the platelet aggregation, both EOs showed different behaviors, the HCL EO showed no antiplatelet activity against the aggregation induced by ADP, nevertheless, an inhibition of the platelet aggregation was observed when Collagen was used. In case of the HCR EO, an inhibition of the platelet aggregation was evidenced when both agonists were used. Among the different compounds identify in these EOs, highlight the relatively high amount of α -pinene

and carvacrol, from which has been described an inhibition of the platelet aggregation. The HCL EO showed no antibacterial activity at the concentrations used, meanwhile the HCR EO showed antibacterial activity against the Gram-positive bacteria *S. aureus* and *E. faecalis*, possibly due to the high amount of Tricyclene (> 33%) found in this fraction.

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