Rev Bras Farmacogn 24(2014): 408-412

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.journals.elsevier.com/revista-brasileira-de-farmacognosia

Original article

Cancer chemoprevention activity of labdane diterpenes from rhizomes of *Hedychium coronarium*

Denise C. Endringer[a,*], *Francisca S.N. Taveira*[b], *Tamara P. Kondratyuk*[c], *John M. Pezzuto*[c], *Fernão C. Braga*[a,*]

[a]*Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[b]*Departamento de Química, Universidade Federal do Maranhão, São Luís, MA, Brazil*
[c]*The Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, USA*

ARTICLE INFO

Article history:
Received 23 April 2014
Accepted 8 August 2014

Keywords:
Hedychium coronarium
Zingiberaceae
COX-1 and -2
NF-κB
Labdane diterpenes

ABSTRACT

Hedychium coronarium J. Koenig, Zingiberaceae, is a medicinal plant popularly used to treat inflammatory conditions in different countries. Three labdane diterpenes [isocoronarin D (**1**), methoxycoronarin D (**2**), ethoxycoronarin D (**3**)] and benzoyl eugenol (**4**) were isolated from rhizomes and their chemopreventive potential was evaluated using *in vitro* assays, namely the inhibition of NF-κB, COX-1 and -2, the induction of antioxidant response element (ARE), and the inhibition of cell proliferation. Diterpene **1** activated ARE (EC_{50} 57.6 ± 2.4 μM), while **2**, **3** and **4** significantly inhibited NF-κB (IC_{50} of 7.3 ± 0.3, 3.2 ± 0.3 and 32.5 ± 4.9 μM, respectively). In addition, **2** and **3** selectively inhibited COX-1 (IC_{50} values of 0.9 ± 0.0 and 3.8 ± 0.0 μM, respectively). These data support the potential chemopreventive activity of constituents from H. coronarium rhizomes.

Introduction

Hedychium coronarium J. Koenig, Zingiberaceae, popularly known as *butterfly ginger, butterfly lily, cinnamon jasmine, garland flower* and *ginger lily*, is a medicinal plant cultivated throughout India, Southeast Asian countries, China, Japan and Brazil (Manish, 2013). Its rhizomes are widely used in Brazil to treat pain, wounds, infections and rheumatism (Pio Corrêa, 1974; Ribeiro et al., 1988). Previous studies have demonstrated the anti-inflammatory, analgesic and leishmanicidal activities of different extracts from the rizhomes of *H. coronarium* (Braga et al., 1999; Shrotriya et al., 2007; Valadeau et al., 2009). Labdane diterpenes, farnesane sesquiterpenes and essential oil constituents such as 1,8-cineole, β-pinene and α-terpineol have been isolated from the rhizomes of this species and anti-inflammatory properties have been described (Morikawa et al., 2002; Taveira et al., 2005; Joy et al., 2007; Chimnoi et al., 2008; Manish, 2013).

A broad spectrum of biological activities has been described for labdane diterpenes, including antibacterial, antiviral, anti-inflammatory and anti-tumor activities (Ghosh and Karin, 2002; Pezzuto et al., 2005; Maiti et al., 2007; Girón et al., 2008; Hegazy et al., 2008; Kunnumakkara et al., 2008; Suresh et al., 2010; Zhan et al., 2012). Remarkable inhibitory activity of nitric oxide (NO) production in LPS-activated mouse peritoneal macrophages has been reported for coronarin D and hedychilactone A, labdane diterpenes isolated from the rizhomes of *H. coronarium* (Matsuda et al., 2002; Kiem et al., 2012).

* *Corresponding author.*
E-mail: fernao@netuno.lcc.ufmg.br (F.C. Braga); denise.endringer@ifes.edu.br (D.C. Endringer).
0102-695X/$ - see front matter
http://dx.doi.org/10.1016/j.bjp.2014.08.002

One of the most important steps in the inflammatory response is the activation of the nuclear transcription factor-κB (NF-κB) (Ghosh and Karin, 2002; Kunnumakkara et al., 2008). NF-κB activation is involved in inflammation, cell proliferation and oncogenic processes (Skehan et al., 1990; Matsuda et al., 2002; Pezzuto et al., 2005; Maiti et al., 2007; Kang et al., 2009; Kiem et al., 2012). Thus, agents that suppress NF-κB activation can abrogate carcinogenesis. This finding has encouraged the search for specific inhibitors of NF-κB activation from natural sources, which might lead to new candidates for cancer chemoprevention (Skehan et al., 1990; Pezzuto et al., 2005). In addition, COX-2 is induced during the inflammatory response and has been implicated in the etiology of cancer (Ghosh and Karin, 2002; Maiti et al., 2007; Kunnumakkara et al., 2008). Coronarin D has been shown to inhibit the NF-κB activation pathway induced by different carcinogens and pro-inflammatory molecules, and also to impair the expression of constitutive NF-κB (Girón et al., 2008; Kunnumakkara et al., 2008). It also inhibits NF-κB by down regulating IKK activation (Girón et al., 2008; Kunnumakkara et al., 2008).

As part of our continuing search for bioactive molecules from medicinal plants, herein we report the potential cancer chemopreventive activity of labdane diterpenes isolated from rhizomes of *H. coronarium*. Chemopreventive activity was assessed by examining *in vitro* inhibition of NF-κB, COX-1 and -2, ARE induction, and cell proliferation inhibition.

Materials and methods

Plant material

The rhizomes of *Hedychium coronarium* J. Koenig, Zingiberaceae, were collected in June 2002, at UFMG campus, in Belo Horizonte, Minas Gerais state, Brazil. The species was identified by Dr. J. A. Lombardi, from the Departamento de Botânica, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Brazil, where a voucher specimen was deposited (BHCB 68447).

Reagents and apparatus

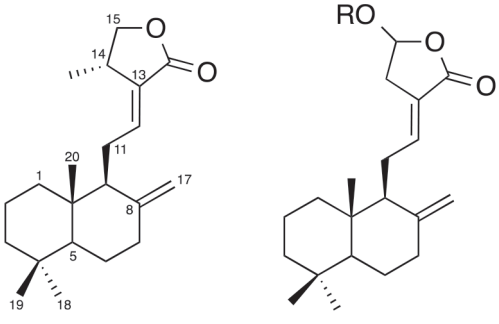
All chemicals, unless specified otherwise, were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Cell lines

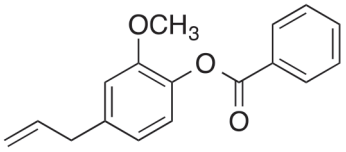
Cell culture media and supplements were obtained from Gibco (Life Technologies, Grand Island, NY). Wild-type HepG2 and HepG2 transfected with ARE-luciferase plasmid, human hepatoma cells, were supplied by Dr. Hong-Jie Zhang (University of Illinois at Chicago, Chicago, IL); 293/NF-κB, human embryonic kidney cells, transfected with NF-κB-luciferase plasmid and Hygromycin (RC0014) were purchased from Panomics (Fremont, CA); LU1, human lung carcinoma cells were supplied by the Department of Surgical Oncology, University of Illinois at Chicago, Chicago, IL; MCF-7, human breast carcinoma (ATCC HTB-22) and hormone-dependent human prostate carcinoma LNCaP (ATCC-CRL-1740) were purchased from American Type Culture Collection (Rockville, MD). All media were supplemented with 10% fetal bovine serum (FBS) (HyClone*Thermo Scientific, VWR, Atlanta, GA) containing 100 U/ml penicillin and 0.1 mg/ml streptomycin. Cells were incubated at 37°C with 5% CO_2. All cells were kept under twenty passages and used in experiments during the linear phase of growth. COX-1 enzyme was purchased from Animal Technologies Tyler (Texas). For the assays, the compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted to their final concentrations. An equivalent volume of DMSO was added to control wells and showed no measurable effect on the assayed culture cells or enzymes.

Isolation and identification of constituents of H. coronarium

After drying at 40°C, for 72 h, the rhizomes of *H. coronarium* were powdered (706.3 g) and percolated with 96% EtOH at room temperature. The solvent was removed under reduced pressure yielding 81.9 g of a dark residue that was successively extracted by partition with *n*-hexane, dichlorometane and methanol yielding three main fractions *n*-hexane (6.5 g), dichloromethane (26.0g) and methanol (15.0 g). The dichloromethane fraction was re-chromatographed on a silica gel column (70-230 mesh, Merck), eluting with *n*-hexane-ethyl acetate (95:5-1:1) gradient. The sub-fraction eluted with *n*-hexane-ethyl acetate (9:1) afforded benzoyleugenol (48.8 mg, 4). The sub-fraction eluted with *n*-hexane-ethyl acetate (85:15) was re-chromatographed on a silica gel column eluting with dichloromethane-ethyl acetate (100:0-0:100) gradient. The sub-fraction eluted with 100% of dichloromethane yielded isocoronarin D (**1**, 311.2 mg) and the one eluted with DCM:EtOAc (98:2) yielded ethoxycoronarin D (epimers, **3a/3b**, 44.0 mg). The methanol fraction was re-chromatographed on a silica gel column, eluting with dichloromethane-ethyl acetate (100:0-0:100) gradient. The sub-fraction eluted with 100% of dichloromethane yield methoxycoronarin D (epimers, **2a/2b**, 5.8 mg). Isocoronarin D was isolated as a pure compound. Structure identification was accomplished by spectroscopic methods (Taveira et al., 2005).



1 **2a/2b** R=CH_3 **3a/3b** R=CH_2CH_3



4

Luciferase assays

Luciferase assays were conducted as previously described (Pezzuto et al., 2005). In brief, transfected cells were incubated at an initial density of 1.5×10^5 cells per well for 48 h in 96-well plates. After a 6 h incubation with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (100 nM) and test compounds, cells were analyzed for luciferase activity. Cells were washed with PBS and lysed using 50 µl 1X Reporter Lysis Buffer for 10 min, and the luciferase determination was performed according to the manufacturer's protocol (Promega, Madison, WI). Data for ARE induction (EC_{50} values) were expressed as the concentration of compound that provoked activation halfway between baseline (DMSO control) and maximum response at a concentration of 20 µg/ml. NF-kB constructs data were expressed as IC_{50} values (the concentration required to inhibit TPA-activated NF-kB activity by 50%). For ARE induction, sulforaphane (EC_{50} 4-6 µM) was used as standard inducer. Resveratrol was used as a standard TPA-activated NF-kB activity inhibitor (IC_{50} 25 mM). IC_{50} values were generated from the results of four serial dilutions of the active samples tested in triplicate (Tablecurve 2D, AISN Software, USA, 1996). No signs of overt cellular toxicity were observed under the employed experimental conditions.

Assay for inhibition of COX-1 and -2 activities

The effect of test compounds on COX activity was determined by measuring PGE_2 production as described previously (Kang et al., 2009).

Cell proliferation assay

Cell density determinations were performed as described by Skehan et al. (1990). This assay is based on the selective binding of sulforhodamine B with cellular proteins (Ahmad et al., 2006). The human cancer cell panel was comprised of LU-1, LNCaP, HepG2, and MCF-7 cells. The cells were seeded (1×10^4 cells/well) in 96-well plates; test samples were added at various concentrations at day 1 and cell growth was estimated at day 4. After the incubation period, cell monolayers were fixed with 10% (w/v) trichloroacetic acid and stained for 30 min. Excess dye was removed by repeated washing with 1% acetic acid. Protein-bound dye was dissolved in 10 mM Tris base solution and measurements were performed at 510 nm using a microplate reader. For each cell line, four concentrations were tested in triplicate.

Statistical analyses

Results are presented as means ± standard error. Statistical comparisons were performed using the Student's *t*-test, and the level of $p < 0.05$ was considered as significantly different.

Results and discussion

The potential cancer chemopreventive activity elicited by compounds isolated from the ethanol extract of *Hedychium coronarium* J. Koenig, Zingiberaceae, rhizomes (EHC) was assayed using a battery of *in vitro* tests (inhibition of NF-κB, COX-1 and -2, ARE induction, and cell proliferation). Compounds showing at least 50% inhibition at a concentration of 10 µg/ml (COX-1 and -2) or 20 µg/ml (NF-κB, ARE and cell proliferation) were tested in triplicate and dose-response curves were used to determine IC_{50} values. Inhibition of NF-κB and induction of ARE were carried out using a luciferase reporter gene assay (Kang et al., 2009). The results are shown in Table 1.

Fractionation of EHC by silica gel column chromatography followed by semi-preparative HPLC afforded compounds 1-4 (Taveira et al., 2005), whose chemical structures are depicted in Fig. 1. Compounds **2** and **3** were obtained as epimeric mixtures at C-15 (Taveira et al., 2005). The labdane diterpenes 1-3 differ only in the substitution pattern of the lactone ring, *i.e.*, **1** bears a hydroxyl group at C-14, while **2** and **3** present methoxy and ethoxy groups at C-15. The different biological responses elicited by these compounds suggest the influence of the substituents.

Therefore, ARE induction promoted by compound **1** (EC_{50} 57.6 ± 2.4 µM) decreased significantly by introducing a substituent at C-15 and by removing the hydroxyl group at C-14, as observed with **2** and **3** (EC_{50} > 60.2 and > 57.8 µM, respectively). On the other hand, these features dramatically increased the NF-κB inhibitory potency of **2** and **3** (IC_{50} 7.3 ± 0.3 and 3.2 ± 0.3 µM, respectively), in comparison to **1**, regarded as inactive (IC_{50} > 62.9 µM). The epimeric mixture of coronarin D has been previously reported to inhibit the NF-κB pathway (Kunnumakkara et al., 2008). Our data show a two-fold increase

Table 1
Cancer chemopreventive activity of compounds isolated from *Hedychium coronarium* rhizomes.

Compounds	EC_{50} (µM)	IC_{50} (µM)				
	ARE	NF-κB	COX-1	COX-2	LNCaP	HepG2
Isocoronarin D (**1**)	51.2 ± 5.5	> 62.9	>31.5	> 31.5	64.2 ± 14.2	54.7 ± 0.3
Methoxycoronarin D (**2**)	> 60.2	7.2 ± 0.3	0.9 ± 0.0	> 30.1	> 60.2	79.2 ± 25.6
Ethoxycoronarin D (**3**)	> 57.8	3.2 ± 0.3	3.8 ± 0.1	> 28.9	42.2 ± 11.6	> 57.8
Benzoyl-eugenol (**4**)	> 74.6	32.5 ± 4.9	> 74.6	> 37.3	> 74.6	> 74.6

ARE, induction of antioxidant response element; NF-κB, TPA-induced NF-κB inhibition; COX-1 and -2, inhibition of cyclooxgenase -1 and -2; anti-proliferative effects against LNCaP and HepG2 cell lines.

in the inhibition against this target when the methoxy group at C-15 is replaced by an ethoxy moiety. These changes possibly interfere with the Michael acceptor character of the α, β-unsaturated carbonyl groups of these derivatives. The active Michael acceptor groups have been shown to react with critical cysteines of target proteins, and could interact with the cysteine residue 179 (C179) of IKK-β, a IκB kinase-β which releases NFκB by phosphorylation of IκB (Ahmad et al., 2006; Hee-Juhn et al., 2007; Girón et al., 2008; Kunnumakkara et al., 2008). This assumption is supported by the work of Girón et al. (2008), which demonstrated the potential anti-inflammatory activity of two compounds from a library of hispanolone labdane-type diterpenes. According to these authors, the effects are mediated by the inhibition of IKK activity, which results in stabilization of the NF-κB/IκB complex and consequently impairs the nuclear translocation of NF-κB (Girón et al., 2008; Kunnumakkara et al., 2008). The active compounds described by Girón et al. (2008) bear one or two Michael acceptors as functional groups, whereas this feature is absent in the inactive structurally-related diterpenes. Recently, other labdane diterpenes isolated from *H. coronarium*, hedycoronen A and hedycoronen B, showed moderate inhibitory activity on TNF-α production (IC_{50} 46.0 ± 1.3 and 12.7 ± 0.3 μM, respectively), whereas the corresponding deoxymethyl derivatives were inactive (Kiem et al., 2012). This finding suggests that the inhibitory effect against some nuclear factors increases by introducing a methoxy or ethoxy group at the C-15/16 positions.

Regarding COX-1 and -2 inhibition, the presence of a methoxy group at C-15 (compound 2; IC_{50} 0.9 ± 0.0 μM) led to an increase of potency against COX-1, in a selective manner, in comparison to derivative **3** (IC_{50} 3.8 ± 0.1 μM), which bears an ethoxy group. These compounds did not inhibit COX-2 under these assay conditions.

In addition to labdane diterpenes, fractionation of *H. coronarium* rhizomes afforded benzoyl eugenol (**4**), a derivative of eugenol. This compound showed weak activity (IC_{50} 32.5 ± 4.9 μM) in the NF-κB inhibition assay and was considered inactive in the ARE induction assay as well as in the COX-1 and -2 inhibition assays. Resveratrol is chemically related to **4**, since both compounds have a phenylpropanoid group, but with different location of the second aryl ring. Recently, two libraries of resveratrol derivatives were designed to evaluate structure features that could improve selective inhibition of NF-κB, COX-1 or COX-2 (Nakatani et al., 1994; Pezzuto et al., 2005). Among the COX-1 and -2 inhibitors, the absence of electronic-donating atoms between the two aryl rings seems to be an important structure feature for activity (Skehan et al., 1990; Handler et al., 2007). This requisite is violated by compound **4**, which possess an ester moiety that may account for its inactivity on the COX assay. Additionally, derivatives with a withdrawing substituent were either inactive or weakly active in the NF-κB inhibition assay (Pezzuto et al., 2005). The ester carbonyl group present in compound **4** fulfills this feature, and this is probably related with its low NF-κB inhibitory activity.

To further assess the potential of diterpenes 1-3 as cancer chemopreventive agents, their cytotoxicity was determined against four cell lines (HepG2, LU-1, LNCaP, and MCF-7), at concentrations up to 20 μg/ml. Proliferation suppression with LU-1 and MCF-7 cells was not significant, whereas weak antiproliferative effects against HepG2 (**1** and **2**) and LNCaP (**1** and **3**) was observed (Table 1). The HepG2 antiproliferative activity exhibited by compound **1** could be related to ARE induction activity, since the ARE-luciferase plasmids transfected cells are constructed from the HepG2 cell line. The low cytoxicity of compound 2 in cancer cells MCF-7, A549 and SK-N-SH (Suresh et al., 2010), and Hela and HUMEC has been also reported (Zhan et al., 2012).

Altogether, these results indicate that the cancer chemoprevention effect of the labdane diterpenes from *H. coronarium* most probably involve the inhibition of NF-κB, which may also account for the alleged anti-inflammatory activity of the plant. An increase in the liposolubility of these labdanes increased NF-κB inhibition and decreased cytotoxicity. These data suggest the potential cancer chemopreventive activity of methoxy and ethoxy labdanes from *H. coronarium*.

Authors' contributions

DCE designed the study, contributed in running the laboratory work, analysis of the data and drafted the paper and did a critical reading of the manuscript. FSNT contributed in plant samples collection and identification, confection of herbarium, running the laboratory work. TPK contributed to biological studies. GH contributed in plant identification and herbarium voucher specimen confection. JMP and FCB designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

We thank CNPq/Brazil for a research fellowship (FCB). CAPES/Brazil supported this project with a PhD fellowship. The work was also supported by program project grant [P01 CA48112] awarded by the National Cancer Institute and with funds from FAPEMIG/Brazil and CNPq. We thank Dr. Júlio Antônio Lombardi for collecting the plant material.

REFERENCES

Ahmad, R., Raina, D., Meyer, C., Kharbanda, S., Kufe, D., 2006. Triterpenoid CDDO-Me blocks the NF-κB pathway by direct inhibition of IKKβ on Cys-179. J. Biol. Chem. 281, 35764-35769.

Braga, F.C. Wagner, H. Lombardi, J.A., Oliveira, A.B., 1999. Screening Brazilian plant species for *in vitro* inhibition of 5-lipoxygenase. Phytomedicine 6, 447-452.

Chimnoi, N., Pisutjaroenpong, S., Ngiwsara, L., Dechtrirut, D., Chokchaichamnankit, D., Khunnawutmanotham, N., Mahidol, C., Techasakul, S., 2008. Labdane diterpenes from the rhizomes of *Hedychium coronarium*. Nat. Prod. Res. 22, 1249-1256.

Ghosh, S., Karin, M., 2002. Missing pieces in the NF-κB puzzle. Cell 109, S81-S96.

Girón, N., Través, P.G., Rodríguez, B., López-Fontal, R., Boscá, L., Hortelano, S., las Heras, B., 2008. Suppression of inflammatory responses by labdane-type diterpenoids. Toxicol. Appl. Pharmacol. 228, 179-189.

Handler, N., Brunhofer, G., Studenik, C., Leisser, K., Jaeger, W., Parth, S., Erker, T., 2007. Bridged stilbene derivatives as selective cyclooxygenase-1 inhibitors. Bioorg. Med. Chem, 15, 6109-6118.

Hee-Juhn, P., In-Tae, K., Jong-Heon, W., Seoung-Hee, J., Eun-Young, P., Jung-Hwan, N., Jongwon, C., Kyung-Tae, L., 2007. Anti-inflammatory activities of ent-16αH, 17-hydroxy-kauran-19-oic acid isolated from the roots of Siegesbeckia pubescens are due to the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-kappaB inactivation. Eur. J. Pharmacol. 558, 185-193.

Hegazy, M.E.F., Ohta, S., Abdel-latif, F.F., Albadry, H.A., Ohta, E., Pare, P.W., Hirata, T., 2008. Cyclooxygenase (COX)-1 and -2 inhibitory labdane diterpenes from *Crassocephalum mannii*. J. Nat. Prod. 71, 1070-1073.

Joy, B., Rajan, A., Abraham E., 2007. Antimicrobial activity and chemical composition of essential oil from *Hedychium coronarium*. Phytother. Res, 21, 439-443.

Kang, S.S., Cuendet, M., Endringer, D.C., Croy, V.L., Pezzuto, J.M., Lipton, M.A., 2009. Synthesis and biological evaluation of a library of resveratrol analogues as inhibitors of COX-1, COX-2 and NF-kappaB. Bioorg. Med. Chem. 17, 1044-1054.

Kiem, P.V., Anh Hle, T., Nhiem, N.X., Minh, C.V., Thuy, N.T., Yen, P.H., Hang, D.T., Tai, B.H., Mathema, V.B., Koh, Y.S., Kim, Y.H., 2012. Labdane-type diterpenoids from the rhizomes of *Hedychium coronarium* inhibit lipopolysaccharide-stimulated production of pro-inflammatory cytokines in bone marrow-derived dendritic cells. Chem. Pharm. Bull. 60, 246-250.

Kunnumakkara, A.B., Ichikawa, H., Anand, P., Mohankumar, C.J. Hema, P.S., Nair, M.S., Aggarwal, B.B., 2008. Coronarin D, a labdane diterpene, inhibits both constitutive and inducible nuclear factor-kappa B pathway activation, leading to potentiation of apoptosis, inhibition of invasion, and suppression of osteoclastogenesis. Mol. Cancer Ther. 7, 3306-3317.

Maiti, A., Cuendet, M., Croy, V.L., Endringer, D.C., Pezzuto, J.M., Cushman, M., 2007. Synthesis and biological evaluation of (+/-)-abyssinone II and its analogues as aromatase inhibitors for chemoprevention of breast cancer. J. Med. Chem. 50, 2799-2806.

Manish, M., 2013. Current status of endangered medicinal plant *Hedychium coronarium* and causes of population decline in the natural forests of Anuppur and Dindori districts of Madhya Pradesh, India. Int. Res. J. Bio. Scien. 2, 1-6.

Matsuda, H., Morikawa, T., Sakamoto, Y., Toguchida, I., Yoshikawa M., 2002. Labdane-type diterpenes with inhibitory effects on increase in vascular permeability and nitric oxide production from *Hedychium coronarium*. Bioorg. Med. Chem 10, 2527-2534.

Morikawa, T., Matsuda, H., Sakamoto, Y., Ueda, K., Yoshikawa, M., 2002. New farnesane-type sesquiterpenes, hedychiols A and B 8,9-diacetate, and inhibitors of degranulation in RBL-2H3 cells from the rhizome of *Hedychium coronarium*. Chem. Phar. Bull. 50, 1045-1049.

Nakatani, N., Kikuzaki, H., Yamaji, H., Yoshio, K., Kitora, C., Okada, K., Padolina W.G., 1994. Labdane diterpenes from rhizomes of *Hedychium coronarium*. Phytochemistry 37, 1383-1388.

Pezzuto, J.M., Kosmeder, J.W., Park, E.J., Lee, S.K., Cuendet, M., Baht, K., Grubjesic, S., Park, H.S., Mata-Greenwood, E., Tan, Y., Yu, R., Lantvit, D.D., Kinghorn, A.D., 2005. Strategies for cancer chemoprevention. In: Kelloff, G.J. Hawk, E.T., Sigman C.C. (Eds.). *Cancer Chemoprevention:* Human Press, Totowa, 2005, p. 3-37.

Pio Corrêa, M. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Ministério da Agricultura. Rio de Janeiro, 1974.

Ribeiro, R.A., Barros, F., Melo, M.M.R.F., Muniz, C., Chieira, S., Wanderely, M.G., Gomes, C., Trolin, G., 1988. Acute diuretic effects in conscious rats produced by some medicinal plants used in the state of São Paulo, Brasil. J. Ethnopharmacol. 24, 19-29.

Shrotriya, S., Ali, M.S., Saha, A., Bachar, S.C., Islam, M.S., 2007. Anti-inflammatory and analgesic effects of *Hedychium coronarium* Koen. Pak. J. Pharm. Sci. 20, 47-51.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107-1112.

Suresh, G., Reddy, P.P., Babu, K.S., Shaik, T.B., Kalivendi, S.V., 2010. Two new cytotoxic labdane-diterpenes from the rhizomes of *Hedychium coronarium*. Bioorg. Med. Chem. Lett. 20, 7544–7548.

Taveira, F.N., Oliveira, A.B., Souza Filho, J.D., Braga, F.C., 2005. Epimers of labdane diterpenes from the rhizomes of *Hedychium coronarium* J. Koenig. Rev. Bras. Farmacogn. 15, 55-59.

Valadeau, C., Pabon, A., Deharo, E., Albán-Castillo, J., Estevez, Y., Lores, F.A., Rojas, R., Gamboa, D., Sauvain, M., Castillo, D., Bourdy, G., 2009. Medicinal plants from the Yanesha (Peru): Evaluation of the leishmanicidal and antimalarial activity of selected extracts. J. Ethnopharmacol. 123, 413-422.

Zhan, Z.J., Wen, Y.T., Ren, F.Y., Rao, G.W., Shan, W.G., Li, C.P., 2012. Diterpenoids and a diarylheptanoid from *Hedychium coronarium* with significant anti-angiogenic and cytotoxic activities. Chem. Biodivers. 9, 2754-2760.