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Preliminary Anticancer Potency Evaluation and Phytochemical investigation of Methanol Extract of *Piper claussenianum* (Miq.) C. DC.

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ABSTRACT

Bioassay guided isolation of active natural compounds was performed to investigate the anti-tumor potential of the crude extract and isolated compounds from inflorescences of *Piper claussenianum*. LC-DAD-UV, GC-MS and NMR analyzes revealed the presence of phenolic metabolites in the methanol crude extract. Phytochemical procedures lead to the isolation of the major flavonoids, 2',6'-dihydroxy-4-methoxychalcone, 5,7-dihydroxyflavanone and 5-methoxy-7-hydroxyflavanone that were assayed for inhibition or viability stimulation of the human breast cancer cell line MCF-7. The results suggest the 2',6'-dihydroxy-4-methoxychalcone as the biologically active compound in the crude methanol extract of inflorescences from *P. claussenianum*. The crude extract was found as potential natural source of compounds with breast cancer cell inhibition properties. All isolated compounds have not been described from this species yet.

INTRODUCTION

Natural products have been the mainstay of cancer chemotherapy for the past 30 years. Over 60% of the current anticancer drugs have their origin in one way or another from natural sources (Cragg and Newman, 2009). While relatively few of the actual isolated compounds advance to become clinically effective drugs in their own right, these unique molecules may serve as models for the preparation of more efficacious analogs using chemical methodology such as total or combinatorial (parallel) synthesis, or manipulation of biosynthetic pathways. Thus, many compounds can be used as templates for the construction of novel compounds with enhanced biological properties (Mann, 2002). Due to these facts, nature continues to be the most prolific source of biologically active and chemotypes, showing that plant species are still an important source of new drugs for diseases that continue to lack a cure in the world, such as cancer. In Brazil, plants species have been popularly indicated and traditionally used to treat many conditions, specially in north of the country where trade medicaments are not easily available and drugstores are considerable far from the communities. Although Brazil has the largest diversity of world, with about 60,000 higher plant species cataloged, only 8% were studied for research of bioactive compounds and 1,100 species were assessed in their medicinal properties (Guerra e Nodari, 2001). In this context, Piperaceae family has a representative role in the composition of Brazilian biomes such as Amazonic and Rain Forest regions. Brazil contains between 500 and 700 species of the Piperaceae family having 34.79% of all piperacea species and 83.16% of these being endemic (Monteiro and Guimarães, 2009). Diverse species from this genera have been used for religious purposes and in traditional

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medicine within ethnic groups in regions in the north of Brazil, Central America and South East Asia, for example (Chaveerach *et al.*, 2006). Natural products have first been isolated from a crude extract and structurally characterized prior to analysis of bioactivity. Currently, only 10% of all the *Piper* species, the most representative genus of the Piperaceae family, have undergone phytochemical investigation throughout the world (Myers *et al.*, 2000).

Phytochemical studies of *Piper* species have afforded a diverse wide range of secondary metabilites, many of them flavonoids, specially those with a non-substituted B-ring. (Facundo *et al*., 2004). Many chalcones, dihydrochalcones and flavanones have been isolated from the genus Piper (Parmar *et al.*, 1997; Santos *et al.*, 1999; Danelutte *et al*., 2003; Lago *et al.*, 2007; Portet *et al.*, 2007).

Flavonoids are a group of phenolic compounds, which are widely distributed throughout the plant kingdom. More than 4000 substances have been identified, many of which are responsible for the attractive colours of flowers, fruits, and leaves (Portet *et al*., 2008). Multiple combinations of hydroxyl groups, sugars, oxygen, and methyl groups attached to these structures create the various classes of chalcones, flavonoids; flavonols, flavanones, flavones, flavan-3-ols (catechins), anthocynins and isoflavones. Flavanones, chalcones and dihydrochalcones are biochemically related compounds of restricted occurrence and for this reason they are described as minor flavonoids (Patil *et al*., 2009). Flavanones have a saturated C-ring whereas chalcones as well as dihydrochalcones have an open structure and a carbon skeleton numbered in a different way than other flavonoids.

Naturally occurring chalcones are mostly in the methoxy and hydroxylated forms and many reports have been documented different biological properties such as apoptosis induction, an anti-proliferative action in various cancer cell types (Zi *et al*., 2005), inhibition of pro-inflammatory mediators (Ahmad *et al*., 2006), antiplatelet activity (Zhao *et al*., 2005), antimicobacterial (Yar *et al*., 2007; Avila *et al*., 2008); potential antimalarial activity (Portet *et al., 2007*), leishmanicidal (Santos *et al*., 1999); etc.

The aim of this study was to characterize the chemical composition of the crude inflorescences methanol extract of *Piper claussenianum* and to investigate the anti-proliferative effect of this extract and its chemical marker, the chalcone 2',6'-dihydroxy-4'-methoxychalcone against human breast cancer cell line MCF-7. All of the isolated chalcone and flavanones are being reported here for the first time. In exception the volatile oils (Marques *et al*., 2010; Marques *et al*., 2011), no phytochemical studies have previously been conducted with this species.

MATERIALS AND METHODS

Plant material

The plant was collected at São Manoel, Castelo city, in the Espírito Santo State (Brazil), in February 2009, and was identified by the botanist Dr. Elsie Franklin Guimarães and kept at the Herbarium (HB) of the Rio de Janeiro Botanical Garden (JBRJ), registered under number RB 489043.

Phytochemical Procedures and analysis

General Procedure

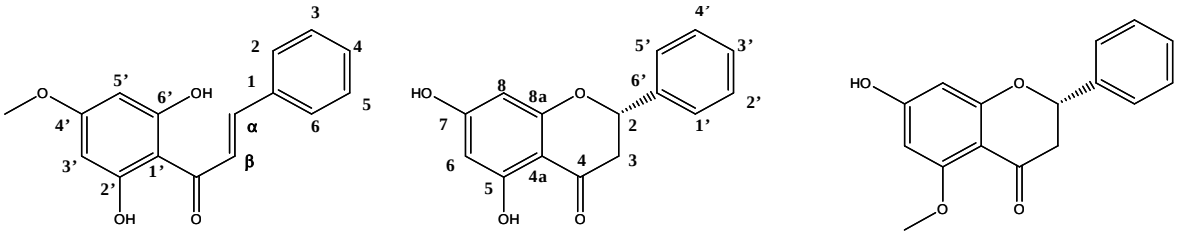
Silica gel (Merck, 60-200 mesh) was employed in the CC separations, whilst analytical TLC was performed using silica gel 60 PF254 layers (Merck).

Preparation of the extract and isolation of pure compounds

Dried and powdered inflorescences of *P. claussenianum* (600.0 g) were extracted by maceration with MeOH at room temperature. The resulting solutions were concentrated *in vacuum* to yield a crude extract (150.0 g), which was evaluated to anti-proliferative effect on human breast cancer cell line MCF-7. Due to the positive activity results of the crude extract, it (5.0 g) was subjected to column chromatography over silica gel and eluted with a (gradient of hexane to EtOAc and from EtOAc to MeOH) yielding several flavonoid rich fractions. Fraction 32 was further recrystallized with methanol affording 140.0 mg of pure compound **1** (2',6'-dihydroxy-4'-methoxychalcone). Compound **2** (5,7-dihydroxy-flavanone) was isolated in pure form from fraction 24 (25.0 mg). The fraction 82 was subjected to column chromatography over silica gel and eluted with a (gradient of EtOAc in hexane and MeOH in EtOAc) yielding 80.0 mg of compound **3** (5-methoxy-7-hydroxy-flavanone). The ^{1}H NMR spectra of the remaining fractions showed a predominance of flavones with one/two sugars and or without methoxyl group. The structures of isolated compounds are shown in Figure1. The compounds were identified by GC-MS, ^{1}H and ^{13}C NMR spectra analysis and confirmed by comparison with the literature data for **1, 2** and **3** (Ching *et al*., 2007; Lago *et al.*, 2007).

GC-FID analysis

Qualitative analysis of non polar compounds were carried out on a GC 2010 Shimadzu machine with a ZB-1MS fused silica capillary column (30 m x 0,25 mm x 0,25 μm film thickness). The operating temperatures used were: injector 260^{o}C, detector 290^{o}C and column oven 60°C up to 290^{o}C (10^{o}C/min). Hydrogen at 1.0 mL x min^{-1} was used as carrier gas. The percentages of the compounds were obtained by GC-FID analysis.



1 2 3

Fig. 1: Isolated compounds from *Piper claussenianum* inflorescences.

GC-MS analysis

Qualitative analyses of non polar compounds were carried out on a GC-QP2010 PLUS Shimadzu with a ZB-5MS fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness). The operating temperatures used were: injector 270°C, detector 290°C and column oven 60°C up to 290°C (10°C/min). Helium at 1.0 mL x min^{-1} was used as carrier gas for GC/MS. The flavonoids were identified by comparison of their mass spectra with published data and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST 3.0) libraries provided with the computer controlling the GC–MS system.

LC-DAD-UV analysis

The inflorescences crude extract (1 mg) was diluted in methanol (HPLC grade, Tedia Brazil) and then filtered prior to analyses. The final volume was adjusted to 1 mL. HPLC-DAD-UV analyses were performed using a Varian ProStar module (Varian, Palo Alto, CA, USA) with ProStar 230 Solvent Delivery and a ProStar 330 HPLC DAD. The analyses were carried out at 240 and 340 nm with a Kromasil 100 C18 reversed phase column (250 mm × 4.6 mm i.d., 5 μm, Akzo Nobel, USA) at an oven temperature of 27°C. The samples were introduced using an injection valve fitted with a 20 μL loop (Rheodyne, California, USA). The mobile phase consisted of aqueous trifluoroacetic acid 1% (v/v) (A) and acetonitrile (B) (HPLC grade, Tedia Brazil) in gradient mode: 5% (A)/ 95% (B) and then 95% (A)/ 5% (B) in 80 mins. The flow rate was 1 mL min^{-1}. Cromatograms were generated with UV detection at 240 and 340 nm.

Nuclear Magnetic Resonance Spectroscopy

The pure compounds obtained from inflorescences of *P. claussenianum* were analyzed by ^{1}H and ^{13}C NMR and recorded on a Varian VNMRS 500 spectrometer. The chemical shifts were determined in DMSO-d_6, using TMS as the internal standard. The signals of the NMR analyses were compared to the literature data (Ching *et al*., 2007; Lago *et al*., 2007).

Biological Tests

Cells and culture conditions

The human breast cancer cell line MCF-7 was grown in Dulbecco's Modified Eagle Medium-DMEM (GIBCO, USA) with penicillin and streptomycin (GIBCO, USA) and supplemented with 10% fetal bovine serum (GIBCO, USA) in disposable plastic bottles, at 37°C, until reaching confluence.

Analysis of cellular viability

For each experiment, cells were seeded in 96-well plates (TPP, Germany), at a concentration of 2×10^4 cells/well. After incubation for 48 h with several concentrations of each compound, the viability was evaluated by MTT (thiazolyl blue) assay (Mosmann 1983). Briefly, MTT (Sigma) at 0.5 mg mL^{-1} was added to each well. After incubation for 3 h at 37°C, the supernatant was removed and 200 μL of dimethyl sulfoxide (Sigma) was added per well to dissolve the formazan crystals. The plates were read on a Thermomax Microplate Reader (Molecular Devices, CA, USA) at 490 nm.

RESULTS AND DISCUSSION

In an effort to investigate Brazilian native *Piper* species, analysis of the methanol extract of inflorescences from *P. claussenianum* was performed. Previous TLC analysis using NP/PEG reagent indicated the presence of free and glycosylflavonoids. As no information is available in the literature regarding the biological activities involving extracts of *P. claussenianum*, the viability of the human breast cancer cell line MCF-7 was evaluated by MTT (thiazolyl blue) assay to investigate the potential of these natural agents to stimulate or inhibit cell viability and growth. The crude extract activity was prior investigated. The crude extract displayed an effect on cell viability, but within the concentration range tested, the lowest concentration ($10mgmL^{-1}$) granted the deepest effect, showing a reduction of about 75% on cell viability when compared with control conditions, whereas treatment with $40mgmL^{-1}$ comparatively wielded a 45% reduction in cell viability. Curiously, the best result was obtained with the lowest concentration of the extract. This was achieved due to the fact the compounds in the mixture were more soluble in low concentrations (Figure 2).

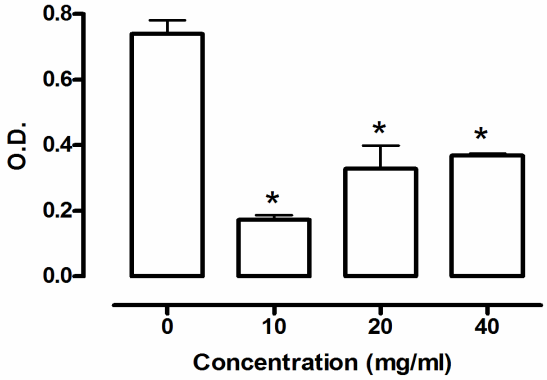


Fig. 2: Effect of a 48 h crude extract treatment on the cell viability of the MCF-7 cell lines. 2 x 10^4 cells were seeded onto 96-well plates and after an initial incubation were treated with different concentrations of a crude extract for 48 hours (n= 5; $p < 0,01$). Statistical analysis: ANOVA test with Tukey post-test.

In order to elucidate the structure of the main active constituents of the active extract, a previous LC-DAD-UV analysis was performed. The UV chromatogram enabled the detection of characteristic groups, due to the absorption pattern. Thus, LC-DAD-UV analysis of the extract showed peaks with UV spectra characteristic for flavonoids [λmax 240, 340 and 360 nm] at 5 and between 30 and 40 minutes. The extraction and separation procedures on the methanol extract of inflorescences of *Piper claussenianum* have led to the isolation and characterization of three flavonoids, which were identified and characterized as

2',6'-dihydroxy-4'-methoxychalcone (**1**), pinocembrin (**2**), alpinetin (**3**) (Figure 1). The compounds were identified by comparison of their spectral data with those reported in the literature. The high field NMR study including HMQC, HMBC, ^{13}C NMR and COSY correlation techniques was therefore undertaken to complement the published NMR data, with respect to the assignment of the peaks. Work-up procedure on fraction 28-34 of methanol extract yielded 2',6'-dihydroxy-4'-methoxychalcone (**1**) as orange needle shaped crystals , $C_{16}H_{14}O_4$. The chalcone was initially obtained as orange needle-shaped crystals from column chromatography of methanol inflorescence extract, which were purified by recrystallization with methanol. Fraction 22-24 yielded pinocembrin (**2**) as yellow needle shaped crystals from column chromatography of methanol inflorescence extract, $C_{15}H_{12}O_4$, while the fraction 82-84 gave alpinetin (**3**) as colorless needle-shaped crystals from methanol inflorescence extract, $C_{16}H_{14}O_4$. The ^1H NMR spectrum showed two doublets at δ 5.87 (*J* = 2.3 Hz, 1H) and 5.92 (*J* = 2.3 Hz, 1H) for compund **1**; δ 5.87 (*J* = 2.1 Hz, 1H) and 5.98 (*J* = 2.1 Hz, 1H) for compound **2** and δ 5.97 (*J* = 2.3 Hz, 1H) and 6.04 (*J* = 2.3 Hz, 1H) for compound **3**. These pairs doublet related with their corresponding carbones (δ 95.25 and 96.90 to **1**; δ 92,40; 96.30 to **2** and δ 83.75 and 96.70 to **3** suggested by HSQC and HMBC which were assigned as H-6 and H-8 in the ring A of flavanones and H-3' and H-5' in the chalcone. The related carbone shifts are in agreement to the oxigenated *meta* substituents. A hydroxyl group chelated to carbonyl group was found in compound **1** and **2** in δ 12.5 and δ 12.2. Broad peaks in δ 10.5 and δ 10.3 were found in the ^1H NMR spectra of **2** and **3**, respectively. These data associated to the HMBC and ^{13}C correlations led us to assign these peaks to hydroxyl groups in C-7 (δ 165.5) and (δ 164.7) in the compounds **2** and **3,** respectively. The ^1H NMR data were associated to the signals at δ 5.55 (dd, *J* = 12.0 and 2.4 Hz, H-2), 2.77 (dd, *J* = 17.1 and 2.2 Hz, H-3a), 3.22 (dd, *J* = 17.2 and 13.5 Hz, H-3b), δ 5.33 (dd, *J* = 13.0 and 2.9 Hz, H-2), 2.69 (dd, *J* = 17.1 and 2.3 Hz, H-3a), 2.98 (dd, *J* = 17.0 and 13.2 Hz, H-3b) corresponding to the compounds **2** and **3** respectively. As no substituition was observed in B ring of all compounds, no AB systems was observed characteristic of *para*-dissustituted aromatic rings. Signals associated to multiplet at δ 7.4 – 7.8 (5H), δ 7.2 – 7.5 (5H), δ 7.2 – 7.5 (5H) were found in agreement corresponding to the B ring to the compounds **1**, **2** and **3** respectively. LREIMS analysis showed molecular ion-peaks at *m/z* 270 ($C_{16}H_{14}O_4$ - compound **1**), *m/z* 256 ($C_{15}H_{12}O_4$ - compound **2**) and *m/z* 270 ($C_{16}H_{14}O_4$ - compound **3**). MS (compound **1**) (*m/z*, rel. int.): *m/z* 270 (52%), 269 (57), 253 (9), 193 (100), 167 (39), 131 (7), 103 (22), 77 (31). MS (compound **2**) (*m/z*, rel. int.): *m/z* 256 (100%), 238 (14), 179 (92), 152 (77), 124 (52), 103 (18), 96 (20), 78 (29), 69 (31), 51 (16). MS (compound **3**) (*m/z*, rel. int.): *m/z* 270 (51%), 269 (14), 252 (1), 242 (2), 193 (22), 166 (100), 138 (51), 104 (15), 95 (16). Finally, compounds **1**, **2** and **3** were identified as 2',6'-dihydroxy-4'-methoxychalcone, 5,7-dihydroxy-flavanone and 5-methoxy-7-hydroxy-flavanone, respectively. The inhibitor effect of the major compound of this extract, the chalcone 2',6'-dihydroxy-4'-methoxychalcone was furthermore investigated. Figure 3 shows that the pure chalcone presents a toxic effect on the breast cancer MCF-7 cell line. The effect is dose-dependent and is at its maximum at a 100μM concentration, with a 50% reduction of cell viability when compared with control conditions. The comparison between the effects of the tested chalcone and doxorubicin, a drug widely used in breast cancer treatment (Gianni *et al*., 2009), showed that our results presented no significant difference in regards to cell viability between the effects caused by 100μg of the chalcone and 400nM of doxorubicin (Figure 4). Doxorubicin is a substrate to ABCC1, a member of the ABC superfamily of transporters. The MCF-7 constitutively expresses ABCC1, therefore exhibiting the multidrug resistance (MDR) phenotype, making it less susceptible to drugs such as doxorubicin itself (Deeley and Cole, 2006). Whether or not this chalcone is a substrate for ABCC1 remains to be investigated.

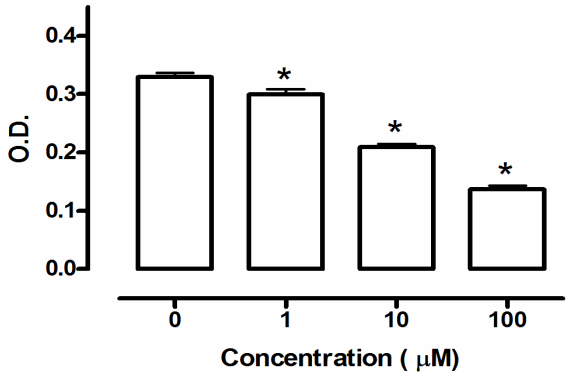


Fig. 3: Effect of a 48 h chalcone treatment on the cell viability of the MCF-7 cell lines. 2 x 10^4 cells were seeded onto 96-well plates and after an initial incubation were treated with different concentrations of a chalcone preparation for 48 hours (n= 5; $p < 0,05$). Statistical analysis: ANOVA test with Tukey post-test.

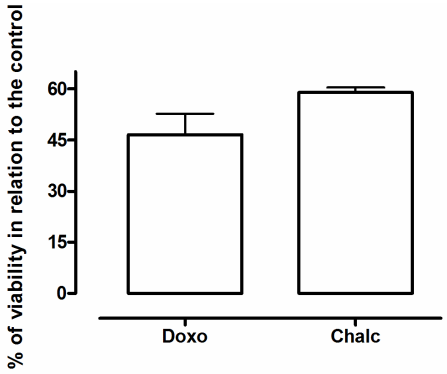


Fig. 4: Comparison between the effects of a 48 h chalcone or doxorubicin treatment on the cell viability of the MCF-7 cell lines. 2 x 10^4 cells were seeded onto 96-well plates and after an initial incubation were treated with either 100 μM of a chalcone preparation or 400 nM of doxorubicin for 48 hours (n= 5). Statistical analysis: ANOVA test.

The presence of the chalcone and related derivatives in the methanol flower extract from *P. claussenianum* suggest these flavonoids, especially the chalcone as a potential chemical marker for this species, enhancing the biological and ecological role of these compounds to the species. Flavonoids are known to exhibit

an important role in the interaction between plants and their environment, not only protect the plant from the harmful effects of UV irradiation but also play a crucial role in the sexual reproduction process (Derksen *et al*., 1999). The intense yellow color of the major isolated compound could be implicated in the attraction of some pollinators as well as in the insect's attraction through complex patterns in ultraviolet light. The remarkable presence of this chalcone as major compound in the extract is in agreement of the flavonoids biosynthesis pathway leading to correspondent flavanones derivatives. The equilibrium between the open chalcone form and the cyclic flavanone isomer is the key step at the origin of the skeletal modifications of the biosynthetic pathway. The chalcone isomerase catalyzes the intramolecular cyclization of chalcones into (*S*)-flavanones and the formation of the C ring (Jez *et al*., 2002). Naturally occurring chalcones have been documented with different biological properties such as apoptosis induction, an anti-proliferative action in various cancer cell types, inhibition of pro-inflammatory mediators, antiplatelet activity, and potential antimalarial activity, among others (Zi and Simoneau, 2006). These innumerous pharmacological activities rise from the multiple possibility of chemical interactions, due to the versatility of these secondary metabolites and are based, in most of times, on the potential antioxidant activity of chalconoids. A number of different mechanisms have been describe for justify these pharmacological properties, such as: free radical scavenging, hydrogen donation singlet oxygen quenching, metal ion chelation and acting as a substrate for free radicals such as superoxide and hydroxide (Patil *et al*., 2009). Therefore, chalcones are less likely to interact with nucleic acids and then avoid the problems of mutagenicity and carcinogenicity associated with certain alkylating agents used in cancer chemotherapy. A variety of chalcones have shown several anticancer activities as inhibitors of cancer cell proliferation, carcinogenesis, and metastasis (Jez *et al*. 2002) and can be found in many Piperaceae species (Parmar *et al*., 1997; Santos *et al*., 1999). The occurrence of chalcones and flavanones in *Piper claussenianum* species is of particular interest since to date no flavonoid isolation has been described for this species. In Piperaceae species, the literature survey describes particularly the presence of this 2',6'-dihydroxy-4'-methoxychalcone in *P. aduncum* dichloromethane inflorescences extract (Santos *et al*., 1999). Recently, the cytotoxic effects of various chemicals and natural substances on malignant tumor cells in culture have been extensively studied as a primary screening for their anti-tumor activities, and hence it seems important and necessary to confirm the connection between the in vitro cytotoxic and in vivo anti-tumor activities (Morita *et al*., 2003). Thus, our preliminary findings are very consistent with others demonstrating the huge potential of the Piperaceae family as a source for new drug development.

CONCLUSION

The presence of flavonoids found in methanol inflorescence extract from *P. claussenianum* suggest these flavonoids, especially the 2',6'-dihydroxy-4'-methoxychalcone as a potential chemical marker and a possibly noteworthy role in the pollination interactions due to the remarkable color and high UV absorption pattern of the isolated constituents. This is the first occurrence report of chalcones and flavanones in *Piper claussenianum* species. Our preliminary findings should encourage not only the phytochemical investigation but also development of more potent chalcone derivatives for prevention and treatment of different types of cancer.

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REFERENCES

Ahmad S., Israf D.A., Lajis N.H., Shaari H.M., Mohamed H., Wahab A.A., Ariffin K.T. Cardamonin, inhibits pro-inflammatory mediators in activated RAW 264.7 cells and whole blood. Eur J Pharmacol. 2006; 538: 188–194.

Avila H.P., Smânia E.F.A., Monache F.D., Smânia Junior A. Structure-activity relationship of antibacterial chalcones. Biorgan Med chem. 2008; 16: 9790-9794.

Chaveerach A., Mokkamul P., Tanee T. Ethnobotany of the Genus *Piper* in Thailand. Ethnobot Res Appl. 2006; 4: 223-232.

Ching A.Y.L., Wah T.S., Sukari M.A., Lian G.E.C., Rahmani M., Khalid K. Characterization of flavonoid derivatives from *Boesenbergia rotunda*. Malaysian J Anal Sci. 2007; 11: 154-159.

Cragg G.M., Newman D. J. Nature: a vital source of leads for anticancer drug Development. Phytochem Rev. 2009; 8: 313–331.

Danelutte A.P., Lago J.H.G., Young M.C.M., Kato M.J. 2003. Antifungal flavanones and prenylated hydroquinones from *Piper crassinervium* Kunth. Phytochemistry. 2003; 64: 555-559.

Deeley R.G., Cole S.P.C. Substrate recognition and transport by multidrug resistance protein 1 (ABCC1). Fed Eur Biochem Soc. 2006; 580: 1103-1111.

Derksen J., Wezel R., Knuiman B., Ylstra B. Pollen tubes of Flavonol-defcient *Petunia* show striking alterations in wall structure leading to tube disruption. Planta; 1999; 207: 575-581.

Facundo V.A., Braz-Filho R. C-methylated flavonoids from the roots of *Piper carniconnectivum*. Biochem Syst Ecol. 2004; 32: 1215-1217.

Guerra, P.M., Nodari, O.R. (2001). Biodiversidade: aspectos biológicos, geográficos, legais e éticos. In: Simões, M. O. et al. (3 Ed.) Farmacognosia: da planta ao medicamento (p15). Porto Alegre: UFRGS.

Gianni L., Norton L., Wolmark N., Bonadonna G., Hortobagyi G. Role of Anthracyclines in the Treatment of Early Breast Cancer. J Clin Oncol. 2009; 27: 4798-4808.

Jez J.M., Bowman M.E., Noel J.P. Role of Hydrogen Bonds in the reaction Mechanism of Chalcone Isomerase. Biochemistry. 2002; 41: 5168-5176.

Lago J.H.G., Young M.C.M., Reigada J.B., Soares M.G., Kato M.J. Antifungical derivatives from *Piper mollicomum* and *P. lhotzyanum*. Quím Nova. 2007; 30: 1222-1224.

Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer. 2002; 2: 143-148.

Marques A.M., Barreto A.L.S., Batista E.M., Curvelo J.A.R., Veloso L.S.M., Moreira D. L., Guimarães E.F., Soares R.M.A., Kaplan M.A.C. Chemistry and biological activity of essential oils from *Piper claussenianum* (Piperaceae). Nat Prod Commun. 2010; 5: 1837–1840.

Marques A.M., Barreto A.L.S., Curvelo J.A.R., Romanos M.T.V., Soares R.M.A., Kaplan M.A.C. Antileishmanial activity of nerolidol-rich essential oil from *Piper claussenianum*. Braz. J. Pharmacog. 2011; 21: 908–914.

Myers N., Mittermeier R.A.; Mittermeier C.G.; Fonseca G.A.B., Kent J. Biodiversity Hotspots for Conservation Priorities. Nature. 2000; 403: 853-858.

Monteiro D., Guimarães E.F. Flora do Parque Nacional do Itatiaia-Brasil: *Manekia* e *Piper* (Piperaceae). Rodriguésia. 2009; 60(4): 999-1024, 2009.

Morita K., Arimochi H. and Ohnishi Y. In Vitro Cytotoxicity of 4-Methylcatechol in Murine Tumor Cells: Induction of Apoptotic Cell Death by Extracellular Pro-Oxidant Action. J Pharmacol Exp Ther. 2003; 306: 317–323.

Parmar S.V., Jain C.S., Bisht S.K., Jain R., Taneja P., Jha A. Phytochemistry of the Genus *Piper*. Phytochemistry. 1997. 46: 597-673.

Patil C.B., Mahajan S.K., Katti S.A. Chalcone: A versatile Molecule. J Pharm Sci & Res. 2009; 1: 11-22.

Portet B., Fabre N., Roumy V., Gornitzka H., Bourdy G., Chevalley S. Activity-guided isolation of antiplasmodial dihydrochalcones and flavanones from *Piper hostmannianum* var. *berbicense*. Phytochemistry. 2007; 68: 1312-1320.

Portet B., Fabre N., Rozenberg R., Jiwan J.L.H., Moulis C., Leclercq J.Q. Analysis of minor flavonoids in *Piper hostmannianum* var. berbicense using liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry. J Chromatogr A. 2008; 1210: 45-54.

Santos E.C.T., Moreira D.L., Kaplan M.A.C., Bergmann B.R. Selective Effect of 2',6'-dihydroxy-4'-methoxychalcone Isolated from *Piper aduncum* on *Leishmania amazonensis*. Antimicrob Agents Ch. 1999; 43: 1234-1241.

Yar M.S., Siddiqui A.A., Ali M.A. Synthesis and antimycobacterial activity of novel heterocycles. J Serb Chem Soc. 2007; 72: 5-11.

Zhao L.M., Jin H.S., Sun L.P, Piao H.R., Quan Z.S. Synthesis and evaluation of antiplatelet activity of trihydroxychalcone derivatives. Bioorg Med Chem Lett. 2005; 15: 5027–5029.

Zi X., Simoneau A.R. Flavokawain A, a Novel Chacone from Kava Extract, induces apoptosis in Bladder Cancer Cells by involvement of Bax Protein-dependent and Mitochondria-dependent Apoptotic Pathway and Suppresses Tumor Growth in Mice. Cancer Res. 2005; 65: 3479-3486.

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