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Brief communication

Antiproliferative activity of the hexanic extract and phloroglucinols from *Hypericum brasiliense*

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ABSTRACT

Natural products are regarded as major and important sources of molecules used in chemotherapy. *Hypericum brasiliense* Choisy, Hypericaceae, is an annual bush, native in the southern and southeastern Brazil. This species has been used in Brazilian folk medicine the anti-spasmodic and for the treatment of infectious diseases. *H. brasiliense* is chemically composed by flavonoids and xanthones. In addition, this species contain phloroglucinols, a class of substances with citotoxity effects against tumor cells lines. On the present study, hexanic extract and derivatives phloroglucinols obtained from *H. brasiliense* were tested against some human tumor cell lines. Hexanic extract presented a potent antiproliferative activity, with selective action on OVCAR-03 (ovarian), NCI-ADR/RES (ovarian resistant) and UACC-62 (melanoma) tumor cell lines. Uliginosin B was the most active derivative phloroglucinol, presenting selectivity against NCI-ADR/RES (resistant ovarian) and OVCAR-03 (ovarian) tumor cell lines. Analysis of the results suggests that phloroglucinol derivatives with isoprenyl unit closed in the 9' position increases antiproliferative activity. Furthermore, this study contributes to identification of anti-tumor molecules and valorization of *Hypericum brasiliense*.

Introduction

Natural products are regarded as major and important sources of molecules used in chemotherapy. Furthermore, approximately 60% of drugs used to treat currently prevent cancer and are derived from natural products, such as Taxol, isolated from *Taxus brevifolia*, vincristine and vinblastine from *Catharantus roseus* and camptothecin from *Camptotheca acuminate* (Newman and Cragg, 2012).

Hypericaceae comprehend 316 species, genus belonging to ten, including *Hypericum* spp. The most known species from this genus is *Hypericum perforatum* L., due to its use in mild to moderate depression treatment. The mechanism involved suggests an action on norepinephrine and serotonin reuptake

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(Blad and Wagner, 1994; Bombardelli and Morazzoni, 1997; Rodriguez-Landa and Contreras, 2003; Pilkington et al., 2006). Other species, such as *H. sampson* (Zeng et al., 2006), *H. hookerianum* (Dongre at al., 2007; Dongre at al., 2008), *H. caprifoliatum, H. carinatum, H. connatum, H. cordatum, H. myrianthum, H. piriai and H. polyanthemum* (Ferraz et al., 2005; Pinhatti at al., 2013) have been the presenting reported significant results in growth inhibition of tumor cells lines. These cytotoxic activities have been related to benzopyrones and phloroglucinols derivatives present in the nonpolar extracts (Ferraz et al., 2005; Pinhatti at al., 2013).

Hypericum brasiliense Choisy, Hypericaceae, is an annual bush, native in the southern and southeastern Brazil. This species has been used in Brazilian folk medicine as antispasmodic and for infectious diseases treatment (Robson, 1990; Corrêa, 1984). *H. brasiliense* is chemically composed by flavonoids, xanthones and phloroglucinols derivatives. The xanthones showed inhibitory effects on the enzyme monoamine oxidase (MAO) (Rocha et al., 1994), as well as antifungal activity (Rocha et al., 1995). Hydroalcoholic extract from leaves showed analgesic activity (Rocha et al., 1991; Perazzo et al., 2008a), antidepressant effects in mice (Perazzo et al., 2008b) and antiophidic properties (Assafim et al., 2011). Phloroglucinols derivatives isolates from *H. brasiliense* were able to inhibit bacterial growth of different clones and strains of *Staphylococcus aureus* (França et al., 2009). Although several *Hypericum* species exhibited inhibitory activity against tumor cells lines, with remarkable correlation to phloroglucinols (Zeng et al., 2006; Dongre at al., 2007; Ferraz et al., 2005; Pinhatti at al., 2013), *H. brasiliense* the remains an almost unexplored for these species approach. The aim of this study was to evaluate antiproliferative activity of hexanic extract and three phloroglucinols obtained from aerial parts *H. brasiliense* against human tumor cells lines.

Materials and methods

Plant

The aerial parts *Hypericum brasiliense* Choysi, Hypericaceae, were collected in Nova Friburgo, Brazil in 2001. The voucher specimen was deposited in the herbarium of the National Museum at the Federal University of Rio de Janeiro (N° 199820).

Assays

Dried, powdered aerial parts of *H. brasiliense* (1000 g) were exhaustively extracted with *n*-hexane, filtered and concentrated using a rotary evaporator in order to obtain the hexanic extract (HE), which was stored and kept in a refrigerator (4°C). The phloroglucinols before were already isolated from *n*-hexanic extract (França at al., 2009) and identified unequivocally as japonicin A (**1**), uliginosin B (**2**) and isouliginosin B (**3**), respectively (Ferraz et al., 2005; Rocha et al., 1995; França at al., 2009).

Cell cultures

For *in vitro* antiproliferative screening, the following human tumor cell lines were tested: UACC-62 (melanoma), MCF-7 (mammary), NCI-ADR/RES (drug resistant ovary), 786-0 (kidney), NCI-H460 (lung), PC-3 (prostate), OVCAR-3 (ovary) and HT-29 (colon). These cell lines were kindly provided by Frederick Cancer Research & Development Center, National Cancer Institute, Frederick, MA, USA. All cell lines were cultured in RPMI 1640 medium supplemented with 5% of fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37°C in humid atmosphere containing 5% CO_2.

Antiproliferative assay

Briefly, all the cells (100 µl/well, at a density of 3-6 × 10^4 cell/ml) were plated in 96-well plates and incubated with samples at various concentrations (0.25 to 250 µg/ml, 100 µl/well) in DMSO/RPMI 1640/FBS 5% at 37°C and 5% of CO_2 in air for 48 h. Final DMSO concentration did not affect cell viability. Cells were then fixed with 50% trichloroacetic acid and cell proliferation was determined by spectrophotometric quantification of cellular protein content at 540 nm, using the sulforhodamine B assay (Monks et al., 1991). Doxorubicin (0.025-25 µg/ml) used was the positive control. Three measurements were obtained at the beginning of incubation (time zero, T0) and 48 h post-incubation for compound-free (C) and tested (T) cells. Cell proliferation was determined according to the equation 100 x [(T-T0)/C-T0], for T0 < T ≤ C, and 100 x [(T-T0)/T0], for T ≤ T0 and the concentration-response curve for each cell line was plotted using ORIGIN 7.5 software (OriginLab Corporation) (Monks et al., 1991).

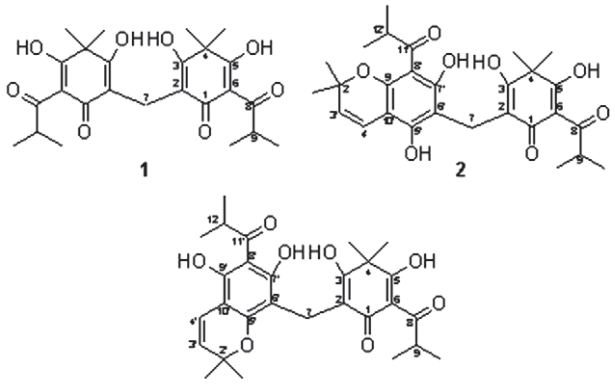
Date analysis

Using the concentration-response curve for each cell line, TGI (concentration causing 0% growth inhibition) (Shoemaker, 2006) was determined by means of non-linear regression analysis, using software ORIGIN 7.5 (OriginLab Corporation). The average activity (mean of TGI) of the extracts also tested was determined using MS Excel software. Extracts were regarded as follows: inactive (mean TGI > 50 µg/ml), weak activity (15 µg/ml < mean TGI < 50 µg/ml), moderate activity (6.25 µg/ml < mean TGI < 15 µg/ml) or potent activity (mean TGI < 6.25 µg/ml) based on the Council for Scientific and Industrial Research (CSIR) criteria (Fouche et al., 2008).

Results and discussion

Several studies revealed *Hypericum* species and its phloroglucinols the potential antiproliferative agents (Zeng et al., 2006; Dongre at al., 2007; Ferraz et al., 2005; Pinhatti at al., 2013). Since the phloroglucinols of *H. brasiliense* have been isolated from the apolar extract of this species (Rocha et al., 1995; Rocha et al, 1996; França at al., 2009) the *n*-hexanic extract (HE) of *H. brasiliense* and purified substances japonicin A (**1**), uliginosin B (**2**) and isouliginosin B (**3**) were evaluated against the proliferation of tumor cells from various histological

origins, such as melanoma (UACC-62), breast (MCF-7), ovarian expressing the resistance phenotype for adryamycin (NCI-ADR/RES), kidney (786–0) non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-03) and colon (HT-29).



According to criteria CSIR (Fouche et al., 2008) HE presented to potent antiproliferative activity (TGI mean 4.84 µg/ml), with a selective action on OVCAR-03 (ovarian, TGI = 0.56 µg/ml), NCI-ADR/RES (ovarian resistent, TGI = 1.13 µg/ml) and UACC-62 (melanoma, TGI = 2.41 µg/ml) tumor cell lines (Table 1). Among the three substances, japonicin A (**1**) considered was inactive (mean TGI = 148.44 µg/ml) and uliginosin B (**2**) was the most active (mean TGI = 3.91 µg/ml), presenting selectivity against NCI-ADR/RES (ovarian resistent, TGI = 0.72 µg/ml) and OVCAR-03 (ovarian, TGI = 1.49 µg/ml) tumor cell lines. Isouliginosin B (**3**) presented a weak antiproliferative activity (mean TGI = 21.03 µg/ml) and its activity was better against NCI-ADR/RES observed (ovarian resistent, TGI = 7.04 µg/ml) despites been almost ten times less active than uliginosin B.

Antiproliferative activity of HE indicated TGI values close to that observed for uliginosin B (**2**). Since the HE is a complex matrice, with compounds in low concentration, the synergic effect achieved may induce a positive effect on the antiproliferative activity. Studies with *H. caprifoliatum, H. myrianthum* and *H. ternun* also indicated positive results for nonpolar fractions of these species (Ferraz et al., 2005). Compounds uliginosin B and isouliginosin B are derivatives of filicinic acid and phloroglucinol jointed by the methylenic bond, while compound japonicin A is a filicinic acid dimer. The antiproliferative obtained results in this study suggests that phloroglucinol portion may play an important role on the antiproliferative activity, since japonicin A, which do not present the phloroglucinol portion in the molecule was inactive considered.

Comparison of substances uliginosin B and isouliginosin B suggests that isoprenyl unit at 9' position (uliginosin B) instead of at 5' position (isouliginosin B) was probably responsible for the observed increased antiproliferative activity is uliginosin B (mean TGI = 3.91 µg/ml, potent activity) when compared to isouliginosin B (mean TGI = 21.03 µg/ml, weak activity). These results were in accordance with literature date for observed filicinic acid and phloroglucinol derivatives isolated from *H. drummondii*, where phloroglucinol derivatives with cyclization of isoprenyl unit at the 9' position were more active then filicinic acid and phloroglucinol derivatives with open chain form of isoprenyl unit (Jayasuriya and McChesney, 1989). Also it is interesting to notice that HE, uliginosin B and isouliginosin B showed a marked selectivity to ovarian tumor cell lines (NCI-ADR/RES and OVCAR-03). In the works of the Pinhatti at al. (2013), japonicin A and uliginosin B showed a significant effect only against the ovarian carcinoma cell line (OVCAR-3) in the dose of 50 µg/ml.

This study demonstrated the antiproliferative activity of three phloroglucinols and hexanic obtain the extract *H. brasiliense*. In addition, analysis of the results together with literature date, suggests that phloroglucinol derivatives with isoprenyl unit closed in the 9' position increases antiproliferative activity against some tumor cells, contributing to the identification of antiproliferative molecules or prototypes

Table 1
TGI values (µg/ml) for hexanic *H. brasiliense* extract (HE), japonicin A, uliginosin B and isouliginosin B.

Cell line[a]	HE	Japonicin A	Uliginosin B	Isouliginosin B	Doxorubicine[b]
UACC-62	2.41	93.81	3.47	18.8	0.08
MCF-7	5.38	103.21	3.82	10.89	3.09
NCI-ADR/RES	1.13	77.39	0.72	7.04	0.06
786-0	5.04	74.10	3.34	12.39	0.10
NCI-H460	5.90	162.98	2.18	44.61	0.19
PC-3	6.74	250.00	6.61	30.64	0.57
OVCAR-03	0.56	180.28	1.49	13.92	2.20
HT29	11.60	245.76	9.70	29.86	0.81
Mean TGI[c]	4.84	148.44	3.91	21.03	0.88

[a]Melanoma cell lines (UACC-62), breast (MCF-7), ovarian expressing the resistance phenotype is adryamycin (NCI-ADR/RES), kidney (786–0) non-small cells lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-03) and colon (HT-29)].
[b]Positive control.
[c]The Council for Industrial and Scientific Research (CSIR) criteria: inactive (I, mean TGI > 50 µg/ml), weak activity (W, 15 µg/ml < mean TGI < 50 µg/ml), moderate activity (M, 6.25 µg/ml < mean TGI < 15 µg/ml) and potent activity (P, mean TGI < 6.25 µg/ml).

for this approach. Furthermore, this study contributes to the valorization of *Hypericum brasiliense*, the species used in the Brazilian folk medicine.

Authorship

CPF (PhD student), LR and HSF contributed in collecting plant and sample identification, confection of herbarium, running the laboratory work, analysis of the date and drafted the paper. ALTGR, JEC contributed to biological studies. LR and HSF contributed in plant identification and herbarium confection. CPF, LR and HSF contributed to chromatographic analysis. CPF, LR and ALTGR contributed to critical reading of the manuscript. HSF and LR contributed to plant collection. LR and HSF 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.

REFERENCES

Assafim, M., De Coriolano, E.C., Benedito, S.E., Fernandes, C.P., Lobo, J.F.R., Sanchez, E.F., Rocha, L.M., Fuly, A.L., 2011. *Hypericum brasiliense* plant extract neutralizes some biological effects of *Bothrops jararaca* snake venom. J. Venom. Res. 2, 11-16

Bladt, S., Wagner, H., 1994. Inhibition of MAO by fractions and constituents of *Hypericum* extract. J Geriatr. Psychiatry Neurol. 7, S57-S59.

Bombardelli, E., Morazzoni, P., 1995. *Hypericum perforatum*. Fitoterapia 66, 43-68.

Corrêa, M.P., 1984. *Dicionário de Plantas Úteis do Brasil e das Exóticas Cultivadas*. Imprensa Nacional, Rio de Janeiro.

Dongre, S.H., Badami, S., Godavarthi, A., 2008. Antitumor activity of *Hypericum hookerianum* against DLA induced tumor in mice and its possible mechanism of action. Phytother Res. 22, 23-29.

Dongre, S.H., Badami, S., Natesan, S., Raghu, C.H., 2007. Antitumor activity of the methanol extract of *Hypericum hookerianum* stem against Ehrlich ascites carcinoma in swiss albino mice. J. Pharmacol. Sci. 103, 354-359.

Ferraz, A., Faria, D.H., Benneti, M.N., Rocha, A.B., Schwartsmann, G., Henriques, A., von Poser, G.L., 2005. Screening for antiproliferative activity of six southern Brazilian species of *Hypericum*. Phytomedicine. 12, 112-115.

Fouche, G., Cragg, G.M., Pillay, P., Kolesnikova, N., Maharaj, V.J., Senabe, J., 2008. *In vitro* anticancer screening of South African plants. J. Ethnopharmacol. 119, 455-461.

França, H.S., Kuster, R.M., Rito, P.D., Oliveira, A.P., Teixeira, L.A., Rocha, L., 2009. Antibacterial activity of the phloroglucinols and hexanic extract from *Hypericum brasiliense* Choysi. Quim. Nova. 32, 1103-1106.

Jayasuriya, H., McChesney, J.D., 1989. Antimicrobial and cytotoxic activity of rottlerin-type compounds from *Hypericum drummondii*. J. Nat. Prod. 52, 325-331.

Monks, A., Scudeiro, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, M., 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J. Natl. Cancer Inst. 83, 757-766.

Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the last 30 years from 1981 to 2010. J. Nat. Prod. 75, 311-335.

Perazzo, F.P., Lima, L.M., Padilha, M.M., Rocha, L.M., Sousa, P.J.C., Carvalho, J.C.T., 2008a. Anti-inflammatory and analgesic activities of *Hypericum brasiliense* (Willd) standardized extract. Rev. Bras. Farmacogn. 18, 320-325.

Perazzo F.P., Lima, L.M., Rocha, L., França, H.S., Carvalho, J.C.T., 2008b. Antidepressant activity evaluation of *Hypericum brasiliense* standardized extract. Phcog. Mag. 4, 155-158.

Pilkington, K., Boshnakova, A., Richardson, J., 2006. St John's wort for depression: Time for a different perspective? Complement Ther. Med. 14, 268-281.

Pinhatti, A.V., de Barros, F.M.C., de Farias, C.B., Schwartsmann, G., von Poser, G.L., Abujamra, A.L., 2013. Antiproliferative activity of the dimeric phloroglucinol and benzophenone derivatives of *Hypericum* spp. Native to southern Brazil. Anticancer Drugs. 24, 699-703

Robson, N.K.B., 1990. *Bulletin of the British Museum (Natural History)* 20, 1.

Rocha, L., Kaplan, M.A.C., Ruppelt, B.M., Pereira, N.A., 1991. Atividade biológica de *Hypericum brasiliense*. Rev. Bras. Farm. 72, 67-69.

Rocha, L., Marston, A., Kaplan, M.A.C., Stoeckli-Evans, H., Thull, U., Testa, B., Hostettmann, K., 1994. An antifungal γ-pyrone and xanthones with monoamine oxidase inhibitory activity from *Hypericum brasiliense*. Phytochemistry. 36, 1381-1385.

Rocha, L., Marston, A., Potterat, O., Kaplan, M.A.C., Stoeckli-Evans, H., Hostettmann, K., 1995. Antibacterial phloroglucinols and flavonoids from *Hypericum brasiliense* Phytochemistry. 40, 1447-1452.

Rocha, L., Marston, A., Potterat O., Kaplan, M.A.C., Stoeckli-Evans, H., Hostettmann, K., 1996. More phloroglucinols from *Hypericum brasiliense*. Phytochemistry. 42, 185-188.

Rodríguez-Landa, J.F., Contreras, C.M., 2003. A review of clinical and experimental observations about antidepressant actions and side effects produced by *Hypericum perforatum* extracts. Phytomedicine. 10, 688-699.

Shoemaker, R.H., 2006. The NCI60 human tumour cell line anticancer drug screen. Nat. Rev. Cancer. 6, 813-823.

Zeng, J.Z., Sun, D.F., Wang, L., Cao, X., Qi, J.B., Yang, T., Hu, C.Q., Liu, W., Zhang, X.K., 2006. *Hypericum sampsonii* induces apoptosis and nuclear export of retinoid X receptor-alpha. Carcinogenesis. 27, 1991-2000.