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# Cytotoxic activity of different polarity fractions obtained from methanolic extracts of *Vismia baccifera* and *Vismia macrophylla* (Hypericaceae) collected in Venezuela

[Actividad citotóxica de fracciones de diferentes polaridades obtenidas a partir de extractos metanólicos de *Vismia baccifera* y *Vismia macrophylla* (Hypericaceae) colectadas en Venezuela]

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**Abstract**

*Context*: Cancer is a complex disease involving numerous changes in cell physiology and abnormal cell growth, which lead to malignant tumors. Many investigations are still carrying on in different areas including, natural products, to find a possible break point to this pathology.

*Aims*: To evaluate the cytotoxic activity on different polar extracts from *Vismia baccifera* and *Vismia macrophylla* collected in two locations of the Venezuelan Andes.

*Methods*: Cytotoxic activity assay was carried out following the colorimetric (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) MTT assay. Human tumor cell Lines from breast carcinoma without gene over-expression (MCF-7), breast carcinoma with overexpressed gene (SKBr3), prostate carcinoma (PC3) and cervix epithelial carcinoma (HeLa) were tested with different polarity solvent extracts (hexane, dichloromethane, ethyl acetate, butanol, water) from the two species under investigation. Human dermis fibroblasts were used as control cells. Mean inhibitory concentration ($IC_{50}$) was calculated.

*Results*: Extracts from *V. macrophylla* showed significant inhibition of cervix epithelial carcinoma with values ranging from 6.09 µg/mL to 17.51 µg/mL; breast carcinoma with an overexpressed gene with values from 12.14 µg/mL to 16.90 µg/mL and prostate carcinoma from 10.91 µg/mL to 17.70 µg/mL. *V. baccifera* extracts showed the strongest activity against prostate carcinoma with an IC50 value of 2.92 µg/mL.

*Conclusions*: The present study showed evidence for the anticancer activity of *Vismia baccifera* and *Vismia macrophylla* extracts since caused growth inhibition in different cell lines at low concentrations, thus, it is considered not only an important contribution to the natural products research but bring supportive data for further investigations on cancer research.

*Keywords*: cytotoxic activity; HeLa; MCF-7; PC3; SKBr3; *Vismia baccifera*; *Vismia macrophylla*.

**Resumen**

*Contexto*: El cáncer es una enfermedad que envuelve cambios en la fisiología y crecimiento anormal de las células, conduciendo a la aparición de tumores malignos. Investigaciones están realizándose en diferentes áreas, incluyendo productos naturales, para conseguir el tratamiento que elimine en forma definitiva esta patología.

*Objetivos*: Evaluar la actividad citotóxica de extractos de diferentes polaridades obtenidos de *Vismia baccifera* y *Vismia macrophylla* colectadas en dos regiones de los andes venezolanos.

*Métodos*: La actividad citotóxica se realizó siguiendo el método colorimétrico del MTT. Líneas celulares tumorales de carcinoma de mama sin sobreexpresión del oncogen (MCF-7), carcinoma de mama con sobreexpresión del oncogen (SKBr3), carcinoma de próstata (PC3) y carcinoma de cuello uterino (HeLa) fueron probadas con extractos de diferentes polaridades (hexano, diclorometano, acetato de etilo, butanol, agua) obtenidos de las dos especies en estudio. Fibroblastos de la dermis humana fueron usados como células de control. Se calculó la concentración mínima inhibitoria ($IC_{50}$).

*Resultados*: Los extractos de *V. macrophylla* mostraron inhibición del carcinoma de cuello uterino con valores de $IC_{50}$ entre 6,09 a 17,51 µg/mL; carcinoma de mama con sobreexpresión del oncogen (12,14 a 16,90 µg/mL) y carcinoma de próstata (10,91 a 17,70 µg/mL). Los extractos de *V. baccifera* mostraron la mayor actividad frente al carcinoma de próstata con una $IC_{50}$ de 2,92 µg/mL.

*Conclusiones*: El presente estudio mostró actividad anticancerígena de los extractos de *V. baccifera* y *V. macrophylla*, al causar inhibición del crecimiento en las líneas celulares a bajas concentraciones. Se considera una contribución a la investigación de productos naturales ya que se aportan datos para futuras investigaciones.

*Palabras Clave*: actividad citotóxica; HeLa; MCF-7; PC3; SKBr3; *Vismia baccifera*; *Vismia macrophylla*.

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## INTRODUCTION

Cancer is a complex disease involving numerous changes in cell physiology and abnormal cell growth, which lead to malignant tumors. This uncontrolled cell growth is known as neoplasia and it is considered as the disease endpoint. The biological process by which normal cells are transformed into malignant cancer cells has been the subject to numerous investigations in the biomedical sciences for many decades. However, despite all this effort, cancer is still challenging researcher due to the metastasis, where, cancer cells break away from where they first formed (primary cancer), travel through the blood or lymph system and form new tumors in other parts of the body (Arvelo et al., 2016a).

Furthermore, without a clear idea on cancer origins, it becomes difficult to formulate a clear strategy for effective management. Although very specific processes cause malignant transformation, many unspecific influences can initiate the disease including radiation, chemicals, viruses, inflammation, among others. It seems that prolonged exposure to almost any provocative agent in the environment can potentially cause cancer (Arvelo et al., 2016b).

However, many investigations are still carrying on in different areas including, natural products, to find a possible break point to this pathology. Regarding plants, there is a huge potential since plantae kingdom offers a wide range of species for the search of secondary metabolites that might show cytotoxic activity. Nevertheless, a medicinal plant before is launched to community or used in the formal health services should be evaluated for its efficacy, safety and acceptability.

Present study aims to find this activity in two *Vismia* species collected in Venezuela. In this respect, *Vismia* genus belongs to Hypericaceae family and comprises around 55 species distributed in tropical and subtropical areas of Central and South America, although there are some species reported in Africa as well (Hokche et al., 2008; Crocketta and Robson, 2011). Phytochemical studies have revealed the presence of about 161 chemical compounds isolated from different *Vismia* species being represented mainly by anthrones, xanthones and anthraquinones (Hussain et al., 2012; Vizcaya et al., 2014).

On the other hand, many of *Vismia* species have been used in traditional medicine for the treatment of skin ulcerations, fungus, herpes, as well as laxative and to alleviate high fever (Vizcaya et al., 2012). However, different investigations have proved antimicrobial (Vizcaya et al., 2014; Buitrago et al., 2015; Ferreira et al., 2015), antioxidant (Buitrago et al., 2016), leishmanicide, tripanomicide (Weniger et al., 2001), antitumor (Salas et al., 2008; Lizcano et. al., 2015), among other biological activities. Present investigation was conducted to find cytotoxic activity on different polarity solvent extracts from *Vismia baccifera* L. Triana & Planch and *Vismia macrophylla* Kunth collected in two locations of the Venezuelan Andes.

## MATERIAL AND METHODS

### Plant material

*V. macrophylla* Kunth (VM) was collected from Michelena, Táchira State, at 1200 m.a.s.l. (7°56'30" N-72°14'33" W) and *V. baccifera* L. Triana & Planch (VB) was harvested from La Hechicera, Mérida State at 1800 m.a.s.l. (8°37'41" N-71°09'34" W). Both species were collected in February 2015, during rainy season and flowering stage. Botanical identification was carried out by Dr. Pablo Meléndez, MERF Herbarium, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida, Venezuela. Voucher specimens were deposited under the following codes: VM-JR39 and VB-JR25.

### Extraction

Leaves of both species were placed into an oven at 45°C for three days. Dry material was ground in a mill to obtain a fine powder able to penetrate a Nº 20 mesh and weighs were measured, VM 1850 g and VB 1650 g. Room temperature methanol extraction (Met: 4 L, Sigma-Aldrich, anhydrous 99.8%) was carried out with both species under investigation for five days, changing the solvent for fresh and leaving the extraction for another five days with same plant material to achieve an exhaustive extraction. The two extracts were concentrated to dryness by using a rotaevaporator under reduced pressure (Rotavapor® R-300, Buchi, Brazil); weights

of concentrated extracts were also measured (250 g; 13.51% Met-VM and 200 g; 12.12% Met-VB).

### Liquid-liquid extracts fractionation

Met-VM and Met-VB were submitted to liquid-liquid fractionation using hexane (Hx: 1 L, Sigma-Aldrich, anhydrous 95%), dichloromethane (DCl: 1 L, Sigma-Aldrich, anhydrous 99.8%), ethyl acetate (EtAc: 1 L, Sigma-Aldrich, anhydrous 99.8%), and butanol (But: 1 L, Sigma-Aldrich, anhydrous 99.8%) as solvents. A volume of 500 mL of distilled water (Wt) were added to each extract in a separation funnel of 1 L capacity, and three consecutive extractions were carried out for every single solvent by increasing polarity. All samples were concentrated in a rotaevaporator to dryness and then weighed; Hx (34 g VM; 40 g VB), DCl (10 g VM; 22 g VB), EtAc (39 g VM; 30 g VB), But (65 g VM; 40 g VB) and Wt (50 g VM; 45 g VB). Concentrated extracts were maintained in the dark at 4°C until cytotoxic activity evaluation.

### Human tumor cell lines and culture media

Human tumor cell lines from MCF-7 (breast carcinoma, without over-expression of the HER2/c-erb-2), PC3 (prostate carcinoma) and HeLa (cervix epithelial carcinoma) were provided by Dr Marie France Poupon, Curie Institute, Paris- France. SKBr3 (breast carcinoma, in which the HER2/c-erb-2 gene is overexpressed were provided by Dr Manuel Rieber, IVIC Caracas, Venezuela. Human dermis fibroblasts (Hdf), used as control cells, were obtained from Laboratory of Tissue Culture and Tumor Biology of the Institute of Experimental Biology (Caracas, Venezuela).

MCF-7, SKBr3, and fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS; Gibco), 1% of L-glutamine, 1% streptomicyn®/100 units/mL penicillin® (Gibco) and PC3, HeLa were grown in RPMI 1640 medium (Gibco) supplemented with 10% heat inactivated fetal bovine serum, 1% of L-glutamine, 1% streptomicyn®/100 units/mL penicillin (Gibco) all were incubated at 37°C with an atmosphere composed by 5% of $CO_2$ and 95% humidity.

### Cytotoxic activity assay

This activity was carried out following the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. This method is based on the conversion of MTT (yellow tetrazolium salt) to purple formazan crystals by living cells that determines mitochondrial reductases activity (Mosmann, 1983; van Meerloo et al., 2011). Cell lines were used to determine the cytotoxic activity from different solvent extracts of VM and VB collected from two different locations in Venezuela. Samples at concentrations of 0.001; 0.01; 0.1; 1; 5; 10; 15; 25 y 100 µg/mL were placed along with a positive control (Taxol®) in 96-well plates ($5 \times 10^3$ cells/well) and incubated at 37°C with 5% $CO_2$ for 24 h, to allow cells adhesion. After incubation time, 50 µL of sample dilutions and reference drug were added and re-incubated at 37°C with 5% $CO_2$ for 3 days. After 72 h of incubation, culture medium was removed and cells were treated with 50 µL of MTT at concentration of 0.4 mg/mL allowing formation of formazan crystals during 3 hours. These solid crystals were dissolved in 50 µL of DMSO and optical density was measured at 570 nm (DO570) by using an ELISA spectrophotometer (Sunrise™ TECAN, Switzerland) (Raybaudi-Massilia et al., 2015; Suárez et al., 2016). Assays were conducted by triplicate and results were expressed as cytotoxicity percentages (%C), calculated using the following equation:

$$(\%C) = 1 - \frac{(DO570\ \text{tumor cells})}{(DO570\ \text{control cells})} \times 100$$

### Selectivity index (SI)

Selectivity index was expressed as the $IC_{50}$ (control cells)/$IC_{50}$ (tumor cell line) ratio. A selectivity index > 1 indicates that cytotoxicity on tumor cell lines surpassed that on healthy non-tumor cells (Nugroho et al., 2013).

### Statistical analysis

Mean inhibitory concentration ($IC_{50}$) was calculated through 95% of confidential interval by a linear regression equation and statistical analysis was performed using Graph-Pad Prism 5.0 software. The global comparison was performed using two-way variance analysis (ANOVA) followed by Bonfer-

roni's test for multiple comparisons. $P<0.001$ and $p<0.05$ were considered statistically significant.

## RESULTS

Different polarity solvent extracts (Hx, DCl, AcEt, Met, But, Wt) from VB and VM were analyzed for *in vitro* cytotoxic activity by using the MTT assay. Results (Table 1) showed that VM have a wider range of activity comparing to VB since, VM inhibited more variety of tumor cell lines. According to data obtained from assays, extracts from VM showed inhibition of HeLa (VM-Hx, 6.09 µg/mL; VM-Wt 8.18 µg/mL; VM-EtAc/But 17.51 µg/mL), SKBr3 (VM-Wt 12.14 µg/mL; VM-But 16.90 µg/mL) and PC3 (VM-Wt 10.91 µg/mL; VM-DCl 17.70 µg/mL). According to the statistical analysis there was significant differences ($p<0.001$) with respect to VB and VM group and different tumor cell lines in comparison to different polarity solvent extracts. Furthermore, significant differences ($p<0.05$) were also observed for HeLa (VM-Hx 6.09 µg/mL, VM-EtAc/But 17.51 µg/mL) between the different polarity solvent extracts.

Moderate activity was also observed in VM extracts; PC3 (VM-Hx 22.41 µg/mL), Hela (VM-DCl 32.22 µg/mL; VM-EtAc 37.02 µg/mL), MCF-7 (VM-Wt 36.25 µg/mL) and SKBr3 (VM-EtAc/But 53.60 µg/mL; VM-EtAc 57.51 µg/mL). Regarding VB extracts, the strongest activity was observed in VB-DCl 2.92 µg/mL against PC3 cell line ($p<0.001$, $p<0.05$), while for the same PC3, VB-EtAc and VB-EtAc/But showed moderate inhibition with $IC_{50}$ values of 48.08 µg/mL and 66.83 µg/mL, with significant difference ($p<0.001$), respectively.

Concerning HeLa cell line, VB extracts demonstrated a rather moderate activity; with significant difference ($p<0.001$, $p<0.05$) in VB-Hx 28.81 µg/mL; VB-DCl 30.44 µg/mL and VB-EtAc/But 30.85 µg/mL with ($p<0.001$), however, VB extracts showed no activity for MCF-7 and SKBr3 cell lines. It is important to state that some VM extracts such as VM-EtAc (37.65 µg/mL) and VM-But (17.12 µg/mL) showed moderate impact on Hdf but VM-EtAc/But (5.78 µg/mL) showed a very low impact on these non-tumor cells showing a significant difference of ($p<0.001$).

Table 1 shows more details about results observed in this investigation. On the other hand, extracts obtained in water for VM-Wt were found to be selective for all cell lines evaluated, but VB-Wt did not showed same behavior; while extracts obtained with low to medium solvent polarities VB-Hex, VM-Hex, VB-DCl and VM-DCl were found to be highly selective for PC3 and Hela cell lines with respect to control cells. Likewise, extraction with AcEt/But solvent mixture in VB and VM, only exhibited selectivity for HeLa cell line, showing a high specificity for this tumor line. Nonetheless, AcEt and But, extracted by separately, either for VB or VM, did not show any selectivity for the tumor lines, as shown in Table 2.

## DISCUSSION

Present investigation revealed that *Vismia baccifera* and *Vismia macrophylla* extracts showed selective cytotoxic activity for some of the cell lines used in this study. The strongest activity was observed in VB-DCl (2.92 µg/mL) for prostate carcinoma (PC3) being important to state that according to SI, this extract is selective to this tumor cell line and has no activity against Hdf. Other extracts such as VM-Hx (6.09 µg/mL); VM-Wt (8.18 µg/mL) exhibited a selective activity against Hela at low concentrations and VM-Wt (12.14 µg/mL) also displayed a selective activity against SKBR3 cell line. Regarding SKBr3 (breast carcinoma, in which the HER2/c-erb-2 gene is overexpressed) it is important to explain that this type of breast cancer it is more aggressive and fast growing than other carcinomas (Suárez et al., 2016). Thus, results observed in present investigation are considered a contribution to the natural products research.

Previous investigations have also been conducted on several *Vismia* species; Lizcano et al. (2015), studied the effect of aqueous extracts from *Vismia baccifera* collected in Colombian Amazonia, against human liver cancer (HepG2). Researchers observed a selective growth inhibition of HepG2.

**Table 1.** $IC_{50}$ values of different polarity fractions obtained from methanolic extracts of *V. baccifera* and *V. macrophylla* on cell lines.

| Treatment | Cell lines | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MCF-7 | | SKBr3 | | PC3 | | HeLa | | Hdf | |
| Extracts | VB | VM | VB | VM | VB | VM | VB | VM | VB | VM |
| Hexane | $NA^{C}$ | $81.95 \pm 1.19^{ABCD}$ | $NA^{DE}$ | $NA^{DG}$ | $NA^{DF}$ | $22.41 \pm 1.06^{ABCDEFGH}$ | $28.81 \pm 1.02^{ABCDEFGHI\ a}$ | $6.09 \pm 1.02^{ABCDEFGHI\ a}$ | $NA^{A}$ | $NA^{B}$ |
| Dichloromethane | $NA^{C}$ | $NA^{D}$ | $NA^{E}$ | $NA^{G}$ | $2.92 \pm 1.52^{ABCDEFG\ b}$ | $17.70 \pm 1.04^{ABCDEFGH\ b}$ | $30.44 \pm 1.04^{ABCDEFGH}$ | $32.22 \pm 1.05^{ABCDEFGH}$ | $NA^{A}$ | $NA^{B}$ |
| Ethyl acetate | $NA^{BC}$ | $NA^{BD}$ | $NA^{BE}$ | $57.51 \pm 1.08^{ABCDEG}$ | $48.08 \pm 1.09^{ABCDEFG}$ | $NA^{BGF}$ | $NA^{BGFI}$ | $37.02 \pm 1.05^{ACDEFGHI}$ | $NA^{A}$ | $37.65 \pm 1.09^{B}$ |
| Butanol | $NA^{BC}$ | $92.70 \pm 1.10^{ABCD}$ | $NA^{BDE}$ | $16.90 \pm 1.04^{ACDEG}$ | $NA^{BDG}$ | $NA^{BDG}$ | $NA^{BDG}$ | $NA^{BDG}$ | $NA^{A}$ | $17.12 \pm 1.03^{B}$ |
| Water | $NA^{C}$ | $36.25 \pm 1.02^{ABCD}$ | $NA^{DE}$ | $12.14 \pm 1.05^{ABCDEG}$ | $NA^{DGF}$ | $10.91 \pm 1.00^{ABCDEFH}$ | $NA^{DGHI}$ | $8.18 \pm 1.02^{ABCDEFGHI}$ | $NA^{A}$ | $NA^{B}$ |
| Ethyl acetate/ Butanol | $NA^{BC}$ | $NA^{BD}$ | $NA^{BE}$ | $53.60 \pm 1.06^{ACDEG}$ | $66.83 \pm 1.50^{ABCDEFG}$ | $99.36 \pm 1.18^{BGHF}$ | $30.85 \pm 1.00^{ABCDEFGHI\ f}$ | $17.51 \pm 1.08^{ABCDEFGHI\ f}$ | $NA^{A}$ | $51.78 \pm 1.18^{B}$ |
| Taxol® | $0.14 \pm 0.03$ | | $1.03 \pm 0.22$ | | $0.025 \pm 1.13$ | | $0.40 \pm 1.12$ | | $0.76 \pm 1.23$ | |

Values (µg/mL) represent the mean ± standard deviation (n=8). Different capital letters ($^{A,B,C,D,E,F,EG,H,I}$) in the same row indicate significant differences ($p<0.001$) between control cell (Hdf) detected in VB: *Vismia baccifera* and VM: *Vismia macrophylla* group with respect to different tumor cells line *vs.* different polarity solvents. Different lower case letters ($^{a,b,c,d,e,f}$) in the same column indicate significant differences ($p<0.05$) between different polarity solvent extracts of VB: *Vismia baccifera* and VM: *Vismia macrophylla*. Two-way ANOVA followed by Bonferroni's test were carried out for multiple comparisons. Taxol was used as reference drug. NA: values over 100 µg/mL were considered as no active.

**Table 2.** Selectivity index of different polarity fractions obtained from methanolic extracts of *V. baccifera* and *V. macrophylla* on cell lines.

| Treatment | Cell lines | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MCF-7 | | SKBr3 | | PC3 | | HeLa | |
| Extracts | VB | VM | VB | VM | VB | VM | VB | VM |
| Hexane | 1.0 | 1.2 | 1.0 | 1.0 | 1.0 | **4.5** | **3.5** | **16.4** |
| Dichloromethane | 1.0 | 1.0 | 1.0 | 1.0 | **34** | **5.6** | **3.3** | **3.1** |
| Ethyl acetate | 1.0 | 0.4 | 1.0 | 0.6 | **2.0** | 0.4 | 1.0 | 1.02 |
| Butanol | 1.0 | 0.2 | 1.0 | 1.01 | 1.0 | 0.17 | 1.0 | 0.17 |
| Water | 1.0 | **2.8** | 1.0 | **8.2** | 1.0 | **9.2** | 1.0 | **12.2** |
| Ethyl acetate / Butanol | 1.0 | 0.52 | 1.0 | 0.97 | **1.49** | 0.52 | **3.24** | **2.95** |
| *Taxol® | 5.42 | | 0.74 | | 30.4 | | 1.9 | |

VB: *Vismia baccifera*, VM: *Vismia macrophylla*, *Reference drug. Values < 1 selective for normal cells, > 1 selective for tumor cells, 1 no selectivity.

Another investigation reported the effect of sesamin, a secondary metabolite isolated from *Vismia baccifera* var. *dealbata*, species collected in Chiguará, Merida state, Venezuela, against SK-OV-3 (ovary carcinoma), PC3 (prostate carcinoma) and NCI-H292 (lung carcinoma). The investigation showed a rather low effect against the assayed cells (Salas et al., 2008).

Other investigation carried out with methanolic extracts of *V. baccifera, V. jefensis* and *V. macrophylla* leaves showed cytotoxic activity against (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines according to the method given by Monks et al. (1997). Furthermore, five active compounds were isolated from *V. macrophylla* leaves; ferruginin C, ferruginins A and B, vismin and harunganin while from *V. baccifera,* vismione B, deacetylvismione H and deacetylvismione A, were also isolated. Results of this investigation revealed the potential of either extracts or isolated compounds, since activity of the extracts ranged from 1 to 4 µg/mL, while activity of isolated compounds was observed between 0.4 to 7.3 µg/mL. According to results *V. jefensis* proved to have the strongest activity against MCF7 cell line (1 µg/mL) while deacetylvismione H, isolated from *V. baccifera*, showed a very good activity against MCF-7 (0.4 µg/mL), H-460 (0.6 µg/mL) and SF-268 (0.6 µg/mL) (Hussein et al., 2003). A study from Brazil showed that aqueous and organic extracts from the stem and fruits of *Vismia guianensis* likewise showed activity against breast cancer cell line MCF-7 at a dose of 38.03 µg/mL (Suffredini et al., 2007).

On the other hand, *Vismia laurentii* collected from Cameroon was evaluated for anticancer activity against A431 (squamous epidermal carcinoma), WM35 (melanoma), A2780 (ovary carcinoma) and cisplatin-resistant A2780 cells, using a direct colorimetric assay adapted from the Mossman MTT method (Mosmann, 1983). Results showed that *V. laurentii* extract inhibited successfully the growth of melanoma cell WM35, an aggressive malignant type of cell and also caused inhibition on A431 cell line. According to researchers *V. laurentii* extract is able to induce early apoptotic processes in treated cells that will conduct later to a massive cell loss (Tamokou et al., 2013).

## CONCLUSIONS

The overall results of present study showed evidence for anticancer activity of *Vismia baccifera* and *Vismia macrophylla* extracts. To be able to cause cytotoxic effect in cell lines such as prostate carcinoma, cervix epithelial carcinoma and breast carcinoma, in which the HER2/c-erb-2 gene is overexpressed at low concentrations between 2.92 µg/mL to 12.14 µg/mL, is considered not only an important contribution to the natural products research but bring likewise supportive data for further investigations that might be useful on cancer research.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**Author contribution:**

| Contribution | Rojas JC | Buitrago AA | Arvelo FA | Sojo FJ | Suarez AI |
|---|---|---|---|---|---|
| Concepts or ideas | X | X | X | X | X |
| Design | X | X | | | |
| Definition of intellectual content | X | X | X | X | X |
| Literature search | | X | | | |
| Experimental studies | X | X | X | X | |
| Data acquisition | X | X | X | X | X |
| Data analysis | X | X | X | X | X |
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| Manuscript preparation | X | | | | |
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