Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Antiproliferative Activity of *Haematoxylum brasiletto* H. Karst

Bello-Martínez J[1], Jiménez-Estrada M[2], Rosas-Acevedo JL[3], Avila-Caballero LP[1], Vidal-Gutierrez M[4], Patiño-Morales C[5], Ortiz-Sánchez E[5], Robles-Zepeda RE[4]

[1]Laboratory of Chemistry of Natural Products, School of Chemical and Biological Sciences, Guerrero State University, Chilpancingo, Guerrero, [2]Department of Natural Products, Institute of Chemistry, Mexico National Autonomous University (UNAM), Ciudad de México, [3]School of Regional Development Sciences, Guerrero State University, Acapulco, Guerrero, [4]Department of Chemical and Biological Sciences, Sonora State University, Blvd. Luis Donaldo Colosio esq. Rosales s/n Col. Centro, Hermosillo, Sonora, [5]National Institute of Cancerology, Tlalpan, Ciudad de México, Mexico

Submitted: 19-10-2016 Revised: 17-11-2016 Published: 11-07-2017

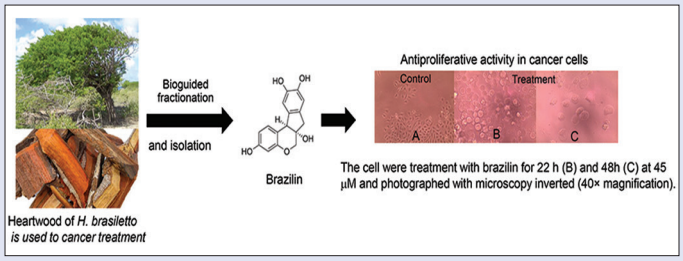
ABSTRACT

Background: *Haematoxylum brasiletto* is a tree that grows in Central America, commonly known as "Palo de Brasil," which is used in the traditional medicine for the treatment of cancer and gastric ulcers. **Objective:** The aim of this study was to isolate the compounds responsible for antiproliferative activity of *H. brasiletto*. **Materials and Methods:** A bioassay-guided fractionation of ethanol extract of *H. brasiletto* was performed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide cell proliferation assay to measure the antiproliferative activity on six human cancer cell lines (A549, LS180, HeLa, SiHa, MDA-MB-231, and NCI-H1299) and one human noncancer cell line (ARPE-19). The ethanol extract was partitioned with hexane, dichloromethane, and ethyl acetate. The active dichloromethane fraction was fractioned by silica-column chromatography, and active subfractions were separated using preparative-thin layer chromatography. The chemical structure of an isolated compound was elucidated with different chemical and spectroscopic methods. **Results:** The flavonoid brazilin (1) was isolated from the heartwood of *H. brasiletto*. The measurement of antiproliferative activity showed that brazilin can inhibit the growth of SiHa, MDA-MB-231, A549, and NCI-H1299 cell lines by 50% at doses of 44.3, 48.7, 45.4, and 48.7 μM, respectively. Furthermore, the flavonoid showed a high antiproliferative activity on LS 180 and HeLa with IC50 values of 62.2 and 71.9 μM, respectively. Brazilin also exhibited a high antiproliferative activity on the human noncancer cell line ARPE-19 with an IC50 value of 37.9 μM. **Conclusions:** Brazilin: (6a*S*,11b*R*)-7,11b-Dihidro-6*H*-indeno[2, 1-*c*] cromeno-3,6a, 9,10-tetrol was isolated; this compound demonstrated antiproliferative activity against several human cancer cell lines. This work demonstrated that brazilin, a flavonoid isolated and characterized of *H. brasiletto*, has antiproliferative activity against cancer cell lines.

Key words: Antiproliferative activity, flavonoids, *Haematoxylum brasiletto*

SUMMARY

- The flavonoid brazilin was isolated from the heartwood of *H. brasiletto*
- Brazilin is able to inhibit the growth of SiHa, MDA-MB-231, A549 and NCI-H1299 cancerous cell lines
- Brazilin exhibited a moderate antiproliferative activity on the human non-cancer cell line ARPE-19
- Brazilin demonstrated to have antiproliferative activity against human cancer cell lines and could be a potential source of anticancer agents.



Abbreviations used: MTT: [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium]; FBS: Fetal bovine serum; TLC: Thin layer chromatography.

Correspondence:

Dr. Robles-Zepeda RE,
Department of Chemical and Biological Sciences, Sonora State University, Blvd. Luis Donaldo Colosio esq. Rosales s/n Col. Centro, Hermosillo, Sonora, Mexico.
E-mail: rrobles@guayacan.uson.mx

DOI: 10.4103/pm.pm_466_16

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Cancer is a group of diseases that represent a serious public health problem.[1] Cancer is the second highest cause of morbidity and mortality worldwide, with approximately 14.1 million new cases and 8.2 million cancer-related deaths. In addition, 32.6 million people are currently living with some kind of cancer.[2] Thus, cancer is one of the main causes of death in developed countries.[3] Some of the most common cancer types, such as breast, cervical, oral, and colorectal cancers have high cure rates when detected early and treated according to effective means, including surgery, radiation therapy, and chemotherapy. However, all these treatments are also accompanied of severe side effects such as tingling, burning, weakness or numbness in the hands, feet, or both, weak, sore, tired, or achy muscles, loss of balance, and shaking or trembling.[4]

The use of natural products derived from plants, animals, or microorganisms for medicinal purposes has a long history.[5] Medicinal plants constitute an important natural source of bioactive compounds with multiple applications that can be used for therapeutic purposes. Their chemical components, with possibly novel mechanisms of action, provide the basis for the synthesis of pharmaceutical products.[6] Plants are the major source of anticancer drugs.[7] In this way, ethnobotanical

Cite this article as: Bello-Martinez J, Jiménez-Estrada M, Rosas-Acevedo JL, Avila-Caballero LP, Vidal-Gutierrez M, Patiño-Morales C, *et al*. Antiproliferative activity of *Haematoxylum brasiletto* H. Karst. Phcog Mag 2017;13:S289-93.

knowledge of medicinal plants represents an alternative for the identification of secondary metabolites with antiproliferative activity that could be a promising source of future anticancer drugs.[8,9]

Haematoxylum brasiletto H. Karst (Fabaceae), native from Mexico and extending into Central America, commonly known as "Palo de Brasil," is used by rural communities in the State of Guerrero, Mexico, as traditional treatment for hypertension, stomach upsets, mouth infections, diarrhea, gastric ulcers, and cancer.[10] An ethanolic extract of the stem bark of *H. brasiletto* was found to inhibit the growth of *Escherichia coli* O157:H7 (EHEC), verotoxin production, and adhesion of *E. coli* O157:H7 to HeLa cells.[11] *H. brasiletto* has shown antimicrobial activities against *Staphylococcus aureus* 375, *S. aureus* ATCC 25923, and *Enterococcus faecium* 379.[12]

To provide scientific validation of traditional medicinal use of *H. brasiletto* for the treatment of cancer, in the present study, We evaluated the bioguided antiproliferative activity of *H. brasiletto*.

MATERIALS AND METHODS

Chemicals and reagents

All solvents used were of analytical grade. Methanol (PubChem CID: 887), ethanol (PubChem CID: 702), n-hexane (PubChem CID: 8058), dichloromethane (PubChem CID: 6344), ethyl acetate (PubChem CID: 8857), and sulfuric acid (PubChem CID 1118) were purchased from Fermont chemicals (Monterrey, NL, Mexico). CD_3OD was purchased from Cambridge Isotopes Laboratories, Inc., (Tewksbury, MA, USA). Water was purified by Milli-Q instrument (Millipore, Bedford, MA, USA). Dulbecco's Modified Eagle's Medium (DMEM) high-glucose, L-glutamine solution 200 mM (PubChem CID: 24895310), L-arginine monohydrochloride (PubChem CID: 87640969), L-asparagine (PubChem CID: 24890831), sodium pyruvate solution 100 mM (PubChem CID: 24899804), penicillin–streptomycin solution (PubChem CID: 86591708), doxorubicin hydrochloride (PubChem CID: 31703), dimethyl sulfoxide (DMSO) (PubChem CID: 679), trypsin–EDTA solution 0.25%, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (PubChem CID: 64965), phosphomolybdic acid (PubChem CID 24845315), and ceric sulfate (PubChem CID 159684) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum was obtained from Gibco Life Technologies (Grand Island, NY, USA). Silica gel 60 (70–230) mesh and silica gel 200–400 mesh were purchased from Sigma-Aldrich (St. Louis, MO, USA). Preparative-thin layer chromatography (TLC) glass-backed 20 cm × 20 cm silica gel plates (2.0 mm thickness) and TLC aluminum-backed silica gel plates (0.2 mm thickness) were obtained from E.M. Merck (Germany).

Plant material

The heartwood of *H. brasiletto* was collected at Mochitlán, Guerrero, Mexico. 99°21'19.03" W; 17°29'03.27" N to 1042 msnm in March 2015. The specimens were taxonomically identified by Professor María de los Angeles Venalonzo Martínez, a voucher of classification was assigned (UAGROHBH15) and was deposited in the Herbarium of Universidad Autónoma de Guerrero. All plant materials were air-dried in the shade at room temperature. The dried samples were powdered and stored at 4°C.

Preparation of ethanolic extract and solvent fractions

The plant extract was obtained based on the methodology described by González-Salvatierra *et al.*[13] In brief, the ethanolic extract of the powder of heartwood of *H. brasiletto* (1 kg) was obtained by maceration with 96% ethanol (EtOH) at room temperature for 10 days with regular manual stirring twice daily. The combined EtOH extracts were evaporated under reduced pressure in a rotatory evaporator to yield the crude extract (50 g). The crude extract (HBM-1) was suspended in 250 mL of an aqueous (3:2 water [H_2O]/methanol [MeOH]) mixture and the resulting suspension was fractioned by successive liquid–liquid partition with *n*-hexane (Hx), dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc) to produce the corresponding low (HBM-2A [500 mg]), low-medium (HBM-2B [5 g]), and medium (HBM-2C [20 g]) polarity fractions, respectively. The ethanol crude extracts and their fractions were analyzed by TLC. A sample of 100 µg is dissolved in 100 µL of CH_2Cl_2 and is applied to the plate using a capillary tube, and then placed in a chromatographic chamber and eluted with a suitable system. The plate is observed under ultraviolet (UV) light and the bands of interest were evaluated for their retention factor (Rf). Chromatographic analyses were performed on 5.5 cm^2, 0.2 mm thick silica gel plates (E.M. Merck DC Alufolien). All extracts were stored at −4°C in amber glass vials until use.

Isolation of bioactive metabolites of *Haematoxylum brasiletto*

Column chromatography (CC) was performed using silica gel 60 (70–230 mesh, Sigma). While silica gel 200–400 mesh (Sigma) was used for flash CC, vacuum liquid chromatography (VLC) purifications were carried out using TLC-grade silica gel (Merck). Gel-preparative TLC purifications were performed using glass-backed 20 cm × 20 cm silica gel plates (2.0 mm thickness, Merck). For analytical TLC analyses, aluminum-backed silica gel plates (E.M. Merck, 0.2 mm thickness) were used. Chromatograms were examined under UV light and then visualized by dipping the plates in a solution of phosphomolybdic acid (20 g) and ceric sulfate (2.5 g) in 500 mL of sulfuric acid (5%), followed by drying and heating to 100°C.[14]

VLC purification of the bioactive dichloromethane fraction (5 g), using a gradient elution with mixtures of CH_2Cl_2:EtOAc: MeOH, produced 11 major fractions (3A-3K). Fraction 3B (1.45 g) was purified by CC eluting with *n*-hexane: EtOAc: MeOH (45:50:5) to produce 8 new fractions (4A-4H). Final purification of fraction 4C (152.5 mg) using a multiple elution (3×) preparative-TLC eluting with EtOAc: $CHCl_3$:MeOH (70:30:10 + 50 µL of formic acid/10 mL of solution) resulted in the isolation of brazilin (1) (54 mg), which was identified by comparing its spectroscopic and spectrometric data with those in literature.[15]

Structure elucidation

The structure of isolated compound was determined by different spectroscopic analyses such as IR, NMR spectra (^{1}H NMR, ^{13}C DEPT 135 and DEPT 90), and two-dimensional experiments, such as hydrogen–hydrogen correlation (H–H COSY), heteronuclear multiple bond coherence (HMBC), heteronuclear single quantum coherence (HSQC), mass spectrometry, and also by comparison with literature data. For obtaining IR spectrum, we used a Bruker Tensor 27 spectrometer coupled with ATR. ^{1}H NMR and ^{13}C NMR were acquired on a Bruker Avance III, operating in 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Samples were dissolved in CD_3OD. Chemical shifts were given in δ (ppm), and coupling constants (J) are expressed in Hertz (Hz). Residual not deuterated solvent peak (δ_H 4.87, 3.31 and δ_C 49.15) was set as reference, and tetramethylsilane (TMS) was used as an internal standard. DART-MS (Direct Analysis in Real Time Mass Spectrometry) was measured using a Joel AccuTOF JMS-T100 LC Mass Spectrometer (Japan) and positive ion [M^+ H^+] was identified. Fourier-transform infrared (FT-IR) spectra were taken on a Bruker Tensor 27 spectrometer with photodiode detector using KBr pellets method for sample preparation.

Cell lines and cell culture

Cell lines such as ARPE-19 (human retinal pigmented epithelium), HeLa (human cervix carcinoma), SiHa (human cérvix squamous cell carcinoma), MDA-MB-231 (human mammary gland epithelial adenocarcinoma), NCI-H1299 (human lung carcinoma; nonsmall cancer cell), A549 (human alveolar adenocarcinoma), and LS 180 (human colorectal adenocarcinoma) were purchased from the American Type Culture Collection (ATCC, Rockville, MD).[16] All cell cultures were maintained in DMEM supplemented with 5% heat-inactivated fetal calf serum (D5F) and grown at 37°C at an atmosphere of 5% CO_2.

Cell proliferation assay

To evaluate the effect of plant extracts on the proliferation of seven cell lines, cell proliferation was determined using the standard MTT assay.[17] In brief, 10,000 cells (50 µL) were added into each well of a flat 96-well plate. After 12 h incubation at 37°C at an atmosphere of 5% CO_2 to allow cell attachment, the cell cultures were incubated with 50 µL of medium containing different concentrations of either crude extract or fractions, and the cell cultures were incubated for 48 h. The crude extract or fraction was first dissolved in DMSO and then diluted in D5F. Control cell cultures were incubated with DMSO (final concentrations of DMSO: 0.06%–0.5%). The antitumor drug doxorubicin was used as a positive control due to its wide use in the clinic for the treatment of a broad spectrum of cancers.[18]

In the last 4 h of the cell culture, 10 µL of MTT stock solution (5 mg/mL) was added to each well. Formazan crystals were dissolved with acidic isopropanol, and the plates were read in an ELISA plate reader, using a test wavelength of 570 nm and a reference wavelength of 630 nm. The absorbance of the wells was read within 15 min of adding isopropanol. The antiproliferative activity of extracts was reported as IC_{50} values (IC_{50} was defined as the concentration of extract evaluated which inhibits cell proliferation by 50%).[19] Values of proliferation of at least three experiments, in triplicate, were log transformed, normalized, and nonlinear regression analysis was used to generate a dose-response curve to calculate IC_{50} values. The differences in means were analyzed using one-way analysis of variance (one-way ANOVA) followed by Tukey's test GraphPad Prism 5 (GraphPad Software, Inc., CA, USA).

RESULTS

Flavonoid isolated from ethanol extract of *Haematoxylum brasiletto*

In this study, the *in vitro* antiproliferative activity of the ethanol extract and isolated compound from *H. brasiletto* against seven cell lines was evaluated. Purification of the dichloromethane partition of ethanol extract of the heartwood of *H. brasiletto* led to the isolation of one known compound: brazilin, ($C_{16}H_{14}O_5$) (6a*S*,11b*R*)-7,11b-Dihidro-6*H*-indeno[2,1-*c*] cromeno-3,6a, 9,10-tetrol [Figure 1]. brazilin was characterized and identified by its spectroscopic data (^{1}H NMR, ^{13}C NMR, DEPT, COSY HSQC, HMBC, and IR) and by comparison with published values [Table 1], this compound was previously described.[15] The flavonoid brazilin has been previously isolated from *H. brasiletto*[20,21] and *Caesalpinia sappan*.[22]

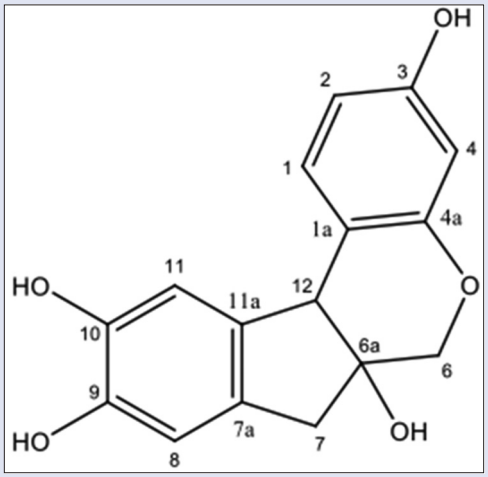


Figure 1: Chemical structure of the flavonoid brazilin

Table 1: Spectroscopic data analysis of Brazilin from *Haematoxylum brasiletto*

Carbon	$^{13}C^a$	$^1H^b$ (m, J in Hz)
1	131.35	7.17 (dd, 8.3, 0.8)
1a	115.58	
2	112.45	6.70 (s)
3	157.86	
4	109.96	6.46 (dd, 8.3, 2.5)
4a	155.72	
6	78.09	3.68 (d, 11.3)
		3.92 (d, 11.3)
6a	70.87	
7	42.92	2.76 (d, 15.7)
		3.01 (d, 15.5)
7a	132.21	
8	112.86	6.59 (s)
9	137.46	
10	145.34	
11	104.28	6.28 (d, 2.5)
11a	145.65	
12	51.09	3.95 (s)

[a]The chemical shifts are expressed in δ values (ppm). Spectra recorded in CD_3OD at 100.6 MHz. [b]The chemical shifts are expressed in δ values (ppm), m, and coupling constants are in Hz. Spectra recorded in CD_3OD at 400 MHz. m: Multiplicity; Hz: Hertz

Antiproliferative activity

Recently, the antiproliferative activity of the extracts of *H. brasiletto* against A549, RAW 264, and L-929 cells was evaluated using the MTT assay, which demonstrates mitochondrial activity of cells and is commonly used to measure the cell viability. These previous results prompted us to perform the present study in which the aim was to isolate the compounds responsible for antiproliferative activity of the ethanol extract of *H. brasiletto.*

The flavonoid isolated of chromatographic fraction of dichloromethane fraction of *H. brasiletto* was evaluated for its effects on proliferation of a panel of six human cancer cell lines (A549, LS180, H1299, HeLa, SiHa, and MDA-MB-231) and a normal (noncancer) human cell line (ARPE-19). The broad-spectrum chemotherapeutic agent, doxorubicin, was included as a positive control and for comparison purposes as it can induce apoptosis for intercalation into DNA and disruption of topoisomerase-II DNA repair.[23] The results are presented in Table 2. The antiproliferative activity of brazilin was evaluated [Table 2], demonstrating that it causes a moderate inhibitory effect on the growth in the human SiHa, MDA, A549, and H1299 cell lines at IC_{50} values of 44.3, 48.7, 45.4, and 48.7 µM, respectively. In LS180 and HeLa, a low effect at IC_{50} 62.2 and 71.9 µM, respectively, was observed.

Brazilin showed a moderate antiproliferative effect on noncancer ARPE-19 cell line with IC_{50} value of 37.9 µM, suggesting that the antiproliferative activity of brazilin is nonselective.

Table 2: *In vitro* antiproliferative activity of flavonoid brazilin from *Haematoxylum brasiletto* on seven cell lines measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

	Cell lines IC_{50} (μM)						
	A549	LS 180	H1299	HeLa	SiHa	MDA	ARPE 19
Brazilin[a]	45.4±2.5	62.2±2.9	48.7±1.9	71.9±2.3	44.3±0.7	48.7±1.9	37.9±0.7
Doxorubicin[a]	8.3±0.2	4.7±0.3	6.5±0.8	5.6±0.2	4.1±0.13	4.6±0.4	7.77±0.4

IC_{50} values represent a mean±SD (n=3) of three independent experiments. SD: Standard deviation. [a]Doxorubicin was used as a control positive

DISCUSSION

In this work, we demonstrated that brazilin, a flavonoid isolated and characterized of *H. brasiletto*, has antiproliferative activity against cancer cell lines. Rivero-Cruz[12] reported that brazilin inhibited the growth of *S. aureus* 375, *S. aureus* ATCC 25923, and *E. faecium* 379. Moreover, brazilin was described as an inhibitor of NO synthase,[24] xanthine oxidase,[22] protein kinase C,[25] and aldose reductase enzymes.[26] However, there are no reports of research of antiproliferative activity induced by brazilin on cell lines.

Flavonoids with antiproliferative effect, such as kaempferitrin and curcumin, have previously been described.[27] Several studies indicate that curcumin possesses reactive oxygen species (ROS)-inducing or pro-oxidant activity.[28] It is known that ROS, including the superoxide anion, hydrogen peroxide, and hydroxyl radical, are known to mediate apoptosis induced by some cancer chemopreventive and therapeutic agents.[29] Moreover, curcumin induces hypomethylation of the miR-203 promoter and subsequent upregulation of miR-203 expression. This leads to downregulation of miR-203 target genes Akt2 and Src that culminates in decreased proliferation and increased apoptosis of bladder cancer cells.[30] Kaempferitrin may induce both transcription-independent and transcription-dependent pathways of p53 as it upregulates pro-apoptotic proteins and downregulates antiapoptotic proteins. Moreover, it has been described that an increase in p53 levels leads to cell cycle arrest at G1 phase.[31]

However, brazilin is more active than other compounds, such as a 5-fluorouracil, usually used in anti-tumoral clinical therapy which has IC_{50} values >100 μM in HeLa. Likewise, other natural products isolated from Sonora propolis in Mexico, such as CAPE, galangin, and xanthomicrol, exhibit an IC_{50} ≥60 μM in HeLa and A549 cell lines.[19] In the same way, kaempferitrin, isolated from *Justicia spicigera*, shows IC_{50} >45 μM in HeLa.[32]

CONCLUSIONS

In this study, using a bioassay-guided method, we isolated one flavonoid with antiproliferative activity from *H. brasiletto*: brazilin (6a*S*,11b*R*)-7,11b-Dihidro-6*H*-indeno[2,1-*c*] cromeno-3,6a, 9,10-tetrol; this compound demonstrated antiproliferative activity against selected human cancer cells.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Bergstrom R. Drivers of the cost of cancer care. Lancet Oncol 2012;13:14-5.
2. World Health Organization, Prevalence Data of Cancer Worldwide. Available from: http://www.who.int/features/factfiles/cancer/en/index.htmlS. [Last accessed on 2016 Sep 06].
3. Ueda JY, Tezuka Y, Banskota AH, Le Tran Q, Tran QK, Harimaya Y, *et al*. Antiproliferative activity of Vietnamese medicinal plants. Biol Pharm Bull 2002;25:753-60.
4. Kintzios SE, Barberaki MG. Plants that Fight Cancer. Boca Raton: CRC Press; 2004.
5. Jiménez-Estrada M, Velázquez-Contreras C, Garibay-Escobar A, Sierras-Canchola D, Lapizco-Vázquez R, Ortiz-Sandoval C, *et al*. *In vitro* antioxidant and antiproliferative activities of plants of the ethnopharmacopeia from Northwest of Mexico. BMC Complement Altern Med 2013;13:12.
6. Butler MS. The role of natural product chemistry in drug discovery. J Nat Prod 2004;67:2141-53.
7. Gordaliza M. Natural products as leads to anticancer drugs. Clin Transl Oncol 2007;9:767-76.
8. Kalekar S, Munshi R, Thatte U. Do plants mediate their antidiabetic effects through antioxidant and anti-apoptotic actions? An *in vitro* assay of 3 Indian medicinal plants. Complement Alternat Med 2013;13:257.
9. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Med Res Rev 2003;23:519-34.
10. Argueta A. Atlas De Las Plantas De La Medicina Tradicional Mexicana. Vol. 1. México City, México: Instituto Nacional Indigenista; 2004.
11. Heredia N, Escobar M, Rodríguez-Padilla C, García S. Extracts of *Haematoxylum brasiletto* inhibit growth, verotoxin production, and adhesion of enterohemorrhagic *Escherichia coli* O157:H7 to HeLa cells. J Food Prot 2005;68:1346-51.
12. Rivero-Cruz JF. Antimicrobial compounds isolated from *Haematoxylum brasiletto*. J Ethnopharmacol 2008;119:99-103.
13. González-Salvatierra C, Luis Andrade J, Escalante-Erosa F, García-Sosa K, Manuel Peña-Rodríguez L. Antioxidant content in two CAM bromeliad species as a response to seasonal light changes in a tropical dry deciduous forest. J Plant Physiol 2010;167:792-9.
14. Zapata-Estrella HE, Sánchez-Pardenilla AD, García-Sosa K, Escalante-Erosa F, de Campos-Buzzi F, Meira-Quintão NL, *et al*. Bioactive metabolites from *Cnidoscolus souzae* and *Acmella pilosa*. Nat Prod Commun 2014;9:1319-21.
15. Craig JC, Naik AR, Pratt R, Johnson E, Bhacca NS. Nuclear magnetic resonance spectra and stereochemistry of the antibacterial principle from *Haematoxylum brasiletto*. J Org Chem 1965;30:1573-6.
16. Rascón-Valenzuela L, Jiménez-Estrada M, Velázquez-Contreras C, Garibay-Escobar A, Vilegas W, Campaner L, *et al*. Chemical composition and antiproliferative activity of *Acalypha califórnica*. Ind Crops Prod 2015;69:48-54.
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
18. Rascón-Valenzuela L, Velázquez C, Garibay-Escobar A, Medina-Juárez LA, Vilegas W, Robles-Zepeda RE. Antiproliferative activity of cardenolide glycosides from *Asclepias subulata*. J Ethnopharmacol 2015;171:280-6.
19. Torres-Moreno H, Velázquez CA, Garibay-Escobar A, Massimo C, Marcotullio MC, Robles-Zepeda RE. Antiproliferative and apoptosis induction of cucurbitacin-type triterpenes from *Ibervillea sonorae*. Ind Crops Prod 2015;77:895-900.
20. Sanchez-Marroquin A, Garcia L, Mendez M. Brazilin, antibacterial substance from *Haematoxylum brasiletto*. Rev Latinoam Microbiol 1958;1:225-32.
21. Pratt R, Yuzuriha Y. Antibacterial activity of the heartwood of *Haematoxylum brasiletto*. J Am Pharm Assoc 1959;48:69-72.
22. Nguyen MT, Awale S, Tezuka Y, Tran QL, Kadota S. Xanthine oxidase inhibitors from the heartwood of Vietnamese *Caesalpinia sappan*. Chem Pharm Bull (Tokyo) 2005;53:984-8.
23. Sasaki Y, Hosokawa T, Nagai M, Nagumo S. *In vitro* study for inhibition of NO production about constituents of *Sappan lignum*. Biol Pharm Bull 2007;30:193-6.
24. Kim GG, Kim YM, Khil LY, Jeon SD, So DS, Moon CH, *et al*. Brazilin inhibits activities of protein kinase C and insulin receptor serine kinase in rat liver. Arch Pharm Res 1998;21:140-6.
25. Moon CK, Yun YP, Lee JH, Wagner H, Shin YS. Inhibition of lens-aldose reductase activity by brazilin and haematoxylin. Planta Med 1985;51:66-7.
26. Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, *et al*. Doxorubicin pathways: Pharmacodynamics and adverse effects. Pharmacogenet Genomics 2011;21:440-6.
27. Alonso-Castro AJ, Ortiz-Sánchez E, García-Regalado A, Ruiz G, Núñez-Martínez JM,

González-Sánchez I, *et al.* Kaempferitrin induces apoptosis via intrinsic pathway in HeLa cells and exerts antitumor effects. J Ethnopharmacol 2013;145:476-89.

28. Javvadi P, Segan AT, Tuttle SW, Koumenis C. The chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase pathway. Mol Pharmacol 2008;73:1491-501.
29. Davis W Jr., Ronai Z, Tew KD. Cellular thiols and reactive oxygen species in drug-induced apoptosis. J Pharmacol Exp Ther 2001;296:1-6.
30. Saini S, Arora S, Majid S, Shahryari V, Chen Y, Deng G, *et al.* Curcumin modulates microRNA-203-mediated regulation of the Src-Akt axis in bladder cancer. Cancer Prev Res (Phila) 2011;4:1698-709.
31. Haupt S, Berger M, Goldberg Z, Haupt Y. Apoptosis – The p53 network. J Cell Sci 2003;116:4077-85.
32. Alonso-Castro AJ, Ortiz-Sánchez E, Domínguez F, Arana-Argáez V, Juárez-Vázquez Mdel C, Chávez M, *et al.* Antitumor and immunomodulatory effects of *Justicia spicigera* Schltdl (Acanthaceae). J Ethnopharmacol 2012;141:888-94.