In Vitro Evaluation of the Antiinflammatory and Anticancer Activities of Compounds from the Plants *Casearia sylvestris* and *Zanthoxylum monophyllum*

Omar A. Dupuy L.[1,*], José A. Bonilla V.[2], Renato Murillo M.[3], Peter Taylor[4], María J. Abad[4], Lorena I. González P.[1], Olmedo Otero[1] and Johanna Juliao A.[1]

[1]*Instituto de Investigaciones en Biotecnología y Ciencias Biomédicas, Escuela de Biotecnología, Facultad de Ciencias de la Salud, Universidad Latina de Panamá, Panamá, Panamá*

[2]*Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San José, Costa Rica*

[3]*Escuela de Química y CIPRONA, Universidad de Costa Rica, San José, Costa Rica*

[4]*Laboratorio de Patología Celular y Molecular, Centro de Medicina Experimental, Instituto Venezolano de Investigaciones Científicas, Apartado 21827, Caracas 1020-A, Venezuela*

Abstract: The anthocyanins, coumarins, and casearins are interesting compounds due to their chemical and biological properties. In the present study, the *in vitro* effect of 4´-O-methyl-gallocatechin (an anthocyanin), columbianatin (a coumarin), and casearin G on the mitogenic response and phagocytic activity from whole blood cells, was evaluated. In addition, the cytotoxic effect of these compounds on the cell lines B16/BL6 (murine melanoma) and COS-7 (kidney fibroblasts transfected with SV40 virus) was measured by a colorimetric assay (MTS/PMS, IC_{50}: inhibitory concentration) and the sulphorhodamine B assay (GI_{50}: growth inhibition, TGI: total growth inhibition, LC_{50}: lethal concentration). The results show that 4´-O-methyl-gallocatechin and columbianatin reduced lymphoproliferation. Columbianatin reduced both the phagocytic index and the percentage of phagocytic monocytes/macrophages. Casearin G showed both cytotoxic (IC_{50}, LC_{50}) and cytostatic (GI_{50}) effects against the tumor cells, B16/BL6 (IC_{50} = 30.8 µM; GI_{50} = 12.4 µM; LC_{50} = 34.7 µM) and COS-7 (IC_{50} = 137.3 µM; GI_{50} = 3.8 µM; LC_{50} = 29.9 µM). In conclusion, 4´-O-methyl-gallocatechin and columbianatin showed immunosuppressive properties *in vitro* while casearin G was the most cytotoxic.

Keywords: 4´-O-methyl-gallocatechin, columbianatin, casearin G, peripheral blood mononuclear cells, B16/BL6 melanoma, COS-7.

INTRODUCTION

Investigations on medicinal plants led to the isolation and purification of various compounds that have properties of biomedical interest, from plants like *Casearia sylvestris* (Salicaceae) [1-4], *Decachaeta thieleana*, *Viguiera sylvatica* (Asteraceae) [5-7], and *Zanthoxylum monophyllum* (Rutaceae) [8]. Some of these compounds, such as anthocyanins, coumarins and casearins, show anti-inflammatory activity. Investigations with extracts rich in anthocyanins, have shown that they can inhibit mitogen-induced liver injury, decrease both lipid peroxidation and oxidative damage, and increase manganese superoxide dismutase activity showing antiinflammatory effects [9]. In addition to their antioxidant properties, anthocyanins have an ability to adhere to macromolecules such as proteins and polysaccharides [10]. Coumarin possesses antithrombotic and antiallergic activity [11]. Hydroxycoumarin is a typical phenolic compound that acts as a powerful antioxidant. Coumarin derivatives inhibit the edema and the formation of granuloma in animal models of inflammation and have an effect comparable to drugs such as diclofenac [12] and indomethacin [13]. On the other hand, the casearins show anti-inflammatory, antiofidic [1, 3], antiulcer [14], and antitumoral [2-4, 15, 16] activities. However, more studies about the effect of the anthocyanins, coumarins and casearins on different cell types are required, in order to better understand their therapeutic potential and possible interactions between these compounds and human cells are required.

The aim of this research was to study the casearin G, 4´-O-methyl-gallocatechin and the columbianatin from *C. sylvestris* and *Z. monophyllum,* in terms of their possible cytotoxic effects against the tumor cell lines B16/BL6 and COS-7, and their effect on lymphocytes proliferation and phagocytic activity by monocytes/macrophages.

MATERIALS AND METHODS

Compounds

4´-O-methyl-gallocatechin and columbianatin, (Figure **1A**, **B**) were isolated from *Z. monophyllum*

*Address correspondence to this author at the Instituto de Investigaciones en Biotecnología y Ciencias Biomédicas, Escuela de Biotecnología, Facultad de Ciencias de la Salud, Universidad Latina de Panamá, Panamá, Panamá; Tel: +507 207 6728; E-mail: omarielsag@yahoo.com

ISSN: 1929-2260 / E-ISSN: 1929-2279/15

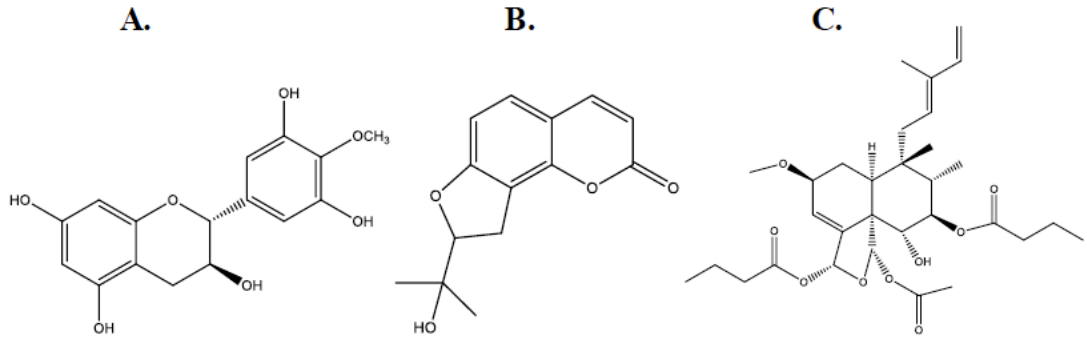


Figure 1: Chemical structure of **A.** 4´-O-methyl-gallocatechin, an anthocyanin isolated from *Z. monophyllum*; **B.** Columbianatin, a coumarin isolated from *Z. monophyllum* and **C.** 18-butanoyl-6-α-hydroxy-Casearin G, a diterpene isolated from *C. sylvestris*.

(code: JVR 13509) and 18-butanoyl-6-α-hydroxi-Casearin G (Figure **1C**) from *C. sylvestris* (code: JVR 13508). Both plants were collected in El Rodeo, San José, Costa Rica, and identified by Luis Poveda (botanist of the Universidad Nacional, Costa Rica, where control samples were deposited). The compounds were isolated and purified using extraction techniques described by other authors [8] and their chemical structures were determined by one- and two-dimensional nuclear magnetic resonance techniques. One milligram of each compound was dissolved in 0.1 mL of dimethylsulfoxide (DMSO) and further diluted in 0.9 mL of culture medium RPMI 1640 (Roswell Park Memorial Institute 1640) (Sigma, no Cat. R7388), to obtain solutions of 4´-O-methyl-gallocatechin (3.12 mM), columbianatin (4.06 mM) or casearin G (1.82 mM).

Cells

B16/BL6 (murine melanoma) and COS-7 (kidney fibroblasts transfected with SV40 virus) cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with fetal bovine serum (FBS) to 5% (Gibco, USA), 100 U/mL of penicillin and streptomycin (Sigma Chemical Corp., USA) 100 µg/mL. The cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

Peripheral Blood Mononuclear Cells (PBMC)

The whole blood samples from apparently healthy volunteers (five women and three men, aged 20 to 45 years old), were collected in sterile vacuum tubes, with heparin as an anticoagulant. The PBMC were separated on a Ficoll-Hypaque gradient (Sigma) according to the manufacturer's instructions. Briefly, each blood sample was diluted to 1:2 in sterile phosphate buffered saline (PBS) and placed on 2 mL of Ficoll-Hypaque (density 1044). The sample was then centrifuged at 1800 rpm for 45 min at 20°C. Mononuclear cells were extracted with a pipette and washed with sterile PBS, pH 7.2, by centrifugation. Finally, the cells were re-suspended in RPMI 1640, supplemented with 10% FBS, 50 µg/mL streptomycin, 100 UI/mL of penicillin, 2 mM glutamine, 5 mM 2-mercaptoethanol, 10 mM solution MEM of essential amine acids, 45 mM bicarbonate of sodium, 0.8 mM of glucose and 25 mM of N-(2-hydroxyethyl) piperazin-N'-(2-ethanesulfonic acid) (HEPES) as a buffer. Cell viability was assessed by trypan blue dye exclusion and the concentration was adjusted to 1 x 10^6 cells/mL.

Cytotoxicity

PBMC were plated at 1 x 10^5 cells/well in 100 µL of RPMI 1640 in round-bottomed 96-well plates. Different concentrations of the compounds were added in 100 µL of RPMI 1640. Control wells were set up containing equivalent quantities of DMSO, which in no case exceeded 1%. Then, PBMC were incubated at 37°C in a humidified 5% CO_2 atmosphere for 72 h. Subsequently, the toxic effect of the compounds on the cells was assessed using the method described by Mosmann [17] modified by Bonilla *et al*. [18]. Briefly, 50 µL of a tetrazolium salt solution 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-(phenylamine-carbonyl)-2H-tetrazol hydroxide (XTT) (1 mg/mL) and N-methylphenacinemetasulphate (PMS) (0.01 M) were added to each well. The cells were incubated for 2 h at 37°C in darkness. Subsequently, 100 µL of the supernatant of each well were transferred to another 96-well plate and the change of color was quantified on a microplate reader, at a wavelength of 450 nm and with a 650 nm reference filter. In addition, cell viability was assessed by trypan blue dye exclusion. In subsequent immunological assays, non-toxic concentrations of each compound were used.

B16/BL6 and COS-7 cells were seeded at 2.5 - 5 x 10^4 cells/well in 100 µL of DMEM in flat-bottomed 96-

well plates (Linbro, Flow Laboratories, VA, USA) and allowed to attach for 24 h. Different concentrations of the compounds were added in 100 µL of DMEM. Control wells were set up containing equivalent amounts of DMSO, which in no case exceeded 1%. After 48 h of incubation at 37°C, the number of viable cells was measured with two different assays, the chromogenic assay 5 - (3 - carboxymethoxyphenyl) - 2 - (4, 5-dimethylthiazolyl) - 3 - (4-sulphophenyl) (MTS/PMS) and the sulphorhodamine B (SRB) assay (Promega Corp., USA). In the case of the MTS/PMS assay, after followinged the manufacturer's instructions, the IC_{50} (50% inhibitory concentration) was calculated from the dose-response curve using linear interpolation (Excel, Microsoft Corp., USA). The SRB assay is based on the binding of negatively charged sulphorhodamine to basic amine acids in the cell, thus enabling an estimate of cell mass [19]. Measurements of the cell mass at T = 0 and T = 48 h were used to calculate three parameters, GI_{50} (the concentration of the drug that inhibited the growth of cells by 50%), TGI (the concentration of the drug that caused total growth inhibition) and the LC_{50} (lethal concentration, the concentration of the drug which induced 50% cytotoxicity. As for the IC_{50}, these values were obtained by linear interpolation of the dose-response curve.

Lymphoproliferation

One hundred µL of the PBMC (1 x 10^6 cells/mL) obtained as described previously, were added to each well of a 96-well round bottom plate, followed by phytohemagglutinin-M (PHA-M) as mitogen to a final concentration of 10 µg/mL. The 4´-O-methyl-gallocatechin, columbianatin and casearin G were added, in quintuplicate, at different final concentrations.

The final volume in each well was 200 µL. Control wells were set up containing PHA-M and equivalent amounts of DMSO, which in no case exceeded 1%. The plates were incubated at 37°C and 5% CO_2 for 72 h. The determination of cell proliferation was performed using the XTT assay (see above). The results were expressed as a percentage of stimulation, where 100% of stimulation corresponds to PBMC in the presence of PHA-M+DMSO without compounds (control).

Phagocytic Activity

The effect of the compounds on phagocytic activity was evaluated using whole blood samples from apparently healthy volunteers (five women and three men, aged 20-45 years old). Heparinized venous blood (125 µL) was treated in triplicate with the compounds at different concentrations for one hour at 37°C and 5% CO_2. Then 125 µL of a suspension of *Bacillus subtilis* (1 x 10^9 CFU/mL) inactivated previously for 1 h at 80°C was added. The samples were incubated at 37°C and 5% CO_2 during 30 min. They were placed on slides and dyed with Wright stain. The plates were inspected under a microscope to find 50 monocytes and determine the phagocytic activity (percentage of phagocytic monocytes), by means of the following formula: percentage of phagocytic monocytes = number of monocytes with bacteria inside / 50 X 100. Then 30 monocytes with bacteria inside were selected and the phagocytic index was determined (average amount of phagocyted bacteria by monocytes), by means of the following formula: phagocytic index = sum of the quantity of bacteria on the inside of each monocyte / 30.

Statistical Analysis

The data was assessed by means of an one-way analysis of variance (ANOVA) using the VassarStats web site for statistical computation (http://vassarstats.net/index.html) to compare experimental *versus* control. The results are expressed as mean ± standard deviation. Sgnificance of the results was calculate using the Student t test with the Bonferroni correction for multiple comparisons. $P < 0.05$ was considered to be level of statistical significance.

RESULTS

The number of viable PBMC significantly decreased with casearin G at 64 µM. At lower concentrations, cytotoxic effects on the PBMC were not observed. 4'-O-methyl-gallocatechin and columbianatin did not significantly affect cell viability (Figure **2**). In the subsequent immunological assays, only lower non-cytotoxic concentrations were employed.

The cytotoxicity assays on B16/BL6 tumor cells showed values of IC_{50} = 30.8 µM, GI_{50} = 12.4 µM and LC_{50} = 34.7 µM with casearin G, whereas with COS-7 cells the values were IC_{50} = 137.3 µM, GI_{50} = 3.8 µM and LC_{50} = 29.9 µM. No cytotoxic effect of 4'-O-methyl-gallocatechin and columbianatin on the B16/BL6 and COS-7 cells was observed (Tables **1**, **2**). No effect due to the DMSO was observed. The lymphoproliferative response to PHA-M significantly decreased in PBMC cultures treated with 4'-O-methyl-gallocatechin (≥8 µM) and columbianatin (≥10 µM), whereas casearin G did

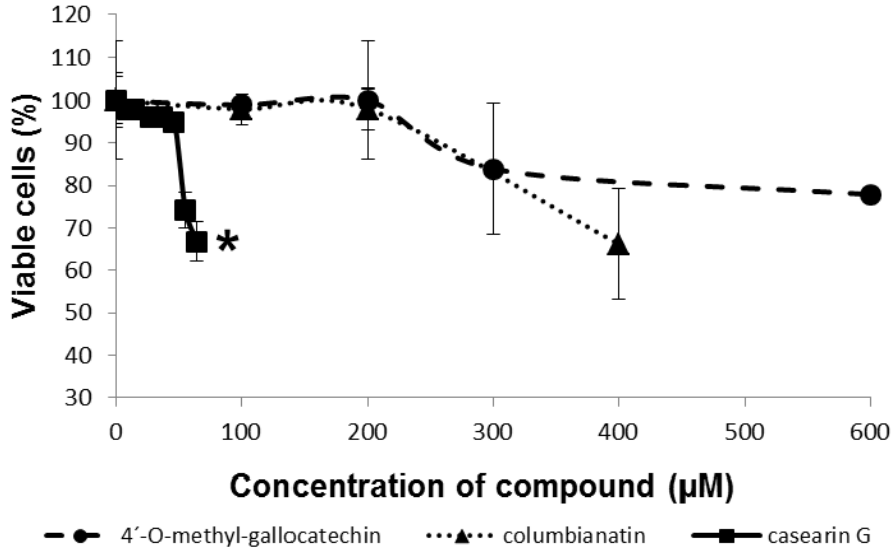


Figure 2: Effect of the 4´-O-methyl-gallocatechin, columbianatin and casearin G on the viability of peripheral blood mononuclear cells (PBMC). Each point represents mean ± SD of quintuplicate measure. *p<0.05 with respect to the control (no compound).

Table 1: Cytotoxic Effect of Casearin G, 4´-O-methyl-gallocatechin and Columbianatin on B16/BL6 and COS-7 Cells, Measured by the MTS/PMS Assay, after 48 h of Incubation

Compounds	Cell Lines	
	B16/BL6 IC_{50}	COS-7 IC_{50}
Casearin G	30.8	137.3
4´-O-methyl-gallocatechin	>312	>312
Columbianatin	>406	>406

B16/BL6: murine melanoma.
COS-7: kidney fibroblasts transfected with SV40 virus.
IC_{50} (inhibitory concentration): inhibitory concentration of 50% cell viability. Concentrations in µM.

Table 2: Cytotoxic and Cytostatic Effect of Casearin G, 4´-O-methyl-gallocatechin and Columbianatin on B16/BL6 and COS-7 Cells, Measured by the Sulphorhodamine B Assay, after 48 h of Incubation

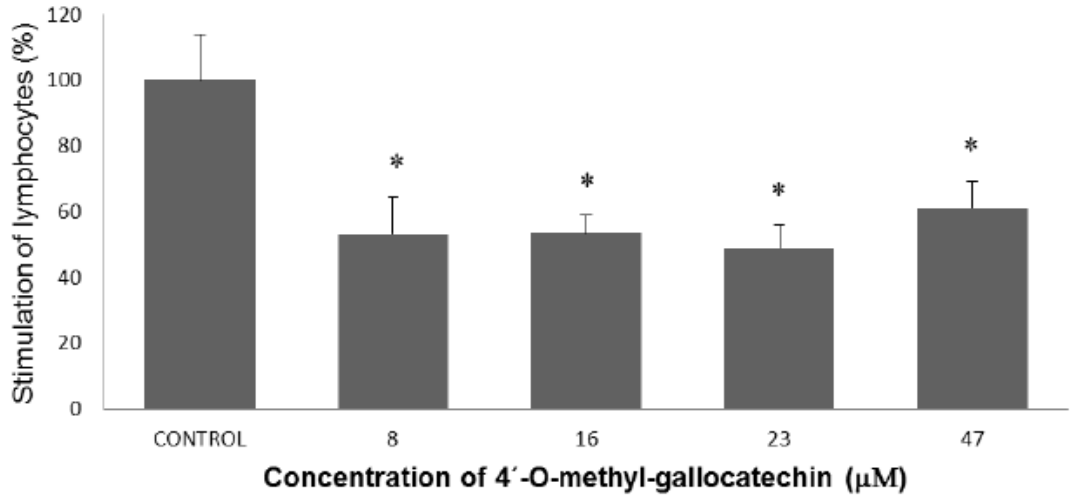
Compounds	Cell Lines					
	B16/BL6			COS-7		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
Casearin G	12.4	18.5	34.7	3.8	5.5	29.9
4´-O-methyl-gallocatechin	>312	>312	>312	137.8	214.1	>312
Columbianatin	>406	>406	>406	268.1	345.6	>406

B16/BL6: murine melanoma.
COS-7: kidney fibroblasts transfected with SV40 virus.
GI_{50} (growth inhibition): the concentration of the drug that inhibited the growth of cells by 50%, TGI (total growth inhibition): the concentration of the drug that inhibited the growth of cells in a 100%, LC_{50} (lethal concentration): the concentration of the drug which induced 50% cytotoxicity. Concentrations in µM.

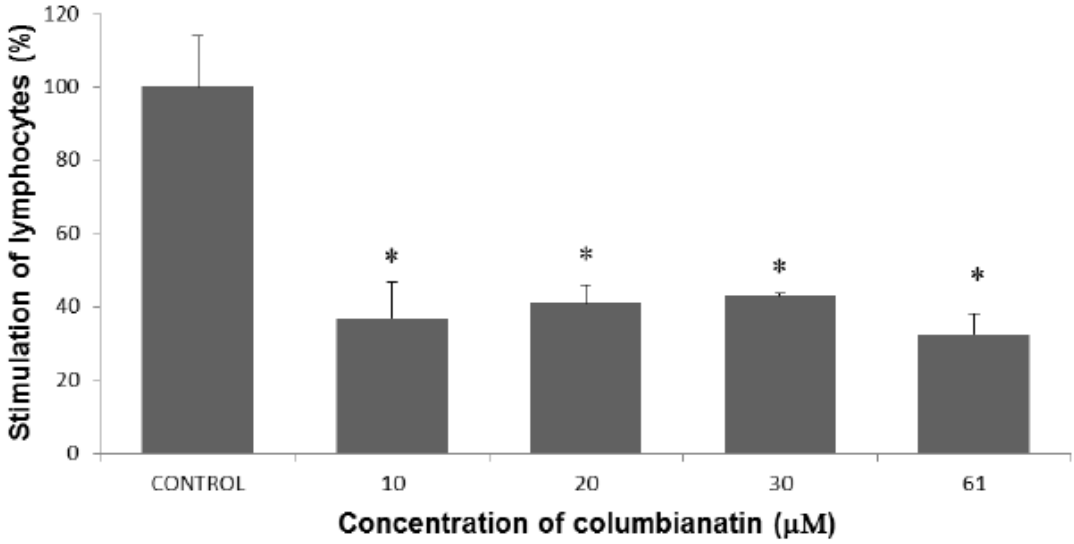
not affect the response to mitogen (Figure **3**). A decrease of the phagocytic activity was observed in the whole blood samples treated with columbianatin at 4 mM whereas 4'-O-methyl-gallocatechin and casearin G did not significantly affect phagocytic activity (Figure **4**).

DISCUSSION

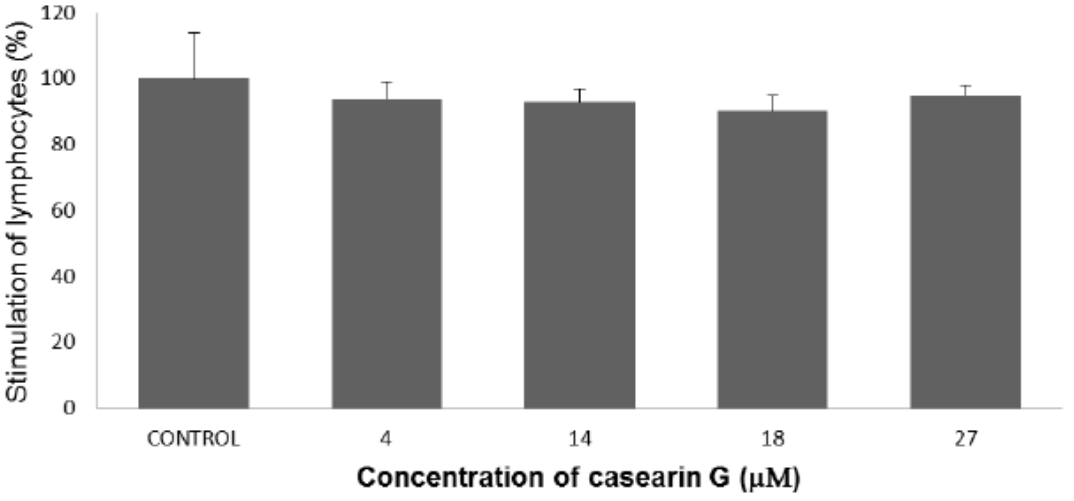
The genus *Zanthoxylum* includes many plants used in traditional medicine specially in America, Africa and Asia. Both antioxidant and anti-inflammatory activities have been identified in this genus [20], and *Z.*



A

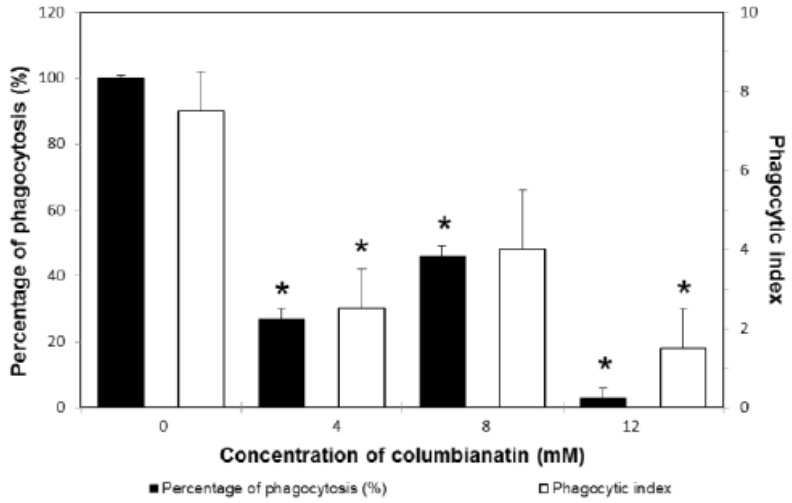


B

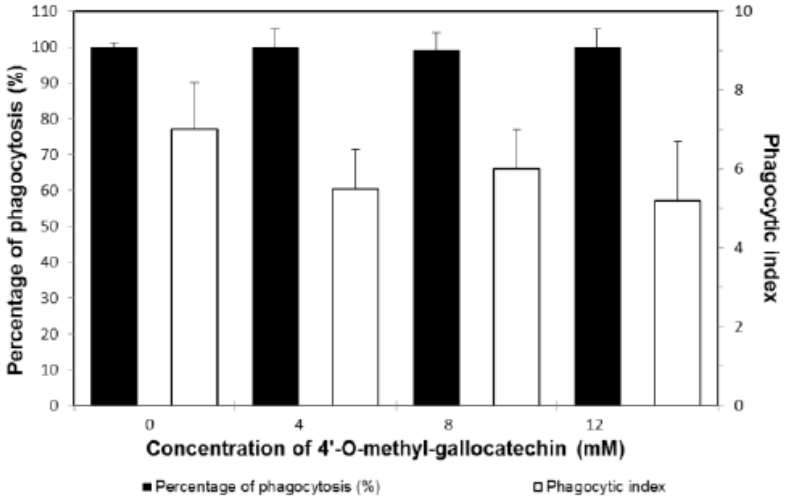


C

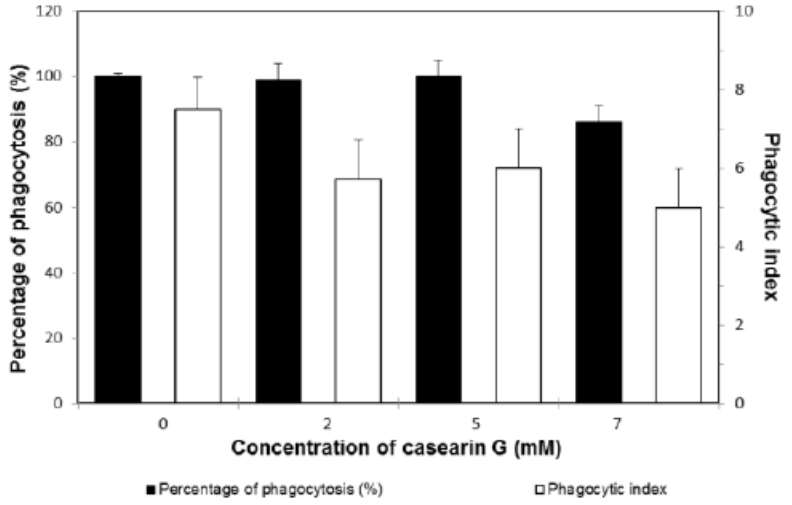
Figure 3: *In vitro* effect of **A.** The 4´-O-methyl-gallocatechin, **B.** The columbianatin and **C.** The casearin G on lymphoproliferation. Each column represents the mean ± SD of quintuplicate measure. *p<0.05 with respect to 100% stimulation (PHA-M+DMSO without treatment).



A



B



C

Figure 4: *In vitro* effect of **A.** The columbianatin, **B.** The 4´-O-methyl-gallocatechin and **C.** The casearin G on phagocytic activity using whole blood. Each column represents the mean ± SD of triplicate measure. *p<0.05 with respect to the control (no compound)..

monophyllum, the object of this study, is used as an analgesic to treat the inflammation [21].

4'-O-methyl-gallocatechin and columbianatin inhibited the lymphoproliferative response to mitogen. These effects were observed at concentrations well below their cytotoxic concentrations, indicating true inhibition, rather than a nonspecific effect.

A close relationship exists between reactive oxygen species and the inflammation [22], demonstrated for example, by the inhibitory effect of antioxidants on NO production [23]. The coumarins act as antioxidants and enzymatical inhibitors since they inhibit the biosyntesis of prostaglandins, involving cyclooxygenase and lipoxygenase and the generation of the neutrophile-dependent superoxide anion [11]. Coumarin derivatives show an effect comparable to that of drugs as diclofenac [12] and indomethacin [13]. In view of our results, and of others, which show activity of the anthocyanins and coumarins on related processes (redox cellular condition, NO production, lymphoproliferation, phagocytosis), the question arises as to whether different targets are involved, or if there is a common key target, for example the nuclear factor NF-*κ*B, that certainly plays a central role in all the mentioned processes.

It was notable that the columbianatin showed its effects at much lower concentrations in purified cell culture that in the assays using whole blood. It is well-known that the interaction of drugs with serum proteins may affect their activity and toxicity, and it is very probable that chelation of free columbianatin is the reason of this discrepancy in the results.

C. sylvestris is used by natives of South America for the treatment of the cancer [24]. Our results might help to explain, at least partly, the antitumoral properties ascribed to this plant, since the casearin G is the most abundant clerodane-like diterpene in *C. sylvestris* (data to be published).

Using two different viability assays, MTS, which measures mitochondrial metabolic activity, and SRB, which measures cell mass, we measured the *in vitro* activity of casearin G against tumor cells. The SRB assay is useful in that it discriminates between cytostatic and cytotoxic effects, rather than a general “inhibitory” effect as provided by the MTS assay. The IC_{50} of casearin G with B16/BL6 was lower than that of millerenolide, a sesquiterpene lactone that has been reported to show an antitumor effect in a murine model [7].

Nevertheless, the COS-7 cells were less sensitive to the casearin G that the B16/BL6 cells. The casearins possess a diacetal system in the C ring at positions C-18 and C-19 which may possibly explain its cytotoxic properties [2, 15]. Future studies in animal models might help to evaluate the *in vivo* anticancer potential of casearin G. In fact, there are data suggesting that *C. sylvestris* could be safe even when used therapeutically over long periods [25].

In conclusion, our results showed that 4'-O-methyl-gallocatechin and columbianatin possess immuno-suppressive properties *in vitro*, whereas casearin G is cytotoxic for the tumor cells B16/BL6 and COS-7.

ACKNOWLEDGEMENTS

This work was supported by the National Secretariat of Science, Technology and Innovation (SENACYT) of Panama.

REFERENCES

[1] Borges MH, Soares AM, Rodrigues VM, *et al.* Effects of aqueous extract of *Casearia sylvestris* (Flacourtiaceae) on actions of snake and bee venoms and on activity of phospholipases A2. Comp Biochem Physiol B Biochem Mol Biol 2000; 127: 21-30. http://dx.doi.org/10.1016/S0305-0491(00)00237-6

[2] Oberlies NH, Burgess JP, Navarro HA, *et al.* Novel bioactive clerodane diterpenoids from the leaves and twigs of *Casearia sylvestris*. J Nat Prod 2002; 65: 95-9. http://dx.doi.org/10.1021/np010459m

[3] Ferreira PM, Costa-Lotufo LV, Moraes MO, *et al.* Folk uses and pharmacological properties of *Casearia sylvestris*: a medicinal review. An Acad Bras Cienc 2011; 83(4): 1373-84. http://dx.doi.org/10.1590/S0001-37652011005000040

[4] Bou DD, Lago JH, Figueiredo CR, *et al.* Chemical composition and cytotoxicity evaluation of essential oil from leaves of *Casearia sylvestris*, its main compound α-zingiberene and derivatives. Molecules 2013; 18: 9477-87. http://dx.doi.org/10.3390/molecules18089477

[5] Dupuy OA, Murillo R, Bonilla JA. Actividad supresora del millerenólido sobre células mononucleares de sangre periférica humana. Rev Med Chil 2008; 136: 64-72. http://dx.doi.org/10.4067/S0034-98872008000100008

[6] Dupuy OA, Murillo R, Bonilla JA. Lactonas sesquiterpénicas de las plantas *Viguiera sylvatica* y *Decachaeta thieleana* (Asteraceae) modulan la producción de óxido nítrico y la fagocitosis de macrófagos RAW. Rev Biol Trop 2008; 56: 1063-73.

[7] Taylor PG, Dupuy OA, Bonilla JA, Murillo R. Anticancer activities of two sesquiterpene lactones, millerenolide and thieleanin isolated from *Viguiera sylvatica* and *Decachaeta thieleana*. Fitoterapia 2008; 79: 428-32. http://dx.doi.org/10.1016/j.fitote.2007.07.019

[8] Rodríguez-Guzmán R, Fulks LC, Radwan MM, Burandt CL, Ross SA. Chemical constituents, antimicrobial and antimalarial activities of *Zanthoxylum monophyllum*. Planta Med 2011; 77: 1542-44. Supporting information for this article is available online at https://www.thieme-connect.de/media/plantamedica/201113/supmat/pmleu1147sup_10-1055-s-0030-1270782.pdf

[9] Li X, Li Y, States VA, Li S, Zhang X, Martin RC. The effect of black raspberry extracts on MnSOD activity in protection against concanavalin A induced liver injury. Nutr Cancer 2014; 66(6): 930-7. http://dx.doi.org/10.1080/01635581.2014.922201

[10] Toledo de Oliveira T, Nagem TJ, Rocha da Costa M, *et al.* Propiedades biológicas de los tintes naturales. Ars Pharmaceutica 2004; 45: 5-20.

[11] Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN. Natural and synthetic coumarin derivatives with anti-inflammatory/ antioxidant activities. Curr Pharm Des 2004; 10: 3813-33. http://dx.doi.org/10.2174/1381612043382710

[12] Ahmad R, Asad M, Siddiqui ZN, Kumar A. Screening of synthetic new heterocyclic derivatives of 3-Formyl-4-Hydroxycoumarin for anti-inflammatory activity in albino rats. JPRHC 2009; 1: 46-62.

[13] Menghini L, Epifano F, Genovese S, Marcotullio MC, Sosa S, Tubaro A. Antiinflammatory activity of coumarins from *Ligusticum lucidum* Mill. subsp. *cuneifolium* (Guss.) Tammaro (Apiaceae). Phytother Res 2010; 24: 1697-99. http://dx.doi.org/10.1002/ptr.3170

[14] Sertié JA, Carvalho JC, Panizza S. Antiulcer activity of crude extracts from leaves of *Casearia sylvestris*. Pharm Biol 2000; 38: 112-9. http://dx.doi.org/10.1076/1388-0209(200004)3821-1FT112

[15] Carvalho PR, Furlan M, Young MC, Kingston DG, Bolzani VS. Acetylated DNA-damaging clerodane diterpenes from *Casearia sylvestris*. Phytochemistry 1998; 49: 1659-62.

[16] Dupuy OA, Bonilla JA, Murillo R, *et al.* Efecto *in vitro* de los terpenos lupeol y casearina G sobre células sanguíneas y tumorales. Rev Med Chil 2013; 141: 1150-57. http://dx.doi.org/10.4067/S0034-98872013000900007

[17] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4

[18] Bonilla JA, Mesén MG, Cartín W. Modificación de un método colorimétrico que usa XTT, para la determinación de linfoproliferación. Rev Cost Cienc Med 1993; 14: 25-31.

[19] Skehan P, Storeng R, Scudiero D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990; 82: 1107-12. http://dx.doi.org/10.1093/jnci/82.13.1107

[20] Dongmo PMJ, Tchoumbougnang F, Sonwa ET, Kenfack SM, Zollo PHA, Menut C. Antioxidant and anti-inflammatory potential of essential oils of some Zanthoxylum (Rutaceae) of Cameroon. Int J Essential Oil Ther 2008; 2: 82-8.

[21] Díaz W, Ortega F. Inventario de recursos botánicos útiles y potenciales de la cuenca del río Morón, estado Carabobo, Venezuela. Ernstia 2006; 16: 31-67.

[22] Guzik TJ, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol 2003; 54: 469-87.

[23] Bor JY, Chen HY, Yen GC. Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. J Agric Food Chem 2006; 54: 1680-6. http://dx.doi.org/10.1021/jf0527448

[24] Da Silva SL, Chaar J, Figueiredo P, Yano T. Cytotoxic evaluation of essential oil from *Casearia sylvestris* Sw on human cancer cells and erythrocytes. Acta Amaz 2008; 38: 107-12. http://dx.doi.org/10.1590/S0044-59672008000100012

[25] Ameni A, Latorre A, Torres L, Górniak S. Risk assessment of medicinal plant *Casearia sylvestris* Sw (Salicaceae). Toxicol Lett 2014; 229: S129. http://dx.doi.org/10.1016/j.toxlet.2014.06.459

Received on 19-06-2015 Accepted on 09-07-2015 Published on 13-08-2015

DOI: http://dx.doi.org/10.6000/1929-2279.2015.04.03.2