© 2012 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 11 (2): 127 - 137
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Study of the Effects of *Psidium cattleyanum* on Gene Expression from Senescent Mouse Hippocampus

[Perfil de Expresión Génica en Hipocampo de Ratones Idosos Tratados con *Psidium cattleyanum*]

Maria Rosana RAMIREZ[1]*, Nilson Ivo TONIN ZANCHIN[2]*, Amelia Teresinha HENRIQUES[1] & José Ângelo SILVEYRA ZUANAZZI[1]*

[1]Faculdade de Farmácia, UFRGS, Porto Alegre, RS.
[2]Laboratório Nacional de Biociências, CNPEM, Campinas-SP, Brazil
Contactos | Contacts: María Rosana Ramirez - E-mail address*: *mariarosanar@yahoo.com.br

Abstract
In this work, we report a characterization of gene expression profiles of mice hippocampus by use microarray after treatment with *Psidium cattleyanum* fruit (1000 mg/kg oral). After eight months, no toxic effects were detected with the supplementation. Genes differentially expressed include genes involved in a wide range of physiological functions, such as metabolism, transport, signal transduction and a group of genes whose function has not yet been identified. These results suggest that global analysis of gene expression might be useful to elucidate the mechanisms of beneficial phytochemical action and may also help to identify potential targets for further investigation.

Keywords: Araçá, hippocampus, aging, polyphenols, microarray

Resumen
El objetivo de éste trabajo fue evaluar el efecto de la administración oral prolongada (1000 mg/kg, 8 meses) del extracto de *Psidium cattleyanum* Sabine (Myrtaceae) en el perfil de la expresión génica en hipocampo de ratones idosos (Swiss). Después de 8 meses de suplementacion, no se detectaron efectos tóxicos en los animales tratados con relación al grupo control. Los genes con expresión diferencial incluyen, genes que codifican proteínas relacionadas con procesos de señalización, transcripción, metabolismo, así como genes con función desconocida. Los resultados demuestran la importancia de los microarray como herramienta para el estudio del mecanismo de acción de los compuestos fitoquímicos.

Palabras Claves: Araçá, hipocampo, envejecimiento, polifenoles, microarray

Recibido | Received: September 1, 2011.
Aceptado en versión corregida | Accepted in revised form: January 10, 2012.
Publicado en línea | Published online: March 30, 2012.
Declaración de intereses | Declaration of interests: This work was supported by CNPq and FAPESP grants.
Este artículo puede ser citado como / This article must be cited as: Maria R. Ramírez, Nilson Ivo Tonin Zanchin, Amelia T. Henriques, José Ângelo Silveyra Zuanazzi. 2012. Study of the Effects of *Psidium cattleyanum* on Gene Expression from Senescent Mouse Hippocampus. **Bol Latinoam Caribe Plant Med Aromat** 11(2): 127 – 137.

INTRODUCTION

Psidium cattleyanum (Myrtaceae), known as "strawberry guava", is a plant native to tropical forests of Central and South America. *P. cattleyanum* is a small bushy tree (3-8 m tall) and is known in Brazil by various popular names, including "araçá-rosa and araçá-da-praia". The guava fruit is a red o yellow strawberry, which consists of a fleshy pericarp and a cavity with fleshy pulp and abundant little seeds. The fruit is used in jams, jellies and juice, or are consumed fresh. The chemical composition of *P. cattleyanum* fruit has been published elsewhere (Pino *et al*., 2001, Ramirez *et al*., 2009a).

Previous studies indicated that plant belonging to the *Psidium* genus possess anti-inflammatory, analgesic, antimutagenic and central nervous system-depressant activities, (Teixeira *et al*., 2003; Begum *et al*., 2002). Pharmacological reports about *P. cattleyanum* leaves extracts can be found in the literature; this plant possesses antimicrobial (de Souza *et al*., 2004), antigenotoxic and antimutagenic properties (Costa *et al*., 2008). *P. cattleyanum* fruits extract displayed antioxidant and anti-inflammatory properties in rodents (Ramirez *et al*., 2009a; Ramirez *et al*., 2009b).

Recent literature suggests that stress oxidative and inflammation are important sources contributing to the deleterious effects of aging and the development of age-related neurodegenerative diseases, and plant food phytochemicals may exert their beneficial effects either through their capacity to lower oxidative stress and inflammation or by altering the neuroprotective stress shock proteins, plasticity, and stress signaling pathways (Bu *et al*., 2011). Suggesting that preventive use of plant product may exert protection against age-related changes in cognitive and motor function.

Therefore, in order to obtain information about the mechanism of action of *Psidium cattleyanum* fruit, on the hippocampus we investigated the effects of strawberry guava administered orally on putatively important factors in controlling the brain aging process, i.e. on gene expression profiles.

MATERIALS AND METHODS

P. cattleyanum (araçás) were produced by EMBRAPA DE CLIMA TEMPERADO, Pelotas, RS, Brazil. Chemicals and Reagents Standards were purchased from ChromaDex (USA), acetonitrile (HPLC grade) from Merck (Darmstadt, Germany) and trifluoroacetic acid (analytical grade) from Nuclear (Diadema, Brazil). RNeasy Mini Kit, from Qiagen (SP, Brazil). GeneChip from *Affymetrix* (Mouse Gene ST 1.0 Arrays) (SP, Brazil).

Compositional analyses

Determination of Total Polyphenols and Flavonoids were estimated by the method of Brazilian Pharmacopoeia (2003). Identification and quantification of anthocyanins was based on retention time and UV/VIS spectra in HPLC-DAD (Waters), by comparison with commercial standards of known concentrations (Ramirez *et al*., 2010a).

Animals

Experiments were carried out using adult male mice (12 months). All procedures were carried out according to the institutional policies on animal experimental handling designed to minimize suffering and to limit the number of animals used. Animals were subsequently divided into a control group, which drank water *ad libitum*, and a group which drank water supplemented with different concentrations of total araça extract orally for 8 months (n = 6 animals/group). For the experiments, *P. cattleyanum* juice was performed as described by Barros *et al*., (2006). The daily quantity of extract offered to the animals was calculated to provide 1000 mg/kg/day of total araçá extract (Costa *et al*., 2008).

Collection of serum and tissues

Finishing the supplementation period, all the mice were weighted and sacrificed by decapitation. For any indications of toxicity effect, vital organs such as heart, kidneys, liver and hippocampus were dissected out and weighed and their weights were compared between both treated and control groups. Blood samples from all animals were collected for separation of serum and used for cholinesterase quantification (Dietz *et al*., 1973). Hippocampus samples were kept in RNA Later (Qiagen). Total RNA was extracted using the RNeasy Mini kit (Qiagen, SP, Brazil).

RNA preparation

The transcriptional profile microarray analysis was performed with 1 μg of total poly-adenylated-mRNA (polyA+-mRNA), which was isolated from 100 μg of total RNA using the Oligotex mRNA purification system (Qiagen). Microarray experiments using GeneChip Mouse Gene ST 1.0 Arrays (Affymetrix) were performed according to the manufacturer's instructions. The stained arrays were scanned on an Affymetrix Gene Chip Scanner 3000-7G using the

Gene Chip Operating Software version 1.4. Average signal to noise normalization and background subtraction were obtained using the RMA (Robust Multichip Averaging) algorithm from the GeneSpring GX software package (Agilent Technologies, Santa Clara, CA, USA). Unpaired t-test and asymptotic p-value computation were used for the statistical analysis with a Benjamini–Hochberg correction type (GeneSpring GX,Agilent Technologies, Santa Clara, CA, USA) (Hesling *et al*., 2007). For the analysis we also used, GenBank databases at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/), BLASTN and SMART/EMBL.

RESULTS

Polyphenolic compounds and flavonoids including anthocyanins were all detected in a preliminary phytochemical screening of the dried extract of araçá fruit. *P cattleyanum* extract presented the lowest amount of total polyphenols at 500 mg/100 g of LF. In this study, all flavonoids, anthocyanins, and nonflavonoid phenolic compounds are estimated in this parameter. Total flavonoids in *P cattleyanum* samples, are 20 mg/100 g of fruit lyophilized (LF). Total anthocyanins in araçá red fruit are 8 mg/100 g of LF. Identities of main anthocyanin were confirmed in *P cattleyanum* extract, the cyanidin is the primary anthocyanin.

No toxic symptoms or mortality were observed in any animals, which lived up to 24 h after the administration of fruit extract at single dose level of 1000 mg/kg body weight. There were no significant changes in body weight between both control and treated groups. The results also revealed that, the essential organs such as kidney, liver, heart and hippocampus as well as plasma cholinesterase quantification were not adversely affected throughout the treatment ($P < 0.05$). The present results show that fruit extract of *P. cattleyanum* does not cause any apparent *in vivo* toxicity in this animal model.

Gene expression profiles of 6 treated old mice hippocampus tissues were compared to those of 6 control tissues. We identified 5000 genes that showed statistically altered expression in this tissue (unpaired T test, false discovery rate 0.05 and fold change 1), and 179 genes expressed with a fold change of ± 1.3 (unpaired T test, false discovery rate 0.05) in hippocampus treated as compared to controls. Only ten genes showed fold change greater than ± 2. Interestingly, most of these 179 genes were downregulated (128 genes) while only about 28% were upregulated.

The genes with known function in this group are listed in supplementary data (Table 1) and represent genes whose expression was significantly up/down-regulated in hippocampus following araçá treatment. In this study, there was also a larger portion of unclassified genes in hippocampus (60%, Supplemental Table 2). Determining the altered levels of expression of these genes in the old hippocampus may help identify the roles they engage in maintenance and plasticity of this tissue.

DISCUSSION

During brain aging even in the absence de neurodegenerative disease, numerous alterations develop in the physiology, biochemistry and structure of neurons and glia. Aging changes occur in the majority brain regions and, in the hippocampus, have been associated to declining cognitive performance in both humans and rodents (Wang *et al*., 2004). However, the mechanisms involved in the cognitive and behavioral deficits during aging remain to be discerned. Previous studies using genome microarrays, have identify several processes associated with brain aging, such as down-regulated structural synaptic plasticity, activity regulated signaling and transcription factors, as well as upregulated myelin turnover, cholesterol synthesis/transport, protein processing, inflammatory response and various signal transduction, among others (Blalock *et al*., 2005).

Other studies have also addressed the role of antioxidant intake in aging associated diseases. These findings concur with epidemiologic and animal studies in building up the hypothesis that high intake of specific food matrices such as polyphenolic compounds is associated with a reduced risk of stroke, cancer, cardiovascular disease, age-related degenerative diseases, brain dysfunction the leading causes of morbidity and mortality among the elderly (Bastianetto and Quirion, 2002).

In this study, a long term experiment was performed to analyze how *P cattleyanum* fruit polyphenols modulates the gene expression profile of the hippocampus. We found that 38 genes encoding for signal transduction and 29 genes related to protein synthesis (6 genes for protein degradation) were up or down-regulated by *P cattleyanum* extract. These included ribosomal proteins, Serpinb1a, PEX1, PGAP1, DIP2c and Usp47, a protein associated with regulation of many essential cellular processes usually by the degradation of regulators of these processes

such as cell cycle, proliferation, differentiation, and signal transduction among others (Inoue *et al*., 2003, Voges *et al*., 1999).

Results similar to ours have been reported for other phenolic compounds found in other vegetal

Table 1

List of genes with known function found to be differently expressed after *P cattleyanum* supplementation.

Major function
Transmembrane/signal transduction

Probe set ID	FC	Symbol	Description
Up-regulated			
10502772	2,59	Lphn2	Latrophilin 2
10489041	1,58	Pdcd10	Programmed cell death 10
10539769	1,54	fu1	NFU1 iron-sulfur cluster scaffold homolog
10357103	1,50	Cdh19	Cadherin 19, type 2
10460102	1,49	1700034H14Rik	RIKEN cDNA 1700034H14 gene
10508663	1,40	Laptm5	Lysosomal-associated prot. transmembrane 5
10594971	1,35	Unc13c	Unc-13 homolog C
10536363	1,33	Tac1	Tachykinin 1
10386821	1,33	A530017D24Rik	RIKEN cDNA A530017D24
10599884	1,33	Slitrk2 SLIT and NTRK-like family	Uknown
10571829	1,32	Glra3	Glycine receptor, alpha 3 subunit
10519527	1,32	Abcb1a	ATP-binding cassette, (MDR/TAP)
10601473	1,31	Apool	Apolipoprotein O-like
10603833	1,31	Usmg5	up regulated during skeletal muscle growth 5
10607792	1,31	Glra2	Glycine receptor, alpha 2 subunit
10530393	1,31	Gabrg1	Gamma-aminobutyric acid receptor
10498710	1,31	Bche	Butyrylcholinesterase
Down-regulated			
10479973	2,16	ENSMUSG00000062319	Uknown
10529953	1,76	LOC625026	Uknown
10506431	1,62	ENSMUSG00000070886	Uknown
10352916	1,44	C030002C11Rik	RIKEN cDNA D930030D11
10373610	1,43	Olfr767	Olfactory receptor 767
10436658	1,37	ENSMUSG00000074937	Uknown
10432263	1,36	5830427D03Rik	RIKEN cDNA 5830427D03 gene
10484733	1,36	Olfr1174	Olfactory receptor 1174
10586718	1,35	9530091C08Rik	RIKEN cDNA 9530091C08 gene
10373692	1,35	Vmn2r87	Vomeronasal 2, receptor 87
10477001	1,35	LOC675602	Similar to Glyceraldehyde-3-phosphate dehydrogenase
10414920	1,34	Trav7d-2	T-cell receptor alpha variable region 7D-2
10469577	1,34	ENSMUSG00000066088	Uknown
10506452	1,34	ENSMUSG00000063691	Uknown
10583203	1,33	Phxr4	Per-hexamer repeat gene 4
10354816	1,32	Clk1	CDC-like kinase 1 (encodes a member of the CDC2-like)
10459602	1,32	Ptpn2	Protein tyrosine phosphatase non-receptor type 2
10474369	1,32	C130023O10Rik	RIKENcDNA C130023O10

10423379	1,31	Tiaf2	TGF-beta1-induced anti-apoptotic factor 2
10549615	1,31	Leng8	Leukocyte receptor cluster (LRC) member 8
10504201	1,31	4933409K07Rik	ENSMUSG00000073868RIKENcDNA 4933409K07

Metabolism:
Protein synthesis

Up-regulated

10356269	2,15	A530032D15Rik	Uknown
10603706	1,92	LOC668990	Similar to vitamin D receptor interacting protein
10572730	1,46	Zfp617	Zinc finger protein 617
10435980	1,46	Rps24|LOC677113	Ribosomal protein S24 | similar to ribosomal protein S24
10601519	1,42	Klhl4	Kelch-like 4 (Drosophila)
10596259	1,41	Dnajc13	DnaJ (Hsp40) homolog, subfamily C, member 13
10569069	1,40	LOC546015	Similar to ribosomal protein S9
10466923	1,38	Rpl26|LOC672214|LOC100034726	Ribosomal protein L26 pseudogene
10548976	1,38	Rpl38|LOC638514|EG664868	Ribosomal protein L38 | similar to ribosomal Protein L38 | predicted gene, EG664868
10503212	1,37	Chd7	Chromodomain helicase DNA binding protein 7
10502029	1,37	Larp7	Ribonucleoprotein domain family, member 7
10553537	1,37	Luzp2	Leucine zipper protein 2
10349049	1,34	Zfp706	Zinc finger protein 706
10549276	1,35	Bhlhb3	Basic helix-loop-helix domain containing, class B3
1047893	1,35	Teashirt zinc finger family member 2	Nuclear, zinc ion binding**,** transcription factor
10527886	1,33	Gatad1	GATA zinc finger domain containing 1
10366186	1,32	Ccdc59	Coiled-coil domain containing 59
10518833	1,31	Camta1	Calmodulin binding transcription activator 1
10476590	1,31	Macrod2	MACRO domain containing 2
10486041	1,30	Meis2	Meis homeobox 2
10499121	1,30	Rps3a	Ribosomal protein S3a

Down-regulated

10479971	1,70	ENSMUSG00000075538	Uknown
10398362	1,51	Rian	RNA imprinted and accumulated in nucleus
10446617	1,45	XR_033984	Similar to ribosomal protein L7a
10514070	1,39	2310067E19Rik	RIKEN cDNA 2310067E19 gene
10478066	1,39	A930034L06Rik	RIKEN cDNA A930034L06 gene
10398326	1,38	Meg3	Maternally expressed 3
10544640	1,38	Tra2a	Transformer 2 alpha homolog (Drosophila)
10428002	1,31	5730557B15Rik	RIKENcDNA 5730557B15 gene

Protein Degradation

Up-regulated

10408557	1,43	Serpinb1a	Serine (or cysteine) peptidase inhibitor, clade B, member 1a
10357300	1,34	Dpp10	Dipeptidylpeptidase 10
10556350	1,30	Usp4	Ubiquitin specific peptidase 47

Down-regulated

10586967	1,41	EG639396	Uknown
10435501	1,41	Stfa1|Stfa3	Cysteine-type endopeptidase inhibitor activity
10557311	1,38	ENSMUSG00000066185	Uknown

Response to oxidative stress

Up-regulated			
10350516	1,40	Ptgs2	Prostaglandin-endoperoxide synthase 2
10494428	1,40	Txnip	Thioredoxin interacting protein
10450372	1,38	Hspa1l	Heat shock protein 1-like
Down-regulated			
10584578	1,59	Hspa8	Heat shock protein 8
10450369	1,32	Hspa1a	Heat shock protein 1A

Other Metabolic processes

Up-regulated			
10354647	1,37	Pgap1	Post-GPI attachment to proteins 1
10527886	1,33	Pex1	Peroxisomal biogenesis factor 1
10403464	1,30	Dip2c	DIP2 disco-interacting protein 2 homolog C (Drosophila)

List of genes with known function differently expressed in the hippocampus of old mice subjected relative to treated animals, simply left on their cage during the experimental period, as indicated by high-density microarray analysis (GeneChip® Mouse Gene ST 1.0 Arrays Affymetrix Inc., Santa Clara, CA, USA). Statistical analysis based on present call in all array sets, > 1.3-fold change (FC), *t*-test $p < 0.05$ with a Benjamini–Hochberg correction type (GeneSpring GX, Agilent Technologies, Santa Clara, CA, USA).

species, including berries containing quercetin, resveratrol and some anthocyanins (Wung *et al*., 2005; de Boer *et al*., 2006; Fujishita *et al*., 2009), which would be consistent with the proposed role of polyphenols in neuroprotective and neurorestorative signal transduction mechanisms (Williams *et al*., 2004). However, more information is needed before any suggestions regarding functional effects can be made.

Further, both *in vitro* and *in vivo* models have demonstrated that polyphenolic compounds can inhibit the inflammatory process. In general the anti-inflammatory effects of polyphenols appear to regulate signal transduction processes/transcription factors implicated in the regulation of inflammatory genes (Fujishita *et al*., 2009). In this study significant changes were recorded in the LAPTM5, Ptgs2 and Serpinb1a inhibitor. Similarly to other reported experiments, we found a up-regulation of various genes encoding for proteins involved in radical scavenging (Txnip, Hspa, Lphn2), possibly as part of a coordinated attempt to overcome the damaging effects of several changes associated with brain aging, such as inflammatory and oxidative stress process (Wung *et al*., 2005, Patwari *et al*., 2009, Ramirez *et al*., 2010b).

The list of differentially expressed genes includes also Tac1, Glycine and GABA-A receptor, Cadherins and PDCD10 among others. Genes that are highly conserved in evolution may be required for viability or other essential functions of the cell (Qi *et al*., 2003). In addition, we identified various genes that have not been characterized in mammals yet, which have highly conserved homolog in non-mammalian organisms and show higher levels of expression in the treated hippocampus as compared to controls (Klhl4, DIP2c, fu1, unc-13, and Abcb1a). Consistent with their high conservation, these genes encode proteins of important biological functions, such as transcription, signal transduction, and intercellular communication among others (Brose *et al*., 1995; Liang *et al*., 2006). Expression profiles of these genes in the hippocampus of old mice treated, along with their possible cellular functions open up new ways for studying the functions of these evolutionarily conserved genes.

In conclusion, the reported array expression pattern in hippocampus of mice after treatment with *P cattleyanum* point in the same direction as effects described in available literature reports, but also has specific characteristics possibly related to the chemical profile of extract. However, the gene expression data obtained by microarray experiments are preliminary finding and allow us to have a general view of the putative genes whose expression is altered. These results should be confirmed by real-time PCR in order

to have more precise data that allow us to identify changes in the expression of genes whose products are involved in the brain aging process.

ACKNOWLEDGEMENTS

We thank EMBRAPA (Pelotas, RS) and Roselaine Santos de Quadros, Adriana C. Alves, Tereza C. Lima Silva, for technical assistance. This work was supported by CNPq and FAPESP grants.

REFERENCES

Barros DM, Amaral OB, Izquierdo I, Geracitano L, Raseira MCB, Henriques, AT, Ramirez MR 2006. Behavioral and genoprotective effects of *Vaccinium* berries intake in mice. **Pharmacol Biochem Behav** 84: 229 - 234.

Bastianetto S, Quirion R. 2002. Natural extracts as possible protective agents of brain aging. **Neurobiol Aging** 23: 891 - 897.

Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, Gilani AH. 2002. Triterpenoids from the leaves of *Psidium guajava*. **Phytochemistry** 61: 399 - 403.

Blalock EM, Chen KC, Stromberg AJ, Norris CM, Kadish I, Kraner SD, Porter NM, Landfield PW. 2005. Harnessing the power of gene microarrays for the study of brain aging and Alzheimer's disease: Statistical Reliability and Functional Correlation. **Ageing Res Rev** 4: 481 - 512.

Brazilian Pharmacopoeia 5 fasciculo 2003. CD ROM.

Brose N, Hofmann K, Hata Y, Sudhof TC. 1995. Mammalian homologues of *Caenorhabditis elegans* unc-13 gene define novel family of C2-domain proteins. **J Biol Chem** 270: 25273 - 25280.

Bu M, Sanchez N, Perez-Saad H, Lara G, Scull I. 2011**.** Efecto neurofarmacológico del zumo de *Morinda citrifolia*. **Bol Latinoam Caribe Plants Med Aromat** 10: 159 - 166.

Costa TDA, Vieira S, Andrade SF, Maistro EL. 2008. Absence of mutagenicity effects of *Psidium cattleyanum* Sabine (Myrtaceae) extract on peripheral blood and bone marrow cells of mice. **Genet Mol Res** 7: 679 - 686.

de Boer VC, van Schothorst EM, Dihal AA, van der Woude H, Arts IC, Rietjens IM, Hollman PC, Keijer J. 2006. Chronic quercetin exposure affects fatty acid catabolism in rat lung. **Cell Mol Life Sci** 63: 2847 - 2858.

de Souza GC, Haas AP, von Poser GL, Schapoval EE, Elisabetsky E. 2004. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. **J Ethnopharmacol** 90: 135 - 143.

Dietz AA, Rubinstein HM, Lubrano T. 1973. Colorimetric determination of serum cholinesterase and its genetic variant by the propionylthiocholine-dithiobis (nitrobenzoic acid) procedure. **Clin Chem** 19: 1309 - 1313.

Fujishita K, Ozawa T, Shibata K, Tanabe S, Sato Y, Hisamoto M, Okuda T, Koizumi S. 2009. Grape seed extract acting on astrocytes reveals neuronal protection against oxidative stress via Interleukin-6-mediated mechanisms. **Cell Mol Neurobiol** 29: 1121 - 1129.

Hesling C, Oliveira CC, Castilho BA, Zanchin NIT. 2007. The Shwachman–Bodian–Diamond syndrome associated protein interacts with HsNip7 and its down-regulation affects gene expression at the transcriptional and translational levels. **Exp Cell Res** 313: 4180 - 4195.

Inoue H, Jiang XF, Katayama T. 2003. Brain protection by resveratrol and fenofibrate against stroke requires peroxisome proliferator activated receptor alpha in mice. **Neurosci Lett** 352: 203 - 206.

Liang DY, Liao G, Lighthall GK, Peltz G, Clark DJ. 2006. Genetic variants of the P glycoprotein gene Abcb1b modulate opioid-induced hyperalgesia, tolerance and dependence. **Pharmacogenet Genomics** 16: 825 - 835.

Patwari P, Higgins LJ, Chutkow WA, Yoshioka J, Lee RT. 2006. The Interaction of thioredoxin with Txnip: Evidence for formation of a mixed disulfide by disulfide exchange. **J Biol Chem** 281: 21884 - 21891.

Pino JA, Marbot R, Vazquez C. 2001. Characterization of volatiles in strawberry guava (*Psidium cattleianum* Sabine) Fruit. **J Agric Food Chem** 49: 5883 - 5887.

Qi SY, Riviere PJ, Trojnar J, Junien JL, Akinsanya KO. 2003. Cloning and characterization of dipeptidyl peptidase 10, a new member of an emerging subgroup of serine proteases. **Biochem J** 373: 179 - 189

Ramirez MR, Zanchin NI, Henriques AT, Zuanazzi JA. 2009a. Composición química y actividad antioxidante de frutas de *Psidium Cattleyanum* Sabine e *Eugenia Pyriformis* Cambess. **Summaries of the Congreso Argentino de**

Nutrición, Mar Del Plata, Buenos Aires, Argentina.

Ramirez MR, Zanchin NI, Zuanazzi JA, Henriques AT. 2009b. Phenolic compounds: further investigation of its antichemotactic properties. Summaries of the **XVIII Congresso Ítalo-Latinoamericano de Etnomedicina**, SILAE. La Habana, Cuba.

Ramirez MR, Apel M, Zuanazzi SJA, Henriques AT. 2010a. Polyphenols content and evaluation of antichemotactic, antiedematogenic and antioxidant activities of *Rubus* sp. cultivars. **J Food Biochem** 35: 1389 - 1397.

Ramirez MR, Guterres L, Dickel OE, de Castro MR, Henriques AT, de Souza MM, Marti-Barros D. 2010b. Preliminary studies on the antinociceptive activity of *Vaccinium ashei* berry in experimental animal models. **J Med Food** 13: 1 - 7.

Teixeira RO, Camparoto ML, Mantovani MS, Vicentini VEP. 2003. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* assays. Genet. **Mol Biol** 26: 551 - 555.

Tsuda T, Ueno Y, Aoki H. 2004. Anthocyanin enhances adipocytokine secretion and adipocyte specific gene expression in isolated rat adipocytes. **Biochem Biophys Res Commun** 316: 149 - 157.

Voges D, Zwickl P, Baumeister W. (1999). The 26S proteasome: a molecular machine designed for controlled proteolysis. **Annu Rev Biochem** 68: 1015 - 1068.

Wang Z, Du Q, Wang F, Liu Z, Li B, Wang A, Wang Y. 2004. Microarray analysis of gene expression on herbal glycoside recipes improving deficient ability of spatial learning memory in ischemic mice. **J Neurochem** 88: 1406 - 1415.

Williams RJ, Spencer JP, Rice-Evans C. 2004. Flavonoids: antioxidants or signaling molecules? **Free Radical Biol Med** 36**:** 838 - 849.

Wung BS, Hsu MC, Wu CC, Hsieh CW. 2005. Resveratrol suppresses IL-6-induced ICAM-1 gene expression in endothelial cells: effects on the inhibition of STAT3 phosphorylation. **Life Sci** 78: 389 - 397.

Supplemental Table 2
List of genes unknown found to be differently expressed after araçá supplementation.

a) Genes partially unknown with homology with other vertebrates

ID	FC	Symbol	Homology
Up-regulated			
10350590	1,36	EG625534	Homo sapiens, chimpanzee, cow, rat, chicken, zebrafish
10373452	1,36	Gm129	Homo sapiens, Canis familiaris, Rattus norvegicus, Cow Equus caballus
10598018	1,34	Transmembrane	Mus musculus castaneus

ID	FC	Probable Function	Homology
Down-regulated			
10367475	1,67	Extracellular signaling	Rattus norvegicus
10504912	1,59	Transmembrane	Tupaia belangeri, Canis Familiaris, Homo sapiens, Rattus norvegicus, Pan troglodytes, Pig, Papio anubis
10435787	1,53	Signal transduction	Homo sapiens, Pig, Tupaia belangeri, Pan troglodytes
10385234	1,50	Transmembrane	Homo sapiens, Rat, Zebrafish
10492165	1,49	Ubiquitin proteolytic	Homo sapiens, Tupaia belangeri, Bos taurus, Rattus norvegicus, Zebrafish, Pig
10408898	1,48	Microtubule-based	Homo sapiens Tupaia belangeri, Bos taurus, Felis catus Canis familiaris, Rattus norvegicus, Zebrafish, Pig, Equus caballus, Pongo abelii,
10352926	1,48	Transmembrane	Rattus norvegicus, sapiens, Monodelphis domestica
10360542	1,46	Transmembrane	Rattus norvegicus Feliscatus,Homo sapiens
10349510	1,45	Regulation the actin cytoskeleton	Pan paniscus, Pan troglodytes, Macaca mulatta,

			Zebrafish, Danio rerio, Homo sapiens
10479975	1,44	Posttranscriptional regulation	Rat, Homo sapiens, Canis familiaris, Pig, Gallus gallus, Felis catus, Xenopus laevis, Pan troglodytes
1047629	1,43	Biosynthesis of coenzyme A	Homo sapiens, Zebrafish, Bos taurus , Gallus gallus
10480314	1,43	Cytoskeletal activities, cell motility	Pan troglodytes, Homo sapiens, Rattus norvegicus, Ornithorhynchus anatinus
10549445	1,43	Ribosomal protein	Ovis áries, Pongo abelii, Homo sapiens
10422942	1,42	Transmembrane	Homo sapiens, Tupaia belangeri, Canis familiaris, Rattus norvegicus , Pan Troglodytes, Pig, Rat
10454731	1,40	Sphingolipid biosynthesis	Homo sapiens, Macaca mulatta, Pan troglodytes, Zebrafish, X.laevis
10350853	1,39	Detoxifying enzymes	Rattus norvegicus, Canis familiaris, Pan troglodytes, Homo sapiens
10564209	1,38	Small nucleolar RNA	Homo sapiens
10492888	1,36	Small nucleolar RNA	Homo sapiens, Rattus norvegicus, Pan troglodytes, Ornithorhynchus anatinus
10415013	1,35	Transmembrane	Drosophila erecta, Ginglymostoma cirratum, Bos Taurus, Rhesus Macaque, Monodelphis domestica, Macaca mulatta ,Pongo abelii , Xenopus laevis
10470444	1,35	Pro-nociceptive	Rattus norvegicus
10500089	1,34	Transmembrane	Ornithorhynchus anatinus, Homo sapiens, Pig
10555718	1,34	Transmembrane	Rattus norvegicus, Equus caballus , Canis familiaris, Bos taurus, Macaca mulatta, Alouatta caraya,Pan troglodytes
10500089	1,34	Transmembrane	Ornithorhynchus anatinus, Homo sapiens, Pig
10555718	1,34	Transmembrane	Rattus norvegicus, Equus caballus, Canis familiaris, Bos taurus, Macaca mulatta, Alouatta caraya, Pan troglodytes
10554468	1,34	Glycine hydroxymethyltransferase	Equus caballus, Homo sapiens, Macaca fascicularis, Macaca mulatta, Rattus norvegicus, Bos taurus, Pongo abelii, Canis familiaris
10398344	1,33	Retrotransposon-like	Rattus norvegicus, Equus caballus, Pan troglodytes, Canis familiaris, Pongo pygmaeus, Bos taurus, Macaca nemestrina, Macaca mulatta, Homo sapiens
10394344	1,33	Expressed in fetal brain	Xenopus laevis, Equus caballus, Rattus norvegicus, Chinese hamster, Meriones unguiculatus
10463643	1,33	Detoxification	Rattus norvegicus, Pan troglodytes, Homo sapiens
10438169	1,32	Regulation of transcription	Canis familiaris, Homo sapiens, Equus caballus, Pongo abelii, Bos taurus, Felis catus, Rattus norvegicus, Tupaia belangeri, Canis Familiaris
10592531	1,32	Vasoactive peptides	Pan troglodytes, Macaca mulatta, Pongo abelii, Rhesus Macaque, Homo sapiens
10470909	1,31	Transmembrane	Macaca Mulatta, Homo sapiens, Rattus norvegicus, Pig, Zebrafish, Canis familiaris, Rhesus Macaque, Monodelphis Domestica

b) Genes unknown with homology with nonvertebrate animal

ID	**FC**	**Probable Function**	**Homology**
Down-regulated			
10582888	3,68	TRANSMEMBRANE	Plasmodium berghei, Plasmodium yoelii yoelii
10582916	3,21	Transmembrane	Plasmodium berghei, Plasmodium yoelii yoelii, Plasmodium chabaudi, Schistosoma mansoni

10582896	3,20	Transmembrane	Plasmodium	berghei,	Plasmodium	yoelii	yoelii,	Plasmodium chabaudi, Schistosoma mansoni
10582890	2,85	Transmembrane	Plasmodium	berghei,	Plasmodium	yoelii	yoelii,	Plasmodium chabaudi, Schistosoma mansoni
10582882	2,83	Transmembrane	Plasmodium	berghei,	Plasmodium	yoelii	yoelii,	Plasmodium chabaudi, Schistosoma mansoni
10485357	2,53	Transmembrane	Plasmodium	berghei,	Plasmodium	yoelii	yoelii,	Plasmodium chabaudi, Schistosoma mansoni
10582899	2,23	Transmembrane	Plasmodium	berghei,	Plasmodium	yoelii	yoelii,	Plasmodium chabaudi, Schistosoma mansoni
10582884	1,66	Transmembrane	Plasmodium	berghei,	Plasmodium	yoelii	yoelii,	Plasmodium chabaudi, Schistosoma mansoni

c) ***Genes unknown and without homology***

ID	**FC**	**Probable Function**
Down-regulated		
10435765	1,78	Transmembrane
10551250	1,33	Modulates neuron-specific apoptosis
10389625	1,55	Negative regulators of cell stress response pathways
10602221	1,46	Cell-cell interactions
10537880	1,45	Transmembrane
10548661	1,44	Transmembrane
10394850	1,40	Negative Cell Cycle Regulator, oxidation of LDL
10425761	1,39	Trasmembrane
10562729	1,35	Trasmembrane
10357001	1,34	Trasmembrane
10510872	1,33	Regulation of signal transduction pathways
10410654	1,32	Arachidonic acid metabolism. Linoleic acid metabolism
10506296	1,32	Regulate molecular chaperone activity by stimulating ATPase activity
10562397	1,32	Protein binding
10595791	1,59	Detection of odorant molecules

d) Genes with function Unknown and with homology with other vertebrates

ID	**FC**	**Homology**
Down-regulated		
10564157	1,93	Pongo abelii, Homo sapiens
10529953	1,76	Rattus norvegicus
10438813	1,75	Rattus norvegicus
10435767	1,75	Homo sapiens
10398418	1,48	Homo sapiens
10580953	1,46	Homo sapiens
10555009	1,46	Homo sapiens
10398390	1,44	Homo sapiens, Bos taurus
10447617	1,40	Rattus norvegicus
10398432	1,40	Homo sapiens
10398414	1,40	Homo sapiens
10398384	1,38	Homo sapiens
10432471	1,37	Rattus norvegicus, Tupaia belangeri, Canis familiaris, Pig, Pan troglodytes, Homo sapiens
10564203	1,36	Homo sapiens

10398416	1,36	Homo sapiens
10436594	1,35	Pan troglodytes, Homo sapiens
10398440	1,34	Homo sapiens
10398428	1,34	Pan paniscus, Macaca nemestrina, Pongo pygmaeus, Gorilla gorilla, Pan troglodytes
10398400	1,33	Homo sapiens
10415873	1,33	Gallus gallus
10416271	1,31	Homo sapiens
10398420	1,31	Pan paniscus, Macaca nemestrina, Gorilla gorilla, Pongo pygmaeus
10560399	1,31	Canis familiaris, Homo sapiens, Rattus norvegicus, Pan troglodytes, Pig
10563929	1,33	Pongo abelii, Homo sapiens

e) Genes Completely Unknown

ID	**FC**
Up-regulated	
10424555	1,36
Down-regulated	
10433428	1,84
10578681	1,76
10564235	1,64
10564233	1,62
10398396	1,55
10398364	1,54
10398360	1,53
10398356	1,51
10398388	1,51
10398378	1,49
10394829	1,47
10398368	1,46
10398392	1,46
10469243	1,44
10398354	1,42
10478744	1,42
10398366	1,40
10398338	1,40
10563899	1,38
10398358	1,36
10606257	1,35
10412699	1,34
10398350	1,33
10398404	1,33
10400004	1,31
10398408	1,31

List of unknown genes differently expressed in the hippocampus of old mice subjected relative to treated animals, as indicated by high-density microarray analysis (GeneChip® Mouse Gene ST 1.0 Affymetrix Inc., Santa Clara, CA, USA). Statistical analysis based on present call in all array sets, > 1.3-fold change (FC), *t*-test $p < 0.05$ with a Benjamini–Hochberg correction type (GeneSpring GX,Agilent Technologies, Santa Clara, CA, USA).