



**3^{er} Simposio
FRAG Chile
2013**

**ESPECIES REACTIVAS Y SU RELACION CON
PRODUCTOS NATURALES, ANTIOXIDANTES POLIFENOLICOS,
MENBRANAS BIOLÓGICAS, OXIDACION DE FARMACOS
Y MODELAMIENTO MOLECULAR.**

**Auditorium Jaime Baeza Hernández,
Edificio Emprende UdeC,
Universidad de Concepción,
Concepción.**

Viernes 06 de Diciembre, 2013.

Free Radicals and Antioxidants Group, Chile
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3^{er} Simposio

FRAG Chile

2013

COMITÉ FRAG

Dr. Edgard Pastene	Universidad de Concepción
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Dr. Eduardo Pino	Universidad de Santiago
Dra. Margarita E. Aliaga	Pontificia Universidad Católica
Dr. Felipe Simon	Universidad Andrés Bello
Dr. Rodrigo López	Universidad de Chile

PROGRAMA:

09:00 - 09:30 hrs:

Inscripción y bienvenida (Dra. Victoria Velarde)

9:30 – 10:30 hrs:

Dr. Mario Suwalsky (Radicales libres y daño a membranas biológicas)

10:30 - 11:30 hrs:

Dr. Hector Mancilla (Eliminación de residuos peligrosos empleando radicales libres generados por radiación visible)

11:30 - 12:00 hrs: Café

12:00 - 13:00 hrs:

Dr. Carlos Cespedes (Antioxidant Natural Compounds can play a role as a model for search of new botanical pesticides)

13:00 - 15:00 hrs: Tiempo para Almuerzo

15:00 – 16:00 hrs:

Mesa redonda 1:

Dra. Marcia Abello ("Efecto antioxidante de polifenoles en seres humanos: Evidencia clínica, significado y estrategias experimentales").

Dra. Catalina Carrasco (Modelos celulares y animales para evaluar efectos protectores de antioxidantes polifenólicos)

16:00 – 17:00 hrs:

Mesa redonda 2:

Dr. David Contreras (Modelamiento, mediante métodos quimiométricos, de la modulación de la reacción de Fenton por ligandos de hierro del tipo 1,2-dihydroxybenzenos y sus aplicaciones como procesos de oxidación avanzada)

Dr. Claudio Aguayo (Especies Reactivas y disfunción endotelial)

17:00 – 19:00 hrs: Con Coffee

POSTER

ANTIOXIDANT ACTIVITY OF SKYTANTUS ACUTUS EXTRACTS.

Aballay A.¹, Paz C.², Fernández M.², Ulloa V.¹, Sepulveda B.³, Astuya A.¹ (1) Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences. (2) Laboratory of chemistry natural products, University of Concepcion. (3) University of Atacama, Copiapo. aastuya@udec.cl

The aim of this study was to compare the antioxidant activity of acidic, basic, and syringetin extracts from *Skytanthus acutus*, an endogenous plant from the north of Chile and Argentina. The antioxidant activity was determined by DPPH and DCFH-DA assays. Prior to DCFH-DA assay the cytotoxic potential of extracts was evaluated by MTT assay. DPPH colorimetric assay was used to measure the antiradical activity at different concentrations of the extracts (0,4-200µg/ml). The intracellular antioxidant activity was evaluated using Neuro2a cells with DCFH-DA fluorescent probe, by measuring the production of intracellular ROS when the cells are exposed to 100 µM H₂O₂. Quercetin extract from *Aristotelia chilensis* was used as a positive control. The results showed that acidic extract from *Skytanthus acutus* had the highest intracellular antioxidant activity by reducing 45% the intracellular ROS production at 50µg/ml and showed 90% of antiradical activity at the same concentration. The syringetin compound was isolated from the acidic extract, and presented a reduction of 10% of intracellular ROS production and only 25% of antiradical activity at 50µg/mL. Finally, the basic extract showed a reduction of 25% of intracellular ROS production and 20% of antiradical activity at 50µg/mL. The difference between the acidic extract and Syringetin could be explained by a synergistic effect of the other molecules present in the acidic extract. Regarding the antiradical activity measured in the DPPH assay, the results are consistent with those found with the cell assay.

Acknowledgment. Project FICETA and University of Concepción.

ANTIOXIDANTE ACTIVITY OF BIOACTIVE EXTRACTS OBTAINED FROM CYPERUS DIGITATUS.

Forero-Doria O., Fuentes E., Castro R., Astudillo L., Gutiérrez M., Salgado M., Palomo I., Guzmán-Jofre L., Universidad de Talca, Instituto de Química de Recursos Naturales, Talca, Chile. oforero@utalca.cl

Members of the family Cyperaceae such as *C. alopecuroides*, *C. articulatus*, *C. scariosus* and *C. rotundus* possess significant amount of studies about their antioxidant activities and other properties. Nevertheless, the plant *C. digitatus* belonging to the genus *Cyperus* lacks of studies about any kind of intrinsic activity. That is why, we made different extracts and fractions of this plant and a phytochemical screening and the antioxidant property was studied. Different extracts and fractions were obtained from the rhizomes of *C. digitatus*, and a Phytochemical screening and the content of phenols and flavonoids were quantified in each of the extracts and fractions, also we studied the antioxidant activity on different in vitro models like 1,1-diphenyl-picryl hydrazyl (DPPH) Assay, Ferric Reducing Antioxidant Power (FRAP) assay and β-Carotene bleaching assay. Of all the extracts obtained, the butanol and aqueous extracts showed the best antioxidant potential, meanwhile, none of the fractions obtained from the ethyl acetate extract show a relevant activity. Antioxidant potential might be helpful in preventing the progress of various oxidative stress related disorders.

Acknowledgment. This work was funded by the CONICYT REGIONAL / GORE MAULE / CEAP / R09I2001.

GASTROPROTECTIVE AND ANTIOXIDANT ACTIVITY OF MERODITERPENOIDIS ISOLATED FROM STYPOPODIUM FLABELLIFORME.

Areche C., Sepulveda B., Gutierrez M. Universidad de Chile, Facultad de Ciencias, Santiago, Chile. areche@uchile.cl

Natural products from marine environments have been increasingly used in human health. Due to low temperatures and light availability and high pressure, among others, seaweeds respond by producing secondary metabolites with potential pharmacological activities. **Objectives:** to isolate epitaondiol **1**, isoeptaondiol **2** and taondiol **3** from the brown alga *S. flabelliforme* and evaluate their gastroprotective and antioxidant activity. **Results:** The compounds **1-3** at 50 mg/kg showed gastroprotective activity in the same range (60-70%) and it was similar to that observed with lansoprazole at 20 mg/kg (61%) on gastric lesions induced by HCl/EtOH in mice. However, in DPPH studies some differences were observed. Epitaondiol, isoeptaondiol and taondiol showed an IC₅₀ (µM) 0.81, 0.62 and 0.37 respectively. In this work, we will discuss the gastroprotective and antioxidant activity of these compounds.

Acknowledgment. Financial support came from Fondecyt Iniciación N° 11110241.

VASCULAR PROTECTION INDUCED BY GRAPE EXTRACTS IS RELATED WITH IMPROVED LEVELS OF NITRIC OXIDE IN HUMAN ENDOTHELIAL CELLS.

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Monomeric and polymeric proanthocyanidins (PAs) from seeds and skins of grapes have been associated with a decreased of angiotensin I-converting enzyme activity *in vitro*. We evaluated the effects of PAs with different molecular size present in both seeds and skins of *Pais* grape, on cell viability, reactive oxygen species (ROS) and nitric oxide (NO) levels in human umbilical vein endothelial cells (HUVECs).

Extracts purification was performed in a medium size exclusion (Toyopearl HW-40 F) to obtain rich PAs fractions of both seeds and skins *Pais* grapes (Quillón, Chile), which were submitted to degradation by acid catalysis in the presence of phloroglucinol and subsequent analysis by reverse-phase high-performance liquid (RP-HPLC). For different concentrations of extracts, viability and baseline measurement of ROS and NO of these fractions were carried out in HUVECs. A total of 9 purified fractions (F1S to F6S from seeds and F4P to F6P from skins) were obtained and characterized.

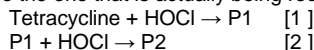
The results showed that monomeric and polymeric PAs fractions had a degree of polymerization (mDP) ranging from 2 to 18 and 6 to 45, in seeds and skins; respectively. The extracts did not cause significant increases in ROS and induced significant increases in the levels of endothelial NO. Skin polymeric fractions (F5P and F6P) could have the best vasoprotective effect with a mechanism that could be related with moderate levels of ROS and increased NO synthesis in HUVECs. This suggests that F5P and F6P might be of interest to be used as alternative antioxidant and hypertensive.

Acknowledgments. Supported by Fondecyt N°1120148 & Fondecyt N°11100192

CONSUMPTION OF TETRACYCLINE AND OMEPRAZOL INDUCED BY HYPOCHLOROUS ACID.

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The reaction between Tetracycline and Omeprazole with HOCl and the effect that polyphenols have in it, was studied. The study estimated the consumption of the drugs, mediated by reactive oxygen species in the presence and absence of antioxidants. The addition of HOCl generated drug consumption thereof, resulting in the decrease in intensity of corresponding characteristic bands. Large differences were found when comparing reaction rates. Showing that the HOCl - Tetracycline interaction is much faster than HOCl - Omeprazole where obtaining products depends on the reaction time and concentration of HOCl provided. The behavior described for each drug was confirmed by HPLC, confirming the appearance of a reaction product associated with tetracycline and two for omeprazole. It was found that for tetracycline reaction stoichiometry obeys 1:2 (tetracycline:HOCl, respectively). By stopped-flow method, it was demonstrated that the reaction is given in two steps. First a product P1 is generated, which reacts immediately with HOCl leading to a product P2 (equations 1 and 2) that would be the one that is actually being recorded.



Finally is noted that, in the presence of phenol, the consumption of tetracycline is inhibited. These results would be an indication of the beneficial role of antioxidants against phenomena associated with gastric ulcer.

Acknowledgements. FONDECYT (project 1100659), VRI, Pontificia Universidad Católica (doctoral scholarship J. Benavides) and CONICYT doctoral scholarship – AGCI

NUTRITIONAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF *Macleania rupestris* (joyapa) AND *Vaccinium floribundum* kunth (mortiño).

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Several endemic fruits grow in wild state, and have been used since our ancestors for food and ceremonials. Species are two plants in the family Ericaceae, species usually grow in the Sierra in the wild, whose edible fruits have been used since ancient times in Ecuador for the preparation of traditional foods consumed fresh.

The nutritional potential and biological activity of this species is a main field of study, due to scarce studies of plants as joyapa (*Macleania rupestris*) and mortiño (*Vaccinium floribundum* kunth).

The present work aimed to evaluate the antioxidant activity using DPPH assays and present the nutritional characterization of the included species, by means of the phenolic content, humidity ashes and ascorbic acid concentration. Species were collected in the Province of Azuay Ecuador and processed in the laboratory of Natural Product Biotechnology, University of Azuay. These parameters bromatological were related with the antioxidant activity of the studied fruits. To our best knowledge, this is the first report of nutritional parameters and biological activity of *Macleania rupestris*.

VITIS VINIFERA CANES, A PROMISING SOURCE OF *trans*-RESVERATROL AND OTHER STILBENES: EFFECT OF POST-PRUNING STORAGE. Gorena T., von Baer D., Mardones C, Sáez V, Vergara C. Univ. Concepción, Facultad de Farmacia, Departamento de Análisis Instrumental, Concepción, Chile. tgorena@udec.cl

The main aim of this research was to determine the effect of post-pruning storage on stilbene levels in grape cane. Stilbenes were extracted with an ethanol/water mixture 80:20 (v/v), using an ultrasonic bar for 5 min. Then, the extracts were analyzed by HPLC-DAD-ESI-MS/MS. In all samples, the predominant stilbene was *trans*-resveratrol, followed by ϵ -viniferin. In whole canes (cv. Pinot Noir) stored after pruning at room temperature, stilbenes presented a significant increase after two months. The yield raised up 5.2 times, reaching 4.777 mg/kg dry matter. This effect does not occur in frozen, lyophilized or milled material. Branches obtained directly from the plants and remaining on the field without storage after pruning, showed no significant change in stilbene levels, containing only 611 mg kg⁻¹ dry matter.

One possible explanation is that storage at room temperature and injury caused by pruning triggers stilbene biosynthesis, but the involved biochemical mechanism is still unknown for grape cane. Another alternative is that in fresh non-aged grape cane stilbenes are bound to other components of canes and thus are less extractable. In both cases, storage of whole canes at room temperature for at least 3 months is advisable to increase the extraction yield.

Acknowledgments. FONDECYT 1110767 and CONICYT Doctoral Fellowship

CONSUMPTION OF TRYPTOPHAN INDUCED BY AZO-COMPOUNDS-DERIVED PEROXYL RADICALS.

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Proteins are one of the main targets of free radicals and oxidants in biological environments. It has been proposed that tryptophan (Trp) residues play a relevant role in protein oxidation leading to aggregation and fragmentation processes. In these processes, chain reactions involving Trp free radicals (Trp=, Trp-OO=) could be present. In the present work we studied the kinetic of the reaction between free Trp, as well as included in the dipeptide Gly-L-Trp, towards cationic and anionic peroxy radicals. Free Trp was efficiently consumed by peroxy radicals. At low initial concentrations (lower than 50 μ M) one Trp molecule was oxidized by two peroxy radicals generated from the pyrolysis of AAPH ((2,2-azobis(2-amidinopropane) dihydrochloride). In contrast, at high Trp concentrations (higher than 250 μ M), chain reactions were evidenced, involving the consumption of three Trp per each peroxy radical added to the system. The rate of Trp consumption included in the dipeptide Gly-L-Trp was two times higher than that of free Trp (at lower concentrations than 250 μ M). However, at high concentrations, free Trp and Gly-L-Trp were oxidized at similar reaction rates. The charge of peroxy radicals did not affect chain reactions of Trp. Nonetheless, at low Trp concentrations, cationic peroxy radicals showed a faster consumption of free Trp than anionic free radicals (derived from thermolysis of ABCPA, (4,4'-Azobis(4-cyanovaleic acid)). This result could be explained by ionic interactions between free Trp with peroxy radicals or by different rates of peroxy radical formation from azo compounds.

Acknowledgment: FONDECYT grant (n° 1100659).

LASER FLASH PHOTOLYSIS APPLIED TO PHOTOINDUCED FREE RADICALS GENERATION AND PHOTOCATALYSIS.

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Laser flash photolysis coupled to OPO system (optical parametric oscillator) is a powerful technique that permits to irradiate samples with photons at different wavelengths between 410 and 710 nm. After the irradiation, organic molecules with photochemical properties arise excited states experimenting reactions as electron photo-ejection, electron transfer, hydrogen transfer and energy transfer. In this case, the molecular dynamic and reactivity of the free radical intermediaries generated with biological relevant substrates can be studied in compartmentalized systems such micelles and liposomes from kinetic traces of the transient decay. On the other hand, when the TiO₂, SiO₂ or metal nanoparticles are irradiated with laser visible wavelength, it triggers free radical production and redox reactions that permits to catalyze different processes (photocatalysis) such as photodegradation of pollutants.

In the present work, we discuss the use of this methodology and also the chemical treatment of data to evaluate free radical and also excited triplet states reaction with biological substrates determining kinetic parameters following transients by nanosecond transient absorption spectroscopy. It is also discussed quantification of free radical or triplet excited states generation, and charge separation in complex molecules.

Acknowledgments. Financial support from FONDECYT (3130697, 1110809 and 1130701).

ANTIOXIDANT CAPACITY IN DIFFERENT FRACTIONS OF CALAFATE (*BERBERIS MICROPHYLLA*) AND TABLE GRAPES.

Bustamante L.¹, Oporto K¹, Ruiz A¹, Pastene E², von Baer D¹, Mardones C.¹ ¹Departamento de Análisis Instrumental, Universidad de Concepción, Concepción, Chile, ²Laboratorio de Farmacognosia, Universidad de Concepción, Concepción, Chile, lbustamante@udec.cl

Calafate (*Berberis microphylla*) is an underexploited endemic fruit growing in Chile and Argentine Patagonia. A recent research demonstrated their great nutraceutical potential, due to their high concentrations of anthocyanins and hydroxycinnamic acids. In this research, antioxidant capacity in different fractions of anthocyanins and flavonols-phenolic acids of calafate were tested. The results were compared with fractions of same families of compounds from *Vitis vinifera* L. "Red Globe" and "Pink Globe". Several calafate berries were analyzed, associating each fraction with their antioxidant capacity. Concentration of fractions varied between 0.60–31.68 µmol/g for total anthocyanins, 0.26–1.78 µmol/g for total flavonols and 0.68–4.94 µmol/g for total hydroxycinnamic acids. CUPRAC and ABTS antioxidant capacity were higher for anthocyanins fractions of calafate (between 13 to 50% and 4 to 30% respectively) than for flavonols-phenolic acids fractions (between 23 to 30% and 3 to 10% respectively). A tendency of higher antioxidant capacity was observed for the most concentrated extracts. The sum of both fractions represents 20-66% of total antioxidant capacity, demonstrating that other bioactive or reducing molecules are present in the whole calafate extract. Comparatively, the values obtained are between 2 and 10 times higher than those for *Vitis vinifera* L. berries.

Acknowledgement. Proyecto Fondecyt 1100944; Beca Conicyt-PCHA.

ANTIOXIDANT ACTIVITY OF *POPULUS*.

Cornejo J., ¹Baettig R., ²Gutierrez M., ²Astudillo L. ¹Centro Tecnológico del Álamo, Facultad de Ciencias Forestales, Universidad de Talca, Chile. ²Instituto de Química de Recursos Naturales, Universidad de Talca, Chile. jocornejo@utalca.cl

The *Populus* species possess great potential for therapeutical applications, especially for their known anti-inflammatory properties. The collective antioxidant properties and transcriptional effect of this extract previously reported suggest potential antiaging properties which could be utilized in cosmetic and nutraceutical formulations.

Here is reported the characterization of the antioxidant properties of different poplar bud (*Populus*) extract. The vegetable material was collected from trials for the determination of clonal adaptability established by the Poplar Technology Center at the University of Talca and was macerated in organic solvent obtained extracts with different polarity, then was determined the content of phenolic and flavonoids totals and analyzed the antioxidant activity. All extracts obtained was evaluated using free radical-scavenging activity on DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. Free radical-scavenging activity showed for sample can be attributed to a high amount of hydrophilic phenolics present in the species. The presence of antioxidant metabolites in hybrids evaluated its potential use as a source of natural antioxidants for industrial application providing added value to any forest industry waste. The data showed a relationship of free radical-scavenging activity of extracts with the total contents of phenolic present in the different sample studied.

ANTIOXIDANT EFFECT OF *Pinus radiata* BARK EXTRACT IN FISH CELL CULTURES.

Ulloa V.¹, Roa J.¹, Ortiz S.¹, Roeckel M.², Aspé E.², Astuya A.¹, Fernández K.², ¹Cell Culture and Marine Genomics Laboratory, Marine Biotechnology Unit, Faculty of Natural and Oceanographic Sciences. ²Laboratory of Bioengineering, Chemical Engineering Department, Faculty of Engineering, University of Concepcion, Chile. viv.ulloa@gmail.com

Fish enteritis is one of the inflammatory diseases associated with the presence of soybean meal in aquatic feed. The inflammation in the enteritis increases the levels of reactive oxygen species (ROS) producing the deterioration of the fish digestive mucosa. In this study, we evaluated the bioactive properties of the *P. radiata* bark extract *in vitro*, through the determination of cytotoxicity and antioxidant activity of the extract. Cytotoxicity of the *P. radiata* bark extract was measured in CHSE-214 cells, a salmonid cell line, using the MTT cell viability assay. The extract demonstrated to be innocuous up to 100µg/mL. The antioxidant extract capacity was evaluated using the 2',7'-dichlorofluorescein diacetate (DCFH-DA) method in two salmonid cell lines, SHK-1 and ASK, to determine intracellular levels of ROS. We determined an antioxidant activity close to 90% at 50µg/mL, higher than vitamin C and the commercial *P. pinaster* extract Pycnogenol at the same concentrations. In addition, the capacity of the extract to protect DNA from oxidative damage was assessed using the comet assay. A protective effect against DNA damage in SHK-1 cells exposed to hydrogen peroxide (50µM-100µM) was determined at 100µg/mL of the extract. The preincubation with the extract decreased comet assay parameters as tail DNA, tail moment, and Olive tail moment. The protective antioxidant properties of the *P. radiata* bark extract suggest that it could be used as a potential phyto-drug to decrease the noxious effects caused by ROS in soybean meal-induced enteritis.

Acknowledgment. INNOVA-Chile 12IDL2-13353.

ANTIOXIDANTE ACTIVITY OF BIOACTIVE EXTRACTS OBTAINED FROM *CYPERUS DIGITATUS*. Forero-Doria O., Fuentes E., Castro R., Astudillo L., Gutiérrez M., Salgado M., Palomo I., Guzmán-Jofre L., Universidad de Talca, Instituto de Química de Recursos Naturales, Talca, Chile. oforero@utalca.cl

Members of the family Cyperaceae such as *C. alopecuroides*, *C. articulatus*, *C. scariosus* and *C. rotundus* possess significant amount of studies about their antioxidant activities and other properties. Nevertheless, the plant *C. digitatus* belonging to the genus *Cyperus* lacks of studied about any kind of intrinsic activity. That is why, we made different extracts and fractions of this plant and a phytochemical screening and the antioxidant property was studied. Different extracts and fractions were obtained from the rhizomes of *C. digitatus*, and a Phytochemical screening and the content of phenols and flavonoids were quantified in each of the extracts and fractions, also we studied the antioxidant activity on different in vitro models like 1,1-diphenyl-picryl hydrazyl (DPPH) Assay, Ferric Reducing Antioxidant Power (FRAP) assay and β -Carotene bleaching assay. Of all the extracts obtained, the butanol and aqueous extracts showed the best antioxidant potential, meanwhile, none of the fractions obtained from the ethyl acetate extract show a relevant activity. Antioxidant potential might be helpful in preventing the progress of various oxidative stress related disorders.

Acknowledgment. This work was funded by the CONICYT REGIONAL / GORE MAULE / CEAP / R09I2001.

IN VITRO SCREENING FOR CHOLINESTERASE INHIBITION AND ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS FROM ECUADOR. ¹Jara A, ^{1,2}Astudillo L, ^{1,2}Gutiérrez M.

¹Laboratory of Organic Synthesis, Institute of Chemistry of Natural Resources ²Interdisciplinary Excellence Research Program on Healthy Aging, Universidad de Talca, Talca, Chile. adjara@utalca.cl

Due to climatic factors and topography, Ecuador is a highly vegetable species rich country. 15,000 species of vascular plants have been documented where as 4,857 (31.7%) are native to Ecuador's Amazon region. Ecuador, a megadiverse country has extensive flora which highlights some species for use in traditional medicine, knowledge that has been passed down from generation to generation in indigenous communities. Using this information ethnomedicinal of such plants was selected and collected six plants used in traditional medicine in Amazonia of Ecuador; the materials vegetal were collected in the provinces of Cañar and Morona Santiago from the Ecuador in 2011 and 2012. The plant material was botanically identified by Herbario Azuay of University of Azuay, Ecuador. The material were collected, garbled and dried under sunlight. The dried material was powdered coarsely. The powdered materials were extracted for maceration with organic solvents of different polarity, filtered and concentrated to reduced pressure. The solvents used for preparing the extracts were ethanol, ethyl acetate and dichloromethane, was determinated content of phenolic and flavonoids totals in all extracts and analyzed the capacity of inhibition of cholinesterase enzymes and antioxidant activity.

The ethanolics extracts were the most active with high phenolics and flavonoids content. The results revealed that amazonic plants are good resource with antioxidant activity and cholinesterasic effects with potential application in neurodegenerative disease, the data obtained can be used in futures studies for know phytochemical composition. The free radical-scavenging activity showed for extracts relationship with the total contents of phenolics and flavonoids.

REACTION OF PHENOLS WITH NITROUS ACID IN ACIDIC MEDIUM: ROLE OF NITROGEN DIOXIDE IN THE NITRIC OXIDE GENERATION. Reyes J.,¹ Lissi, E.,² Rubio, M. A.,² López-Alarcón, C.^{1, 1} Pontificia Universidad Católica de Chile, Facultad de Química, Departamento de Farmacia. e-mail: jcreyes@uc.cl.² Universidad de Santiago de Chile, Facultad de Química y Biología.

The reaction of phenols with nitrous acid (HONO) is a matter of current interest due to its occurrence in the gastrointestinal tract and a concomitant release of nitric oxide (NO). In acidic conditions HONO is in equilibrium with different species including nitrogen dioxide (NO₂), and dinitrogen trioxide (N₂O₃), among others. In particular, the reaction of phenols with NO₂ could be associated with the release of NO in the presence of HONO. The present work addressed the reaction of phenols with HONO and NO₂ as well as the release of NO during such processes. Phenol, 3-hidroxyphenol, 4-nitrophenol, 4-metoxiphyenol, sinapic acid, pyrogallol red (a colored phenol) were selected as phenols. Amongst these compounds, sinapic acid, pyrogallol red, and 4-metoxiphyenol reacted efficiently with NO₂ and HONO. Pyrogallol red, the most reactive phenol, was totally consumed after 20 and 300 minutes of incubation with a constant flux of NO₂ and in the presence of HONO (20 micromolar), respectively. However, the rate of these reactions was not in agreement with the rate of NO release. These results were interpreted in terms of a first step of the HONO-phenol reaction involving one electron reduction of HONO, generating a phenolic secondary free radical which after reacts with NO₂.

Acknowledgement: FONDECYT (n°1100659). J. Reyes acknowledges CONICYT for doctorate fellowship and DIPOG/Facultad de Química/UC.

CHARACTERIZATION OF ANTIOXIDANT ACTIVITY OF PLANT SPECIES OF THE GENUS AZORELLA OF ECUADOR. Romero J.¹, Gutiérrez M.¹, Astudillo L.¹, San Martín A.²

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Azorella Lam. is a South American genus of Apiaceae (Umbellifereae) represent by 26 species growing in the Andean mountains and Patagonia. Most of the *Azorella* species are commonly known as "yareta" and some are used in folk medicine as a blood depurative and as a gastric stimulant. Studies of *Azorella* species growing in the Chilean High Andes have shown that its petrol extracts are a rich and exclusive source of mulinane and azorellane, both type of diterpenoids. These skeletons are characterized by a wide range of biological activities including antibacterial for mulin-12,14-dien-11-on-20-oiic acid, hypoglycemic forazorellanol, anti-tuberculosis for 13 β -hydroxyazorellane and anti-inflammatory for 13-epiazorellanol. Extracts from *A. spinosa* and *A. monantha* of Chile has been reported as free radical-scavenging activity.

So far only *Azorella* species of Argentina and Chile have been investigated chemically and biologically, due to scarce studies of this genus other Andean country.

The present work aimed to evaluate the antioxidant activity using DPPH assay and present the characterization chemistry by means of the phenolic content and flavonoids overall of extracts, methanol hexane and ethyl acetate of *Azorellas* collected in Ecuadorian. The datas showed a relationship of free radical-scavenging activity of extracts with the total contents of phenolic present in the different sample studied.

To our best knowledge, this is the first report to study species of genus *Azorella* of Ecuador.

Acknowledgment. The international academic mobility program at the University of Antioquia Colombia and the University of Talca, Chile.

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ANTIOXIDANT CAPACITY AND STILBENES CONCENTRATION OF A GRAPE CANE EXTRACT.

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The evidence of the health benefits of *trans*-resveratrol has increased during the last twenty years. This fact has triggered the interest in studying the levels of this and other stilbenes in grapes, wine and winemaking residues. In Chile, more than 100.000 tons of winemaking residues are produced each year, including pomace, seeds, stalks and canes. In this research, profiles and levels of stilbenes in these waste products are studied by using liquid-solid extraction with ethanol-water 80% and HPLC-DAD-ESI-MS/MS in negative ionization mode. Main results show that α -viniferin is the main stilbene in these matrixes, followed by *trans*-resveratrol, except for canes, where *trans*-resveratrol was higher. Total stilbenes (expressed as *trans*-resveratrol equivalents in dry matter) were 22.81 \pm 10.59 mg/kg; 5.17 \pm 2.02 mg/kg; 0.93 \pm 0.44 mg/kg and 4485.87 \pm 200.28 mg/kg in stalks, pomace, seed and cane, respectively. Considering these results, a stilbene rich extract was produced at pilot scale (7 L reactor) using canes, obtaining a dry extract which contained 11 % of phenolic compounds (mainly stilbenoids and procyanidins). The antioxidant capacity of this extract was studied using cell free and cell based antioxidant assays (TEAC, ORAC, CUPRAC and endothelial cell ECV-304). The results show antioxidant capacity of the extract, however, depending of the method, synergic or antagonistic effect can be observed.

Acknowledgement: Proyecto Fondef D10I1104.

SITE DIRECTED PROTEIN OXIDATION IN HUMAN SERUM ALBUMIN CONDUCTED COUMARIN VISIBLE LIGHT IRRADIATION.

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Protein oxidation induced by free radicals and also non-radical species such as hypochlorite are extremely complex processes. The initial target amino acid in the protein and the extension of the damage depends on the attacking species conducting to different amino acid oxidation, fragmentation and/or aggregation patterns. We have been developing photochemical techniques for producing specific oxidation in proteins in order to study intra-protein free radical migration from the initial damage to remote sites using anchoring dyes in proteins.

In this work, we study the use of coumarin C314 to specific oxidize the tryptophan residue (Trp) in human serum albumin (HSA) after stationary visible light irradiation and laser pulse irradiation. The irradiation of C314 in solution by visible wavelengths produce consumption of the C314 and the formation of mainly one product associated to C314 dimer. The presence of HSA protected C314 and reduced the formation of the C314 photoproduct, but also induced degradation of Trp in HSA, in agreement with the C314 association to binding site 1 close to the Trp residue. We discuss these results based on the photochemistry of coumarin 314 to produce photoinduced free radicals and triplet species by laser irradiation at wavelengths in visible range ($\lambda=420$ nm).

Acknowledgment. Financial Support from FONDECYT 1110809 is greatly acknowledged.

ANTIOXIDANT ROLE OF HBU IN RATS WITH HIGH FRUCTOSE DIET.

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Introduction: We have prepared flour by milling Grape pomace, rich in fiber and polyphenols (HBU). Currently no functional properties are known for HBU, for this reason we wanted to evaluate its benefits in health in a rat model of metabolic syndrome induced by a high fructose diet. We propose that "HBU, improves markers of metabolic syndrome and decreases oxidative stress in rats with a high fructose diet."

Methods: Male Sprague Dawley rats of 180 g, were divided into four groups according to their feeding treatments: Control (C), 20% HBU (C+HBU), 60% fructose (F) and 60% fructose+20% HBU (F+HBU) for 16 weeks. Weight, blood glucose, triglycerides and systolic blood pressure were measured. HOMA index was calculated using insulin and glucose values. A glucose tolerance test was performed before the end of the experiment. TBARS were quantified in plasma and renal tissue as a measurement of oxidative stress.

Results: We did not observe any significant change in weight or systolic blood pressure in any of the groups. In F the area under the curve (AUC) of the glucose tolerance test (9915 \pm 599AU), fasting blood glucose (104.3 \pm 2mg/dl), plasma insulin (12 \pm 1mU/L), HOMA index (3.2 \pm 0.4AU) and TBARs in plasma (0.05 \pm 0.01 μ M/g) and renal tissue (0.40 \pm 0.01 μ M/100mg), were increased compared to C (AUC:7638 \pm 382AU; glycemia:91 \pm 3mg/dl, insulinemia:8 \pm 2mU/L, HOMA: 1.8 \pm 0.4AU, TBARs in plasma:0.02 \pm 0.01 μ M/g, TBARs in kidney:0.15 \pm 0.01 μ M/100mg.).

The increase in all these parameters was prevented in F+HBU (AUC:7479 \pm 627 AU, glycemia:91 \pm 2mg/dl, insulinemia: 7 \pm 1mU/L, HOMA:1.6 \pm 0.2AU, TBARs in plasma: 0.02 \pm 0.01 μ M/g, TBARs in kidney: 0.21 \pm 0.01 μ M/100mg).

Conclusions: According to these results, we propose that HBU prevents hyperglycemia, and reduces oxidative stress in animals with a high fructose diet. We propose that HBU could be used as a supplement for humans as well.

Acknowledgements: Puente #20/2013, Fondef AF 10i1014.