

NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all
Aspects of Natural Products Research



**This Issue is Dedicated to
Dr. Pawan K. Agrawal
On the Occasion of his 60th Birthday**

Volume 10. Issue 6. Pages 823-1140. 2015
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

EDITOR-IN-CHIEF**DR. PAWAN K AGRAWAL**

Natural Product Inc.
7963, Anderson Park Lane,
Westerville, Ohio 43081, USA
agrawal@naturalproduct.us

EDITORS**PROFESSOR ALEJANDRO F. BARRERO**

Department of Organic Chemistry,
University of Granada,
Campus de Fuente Nueva, s/n, 18071, Granada, Spain
afbarre@ugr.es

PROFESSOR ALESSANDRA BRACA

Dipartimento di Chimica Bioorganica e Biofarmacia,
Università di Pisa,
via Bonanno 33, 56126 Pisa, Italy
braca@farm.unipi.it

PROFESSOR DE-AN GUO

State Key Laboratory of Natural and Biomimetic Drugs,
School of Pharmaceutical Sciences,
Peking University,
Beijing 100083, China
gda5958@163.com

PROFESSOR YOSHIHIRO MIMAKI

School of Pharmacy,
Tokyo University of Pharmacy and Life Sciences,
Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan
mimakiy@ps.toyaku.ac.jp

PROFESSOR STEPHEN G. PYNE

Department of Chemistry
University of Wollongong
Wollongong, New South Wales, 2522, Australia
spyne@uow.edu.au

PROFESSOR MANFRED G. REINECKE

Department of Chemistry,
Texas Christian University,
Forts Worth, TX 76129, USA
m.reinecke@tcu.edu

PROFESSOR WILLIAM N. SETZER

Department of Chemistry
The University of Alabama in Huntsville
Huntsville, AL 35809, USA
wssetzer@chemistry.uah.edu

PROFESSOR YASUHIRO TEZUKA

Faculty of Pharmaceutical Sciences
Hokuriku University
Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan
y-tezuka@hokuriku-u.ac.jp

PROFESSOR DAVID E. THURSTON

Department of Pharmacy and Forensic Science,
King's College London,
Britannia House, 7 Trinity Street,
London SE1 1DB, UK.
david.thurston@kcl.ac.uk

HONORARY EDITOR**PROFESSOR GERALD BLUNDEN**

The School of Pharmacy & Biomedical Sciences,
University of Portsmouth,
Portsmouth, PO1 2DT U.K.
axuf64@dsl.pipex.com

ADVISORY BOARD

Prof. Viqar Uddin Ahmad
Karachi, Pakistan

Prof. Giovanni Appendino
Novara, Italy

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Roberto G. S. Berlinck
São Carlos, Brazil

Prof. Anna R. Bilia
Florence, Italy

Prof. Maurizio Bruno
Palermo, Italy

Prof. César A. N. Catalán
Tucumán, Argentina

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Fatih Demirci
Eskişehir, Turkey

Prof. Ana Cristina Figueiredo
Lisbon, Portugal

Prof. Cristina Gracia-Viguera
Murcia, Spain

Dr. Christopher Gray
Saint John, NB, Canada

Prof. Dominique Guillaume
Reims, France

Prof. Duvvuru Gunasekar
Tirupati, India

Prof. Hisahiro Hagiwara
Niigata, Japan

Prof. Tsukasa Iwashina
Tsukuba, Japan

Prof. Leopold Jirovetz
Vienna, Austria

Prof. Vladimir I Kalinin
Vladivostok, Russia

Prof. Phan Van Kiem
Hanoi, Vietnam

Prof. Niel A. Koorbanally
Durban, South Africa

Prof. Chiaki Kuroda
Tokyo, Japan

Prof. Hartmut Laatsch
Göttingen, Germany

Prof. Marie Lacaillé-Dubois
Dijon, France

Prof. Shoen-Sheng Lee
Taipei, Taiwan

Prof. Imre Mathe
Szeged, Hungary

Prof. M. Soledade C. Pedras
Saskatoon, Canada

Prof. Luc Pieters
Antwerp, Belgium

Prof. Peter Proksch
Düsseldorf, Germany

Prof. Phila Raharivelomanana
Tahiti, French Polynesia

Prof. Luca Rastrelli
Fisciano, Italy

Prof. Stefano Serra
Milano, Italy

Prof. Monique Simmonds
Richmond, UK

Dr. Bikram Singh
Palampur, India

Prof. John L. Sorensen
Manitoba, Canada

Prof. Johannes van Staden
Scottsville, South Africa

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Winston F. Tinto
Barbados, West Indies

Prof. Sylvia Urban
Melbourne, Australia

Prof. Karen Valant-Vetschera
Vienna, Austria

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site <http://www.naturalproduct.us>.

Authors may reproduce/republish portions of their published contribution without seeking permission from NPC, provided that any such republication is accompanied by an acknowledgment (original citation)-Reproduced by permission of Natural Product Communications. Any unauthorized reproduction, transmission or storage may result in either civil or criminal liability.

The publication of each of the articles contained herein is protected by copyright. Except as allowed under national "fair use" laws, copying is not permitted by any means or for any purpose, such as for distribution to any third party (whether by sale, loan, gift, or otherwise); as agent (express or implied) of any third party; for purposes of advertising or promotion; or to create collective or derivative works. Such permission requests, or other inquiries, should be addressed to the Natural Product Inc. (NPI). A photocopy license is available from the NPI for institutional subscribers that need to make multiple copies of single articles for internal study or research purposes.

To Subscribe: Natural Product Communications is a journal published monthly. 2015 subscription price: US\$2,595 (Print, ISSN# 1934-578X); US\$2,595 (Web edition, ISSN# 1555-9475); US\$2,995 (Print + single site online); US\$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

Anti-inflammatory, Antioxidant and Antimicrobial Activity Characterization and Toxicity Studies of Flowers of “Jarilla”, a Medicinal Shrub from Argentina

Alejandra Moreno^a, Gabriela Nuño^a, Soledad Cuello^{a,b}, Jorge E. Sayago^{a,b}, María Rosa Alberto^{a,b}, Catiana Zampini^{a,b*} and María Inés Isla^{a,b,*#}

^aInstituto de Química del Noroeste Argentino (INQUINOA), CONICET, San Lorenzo 1469. San Miguel de Tucumán. Tucumán. Argentina

^bFacultad de Ciencias Naturales e IML. Universidad Nacional de Tucumán. San Lorenzo 1469. San Miguel de Tucumán. Tucumán. Argentina.

*Both authors had the same participation as corresponding author

misla@tucbbs.com.ar

Received: January 10th, 2015; Accepted: February 25th, 2015

Zuccagnia punctata Cav. (Fabaceae) is an Argentine medicinal aromatic shrub (jarilla pispito, puspis, lata and jarilla macho). The chalcones were identified as pigments responsible for the yellow color of the flowers. Hydroethanolic extracts were obtained both from fresh flowers and from flowers dried by lyophilization. The extracts were standardized by their phenolic and flavonoids content. Their fingerprints by HPLC-DAD indicated the presence of two chalcones as major compounds (2',4'-dihydroxychalcone and 2',4'-dihydroxy-3'-methoxychalcone). Both extracts showed the same total phenolic, non-flavonoid phenolic and flavonoid phenolic content and their phenolic profiles were similar. The polyphenolic extracts exhibited antioxidant (free radical scavenging and inhibitory activity on lipoperoxidation) and anti-inflammatory (inhibition of lipoxygenase and cyclooxygenase enzymes) activities. The flower extracts were active against six *Candida* species with MIC values between 60 and 120 µg GAE.mL⁻¹ and were also active on methicillin-resistant *Staphylococcus aureus* (MIC: 250 µg GAE.mL⁻¹) and *Enterococcus faecalis* (MIC: 500 µg GAE.mL⁻¹). The extracts were neither toxic (*Artemia salina* test) nor mutagenic (Ames test). Jarilla flowers could be considered as a new dietary supplement that could help to prevent pathologies associated with oxidative stress and the polyphenolic extract obtained from them could be considered as a standardized phytotherapeutic product with antimicrobial, antioxidant and anti-inflammatory activities.

Keywords: *Zuccagnia punctata*, Flowers, Functional food, Phytotherapeutic product, Bioactive chalcones.

Zuccagnia punctata Cav. (Fabaceae) is a monotypic species, commonly known as jarilla pispito, puspis, lata and jarilla macho. It is an Argentine medicinal endemic shrub widely distributed in the biogeographic region of Monte de Sierras y Bolsones from western Argentina (provinces of Jujuy, Salta, Tucumán, Catamarca, La Rioja, San Juan, Mendoza and San Luis) [1a] and it shows resistance to diseases and pests. It is a glutinous and aromatic shrub with pinnate resinous leaves of 3-5 cm. The plants bloom from August to March. The yellow flowers, born in erect racemes, have a funnel-shaped calyx, with 5 sepals, corolla with 5 free petals and 10 free stamens [1a-1c]. The aerial parts of *Z. punctata* with and without flowers or fruits have been used extensively as a traditional medicine in Argentina for the treatment of bacterial and fungal infections, asthma, arthritis and rheumatism [2a,2b].

Previous reports showed the biological activities (antioxidant, antibacterial, antifungal, antiulcer, antigenotoxic, chemopreventive) and the chemical composition (flavanones, flavones, chalcones and caffeoyl esters) of the aerial parts (fruit, stem and leaves) of *Z. punctata* [3a-3k]. However, the biological activities and chemical composition of *Z. punctata* flowers have not yet been analyzed.

The aim of this work was to determine the pigments responsible for the yellow color of the flowers of *Z. punctata* and to evaluate the functional properties of the polyphenolic extract of the flowers. The toxicity (*Artemia salina*) and mutagenic activity (Ames test) of the extract were also evaluated.

Table 1: Total phenolic, non-flavonoid and flavonoid phenolic content of aerial parts (Ap), fresh and dried flowers (FF and DF) of *Z. punctata*.

Z. p.	Total phenolic compounds (TP)	Non flavonoid phenolics (NFP)	Flavonoid phenolics (FP)	Flavone and flavonol
	mg GAE.g ⁻¹ plant material			mg QE. g ⁻¹
A.p.	168.0±2.0 ^a	34.4±1.0 ^a	133.6±2.0 ^a	16.0 ± 1.0 ^a
F.F	166.0±2.0 ^a	30.8±2.0 ^a	135.2±2.0 ^a	9.0±0.9 ^b
D.F.	166.7±2.0 ^a	31.0±2.0 ^a	135.7±2.0 ^a	8.8±0.9 ^b

Values are reported as mean ± standard deviation of triplicates. Similar letters (a, b) in the same column show no significant differences between different samples according to Tukey's test ($p \leq 0.05$).

The flowers were used either directly after harvesting or as dried material by lyophilization. Hydroethanolic extracts were obtained from fresh and dried flowers. The content of phenolic compounds (166.7±15 mg GAE.g⁻¹ flowers) did not show a significant difference between them and was similar to the content of phenolic compounds in the aerial parts of *Zuccagnia punctata* (168.0±15 mg GAE.g⁻¹ aerial parts). Both fresh and dried flowers contained principally flavonoid phenolics (Table 1). The content of flavones and flavonols was higher in aerial parts (16.0 ± 1.0 mg QE.g⁻¹) than in flowers (9.0±0.9 mg QE. g⁻¹ flowers) (Table 1).

HPLC-DAD analysis of both flower hydroethanolic extracts showed the presence of cinnamic acid (C1), galangin (C2), carysin (C3), 2',4'-dihydroxychalcone (C4) and 2',4'-dihydroxy-3'-methoxychalcone (C5). These results were confirmed by standard compound retention time, UV spectral analysis and by co-injection (standard plus flower extract). 2',4'-Dihydroxy-3'-methoxychalcone (C5) was the major component of flower extracts.

Carotenoid pigments were not detected in flowers by qualitative and quantitative methodology. Consequently, the yellow pigmentation of *Z. punctata* flowers could be mainly due to chalcones. These pigments confer a striking yellow color to the flowers of different species such as *Bidens*, *Coreopsis*, *Cosmos*, *Dahlia*, *Acacia* and *Dianthus* and are useful as attractants of pollinators [4a,4b].

Both samples (fresh and dried) exhibited ABTS^{•+} reducing capacity, with similar SC₅₀ values (3.8±0.2 µgGAE.mL⁻¹). A dose-response relationship between the antioxidant activity percentage and phenolic compound content was observed (R²=0.98). The scavenging activity was higher than the commercial natural and synthetic antioxidants used by the food industry such as quercetin (SC₅₀= 6.7±0.3 µg.mL⁻¹) and BHT (SC₅₀= 7.7±0.4 µg.mL⁻¹). The autographical assays showed that both chalcones should be responsible for the antioxidant activity. This clearly suggests that antioxidant mechanisms by both chalcones could be related to their direct antioxidant properties (reducing capacity).

In addition, the flower extracts were able to protect lipids from oxidation, showing an IC₅₀ value of 14.7±0.2 µgGAE.mL⁻¹. A dose-response relationship between antioxidant activity percentage and phenolic compound content was observed (R² = 0.95). Nevertheless, standards such as quercetin (IC₅₀=7.3±0.3 µg.mL⁻¹) and BHT (IC₅₀ = 4.0±0.2 µg.mL⁻¹) inhibited the lipid oxidation more efficiently in a concentration-dependent manner.

It is known that many anti-inflammatory drugs have various and severe adverse effects. Therefore, natural agents with very little side-effects are required to substitute chemical therapeutics. The *in vitro* inhibitory activity of two enzymes, cyclooxygenase (COX-2) and lipoxygenase (LOX), responsible for the biosynthesis of inflammatory mediators, was used as an indication of the anti-inflammatory activity of the samples. COX products are well known to play an important role in pain and inflammation and LOX products are also involved in inflammatory processes, being important mediators in bronchial constriction and in hypersensitivity reactions. The phenolic extracts obtained from *Z. punctata* flowers were able to inhibit COX-2 and LOX in an equipotent manner (Table 2) behaving as dual inhibitors that block both routes of biosynthesis of mediators of the inflammatory process. Nevertheless, 2',4'-dihydroxychalcone exhibited an inhibitory effect on LOX activity, but did not show any effect on COX-2 activity until a concentration of 150 µg.mL⁻¹. 2',4'-dihydroxy-3'-methoxychalcone was able to inhibit PG biosynthesis (IC₅₀ value for COX-2 of 1.72 µg.mL⁻¹), being 58 fold more active on COX-2 than on LOX enzyme. According to our results, *Z. punctata* flower extracts and metabolites isolated from them may reduce the risk of inflammation-related diseases via either inhibition of COX-2 and LOX pathways and by scavenging free radicals and suppressing lipid peroxidation.

Candida species are opportunistic pathogens that produce mucosal to systemic infections such as oral candidiasis, vulvovaginitis and candidemia [5a,b]. In recent years, the number of infections worldwide by *Candida* species has increased considerably and resistance to traditional antifungal therapies is also rising. The current therapeutic options appear to be highly toxic and there are a lot of drug interactions. Furthermore, Gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* have developed resistance mechanisms against several commercial antimicrobial drugs [5c]. For this reason natural products could be a new alternative therapy.

Table 2: Effect of *Zuccagnia punctata* flower extract and pure compounds on the pro-inflammatory enzymes, lipoxygenase (LOX) and cyclooxygenase-2 (COX-2).

Samples	LOX	COX-2
	IC ₅₀ (µg.mL ⁻¹)	
Flower extract	49.6 ± 2.5	59.7 ± 3.1
DHC	99.5 ± 5.0	1.7 ± 0.01
DHMC	63.4 ± 3.3	ND
Naproxen	14.0 ± 0.7	-
Nimesulide	-	0.39 ± 0.03

DHC: 2',4'-dihydroxychalcone; DHMC: 2',4'-dihydroxy-3'-methoxychalcone.

Values are reported as mean ± standard deviation of triplicates.

ND: IC₅₀ not determined. 50% inhibition was not observed until 150 µg.mL⁻¹.

Table 3: *In vitro* determination of antifungal activity (MIC values) of flower extract against clinical strains of *Candida* species.

Yeast	Voucher number	MIC (µg GAE.mL ⁻¹)
<i>C. albicans</i>	F-100	60±10
<i>C. albicans</i>	F-101	60±10
<i>C. tropicalis</i>	F-300	60±10
<i>C. tropicalis</i>	F-301	120±20
<i>C. krusei</i>	F-400	60±10
<i>C. parapsilosis</i>	F-500	120±10
<i>C. glabrata</i>	F-200	60±10
<i>C. guilliermondii</i>	F-600	60±10
<i>C. parapsilosis</i>	ATCC 22019	60±10
<i>C. albicans</i>	ATCC10231	60±10

Values are mean ± standard deviation of triplicates.

Table 4: Minimal inhibitory concentration (MIC) values of flowers extract against antibiotic-resistant Gram-positive bacteria.

Microorganism	Voucher number/strain	Phenotype	MIC (µg GAE.mL ⁻¹)
<i>Staphylococcus aureus</i>	F2/ MRSA	Met ^r , Oxa ^r , Gen ^r	250±50
	F7/ MRSA	Met ^r , Oxa ^r , Gen ^r	250±50
	F22/ MRSCN	Met ^r Oxa ^r Gen ^r Van ^s	250±50
	F24/ MRSCN	Met ^r Oxa ^r Gen ^r Van ^s	250±25
	ATCC 29213	Control strain	125±25
<i>Enterococcus faecalis</i>	F201	Van ^r Amp ^r Gen ^s Str ^r	500±50
	F202	Gen ^r Str ^r Van ^s Amp ^s	>500
	F203	Gen ^r Str ^r Van ^s Amp ^s	>500
	F204	Van ^r Amp ^r Gen ^s Str ^r	500±50
	ATCC 29212	Control strain	250±25

^rResistant, ^sSusceptible; Vancomycin (Van), Ampicillin (Amp), Gentamycin (Gen), Streptomycin (Str), Methicillin (Met), Oxacillin (Oxa). Values are mean ± standard deviation of triplicates.

Flower extracts were active on six *Candida* species (MIC values between 60 to 120 µg GAE.mL⁻¹; Table 3) and on clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *S. aureus* coagulase negative (MRSCN) (MIC values of 250 µg GAE.mL⁻¹; Table 4). The MIC values against *Enterococcus faecalis* were two-fold higher (MIC values: 500 µg GAE.mL⁻¹) than those against *S. aureus*. The bioautographical assay of *Z. punctata* flower extract on MRSA showed that the extract had two bands with antibacterial and anti-*Candida* activity coincident with the Rfs of C4 and C5.

The flower extracts showed no toxic effect on *Artemia salina*. None of the doses was mutagenic to TA98 or TA100 strains under the conditions used in this assay. This result indicates the absence of mutagens that cause base pair substitution (detected in TA100) and frame-shift (detected in TA98) mutations. The absence of mutagenicity of the extract studied in the test against *Salmonella* strains indicates that DNA does not seem to be a relevant target.

Conclusions: The present study indicates that *Z. punctata* flowers could be a significant source of natural compounds with beneficial health effects (antifungal, antibacterial, antioxidant and anti-inflammatory) and may be useful as either a dietary supplement or for phytotherapy.

Experimental

Plant material: *Zuccagnia punctata* flowers were collected in December 2013 at 2000 m above sea level (masl) in Amaicha del

Valle, Tucumán, Argentina. Voucher specimens (IML 605935) were deposited at the Herbarium of Fundación Miguel Lillo, Tucumán, Argentina. *Z. punctata* was authenticated by Dra Soledad Cuello, INQUINOA (CONICET). The flowers were used either immediately after harvesting or dried by lyophilization.

Sample preparation: The flowers (10 g), fresh or dried, were extracted with 300 mL of ethanol: H₂O (60:40, v:v) for 1 h with sonication. Then, the extracts were filtered through Whatman N° 1 filter paper. The filtrate was evaporated to dryness and then lyophilized.

Total polyphenols, non-flavonoids and flavonoids: Total polyphenols (TP) of the extracts were determined by Folin-Ciocalteu's reagent [6a]. Non-flavonoid phenols (NFP) were measured by determination of total phenols content remaining after precipitation of the flavonoids with acidic formaldehyde [6b]. Flavonoid phenolics (FP) was determined as TP-NFP=FP. In all cases the results are expressed in mg gallic equivalents (mgGAE) per g of flowers. Flavones and flavonols were determined with aluminum chloride (AlCl₃) according to Lamaison and Carnet [6c]. Flavonoid content was expressed as mg quercetin equivalents (mg QE) per g of flowers.

Total carotenoids: The samples (1 g of flowers) were extracted with 6 mL of light petroleum: acetone (50:50, v/v). The total carotenoid content was calculated according to Rodríguez-Amaya [6d].

HPLC fingerprints of flower extracts: The HPLC system used consisted of a Waters 1525 Binary HPLC Pumps system with a 1500 Series Column Heater, a manual injection valve with a 20 μ L loop (Rheodyne Inc., Cotati, CA), a Waters 2998 photodiode array detector (PDA) and a XBridgeTM C18 column (4.6 x 150 mm, 5 μ m; Waters Corporation, Milford, MA). The solvent system for the separation of components from extracts was composed of solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in methanol) (conditions: 10–57% B from 0 to 45 min and kept at 100% B from 45 to 60 min). The flow rate was set at 0.5 mL.min⁻¹. A solution of 4 mg DW.mL⁻¹ was used. Data collection was carried out with EmpowerTM 2 software. The identification of phenolic compounds present in the extract was carried out by comparing the retention times and spectral data (220–600 nm) of each peak with those of standards from Sigma-Aldrich (MO, USA), Fluka Chemical Corp. (USA) and Indofine Chemical Company, Inc.

Measurement of antioxidant capacity

ABTS free radical scavenging activity: The antioxidant capacity assay was carried out by the ABTS^{•+} spectrophotometric method as described by Re *et al.* [6e] and the autographic assay as described by Zampini *et al.* [6f]. Commercial antioxidants (BHT and quercetin) were used as positive controls. SC₅₀ values denote the μ g GAE.mL⁻¹ required to scavenge 50% ABTS free radicals.

β -Carotene bleaching assay: Antioxidant activity was determined according to the β -carotene bleaching method following the procedure described by Ordoñez *et al.* [6g]. Quercetin and BHT were used as standards.

Measurement of anti-inflammatory activity

Cyclooxygenase inhibition assay: The ability of the extracts to inhibit the conversion of arachidonic acid to prostaglandin (PG) by human recombinant cyclooxygenase 2 (COX-2) was determined using a COX inhibitor screening assay kit (No. 560131; Cayman Chemical, Ann Arbor, MI, USA). PG produced was measured by

enzyme immunoassay (EIA). The inhibitory assays were performed in the presence of 25 to 150 μ g.mL⁻¹ of *Z. punctata* extract, 1 to 4 μ g.mL⁻¹ of 2',4'-dihydroxychalcone and 10 to 150 μ g.mL⁻¹ of 2',4'-dihydroxy-3'-methoxychalcone. The commercial anti-inflammatory drug nimesulide was used as reference.

Lipoxygenase inhibition assay: Lipoxygenase (LOX) activity was determined using a continuous spectrophotometric method, based on the enzymatic oxidation of linoleic acid to the corresponding hydroperoxide [6h]. The inhibitory assays were performed in the presence of either *Z. punctata* extract (50-150 μ g.mL⁻¹) or pure compounds (25-150 μ g.mL⁻¹). The anti-inflammatory effect of test compounds was evaluated by calculating the percentage inhibition of hydroperoxide production from the Δ OD values at 234 nm at the end of incubation. The anti-inflammatory drug naproxen was employed as reference compound.

Antimicrobial assays

Microorganisms: The following reference microorganisms were obtained from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Candida parapsilosis* ATCC 22019 and *C. albicans* ATCC10231. Clinical isolates of antibiotic methicillin-resistant *Staphylococcus aureus* (n=2, MRSA), *S. aureus* coagulase negative (n=2, MRSCN) and *Enterococcus faecalis* (n=2) were obtained from Hospital Dr Nicolás Avellaneda, San Miguel de Tucumán, Tucumán, Argentina. The yeasts *Candida albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. guilliermondii* were obtained from clinical samples from Hospital del Niño Jesús, San Miguel de Tucumán, Tucumán, Argentina. Bacterial strains were identified by the biochemical profiles according to [6i].

Minimal inhibitory concentration: This was determined by the agar macrodilution technique according to the guidelines of the Clinical and Laboratory Standard Institute for yeasts and bacteria [6j, 6k]. Concentrations were tested up to 500 μ g GAE.mL⁻¹. The inoculum (2 μ L) containing 5 \times 10⁵ CFU.mL⁻¹ (bacteria) and 5 \times 10³ CFU.mL⁻¹ (yeasts) was added to each medium. Plates were aerobically incubated at 37°C for bacteria and 35°C for yeasts for 24 h and 48 h, respectively. MIC was defined as the lowest extract concentration without macroscopically visible growth. As references, several antibiotics were used.

Bioautographic assays: The assay was realized on TLC developed with toluene: acetone: chloroform (4.5:3.5:2.5; v/v/v) by the method described by Zampini *et al.* [6i]. The R_f values of growth inhibition areas were compared with the R_f values of the related spots on the TLC plate observed under UV light at 365 nm before and after spraying with Neu's reagent, 2-aminoethyl-diphenylborinate (Sigma) [6l].

Mutagenicity assay: The mutagenicity assay with *S. typhimurium* was performed as described by Maron and Ames [6m]. The extract was considered mutagenic if the number of revertants per plate was more than two-fold the number of colonies produced on the solvent control plates (spontaneous revertant frequency). Different concentrations (25-500 μ g/plate) were tested in duplicate with two replicates. DMSO was used as the negative control, 100 μ L/plate, and the positive control employed was 4-nitro-o-phenylenediamine (4-NPD), 10 μ g/plate.

General toxicity using *Artemia salina*: Ten nauplii were added to a well of a 24-well plate containing an extract dilution (between 125 and 500 μ g.mL⁻¹). After 24 h incubation under light, the number of dead and surviving brine shrimps in each well was counted. The

LC₅₀ value was defined as the amount of phenolic compound that caused the death of 50% of nauplii.

Statistical analysis: Sampling and analyses were performed in triplicate, and the data are presented as mean ± standard deviation (S.D.). The Pearson test correlation coefficient with 95% confidence was used. Statistical analysis was performed by one way

ANOVA followed by Tukey's multiple comparison test ($p < 0.05$). All statistical analyses were carried out using Infostat software.

Acknowledgments - The authors acknowledge the financial support from Secretaría de Ciencia, Arte e Innovación Tecnológica (SCAIT), Argentina, Agencia Nacional de Promoción Científica y Técnica (ANPCyT) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

References

- [1] (a) Burkart R, Bárbaro NO, Sánchez RO, Gómez DA. (1999) Eco-regiones de la Argentina. Buenos Aires. Secretaría de Recursos Naturales y Desarrollo Sustentable 42; (b) Ulibarri EA. (2005) *Zuccagnia punctata* (Leguminosae): ¿nuevo o viejo endemismo argentino? *Darwiniana*, **43**, 212-215; (c) Zuloaga FO, Morrone O. (1999) *Catálogo de las Plantas Vasculares de la República Argentina. II. Fabaceae-Zygophyllaceae (Dicotyledoneae)*. Monographs in Systematic Botany from the Missouri Botanical Garden 74.
- [2] (a) Ratera EL, Ratera MO. (1980) *Plantas de la flora argentina empleadas en medicina popular*. Hemisferio S. (Ed), Buenos Aires, Argentina 189; (b) Toursarkissian M. (1980) *Plantas medicinales de la Argentina; sus nombres botánicos, vulgares, usos y distribución geográfica*. Hemisferio Sur, S. A., Buenos Aires, Argentina 178.
- [3] (a) Agüero MB, González M, Lima B, Svetaz L, Sanchez M, Zacchino S, Feresin G, Schmeda-Hirschmann G, Palermo J, Wunderlin D, Tapia A. (2010) Argentinian propolis from *Zuccagnia punctata* Cav. (Caesalpinieae) exudates: Phytochemical characterization and antifungal activity. *Journal of Agricultural and Food Chemistry*, **58**, 194-201; (b) Chieli E, Romiti N, Zampini IC, Garrido G, Isla MI. (2012) Effects of *Zuccagnia punctata* extracts and their flavonoids on the function and expression of ABCB1/P-glycoprotein multidrug transporter. *Journal of Ethnopharmacology*, **144**, 797-801; (c) De la Rocha N, María A, Gianello J, Pelzer L. (2003) Cytoprotective effects of chalcones from *Zuccagnia punctata* and melatonin on gastroduodenal tract in rats. *Pharmacological Research*, **48**, 97-99; (d) Morán-Vieyra F, Boggetti H, Zampini I, Ordoñez R, Isla M, Alvarez R, De Rosso V, Mercadante A, Borsarelli C. (2009) Singlet oxygen quenching and radical scavenging capacities of structurally related flavonoids present in *Zuccagnia punctata* Cav. *Free Radical Research*, **43**, 553-564; (e) Nuño G, Alberto M, Zampini I, Cuello S, Ordoñez R, Sayago J, Baroni V, Wunderlin D, Isla MI. (2014) The effect of *Zuccagnia punctata* Cav, an Argentine medicinal plant, on virulence factors from *Candida* species. *Natural Product Communications*, **9**, 933-936; (f) Quiroga EN, Sampietro AR., Vattuone MA. (2001) Screening antifungal activities of selected medicinal plants. *Journal of Ethnopharmacology*, **74**, 89-96; (g) Svetaz L, Tapia A, López S, Furlán R, Petenatti E, Pioli R, Schmeda-Hirschmann G, Zacchino S. (2004) Antifungal chalcones and new caffeic acid esters from *Zuccagnia punctata* acting against soybean infecting fungi. *Journal of Agricultural and Food Chemistry*, **52**, 3297-3300; (h) Svetaz L, Agüero MB, Alvarez S, Luna L, Feresin G, Derita M, Tapia A, Zacchino S. (2007) Antifungal activity of chalcones from *Zuccagnia punctata* Cav. acting against clinically important fungi and studies of mechanisms of action. *Planta Medica*, **73**, 1074-1080; (i) Zampini IC, Vattuone MA., Isla MI. (2005) Antibacterial activity of *Zuccagnia punctata* Cav. ethanolic extracts. *Journal of Ethnopharmacology*, **102**, 450-456; (j) Zampini IC, Villarini M, Moretti M, Dominici L, Isla MI. (2008) Evaluation of genotoxic and antigenotoxic effects of hydroalcoholic extracts of *Zuccagnia punctata* Cav. *Journal of Ethnopharmacology*, **115**, 330-335; (k) Zampini IC, Villena J, Salva S, Herrera M, Isla MI, Alvarez S. (2012) Potentiality of standardized extract and isolated flavonoids from *Zuccagnia punctata* for the treatment of respiratory infections by *Streptococcus pneumoniae*: *In vitro* and *in vivo* studies. *Journal of Ethnopharmacology*, **140**, 287-292
- [4] (a) Andersen M, Jordheim M. (2010) Chemistry of flavonoid-based colors in plants. Module in chemistry, molecular sciences and chemical engineering. *Comprehensive Natural Products II*. 574-614; (b) Harborne JB. (1966) Comparative biochemistry of flavonoids. I. Distribution of chalcone and aureone pigments in plants. *Phytochemistry*, **5**, 111-115.
- [5] (a) Davies AN, Brailsford SR, Beighton D. (2006) Oral candidosis in patients with advanced cancer. *Oral Oncology*, **42**, 698-702; (b) Sobel J D. (2007) Vulvovaginal candidosis. *Lancet*, **369**, 1961-1971; (c) Quave CL, Plano LR, Pantuso T, Bennett BC. (2008) Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology*, **118**, 418-428.
- [6] (a) Singleton VL, Orthofer R, Lamuela-Raventos RM. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, **299**, 152-178; (b) Isla MI, Salas A, Danert FC, Zampini IC, Ordoñez RM. (2013) Analytical methodology optimization to estimate the content of non-flavonoid phenolic compounds in Argentine propolis extracts. *Pharmaceutical Biology*, **52**, 835-840; (c) Lamaison JL, Carnet A. (1990) Teneurs en principaux flavonoïdes des fleurs de *Crataegus monogyna* Jacq et de *Crataegus laevigata* (Poiret D. C) en fonction de la végétation. *Pharmaceutica Acta Helvetica*, **65**, 315-320; (d) Rodríguez-Amaya DB. (1999) *A guide to carotenoid analysis in foods*. ILDI Press, Washington DC; (e) Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decoloration assay. *Free Radical Biology and Medicine*, **26**, 1231-1237; (f) Zampini I, Ordoñez R, Isla MI. (2011) Autographic assay for the rapid detection of antioxidant capacity of liquid and semisolid pharmaceutical formulations using ABTS^{•+} immobilized by gel entrapment. *American Association of Pharmaceutical Scientists*, **11**, 1159-1163; (g) Ordoñez RM, Cardozo ML, Zampini IC, Isla MI. (2010) Evaluation of antioxidant activity and genotoxicity of alcoholic and aqueous beverages and pomace derived from ripe fruits of *Cyphomandra betacea* Sendt. *Journal of Agricultural and Food Chemistry*, **58**, 331-337; (h) Taraporewala, IB, Kauffman JM. (1990) Synthesis and structure-activity relationship of anti-inflammatory 9, 10-dihydro 9-oxo-2-acridine alkanic acids and 4-(2-carboxyphenyl) aminobenzenealkanoic acids. *Journal of Pharmaceutical Sciences*, **79**, 173-178; (i) Murray PR, Baron EJ, Pfaller MA, Tenoer FC, Tenover RH. (1999) *Manual of Clinical Microbiology*, Vol. 6. ASM, Washington, DC; (j) CLSI (2012) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility test for bacteria that grow aerobically; Approved Standard. Ninth Edition M7-A9 Wayne, USA; (k) CLSI (2013) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty-third Informational Supplement M100-S23. Wayne, USA; (l) Neu R. (1957) A new reagent for differentiating and determining flavones on paper chromatograms. *Naturwissenschaften*, **43**, 147-156; (m) Maron DM, Ames BN. (1983) Revised methods for the *Salmonella* mutagenicity test. *Mutation Research*, **113**, 173-215.

A New Aromatic Compound from the Stem Bark of <i>Terminalia catappa</i> David Pertuit, Anne-Claire Mitaine-Offer, Tomofumi Miyamoto, Chiaki Tanaka, Stéphanie Delemasure, Patrick Dutartre and Marie-Aleth Lacaille-Dubois	1005
Effect of Non-psychotropic Plant-derived Cannabinoids on Bladder Contractility: Focus on Cannabigerol Ester Pagano, Vittorino Montanaro, Antonio di Girolamo, Antonio Pistone, Vincenzo Altieri, Jordan K. Zjawiony, Angelo A. Izzo and Raffaele Capasso	1009
In Cell Interactome of Oleocanthal, an Extra Virgin Olive Oil Bioactive Component Chiara Cassiano, Agostino Casapullo, Alessandra Tosco, Maria Chiara Monti and Raffaele Riccio	1013
Synthesis of β-Viniferin Glycosides by Glucosyltransferase from <i>Phytolacca americana</i> and their Inhibitory Activity on Histamine Release from Rat Peritoneal Mast Cells Hiroki Hamada, Hatsuyuki Hamada and Kei Shimoda	1017
Stability of the Ellagitannin Fraction and Antioxidant Capacity of Varietal Pomegranate Juices Pedro Mena and Cristina García-Viguera	1019
Phthalide Anions in Organic Synthesis. A Direct Total Synthesis of Furomollugin George A. Kraus and Pengfei Dong	1025
Absolute Configuration Assignment of 3',4'-di-<i>O</i>-acylkhellactones Using Vibrational Circular Dichroism Exciton Chirality Abigail I. Buendía-Trujillo, J. Martín Torres-Valencia, Pedro Joseph-Nathan and Eleuterio Burguero-Tapia	1027
Antifouling Compounds from the Marine-Derived Fungus <i>Aspergillus terreus</i> SCSGAF0162 Xu-Hua Nong, Xiao-Yong Zhang, Xin-Ya Xu and Shu-Hua Qi	1033
Goji Berry: Quality Assessment and Crop Adaptation of Plants Cultivated in Tuscany (Italy) by Combination of Carotenoid and DNA Analyses Giada Capecci, Emanuele Goti, Elena Nicolai, Maria Camilla Bergonzi, Roberto Monnanni and Anna Rita Bilia	1035
Activity of <i>Vitis vinifera</i> Tendrils Extract Against Phytopathogenic Fungi Daniele Fraternali, Donata Ricci, Giancarlo Verardo, Andrea Gorassini, Vilberto Stocchi and Piero Sestili	1037
Long-chain Glucosinolates from <i>Arabis turrita</i>: Enzymatic and Non-enzymatic Degradations Ivica Blažević, Sabine Montaut, Gina Rosalinda De Nicola and Patrick Rollin	1043
Aroma of Turmeric: Dependence on the Combination of Groups of Several Odor Constituents Toshio Hasegawa, Kenta Nakatani, Takashi Fujihara and Hideo Yamada	1047
Terpenoids Preserved in Fossils from Miocene-aged Japanese Conifer Wood Agnieszka Ludwiczuk and Yoshinori Asakawa	1051
Can Ozone Alter the Terpenoid Composition and Membrane Integrity of <i>in vitro</i> <i>Melissa officinalis</i> Shoots? Francesca D'Angiolillo, Mariagrazia Tonelli, Elisa Pellegrini, Cristina Nali, Giacomo Lorenzini, Luisa Pistelli and Laura Pistelli	1055
Composition and Chemical Variability of Ivoirian <i>Xytopia staudtii</i> Leaf Oil Thierry Acafou Yapi, Jean Brice Boti, Antoine Coffy Ahibo, Sylvain Sutour, Ange Bighelli, Joseph Casanova and Félix Tomi	1059
Chemoinformatics Approach to Antibacterial Studies of Essential Oils Dragoljub L. Miladinović, Budimir S. Ilić and Branislava D. Kocić	1063
Chemical Composition of <i>Nardostachys grandiflora</i> Rhizome Oil from Nepal – A Contribution to the Chemotaxonomy and Bioactivity of <i>Nardostachys</i> Prabodh Satyal, Bhuwan K. Chhetri, Noura S. Dosoky, Ambika Poudel and William N. Setzer	1067
Chemical Composition and Biological Activity of Essential Oils from Wild Growing Aromatic Plant Species of <i>Skimmia laurolela</i> and <i>Juniperus macropoda</i> from Western Himalaya Iris Stappen, Nurhayat Tabanca, Abbas Ali, David E. Wedge, Jürgen Wanner, Vijay K. Kaul, Brij Lal, Vikas Jaitak, Velizar K. Gochev, Erich Schmidt and Leopold Jirovetz	1071
Comparative Chemical Composition and Antioxidant Properties of the Essential Oils of three <i>Sideritis libanotica</i> Subspecies Carmen Formisano, Filomena Oliviero, Daniela Rigano, Nelly Apostolides Arnold and Felice Senatore	1075
<i>Asplenoideae</i> Species as a Reservoir of Volatile Organic Compounds with Potential Therapeutic Properties Didier Froissard, Sylvie Rapior, Jean-Marie Bessière, Bruno Buatois, Alain Fruchier, Vincent Sol and Françoise Fons	1079
Composition and Comprehensive Antioxidant Activity of Ginger (<i>Zingiber officinale</i>) Essential Oil from Ecuador Martina Höferl, Ivanka Stoilova, Juergen Wanner, Erich Schmidt, Leopold Jirovetz, Dora Trifonova, Veselin Stanchev and Albert Krastanov	1085
Chemical Components of Four Essential Oils in Aromatherapy Recipe Sarin Tadtong, Narisa Kamkaen, Rith Watthanachaiyingcharoen and Nijisiri Ruangrunsi	1091
<u>Accounts/Reivews</u>	
Recent Advances in the Synthesis of <i>Stemona</i> Alkaloids Xiao-Yu Liu and Feng-Peng Wang	1093
Flavonoid Properties in Plant Families Synthesizing Betalain Pigments (Review) Tsukasa Iwashina	1103
Phytochemistry and Pharmacology of the Genus <i>Tovomita</i> Francesco Epifano, Maria Carmela Specchiulli, Vito Alessandro Taddeo, Serena Fiorito and Salvatore Genovese	1115
Fungal Phytotoxins with Potential Herbicidal Activity to Control <i>Chenopodium album</i> Alessio Cimmino, Marco Masi, Marco Evidente and Antonio Evidente	1119
Essential Oils as “A Cry for Help”. A Review Christine Zitzelsberger and Gerhard Buchbauer	1127

Anti-Acetylcholinesterase Alkaloids from <i>Annona glabra</i> Leaf Shoei-Sheng Lee, Dong-Yi Wu, Sheng-Fa Tsai, and Chien-Kuang Chen	891
Increased Oxidative Stress in Cultured 3T3-L1 Cells was Attenuated by Berberine Treatment Shi-fen Dong, Naomi Yasui, Hiroko Negishi, Aya Kishimoto, Jian-ning Sun and Katsumi Ikeda	895
Synthesis and Antimicrobial Activities of 3-Methyl-β-Carboline Derivatives Jiwen Zhang, Longbo Li, Wenjia Dan, Jian Li, Qianliang Zhang, Hongjin Bai and Junru Wang	899
A Novel One-step Synthesis of Quinoline-2(1H)-thiones and Selones by Treating 3-Aryl-3-(2-aminophenyl)-1-propyn-3-ols with a Base and Elemental Sulfur or Selenium Kazuaki Shimada, Hironori Izumi, Koki Otashiro, Kensuke Noro, Shigenobu Aoyagi, Yuji Takikawa and Toshinobu Korenaga	903
Normonanchocidins A, B and D, New Pentacyclic Guanidine Alkaloids from the Far-Eastern Marine Sponge <i>Monanchora pulchra</i> Ksenya M. Tabakmakher, Tatyana N. Makarieva, Vladimir A. Denisenko, Alla G. Guzii, Pavel S. Dmitrenok, Aleksandra S. Kuzmich and Valentin A. Stonik	913
Computational and Investigative Study of Flavonoids Active Against <i>Trypanosoma cruzi</i> and <i>Leishmania</i> spp Frederico F. Ribeiro, Francisco J.B.M. Junior, Marcelo S. da Silva, Marcus Tullius Scotti and Luciana Scotti	917
Two New Secondary Metabolites from <i>Tephrosia purpurea</i> Yin-Ning Chen, Yan Peng, Cheng-Hai Gao, Tao Yan, Zhi-Fang Xu, Samuel X. Qiu, Wen-Hao Cao, Ligao Deng and Ri-Ming Huang	921
Regioselective Glycosylation of 3-, 5-, 6-, and 7-Hydroxyflavones by Cultured Plant Cells Kei Shimoda, Naoji Kubota, Daisuke Uesugi, Yuuya Fujitaka, Shouta Okada, Masato Tanigawa and Hiroki Hamada	923
Unusual Flavonoid Glycosides from the Hawaiian Tree <i>Metrosideros polymorpha</i> Benjamin R. Clark, Swapan Pramanick, Norman Arancon and Robert P. Borris	925
Anti-inflammatory Flavonoids Isolated from <i>Passiflora foetida</i> Thi Yen Nguyen, Dao Cuong To, Manh Hung Tran, Joo Sang Lee, Jeong Hyung Lee, Jeong Ah Kim, Mi Hee Woo and Byung Sun Min	929
Clovamide and Flavonoids from Leaves of <i>Trifolium pratense</i> and <i>T. pratense</i> subsp. <i>nivale</i> Grown in Italy Aldo Tava, Lukasz Pecio, Anna Stochmal and Luciano Pecetti	933
Water Extract of <i>Mentha</i> \times <i>villosa</i>: Phenolic Fingerprint and Effect on Ischemia-Reperfusion Injury Silvia Fialova, Lucia Veizerova, Viera Nosalova, Katarina Drabikova, Daniela Tekelova, Daniel Grancai and Ruzena Sotnikova	937
Distribution and Taxonomic Significance of Secondary Metabolites Occurring in the Methanol Extracts of the Stonecrops (<i>Sedum</i> L., Crassulaceae) from the Central Balkan Peninsula Gordana S. Stojanović, Snežana Č. Jovanović and Bojan K. Zlatković	941
In vitro Xanthine Oxidase Inhibitory Studies of <i>Lippia nodiflora</i> and Isolated Flavonoids and Phenylethanoid Glycosides as Potential Uric Acid-lowering Agents Lee-Chuen Cheng, Vikneswaran Murugaiyah and Kit-Lam Chan	945
Enzymatic Synthesis of Quercetin Monoglucopyranoside and Maltooligosaccharides Ryo Yasukawa, Natsumi Moriwaki, Daisuke Uesugi, Fuya Kaneko, Hiroki Hamada and Shin-ichi Ozaki	949
Polyurethane Microstructures—a Good or Bad in vitro Partner for the Isoflavone Genistein? Corina Danciu, Florin Borcan, Codruta Soica, Istvan Zupko, Erzsébet Csányi, Rita Ambrus, Delia Muntean, Camelia Sass, Diana Antal, Claudia Toma and Cristina Dehelean	951
Chemical Constituents of the Underground Parts of <i>Iris florentina</i> and their Cytotoxic Activity Akihito Yokosuka, Yoshikazu Koyama and Yoshihiro Mimaki	955
Synthesis of Arecatannin A1 from Dimeric Epicatechin Electrophile Manato Suda, Kohki Takanashi, Miyuki Katoh, Kiriko Matsumoto, Koichiro Kawaguchi, Sei-ichi Kawahara, Hiroshi Fujii and Hidefumi Makabe	959
Anthocyanin Profile and Antioxidant Activity of Various Berries Cultivated in Korea Hong-Sook Bae, Hyun Ju Kim, Jin Hee Kang, Rika Kudo, Takahiro Hosoya, Shigenori Kumazawa, Mira Jun, Oh-Yoen Kim and Mok-Ryeon Ahn	963
Metabolite Fingerprinting of <i>Eugenia jambolana</i> Fruit Pulp Extracts using NMR, HPLC-PDA-MS, GC-MS, MALDI-TOF-MS and ESI-MS/MS Spectrometry Ram Jee Sharma, Ramesh C. Gupta, Arvind Kumar Bansal and Inder Pal Singh	969
Flavonoids and Phenolic Acids in Methanolic Extracts, Infusions and Tinctures from Commercial Samples of Lemon Balm Agnieszka Arceusz, Marek Wesolowski and Beata Ulewicz-Magulska	977
RP-HPLC-DAD-MSⁿ Analysis and Butyrylcholinesterase Inhibitory Activity of <i>Barbacenia blanchetii</i> Extracts Jósquia S Barbosa, Verônica M Almeida, Rosilene M Marçal and Alessandro Branco	983
Flavonoids and Other Phenolic Compounds in Needles of <i>Pinus peuce</i> and Other Pine Species from the Macedonian Flora Marija Karapandzova, Gjose Stefkov, Ivana Cvetkovikj, Jasmina Petreska Stanoeva, Marina Stefova and Svetlana Kulevanova	987
Anti-inflammatory, Antioxidant and Antimicrobial Activity Characterization and Toxicity Studies of Flowers of “Jarilla”, a Medicinal Shrub from Argentina Alejandra Moreno, Gabriela Nuño, Soledad Cuello, Jorge E. Sayago, María Rosa Alberto, Catiana Zampini and María Inés Isla	991
Synthesis of Resveratrol Glycosides by Plant Glucosyltransferase and Cyclodextrin Glucanotransferase and Their Neuroprotective Activity Kei Shimoda, Naoji Kubota, Hatsuyuki Hamada and Hiroki Hamada	995
Anti-austeritic Constituents of the Congolese Medicinal Plant <i>Aframomum melegueta</i> Dya Fita Dibwe, Suresh Awale, Hiroyuki Morita and Yasuhiro Tezuka	997
The Lignan-containing Extract of <i>Schisandra chinensis</i> Berries Inhibits the Growth of <i>Chlamydia pneumoniae</i> Elina Hakala, Leena L. Hanski, Teijo Yrjönen, Heikki J. Vuorela and Pia M. Vuorela	1001

Natural Product Communications

2015

Volume 10, Number 6

Contents

<u>Editorial</u>	<i>i</i>
<u>Preface</u>	<i>iii</i>
<u>Original Paper</u>	
Chemical and Genetic Diversity of <i>Ligularia hodgsonii</i> in China Chiaki Kuroda, Kou Inagaki, Xun Chao, Kyosuke Inoue, Yasuko Okamoto, Motoo Tori, Xun Gong, and Ryo Hanai	823
Constituents of <i>Ligularia brassicoides</i> Collected in China: A New Diels-Alder Adduct of Eremophilan-10β-ol and Methacrylic Acid Mizuho Taniguchi, Katsuyuki Nakashima, Yasuko Okamoto, Xun Gong, Chiaki Kuroda and Motoo Tori	827
Four New Sesquiterpenoids from <i>Ligularia subspicata</i> Collected in China; Isolation of a Bakkane-type Lactone, an Eremophilane-type Lactone, and Two Ortho Esters Yoshinori Saito, Takanori Otsubo, Yuko Iwamoto, Katsuyuki Nakashima, Yasuko Okamoto, Xun Gong, Chiaki Kuroda and Motoo Tori	831
Natural Caryophyllane Sesquiterpenoids from <i>Rumphella antipathies</i> Hsu-Ming Chung, Wei-Hsien Wang, Tsong-Long Hwang, Yang-Chang Wu and Ping-Jyun Sung	835
Bioactive Compounds in Wild, <i>In vitro</i> Obtained, <i>Ex vitro</i> Adapted, and Acclimated Plants of <i>Centaurea davidovii</i> (Asteraceae) Antoaneta Trendafilova, Milka Jadrantin, Rossen Gorgorov and Marina Stanilova	839
New Laurene-type Sesquiterpene from Bornean <i>Laurencia nangii</i> Takashi Kamada and Charles Santharaju Vairappan	843
New Furanone and Sesquiterpene from the Pericarp of <i>Calocedrus formosana</i> Tzong-Huei Lee, Ming-Shian Lee, Horng-Huey Ko, Jih-Jung Chen, Hsun-Shuo Chang, Mei-Hwei Tseng, Sheng-Yang Wang, Chien-Chih Chen and Yueh-Hsiung Kuo	845
The Importance of the 5-Alkyl Substituent for the Violet Smell of Ionones: Synthesis of Racemic 5-Demethyl-α-ionone Serena Chierici, Serena Bugoni, Alessio Porta, Giuseppe Zanoni and Giovanni Vidari	847
Antiproliferative Activity of <i>seco</i>-Oxacassanes from <i>Acacia schaffneri</i> J. Martín Torres-Valencia, Virginia Motilva, J. Jesús Manriquez-Torres, Sofía García-Mauriño, Miguel López-Lázaro, Hanaa Zbakh, José M. Calderón-Montaño, Mario A. Gómez-Hurtado, Juan A. Gayosso-De-Lucio, Carlos M. Cerda-García-Rojas and Pedro Joseph-Nathan	853
<i>neo</i>-Clerodane Diterpenoids from <i>Ajuga macrosperma</i> var. <i>breviflora</i> Amaya Castro, Josep Coll, Anil K. Pant and Om Pakrash	857
Three New C₂₀-Diterpenoid Alkaloids from <i>Aconitum tanguticum</i> var. <i>trichocarpum</i> Zhong-Tang Zhang, Xiao-Yu Liu, Dong-Lin Chen, and Feng-Peng Wang	861
Manoalide-related Sesterterpene from the Marine Sponge <i>Luffariella variabilis</i> Toshiyuki Hamada, Daisuke Harada, Mitsunobu Hirata, Keisuke Yamashita, Kishneth Palaniveloo, Hiroaki Okamura, Tetsuo Iwagawa, Naomichi Arima, Toshiyuki Iriguchi, Nicole J. de Voogd and Charles S. Vairappan	863
Oxygenated Terpenes from Indo-Pacific Nudibranchs: Scalarane Sesterterpenes from <i>Glossodoris hikuerensis</i> and 12-Acetoxy Dendrillolide A from <i>Goniobranchus albonares</i> I. Wayan Mudianta, Andrew M. White and Mary J. Garson	865
Germinating Seeds of <i>Citrus aurantium</i> a Good Source of Bioactive Limonoids Marta R. Ariza, M. Mar Herrador del Pino and Alejandro F. Barrero	869
Chemical Constituents of <i>Lecythis pisonis</i> (Lecythydaceae) – A New Saponin and Complete ¹H and ¹³C Chemical Shift Assignments Renné C. Duarte, Carlos R. R. Matos, Raimundo Braz-Filho and Leda Mathias	871
Oleanane-type Triterpene Saponins from <i>Glochidion glomerulatum</i> Vu Kim Thu, Nguyen Van Thang, Nguyen Xuan Nhiem, Hoang Le Tuan Anh, Pham Hai Yen, Chau Van Minh, Phan Van Kiem, NanYoung Kim, Seon Ju Park and Seung Hyun Kim	875
Cucumarioside E from the Far Eastern Sea Cucumber <i>Cucumaria japonica</i> (Cucumariidae, Dendrochirotida), New Minor Monosulfated Holostane Triterpene Pentaoside with Glucose as the Second Monosaccharide Residue Alexandra S. Silchenko, Anatoly I. Kalinovsky, Pavel S. Dmitrenok, Vladimir I. Kalinin, Andrey N. Mazeika, Natalia S. Vorobieva, Nina M. Sanina and Edward Y. Kostetsky	877
Structure Revision of Two Polyoxygenated Sterols from the Marine Sponge <i>Neofibularia nolitangere</i> Yasunori Yaoita, Masao Kikuchi and Koichi Machida	881
Ergosterol of <i>Cordyceps militaris</i> Attenuates LPS Induced Inflammation in BV2 Microglia Cells Neeranjeni Nallathamby, Lee Guan-Serm, Sharmili Vidyadaran, Sri Nurestri Abd Malek, Jegadeesh Raman and Vikineswary Sabaratnam	885
Two Novel Spirostene Glycosides from <i>Selaginella chrysocaulos</i> and their Chemotaxonomic Significance Olaf Kunert, Rumalla Chidananda Swamy, Bobbala Ravi Kumar, Achanta Venkata Narasimha Appa Rao, Owi Ivar Nandi and Wolfgang Schuehly	887

Continued inside backcover