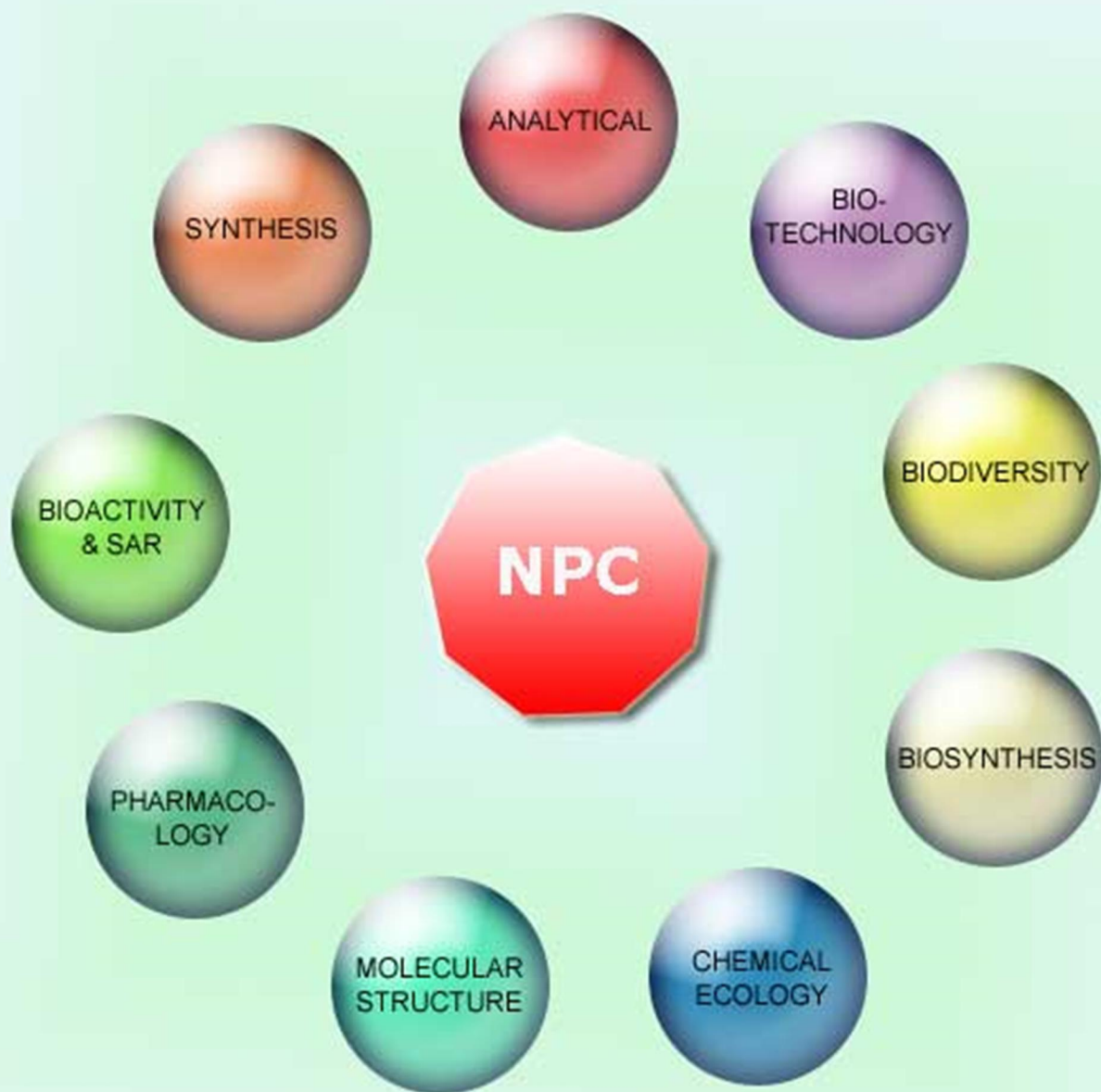


# NATURAL PRODUCT COMMUNICATIONS

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



Volume 11. Issue 1. Pages 1-134. 2016  
ISSN 1934-578X (printed); ISSN 1555-9475 (online)  
[www.naturalproduct.us](http://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,  
Universita di Pisa,  
via Bonanno 33, 56126 Pisa, Italy  
braca@farm.unipi.it

**PROFESSOR DE-AN GUO**

National Engineering Laboratory for TCM Standardization Technology,  
Shanghai Institute of Materia Medica, Chinese Academy of Sciences,  
Shanghai 201203, P. R. China  
gda5958@163.com

**PROFESSOR VLADIMIR I. KALININ**

G.B. Elyakov Pacific Institute of Bioorganic Chemistry,  
Far Eastern Branch, Russian Academy of Sciences,  
Pr. 100-letya Vladivostoka 159, 690022,  
Vladivostok, Russian Federation

**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  
wsetzer@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**

Institute of Pharmaceutical Science  
Faculty of Life Sciences & Medicine  
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. Berlink  
São Carlos, Brazil

Prof. Anna R. Bilia  
Florence, Italy

Prof. Maurizio Bruno  
Palermo, Italy

Prof. Josep Coll  
Barcelona, Spain

Prof. Geoffrey Cordell  
Chicago, IL, USA

Prof. Fatih Demirci  
Eskişehir, Turkey

Prof. Francesco Epifano  
Chieti Scalo, Italy

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. Judith Hohmann  
Szeged, Hungary

Prof. Tsukasa Iwashina  
Tsukuba, Japan

Prof. Leopold Jirovetz  
Vienna, Austria

Prof. Phan Van Kiem  
Hanoi, Vietnam

Prof. Niel A. Koorbanally  
Durban, South Africa

Prof. Chiaki Kuroda  
Tokyo, Japan

Prof. Hartmut Laatsch  
Gottingen, Germany

Prof. Marie Lacaillle-Dubois  
Dijon, France

Prof. Shoei-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

Dr. Bikram Singh  
Palampur, India

Prof. John L. Sorensen  
Manitoba, Canada

Prof. Johannes van Staden  
Scottsville, South Africa

Prof. Valentin Stonik  
Vladivostok, Russia

Prof. Ping-Jyun Sung  
Pingtung, Taiwan

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. 2016 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.

## Polyphenols and Volatile Compounds in Commercial Chokeberry (*Aronia melanocarpa*) Products

Annalisa Romani<sup>a,b</sup>, Pamela Vignolini<sup>a,b,\*</sup>, Francesca Ieri<sup>a,b</sup> and Daniela Heimler<sup>c</sup>

<sup>a</sup>PHYTO LAB laboratory, Scientific and Technological Pole, Via Ugo Schiff, 6 – 50019, Sesto F.no (FI), Italy

<sup>b</sup>DiSIA – Department of Statistics, Computer Science, Applications, University of Florence, Viale Morgagni, 59 – 50134 Florence, Italy

<sup>c</sup>Department of Agri-Food Production and Environmental Sciences - University of Florence, Piazzale Cascine 28 50144 Florence, Italy

pamela.vignolini@unifi.it

Received: July 7<sup>th</sup>, 2015; Accepted: October 21<sup>st</sup>, 2015

*Aronia melanocarpa* (Michx.) Elliott commercial products (dried fruit, juice and compote) were analyzed for their polyphenol content by chromatographic and spectrophotometric analyses in order to ascertain the fate of this group of compounds when fresh fruit is processed and sold in different forms on the market. Different classes of polyphenols were investigated: hydroxycinnamic derivatives ranged from 0.65 mg/g to 4.30 mg/g, flavonoids from 0.36 mg/g to 1.12 mg/g, and anthocyanins from 0.65 to 7.08 mg/g sample. 4-*O*-Caffeoyl-quinic acid was tentatively identified for the first time in *Aronia*. In order to characterize better chokeberry juice, a GC profile of aroma compounds was obtained. The aroma juice compounds belong mainly to the chemical classes of alcohols (48.9%) and ketones (30.28%). The most abundant compound is 3-penthen-2-one (23.6%).

**Keywords:** SPME-GC-MS, VOCs, HPLC-DAD, Spectrophotometric analyses, Chokeberry juice, Chokeberry fruits, Chokeberry compote.

While most berries contain a large number of flavonoids and phenolic acids, black and red berries contain anthocyanins, compounds considered to have a positive effect on a large number of chronic diseases (such as diabetes, cardiovascular disease and obesity) [1,2], as well as protecting against cancer [3]. Chokeberry, *Aronia melanocarpa* (Michx.) Elliott, has been intensively studied in recent years due to its high polyphenol content. Fresh chokeberry fruits, among the richest berries for their anthocyanin content [4-6], have a higher anthocyanin and flavonoid content and antioxidant capacity than cranberries, blueberries and lingonberries [7]. Prevention of gastric damage induced by ethanol in rats is inhibited by the red pigment fraction of the black chokeberry [8].

*A. melanocarpa*, a shrub of the *Rosaceae* family, originally from the eastern parts of North America and Canada, is now cultivated in Eastern European countries, Germany and Slovenia where its characteristics have recently been reviewed [9]. The polyphenol composition of fresh fruit has been extensively studied [10-12]. Since consumption of the fresh fruit is limited, the shelf-life may be increased through processing techniques. In order to assess the presence of polyphenols, dried *Aronia* powders obtained from different drying methods were analyzed [13] and the stability of anthocyanins in berry juices was assessed; in this regard, chokeberry juice exhibited the highest stability [14]. The exploitation of phenolics from industrial *Aronia* by-products was also taken into account [15,16].

The aim of this research was to investigate the polyphenol composition in commercial products in order to ascertain the fate of this group of compounds when fresh fruit is processed and sold in different forms on the market. We took three commercial chokeberry products into account: dried fruit, juice and compote, which were analyzed for their total content of polyphenols, anthocyanins, flavonols and hydroxycinnamic derivatives. Unfortunately, since commercial products were analyzed, it was not

possible to compare the results achieved with those of fresh fruit. In order to characterize more effectively chokeberry juice, a GC profile of aroma compounds was obtained and compared with that of fresh berries [17].

Commercial *A. melanocarpa* products (dried fruit, juice and compote) were analyzed for their polyphenol content by chromatographic and spectrophotometric analyses. The mass spectra were obtained in both positive and negative modes by HPLC-TOF analysis. In particular, the two pairs of isobaric compounds: quercetin 3-*O*-rutinoside and quercetin 3-*O*-rhamnosylgalactoside (compounds 6) and quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside (compounds 7 and 8) were identified based on the order of exit and the mass spectra according to Sliemstad *et al.* [11]. Compound 3 was identified as 4-*O*-caffeoyl-quinic acid based on its typical retention order compared with its isomers [21] and according to the relative intensities of diagnostic fragments in negative ionization mode [22]; the main ion fragment was at  $m/z$  353 (quasimolecular ion), while minor ion fragments were detected at  $m/z$  191 (quinic acid moiety) and  $m/z$  173 ('dehydrated' quinic acid moiety) with an intensity of 15% and 25% respectively. The ion at  $m/z$  173 is characteristic of a substituted isomer in position 4 [22]. To our knowledge this is the first time that the presence of this compound has been reported in *Aronia*.

Table 1 reports the quali-quantitative data of the samples analyzed. In the case of the juice (density = 1.078 g/mL), the data are reported as mg/g. Chlorogenic and neochlorogenic acids are by far the most prevalent compounds in dried fruit, juice and compote, while all the flavonoids are present in about the same amount, as already observed in fresh fruit and juice by Oszmiański and Wojdyło [10]. 4-*O*-Caffeoyl-quinic acid exhibits a peculiar behavior: its amount is practically the same when changing from dried fruit to juice, thus indicating its high water solubility.

**Table 1:** Quali-quantitative composition of dried chokeberry fruit, juice and compote. Data are expressed in mg/g per sample. Standard deviation in brackets.

| Compounds   | Dried fruit                  | Juice                        | Compote                       |
|---|------------------------------|------------------------------|-------------------------------|
| <b>Hydroxycinnamic derivatives</b>  |                              |                              |                               |
| (1) Neo-chlorogenic acid  | 1.82 (0.034)                 | 0.47 (0.009)                 | 0.26 (0.003)                  |
| (2) Chlorogenic acid  | 2.33 (0.043)                 | 0.51 (0.011)                 | 0.37 (0.006)                  |
| (3) 4- <i>O</i> -Caffeoyl-quinic acid   | 0.17 (0.003)                 | 0.13 (0.004)                 | 0.03 (0.3*10 <sup>-3</sup> )  |
| <b>Flavonoids</b>   |                              |                              |                               |
| (4) Quercetin 3- <i>O</i> -arabinoglucoside   | 0.13 (0.002)                 | 0.22 (0.004)                 | 0.02 (0.2*10 <sup>-3</sup> )  |
| (5) Quercetin 3- <i>O</i> -rutinoside + quercetin 3- <i>O</i> -rhamnosylgalactoside | 0.15 (0.003)                 | 0.02 (0.3*10 <sup>-3</sup> ) | 0.023 (0.3*10 <sup>-3</sup> ) |
| (6) Quercetin 3- <i>O</i> -rutinoside + quercetin 3- <i>O</i> -rhamnosylgalactoside | 0.23 (0.003)                 | 0.04 (0.4*10 <sup>-3</sup> ) | 0.03 (0.2*10 <sup>-3</sup> )  |
| (7) Quercetin 3- <i>O</i> -galactoside  | 0.31 (0.003)                 | 0.05 (0.4*10 <sup>-3</sup> ) | 0.06 (0.5*10 <sup>-3</sup> )  |
| (8) Quercetin 3- <i>O</i> -glucoside  | 0.22 (0.004)                 | 0.03 (0.3*10 <sup>-3</sup> ) | 0.03 (0.3*10 <sup>-3</sup> )  |
| (9)   | 0.06 (0.8*10 <sup>-3</sup> ) | 0.01 (0.1*10 <sup>-3</sup> ) | 0.01 (0.1*10 <sup>-3</sup> )  |

Table 2 reports the total HPLC content of hydroxycinnamic derivatives and flavonols, as well as the anthocyanin spectrophotometric content and total polyphenols according to the Folin-Ciocalteu method. Hydroxycinnamic acid derivatives and the flavonoid content decrease when changing from dried fruits to compote. Blueberry jam showed a loss of chlorogenic acid and total anthocyanins, with no change in the total flavonol content [23]. For home-made jams from five different berries (strawberries, raspberries, blackcurrants, bilberries and lingonberries) a considerable loss of flavonols was observed during processing [24]. In the case of red raspberries, while the loss of anthocyanins in the industrial processing of fresh fruit to obtain jam was between 17 and 41% depending on the fruit variety, the flavonol loss was much lower, ranging between 7 and 8% [25].

**Table 2:** Hydroxycinnamic derivatives and flavonoids determined by HPLC analyses (mg/100 g), anthocyanins determined by spectrophotometric analysis (mg/100 g), total phenolics (Folin-Ciocalteu method) expressed as mg gallic acid / 10 g sample. Data are the mean of three determinations.

| Compounds   | Dried fruits | Juice | Compote |
|---|--------------|-------|---------|
| <b>Hydroxycinnamic derivatives (HPLC)</b>                 | 4.3          | 1.1   | 0.7     |
| <b>Flavonoids (HPLC)</b>                                  | 1.1          | 0.2   | 0.12    |
| <b>Anthocyanins (spectrophotometric analyses, 550 nm)</b> | 7.1          | 0.6   | 0.8     |
| <b>Total phenolics (Folin Ciocalteu)</b>                  | 30.9         | 8.7   | 7.7     |

As regards the anthocyanins, which are the compounds generally most investigated in fresh berries, their content changes depending on the cultivar considered [12]: in a survey of the pertinent literature, their content is found between 252 and 460 mg/100 g fresh weight [5,11,12,26], changing from 1041 mg/100 g [27] to 6408 mg/kg [28], dry weight. Our dried commercial fruit maintained 7.08 mg/g (Table 2) of anthocyanins; since the water content of dried fruit is about 17.5%, its content is in the same magnitude as that of fresh fruit, thus indicating that the anthocyanin content is not reduced in the industrial dehydration process. Plums, on the contrary, showed flavonol and anthocyanin degradation after the drying process at different temperatures [29]. A lower anthocyanin content is observed in the juice, since these compounds are mainly found in the external layers of the berry skin and are absent in the flesh tissues, being, however, water soluble pigments [4]. The anthocyanin juice content was similar to that of laboratory-made chokeberry juice, i.e. 62 mg/100 mL [14], which is very close to our datum (0.70 mg/mL, with a density of 1.078 g/mL). The anthocyanin content of compote is similar to that of juice; in this case, however the decrease should be ascribed to the thermal process required to obtain compote [30]. In the case of unprocessed and thermally processed strawberry purées, a 30% decrease of pelargonidin-3-glucoside was observed [30]. Blueberry jam also underwent a total loss of anthocyanins compared with fresh fruit [23]. With regard to the total polyphenols (Folin Ciocalteu method), the content in dried fruit is much higher than that reported for fresh

**Table 3:** Volatiles of chokeberry juice tentatively identified by GC-MS and calculated retention index. RT (Retention time), I value (I: Non-isothermal Kovats retention indices from temperature-programming, using the definition of Van den Dool and Kratz [34] and % of total area are reported. The I values were calculated from the retention time using *n*-alkanes as retention calibration standards according to the Kovats convention (1958).

| no. | Compound   | RT     | I (DB-WAX) | % of total Area |
|-----|--|--------|------------|-----------------|
| 1   | Ethyl acetate  | 5.85   |            | 0.09            |
| 2   | Ethanol  | 6.46   | 937        | 5.7             |
| 3   | 2-Pentanone  | 7.28   | 992        | 3.8             |
| 4   | 3-Penten-2-one, -(E)   | 7.87   | 1032       | 0.9             |
| 5   | 3-Buten-2-ol, 2-methyl-  | 8.01   | 1041       | 0.1             |
| 6   | Undecane   | 8.48   | 1100       | 0.04            |
| 7   | Hexanal  | 8.96   | 1145       | 0.2             |
| 8   | 2-Butanol, 3-methyl-   | 9.39   | 1184       | 0.4             |
| 9   | 3-Penten-2-one, -(Z)   | 9.81   | 1223       | 23.6            |
| 10  | 1-Penten-3-ol  | 10.01  | 1171       | 0.08            |
| 11  | 3-Penten-2-ol  | 10.29  | 1184       | 0.08            |
| 12  | Dodecane   | 10.63  | 1200       | 0.08            |
| 13  | Eucalyptol   | 11.17  | 1225       | 0.2             |
| 14  | 2-Hexenal, (E)-  | 11.30  | 1243       | 0.2             |
| 15  | Styrene  | 11.94  | 1285       | 0.3             |
| 16  | Tridecane  | 12.17  | 1300       | 0.1             |
| 17  | 2-Heptanol   | 12.44  | 1318       | 0.04            |
| 18  | unk 2-Penten-1-ol  | 12.54  | 1325       | 0.1             |
| 19  | 1-Hexanol  | 13.02  | 1358       | 18.2            |
| 20  | 3-Hexen-1-ol, (E)-   | 13.21  | 1372       | 0.3             |
| 21  | 3-Hexen-1-ol, (Z)-   | 13.55  | 1395       | 3.8             |
| 22  | 2-Hexen-1-ol, (E)-   | 13.80  | 1413       | 11.1            |
| 23  | 2-Hexen-1-ol, (Z)-   | 13.94  | 1423       | 0.1             |
| 24  | Acetic acid  | 14.43  | 1458       | 0.4             |
| 25  | cis-Linaloloxide   | 14.53  | 1466       | 0.2             |
| 26  | 2-Pentanone, 4-hydroxy-  | 14.88  | 1491       | 1.7             |
| 27  | 1-Hexanol, 2-ethyl-  | 14.97  | 1497       | 0.3             |
| 28  | Citronellal  | 15.04  | 1502       | 0.08            |
| 29  | 1,6-Octadien-3-ol, 3,7-dimethyl-                                 | 15.63  | 1547       | 0.3             |
| 30  | Benzaldehyde   | 15.98  | 1574       | 2.4             |
| 31  | Isophorone   | 17.006 | 1656       | 0.3             |
| 32  | Butanoic acid, 2-methyl-   | 17.28  | 1678       | 0.9             |
| 33  | alpha-Terpineol  | 17.82  | 1726       | 0.3             |
| 34  | Hexanoic acid  | 19.10  | 1854       | 1.1             |
| 35  | Ethanol, 2-(2-butoxyethoxy)-, acetate                            | 19.16  | 1861       | 0.1             |
| 36  | 2H-Pyran-2-one, tetrahydro-6-methyl-                             | 19.26  | 1872       | 0.6             |
| 37  | 2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)- | 19.29  | 1876       | 0.3             |
| 38  | Benzyl alcohol   | 19.65  | 1917       | 16.7            |
| 39  | Dihydro-3-methylene-5-methyl-2-furanone                          | 19.65  | 1917       |                 |
| 40  | Phenylethyl Alcohol  | 20.01  | 1962       | 0.1             |
| 41  | 2-Hexenoic acid, (E)-  | 20.17  | 1982       | 0.4             |
| 42  | Benzoic acid, 4-ethoxy-, ethyl ester                             | 22.19  |            | 0.2             |
| 43  | Phenol, 3,4,5-trimethyl-   | 23.98  |            | 0.5             |
| 44  | Benzoic acid   | 24.72  |            | 3.0             |

fruit [5,12], while the juice and compote showed a lower content. However, with respect to dried fruit, the loss of anthocyanins is about 90% for the juice and compote, whereas the loss of total polyphenols is 72% and 76% in the case of juice and compote, respectively. Table 3 illustrates the volatile constituents identified, together with I Kovats indices modified according to van den Dool [31,32].

While in the case of two different raspberry cultivars, very slight differences were observed in the aroma composition of fresh fruit and juice [33], in this case, with respect to the volatile constituents determined in fresh berries [17] a lower number of compounds were identified. This occurrence can be ascribed to the different procedures via which the juice was obtained however. In the case of raspberries, it was obtained by pressing frozen fruit without any stabilization processes or the use of enzymes and technological coadjutants. It has been shown in the case of pomegranate juice that different processing methods can change the aromatic composition

of commercial juice compared with fresh-squeezed juice [34]. In the case of fresh chokeberry fruit of two different cultivars [17], 3,9-Epoxy-*p*-menth-1-ene is the most abundant compound, but it was not found in our samples. The most abundant compounds in our juice were 3-penthen-2-one (23.6%), 1-hexanol (18.2%) and 2-hexen-1-ol (11.1%). The juice aroma compounds belong to these chemical classes: alcohols (48.9%), ketones (30.3%), hydrocarbons (0.2%), acids (5.8 %), aldehydes (2.9 %), terpenes (0.6%), esters (0.3%), and others (1.3 %).

Therefore, also considering peaks of 39 and 40 (16.7 %) that were not resolved and contain both an alcohol and a ketone, it was found that the main juice aroma compounds are alcohols and ketones. In the case of crushed dried fruit, few peaks were recorded; however, 3-penthen-2-one was the most abundant compound, as already observed in juice. Both benzaldehyde and acetic acid were found in juice, while 1-ethoxy-2-propanol was not detected in either juice or fresh fruit [17]. On the basis of polyphenol subclasses and volatile compounds, the commercial Aronia products can be regarded as a new source of biologically active compounds that may enhance agricultural products of marginal areas.

## Experimental

**Samples:** Three commercial chokeberry samples (juice, compote and dried fruits) from a Croatian market were analyzed.

### Extraction

**Chokeberry juice:** Fifty mL of 70% ethanol was added to 50 mL of juice and adjusted to pH 2 with formic acid.

**Chokeberry compote:** Forty mg of compote was extracted with 50 mL of 70% ethanol, adjusted to pH 2 with formic acid overnight and then filtered to eliminate residues.

**Chokeberry dried fruit:** Dried fruits (2.5 g) were extracted with 10 mL of 70% ethanol, adjusted to pH 2 with formic acid overnight, and then filtered to eliminate residues.

**Standards and solvents:** Authentic standards of quercetin 3-*O*-glucoside, chlorogenic acid, neo-chlorogenic, gallic acid and kuromanin were purchased from Extrasynthèse S.A. (Lyon, France). All solvents used were of HPLC grade purity.

**HPLC/DAD analysis:** Analyses of flavonols and hydroxycinnamic acids were carried out using an HP 1100L liquid chromatograph equipped with a DAD detector and managed by an HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated using a 150 × 4.6 mm i.d., 5 μm LUNA C18 column (Phenomenex, USA). UV/Vis spectra were recorded in the 190–600 nm range and the chromatograms were acquired at 250, 280, 330, 350 and 520 nm. The samples were analyzed by gradient elution at a flow rate of 0.6 mL/min. The mobile phase was a multi-step linear solvent gradient system, starting from 95% H<sub>2</sub>O (adjusted to pH 3.2 by HCOOH) up to 100% CH<sub>3</sub>CN in 53 min.

**HPLC-TOF analysis:** The HPLC system was interfaced with an Agilent TOF MS equipped with an ESI source (Agilent Corp, Santa Clara, CA, USA). The TOF/MS analysis worked using full-scan mode and the mass range was set to *m/z* 100–1500 in both positive and negative modes. The conditions of the ESI source were as follows: drying gas, high purity nitrogen (N<sub>2</sub>); drying gas temperature, 350°C; drying gas flow-rate, 6 L/min; nebulizer, 20 psi; capillary voltage, 4000 V (negative) 4000 V (positive); fragmentation, 80–150 V, and skimmer, 60 V. The acquisition and data analysis were controlled using Agilent LC-MS TOF Software (Agilent, USA).

**Identification and quantification of individual compounds:** The identity of polyphenols was ascertained using data from HPLC-DAD and HPLC-TOF analyses, by comparison with bibliographic data and combination of retention times, UV/Vis and mass spectra with those of authentic standards. The quantification of individual polyphenolic compounds was performed directly by HPLC-DAD using a five-point regression curve ( $r^2 \geq 0.998$ ) in the range of 0–30 μg on the basis of authentic standards. In particular, flavonols like the quercetin derivatives were determined at 350 nm using quercetin 3-*O*-glucoside as a reference compound, while the hydroxycinnamic acid derivatives were determined at 330 nm using chlorogenic acid as the reference compound. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight. Each sample was analyzed in triplicate, so as to express the analytical results as an average with its standard deviation.

**Spectrophotometric analyses:** The 3 chokeberry extract samples were analyzed using an Agilent 8453, (Agilent Technologies, Palo Alto, CA, USA) spectrophotometer. The absorbance at 550 nm of the hydroethanolic sample solutions was evaluated using a 1 cm pathway quartz cell. The total amount of anthocyanins is expressed as kuromanin through the calibration curve of kuromanin.

**Total phenolic content:** The total phenolic content was determined using the Folin-Ciocalteu method described by Singleton *et al.* [18], and slightly modified according to Dewanto *et al.* [19]. The total phenolics are expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 μg/mL ( $R^2 = 0.9969$ ).

**SPME-GC-MS conditions and VOCs characterization:** The VOCs profile of Aronia samples was determined by SPME (Solid-Phase Micro Extraction)-GC-MS. A tentative compound identification was performed by comparing the mass spectra of each peak with those reported in mass spectral databases after Dynamic Background Compensation by Clear View software, (ALMSCO, UK). Chokeberry juice (0.5 mL) was placed in a 2 mL vial and 0.2 g NaCl added. One g of crushed chokeberry dried fruit was placed in a 2 mL vial. SPME conditions: VOCs profile was determined by absorption of VOCs at 40°C (for 10 min) on a trivalent Carboxen PDMS DVB 1 cm fiber, followed by desorption at 280°C and GC/MS analysis. An Agilent 7890a GC equipped with a 5975C MSD was used. The analyte separation was achieved with an Agilent DB WAX 50 m column, 0.20 μm id, 0.40 μm df. The chromatographic conditions were: initial temperature 40°C, then 10°C min<sup>-1</sup> up to 260°C, and held for 6.6 min.

**Determination of juice density:** The chokeberry juice density was measured with a standard pycnometer (ISO 1183-1:2004) [20]:

$$\rho = \rho_{H_2O} \frac{m_x - m_0}{m_{H_2O} - m_0}$$

$\rho$  = chokeberry juice density

$\rho_{H_2O}$  = water density

$m_x$  = pycnometer + chokeberry juice weight

$m_0$  = empty pycnometer weight

$m_{H_2O}$  = pycnometer + water weight

**Evaluation of moisture content:** The moisture content of the commercial fruits was obtained by keeping the sample in a static oven at 60° for 5 days.

**Acknowledgements** - Part of the work presented was funded by the Regione Toscana with the Tuscany Projects - NATURBEN (PRAF 2012-2015) and GOLICE (POR CREO 2014-2020). Our thanks to Massimo Busdraghi for providing the chokeberry samples.

## References

- [1] Norberto S, Silva S, Meireles M, Faria A, Pintado M, Calhau C. (2013) Blueberry anthocyanins in health promotion: A metabolic overview. *Journal of Functional Foods*, **5**, 1518–1528.
- [2] Tsuda T. (2012) Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies. *Molecular Nutrition & Food Research*, **56**, 159–170.
- [3] Hou DX. (2003) Potential mechanisms of cancer chemoprevention by anthocyanins. *Current Molecular Medicine*, **3**, 149–159.
- [4] Weberic R, Slatnar A, Bizjak J, Stampar F, Mikulic-Petkovset M. (2015) Anthocyanin composition of different wild and cultivated berry species. *LWT Food Science Technology*, **60**, 509–517.
- [5] Benvenuti S, Pellati M, Melegari M, Bertelli D. (2004) Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus*, *Ribes*, and *Aronia*. *Journal of Food Science*, **69**, FCT164–FCT169.
- [6] Wu X, Gu L, Prior RL, McKay S. (2004) Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia* and *Sambucus* and their antioxidant capacity. *Journal of Agricultural Food Chemistry*, **52**, 7846–7856.
- [7] Zheng W, Wang SY. (2003) Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries and lingonberries. *Journal of Agricultural Food Chemistry*, **51**, 502–509.
- [8] Matsumoto M, Hara H, Chiji H, Kasai T. (2004) Gastroprotective effect of red pigments in black chokeberry fruit (*Aronia melanocarpa* Elliot) on acute gastric hemorrhagic lesions in rats. *Journal of Agricultural Food Chemistry*, **52**, 2226–2229.
- [9] Kulling SE, Rawel HM. (2008) Chokeberry (*Aronia melanocarpa*) – A review on the characteristic components and potential health effects. *Planta Medica*, **74**, 1625–1634.
- [10] Oszmianański J, Wojdyło A. (2005) *Aronia melanocarpa* phenolics and their antioxidant activity. *European Food Research and Technology*, **221**, 809–813.
- [11] Slimestad R, Torskangerpoll K, Nateland HS, Johannessen T, Gisked NH. (2005) Flavonoids from black chokeberries, *Aronia melanocarpa*. *Journal of Food Composition and Analysis*, **18**, 61–68.
- [12] Wangenstein H, Bräunlich M, Nikolic V, Malterud KE, Slimestad R, Barsett H. (2014) Anthocyanins, proanthocyanidins and total phenolics in four cultivars of *Aronia*: antioxidant and enzyme inhibitory effects. *Journal of Functional Foods*, **7**, 746–752.
- [13] Horszwald A, Julien H, Andlauer W. (2013) Characterisation of *Aronia* powders obtained by different drying processes. *Food Chemistry*, **141**, 2858–2863.
- [14] Hellström J, Mattila P, Karjalainen R (2013) Stability of anthocyanins in berry juices stored at different temperatures. *Journal of Food Composition and Analysis*, **31**, 12–19.
- [15] Sójka M, Kołodziejczyk K, Milala J. (2013) Polyphenolic and basic chemical composition of black chokeberry industrial by-products. *Industrial Crops Production*, **51**, 77–86.
- [16] Ramić M, Vidović S, Zecović Z, Vladić J, Cvejic A, Pavlić B. (2015) Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from *Aronia melanocarpa* by-products from filter-tea factory. *Ultrasonics Sonochemistry*, **23**, 360–368.
- [17] Kraujalyte V, Leitner E, Rimantas Venskutonis P. (2013) Characterization of *Aronia melanocarpa* volatiles by head-space-solid-phase microextraction (HS-SPME) simultaneous distillation/extraction (SDE) and gas-chromatography-olfactometry (GC-O). *Journal of Agricultural Food Chemistry*, **61**, 4728–4736.
- [18] Singleton VL, Orthofer R, Lamuela-Raventó RM. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of the Folin-Ciocalteu reagent. *Methods in Enzymology*, **299**, 152–178.
- [19] Dewanto V, Wu X, Adom KK, Liu RH. (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural Food Chemistry*, **50**, 3010–3014.
- [20] ISO 1183-1:2004
- [21] Hokkanen J, Mattila S, Jaakola L, Pirttilä AM, Tolonen A. (2009) Identification of phenolic compounds from lingoberry (*Vaccinium vitis-idaea* L.), bilberry (*Vaccinium myrtillus* L.) and hybrid bilberry (*Vaccinium x intermedium* Ruthe L.) leaves. *Journal of Agricultural Food Chemistry*, **57**, 9437–9447.
- [22] Clifford MN, Johnston KL, Knight S, Kuhnert N. (2003) Hierarchical scheme for LC–MSn identification of chlorogenic acids. *Journal of Agricultural Food Chemistry*, **51**, 2900–2911.
- [23] Howard LR, Castrodale C, Brownmiller C, Mauromoustakos A. (2010) Jam processing and storage effects on blueberry polyphenolics and antioxidant capacity. *Journal of Agricultural Food Chemistry*, **58**, 4022–4029.
- [24] Häkkinen SH, Kärenlampi SO, Mykkänen HM, Törrönen AR. (2000) Influence of domestic processing and storage on flavonol contents in berries. *Journal of Agricultural Food Chemistry*, **48**, 2960–2965.
- [25] Garcia-Viguera C, Zafrilla P, Artés F, Romero F, Abellán P, Tomás-Barberán FA (1998) Colour and anthocyanin stability of red raspberry jam. *Journal of the Science of Food and Agriculture*, **78**, 565–573.
- [26] Koponen JM, Happonen AM, Mattila PH, Törrönen AR. (2007) Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *Journal of Agricultural Food Chemistry*, **55**, 1612–1619.
- [27] Kähkönen MP, Hopia AI, Heinonen M. (2001) Berry phenolics and their antioxidant activity. *Journal of Agricultural Food Chemistry*, **49**, 4076–4082.
- [28] Hudec J, Barkoš D, Mravec D, Kobida L, Burdová M, Turianica I, Hlušek J. (2006) Content of phenolic compounds and free polyamine in black chokeberry (*Aronia melanocarpa*) after application of polyamine biosynthesis regulators. *Journal of Agricultural Food Chemistry*, **54**, 3625–3628.
- [29] Piga A, Del Caro A, Corda G. (2003) From plums to prunes: Influence of drying parameters on phenols and antioxidant activity. *Journal of Agricultural Food Chemistry*, **51**, 3675–3681
- [30] Patras A, Brunton NP, Da Pieve S, Butler F. (2009) Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science and Emerging Technology*, **10**, 308–313.
- [31] Kovats E. (1958) Gas-chromatographische Charakterisierung organischer Verbindungen. Part 1: Retentionsindices aliphatischer Halogenide, Alkohol, Aldehyde und Ketone. *Helvetica Chimica Acta*, **206**, 1915–1932.
- [32] Van Den Dool H, Kratz PD. (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, **11**, 463–471.
- [33] Aprea E, Biasioli F, Carlin S, Endrizzi I, Gasperi F. (2009) Investigation of volatile compounds in two raspberry cultivars by two headspace techniques: solid-phase microextraction/gas chromatography-mass spectrometry (SPME/GC-MS) and proton-transfer reaction-mass spectrometry (PTR-MS). *Journal of Agricultural Food Chemistry*, **57**, 4011–4018.
- [34] Vázquez-Araújo L, Chambers IV E, Adhikaria K, Carbonell-Barrachinad AA. (2011) Instrumental and sensory aroma profile of pomegranate juices from the USA: differences between fresh and commercial juice. *Flavour and Fragrance Journal*, **26**, 129–138.

|  |     |
|--|-----|
| <b>Acetophenones Isolated from <i>Acronychia pedunculata</i> and their Anti-proliferative Activities</b><br>Chihiro Ito, Takuya Matsui, Yoshiaki Ban, Tian-Shung Wu and Masataka Itoigawa  | 83  |
| <b>Xanthones from <i>Garcinia propinqua</i> Roots</b><br>Pornphimol Meesakul, Acharavadee Pansanit, Wisanu Maneerat, Tawanun Sripisut, Thunwadee Ritthiwigrom, Theeraphan Machan, Sarot Cheenpracha and Surat Laphookhieo                                      | 87  |
| <b>A New Antibacterial Tetrahydronaphthalene Lignanamide, Foveolatamide, from the Stems of <i>Ficus foveolata</i></b><br>Wirud Meerungrueang and Parkphoom Panichayupakaranant   | 91  |
| <b>Antifungal and Cytotoxic Assessment of Lapachol Derivatives Produced by Fungal Biotransformation</b><br>Eliane O. Silva, Antonio Ruano-González, Raquel A. dos Santos, Rosario Sánchez-Maestre, Nieve A. J. C. Furtado, Isidro G. Collado and Josefina Aleu | 95  |
| <b>Polyphenols and Volatile Compounds in Commercial Chokeberry (<i>Aronia melanocarpa</i>) Products</b><br>Annalisa Romani, Pamela Vignolini, Francesca Ieri and Daniela Heimler   | 99  |
| <b>Volatile Components of the Stressed Liverwort <i>Conocephalum conicum</i></b><br>Nurunajah Ab Ghani, Agnieszka Ludwiczuk, Nor Hadiani Ismail and Yoshinori Asakawa  | 103 |
| <b>Chemical Composition of the Essential Oil of <i>Bupleurum fontanesii</i> (Apiaceae) Growing Wild in Sicily and its Activity on Microorganisms Affecting Historical Art Crafts</b><br>Simona Casiglia, Maurizio Bruno, Federica Senatore and Felice Senatore | 105 |
| <b>Chemical Composition and Antimicrobial Activity of the Essential Oil from Aerial Parts of Algerian <i>Pulicaria mauritanica</i></b><br>Mohammed Gherib, Chahrazed Bekhechi, Fewzia Atik Bekkara, Ange Bighelli, Joseph Casanova and Félix Tomi              | 109 |
| <b><i>Origanum vulgare</i> and <i>Thymbra capitata</i> Essential Oils from Spain: Determination of Aromatic Profile and Bioactivities</b><br>Alejandro Carrasco, Enrique Perez, Ana-Belen Cutillas, Ramiro Martinez-Gutierrez, Virginia Tomas and Jose Tudela  | 113 |
| <b><u>Accounts/Reviews</u></b>   |     |
| <b><i>In vivo</i> Cytotoxicity Studies of Amaryllidaceae Alkaloids</b><br>Jerald J. Nair, Jaume Bastida and Johannes van Staden  | 121 |

# Natural Product Communications

## 2016

Volume 11, Number 1

### Contents

#### Original Paper

|  |    |
|--|----|
| <b>Chemical Constituents and LC-profile of Fresh Formosan <i>Lonicera japonica</i> Flower Buds</b><br>I-Wen Lo, Yuan-Bin Cheng, Yi-Jin Hsieh, Tsong-Long Hwang, Deng-En Shieh, Fang-Rong Chang and Yang-Chang Wu   | 1  |
| <b>Isolation and Characterization of Sclerinenone C from <i>Scleria striatinux</i></b><br>Kennedy D. Nyongbela, Felix L. Makolo, Thomas R. Hoye and Simon MN Efange  | 5  |
| <b>Cytotoxic and Pro-apoptotic Activities of Sesquiterpene Lactones from <i>Inula britannica</i></b><br>Ping Xiang, Xin Guo, Yang-Yang Han, Jin-Ming Gao and Jiang-Jiang Tang  | 7  |
| <b>Influence of Merosesquiterpenoids from Marine Sponges on Seedling Root Growth of Agricultural Plants</b><br>Elena L. Chaikina, Natalia K. Utkina and Mikhail M. Anisimov  | 11 |
| <b>A New Cytotoxic Clerodane Diterpene from <i>Casearia graveolens</i> Twigs</b><br>Pornphimol Meesakul, Thunwadee Ritthiwigrom, Sarot Cheenpracha, Tawanun Sripisut, Wisanu Maneerat, Theeraphan Machan and Surat Laphookhieo   | 13 |
| <b>Influence of Tanshinone IIA on the Apoptosis of Human Esophageal Ec-109 Cells</b><br>Yan-qin Zhu, Bai-Yan Wang, Fang Wu, Yong-kang An and Xin-qiang Zhou  | 17 |
| <b>Trocheliolide B, a New Cembranoid Diterpene from the Octocoral <i>Sarcophyton trocheliophorum</i></b><br>Kuan-Ming Liu, Yu-Hsuan Lan, Ching-Chyuan Su and Ping-Jyun Sung  | 21 |
| <b>Synthesis of a Novel 1,2,4-Oxadiazole Diterpene from the Oxime of the Methyl Ester of 1<math>\beta</math>,13-Epoxydihydroquinopimaric Acid</b><br>Elena V. Tretyakova, Elena V. Salimova, Victor N. Odinkov and Usein M. Dzhemilev  | 23 |
| <b>Phytochemical and Biological Investigations of <i>Conradina canescens</i></b><br>Noura S. Dosoky, Debra M. Moriarity and William N. Setzer  | 25 |
| <b>A New Taraxastane-type Triterpenoid from <i>Cleistocalyx operculatus</i></b><br>Phan Minh Giang, Vu Thi Thu Phuong and Truong Thi To Chinh  | 29 |
| <b>Anti-allergic Inflammatory Triterpenoids Isolated from the Spikes of <i>Prunella vulgaris</i></b><br>Hyun Gyu Choi, Tae Hoon Kim, Sang-Hyun Kim and Jeong Ah Kim  | 31 |
| <b>Inhibition of Alpha-Glucosidase by Synthetic Derivatives of Lupane, Oleanane, Ursane and Dammarane Triterpenoids</b><br>El'mira F. Khusnutdinova, Irina E. Smirnova, Gul'nara V. Giniyatullina, Natal'ya I. Medvedeva, Emil Yu. Yamansarov, Dmitri V. Kazakov, Oxana B. Kazakova, Pham T. Linh, Do Quoc Viet and DoThi Thu Huong  | 33 |
| <b>Cycloartane-Type Saponins from <i>Astragalus tmoleus</i> var. <i>tmoleus</i></b><br>Sibel Avunduk, Anne-Claire Mitaine-Offer, Tomofumi Miyamoto, Chiaki Tanaka and Marie-Aleth Lacaille-Dubois  | 37 |
| <b>Profiling and Metabolism of Sterols in the Weaver Ant Genus <i>Oecophylla</i></b><br>Nanna H. Vidkjær, Karl-Martin V. Jensen, René Gislum and Inge S. Fomsgaard   | 39 |
| <b>Steroidal Glucosides from the Rhizomes of <i>Tacca chantrieri</i> and Their Inhibitory Activities of NO Production in BV2 Cells</b><br>Pham Hai Yen, Vu Thi Quynh Chi, Dong-Cheol Kim, Wonmin Ko, Hyuncheol Oh, Youn-Chul Kim, Duong Thi Dung, Nguyen Thi Viet Thanh, Tran Hong Quang, Nguyen Thi Thanh Ngan, Nguyen Xuan Nhiem, Hoang Le Tuan Anh, Chau Van Minh and Phan Van Kiem | 45 |
| <b>Antimicrobial Metabolites from a Marine-Derived Actinomycete in Vietnam's East Sea</b><br>Quyen Vu Thi, Van Hieu Tran, Huong Doan Thi Mai, Cong Vinh Le, Minh Le Thi Hong, Brian T. Murphy, Van Minh Chau and Van Cuong Pham  | 49 |
| <b>Aspidosperma-type Alkaloids from <i>Melodinus suaveolens</i></b><br>Jian Zhang, Min Song, Zhi-wen Liu, Hua Xiao, Chun-lin Fan, Xiao-qi Zhang and Wen-cai Ye   | 53 |
| <b>Molecular Docking and Binding Mode Analysis of Plant Alkaloids as <i>in vitro</i> and <i>in silico</i> Inhibitors of Trypanothione Reductase from <i>Trypanosoma cruzi</i></b><br>Alonso J. Argüelles, Geoffrey A. Cordell and Helena Maruenda  | 57 |
| <b>Cordycepin, a Natural Antineoplastic Agent, Induces Apoptosis of Breast Cancer Cells via Caspase-dependent Pathways</b><br>Di Wang, Yongfeng Zhang, Jiahui Lu, Yang Wang, Junyue Wang, Qingfan Meng, Robert J. Lee, Di Wang and Lesheng Teng  | 63 |
| <b>Absolute Stereochemistry of the <math>\beta</math>-Hydroxy Acid Unit in Hantupeptins and Trungapeptins</b><br>Deepak Kumar Gupta, Gary Chi Ying Ding, Yong Chua Teo and Lik Tong Tan  | 69 |
| <b>Electron Ionization Mass Spectrometry-based Metabolomics Studies of <i>Sophora flavescens</i> can Identify the Geographical Origin of Root Samples</b><br>Ryuichiro Suzuki, Hisahiro Kai, Yoshihiro Uesawa, Koji Matsuno, Yoshihito Okada and Yoshiaki Shirataki  | 73 |
| <b>Qualitative and Quantitative Analysis of Flower Pigments in Chocolate Cosmos, <i>Cosmos atrosanguineus</i>, and its Hybrids</b><br>Kotarou Amamiya and Tsukasa Iwashina   | 77 |
| <b>A New Geranylated Chalcone from <i>Andrographis lobelioides</i></b><br>Manne Sumalatha, Aluru Rammohan, Duvvuru Gunasekar, Alexandre Deville and Bernard Bodo   | 79 |
| <b>Pterocarpan from <i>Derris laxiflora</i></b><br>Shih-Chang Chien, Hsi-Lin Chiu, Wei-Yi Cheng, Yong-Han Hong, Sheng-Yang Wang, Jyh-Horng Wu, Chun-Ching Shih, Jung-Chun Liao and Yueh-Hsiung Kuo   | 81 |

Continued inside backcover