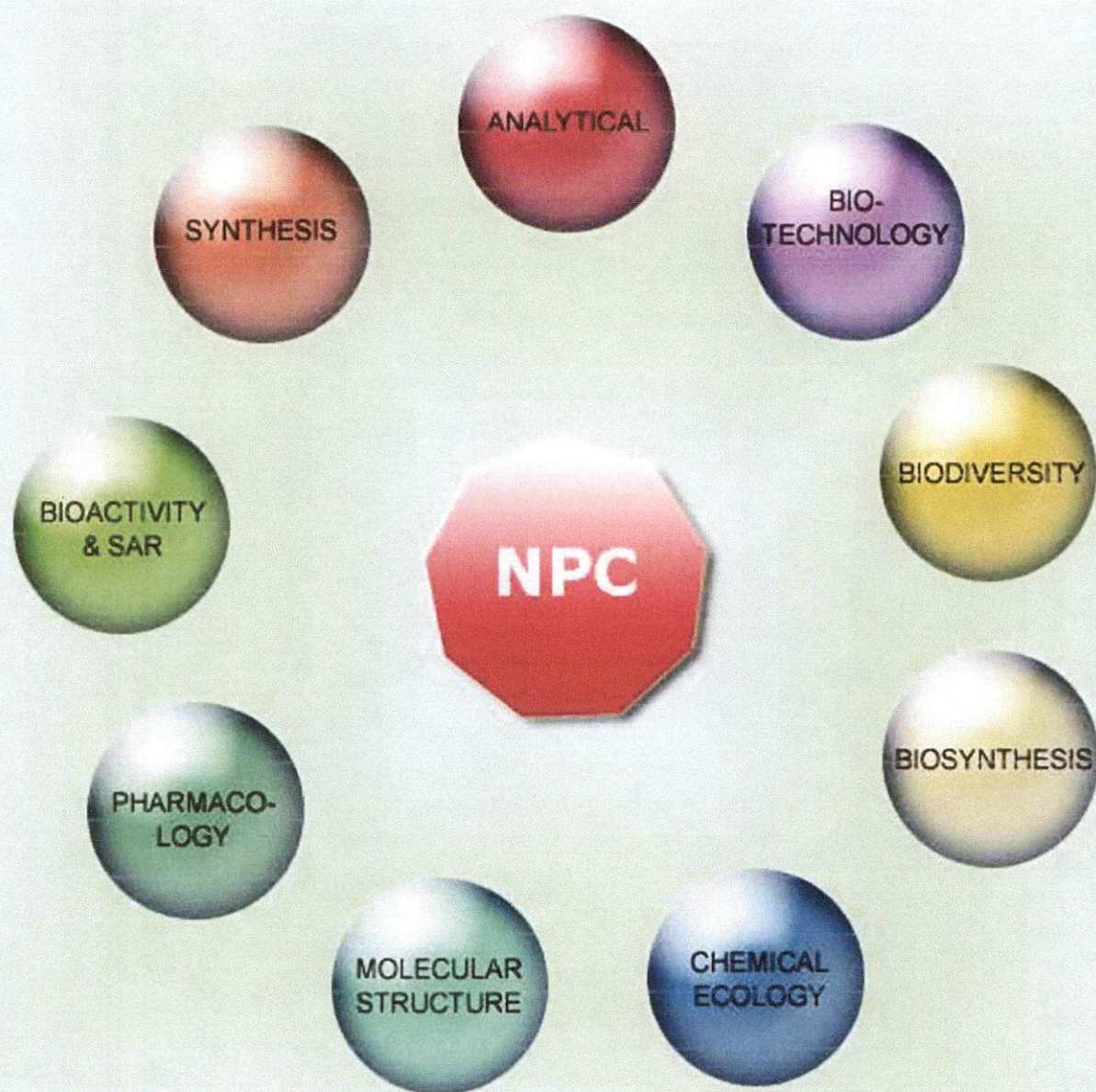


# NATURAL PRODUCT COMMUNICATIONS

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



## **NPC-Bromo Issue**

Volume 13, Issue 12, Pages 1569-1756, 2018  
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 MAURIZIO BRUNO

Department STEBICEF,  
University of Palermo, Viale delle Scienze,  
Parco d'Orleans II - 90128 Palermo, Italy  
maurizio.bruno@unipa.it

## PROFESSOR CARMEN MARTIN-CORDERO

Department of Pharmacology, Faculty of Pharmacy,  
University of Seville, Seville, Spain  
carmenmc@us.es

## 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  
kalininv@pihoc.dvo.ru

## 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 PING-JYUN SUNG

National Museum of Marine Biology and Aquarium  
Checheng, Pingtung 944  
Taiwan  
pjsung@mmba.gov.tw

## 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. Giovanni Appendino  
Novara, Italy

Prof. Norbert Arnold  
Halle, Germany

Prof. Yoshinori Asakawa  
Tokushima, Japan

Prof. Vassaya Bankova  
Sofia, Bulgaria

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

Prof. Anna R. Bilia  
Florence, Italy

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 Lacaille-Dubois  
Dijon, France

Prof. Shoen-Sheng Lee  
Taipei, Taiwan

Prof. M. Soledade C. Pedras  
Saskatoon, Canada

Prof. Luc Pieters  
Antwerp, Belgium

Prof. Peter Proksch  
Düsseldorf, Germany

Prof. Phila Raharivelomanana  
Tahiti, French Polynesia

Prof. Stefano Serra  
Milano, Italy

Dr. Bikram Singh  
Palampur, India

Prof. Marina Stefova  
Skopje, Republic of Macedonia

Prof. Leandros A. Skaltsounis  
Zografou, Greece

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. 2018 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.

## **Editorial**

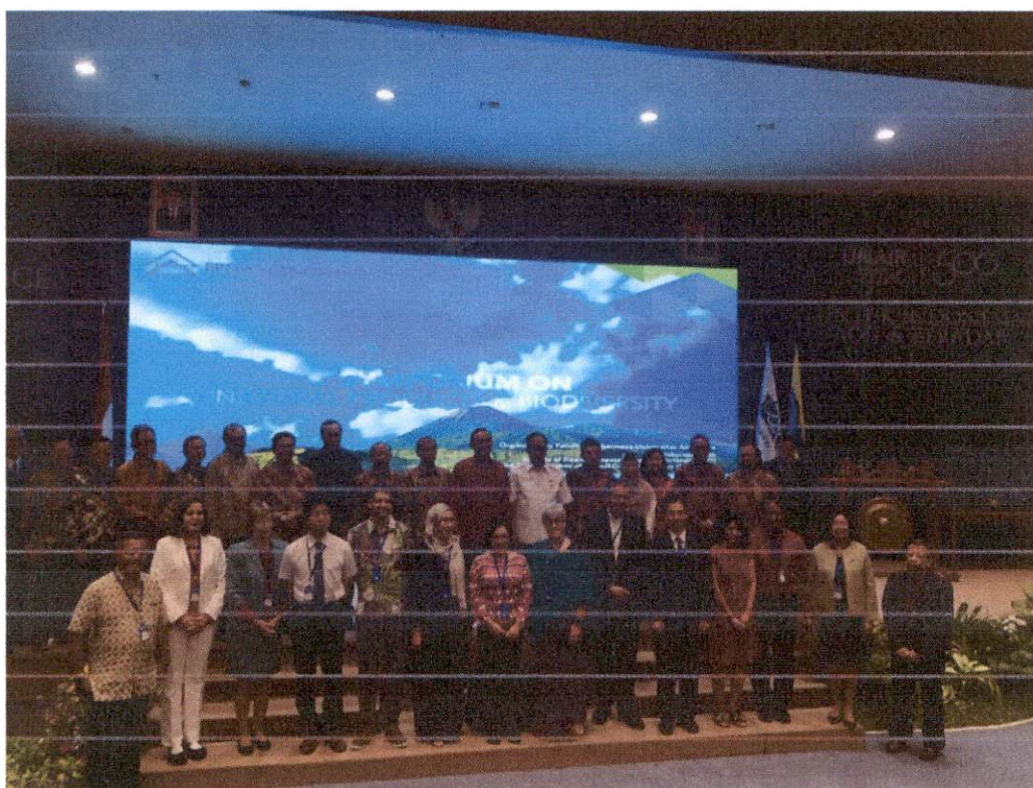
### **NPC-Bromo: Special Issue**

I am very grateful to Prof. Bambang Prajogo, Chairman, Bromo Conference (Symposium on Natural products & Diversity), and Dr. Tutik Sri Wahyuni, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia, and the Organizing Committee for arranging this issue, originating from the Bromo Conference-2018, which was held in Surabaya, Indonesia, from July 11–12, 2018, and attended by a large number of participants.

The first part of the December 2018 edition is devoted to selected manuscripts (8) presented at Bromo-2018. I am very grateful to Profs. Bambang Prajogo and Tutik Sri Wahyuni for extending an invitation to participate in this scientific meeting, as well as for organizing this issue. The editors join me in thanking Profs Prajogo and Wahyuni, the authors, and the reviewers for their efforts that have made this issue possible, and to the production department for putting it into print.

Pawan K. Agrawal  
Editor-in-Chief

## Introduction to NPC Bromo Issue



This special issue contains selected papers previously presented at the Bromo Conference: Symposium of Natural Products and Biodiversity held in Surabaya on July 11-12, 2018. This symposium was organized by Universitas Airlangga, Surabaya, Indonesia in collaboration with the Indonesian Association of Natural Drug Researchers (PERHIPBA) and the Phytochemical Society of Asia (PSA). It was held to commemorate the 10th anniversary of the IOCD seminar in Surabaya. The Bromo Conference provides a forum for the exchange of information on Natural Products within all of the related topics, as well as with the aim to build and strengthen scientific cooperation between the research institutions.

Academic and other researchers, industrial practitioners and students participated in the symposium. The topics of interest covered in the Bromo Conference included ethnomedicine, implementation of the Nagoya Protocol, sustainable valorization of biodiversity, bioactivity of natural products, metabolomics, phytopharmaceutical technology, clinical trials and other related subjects. The manuscripts have been reviewed by the Organizing Committee members, Prof. Katsuyosi Matsunami, Prof. Gunawan Indrayanto, and Prof. Angela Calderon, and edited by Dr Pawan Agrawal. The manuscripts underwent further rigorous peer review and were revised before being accepted for publication.

This special issue of *Natural Product Communications* is intended to help readers gain knowledge from the contributors, as well as to provide an overview of the various fields to improve natural products research.

We would like to present a special thanks to the authors and reviewers. Also, we are grateful to Dr Pawan K Agrawal, the Editor-in-Chief of *Natural Product Communications* and the editorial team for their assistance in the preparation of this issue and for the continued support and collaboration between Universitas Airlangga, Surabaya, Indonesia and *NPC*.

Prof. Bambang Prajogo EW  
Chairman of the Organizing Committee  
Bromo Conference,  
Symposium on Natural Product and Biodiversity  
Faculty of Pharmacy,  
Universitas Airlangga, Surabaya  
Indonesia

# Natural Product Communications

## 2018

Volume 13, Number 12

### Contents

#### Editorial

Pawan K. Agrawal

i

#### Introduction

Bambang Prajogo

iii

#### Original Paper

Page

##### **Optimization of *Clinacanthus nutans* Biodegradable Analgesic Patch**

Em-on Chairateep and Chalerm Sak Thavornwat

1569

##### **A New Antibacterial Polyketide from the Endophytic Fungi *Aspergillus fumigatiaffinis***

Antonius R. B. Ola, Bibiana D Tawo, Henderiana L. L. Belli, Peter Proksch, Dhana Tommy and Euis Holisotan Hakim

1573

##### **Styryl Lactones from Roots and Barks *Goniothalamus lanceolatus***

Nurulfazlina Edayah Rasol, Fasihuddin Badruddin Ahmad, Chun-Wai Mai, Nur Vicky Bihud, Fauziah Abdullah, Khalijah Awang and Nor Hadiani Ismail

1575

##### **Antiviral Activities of *Curcuma* Genus against Hepatitis C Virus**

Tutik Sri Wahyuni, Adita Ayu permatasari, Tri Widiandani, Achmad Fuad, Aty Widyawaruyanti, Chie Aoki-Utsubo and Hak Hotta

1579

##### **Beneficial Effect of Supercritical Carbon Dioxide Extracted (SC-CO<sub>2</sub>) Dabai (*Canarium odontophyllum*) Pulp Oil in Hypercholesterolemia-Induced SPF Sprague-Dawley Rats**

Noor Atiqah Aizan Abdul Kadir, Azrina Azlan, Faridah Abas and Intan Safinar Ismail

1583

##### **Chemical Analysis of Red Ginger (*Zingiber officinale* Roscoe var *rubrum*) Essential Oil and Its Anti-biofilm Activity against *Candida albicans***

Tristia Rinanda, Rizki Puji Isnanda and Zulfitri

1587

#### Accounts/Reviews

##### **Antiplasmodial Anthraquinones from Medicinal Plants: The Chemistry and Possible Mode of Actions**

Che Puteh Osman and Nor Hadiani Ismail

1591

##### **Recent Development of Quality Control Methods for Herbal Derived Drug Preparations**

Gunawan Indrayanto

1599

#### Original Paper

##### **A New Noriridoid and Six Phenolic Compounds from *Rhopalocnemis phalloides***

Nguyen Quang Hung, Nguyen Thi Luyen, Nguyen The Cuong, Tran Huy Thai, Nguyen Thanh Tung and Nguyen Tien Dat

1607

##### **Differential Antifungal Efficiency of Geraniol and Citral**

Roopa Gaonkar, Pramod K Avti and Gurumurthy Hegde

1609

##### **Characterization and Biological Properties of Zederone and Zedoarondiol from Rhizomes of *En-Lueang* (*Curcuma* cf. *amada*)**

Songyot Anuchapreeda, Nattakanwadee Khumpirapang, Sawitree Chiampanichayakul, Wariya Nirachonkul, Aroonchai Saijai, Toyonobu Usuk and Siriporn Okonogi

1615

##### **A New Tocopherol Derivative and Cytotoxicity from the Leaves of *Dalbergia velutina***

Sutin Kaennakam, Thammarat Aree, Kitiya Rassamee, Pongpun Siripong and Santi Tip-pyang

1619

##### **Psolusosides C<sub>1</sub>, C<sub>2</sub>, and D<sub>1</sub>, Novel Triterpene Hexaosides from the Sea Cucumber *Psolus fabricii* (Psolidae, Dendrochirotida)**

Alexandra S. Silchenko, Sergey A. Avilov, Anatoly I. Kalinovskiy, Vladimir I. Kalinin, Pelageya V. Andrijaschenko and Pavel S. Dmitrenok

1623

##### **A New Pentaacyclic Ergosteroid from Fungus *Aspergillus* sp. SCSIO41211 Derived of Mangrove Sediment Sample**

Huaming Tao, Yunqiu Li, Xiuping Lin, Xuefeng Zhou, Junde Dong, Yonghong Liu and Bin Yang

1629

##### **Design, Synthesis and Cytotoxic Evaluation of 4-Anilinoquinazoline-triazole-AZT Hybrids as Anticancer Agents**

Le Nhat Thuy Giang, Nguyen Thi Nga, Dinh Thuy Van, Dang Thi Tuyet Anh, Hoang Thi Phuong, Nguyen Ha Thanh, Le Thi Tu Anh, Vu Quoc Trung, Nguyen Van Tuyen and Phan Van Kiem

1633

##### **Antibacterial Activity of Flavans from *Crinum distichum***

Rom  ol Romain Koagne, Frederick Annang, Mercedes de la Cruz, Gabin Thierry M. Bitchagno, Ignacio Perez-Victoria, Ingrid Simo Konga, Francisca Vicente, Fernando Reyes and Pierre Tane

1637

##### **A New Flavonol Glucoside from the Leaves of *Crypsinus trilobus***

Pham Thi Bich Hanh, Ngo Thi Phuong, Le Ngoc Hung, Nguyen Quoc Dat, Dang Minh Tri, Do Truong Thien and Le Minh Ha

1639

Continued Overleaf

## LIST OF AUTHORS

Abas, F	1583	Gabbia, D	1691	Ma, Q	1655	Stien, D	1731
Abdullah, F	1575	Gao, G	1709	Mai, CW	1575	Stonik, VA	1743
Abubakar, S	1747	Gaonkar, R	1609	Martin, SD	1691	Sugiyama, S	1699
Ahmad, FB	1575	Gerbaux, P	1659	Matsuo, M	1699	Suzuki, M	1699
Ahn, JS	1649	Giang, INT	1633	Mawang, CI	1747		
Andrijaschenko, PV	1623	Giang, LNT	1677	Medina-Ramírez, G	1715	Takahashi, S	1699
Andueza-Leal, F	1715	Giovannini, A	1727	Mittal, N	1673	Tam, KT	1677
Anh, DTT	1633,1677	Gu, Q	1705	Mizuno, T	1641	Tanc, P	1637
Anh, LTT	1633,1677			Molnár, K	1695	Tao, H	1629
Anjos, O	1685	Ha, LM	1639	Moreno, DA	1681	Tava, A	1727
Annang, F	1637	Hakim, EH	1573	Murai, Y	1641	Tawo, BD	1573
Anuchapreeda, S	1615	Hamada, N	1699			Thai, TH	1607
Aoki-Utsubo, C	1579	Hanh, PTB	1639	Nakamura, K	1641	Tham, PT	1677
Aragon-Alencastre, LJ	1725	Hegde, G	1609	Nga, NT	1633	Thanh, HT	1677
Araujo-Baptista, L	1715	Hotta, H	1579	Nirachonkul, W	1615	Thanh, NH	1633
Aree, T	1619	Hu, X	1721			Thanh, NH	1677
Avilov, SA	1623	Hung, LN	1639	Oh, KY	1649	Thavornwat, C	1569
Avti, PK	1609	Hung, NQ	1607	Oh, SR	1649	Thien, DT	1639
Awang, K	1575			Okonogi, S	1615	Tip-pyang, S	1619
Ayu permatasari, A	1579	Indrayanto, G	1599	Ola, ARB	1573	Todoki, K	1699
Azlan, A	1583	Ishola, A	1741	Osman, CP	1591	Tomayila-Cruz, C	1725
Azman, AS	1747	Ismail, IS	1583	Ouaini, N	1731	Tommy, D	1573
		Ismail, NH	1575			Toyama, T	1699
Baenas, N	1681	Ismail, NH	1591	Park, MH	1649	Tri, DM	1639
Belli, HLL	1573	Isnanda, RP	1587	Paula, VB	1685	Trung, VQ	1633
Bihud, NV	1575	Iwashina, T	1641	Perez-Victoria, I	1637	Tung, NT	1607
Bitchagno, GTM	1637			Phuong, HT	1633	Tuyen, NV	1633
Borics, A	1695	Jaramillo-Abril, D	1715	Phuong, HT	1677	Tuyen, NV	1677
Boselli, C	1727	Jun, JH	1667	Phuong, NT	1639		
Brasseur, L	1659			Pino, JA	1725	Usuk, T	1615
		Kadir, NAAA	1583	Ponomarenko, LP	1743		
Caeiro, A	1685	Kaennakam, S	1619	Proksch, P	1573	Valarezo-García, C	1715
Calevo, J	1727	Kalinin, VI	1623			Van, DT	1633
Campos, MG	1685	Kalinovsky, AI	1623	Quynh, DH	1677	Vicente, F	1637
Carpenter, B	1715	Kasi, PB	1695				
Carrara, M	1691	Kassouf, A	1731	Rao, P	1709	Wada-Takahashi, S	1699
Caulier, G	1659	Ke, L	1709	Rasol, NE	1575	Wahyuni, TS	1579
Chaillou, S	1731	Khumpirapang, N	1615	Rassamee, K	1619	Wang, H	1709
Chairatecep, E	1569	Kiem, PV	1633,1677	Retamal-Salgado, J	1681	Watanabe, K	1699
Chiampanichayakul, S	1615	Kim, H	1649	Reyes, F	1637	Wei, M	1721
Chinh, PT	1677	Kim, JH	1649	Rinanda, T	1587	Wei, R	1655
Cruz, M	1637	Knott, MG	1741	Robustelli della Cuna, JS	1727	Widiandani, T	1579
Cuong, NT	1607	Ko, SK	1649	Rodríguez, JL	1725	Widyawaruyanti, A	1579
		Koagne, RR	1637	Rutledge, DN	1731		
Dat, NQ	1639	Kokubugata, G	1641	Ryoo, IJ	1649	Xie, Y	1721
Dat, NT	1607	Konga, IS	1637	Ryu, HW	1649		
Delgado, T	1685	Kotormán, M	1695	Saiai, A	1615	Yang, B	1629
Devkota, HP	1641	Kwon, MC	1649	Saito, Y	1641	Yang, M	1655
Djabayan-Djibeyan, P	1715			Sang, Z	1655	Yokota, M	1641
Djabayan-Russo, A	1715	László, L	1695	Sarkar, R	1673	Yoshida, A	1699
Dmitrenok, PS	1623	Lee, JY	1667	Sasaki, H	1699	Yoshino, F	1699
Dong, J	1629	Lee, SM	1649	Sen, T	1673		
		Lee, TB	1667	Seo, EJ	1667	Zapata, N	1681
Eeckhaut, I	1659	León-Leal, A	1715	Shi, H	1721	Zgheib, R	1731
El Beyrouthy, M	1731	Li, P	1705	Siatka, T	1645	Zhang, Z	1721
Estevinho, LM	1685	Li, Y	1629	Silchenko, AS	1623	Zhong, G	1655
		Lin, X	1629	Siripong, P	1619	Zhou, J	1709
Farinha, N	1685	Lin, Z	1721	Solis-Quispe, JA	1725	Zhou, X	1629
Ferri, N	1691	Liu, Y	1629	Solis-Quispe, L	1725	Zulfutri	1587
Flammang, P	1659	López, MD	1681	Song, D	1705		
Fuad, A	1579	Luyen, NT	1607	Sorensen, J	1673		

Antiviral Activities of *Curcuma* Genus against Hepatitis C VirusTutik Sri Wahyuni<sup>a,b\*</sup>, Adita Ayu permatasari<sup>b</sup>, Tri Widiandani<sup>c</sup>, Achmad Fuad<sup>a,b</sup>, Aty Widyawaruyanti<sup>a,b</sup>, Chie Aoki-Utsubo<sup>d</sup> and Hak Hotta<sup>e</sup><sup>a</sup>Department of Pharmacognocny and Phytochemistry, Faculty of Pharmacy, Airlangga University, Surabaya 60115<sup>b</sup>Institute of Tropical Disease, Airlangga University, Surabaya 60115, Indonesia<sup>c</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya 60115, Indonesia<sup>d</sup>Department of International Health, Kobe University Graduate School of Health Sciences, 7-10-2, Tomogaoka, Suma-ku, Kobe 654-0142, Japan<sup>e</sup>Faculty of Clinical Nutrition and Dietetics, Konan Women's University, 6-2-23 Morikita-machi, Higashinada-ku,, Kobe 658-0001, Japan

wahyuni.tutiksri@yahoo.com; tutik-s-w@ff.unair.ac.id

Received: August 8<sup>th</sup>, 2018; Accepted: November 14<sup>th</sup>, 2018

Hepatitis C virus (HCV) infection is one of the major public health problems in the world. Even though the new agents are shown to increase the sustained virology response, however, there are still many people who cannot access the therapy due to the high cost. Moreover, the emergence of resistance and side effects presented the necessity to develop alternative treatment agents for HCV infection. Plants of the genus of curcuma are popular among traditional medicines in the world, including Indonesia. They have been used for many herb remedies and reported to possess many biological activities. Several plants from the curcuma genus were known as treatment agents in liver disease and jaundice. Our current study determines antiviral activities of *Curcuma domestica*, *Curcuma xanthorrhiza*, and *Curcuma heyneana* against HCV and further examines the mechanism of actions. Antiviral activity was performed by *in vitro* culture cells using Huh 7.5it cells and treated with the mixture of extract and virus JFH1. The effects of extracts in HCV life cycle were determined by mode of action analysis to examine the action of substances in the entry or post entry steps. The results revealed that ethanol extract of *C. domestica*, *C. xanthorrhiza*, and *C. heyneana* showed strong anti-HCV activities with IC<sub>50</sub> values of 1.68 ± 0.05, 4.93 ± 0.42 and 5.49 ± 0.59 µg/mL, respectively without any cytotoxicity effect. Mode of action analysis demonstrated that of *C. domestica* and *C. heyneana* exhibit HCV in the entry step, while *C. xanthorrhiza* inhibit in the entry and post entry steps of HCV life cycle. Docking analysis to predict the interaction of curcumin, the main compound of curcuma genus, revealed a strong interaction between curcumin and 4GAG receptor, a protein involved in the entry step of HCV infection. Moreover, it was also reported to possess good interaction with 4EAW, an HCV NS5B, which plays an important role in HCV replication. These results suggested that *C. domestica*, *C. xanthorrhiza*, and *C. Heyneana* possessed strong inhibition against hepatitis C virus, therefore they may be good candidates for anti-HCV agents.

**Keywords:** Hepatitis C virus, *Curcuma domestica*, *Curcuma xanthorrhiza*, *Curcuma heyneana*, Curcumin, Docking analysis.

Hepatitis C virus (HCV) infection is still a big issue in the world. It is estimated that 71 million people suffer chronic HCV and approximately 400.000 people die each year due to cirrhosis and hepatocellular carcinoma [1]. Direct acting antivirals (DAAs) are currently used to cure HCV infection. Oral interferon (IFN) free regimen by combination of NS3/NS4A or NS5A inhibitor increased the sustained virology response (SVR). However, the emergence of antiviral drug resistance and the limited patients who can access drugs due to the high cost remain the necessities to find new effective anti-viral agents [2, 3].

Medicinal plants are potential resources to search for new drug candidates. They consist of various chemical substances possessing strong biological activities including anti-HCV activities. Secondary metabolites of plants, such as silymarin, epigallocatechin gallate, naringenin that belong to the flavonoid compounds, have been reported to inhibit HCV [4-6]. Our previous study reported anti-HCV activity of Indonesian medicinal plants and obtained active anti-HCV extract of *Toona sureni*, *Melicope latifolia*, *Melanolepis mutiglandulosa* and *Ficus fistulosa* with IC<sub>50</sub> value 3.5-15.0 µg/mL [7]. In another study we evaluated *Ruta angustifolia* leaves and further isolated anti-HCV compounds, chalepin, a coumarin compound and pseudane IX, an alkaloid compound which mediated a strong anti-HCV activity [8]. Exploration of natural sources to search for anti-HCV activity still remained a big chance.

*Curcuma domestica*, *Curcuma xanthorrhiza* and *Curcuma heyneana* belong to the *Zingiberaceae* family. Plants of the genus of curcuma are popular in many areas in the world for several kinds of diseases including their use in traditional herbs [9]. In Indonesia, it has been used for many ingredients of *Jamu*, the traditional medicine of Indonesia [10]. *C. domestica* or *C. longa*, also called turmeric have been used for infection, dermatologic diseases and depression in India and China. Recently, it also shows anti-oxidant, anti-inflammatory, anti-cancer and antibacterial activities [11-13]. *C. xanthorrhiza* is locally known as temulawak in Indonesia. The isolated compound from the fresh rhizome, xanthorrhizol, possesses antimicrobial activities against pathogenic bacteria and fungi [14-16]. It has been reported to have hepatoprotective activities, reduced the fatty liver symptom and inhibit alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and protein content [10, 15]. *C. heyneana* contain oxycurcumenol epoxide, curcumenol and isocurcumenol that have cytotoxicity activity against T-acute lymphoblastic leukemia cells (CEM-SS) with IC<sub>50</sub> values of 11.9, 12.6 and 13.3 µg mL<sup>-1</sup>, respectively [17]. Furthermore, its isolation compounds, heyneanone A, heyneanone C, 4,10-epizedoaronidiol, procurcumenol, aerugidiol, zerumin A, and (E)-15,16-bisnorlabda-8,11-dien-13-one inhibited protein tyrosine phosphatase 1B (PTP1B) with IC<sub>50</sub> values of 42.5, 35.2, 35.1, 45.6, 35.7, 10.4, and 14.7 µM, respectively [18]. Since it has been reported to have many bioactivities, however, there is no report yet for anti-HCV activity.

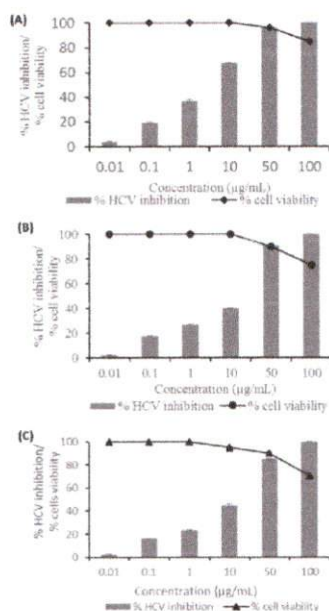
This study examined anti-viral activities of *C. domestica*, *C. xanthorrhiza*, and *C. heyneana* against HCV. Anti-hepatitis C activity was performed by *in vitro* culture cells using Huh 7it and further determine the mode of action of extracts. The cytotoxic effect was accessed by MTT assay.

The results showed that *C. domestica*, *C. xanthorrhiza*, and *C. heyneana* possess potential inhibition against hepatitis C virus without any cytotoxicity (Figure 1). *C. domestica* revealed the strongest anti-HCV activity among the tested extracts and showed a stronger activity than the positive control ribavirin (Table 1).

**Table 1:** Antiviral activity (IC<sub>50</sub>) against HCV and cytotoxicity (CC<sub>50</sub>) of *C. domestica*, *C. xanthorrhiza*, and *C. heyneana*.

Extract	IC <sub>50</sub> (µg/mL)	CC <sub>50</sub> (µg/mL)	SI
<i>C. domestica</i>	1.68 ± 0.05	>100	>59.5
<i>C. xanthorrhiza</i>	4.93 ± 0.42	>100	>20.3
<i>C. heyneana</i>	5.49 ± 0.59	>100	>18.2
Ribavirin (positive control)	2.79 ± 0.3	>50	>10

The data represent means ± SEM of data from three independent experiments.



**Figure 1:** Dose dependent inhibition of extracts and their cytotoxicity. Various concentrations of extracts (A) *Curcuma domestica*, (B) *Curcuma xanthorrhiza*, (C) *Curcuma heyneana*, 100 to 0.01 µg/mL were inoculated to the Huh7it cells (MOI=0.1). After virus adsorption, the cells were cultured with the same concentrations of compounds for 46 hours. The culture supernatants were harvested and titrated for virus infectivity. Percent inhibitions of HCV infectivity by each compound are shown in Figure 1. In parallel, cytotoxicity of the compounds was measured by MTT-1 assay. All three extracts did not show any cytotoxicity effect.

Mode of action analysis was accessed to explore the effect of extracts in the entry or post entry steps of HCV life cycle which divided into: entry steps that include viral attachment and viral entry to the target cells, and post entry steps that include synthesis protein, replication of viral genome, assembly and release of viral particles [19, 20].

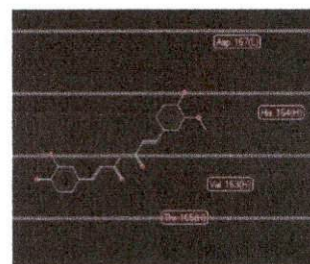
*C. domestica* and *C. heyneana* proved to possess stronger inhibition in the entry step with HCV inhibition higher than 70%, while in the post entry step was around 30% in the concentration of 50 µg/mL. On the other hand, *C. xanthorrhiza* showed a weaker effect in the entry step with 60% inhibition; however, in the post entry step mediated higher inhibition with percentage of HCV inhibition 53.6 ± 3.9% compared to *C. domestica* and *C. heyneana*. Therefore *C. xanthorrhiza* might be conducted to act in the entry and post entry (Table 2).

**Table 2:** Mode of action of *C. domestica*, *C. xanthorrhiza*, and *C. heyneana* extracts.

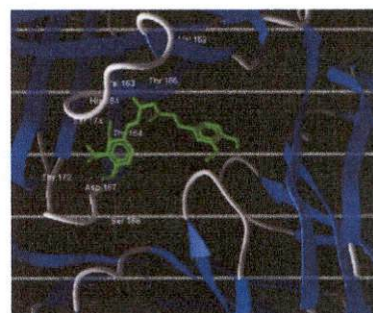
Plant Extract	% HCV inhibition (50µg/mL)			Mode of action
	During +Post infection	During infection	Post infection	
<i>C. domestica</i>	96.2 ± 1.2	75.2 ± 2.1	34.9 ± 2.0	Entry inhibition
<i>C. xanthorrhiza</i>	90.8 ± 0.8	60.8 ± 0.8	53.6 ± 3.9	Entry, Post-entry inhibition
<i>C. heyneana</i>	84.8 ± 1.4	70.2 ± 1.5	30.5 ± 1.4	Entry inhibition

Curcumin, a popular compound in the genus of curcuma, has been identified to have many therapeutic effects including antiviral against HIV, influenza, HPV, H5N1 and all HCV genotypes [10, 21-23]. Curcumin acts as anti-HCV activities by suppressing viral entry step [23] and replication [24].

Further examining to predict the mechanism-of-action of curcumin to the receptors, docking analysis was performed by Molegro Virtual Docking ver 5.5 program to determine the possible interaction of compounds with the protein target. We evaluated several proteins from Protein Data Base which reported to possess interaction with HCV (www.rcsb.org). We found that curcumin has a strong interaction with 4GAG, the protein involved in the entry step of HCV, neutralizing antibody AP33 in complex with E2 epitope [25, 26]. The rerank score of curcumin was -116.94 kcal/mol while the rerank score of ligand was -45 kcal/mol. The lower value of rerank indicated the stronger interaction of curcumin to the receptor. Hydrogen binding of curcumin with Thr 165 and Asp 167, and the steric van der walls between curcumin with Thr 165, Asp 167, His 164 and Val 163 contributed the binding interaction of 4GAG and curcumin (Figure 2). While, the standard ligand revealed hydrogen binding to His 164 and Asp 167, and steric van der walls to Asp 167. The interaction was clearly described in 3D profile (Figure 3).



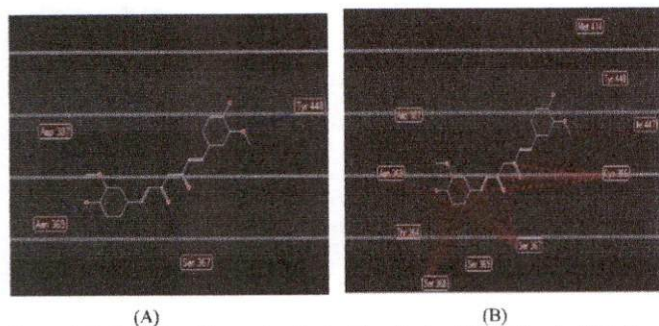
**Figure 2:** Hydrogen bond interaction (dashed blue-line) and Steric-Van der Walls bond interaction (dashed red-line) between Standard Ligand and Curcumin on the active site of HCV protein (4GAG.pdb).



**Figure 3:** The 3D profile of docking interaction of curcumin (green color) with 4GAG protein.

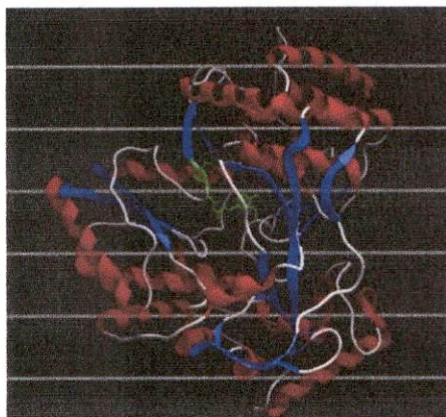
Further analysis was done to observe the possible interaction with other proteins. We obtained a strong interaction between 4EAW.pdb and curcumin. 4EAW is a protein of HCV NS5B, considered to be involved in the replication step of HCV life cycle, a potential therapeutic target in HCV treatment. The rerank score of curcumin was -102.169 kcal/mol, which is similar to the rerank





**Figure 4:** Hydrogen bond interaction (dashed blue-line) and Steric-Van der Walls interaction (dashed red-line) between (A) Standard Ligand and (B) Curcumin on the active site of HCV protein (4EAW.pdb).

score of standard ligand  $-103.24$  kkal/mol. This result indicated a strong interaction between curcumin and receptor and resulting good therapeutic activity. Curcumin revealed more interaction with amino acid of Tyr 448, Ser 367, Asp387, and Asn 369 with hydrogen binding interaction than the standard ligand which only binds with three amino acids, Tyr 448, Asp 318, and Asn 291 (Figure 4). These results indicated that curcumin has strong interaction with 4EAW that might serve as a potential target for HCV inhibition. The 3D profile of curcumin docking interaction is shown in Figure 5.



**Figure 5:** The 3D profile of docking interaction of curcumin (green color) with 4EAW protein.

## Experimental

**Extraction and sample preparation:** The rhizomes of *Curcuma domestica*, *Curcuma xanthorrhiza*, and *Curcuma heyneana* were verified by a licensed botanist of Botanical garden, Purwodadi, Indonesia. The dried powder of the rhizome was extracted with ethanol. The obtained filtrates were evaporated to yield the ethanol extracts *C. domestica*, *C. xanthorrhiza*, and *C. heyneana*. Stock solution was prepared by dissolving the extract in dimethyl sulfoxide (DMSO) to obtain a stock concentration of 100 mg/mL. Serial dilutions of extracts were prepared to yield the concentrations of extracts 100, 50, 10, 1, 0.1 and 0.01  $\mu\text{g/mL}$ .

**Cells and viruses:** Huh7it cells were cultivated in Dulbecco's Modified Eagle Medium (GIBCO Invitrogen, Carlsbad, CS, USA) supplemented with 10% Fetal Bovine Serum (Biowest, Nualle, France), 0.15 mg/mL Kanamycin (Sigma–Aldrich, St. Louis, MO, USA) and non-essential amino acids (GIBCO-Invitrogen) in 5%

CO<sub>2</sub> at 37°C. The culture cells were cultivated and maintained by periodically re-feeding with new medium. The adapted HCV variant was propagated in Huh7it [27]. Culture supernatant from the infected cells was collected at day 2 and day 5 post infection and concentrated using Amicon Ultra centrifugal filter unit. Virus titers were determined for antiviral assay [28, 29].

**Antiviral activity assay:** Antiviral activity assay was conducted as described previously [7, 8, 28, 29]. Huh7it cells ( $5.4 \times 10^4$ ) were seeded for 24 hours. The HCV at multiplication of infection (MOI) of 0.1 in the presence of different concentrations of sample were inoculated to the culture cells. The mixture of extract and virus was incubated for 2 hours. After virus absorption for 2 h, the cells were rinsed with the medium and were further incubated in the medium containing the same sample for 46 hours. Mode of action analysis was performed by time-of-addition experiments. Three series of studies were done. First, the culture was treated with the extract both in pre- and post- inoculation. Second, the culture was only treated with the extract at inoculation steps (2 hours). The third extract was added only after inoculation to examine the action of substance in the post-entry steps of HCV life cycle. Culture supernatants were collected for virus titration. The 50% inhibitory effect (IC<sub>50</sub>) was calculated by SPSS probit analysis [7, 8].

**Virus titration and immunostaining:** Huh7it-1 cells ( $2 \times 10^4$  cells/well) were seeded in a 96-well plate and incubated for 24 hours. Virus supernatants were diluted in the medium and inoculated onto the Huh7it culture cells and incubated for 4 hours. After virus absorption, the cells were cultured with medium containing 0.4% methylcellulose (Sigma–Aldrich) following 41 hours incubation. Infected cells were analyzed with immunostaining using anti-HCV patient anti-serum (250 time dilution on 2% BlockAce/1%BSA/PBS) and HRP-goat antihuman Ig antibody (300x on 2% lockAce/1%BSA/PBS). The HCV antigen positive cells were visualized with Metal Enhanced DAB substrate kits (Thermo Fisher Scientific, Rockford, USA). The infected cells were counted under microscopes and calculated the percentage inhibition.

**MTT assay:** The cytotoxicity analysis was conducted to determine whether the extract mediated any cytotoxicity effects. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was done by inoculating 100, 50, 10, 1 and 0.1  $\mu\text{g/mL}$  of extract in 96 wells plate culture cells which have seeded for 24 hours. After 48 h incubation, the medium was replaced with MTT reagent containing medium and incubated for 4 h. Absorbance sample was evaluated under microplate reader at 450 and 600 nm, which is correlated with the amount of cell viability. The percentage of cell toxicity was calculated by comparing with untreated cells and further determine its 50% cytotoxic concentration (CC<sub>50</sub>) values [7, 8, 27].

**Docking analysis:** The ligand was prepared by making 2D and 3D structures of the curcumin using ChemBioOffice program Ultra 11.0 and its energy was minimized using MMF94. The docking analysis continued by Molegro Virtual Docking ver 5.5 program Ver 5.5, resulted in rerank score describing the minimal energy by the ligand in interaction with the receptor.

**Acknowledgments:** We gratefully acknowledge Research Institute and Innovation, Airlangga University through a *Riset Mandat* Grant.

## References

- [1] Pawlowsky J-M, Negro F, Aghemo A, Berenguer M, Dalgard O, Dusheiko G, Marra F, Puoti M, Wedemeyer H. (2018) EASL Recommendations on Treatment of Hepatitis C. *Journal of Hepatology*, 69, 461-511.

- [2] Teraoka Y, Uchida T, Imamura M, Hiraga N, Osawa M, Kan H, Saito Y, Tsuge M, Abe-Chayama H, Hayes CN, Makokha GN, Aikata H, Miki D, Ochi H, Ishida Y, Tateno C, Chayama K. (2018) Limitations of daclatasvir/asunaprevir plus beclabuvir treatment in cases of NS5A inhibitor treatment failure. *Journal of General Virology*, **99**, 1058-1065.
- [3] Jakobsen JC, Nielsen EE, Koretz RL, Gluud C. (2018) Do direct acting antivirals cure chronic hepatitis C? *British Medical Journal*, **361**, 1382.
- [4] Calland N, Dubuisson J, Rouille Y, Seron K. (2012) Hepatitis C virus and natural compounds: a new antiviral approach? *Viruses*, **4**, 2197-2217.
- [5] Wahyuni TS, Aoki Utsubo C, Hotta H. (2016) Promising anti-hepatitis C virus compounds from natural resources. *Natural Product Communications*, **11**, 1193-1200.
- [6] Khachatoorian R, Arumugaswami V, Raychaudhuri S, Yeh GK, Maloney EM, Wang J, Dasgupta A, French SW. (2012) Divergent antiviral effects of bioflavonoids on the hepatitis C virus life cycle. *Virology*, **433**, 346-355.
- [7] Wahyuni TS, Tumewu L, Permanasari AA, Apriani E, Adianti M, Rahman A, Widyawaruyanti A, Lusida MI, Fuad A, Soetjipto D, Nasronudin D, Fuchino H, Kawahara N, Shoji I, Deng L, Aoki C, Hotta H. (2013) Antiviral activities of Indonesian medicinal plants in the East Java region against hepatitis C virus. *Virology Journal*, **10**, 259.
- [8] Wahyuni TS, Widyawaruyanti A, Lusida MI, Fuad A, Soetjipto, Fuchino H, Kawahara N, Hayashi Y, Aoki C, Hotta H. (2014) Inhibition of hepatitis C virus replication by chalepin and pseudane IX isolated from *Ruta angustifolia* leaves. *Fitoterapia*, **99**, 276-283.
- [9] Omosa LK, Midiwo JO, Kuete V. (2017) *Curcuma longa*. In *Medicinal Spices and Vegetables from Africa*, Academic Press, 425-435.
- [10] Widyowati R, Agil M. (2018) Chemical constituents and bioactivities of several Indonesian plants typically used in Jamu. *Chemical and Pharmaceutical Bulletin*, **66**, 506-518.
- [11] Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, Tajik H, Abubakar S, Zandi K. (2014) A review on antibacterial, antiviral, and antifungal activity of curcumin. *BioMed Research International*, **2014**, 1-12.
- [12] Kim HJ, Yoo HS, Kim JC, Park CS, Choi MS, Kim M, Choi H, Min JS, Kim YS, Yoon SW, Ahn JK. (2009) Antiviral effect of *Curcuma longa* Linn extract against hepatitis B virus replication. *Journal of Ethnopharmacology*, **124**, 189-196.
- [13] Sormpet B, Potha T, Tragoolpua Y, Pringproa K. (2017) Antiviral activity of five Asian medicinal plant crude extracts against highly pathogenic H5N1 avian influenza virus. *Asian Pacific Journal of Tropical Medicine*, **10**, 871-876.
- [14] Mary HPA, Susheela GK, Jayasree S, Nizzy AM, Rajagopal B, Jeeva S. (2012) Phytochemical characterization and antimicrobial activity of *Curcuma xanthorrhiza* Roxb. *Asian Pacific Journal of Tropical Biomedicine*, **2**, S637-S640.
- [15] Hwang JK, Shim JS, Baek NI, Pyun YR. (2000) Xanthorrhizol: a potential antibacterial agent from *Curcuma xanthorrhiza* against *Streptococcus mutans*. *Planta Medica*, **66**, 196-197.
- [16] Lee LY, Shim JS, Rukayadi Y, Hwang JK. (2008) Antibacterial activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. against foodborne pathogens. *Journal of Food Protection*, **71**, 1926-1930.
- [17] Aspollah Sukari M, Wah TS, Saad SM, Rashid NY, Rahmani M, Lajis NH, Hin T-YY. (2010) Bioactive sesquiterpenes from *Curcuma ochrorhiza* and *Curcuma heyneana*. *Natural Product Research*, **24**, 838-845.
- [18] Saifudin A, Tanaka K, Kadota S, Tezuka Y. (2013) Sesquiterpenes from the rhizomes of *Curcuma heyneana*. *Journal of Natural Products*, **76**, 223-229.
- [19] Scheel TK, Rice CM. (2013) Understanding the hepatitis C virus life cycle paves the way for highly effective therapies. *Nature Medicine*, **19**, 837-849.
- [20] Colpitts CC, Baumert TF. (2016) Hepatitis C virus cell entry: a target for novel antiviral strategies to address limitations of direct acting antivirals. *Hepatology International*, **10**, 741-748.
- [21] Lechtenberg M, Quandt B, Nahrstedt A. (2004) Quantitative determination of curcuminoids in *Curcuma* rhizomes and rapid differentiation of *Curcuma domestica* Val. and *Curcuma xanthorrhiza* Roxb. by capillary electrophoresis. *Phytochemical Analysis*, **15**, 152-158.
- [22] Bos R, Windono T, Woerdenbag Herman J, Boersma Ykeliën L, Koulman A, Kayser O. (2007) HPLC-photodiode array detection analysis of curcuminoids in *Curcuma* species indigenous to Indonesia. *Phytochemical Analysis*, **18**, 118-122.
- [23] Anggakusuma, Colpitts CC, Schang LM, Rachmawati H, Frentzen A, Pfaender S, Behrendt P, Brown RJP, Bankwitz D, Steinmann J, Ott M, Meuleman P, Rice CM, Ploss A, Pietschmann T, Steinmann E. (2014) Turmeric curcumin inhibits entry of all hepatitis C virus genotypes into human liver cells. *Gut*, **63**, 1137-1149.
- [24] Ye M-X, Li Y, Yin H, Zhang J. (2012) Curcumin: updated molecular mechanisms and intervention targets in human lung cancer. *International Journal of Molecular Sciences*, **13**, 3959-3978.
- [25] Potter JA, Owsianka AM, Jeffery N, Matthews DJ, Keck ZY, Lau P, Fong SK, Taylor GL, Patel AH. (2012) Toward a hepatitis C virus vaccine: the structural basis of hepatitis C virus neutralization by AP33, a broadly neutralizing antibody. *Journal of Virology*, **86**, 12923-12932.
- [26] Zhu Y-Z, Qian X-J, Zhao P, Qi Z-T. (2014) How hepatitis C virus invades hepatocytes: The mystery of viral entry. *World Journal of Gastroenterology*, **20**, 3457-3467.
- [27] Apriyanto DR, Aoki C, Hartati S, Hanafi M, Kardono LBS, Arsianti A, Louisa M, Sudiro TM, Dewi BE, Sudarmono P, Soebandrio A, Hotta H. (2015) Anti-hepatitis C virus activity of a crude extract from longan (*Dimocarpus longan* Lour.) leaves. *Japanese Journal of Infectious Diseases*, **69**, 213-220.
- [28] Hafid AF, Aoki-Utsubo C, Permanasari AA, Adianti M, Tumewu L, Widyawaruyanti A, Wahyuningsih SPA, Wahyuni TS, Lusida MI, Soetjipto, Hotta H. (2017) Antiviral activity of the dichloromethane extracts from *Artocarpus heterophyllus* leaves against hepatitis C virus. *Asian Pacific Journal of Tropical Biomedicine*, **7**, 633-639.
- [29] Aoki C, Hartati S, Santi MR, Lydwina L, Firdaus R, Hanafi M, Kardono LBS, Shimizu Y, Sudarmono P, Hotta H. (2014) Isolation and identification of substances with anti-hepatitis c virus activities from *Kalanchoe pinnata*. *International Journal of Pharmacy and Pharmaceutical Science*, **6**, 211-215.