



### ANTIHEPATITIS C VIRUS ACTIVITY SCREENING ON *Harpullia arborea* EXTRACTS AND ISOLATED COMPOUND

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#### INTRODUCTION

Hepatitis C is a major healthcare problem worldwide. Available therapy for hepatitis C treatment is very expensive and probably not accessible for all patients. Regarding to this reason, the development of safe and inexpensive antiviral drugs is required. Natural products as a source of new drugs are potential to study. Some antiHCV substances from plants were obtained (Wahyuni, 2013; Adianti, 2014; Aoki, 2014).

*Harpullia arborea* (tulip wood tree) is a member of Sapindaceae family commonly known as kayu pacaat in Indonesia (Basuni, 1997). Traditionally, watery exudates from barks and fruits are used as leech repellent, oil extracted from seeds is a source of antirheumatics (Singh, 2013). *H. arborea* seeds extract also shown antibacterial activities against various strains of bacteria. *H. arborea* seeds contain glycosides, steroids, saponins and resins (Gowri, 2009). A norhopane triterpenoid also isolated from the leaves of *H. arborea* (Poovapathanachart, 2008).

This study was conducted to determine anti-HCV activity of *H. arborea* extracts and isolated compound.

#### MATERIALS AND METHOD

##### Plant material

*Harpullia arborea* was obtained from Alas Purwo National Park at Banyuwangi, East Java. Sample was authenticated by the authority of

Purwodadi Botanical Garden, Pasuruan, East Java.

##### Extraction dan fractionation

*H. arborea* was extracted by ultrasonic assisted extraction method using 80% ethanol as a solvent. Liquid fractionation was conducted using dichloromethane, ethyl acetate and buthanol respectively.

##### AntiHCV activity test

Extract was examined for antiHCV activity against JFH1a and J6/JFH1 in a cell culture system using Huh7 cells at a multiplicity of infection (MOI) of 0.1.

#### RESULTS DAN DISCUSSION

Anti-Hepatitis C Virus (anti-HCV) activity screening of *H. arborea* leaves and stem extract revealed that leaves extract exhibited anti-HCV with IC50 value of 17.5 µg/ml and 12.4 µg/ml against HCV JFH1a and J6/JFH1 respectively, meanwhile stem extract was found to be not active against both HCV type.

Fractionation of leaves extract resulted in 4 fractions which were dichloromethane, ethyl acetate, buthanol and aqueous fraction. Anti-HCV activity screening at a concentration of 30 µg/ml revealed that buthanol fraction inhibited HCV JFH1a growth by 54% in which other fractions only inhibited by 15-30%. Buthanol fraction contains yellow spot on TLC profile as a major compound. Further separation of buthanol fraction using sephadex LH-20 and



methanol 90% as a solvent was obtained 7 fractions (B1-B7). Fraction B5 contain yellow precipitate and by recrystallization process obtained a yellow crystal as a glycosylated flavonoid compound which identified as Kaempferitrin (3,7-di- $\alpha$ -L-rhamnopyranosyl kaempferol). Structure determination of compound was done by nuclear magnetic resonance spectroscopy and data were compared with references (Ouyang Ming-An, 2003; De Souza Menezes, 2007). Kaempferitrin was further tested against JFH1a. Anti-HCV activity test shown that kaempferitrin was not exhibited anti-HCV. It is possible to explain that anti-HCV activity of extract and buthanol fraction was produced by other compounds in the extract and buthanol fraction instead of kaempferitrin or the activity was created by synergism effect of many compounds. Previous studies were reported some activity of kaempferitrin. Like many flavonols, it has antimicrobial, antioxidant and antiinflammatory activities. It is also mimics insulin in stimulating glucose uptake in diabetic rats, but inhibits insulin-stimulated glucose uptake in 3T3-L1 cells (Jorge, 2004; Prasad, 2009). But no report about antiviral activity of kaempferitrin was found. Further study need to be done to investigate the anti-HCV compounds of *H.arborea*.

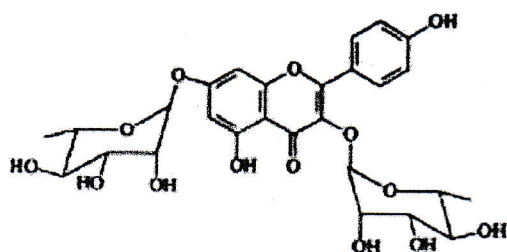


Figure 1. Kaempferitrin  
(3,7-di- $\alpha$ -L-rhamnopyranosyl kaempferol)

#### CONCLUSION

In this study, we concluded that *H.arborea* leaves extract and buthanol fraction were exhibited anti-HCV activity against JFH1a virus,

while the isolated compound, kaempferitrin was not.

#### ACKNOWLEDGEMENT

This study was supported by Science and Technology Research Partnership for Sustainable Development (SATREPS) from Japan International Cooperation Agency (JICA), Japan International Cooperation Agency (JICA), Universitas Airlangga Indonesia.

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# CERTIFICATE

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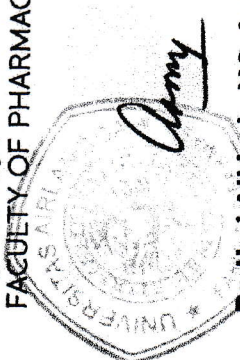
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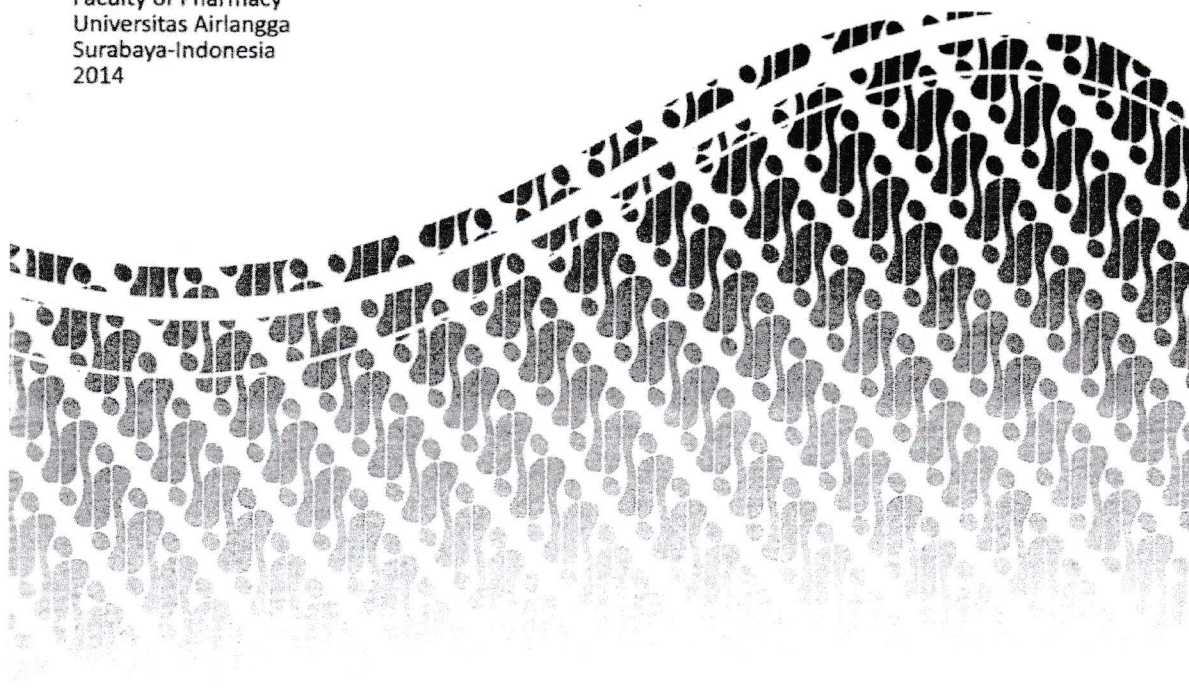
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## **The 1<sup>st</sup> International Conference on Pharmaceutics & Pharmaceutical Sciences**

Published and Organized by  
Faculty of Pharmacy  
Universitas Airlangga  
Surabaya-Indonesia  
2014



# The 1<sup>st</sup> International Conference on Pharmaceutics & Pharmaceutical Sciences Proceedings

ISBN : 978-602-72333-0-0

(Letter of National ISBN Agency No. 4127/E.8/p/03.2015 Date 18 March 2015)

**1st edition Proceeding**

**Published by:**

Faculty of Pharmacy Universitas Airlangga  
Surabaya, Indonesia

**Address:**

Kampus B Jl. Dharmawangsa Dalam  
Surabaya 60286  
Phone +62 31 5033710  
Fax +62 31 5020514  
Website: [www.icpps2014.com](http://www.icpps2014.com) or [www.ff.unair.ac.id](http://www.ff.unair.ac.id)  
Email: [icppinfo@gmail.com](mailto:icppinfo@gmail.com)

ISBN 978-602-72333-0-0



## **PREFACE From Chairman**

It is our pleasure to present you the proceedings of The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS) organized by The Faculty of Pharmacy Universitas Airlangga Surabaya Indonesia.

The proceeding was produced based on papers and posters presented at The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS), held in Surabaya, Indonesia, 14-15 November 2014.

The proceeding clearly reflects broad interest, from the participants that coming from all around the world.

The papers presented were pharmaceutics and biopharmaceutics; requirements on how to evaluate molecules in discovery and their appropriateness for selection as potential candidate; their development in context of challenges and benefits, together with associated time and cost implications and also requirements to progress through pre-clinical and clinical.

In this an opportunity, I would like to express my appreciation to the editorial team of the proceeding who have been working hard to review manuscripts, and making the first edition of this proceeding be possible.

I would like also to thanks to all invited speakers and presenters who participated in The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS) and your contribution to this proceeding.

Finally, I hope this proceeding will give contribution to the Pharmaceutics and Pharmaceutical Sciences research.

Chairman,

Dra. Esti Hendradi, MSI., Ph.D., Apt



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## TABLE of CONTENT

Preface from Chairman	
Committee .....	ii
Table of Contents .....	iii
Author Index .....	iii

## AUTHOR INDEX

COMPARISON OF SODIUM STARCH GLYCOLATE AND CROSSCARMELLOSE SODIUM AS SUPERDISINTEGRANT IN MEFENAMIC ACID FAST DISINTEGRATING TABLET <b>Adeltrudis Adelsa D, Oktavia Eka Puspita, Amalia Ayuningtyas, Marulita Isadora</b> .....	1
STUDY EXPRESSION OF HUMAN ERYTHROPOIETIN EXPRESSION IN MAMMALIAN CELL <b>Adi Santoso, Popi Hadiwisnuwardhani, Yana Rubiana, Yulaika Romadhani, Endah Puji Septisetyani, Dyaningtyas D.P. Putri</b> .....	4
ANTIOXIDANT STABILITY ASSAY OF ALPHA TOCOPHERYL ACETATE IN SOLID LIPID NANOPARTICLE SYSTEM (LIPID BASE BEESWAX AND MONOSTEARIC GLISERYL 50:50) <b>Anggie Widhi, Noorma Rosita, Widji Soeratri</b> .....	8
A BIOACTIVE <i>BOVINE HYDROXYAPATITE</i> -GELATIN IMPLANT FOR IN VITRO GENTAMICIN RELEASE <b>Aniek Setiya Budiatin, M. Zainuddin, Junaidi Khotib, Diah Himawati</b> .....	13
EFFECT OF COMPARISON SURFACTANT AND COSURFACTANT IN WATER/OIL MICROEMULSION IN RELEASE OF OVALBUMIN Microemulsion Water/Oil with Surfactant (Span 80-Tween 80) : Cosurfactant (Ethanol) =5:1, 6:1, and 7:1) <b>Anisa Rizki Amalia, Riesta Primaharinastiti, Esti Hendradi</b> .....	18
ANALYSIS OF MYCOLIC ACIDS CLEAVAGE PRODUCT OF <i>Mycobacterium tuberculosis</i> BY GAS CHROMATOGRAPHY-FLAME IONIZATION DETECTOR <b>Asri Darmawati, Deby Kusumaningrum, Isnaeni, Muhamad Zainuddin</b> .....	21
PERIPLASMIC EXPRESSION OF GENE ENCODING ANTI-EGFRVIII SINGLE-CHAIN VARIABLE FRAGMENT ANTIBODY USING PeIB LEADER SEQUENCE IN <i>ESCHERICHIA COLI</i> <b>Kartika Sari Dewi, Debbie Sofie Retnoningrum, Catur Riani, Asrul Muhamad Fuad</b> .....	24
IN VIVO ANTIMALARIAL ACTIVITY OF ETHANOL EXTRACT AND ETHYL ACETATE FRACTION OF <i>Alectryon serratus</i> LEAVES ON <i>Plasmodium berghei</i> INFECTED MICE <b>Aty Widyawaruyanti, Uswatun Khasanah, Lidya Tumewu, Hilkatul Ilmi, Achmad Fuad Hafid, Indah S Tantular</b> .....	30
PROFILE OF COMMUNITY PHARMACISTS KNOWLEDGE IN PATIENT ASSESSMENT WITH INFLUENZA SYMPTOMS AND ITS PRODUCTS <b>Azza Faturrohmah, Arie Sulistyarini, Ana Yuda</b> .....	33

SOLUBILITY AND DISSOLUTION STUDY OF KETOPROFEN – HIDROXYPROPYL- $\beta$ -CYCLODEXTRIN INCLUSION COMPLEX (Prepared by Kneading Method) <b>Bambang Widjaja, Achmad Radjaram, Arafah Zulhana</b> .....	37
FORMULATION AND STABILITY TESTING OF MELOXICAM SOLID DISPERSION GEL <b>BudiPratiwi Wisudyaningsih, Inka Dewi Nur Anggaraini, Fersiya Wardani</b> .....	40
EFFECT OF MENTHOL AS PENETRATION ENHANCER TO DICLOFENAC SODIUM MEMBRANE-TYPED TRANSDERMAL PATCH CHARACTERIZATION <b>Destria Indah Sari, Esti Hendradi, Junaidi Khotib</b> .....	43
PHYSICAL CHARACTERISTICS AND RELEASE STUDY OF OVALBUMIN FROM ALGINATE MICROSPHERES PREPARED BY DIFFERENT CONCENTRATION OF ALGINATE AND BaCl <sub>2</sub> USING AEROSOLIZATION TECHNIQUE <b>Dewi Melani Hariyadi, Tristiana Erawati, Sisilia Ermawahyuningtyas</b> .....	46
MUCOADHESIVE TABLET OF ETHANOLIC EXTRACT OF SAMBILOTO ( <i>Andrographis paniculata</i> ) AS ANTIDIABETIC USING CHITOSAN <b>Dhadhang Wahyu Kurniawan, Hening Pratiwi, and Lingga Ikaditya</b> .....	50
PHYSICAL INTERACTION STUDY OF IBUPROFEN-STEARIC ACID BINARY MIXTURE <b>Diajeng Putri Paramita, Dwi Setyawan, Dewi Isadiartuti</b> .....	59
MOLECULAR MODELING AND SYNTHESIS OF 1-(3,4-Dichlorobenzoyl)-1,3-dimethylurea <b>Dian Agung Pangaribowo, Siswandono, Bambang Tri Purwanto</b> .....	63
EXPRESSION OF RECOMBINANT HUMAN GRANULOCYTE-COLONY STIMULATING FACTOR WITHIN PERIPLASMIC COMPARTMENT OF <i>Escherichia coli</i> USING PeIB LEADER PEPTIDE <b>Dian Fitria Agustiyanti, Asrul Muhamad Fuad</b> .....	66
EVALUATION OF ANTIHYPERURICEMIC ACTIVITY FROM BULBS OF BAWANG TIWAI ( <i>Eleutherine palmifolia</i> (L.) Merr.) BY IN VITRO AND IN VIVO STUDIES <b>Dian Ratih Laksmiawati, Rininta Firdaus, Yulinda, Mediana Astika</b> .....	72
ANTIOXIDANT ACTIVITY OF 96% ETHANOL EXTRACT OF COMBINATION OF STRAWBERRY FRUIT ( <i>Fragaria x ananassa</i> Duch.) AND STARFRUIT ( <i>Averrhoa carambola</i> L.) USING ABTS FREE RADICAL SCAVENGING METHOD <b>Diana Serlahwaty, Indira Natalia Timang</b> .....	76
ENHANCEMENT OF SOLUBILITY AND DISSOLUTION ATORVASTATIN BY MICROCRYSTALLIZATION METHOD <b>Dolih Gozali, Yoga Windu Wardhana, Ronny Tandela</b> .....	79
<i>IN VITRO</i> ANTIMALARIAL ACTIVITY OF DICHLOROMETHANE SUB-FRACTION OF <i>Eucalyptus globulus</i> L. Stem AGAINST <i>Plasmodium falciparum</i> <b>Elis Suwarni, Achmad Fuad Hafid, Aty Widyawaruyanti</b> .....	86
<i>Arcangelisia flava</i> INCREASES RATS' LEUKOCYTES BUT HAS BIPHASIC EFFECT ON RATS' LYMPHOCYTE <b>Endah Puspitasari, Evi Umayah Ulfa, Vita Ariati, Mohammad SulthonHabibi</b> .....	89

IN VITRO ANTIMALARIAL ACTIVITY OF CHLOROFORM SUBFRACTION OF SALAM BADAK LEAVES ( <i>Acmena acuminatissima</i> ) Erna Cahyaningsih, Achmad Fuad Hafid, Aty Widawaruyanti .....	92
CHARACTERIZATION OF DOSAGE FORM AND PENETRATION DICLOFENAC SODIUM WITH MICROEMULSION SYSTEM IN HPMC 4000 GEL BASE (Microemulsion W/O with ratio use of surfactant Span 80 – Tween 80 : Cosurfactant Ethanol 96% = 6:1) Esti Hendradi, Tutiek Purwanti, Karina Wahyu Irawati .....	95
CONSTRUCTION AND VALIDATION OF THE STRUCTURE-BASED VIRTUAL SCREENING PROTOCOLS WITH PDB CODE OF 3LN1 TO DISCOVER CYCLOOXYGENASE-2 INHIBITORS Mumpuni E, Nurrochmad A, Pranowo HD, Jenie UA, Istyastono EP .....	99
VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ASPIRIN IN RABBIT PLASMA : APPLICATION TO BIOAVAILABILITY STUDY OF ASPIRIN MICROCAPSULE IN RABBIT Faizatun, Novi yantih, Teguh Iman Saputra .....	102
EFFECT OF COMPARISON OF SURFACTANT AND COSURFACTANT W/O MICROEMULSION OVALBUMIN WITH SOYBEAN OIL TO PHYSICOCHEMICAL CHARACTERIZATION (w/o Microemulsion with Surfactant Span 80- Tween 80 : Cosurfactant Ethanol 96% = 5:1; 6:1 and 7:1) Farida Mutiara Sari, Riesta Primaharinastiti, Esti Hendradi .....	105
PH INFLUENCE IN DESALTING PROCESS OF CRUDE PERTUSSIS TOXIN (PT) AND FILAMENTOUS HEMAGGLUTININ (FHA) PURIFICATION FROM <i>Bordetella pertussis</i> BY SEPHADEX G-25 COLUMN CHROMATOGRAPHY Faris Adrianto, Esti Hendradi, Neni Nurainy, Isaeni .....	108
SEPARATION OF COSMETIC PRESERVATIVES USING SILICA-BASED MONOLITHIC COLUMN Febri Annuryanti, Riesta Primaharinastiti, Moch. Yuwono .....	111
PREPARATION AND CHARACTERIZATION OF TELMISARTAN-CITRIC ACID CO-CRYSTAL Fikri Alatas, Hestiary Ratih, Sundani Nurono Soewandhie .....	114
PATIENTS' AND CAREGIVERS' LIQUID MEDICATION ADMINISTRATION ERRORS Gusti Noorrizka Veronika Achmad, Gesnita Nugraheni .....	117
THE POTENCY OF CANARIUM OIL ( <i>Canarium indicum</i> ) AS A MATERIAL FOR STRUCTURED LIPID PRODUCTION Hamidah Rahman, Johnner P Sitompul, Kusnandar Anggadiredja, Tutus Gusdinar .....	121
EFFECT OF TREHALOSE ON THERMAL PROPERTIES OF PHOSPHOLIPID-DDA AND TPGS MIXTURES Helmy Yusuf .....	124
PREPARATION AND CHARACTERIZATION OF FLUKONAZOLE- $\beta$ -CYCLODEXTRIN INCLUSION COMPLEXES Hestiary Ratih, Fikri Alatas, Erin Karlina .....	127
ISOLATION AND IDENTIFICATION OF ANTIOXIDANT COMPOUND BY BIOPRODUCTION OF ENDOPHYTIC FUNGI OF TURMERIC ( <i>Curcuma longa</i> L.) ISOLATE CL.SMI.RF11 Hindra Rahmawati, Bustanussalam, Partomuan Simanjuntak .....	130

MODIFICATION PROCESS OF NATURAL CASSAVA STARCH : THE STUDY OF CHARACTERISTICS AND PHYSICAL PROPERTIES Prasetia, Jemmy A, C.I.S. Arisanti, N.P.P.A. Dewi, G.A.R. Astuti, N.W.N Yulianingsih, I M.A.G. Wirasuta .....	133
DRUG USE PROFILE OF DIABETIC PATIENTS IN EAST SURABAYA PRIMARY HEALTH CARE I Nyoman Wijaya, Azza Faturrohmah, Ana Yuda, Mufarriha, Tesa Geovani Santoso, Dina Kartik, Hikmah Prasasti N, Whanni Wido Agustin .....	136
GLYCINE MAX DETAM II VARIETY AS PREVENTIVE AND CURATIVE ORGAN DAMAGE DUE TO EXPOSURE TO ,LEAD (Pb) Rika Yulia, Sylvan Septian Ressandy, Gusti Ayu Putu Puspikaryani, I Putu Agus Yulyastrawan, D Ayu Kusuma Dewi .....	142
AN ACTIVITY TEST OF MATOA LEAVES EXTRACT AS HEART RATE FREQUENCY REDUCTION WITH ADRENALINE INDUCTION Ika Purwidyaningrum, Elin Yulinah Sukandar, Irda Fidrianny .....	144
EFFORT TO REDUCE COMPRESSIBILITY OF RAMIPRIL THROUGH CRYSTAL ENGINEERING Indra, Sundani N Soewardhi .....	147
IN VITRO ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY OF ETHANOLIC LEAF EXTRACT AND FRACTI OF <i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz Julie Anne D. Bolaños, Ivan L. Lawag .....	150
PERIPLASMIC EXPRESSION OF GENE ENCODING ANTI-EGFRVIII SINGLE-CHAIN VARIABLE FRAGME ANTIBODY USING PeIB LEADER SEQUENCE IN <i>Escherichia coli</i> Kartika Sari Dewi, Debbie Sofie Retnoningrum, Catur Riani, Asrul Muhamad Fuad .....	153
CHARACTERIZATION AND LD <sub>50</sub> VALUE DETERMINATION OF 1,5-bis(3'-ethoxy-4'-hydroxyphenyl)-1,4-pentadiene-3-one (EHP) Lestari Rahayu, Septian, Esti Mumpuni .....	159
DEVELOPMENT OF MELOXICAM TRANSDERMAL MATRIX TYPE PATCH USING POLYVINYLPIRROLIDONE, HYDROXYPROPYL METHYLCELLULOSE, AND ETHYL CELLULOSE COMBINATION Lidya Ameliana, Monica Iwud, Selly Rio .....	162
ANTIHEPATITIS C VIRUS ACTIVITY SCREENING ON <i>Harpullia arborea</i> EXTRACTS AND ISOLATED COMPOUND Lidya Tumewu, Evhy Apryani, Mei Ria Santi, Tutik Sri Wahyuni, Adita Ayu Permanasari, Myrna Adianti, Chie Aoki, Aty Widyawaruyanti, Achmad Fuad Hafid, Maria Inge Lusida, Soetjipto, Hak Hotta.....	165
HPLC METHOD PRECISION TO ASSAY OF A-MANGOSTIN IN Mangosteen ( <i>Garcinia mangostana</i> L. FRUIT RIND EXTRACT FORMULATED IN ORAL SOLUTION Liliek Nurhidayati, Siti Sofiah, Ros Sumarny, Kevin Caesar .....	168