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Menyatakan bahwa makalah berjudul "ANTIHYPERURICEMIA ACITIVITY OF SALAM (*Syzigium Polyanthum Walp.*) AND MENIRAN (*Phyllanthus Niruri Linn.*) HERBS EXTRACTS IN OXONATE-INDUCED MICE" Karya **Muhtadi, Andi Suhendi, Nurcahyanti W., dan EM. Sutrisna** dari Fakultas Farmasi Universitas Muhammadiyah Surakarta telah dipresentasikan pada **9<sup>th</sup> Joint Conference on Chemistry 2014** pada tanggal 12-13 November 2014 di Grand Candi Hotel Semarang.

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Demikian Surat Tugas ini dibuat, untuk dapat dilaksanakan sebagaimana mestinya.

*Wassalamu'alaikum wr wb.*

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**Muhtadi**

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at the 9<sup>th</sup> Joint Conference on Chemistry held on 12-13 November 2014 in Semarang that organised by Chemistry Department, Diponegoro University

Semarang, 13 November 2014

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# Green Chemistry

## Proceeding of 9<sup>th</sup> Joint Conference on Chemistry

12-13 November 2014  
Semarang, Indonesia



# Proceedings of The 9<sup>th</sup> Joint Conference on Chemistry

Diponegoro University (UNDIP),  
Semarang State University (UNNES), Sebelas Maret University (UNS) and  
Jenderal Soedirman University (UNSOED)

Grand Candi Hotel, Semarang, 12-13 November, 2014

## Green Chemistry

### Editors

Dwi Hudyanti

Agustina L.N. Aminin

Adi Darmawan

Yayuk Astuti

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## Preface to The Conference Proceedings

We are very pleased to introduce The 9th Joint Conference on Chemistry (9th JCC) held by Diponegoro University (UNDIP) On behalf of the Chemistry Consortium in Central Java, Indonesia. The JCC is an annual conference organized by the consortium of Chemistry Department of four universities in Central Java: Diponegoro University (UNDIP), Semarang State University (UNNES), Sebelas Maret University (UNS) and Jenderal Soedirman University (UNSOED); since 2006. The growing of environmental problems that persist to escalate worldwide has compelled us to select “**Green Chemistry**” as the leading theme of the 9th JCC.

We had 10 plenary speakers, 10 invited speakers and over 120 suitable papers from 11 countries were submitted for presentation at the conference. This required the program to be organized in five parallel sessions, each on a specific theme, to provide each paper with sufficient time for presentation and to accommodate all of them within the overall time allocated. One of the five sessions contained analytical chemistry. A second session was devoted to the theme of biochemistry. The third and fourth session were dedicated to physical and material chemistry. The fifth session was concerned with chemical education. These were well represented in the program of the conference and were clearly topics which continue to stimulate a global interest. The programs were chaired in a professional and efficient way by the session chairmen who were selected for their international standing in the subject.

All the papers went through a peer-review procedure prior to being accepted for publication in this book. These Proceedings present the permanent documentation of what was presented. They indicated the state of advancement at the time of writing of all aspects of this theme and will be very useful to all people in the field.

As a final point, it is appropriate that we record our thanks to our fellow members of the steering committee, organizing committee, and scientific committee. We are also indebted to those who served as chairmen. Without their support, the conference could not have been the success that it was. We also would like to express our sincere gratitude to all authors for their valuable contributions. We are thankful to the students of Chemistry Department Faculty of Science and Mathematics Diponegoro University especially to Maya and Fuad for their support during preparation of the manuscript.

Dwi Hudiyantri

Agustina L.N. Aminin

Adi Darmawan

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## Table of Contents

Title Page	i
Copyright page	ii
Preface	iii
Board of Reviewers	v
Table of Contents	vii
<b>Section 1: Material Chemistry</b>	<b>1</b>
TiO <sub>2</sub> -SiO <sub>2</sub> Modified on Acrylic Paint with Self-Cleaning Characteristic <i>Agus Ridwan, Sri Wahyuni</i>	3
Synthesis and Characterization of Cellulose Based Superabsorbent Polymer Composites <i>Ahmad Zainal Abidin, N. M. T. P. Sastra, G. Susanto, H.P.R. Graha</i>	8
Synthesis and Characterization of Nano Scale Zero-Valent Iron Supported on Mesoporous Silica <i>Atyaf Khalid Hammed, Nugroho Dewayanto, D. Dongyun, Mohd Ridzuan Nordin</i>	13
Synthesis of 2, 7-Disulfonatonaphthalene-5-Hydroxy-4-Amino-N-Propyl Silica Hybrid by Sol-Gel and Grafting Processes <i>Choiril Azmiyawati, Nuryono, Narsito</i>	21
Modification of Ni/Zn-HZSM-5 Double Promoted Catalyst for Biofuel Production from <i>Cerbera manghas</i> Oil <i>Danawati Hari Prajitno, Agus Budianto, Muhammad Iqbal, Achmad Roesyadi, Victor Purnomo</i>	25
Influences of Ammonia for Synthesis of 8-hydroxyquinoline Copper(II) <i>Suhartana, Laelatri Agustina, Sriatun</i>	29
Influence of Variation Temperature on Phase Composition of Ca-Mg-Al Hydrotalcite <i>Eddy Heraldy, Khoirina Dwi Nugrahaningtyas, Fendry Bangkit Sanjaya, Desi Suci Handayani, Yuniawan Hidayat</i>	34
Synthesis and Characterization of Chitosan – Rice Husk Ash Silica Composite as Polymer Electrolyte Membrane (PEM) <i>Eva Mardiningsih, Ella Kusumastuti</i>	38
Synthesis and Characterization of the Zn(II) Complex with Dimethyl Hydroxyl Pyridine-2,6-Dicarboxylate <i>Fahimah Martak</i>	44

Synthesis and Characterization of $\text{La}_{1-y}\text{Sr}_y\text{Co}_{0.8}\text{Fe}_{0.2}\text{O}_3$ and $\text{La}_{1-y}\text{Ba}_y\text{Co}_{0.8}\text{Fe}_{0.2}\text{O}_3$ ( $0.0 \leq y \leq 0.4$ ) Dense Membranes <i>Hamzah Fansuri, N. Widiastuti, A. Aliyatulmuna, W. P. Utomo, D. Prasetyoko, B. Prijamboedi</i>	50
Synthesis, Characterization and Catalytic Activity of CuO/ZnO on Phenol Oxidation <i>Nuni Widiarti, Sri Wahyuni, S Barokah</i>	56
Characterization of Enzyme Electrode from Nanochitosan Immobilized Glucose Oxidase on Carbon Paste Modified with Nanofiber Polyaniline for Biosensor Application <i>Popi Asri Kurniatin, Laksmi Ambarsari, Inda Setyawati, Puspa Julistia Puspita, Aneisti</i>	60
Andisol Soil Utilization of Mount Lawu as Natural Adsorbent Multi Soil Layering Materials for Domestic Waste <i>Pranoto, R. Sudaryanto, Supriyadi</i>	65
Modifying Surface Charge of Chitosan Membrane by Carboxymethylchitosan Blended with Poly(vinylalcohol) <i>Retno Ariadi Lusiana, Dwi Siswanta, Mudasir, T. Hayashita</i>	72
Effect of N-doped Graphene for Pt/N-doped Graphene Catalyst <i>Rikson Siburian, Minsyahril Bukit</i>	76
The utility of Aqueous Extract of Air-dried <i>Callophyllum inophyllum</i> L. Leaf as Medium/Reduction System for Synthesis of Gold Nanoparticles (AuNPs) <i>Salprima Yudha S., Zulfikri Achid Mardlia, Eka Angasa, Totok Eka Suharto, Yuta Nishina</i>	85
The Impregnated Boron Oxide Catalysts for the Reaction of Dehydrogenation of Ethane <i>Setiadi</i>	89
Preparation of Zn-Ni/TiO <sub>2</sub> Photocatalyst by Sol-gel Method and Its Activity in Water Decomposition <i>Sigit Priatmoko, E. Cahyono, S. Wahyuni, Ella Kusumastuti, Satrio Bekti Uji Prambasto</i>	95
Synthesis of Humic Acid Coated Fe <sub>3</sub> O <sub>4</sub> Magnetic Nanoparticle and Its Application to Adsorp Cu(II) <i>Soerja Koesnarpadi, Daniel</i>	101
Modification of Synthetic Zeolite from Bagasse Ash and Their Characterization <i>Sriatun, Taslimah, Linda Suyati</i>	105
Synthesis and Surface Modification of TiO <sub>2</sub> /Carbon Photocatalyst Produced by Arc Discharge in Ethanol Medium <i>Teguh Endah Saraswati, Isya Fitri Andhika, Astrid Olivia Nandika, Sayekti Wahyuningsih, Candra Purnawana</i>	110
The Effect of Vulcanization Time on Mechanical and Chemical Properties of Liquid Rubber Compound <i>Teja Dwi Sutanto, Bambang Setiaji, Karna Wijaya, Totok Eka Suharto</i>	114
Calcium Phosphate-Chitosan Composite as a Bone Cement Candidate <i>Tri Windarti and Benjamin Horrocks</i>	119

Optimization Process of H-Zeolite Catalyst Preparation with Surface Response Methods <i>Widayat, H. Susanto, H. Satriadi</i>	124
Preparation of Activated Carbon from Oil Palm Shell by Activating ZnCl <sub>2</sub> as Carbon Monoxide Adsorbent <i>Yuliusman, Widodo W. Purwanto, Yulianto S. Nugroho, Randy Anggriany</i>	130
<b>Section 2: Physical Chemistry</b>	<b>135</b>
Effects of Voltage and Number of Cell on Desalination of Brackish Water using Electrodialysis Method <i>Alfan Purnomo, Zakiatul Mirfada, Arseto Yekti Bagastyo</i>	137
Mesostructured Titanosilicates Catalyst for Synthesis of Vitamin K3 <i>Alfa AkustiaWidati, Hamami, Handoko Darmokoesomo, Nada Adhistry Stevany</i>	142
XRD of Synthetic Zeolite for Surfactant Builder: NaOH Concentration Variation in Sodium Silicate Decision of Rice Husk Ash <i>Arnelli, Ahmad Suseno, Teguh Imam Prasetyo</i>	146
Catalytic Conversion of 1-Octadecene to Shorten Chains Alkane (C <sub>6</sub> – C <sub>12</sub> ) <i>D. Setyawan Purwo H, Triyono, Narsito, Tutik Dwi Wahyuni</i>	149
Electrochemical Characterization of Direct Ethanol Fuel Cell (DEFC) with Crude Bioethanol Feed <i>Dwi Kemala Putri, Mitra Eviani, Aditya Yudistira, Isdiriyani M. Nurdin, Hary Devianto, Ardiyan Harimawan</i>	153
Conversion of Glycerol into Polyhydroxybutyrate(PHB) using <i>Escherichia coli</i> <i>Endah Fitriani Rahayu, Wega Trisunaryanti, Karna Wijaya</i>	156
The Effects of Hydrolysis Temperature and Catalyst Concentration on Bio-ethanol Production from Banana Weevil <i>Eni Budiwati and Umar Bandi</i>	161
The Effect of Coconut Oil Concentration on Physical and Chemical Properties of Cosmetic Emulsions <i>Eni Widiyati, AH. Bambang Setiaji, Totok Eka Suharto, Triyono</i>	167
Adsorption of Pb(II) and Co(II) on Adsorbent Clay Immobilized <i>Saccharomyces cerevisiae</i> Biomass <i>Fahmiati, Mashuni, L.D. Syahdam Hamidi, Nasra</i>	171
Utilization of Cassava Peel as Electric Energy Source through Microbial Fuel Cell <i>Linda Suyati, Didik Setiyo Widodo, Abdul Haris, Wuryanti, Rahmad Nuryanto</i>	178
Effect of Activated Bagasse Charcoal Size as Biomaterial Pretreatment on Waste Cooking Oil Biodiesel Characteristics <i>Lizda Johar Mawarani, Tatik Farihah</i>	181

Electrochemical Characterization of Direct Ethanol Fuel Cell (DEFC) with Bioethanol Feed Containing Acetic Acid as Impurity <i>Mitra Eviani, Isdiriyani M. Nurdin, Hary Devianto</i>	185
The in Silico Molecular Interaction of Organoboron Compounds as Curative Measure toward Cervical Cancer <i>Ridla Bakri, Arli Aditya Parikesit, Cipta Priyo Satrianto, Djati Kerami, Usman Sumo Friend Tambunan</i>	189
Catalytic Properties of Bimetallic NiNP-M/AlOH (M = Sn, In, Ga, Ag, Nb, and Zr) on Selective Hydrogenation of Furfural <i>Rodiansono, M. D. Astuti, A. Ghofur, Shogo Shimazu</i>	193
Adsorption Study of 2-mercaptobenzothiazole at Copper Surface as Corrosion Inhibitor in HCl <i>Taamy Alif Firman, Yoki Yulizar</i>	199
Emulsification Ability of Surfactant-Like Peptides Predicted by Coarse Grained Molecular Dynamics Simulations <i>Tegar Nurwahyu Wijaya, Rukman Hertadi</i>	202
Biofuel from Light Tar Resulted from Coconut Shell Pyrolysis by Distillation Process <i>Uswatun Hasanah, Bambang Setiaji, Triyono, Chairil Anwar</i>	205
Adsorption of Cyanide Ion from Aqueous Solutions by <i>Saccharomyces cerevisiae</i> Biomass <i>Venty Suryanti, Fitria Rahmawati, Yudha Anggara Haeqal</i>	209
Biosorption of Cu <sup>2+</sup> , Zn <sup>2+</sup> , and Cd <sup>2+</sup> by <i>Nannochloropsis salina</i> in a Three-Metal System <i>Yusafir Hala, Emma Suryati, Paulina Taba, Nesty MudiTumale</i>	213
The Effect of Annealing Temperature to The X-Ray Diffraction Patterns of The Thin Film of Cardanol Compound from Alor Regency NTT Province <i>Zakarias Seba Ngara, I Gusti M. Budiana, Aliwarsito</i>	217
<b>Section 3: Analytical Chemistry</b>	221
Effect of pH on Cu-S TiO <sub>2</sub> Photocatalytic Performance toward Phenol Photodegradation and Cr(VI) Photoreduction by Visible Light Irradiation <i>Abdul Haris, Didik Setiyo Widodo, Rahmad Nuryanto</i>	223
Electrochemical Impedance Spectroscopy Analysis of Lithium Polymer Batteries during Charge/Discharge Cycle <i>Achmad Rochliadi, Multazam, I Made Arcana, Bunbun Bundjali</i>	226
Influence of C/N Ratio in Activated Sludge to Remove Cr(VI) <i>Arseto Yekti Bagastyo, Natalia Diani Triana</i>	230
Method Development and Validation for Lead (Pb) Analysis in Natural Honey from East Kalimantan <i>Bohari Yusuf, Finqo Aprianto</i>	238

Electroremediation of Polluted Water: Electrodecolorization of Batik Wastewater <i>Didik Setiyo Widodo, Abdul Haris, Gunawan</i>	243
Influence on The Degree of Increase in Natrium Metabisulphite White Bread Flour <i>Heny Kusumayanti, Laila Faizah, R.TD. Wisnu Broto, Hanifah, M. Taqiyuddin</i>	248
Selective Adsorption of Phenol and Vanillin Using Eugenol Based Molecularly Imprinted Polymer <i>M. Cholid Djunaidi, Dwi Siswanta, Jumina</i>	251
The Influence of Ascorbic Acid, Creatinine and Urea on the Analysis of Uric Acid in the Blood Serum by Stripping Voltammetry using Graphite Electrode <i>Miratul Khasanah, Handoko Darmokusumo, Ganden Supriyanto, Ahmad Zaky Pulungan, Putut Satrio Dahono</i>	258
Optimization and Validation of HPLC for Analysis of Rhodamine B in Sponge Cake <i>Novi Yantih, Zuhelmi Aziz, Aditya Dicky Prasetya</i>	263
Analysis of 8 Human Pharmaceuticals in Water Samples Using Solid Phase Extraction Followed by Liquid Chromatography Tandem Mass Spectrometry <i>Samuel Budi Wardhana Kusuma, Ibrahim Al Tarawneh, Robert Kreuzig</i>	267
Analysis of Nitrosodiethylamine (NDEA) in Salted Fish with Hollow Fibre-Liquid Phase Microextraction Gas Chromatography Flame Ionization Detector(HF-LPME-GC-FID) Method <i>Usreg Sri Handajani, Ganden Supriyanto, Yanuardi Raharjo, Gunawan Dwi Saputra</i>	273
Application of Cone Shaped Membrane-Liquid Phase Microextraction for Analysis Nitrosodipropylamine in Salted Fish <i>Yanuardi Raharjo, Usreg Sri Handajani, Eko Aryo Wijaksono</i>	278
<b>Section 4: Organic Chemistry</b>	283
Phytochemical Screening and Toxicity Test BSLT for Ethanol Extract of Agarwood( <i>Aquilaria microcapa Baill</i> ) <i>Ahmad Musir, Risma M. Tambunan, Bambang Triseno</i>	285
Determination of Glabridin in Licorice Root ( <i>Glycyrrhiza glabra L.</i> ) Using High Performance Liquid Chromatography <i>Faridah, Siti Umrah Noor, Rahmawati T.</i>	289
Antidiabetic and Antihypercholesterolemic Activities of Citrus Sinensis Peel in Rats <i>Haryoto, Muhtadi, Tanti Azizah, Andi Suhendi</i>	294
Acute Toxicity for Combination Extract of <i>Terminalia muelleri Benth.</i> and <i>Curcuma xanthorrhiza</i> <i>Khairul Anam, Dewi Kusrini, Ratna Megawati Widharna</i>	298
The Effect of Oil Types on The Characteristics of Solid Soap <i>Mardiyah Kurniasih, Purwati, Anung Riapanitra, Zufahair, Tri Wahyuni</i>	303

Antibacterial Activities Some Compounds Clove Leaf Oil Derivatives <i>Ngadiwiyana, Purbowatiningrum Ria Sarjono, Enny Fachriyah, Nor Basid Adiwibawa Prasetya</i>	308
The Effect of the Addition of Glycerol and Chitosan in the Biodegradable Plastics Production from "Porang" Flour ( <i>Amorphophallus muelleri</i> Blueme) <i>Ninie Fajar Puspita, Saidah Altway, Lizda Johar Mawarani, Dwi Ayu, Dessy Rosita</i>	312
Standardization and $\alpha$ -Glucosidase Inhibitory of Extract from <i>Anredera Cordifolia</i> Leaves <i>Ratna Djamil, Wiwi Winarti, Syamsudin, Merrysca Rasna</i>	317
Determination of Total Flavonoid Content and Standardization <i>Orthosiphon aristatus</i> Leaves Extracts <i>Sarah Zaidan, Ratna Djamil</i>	322
Effect of Reaction Time toward Formation of 1,5-Bis-(2-Furanyl)-1,4-Pentadien-3-One from Claisen-Schmidt Condensation of Furfural and Acetone <i>Siti Mariyah Ulfa, Indah NurPramesti, M. Farid Rahman, Hideki Okamoto</i>	326
Blood Chemistry Data Base of Kedu Chicken;-The Indonesian Indigenous Poultry <i>Siti Susanti, Rina Muryani, Isroli, Hanny Indrat Wahyuni, Agus Sucipto</i>	330
The Potency of Liquorice Extract ( <i>Glycyrrhiza glabra</i> L.) as Skin Whitening <i>Siti Umrah Noor, Faridah, Astri Windi</i>	334
Triterpenoids from Tembelekan( <i>Lantana camara</i> ) Leaf Extract and Its Activity as an Antibacterial ( <i>Escherichia coli</i> ) <i>Sitti Hadijah Sabarwati, Oce Astuti, Indriyani Nur</i>	339
Hydrothermal Methods for Hydrolysis Cellulose to Glucose and/or Oligosaccharide: A Comparative Study with and without Ultrasound Pretreatment <i>Sumari, A. Roesyadi, Sumarno</i>	341
Chemical Constituent of DCM Extract and Neutral-Acid Fraction of <i>Voacangafoetida</i> (Bl.) Rolfe Leaves from Three Locations of Lombok Island on The Basis of GC-MS Analysis <i>Surya Hadi, Lely Kurniawati, Baiq Mariana, Handa Mulasari, Sri Rahayu</i>	345
Preparation and Characterization of Inclusion Complex of Xanthone with Sulfonatocalix[4]arene <i>Triana Kusumaningsih, Maulidan Firdaus, Muhammad Widyo Wartono, Desi Suci Handayani, Sidiq Nugraha, Tegar Parnandi Galih Rosdian</i>	351
Quality Standardization and Determination of in Vitro Antihyperglycemic Activity of Ethanolic Extract of Pacar Kuku ( <i>Lawsonia inermis</i> Linn.) <i>Wiwi Winarti, Syamsudin, Ratna Djamil, Aloysius Sebastian</i>	355
Phenolic Compounds from the Leaves of <i>Kalanchoe blossfeldiana</i> (Crassulaceae) Plant <i>Yenny Febriani Yun, Lilis Siti Aisyah, Tri Reksa Saputra, Arif Rahman Hakim, Tati Herlina, Euis Julaeaha, Achmad Zainuddin, Unang Supratman</i>	359
Bioactive Components and Antioxidant Properties of Stevia Beverage <i>Yohanes Martono, Hartati Soetjipto</i>	363

308	Characteristic of 70% Ethanol Extract from <i>Cyclea barbata</i> Miers leaves and Antioxidant Activity using DPPH Method <i>Yunahara Farida, Erlindha Gangga, Kartiningsih, Elisa, Teguh</i>	369
312	<b>Section 5: Biochemistry</b>	375
317	Isolation and Partial Purification of New Protease form Thermophilic Bacteria <i>Pseudomonas otitidis</i> WN 1 obtained from Indonesian Hot Spring <i>Amin Fatoni, Zufahair</i>	377
322	The Complexity of Molecular Interactions and Bindings between Cyclic Peptide and Inhibit Polymerase A and B1 (PAC-PB1N) H1N1 <i>Arli Aditya Parikesit, Harry Noviard, Djati Kerami, Usman Sumo Friend Tambunan</i>	382
326	Identification and Characterisation of Bioactive Peptides of Fermented Goat Milk <i>Chanif Mahdi, H. Untari, M. Padaga</i>	386
330	Comparative of Biomass for Pretreatment with Biological Process for Efficient Hydrolysis <i>Desy Kurniawati, Muhamad Natsir, Rahmi Febrialis, Prima Endang Susilowati</i>	391
334	Characterization of Immobilized Lipase from Fractionation Result of <i>Azospirillum Sp.</i> Prd1 using Chitosan <i>Dian Riana Ningsih, Zufahair, Santi Nur Handayani, Puji Lestari</i>	397
339	Hydrolysis Enzyme Production $\alpha$ -Amylase and $\beta$ -Glucosidase from <i>Aspergillus niger</i> with Solid State Fermentation Method on Rice Husk, Bagasse and Corn Cob Substrate <i>Heri Hermansyah, Rizky Ramadhani, Adinda Putri Wisman, Rita Arbianti</i>	402
341	Alkaline protease activity of Black Aspergilli isolated from soil of West Sukolilo Madura <i>Isworo Rukmi, Wuryanti, Arina Tri Lunggani</i>	408
345	Anti Hyperuricemia Activity of Salam ( <i>Syzgium Polyanthum Walp.</i> ) and Meniran ( <i>Phyllanthus niruri Linn.</i> ) Herbs Extracts in Oxonate-Induced Mice <i>Muhtadi, Andi Suhendi, Nurcahyanti W., EM. Sutrisna</i>	413
351	Lignocellulolytic Enzyme Complex of Thermophilic Compost for Agriculture Biomass Conversion <i>Nies Suci Mulyani, Octafsari K. Saputri, Agustina L.N. Aminin</i>	420
355	Biogas from the Solid Waste of Dairy Cattle as Renewable Alternative Energy at Mowila and Konda, Konawe Selatan, Sulawesi Tenggara <i>Prima Endang Susilowati, Ahmad Zaeni, Darwis</i>	424
359	Production and Characterization of Biosurfactant from Halophilic Bacteria <i>Pseudomonas stutzeri</i> Strain BK-AB12 <i>Rukman Hertadi, Desyka Sari Sihaloho, Deana Wahyunigrum</i>	428
363	The Ability of Bacterial Isolates of <i>Actinobacillus sp.</i> in Degrading Pollutants p-Cresols and Sunset Yellow <i>Subandi, Muntholib, Eli Hendrik Sanjaya, Prita Olivia Putri</i>	434

<b>Section 6: Chemical Education</b>	439
The Model Development of Chemical Practical Work Approach and Performance Assessment to Increase the Performance of the Laboratory Practitioners <i>Endang Susilaningih, Murbangun Nuswowati</i>	441
Measure Student Teachers' Ability to Implement Authentic Assessment <i>Harjito, Sri Nurhayati</i>	448
Chemical-Science Education Integrated with Religion <i>Kasmadi Imam Supardi</i>	454
Giving Task Designing and Presenting Environmental Problem Solving through Environmental Chemistry Course to Increase Character Values and Knowledge of the Students <i>Murbangun Nuswowati</i>	461
The Application of Discovery Learning with Scientific Approach to Improve the Students' Science Process Skill <i>Naila Ayadiya, Woro Sumarni</i>	466
Science, Environment, Technology and Society (SETS) Oriented Mini-Chem Book <i>Nor Harisah, WoroSumarni</i>	470
Inquiry Learning in Laboratory by HPLC Reversed-Phase Method Development in Taking the Conditions of Heavy Metals Separation <i>Sri Wardani</i>	476



## Anti Hyperuricemiapotential Acitivity of Some Medicinal Plants Extract from Indonesia to Decrease Serum Uric Acid White Male Mice in Balb-C Strain Induced by Potassium Antih Acitivity Of Salam (*Syzigium Polyanthum Walp.*) and Meniran (*Phyllanthus niruri Linn.*) Herbs Extracts in Oxonate-Induced Mice

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### Abstract

The present study was carried out to evaluate in potassium oxonate (250 mg/kgb.wt) induced mice. The water extract of Salam (*Syzigium polyanthum Walp*) and Meniran (*Phyllanthus niruri L.*) administered orally to the hyperuricemic mice, produced significant decrease in the level of uric acid in blood serum. The highest of antihyperuricemia activity in uric acid levels were shown of Salam extract with doses 200 mg/kgb.w and the value of persentage reduction were 0.520 mg/dL, while the negativeand positive control, was 3.100 and 0.200 mg/dL, respectively. Each extract had been analysed the standart test procedure based on the parameters of the general standardize of medicinal plant extracts suggested by The National Agency of Drug and Food Control ofIndonesia. in conclusion, based on the results of standard tests common medicinal plant extracts, both materials under study had met the recommended requirements.

**Keywords:** antihyperuricemia activity, *Syzigium polyanthum Walp*, *Phyllanthus niruri Linn.*, oxonate-induced mice

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### Introduction

Uric acid is the end result of a chemical compound of purine metabolism in the body. Based on the previous reports that 90% of uric acid is the result of purine catabolism assisted by guanase and xanthine oxidase enzyme (Shamley, 2005). Uric acid is carried to the kidneys through the bloodstream to be expelled with the urine. Healthy kidneys will regulate uric acid levels in the blood to keep it under normal circumstances. However, excessive uric acid will not be accommodated and completely metabolized by the body, there will be increased levels of uric acid in the blood known as hyperuricemia. Hyperuricemia which may further develop into gout (Klippel, 2000).

Preliminary observations of the research team, proved that the water extract of Salam leaves at a dose of 1.25 g / kg body weight can reduce blood uric acid levels in white male mice effectively (Handadari, 2007) and Salam leavesinfusion at a dose of 2.5 g / kg BB is able to reduce levels of uric acid with allopurinol equivalent dose of 10 mg / kg (Ariyanti, 2007). There

is also research showing that the ethanol extract of the herb meniran (*Phyllanthus niruri L.*) can reduce levels of uric acid male Leghorn chicken made of hyperuricemia with high purine diet (Susanti, 2005). Another study mentions semi-polar fraction of methanol extract (*Phyllanthus niruri L.*) shows the effect of lowering uric acid levels in rats made hyperuricemic by inducing potassium oxonate (Anonymous, 2008).

In South America meniran is used to treat edema, dealing with the excess uric acid, kidney stones treatment, gallstones, flu, and fever (Anonymous, 2003). Many studies have found meniran as immunostimulator, which is needed by people with infectious diseases, as well as potentan antioxidant and antineoplastic. It is also efficacious as immunotherapy or therapeutic companion other cancer drugs (Novalina, 2003).

The use of traditional medicine in Indonesia is essentially a part of the Indonesian culture. The advantage of the use of the drug (herb) in principle is

a traditional side-effects are relatively smaller than modern medicine. Although empirical traditional medicine can cure various diseases, but efficacy and ability has not been proven scientifically and clinically. Moreover, not much is known what chemical compounds responsible for the efficacy of traditional medicine (Wijayakusuma, 2002). Meniran that contain approximately the same chemistry with regards though there has been no research on the activity decrease uric acid levels in the blood of white mice. Therefore, it is necessary to investigate whether the extract Meniran also have the same activity in reducing blood uric acid levels of white mice and also further research to reveal the leaf extracts as Salam and Meniran standardized herbal medicine (SHM), especially in the treatment of gout, with follow the research methodology recommended by the BPOMRI.

Therefore, in order to explore the competitive advantages of natural ingredients native of Indonesia, by increasing the potential and capacity of natural medicinal materials into standardized herbal medicine (SHM) phytopharmaceutical quality, with its potential to synergize with each other by Industry, Research Institutions (Universities), and full support by the Government, the research for the purpose of raising herbs into a better product is very important to be done.

## Methods

### Preclinical testing

**Tools:** injection volume of 3.0 mL syringe (Terumo), syringes for insulin injection 1.0 ml oral syringe size 15 gauge, flacone, analytical balance of mice with 2610 gram capacity scales (Lark, China) (Presica A-SCS), capillary tube (Assistent), mikrotube centrifuged (eppendorf), centrifuged (mini spin), vortex, micropipette sizes 5-40 mL and 200-1000 mL, blue tip, yellow tip, tools glass (Pyrex), FC StarDust \* 15 (DyaSys) and disposable cuvette.

**Materials:** water extract of Salam leaves and Meniran herbs, Potassium oxonate p.a. (Aldrich Chemical Company), Allopurinol p.a. (Sigma), NaCl 0.9%, distilled water and uric acid reagent kit FS \* TBHBA (DyaSys).

**Animals:** male white mice Balb-C strain with an average weight of 30-40 g and aged 2-3 months.

### Plant material and extraction

Salam (*Syzygium polyanthum* Walp) and Meniran (*Phyllanthus niruri* L.) were collected fresh from the region in Surakarta areas, Central of Java, Indonesia and dried in the shade and then powdered. The plant was identified by experts team from the pharmaceutical biology, Pharmacy Faculty, Muhammadiyah University of Surakarta. Extraction of samples of Salam and Meniran herbs were boiled with water solution until the volume becomes half of its initial volume, then filtered and the filtrates evaporated at the rotary evaporator (RE) in order to obtain a thick extract. Then the condensed extract obtained was dried in a Vacuum Dryers and Vacuum Ovens to be a dried extract.

### Hyperuricemia induction

High uric acid levels (hyperuricemia) is made by the intraperitoneal injection of potassium oxonate 250 mg / kg or 5 mg / 20 g BW in mice (Zhao et al., 2005).

### Introduction preclinical testing

Preliminary tests was conducted for the purpose of obtaining data on the extract dose, blood sampling time, and the single active extracts in lowering uric acid levels.

### Treatment for animal tests

Test animals were divided into treatment groups, which include: a negative control group/hyperuricemia (potassium oxonate dose of 250 mg/kg b.w.), positive control (allopurinol dose of 10 mg/kg b.w.), water extract of Salam leaves and Meniran with single dose (200 mg/kg b.w.). Administration of the test preparation performed one hour after the induction with potassium oxonate dose of 250 mg/ kg b.w.

### Intake of blood serum

Blood sampling is done one hour after giving extract treatment or two hours after induction potassium oxonate. Blood was taken through the eyes of mice by piercing the ophthalmic vein branches located in the median saccus orbitales the capillary tube. Blood flowing through a capillary tube is collected in Eppendorf tubes, centrifuged after a blood clot to obtain serum.

**Table 1-** Preliminary test data modelling hyperuricemia

Treatment	No. HU	Uric acid level (mg/dL)	Mean	SD
normal control(without treatment)	1	1.3	1.433	0.231
	2	1.7		
	3	1.3		
Potassium oxonate dose 250 mg/kg b.w.	1	3.1	3.067	0.950
	2	4		
	3	2.1		

#### Determination of uric acid levels

Uric acid levels are set based on the enzymatic reaction of uric acid reagent FS\*TBHBA. Blood serum was mixed homogeneously with the uric acid reagent FS\*TBHBA incubated for 10 min at 37°C. Then a solution of the sample, standard and blank absorbance is read using a spectrophotometer Start Dust FC\*15 at a wavelength of 546 nm.

#### Standardization of Extract

Standardization of extract were done with following standard procedures based Materia Medika Indonesia and Parameter Extract Plant Medicine General Standards recommended by Badan POM RI, including specific and non-specific parameters.

#### Results and Discussion

Preliminary test was conducted to determine how the model of hyperuricemia in male white mice, is to find an effective dose of potassium oxonate for increasing uric acid levels of normal conditions.

The results of the preliminary testing can be seen that the potassium dose of 250 mg / kg was able raised the levels of uric acid from normal level with an average of 1,433 mg / dL to 3,067 mg / kg. This is consistent with the theory that if the mice said hyperuricemia blood uric acid levels ranged from 1.7 to 3.0 mg / dL. Based on the results of the statistical test result that between normal controls and a group of very different potassium oksonat significant that the significance value of 0.205 ( $p > 0.05$ ). Thus, from the results of the research method can be continued further research is treated by administering test extract preparation.

#### The Results of Preclinical Testing of Extract

The test animals were used in this study were male white mice (*Mus musculus*) which has urikase enzymes that can break down uric acid to allantoin which form the end product is readily soluble in water (Martin, 1987). to minimize biological variation, the researchers conducted some control over variables such as test animals by using more or less the same biological variations that include weighing

approximately 30-40 g, aged 2-3 months, Balb-C strain, male gender and treated equally is placed in cages with each cage the same amount and the same diet and treated before the test animals were fasted beforehand for  $\pm 2$  hours with a fixed given to drink ad libitum. This is done so that the same conditions of test animals and to reduce the influence of food intake on the stocks III given in the study. and to reduce the stress level of test animals adapted to the laboratory conditions for 7 days.

Male sex selection is based on the consideration that male mice do not have the hormone estrogen, jikalau pun there are only relatively few in number and condition of the male hormone more stable when compared with the female mice because female mice experiencing hormonal changes at certain times such as the the estrous cycle, pregnancy and lactation in which these conditions can affect the psychological condition of the test animals. in addition, the level of stress in female mice was higher than the male mice that may be annoying at the time of testing.

Potassium oxonate is used as an inductor hyperuricemia because potassium oxonate is a competitive inhibitor urikase for increase uric acid levels by preventing uric acid into allantoin changes. Where is water soluble allantoin and can be excreted through the urine, so the denial rikase enzyme by potassium oksonat the uric acid will accumulate and are not eliminated in the form of urine.

Allopurinol as a positive control used was the one of the common drug for gout. Allopurinol is the only uricostaticum are currently used therapeutically, which works to reduce the formation of uric acid. While working to increase the elimination of uric acid called uricosurice (Mutschler, 1991). Allopurinol is substrate xantine oxidase and eliminated through the kidneys primarily as oxypurinol (often also referred to as the one that is aloxantine) (Schunack and Mayer, 1990). Allopurinol and oxypurinol, inhibits xantine and uric acid, which in low doses went competitive inhibition mechanism and in high doses it does not work competitively. Allopurinol which has a plasma half of the approximately 40 minutes, is hydrolysed by xantine oxidase into metabolites (Mutschler, 1991). Metabolite of allopurinol-1-ribonucleotide, which can

be expressed in a small organ extracts, may be responsible for the additional inhibition of the de novo purine synthesis (Schunack and Mayer, 1990). Through inhibition of xantine oxidase hypoxantine and xantine then excreted in the urine so that more levels of uric acid in the blood and urine decreased (Mutschler, 1991).

Test preparations are used to lower uric acid levels in this study were water extracts of the leaves starfruit. Penyarian method used is solvent extraction with

water, wherein the method is similar to the use of plant materials as traditional medicine (herbal medicine) is by boiling the material and taking the concentrates to be taken so that the traditional equality of treatment in the study and treatment identical. The difference in this study concentrates obtained after boiling evaporated in a vacuum dryer to dry the extract terbentuk. It is intended to maintain stability during storage as if it is stored in liquid form is very vulnerable to contamination and quickly overgrown by fungi.

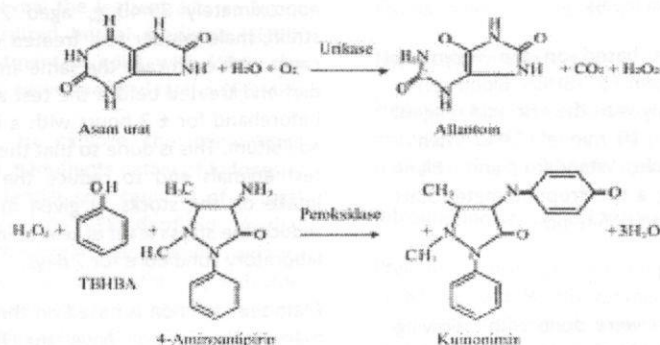


Figure 1- Reaction Mechanism of Formation Kuinonimin (Schunack *et al.*, 1990)

Determination of uric acid levels determined by enzymatic methods using reagents Uric acid TBHBA FS \* (2,4,6-tribromo-3-hydroxybenzoic acid) using a spectrophotometer StarDust FC 15. mechanism that occurs is uric acid is oxidized by the enzyme urikase with the help of H<sub>2</sub>O and O<sub>2</sub> into allantoin, carbon dioxide and hydrogen peroxide. The hydrogen peroxide formed reacts with 4-aminoantipirin and TBHBA be kuinonimin pink where the reaction catalysed by the enzyme peroxidase (POD). The magnitude of the intensity of the colour produced by the kuinonimin equivalent to the levels of uric acid in the blood. The reaction mechanism can be seen in Figure 1.

Data uric acid levels in the serum of mice after induction with potassium oxonate extract the test preparation and administration of a single dose of 200 mg / kg is presented in Table 2.

From the table 2 can be created histograms between treatment groups with an average blood uric acid levels male white mice as follows:

Based on the results of the ANOVA testing can be known that the levels of uric acid of the negative control and the positive control extract group showed significantly different results with a significance value of 0.000. This suggests that allopurinol and Salam leaf extract, or combination Salam-Meniran and Meniran

extract can reduce of blood uric acid levels in white male mice Balb-C strain compared to the negative control (Potassium oxonate).

When compared with the positive control, decrease produced by extracts and extract Meniran greetings differ significantly from the values respectively 0.029 and 0.000 (P < 0.05). from the results obtained it can be said that the extracts with a single dose can lower uric acid levels in white male mice Balb-C strain but decrease significantly different given each other with a significance value of 0.009

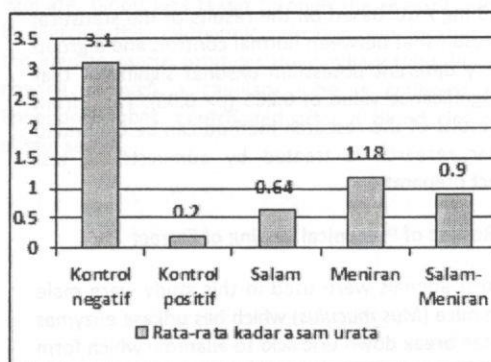


Figure 2. Histogram of treatment groups Vs average blood uric acid levels (mg / dL)

**Table 2-** Data Serum Uric Acid Levels After Extract Treatment

No.	Treatment	BB (Gram)	Uric acid levels		
			Post Testing	Average	SD
1	Negative control(Potasium Oxonate 250 mg/kgBB)	39.3	3.1	3.100	0.3464
2		30.5	3.7		
3		30	2.9		
4		36	2.9		
5		39	2.9		
1	Positive control(Allopurinol 10 mg/kgBW)	37.5	0.1	0.2000	0.1000
2		36.5	0.2		
3		36	0.3		
4		39	0.3		
5		38.5	0.1		
1	Salam leaves extract(200 mg/kgBB)	36	0.5	0.6400	0.1673
2		36.5	0.5		
3		36	0.9		
4		38.5	0.6		
5		39	0.7		
1	Meniran herbs extract (200 mg/kgBB)	35	1.2	1.1800	0.2049
2		36	1.2		
3		37.5	1		
4		39	1		
5		36.5	1.5		
1	Extract ofSalam – Meniran(200 mg/kgBB)1: 1	35	1.5	0.9000	0.4899
2		36.5	0.5		
3		33.5	1.1		
4		36	0.3		
5		36.5	1.1		

While combined, the result is compared with the positive control was also significantly different with  $P_{\text{value}} = 0.001$  ( $P < 0.05$ ), and not significantly different when compared with single Salam leaf extract. This means that the decreasing produced by a combination of Salam extracts and Meniran could not equivalent with allopurinol. Percentage decrease by a combination of extracts Salam-Meniran almost equal to the decrease by Salam as a single extract. Therefore it can be concluded that the Meniran extract less potent in reducing blood uric acid levels male white mice.

#### Results and Discussion of Standardisation Extract

In the determination of standardized extracts, tests performed include analysis of the non-specific analysis of drying shrinkage, specific gravity, moisture content, ash content, the content of residual solvents, pesticide residues, heavy metal contamination, microbial contamination, and specific analysis that includes the identity of the extract, compounds dissolved in certain solvents, also test the chemical content of the extract. Results of standardized test bay leaf extract can be seen in the following table 3.

**Table 3-** The results of standardized extracts of non-specific parameters

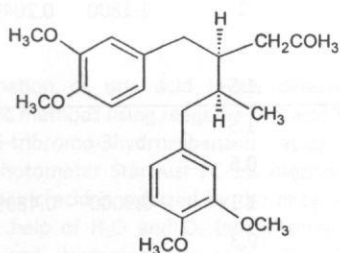
Extract	non-specific parameters					
	drying shrinkage	water content	Ash content	ash content Acid insoluble	Heavy metal contamination (µg/kg)	microbial contamination (aflatoxin)
Salam	12.287	6.733	28.537	22.110	2.782 (Pb) n.d (Cd)	n.d
Meniran	10.206	0.550	28.860	19.636	5.194 (Pb) 0.0096 (Cd)	n.d

Remarks: n.d: no detection

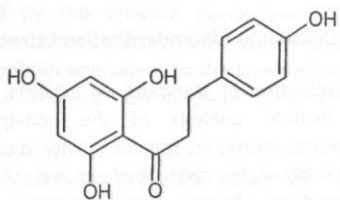
**Table 4-** The results of standardized extract of specific parameters

Extract	specific parameters		
	water-soluble extract	total phenolics	total flavonoid
Salam	64.656	1.083	0.196
Meniran	56.195	7.154	0.786

**Compound Identity** Compound identity: Fluoretine (Salam) and phyllantine (Meniran) Structural formula:



Fluoretine



Phyllantine

## Conclusion

1. Salam and Meniran leaves extract, single dose of 200 mg/kg b.w proved to potentially reduce levels of uric acid in the blood of male white mice Balb-C strain that been induced potassium oxonate where the percentage reduction in uric acid levels provided by Salam extracts approximately 79.35% and 61.94% for Meniran extracts whereas a decrease of 93.55% by allopurinol.

2. The combination of Salam and Meniran extracts with the same ratio (1: 1) gave results not significantly different with positive control is the percentage decrease of 70.97%.
3. Based on the results of standardize common testing medicinal plant extracts, both materials under study had met the recommended requirements.
4. Compound identity of the Salam leaves extract is fluoretine while Meniran extract is phyllantine. The difference is exactly what distinguishes active compound potentiation decreased levels of blood uric acid male white mice.

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