

MAKALAH ILMIAH

**SUPER ANTIOXIDANT FROM INDONESIAN PROPOLIS:  
EFFECT OF PROCESS VARIABLES ON VALUE OF ANTIOXIDANT  
ACTIVITY**



OLEH:

Boy Arief Fachri, Puspita Sari, Sih Yuwanti

Makalah disajikan pada “International Conference on Agromedicine and Tropical Disease “ pada tanggal 20-21 Oktober 2018 di Sapphire Ballroom, Aston Hotel & Conference Center, Jember, Jawa Timur, Indonesia.

Diselenggarakan oleh Fakultas Kedokteran Universitas Jember



## Dear Colleagues,

On behalf of the organizing committee, I am truly honored to take this opportunity to welcome you to the 2nd ICATD 2018, following the success of the 1st ICATD conference in 2016.

The needs for agromedicine research for the improvements on occupational and environmental health and safety in agriculture are growing. The challenges in tropical disease are also increasing that requires a global solution to prevention and elimination. This event would facilitate dissemination of research on this topic, and surely, it will be an outstanding place for networking opportunities to discuss interesting ideas and develop the fruitful project in the future. As a major goal of this event, we hope that it can be an excellent chance for coordinating new partnerships which advance collaboration in the research field as well as the career of all participants.

The ICATD provide an opportunity to network with others with similar interest, to hear and discuss the fuse of acknowledging of experts and to present your own work through an oral and poster presentation.

The 2nd ICATD iss held on October 20th-21st, 2018 in Jember, Indonesia. Jember is a city full of wonder located in the East Java province, Indonesia. Jember has unique culture called "Pandalungan" that is merged from Javanese and Maduranese Culture. Jember area is dominated by farming, gardening, and fishing, therefore Jember has a beautiful view of mountains and beaches. Kawah ijen with blue fire is one of the popular destinations for international tourists. Papuma Beach has beautiful white sands and lovely sunset and you have the opportunity to visit the beach by our city tour program. It is my hope that you get a chance to explore Jember and its surrounding and enjoy the atmosphere of our city.



## INTRODUCTION

Agromedicine is a relatively new concept. It deals with the interdisciplinary application of agriculture, applied chemistry and medicine to the safe global production of food with maintenance of the health conditions of agricultural workers and the general population (Almaida, 2009) In the simple definition, agromedicine is a study of human health related to agriculture in partnership with other agricultural, natural resources and health professionals, in the promotion of health and safety in agriculture, forestry and fisheries. The collaboration involves research, prevention, intervention, education, and outreach by physicians, nurses, and allied health professionals; epidemiologists; Extension and rural health educators; toxicologists; veterinarians; agricultural production specialists; agricultural engineers; family and consumer science specialists; and forestry and fisheries specialists (Hodgso and Cope, 2014). Agromedicine is needed to provide occupational and environmental health safety in agriculture. Agromedicine is a term coined in the 1950s to emphasize interdisciplinary, programmatic approaches which give a greater role for the agricultural professional based upon the equal partnership of the two disciplines (medicine and agriculture) to promote occupational and environmental health and safety in agriculture through research, prevention/intervention, education, and outreach. Agromedicine also was known as the application of medical and agricultural sciences to promote the health and safety of farm families, agricultural workers, and consumers of agricultural products.

In developing countries, particularly in a tropical and subtropical region like Indonesia, many people in agriculture area are dealing with tropical diseases. Most often the disease is transmitted disease caused by an insect bite in agriculture area like Anopheles as malaria vector. Human exploration of tropical rainforests, deforestation, rising immigration and increased international air travel and other tourism to

tropical regions has led to an increased incidence of such diseases. Therefore, agromedicine and tropical disease are an interesting topics to be explored and developed for human welfare.

Communities need a better coordination of agricultural and medical expertise to collaborate across disciplines in so-called basic science. Dedicated teamwork across the countries is required to implement a preventive, educational approach that delivers the best science and the best outreach that our university system can provide to the International community. Agricultural Health Medicine (Agromedicine) is a priority for Faculty of Medicine, University of Jember because Jember area is dominated by farming, forestry, and fishing as well as Indonesia territory.

Studies in the agromedicine and tropical disease field should continue to address the identification of occupational risk factors associated with injuries and illnesses and prevention of spread of tropical disease, as well as develop cost-effective interventions and practices that lead to the minimization or elimination of the injuries and illnesses on a global scale, among the producers and workers in the agriculture field.

Researchers in agromedicine and tropical disease field have to be encouraged. One of the way to encourage the development of agromedicine and tropical disease research in Indonesia, and in all over the world, is to promote scientific forums, where scientists can share their experiences, publish their results, and get new insight/idea for the improvement of their research.

### **HIGHLIGHTS OF THE CONFERENCE**

- Agricultural Health Science (Agromedicine)
- Occupational and Environmental Health for Rural Health Professionals
- Pesticide Related Illness & Health Issues
- Tropical Diseases
- Traditional Medicine
- Biology Molecular
- Marine Biology
- Nutritional science
- Nursing Science



# COMMITTEE ICATD 2018

## International Conference on Agromedicine and Tropical Disease

*“Current Trends, Challenges and Issues in Agriculture Health Medicine :  
from Rural to Urban, Ocean to Island and Molecular to Clinical”*

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### DAY 1 : 20 October 2018

07.30 – 08.30	Registration Day 1	
08.30 – 09.00	Opening Ceremony	Opening Ceremony
		Traditional Dance
		Speech:
		1. Chairwoman of organizing (DR. dr. Yunita Armiyanti, M.Kes)
		2. Dean of Faculty of Medicine University of Jember (dr. Supangat, M.Kes, PhD, SP.BA)
		Prayer
09.00 – 11.30	Plenary Session	Keynote Speaker I (Prof. DR. dr. Nasronudin, Sp.PD., K-PTI FINASIM)
		Keynote Speaker II (Prof. Drs. Bambang Kuswandi, M.Sc., Ph.D)
		Keynote Speaker III (Prof. Susan Alison Brumby)
		Discussion
11.30 – 13.00	Lunch (ISHOMA )	
13.00 – 15.00	Paralel Session	Oral & Poster presentation
15.00 – 15.30	Coffee break	
15.30 – 17.00	Bussiness Meeting	Establishment of Konsorsium Agromedis Indonesia (invitation only)

### DAY 2 : 21 October 2018

07.30 – 08.00	Registration Day 2	
08.00 – 10.40	Plenary Session	Keynote Speaker IV (Prof. Chihaya Koriyama, MD, Ph.D)
		Keynote Speaker V (Dr. Vickneshwaran Muthu)
		Keynote Speaker VI (Dr.rer.nat. Anna Artati, M.Sc., M.Si)
		Discussion
10.40 - 10.55	Coffee break	
10.55 – 12.55	Parallel Session	Oral & Poster presentation
12.55 – 13.30	Lunch	
13.30 – 14.00	Closing ceremony	



**ORAL PRESENTATION**

**DAY 1 : 20 OCTOBER 2018 (13.00 – 15.00 WIB)**

**ROOM 1 : Agriculture and Environment**

No	Name	Code	Institution	Title
1	Esti Utarti, Antonius Suwanto, Maggy T. Suhartono and Anja Meryandini	AEO-1	Bogor Agricultural University, University of Jember	Using Indigenous Actinomycetes Enzyme in Biodegradation of Lignocellulose Through Oxidation Process
2	Dewi Rashati, Mikhania Christiningtyas Eryani	AEO-2	Jember Pharmacy Academy	Viscosity Agent Variation Concentration Effect to Irritation and Physical Properties Katuk Leaf ( <i>Sauropus androgynus</i> (L) Merr) Shampoo
3	Sattya Arimurti	AEO-3	University of Jember	The Potency of Caffeine-Degrading Microbe Indigenous Coffee Pulp Waste
4	Ika Oktavianawati	AEO-4	University of Jember	Brief Review on the Potency of Essential Oil Production of Flowers, Fruits and Leaves of <i>Cananga odorata</i> from Karangpring Village, Jember District
5	Boy Arief Fachri, Puspita Sari, Sih Yuwanti	AEO-5	University of Jember	Super Antioxidant from Indonesian Propolis: Effect of Process Variables on Value of Antioxidant Activity
6	Helda Wika Amini, Istiqomah Rachmawati	AEO-6	University of Jember	Valorization of Coffee Pulp Waste by Esterification Reaction as Antioxidant Potential
7	Rizki Fitria Darmayanti, Bektu Palupi, Afham Kilmi, Ari Susanti	AEO-8	University of Jember	High Strength Linen Suture Thread Production from Pretreated Cocoa Pod Husk Fibre with Enzymatic Scouring
8	Rizki Fitria Darmayanti, Meta Fitri Rizkiana	HMO-17	University of Jember	Pretreatment of Rice Husk for Butanol Production as the Intermediate of Drug Bioplasticizer
9	Azizah, Syafiq Ubaidillah, Adinningtyas Intansari, SarwendahSiswi Winasis, Rudju Winarsa, Kahar Muzakhar and HidayatTeguh Wiyono	AEO-10	University of Jember	Bacterial Identification of Well Water Sample from Sumberwaru Village, District of Banyuputih, Situbondo, East Java as Pre-Indicator of Water Pollution.
10	Nur Indah Julisaniah, Suharjo Suharjo, Retno Mastuti, Estri Laras Arumingtyas	AEO-11	University of Mataram, University of Brawijaya	Character of Peanut Stripe Virus from Lombok, West Nusa Tenggara
11	Sutoyo	AEO-12	University of Jember	A New Species of Keratinolytic Fungi (Pleosporales, Dothideomycetes) From Jember, Indonesia

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## ROOM 2 : Health and Medicine

No	Name	Code	Institution	Title
1	Praba Ginandjar, Lintang Dian Saraswati, Putri Septyarini, Bagoes Widjanarko	HMO-1	Diponegoro University	Community Behavior Towards Filariasis Mass Drug Administration in Tegaldowo Village, Pekalongan District
2	Wantiyah, Muhamad Rifqi Wibowo, Latifa Aini Susumaningrum	HMO-18	University of Jember	Relationship Between Use of Personal Protective Equipments (PPE) with Impaired Skin Integrity of Farmers
3	Julie Ann S. Ng	HMO-3	Riverside College, Inc.	Evaluation of Microcrystalline Cellulose Derived from <i>Saccharum officinarum</i> L. (Sugarcane) Leaves as Disintegrant in Tablet Formulation
4	Rike Oktarianti, Aisyah, Kartika Senjarini	HMO-4	University of Jember	Host Immune Response Against 56 kDa Immunogenic Protein from Salivary Gland of <i>Aedes aegypti</i>
5	Lidia Maziyyatun Nikmah, Susantin Fajariyah, Mahriani	HMO-5	University of Jember	The Effect Of Turmeric ( <i>Curcuma Longa</i> ) Extract On Rectum Histological Structure Of Rat ( <i>Rattus Norvegicus</i> ) Induced Dextran Sodium Sulphate (DSS)
6	Siti Nur Azizah and Mikhanisa	HMO-6	Jember Pharmacy Academy	Isolation and Characterization of Endophyte Actinomycetes from Antituberculosis Medical Plants
7	Wiwien S Utami, Hari Kusnanto, Wayan T Artama, Elsa Herdiana	HMO-7	University of Jember, Gadjah Mada University	Potential Risk Factors For Cryptosporidium Infection Among Farmer Community in Sleman Yogyakarta
8	Heny Arwati, Putu Indah Budi Apsari, Faith Fore, Ramadhani Rusdi Bahalwan and Puspa Wardani	HMO-15	Universitas Airlangga	Suppressive Antimalaria Effect of Goat Bile Against <i>Plasmodium berghei</i> Infection in Mice

**DAY 2 : 21 OCTOBER 2018 (10.40 – 12.40 WIB)**

**ROOM 1 : Health, Medicine and Environment**

No	Name	Code	Institution	Title
1	Muhammad Ihwan Narwanto, Masruroh Rahayu, Setyawati Soeharto, Nurdiana, Moch. Aris Widodo	HMO-8	University of Jember	Identification and in Silico Analysis of Anti Inflammation and Antioxidant Potentials of Polyphenol Compounds in Methanol Extract of Tamarindus Indica Seeds
2	Ancah Caesarina Novi Marchianti, Dwita Aryadina Rachmawati, Ida Srisurani Wiji Astuti, Yohanes Sudarmanto, Angga Mardro Raharjo	HMO-9	University of Jember	Reflection of Maternal Feeding Patterns on Toddlers Towards Stunting in Jember Agroindustrial Community
3	Rosida, Diyan Ajeng Rosetyowati, Yuni Inawati	HMO-10	Pharmacy Academy of Jember	The Effect of Banana Peel Extract (Musa Acuminata) on Total Cholesterol, HDL and LDL Level on Hypercholesterolemia Induced Rats
4	Tantut Susanto, Retno Purwandari; Emi Wuri Wuryaningsih	HMO-11	University of Jember	The Effects of Progressive Muscular Relaxation and Stretching Exercises Combination on Blood Pressure Among Farmers in Rural Areas: A Randomized Controlled Pilot Study
5	Resti Hudayati, Yulia Dwi Setia, Nurdiana, Prasetyo Adi	HMO-12	Universitas Brawijaya	The Effect of Melatonin to the Level of Malondialdehyde (MDA) in the Bronchoalveolar Lavage Fluid (Balf) of Wistar Strain Rats (Rattus Norvegicus) Exposed Subacutely by Coal Fly Ash
6	Dian Mardhiyah, Sophianita, Andrew Rozaan Fadlurrahman., Harliansyah	HMO-13	YARSI University	Increased Malondialdehyde Levels on Saliva Woman Cigarette Smokers
7	Vipi Nurpila, Lintang Dian Saraswati, Martini, Praba Ginandjar	HMO-14	Diponegoro University	Evaluation of Mass Drug Administration (A Cross Sectional Study in Sanggu Village, South Barito, Central Kalimantan, Indonesia)
8	Retno Wimbaningrum, Fefi Ekawardiana, Rendy Setiawan	AEO-7	University of Jember	The Correlation Between The Land Use and The Water Quality of The Rembangan River, Jember, East Java. Indonesia
9	Kiky Chily Arum Dalu, Nurhayati Nurhayati, Jay Jayus	HMO-16	University of Jember	Decreasing of Pathogenic Bacteria to Prevent Tropical Diseases by Using Fermented Probiotic Juice
10	Felix Arie Setiawan, Ari Susanti, Bekti Palupi, Meta Fitri Rizkiana	AEO-9	University of Jember	Natural Sources Screening for Antimicrobial Agent of Herbs, Spices, and Extracts: A Semi-Qualitative Study
11	Reny Indrayani, Erwin Nur Rifah, Ari Satia Nugraha, Hadi Prayitno	HMO-2	University of Jember	Health Seeking Behaviour Among Tengerese Society : An Update
12	Wahyudi Widada <sup>1</sup> , Teddy Ontoseno <sup>2</sup> , Bambang Purwanto <sup>2</sup>	HMO-19	Muhammadiyah University Jember	Potency Wet-Therapy Reduce Apo-B And Total Cholesterol In Hypercholesterolemia Patients

## ROOM 2 : Health and Medicine

No	Name	Code	Institution	Title
1	Anita Dewi Prahastuti Sujoso, Tri Martiana, Santi Martini	HMO-20	Universitas Airlangga	Occupational Pesticide Exposure and Cholinesterase Activity Level Among Tobacco Farmers in Jember
2	Laila Khamsatul M, Fatimatul Munawaroh, Taslim Ersam, Mardi Santoso	HMO-21	Trunojoyo Madura University, Sepuluh Nopember Institute of Technology	Antibacterial Activity of Leaves Extracts from Thaguri Against <i>S. aureus</i> and <i>E.coli</i>
3	Siti Muslichah, EstriLaras Arumingtyas, Rodiyati Azrianingsih, Serafinah Indriyani	HMO-22	University of Jember	Medicinal Plants for Gynecological Problems Used by Madurese
4	Yeni Purnamasari, Agustina Tri Endharti, SofyPermana	HMO-23	Brawijaya University	CFL Synergistically Enhances the Anti- Metastatic Effect of 5-Fluorouracil in Colorectal Cancer Through Modulation of Focal Adhesion Kinase and Intracellular Calcium
5	Candra Bumi, Heni Fatmawati	HMO-24	University of Jember	Normoxia Increase Replicative Senescence Through p21 in Early Passage of Bone Marrow Mesenchymal Stem Cell of New Zealand Rabbit
6	Hans Cendikiawan, Sri Andreani Utomo	HMO-25	Airlangga University	Radiologic Findings for Cerebral Malaria
7	Dina Helianti, Sutjipto, Widjiati	HMO-26	University of Jember	Effect of Catechin and Cacao Powder on Pro- inflammatory Cytokine (IL-1) in Cigarette Smoking Exposed Rat
8	Septarini Dian Anitasari, Dwi Nur Rhikmasari, Ida Ayu Astarini, Made Ria Defiani	HMO-27	Udayana University	Production Potential Pharmaceutical Variety of Sugarcane with Microspore Culture
9	Agustina Tri Endharti, Renata Primasari, Sofy Permana	HMO-28	Brawijaya University	Mango Mistletoe Leaf ( <i>Dendrophthoe pentandra</i> ) Extract Inhibit Par-2 Induces Cell Cycle Arrest in the Colon Cancer via Up- Regulation of P21
10	Erma Sulistyaningsih, Anak Agung Istri Ratnadewi, Rosita Dewi ,Sheilla Rachmania	HMO-29	University of Jember	Expression and Structure Prediction of Cidr1 $\alpha$ - Pfemp1 Recombinant Protein from Indonesian <i>Plasmodium Falciparum</i> Isolate
11	Elyda Akhya Afida Misrohmasari, Hestieyonini Hadnyanawati, Sulistyani, Kiswaluyo, Arwinda Hening Pangestu	HMO-30	University of Jember	Factors Affecting Utilization of National Health Insurance in Dental Health Among Coastal Community in Watu Ulo, Jember

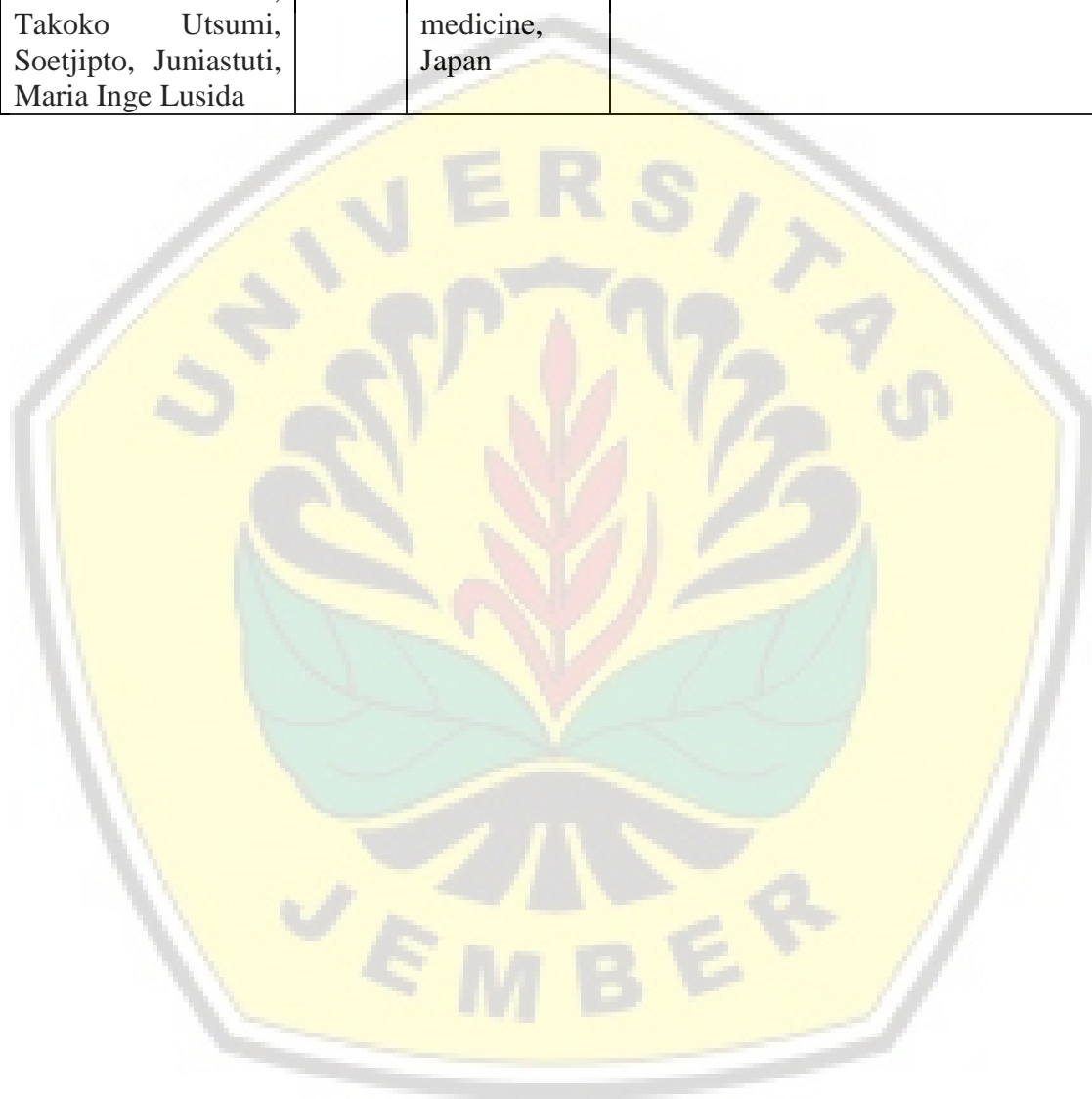
## POSTER PRESENTATION

DAY 1 : 20 October 2018 (13.00 – 15.00 WIB)

No	Name	Code	Institution	Title
1.	Angga M. Raharjo	PP-1	University of Jember	The Effect of Harmonica Exercise on Inspiratory Capacity and Quality of Life of Chronic Obstructive Pulmonary Disease Patients in Agroindustrial Community
2.	PulongWijang Pralampita, Miharu Ushikai, Emi Arimura, Masaharu Abe, Hiroaki Kawaguchi1, Masahisa Horiuchi	PP-2	Kagoshima Prefectural College	Analysis of Age-Dependent Leptin-Signaling in the Leptin Receptor Deficient Mice
3.	Siti Munawaroh, Sujiono, Vivi Yosafianti Pohan	PP-3	Universitas Muhammadiyah Ponorogo	The Influence of Educational Stage on Students' Skill in Performing Physical Examination (a Study in Faculty of Health Science Universitas Muhammadiyah Ponorogo)
4.	Yunita Armiyanti, Widodo <sup>2</sup> , Loeki Enggar Fitri <sup>3</sup> , Teguh Wahju Sardjono	PP-4	University of Jember, Brawijaya University	Identification of Species Composition and Domination of Anopheles Mosquitoes in the Bangsring Beach Banyuwangi
5.	Aris Prasetyo, Enny Suswati, Hairudin, Jauhar Firdaus	PP-5	University of Jember	The Effects of Pesticides Administration on Pregnancy and the Incidence of Congenital Defects in Female Whistar Rat
6.	Elly Nurus Sakinah, Rena Normasari	PP-6	University of Jember	Hepatoprotector Effect of Ethanol Extract of Spinach Leaf (amaranthus tricolor l.) on Mice Hepar Histopathology Induced by Isoniazid
7.	Adinningtyas Intansari, Sarwendah Siswi Winasis, Al Munawir, Angel Yanagihara	PP-7	University of Jember, John A Burns School of Medicine	Identification Species Box Jellyfish at Costal Area Mlandingan Situbondo
8.	Dianita Rifqia Putri, Dian Laila Purwaningroom, Siti Munawaroh, Widodo, Sholihatul Maghfirah, Cholik Harun Rosjidi, Muhaimin Rifa'i	PP-8	Universitas Muhammadiyah Ponorogo, Brawijaya University	Total Phenols Content of Antihypertensive Medicinal Plants used by the Villagers of a Javanese community in Ponorogo East Java, Indonesia
9	Sarwendah Siswi Winasis, Adinningtyas Intansari, Al munawir, Angel Anne Yanagihara	PP-9	University of Jember, John A Burns School of Medicine	Potensial of Cocoa (Theobroma cacao) Ethanol Extract in Inhibiting Nematocyst Tentacle Firing of Physalia utriculus from Papuma Coastal Area
10	Arminatul Jannah, Hidayat Teguh iyono, Purwatiningsih	PP-10	University of Jember	Feeding and Oviposition Preference of <i>Crocidolomia pavonana</i> F. (Lepidoptera: Pyralidae) on Three Host Plants of The <i>Brassicaceae</i> Family

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11	Kristianningrum Dian Sofiana, Provisia Marthalita Y.W, Husnul Khotimah, M. Aris Widodo	PP-11	University of Jember, Universitas Brawijaya	Effects of Low –level Cadmium Exposure on HUVECs (Human Umbilical Vein Endothelial Cells) Cell Viability and Morphology
12	Dewi Setyowati, Teguh Mubawadi, Yudied Agung Mirasa, Didik Purwanto, Mochammad Amin, Takoko Utsumi, Soetjipto, Juniastuti, Maria Inge Lusida	PP-12	Universitas Airlangga, Kobe University Graduate school of medicine, Japan	Hepatitis A virus infections and outbreaks in Two Districs in Indonesia 2018





## Program ICATD

Saturday, October 20, 2018

Sunday, October 21, 2018

Time	Activity	Description
07.30 – 08.30	Registration	
08.30 – 09.00	Opening Ceremony	Opening Ceremony
		Traditional Dance
		Speech:
		1. Chairman of organizing
		2. Rector of the University of Jember: Drs. Moh. Hasan., Ph.D

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09.00 – 09.15	Coffee break and poster session	
09.15 – 11.45	Plenary lecture	Plenary Speaker I
		Plenary Speaker II
		Plenary Speaker III
		Discussion
11.45 – 13.00	Break and poster session	
13.00 – 15.00	Paralel Session	Oral presentation, poster presentation
15.00 – 15.30	Coffe break and poster session	
15.30 – 17.00	Paralel Session	Oral presentation, poster presentation

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## Program ICATD

Saturday, October 20, 2018

Sunday, October 21, 2018

Time	Activity	Description
08.00 – 10.30	Plenary lecture	Plenary Speaker IV
		Plenary Speaker V
		Plenary Speaker VI
		Discussion
10.30 – 11.00	Coffee break and poster session	
11.00 – 12.30	Parallel Session	Oral presentation, poster presentation
12.30 – 13.30	Break and Lunch	
13.30 – 14.00	Closing ceremony	

14.00 - finish

City Tour

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## Super Antioxidant from Indonesian Propolis: Effect of Process Variables on Value of Antioxidant Activity

Boy Arief Fachri<sup>1</sup>, Puspita Sari<sup>2</sup>, Sih Yuwanti<sup>2</sup>

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<sup>2</sup>Department of Agricultural Products Technology, Faculty of Agricultural Technology, University of Jember, Jember, Indonesia

### Abstract

Propolis is a promising source of natural antioxidants containing bioactive compounds such as flavonoids, flavones, phenolic acids, and phenolic acid ester. To isolate propolis from raw material, the method known as extraction is thoroughly needed. The conventional extraction method using organic solvent shows some drawbacks heading to degradation of bioactive constituents. These works are to develop extraction method called supercritical CO<sub>2</sub> extraction method and to observe the effect of process variables to antioxidant activity. The raw material was poured into the reactor vessel. During the extraction, the process variables (temperature, pressure and CO<sub>2</sub> flow rate) are set to constant. When the extraction time was completed, the product was filtered and prepared for analysis using HPLC. To determine the antioxidant activity, the DPPH method was applied to each process conditions. This works claim that (1) supercritical CO<sub>2</sub> extraction can improve antioxidant activity; (2) process variables put the impact on antioxidant activity; (3) the extract containing galangin and caffeic acid phenyl ester.

Keywords: propolis; supercritical the carbon dioxide extraction; antioxidant.

### 1. Introduction

Propolis is one of the products from the activity of bees. It is a resinous substance and colored in dark. The bees collect propolis from various part of the plant. Propolis is accumulated in the hive, mixed with beeswax, making an intensely adhesive matter. Since centuries ago, propolis is used as traditional medicine (Zabaiou et al., 2017). Nowadays, propolis has been reported containing more than 200 chemicals including flavonoids, flavones, phenolic acids, and esterified phenolic acid. These compounds play the role in the activity of propolis such as antioxidant, antimicrobial, anti-inflammatory and antiviral (Al-Ghamdi et al., 2017; Barros et al., 2017; Popova et al., 2017).

Propolis cannot be consumed directly, the process called extraction is needed. The common extraction method is solvent extraction including maceration method (Biscaia and Ferreira, 2009; Trusheva et al., 2007). This method is generally the volume of solvent and time-consuming process. It can cause the degradation of propolis bioactive compounds. This process also produces an extract with strong taste residue. To overcome this issue, this work is to propose an alternative method using supercritical CO<sub>2</sub>. Supercritical CO<sub>2</sub> extraction has some benefits in product separation and reducing extraction time (Del Valle, 2015; Fianco et al., 2018; Kuš et al., 2018; Machado et al., 2015). In this work, the effect of process variables on propolis antioxidant was also observed.

### 2. Methods

#### 2.1. Chemicals

A Trigona Sp raw propolis was purchased from the honey home industry in Garut, West Jawa, Indonesia and collected from September 2017 until February 2018. The liquid carbon dioxide (purity 99.99%) in food grade was supplied in cylinder tube by PT Inter Gas Mandiri (Cikarang, Indonesia).

#### 2.2. Experimental Procedure

The extraction was conducted in extractor vessel for 240 min. During the extraction, the pressure, temperature and CO<sub>2</sub> flow rate were varied following central composite design. The extract of propolis was purified by centrifuge and filtered using membrane filter. The liquid products were qualitatively determined using HPLC (Waters Alliance) using a C-18 column. Some external standard solutions were occupied to identify some compounds.

To observe the effect of process variables on antioxidant activity, the DPPH (2, 2-diphenyl-1-picrylhydrazyl) methods were applied in this work. Further, the antioxidant activity was implicitly determined by IC<sub>50</sub> value.

#### 2.3. Statistical Analysis

Design Expert 10 software (Stat-Ease) was used to run the statistical analysis. The model of IC value as a function of process variables was mathematically formulated in equation 1.

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University of Jember, Indonesia



# 2nd International Conference on Agromedicine and Tropical Disease

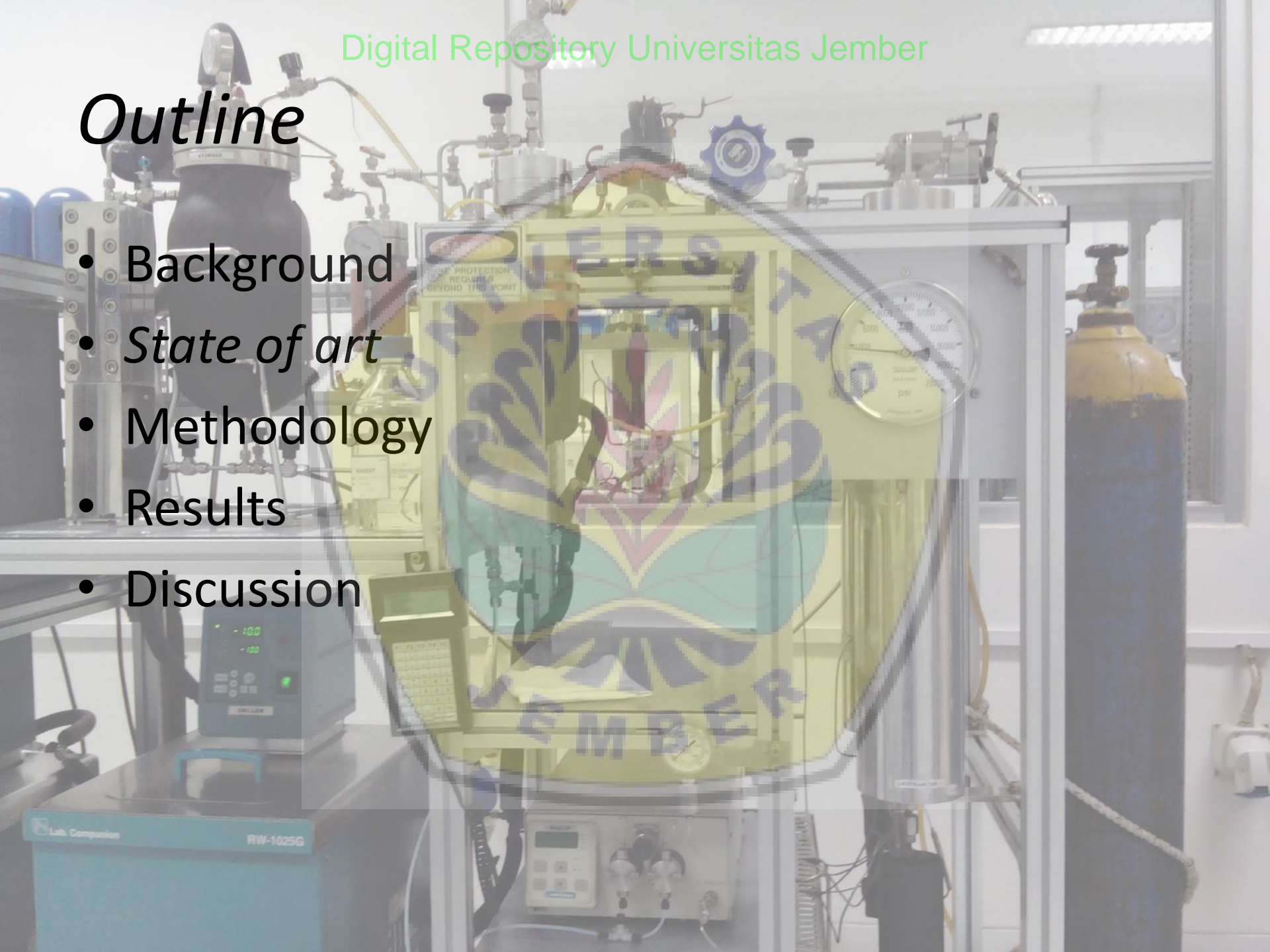
*“Current Trends, Challenges, and Issues in Agricultural Health Medicine : from Rural to Urban, Ocean to Island and Molecular to Clinical”*

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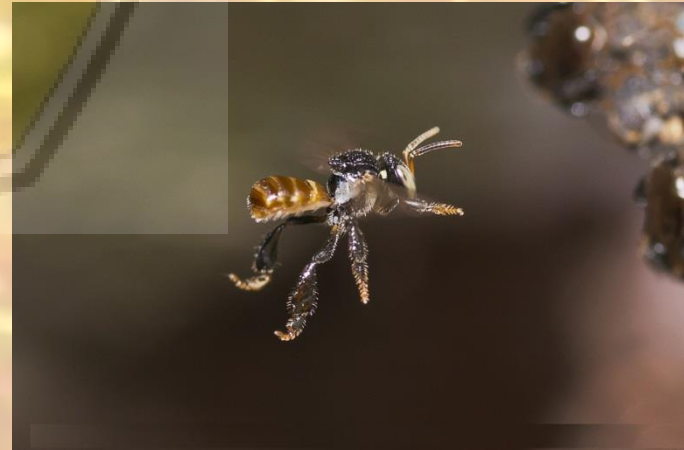
# Outline

- Background
- *State of art*
- Methodology
- Results
- Discussion



# Introduction

- Propolis contains antioxidant
- Conventional extraction: *time-consuming* and using organic solvent
- Optimum condition providing  $IC_{50}$  value is not available yet



# State of art

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materials	wt%
Resin (flavonoid, phenolic acids, ester)	45 -55
wax and fatty acids	25 -35
essential oil	10
bee Pollen (protein)	5
Minerals (Fe, Zn), vitamin (B3), others (organic compounds)	5

Wpropolis, g	solvent	methods	condition	Yield, %w/w	Ref.
20	Ethanol absolute	maceration	T= RT, t=20 d	40,43	Cunha et al, 2004
90	Ethanol 190 mL	maceration	T=RT, t=90 d	38,34	Funari et al, 2007
5	H <sub>2</sub> O 150 mL	soxhlet	T=60oC, t=6 h	14,3	Biscaia &Ferreira, 2009

5	Ethyl acetate	soxhlet	T=60oC, t=6 h	59,7	Biscaia &Ferreira, 2009
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5	CHCl <sub>3</sub> 150 mL	soxhlet	T=60oC, t=6 h	73	Biscaia &Ferreira, 2009
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5	n-Hexane	soxhlet	T=60oC, t=6 h	17	Biscaia &Ferreira, 2009
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1	Ethyl acetate 120 mL	soxhlet	t=16 h	55,6	Chen et al, 2009
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25	Olive oil	solvent	T=40oC, t=7 d	n/a	Pujirahayu et al, 2015
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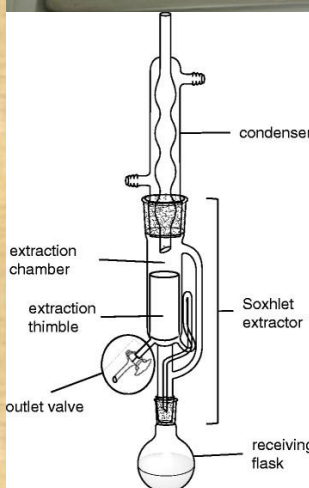
25	VCO	solvent	T=40oC, t=7 d	n/a	Pujirahayu et al
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25	Propylene glycol		T=40oC, t=7 d	n/a	Pujirahayu et al, 2015
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10	Ethanol 70 % 100 mL	solvent	T=RT, t=5 h	12,7 mg/mL GAE	Kubiliene et al, 2015
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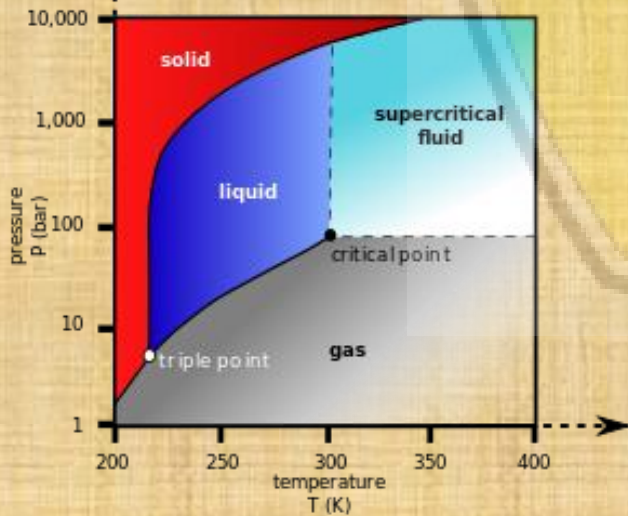
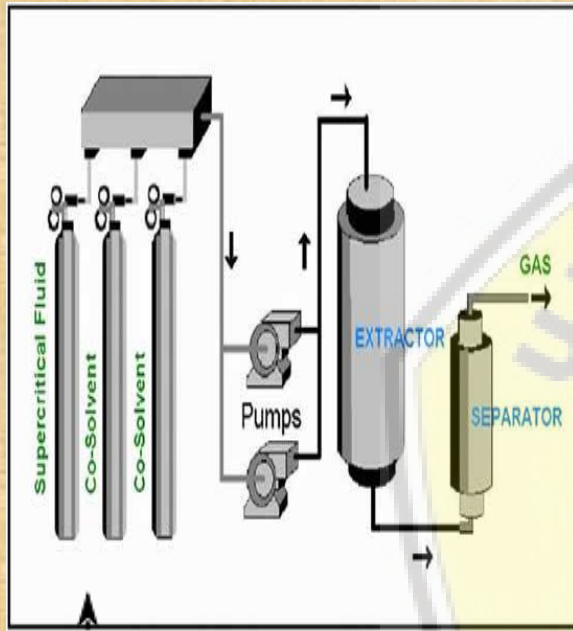
2	Ethanol 15 mL	solvent	T=70oC, t=30 m	n/a	Machado at al 2016
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0.5	Ethanol 4 mL	solvent	T= RT, t=5 m	n/a	Jerkovic et al, 2016
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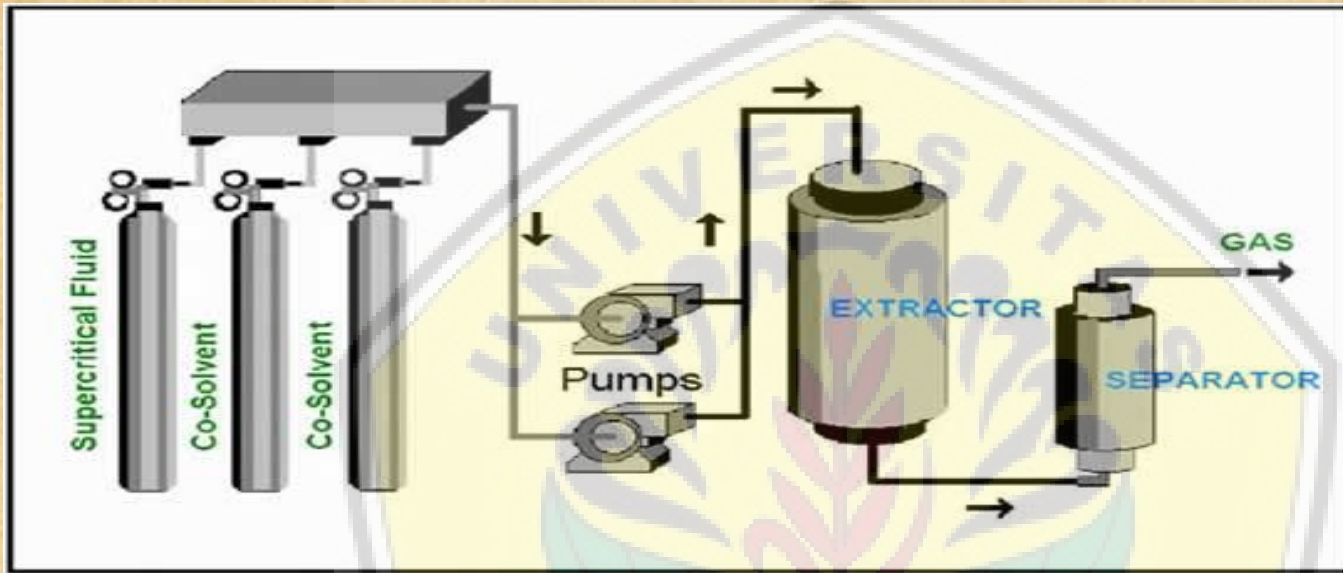


# Supercritical Fluid Extraction



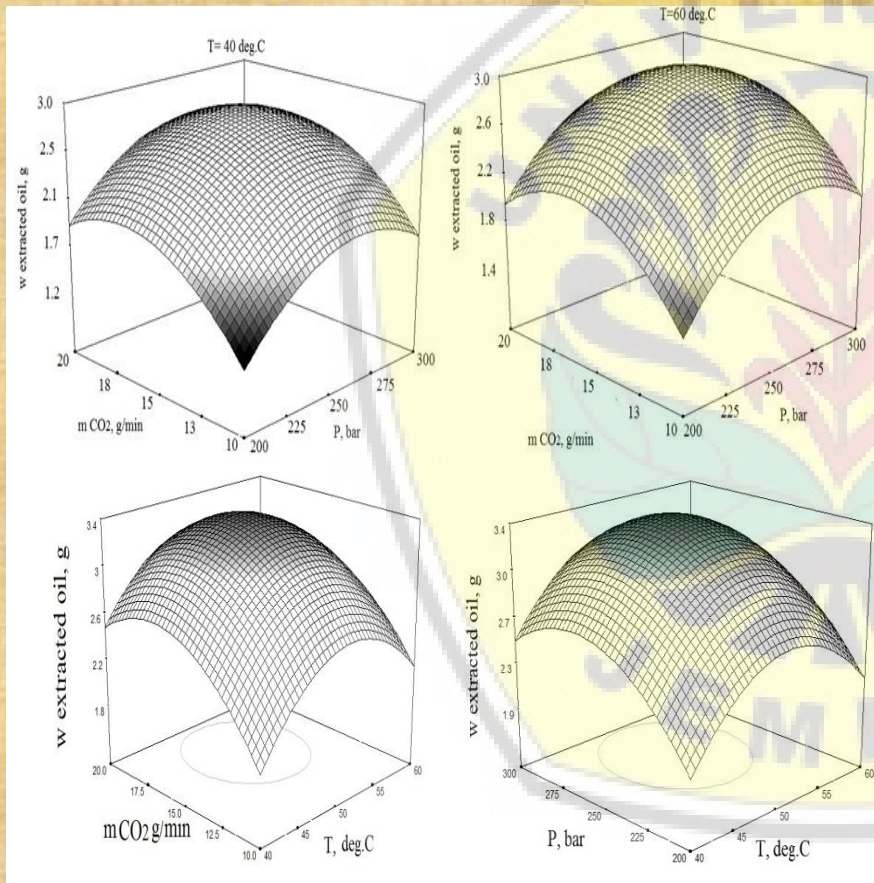
W propolis, g	Supercritical fluid	Co-solvent	Process variable	Yield, wt%	Ref.
5 g EEP	CO <sub>2</sub> 1 g/min	-	<ul style="list-style-type: none"> <li>• P= (150-350) bar</li> <li>• T=(20-50)°C</li> <li>• t=(1-2) h</li> </ul>	13.07 (P=350 bar, T=60C)	Paviani dkk, 2010
10	CO <sub>2</sub>	-	<ul style="list-style-type: none"> <li>• P= (13.8-27.6)MPa</li> <li>• T= (40-60) C</li> </ul>	0.6 (P=20.7 MPa, T=50C)	Chen dkk, 2009
20	CO <sub>2</sub>	-	<ul style="list-style-type: none"> <li>• P= (100-200) bar</li> <li>• T= (30-50)°C</li> <li>• t= (3-5) h</li> </ul>	12 (P=250 bar, T=40C, CO <sub>2</sub> = 5g/min)	Biscaia &Ferreira, 2009
20	CO <sub>2</sub>	-	<ul style="list-style-type: none"> <li>• P= (100-200) bar</li> <li>• T= (30-50)°C</li> <li>• t= (3-5) h</li> </ul>	8.9 (P=250 bar, T=40C, two stage process)	Biscaia &Ferreira, 2009
5 (2 g EEP)	CO <sub>2</sub>	-	<ul style="list-style-type: none"> <li>• P= (150-250) bar</li> <li>• T=(20-50)°C</li> </ul>	7.3 (P=250 bar, T=50C)	Paviani dkk 2012
32	CO <sub>2</sub>	-	<ul style="list-style-type: none"> <li>• P= (82-320) bar</li> <li>• T=(31-50)°C</li> <li>• t=(1.5-6.5) h</li> </ul>	14.3 (P=317 bar, T=45C, ratio EtOH=15 wt%)	De Zordi dkk, 2014
10	CO <sub>2</sub>	Ethyl Acetate	<ul style="list-style-type: none"> <li>• P= (13.9-27.7)kPa</li> <li>• T= (35-60) bar</li> <li>• EtOAc =(0-6.3)wt%</li> </ul>	31.1 mg/g (P=20.7 kPa, T=60C, min, EtOAc= 6 wt%)	Lee dkk, 2007
20	CO <sub>2</sub>	Ethanol (2-7) wt%	<ul style="list-style-type: none"> <li>• P= (100-200) bar</li> <li>• T= (30-50)°C</li> <li>• t= (3-5) h</li> </ul>	24.8 (P=150 bar, T=40C, EtOH= 5 wt%, one stage process)	Biscaia &Ferreira, 2009

# Methodology



# Results

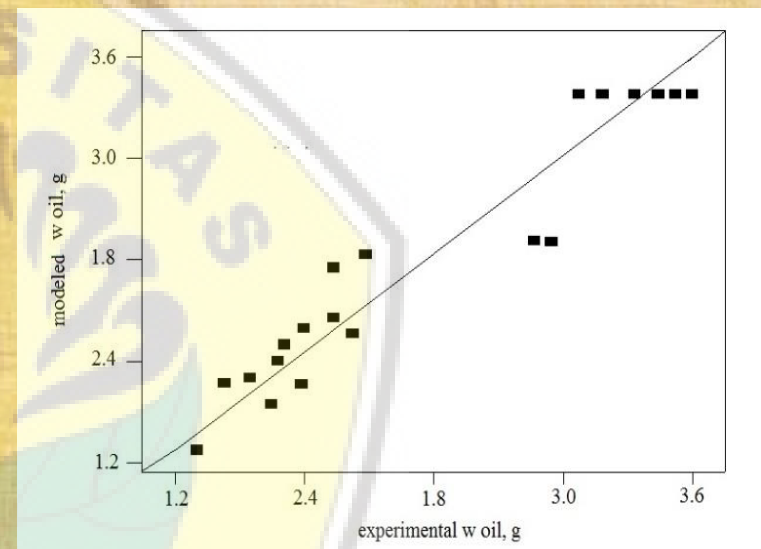
Effect of process variables on propolis yield



Run	T, °C	P, bar	m CO <sub>2</sub> , g/min	W <sub>extracted oil</sub> , g
1	66.8	250	15	2.01
2	40	200	20	1.69
3	50	1	15	1.53
4	50	9	15	2.86
5	50	250	15	3.44
6	60	300	10	1.92
7	50	250	6.59	1.41
8	40	300	20	1.92
9	50	250	15	3.33
10	50	250	15	3.52
11	50	250	15	3.07
12	50	250	23.41	2.94
13	60	200	10	1.63
14	40	200	10	1.28
15	40	300	10	1.66
16	60	300	20	2.07
17	50	250	15	3.6
18	33.2	250	15	1.77
19	60	200	20	1.78
20	50	250	15	3.18

## Analysis variance of extracted oil model

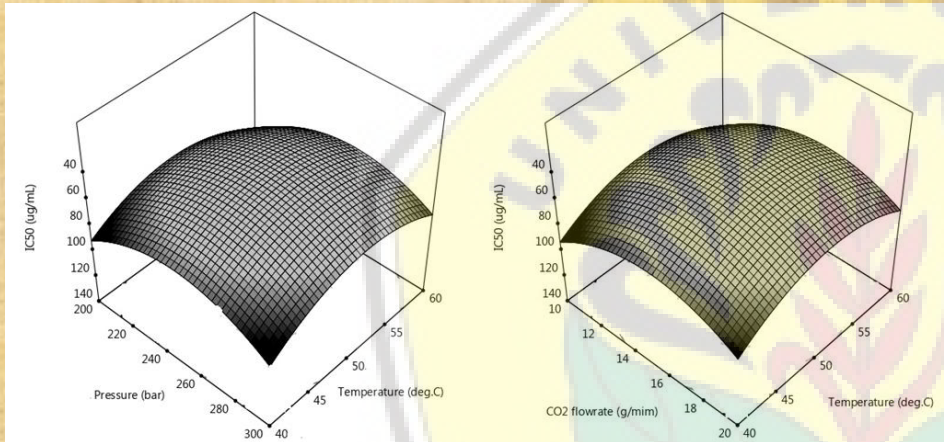
Source	Sum of Squares	df	Mean Square	F value	p-value
					Prob > F
Model	11.23	9	1.25	12.36	0.0003
A-T	0.12	1	0.12	1.14	0.3109
B-P	0.86	1	0.86	8.51	0.0154
C-m	0.92	1	0.92	9.1	0.0130
AB	$1.13 \times 10^{-4}$	1	$1.13 \times 10^{-4}$	$1.11 \times 10^{-3}$	0.9740
AC	0.017	1	0.017	0.17	0.6893
BC	$2.81 \times 10^{-3}$	1	$2.81 \times 10^{-3}$	0.028	0.8708
A <sup>2</sup>	4.76	1	4.76	47.12	< 0.0001
B <sup>2</sup>	3.14	1	3.14	31.09	0.0002
C <sup>2</sup>	3.24	1	3.24	32.04	0.0002
Residual	1.01	10	0.1		



$$W_{\text{extracted oil}} = -29.44 + 0.58 (T) + 0.098 (P) + 0.62 (m) - 5.746 \times 10^{-3} (T^2) - 1.867 \times 10^{-4} (P^2) - 0.0189 (m^2)$$

The R-squared of the model is 0.9159

Effect of process variables (T, P, and CO<sub>2</sub> flow rate) on IC<sub>50</sub> value

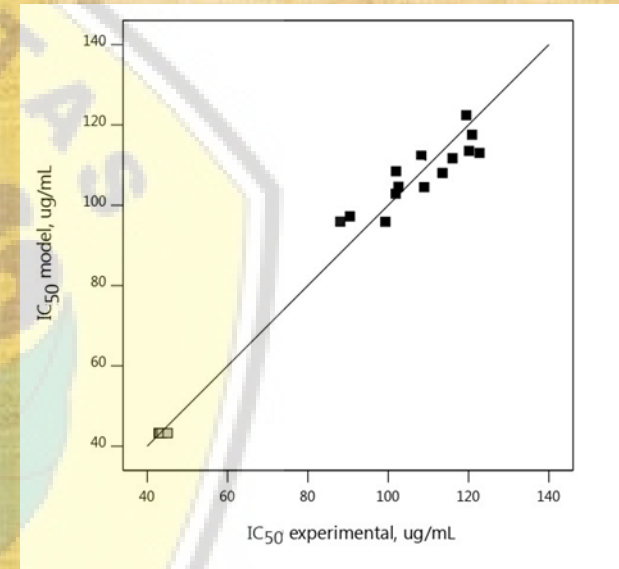


Run	T <sub>dynamic</sub> °C	P <sub>dynamic</sub> bar	m CO <sub>2</sub> , g/min	IC <sub>50</sub> , µg/mL
1	66.82	250	15	82.77
2	40	200	20	102.55
3	50	165.91	15	103.41
4	50	334.09	15	101.86
5	50	250	15	43.05
6	60	300	10	112.09
7	50	250	6.59	120.11
8	40	300	20	122.74
9	50	250	15	42.88
10	50	250	15	43.55
11	50	250	15	42.87
12	50	250	23.41	80.35
13	60	200	10	119.44
14	40	200	10	120.88
15	40	300	10	97.62
16	60	300	20	99.28
17	50	250	15	43.77
18	33.18	250	15	113.51
19	60	200	20	122.77
20	50	250	15	42.95

$$IC_{50} = b_0 + \sum_{i=1}^3 b_i x_i + \sum_{i=1}^3 \sum_{j=1}^3 b_{ij} x_i x_j$$

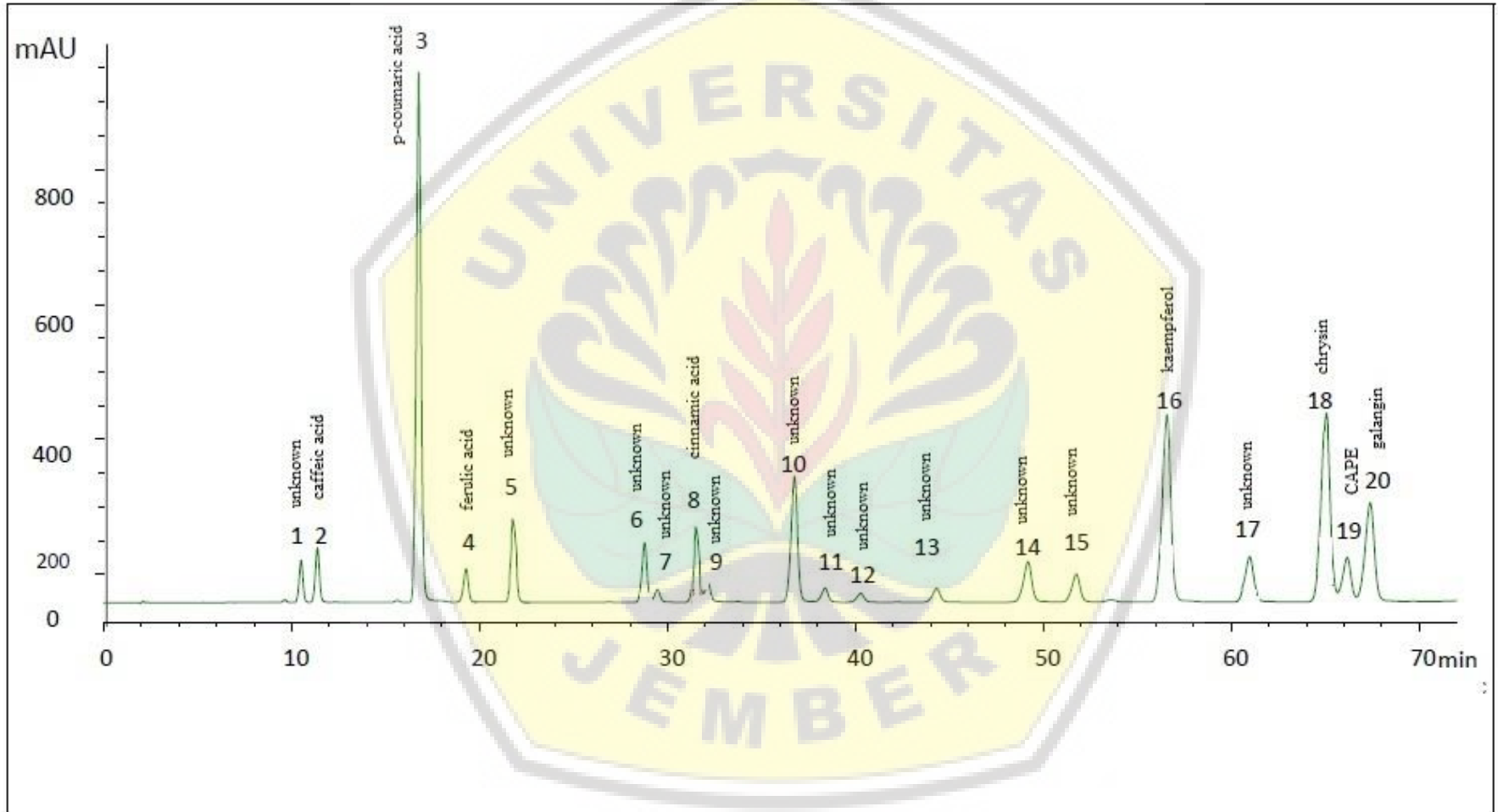
## Analysis variance of IC<sub>50</sub>

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	18047.83	9	2005.31	13.25	0.0002	significant
A-Temperature	128.63	1	128.63	0.8501	0.3782	
B-Pressure	97.64	1	97.64	0.6453	0.4405	
C-CO <sub>2</sub> flowrate	354.33	1	354.33	2.34	0.1569	
AB	96.40	1	96.40	0.6371	0.4433	
AC	33.09	1	33.09	0.2187	0.6501	
BC	93.23	1	93.23	0.6162	0.4507	
A <sup>2</sup>	6402.06	1	6402.06	42.31	< 0.0001	
B <sup>2</sup>	7404.77	1	7404.77	48.94	< 0.0001	
C <sup>2</sup>	6858.80	1	6858.80	45.33	< 0.0001	



$$\begin{aligned}
 IC_{50} = & 1310.99 - 19.03 [T] - 4.44 [P] - 28.57 [m] - \\
 & 0.0079 [T] [P] - 0.04 [T] [m] + 0.013 [P][m] + 0.21 \\
 & [T^2] + 0.009 [P^2] + 0.87 [m^2]
 \end{aligned}$$

# Chromatogram Profile







# Conclusion

These works conclude that (1) antioxidant activity was influenced by the process variables such temperature, pressure, and CO<sub>2</sub> flow rate; (2) propolis extract contains galangin and caffeic acid phenyl ester; (3) propolis shows promising as super antioxidant.

# Acknowledgments

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- The author would also like to thank Dexa Medica and Dexa Laboratories and Biomoleculars Science (DLBS) for facilitating this work.for supporting the research and giving financial assistance.
- The University of Jember





Dear Mr. Boy Arief Fachri

Jember, 2018, 11<sup>th</sup> October

On behalf of The ICATD committee, it is our pleasure to inform you that your paper entitled: **“Super Antioxidant from Indonesia Propolis: Effect of Process Variables on Value of Antioxidant Activity”** has been accepted to be presented in plenary session of The 2<sup>nd</sup> ICATD 2018. You are requested to submit the full paper through “Online Paper Submission” in ICATD homepage by October 15<sup>th</sup> 2018 and to guarantee your abstract is included in the seminar program we ask you to complete seminar registration by completing the payment. Please follow the guidelines as mentioned in our website [icatd.fk.unej.id](http://icatd.fk.unej.id).

We look forward to having you participate in this upcoming seminar and present your work.

Sincerely,

**DR.dr Yunita Armiyanti, M.Kes**

Chairman of Organizing Committee



# CERTIFICATE

OF APPRECIATION

THIS CERTIFICATE IS PROUDLY PRESENTED TO

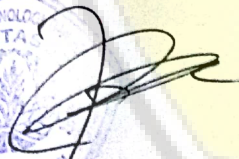
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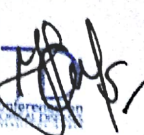
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