

Dear Participant of the 2nd ICWEB 2021

Department of Biology Faculty of Mathematics and Natural Sciences Universitas Lambung Mangkurat with 9 collaborators will virtually hold **the 2nd International Conference on Tropical Wetland Biodiversity and Conservation (ICWEB) on October 23-24, 2021.** The theme of the conference will be *"Enhancing Education and Research in Tropical Wetland Biodiversity and Conservation for Better Development."*

On behalf of the 2nd ICWEB 2021 committee, we would like to invite you to attend the Opening Ceremony, Plenary Session, and Parallel Session that will be held on:

Date : October 23-24, 2021 Time : 09.00-16.20 and 09.00-13.30 Central Indonesia Time (UTC/GMT +8 hours) Via : Zoom meeting https://linktr.ee/icweb

The details of the conference are presented in the conference program. We are expecting your attendance of this occasion and thank you for your attention and precious time.

Sincerely,

ICWEB

Anni Nurliani, S.Si., M.Sc., Ph.D. Chairperson





Conference Program of The 2nd International Conference on Tropical Wetland Biodiversity and Conservation (ICWEB) 2021

	DAY 1: 23 OCTOBER 2021	
Time	Activities	Description
08.00-09.00	Registration Open: Traditional Music	
	Opening Ceremony:	Master of Ceremony (MC):
		1. Rinta Dwi Takarini
		2. Iqbal Amanullah Putra Gazali
09.00-09.05	Wit Opening	Undergraduate Students, Department of Biology, FMIPA, Universitas Lambung Mangkurat
09.05-09.20	Radap Rahayu Traditional Dance	Undergraduate Students, Department of Biology, FMIPA, Universitas Lambung Mangkurat
09.20-09.30	Indonesian National Anthem (Indonesia Raya)	
09.30-09.45	Opening Speech: Dean, Faculty of Mathematics and Natural Sciences	Drs. Abdul Gafur, M.Si., M.Sc., Ph.D.
09.45-10.00	Opening Speech and Official Opening The 2nd ICWEB 2021:	Prof. Dr. H. Sutarto Hadi, M.Si, M.Sc.
	Rector of Universitas Lambung Mangkurat	
	Plenary Session I	Moderator:
	1. Prof. Dr. Budi Setiadi Daryono, M.Agr.Sc.	Dr. Drs. Krisdianto, M.Sc.
10.00-10.45	(Head of Indonesian Biology Consortium/Konsorsium Biologi Indonesia)	(Department of Biology EMIPA Universitas
1000 1000	Title: "Contribution and Effort of the Indonesian Biology Consortium (KOBI) in Bending the Curve of Indonesia's Biodiversity Loss".	Lambung Mangkurat, Indonesia)
	2. Dr. Atit Kanti, S.Si., M.Sc.	
10.45-11.30	(Senior Researcher in Biologi research center- BRIN)	
	Title: "Mainstreaming Biodiversity and Management for Sustainable Development".	
11.30-11.45	Q and A Session	
11.45-12.45	Break	MC
	Plenary Session II	Moderator:
	1. Assoc. Prof. Dr. Ramesh Boonratana	Dr. drh. Hery Wijayanto, M.P.
12.45-13.30	(Biodiversity Conservation & Ecosystem Protection, Mahidol University, Thailand)	(Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia)
	Title: "Education and Research as Tools for Enhancing Tropical Wetlands Conservation for Climate Adaptation and Mitigation".	
	2. Prof. Matthew Hayward	
12 20 14 15	(School of Environmental and Life Sciences, The University of Newcastle, Australia)	
13.30-14.15	Title: "Conservation Works! A Story of Conservation Success and Lessons from Around the World"	
14.15-14.30	Q and A Session	
	Plenary Session III	Moderator:
	1. Assistant Prof. Dr. Daniele Cicuzza	Hasrul Satria Nur, S.Si., M.Si.
14.30-15.15	(Faculty of Science, Universiti Brunei Darussalam, Brunei)	(Department of Biology, FMIPA, Universitas
	Title: "The Mosaic Structure of Wetland Forest Tells Us to See Them Differently".	Lambung Mangkurat, Indonesia)
	2. Prof. Toshio Tsubota	
15.15-16.00	(Graduate School of Veterinary Medicine, Hokkaido University, Japan)	
	Title: "One Health Approach - Disease Ecology in Hokkaido Wildlife".	
16.00-16.15	Q and A Session	
16.15-16.20	Closing Plenary Session Day 1	MC



	DAY 2: 24 OCTOBER .	2021
Time	Activities	Description
(Central Indonesian Time)		
08.45-09.00	Opening and Moderator Introduction	MC: Hasrul Satria Nur, S.Si, M.Si
Break Room 1	Room 1: Environmental Science	Code:
09.00-09.35	Invited Speaker:	Moderator:
	Drs. Abdul Gafur, M.Si., M.Sc., Ph.D.	Amalia Rezeki, S.Pd., M.Pd.
	(Department of Biology, FMIPA, Universitas Lambung Mangkurat, Indonesia)	(Sahabat Bekantan Indonesia)
	Title: "Nematodes as Bioindicator in Wetlands: Prospects and Challanges".	
09.35-09.45	Q and A Session	
Break Room 2	Room 2: Animal Biodiversity	Code:
09.00-09.35	Invited Speaker:	Moderator:
	Dr. drh. Hery Wijayanto, M.P.	Dr. drh. Hevi Wihadmadyatami, M.Sc
	(Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia)	(Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia)
	Title: How the Religion Contributes to the Wild Life	(inconcerce)
09.35-09.45	Q and A Session	
Break Room 3	Room 3: Plant Biodiversity	Code:
09.00-09.35	Invited Speaker:	Moderator:
	Dr. Dra. Rusmiati, M.Si.	Sasi Gendro Sari, S.Si., M.Sc.
	(Department of Biology, FMIPA, Universitas Lambung Mangkurat, Indonesia)	(Department of Biology, FMIPA, Universitas Lambung Mangkurat, Indonesia)
	Title: "Potential of Exotic Durians from South Kalimantan's	
09.35-09.45	Q and A Session	
Break Room 4	Room 4: Microbe Biodiversity	Code:
09.00-09.35	Invited Speaker:	Moderator:
	Prof. Dr. Liswara Neneng, S.Pd., M.Si.	Witiyasti Imaningsih, S.Si., M.Si.
	(Department of Biology Education, Palangkaraya University, Indonesia)	(Department of Biology, FMIPA, Universitas Lambung Mangkurat, Indonesia)
	Title: "Potential Microorganisms for Mercury	
09.35-09.45	Q and A Session	
09.45 -10.00	Presentation Guidelines and Moderator Introduction	Each Moderator of Room
10.00 - 12.00	Parallel Session:	Break room
	Enviromental Science (ES) A and B	ES-A ; ES-B
	Animal Biodiversity (AB) A and B	AB-A ; AB-B
	Plant Biodiversity (PB) A, B and C	PB-A ; PB-B ; PB-C
	Microbe Biodiversity (MB) A and B	MB-A ; MB-B
12.00-13.00	Break	
13.00 -13.10	Announcement of The Best Presenter	MC: Hasrul Saria Nur, S.Si, M.Si,
13.10-13.20	Publication Information	Editor: Dr. Ir. Badruzsaufari, M.Sc.
13.20-13.30	Closing Speech	Chairperson: Anni Nurliani, S.Si, M.Sc, Ph.D.





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PARALLEL SESSION: ENVIRONMENTAL SCIENCE A (ES_A)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Dr. Gunawan, S. Si, M.Si

Ope	erator: Muhammad Riyan Firnanda			
No.	Time	Title	Presenter	
1	10.00-10.10	Comparison of Dissolved Organic Carbon and Nutrients Content in Papua Peatland	Siti Sundari	
2	10.10-10.20	Interactive Governance Framework and Its Potential for Governing Protected Area Landscape	Endratno Budi Santoso	
3	10.20-10.30	Effect of Land Use On Water Quality Around Lake Toba Catchment	Asep Sukmana	
	10.30-10.40	Discussion		
4	10.40-10.50	Abundance of Seedlings in the Process of Mangrove Ecosystem Development on Ajkwa Island, Mimika Regency, Papua	Aditya Sukma Bahari	
5	10.50-11.00	Institutional Network of the Peat Ecosystem Restoration Plan in Riau Province: Hierarchy and Classification Approached	Laila Febrina	
6	11.00-11.10	Increases in pH of Acid Mine Drainage With Coal Fly-Ash Application	Bambang Joko Priatmadi	
	11.10-11.20	Discussion		
7	11.20-11.30	Decreasing Concentration of Textile Dye Congo Red Using Fenton Reagent/TiO2/UV	Dyndie Maulidia	
8	11.30-11.40	Reduction in Carbon Dioxide and Methane Production of Tropical Peatlands Due to Coal Fly-Ash Application	Akhmad R. Saidy	
9	11.40-11.50	Wetlands Utilization Through the Social Forestry Program in the Kayan Sembakung Delta, North Kalimantan Province, Indonesia	Catur Budi Wiati	
	11.50-12.00	Discussion		
	12.00-13.00	Break		

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PARALLEL SESSION: ENVIRONMENTAL SCIENCE B (ES_B)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Agustina Ambar Pertiwi, M.Pd

Opera	Operator: Akbar Setiawan			
No.	Time	Title	Presenter	
1	10.00-10.10	Community Dependence on Biodiversity Around the Gunung Jampang Forest Area, South Garut Regency as a Form of Conservation and Sustainable Development	Wardah	
2	10.10-10.20	Analysis of High Conservation Value Ecotourism Areas: A Case Study in Tanah Laut District, South Kalimantan, Indonesia	Meldayanoor	
3	10.20-10.30	Vegetation in Ex-Tin Mining In Mempayak Village in Manggar District, Belitung Regency and Its Utilization	Priyanti	
	10.30-10.40	Discussion		
4	10.40-10.50	Analysis of Soil Erosion Change and Its Relationships With Land Use/Cover Change in Tabunio Watershed	Nurlina Abdullah	
5	10.50-11.00	Involvement and Roles of Stakeholders in Mahakam Delta Management to Support Mitigation Adaptation Effort of Climate Change in East Kalimantan	Tien Wahyuni	
6	11.00-11.10	Nutrients Removal from Inergrated Multi-Thropic Aquaculture (IMTA) Water Using Waste Stabilization Ponds (WSP)	Guruh Satria Adjie	
	11.10-11.20	Discussion		
7	11.20-11.30	A Mapping of Peatland Fire Hazard in Central Kalimantan Province Based on Hotspot Distributions in 2019	Titin Alfiani	
8	11.30-11.40	Estimation of Carbon Storage Loss and Carbon Dioxide Emission Increase Due to Deforestation Forest Degradation on Peatlands in Central Kalimantan, 1990-2019	Putri Risa Fatmawati	
9	11.40-11.50	Increasing Biology Learning Achievement Through The Assignment Method of DNA Model Development In Class XII IPA 1 Academic Year 2017/2018 MAN 1 Tuban	Chotimahwati	
10	11.50-12.00	The Influence of Rise Husk Ash Adsorbent Mass on Decreasing Mercury Levels in Liquid Waste Using Column Adsorption Process	Desi Nurandini	
	12.00-12.10	Discussion		

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PARALLEL SESSION: ANIMAL BIODIVERSITY A (AB_A)

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OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Dr. drh. Vista Budiarti, M.Si

No.	Time	Title	Presenter
1	10.00 - 10.10	In Silico Restriction Site Analysis for Characterization of Toxoplasma gondii Isolate	Fitrine Ekaswati
2	10.10-10.20	Molecular Sexing in Bos tourus Using Quantitative Polymerase Chain Reaction (qPCR) Method	Asmarani Kusumawati
3	10.20-10.30	The Genetic Variation Analysis of Sandfish (Holothuria scabra) Populations Using Simple Sequence Repeats (SSR)	Sari Budi Moria Sembiring
	10.30-10.40	Discussion	
4	10.40-10.50	Distribution of the Critically Endangered Javan Blue-banded Kingfisher Alcedo euryzona Along the Welo River Flow in the Petungkriyono Forest	Yeni Rachmawati
5	10.50-11.00	Community Structure of Dragonfly (Insecta: Odonata) in Pond Habitat Type with Canopied and Non-Canopied at Sumur Panguripan Cultural Reserve, Surabaya, East Java, Indonesia	Muhammad Azmi Dwi
6	11.00-11.10	Termite Attack on Rubber Plantation on Peat Soil: Level of Damage and Identification of Pest Species	Yuliati Indrayani
	11.10-11.20	Discussion	
7	11.10-11.20	Reference of Spodoptero pectinicornis as a Biocontrol Agent Of Water Lettuce (Pistia Stratiotes L.) a Wetland Weed to Some Forms of Feed Stocks	Lyswiana Aphrodyanti
8	11.20-11.40	Development of Duplex PCR Assays for Detect Pathogen Eimeria Species in Cattle	Fitrine Ekawasti
9	11.40-11.50	The Study of Important Value of Mangrove Crabs Base on Sediment Conditions at the Estuary of Asam-Asam River in Tanah Laut Regency	Bunda Halang
10	11.50-12.00	Case Study: Surgery Treatment of Bladder Stone in a Sulculata Tortoise (Centrochelys sulculata)	Slamet Raharjo
	12.00-12.10	Discussion	

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PARALLEL SESSION: ANIMAL BIODIVERSITY B (AB_B)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: drh. Marla Anggita, M.Sc.

No.	Time	Title	Presenter
1	10.00-10.10	Predation Ability Toxorhynchites splendens Larvae From Banjarbaru	Muhammat
2	10.10-10.20	Cat Viral Diseases Pattern in Prof. Soeparwi Hospital Animal Hospital in 2017-2019	Widagdo Sri Nugroho
3	10.20-10.30	Daily Behavioral Activities of the Proboscis Monkey (Nasalis farvatus Wrumb) in the Bekantan Rescue Center, Sahabat Bekantan Indonesia Foundation	Siti Istiqomah
	10.30-10.40	Discussion	
4	10.40-10.50	Activity Test of Chitosan Haruan (Chana Striata) Fish Scales as Antibiofilm Agent Againts Biofilm of Potphyromonas Gingivalis	Deby Kania Tri Putri
5	10.50-11.00	Molecular Fish Sexing on Kohaku Koi (Cyprinus carpio) Based on Ar5.9-15 Gene Amplification by PCR Method	Aris Haryanto
6	11.00-11.10	Sexual Behavior of the Proboscis Monkey (Nasalis larvatus Wrumb) in the Bekantan Rescue Center, Sahabat Bekantan Indonesia	Amalia Rezeki
	11.10-11.20	Discussion	
7	11.20-11.30	Gross Anatomical Study of Vertebrae Column of the Musang Luwak (Paradoxurus hermaphroditus)	Ahmad Naufal Trifayoga
8	11.30-11.40	Skin Histology of the Musang Luwak (Paradoxurus hermaphroditus)	Chew Pei Yi
9	11.40-11.50	Sexual Dimorphism Characters on Painted Terrapin (Batagur borneonsis) from Borneo Island	Anni Nurliani
	11.50-12.00	Discussion	
10	12.00-12.10	The Relationship of Wheater and Daily Activities of Nasalis Larvatus at Bekantan Research Station Curiak South Kalimantan	Hery Wijayanto
11	12.10-12.20	Sequence Variation of Proboscis Monkey (Nasalis larvatus, Wurmb) from Hulu Sungai Tengah Based the COX2 Gene	Rani Sasmita
	12.20-12.30	Discussion	

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PARALLEL SESSION: PLANT BIODIVERSITY A (PB_A)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Dr. Bayu Adjie, M.Sc.

Opera	ator: Muhammad	Rasyid Azkia	
No.	Time	Title	Presenter
1	10.00-10.10	Palm Species Diversity on Mount Slamet, Java, Indonesia	Rizmoon Nurul Zulkarnaen
2	10.10-10.20	Effect of Fixator From Bark Extract of Three Tropical Wetland Species for Fabric	Saefuddin
3	10.20-10.30	Molecular Docking Evaluation of 4-ethyl-2-metoxyphenol and 1,3-cyclopentanedione Compounds from Gemor (Nothaphoebe coriacea) with Glucagon like-Peptide-1 (GLP-1) Receptor	Eko Suhartono
	10.30-10.40	Discussion	
4	10.40-10.50	Production of Recombinant SARS-CoV-2 3CL-protease: The Key for the Development of Protease Inhibitors Screening Kit in Search of Potential Herb Cure for COVID-19	Haniyya
5	10.50-11.00	Strategies Control to Late Blight and Virus Diseases on Some New Local Varieties Use Fungicides in Indonesian	Ineu Sulastrini
6	11.00-11.10	Effect of Extraction Method on Antioxidant Activity of Ethanol Extract of Pasak Bumi Root (Eurycoma Iongifolia Jack.)	Samsul Hadi
	11.10-11.20	Discussion	
7	11.20-11.30	Allelopathic Potential of Root Exudates from Perennial Herbaceous Plants Against Ganoderma boninense	Suwandi
8	11.30-11.40	Inhibition Protease of Sars Cov-2 Using Medicinal Plant Bioactive Constituents : Molecular Docking Simulation Approach	Firdayani
9	11.40-11.50	Study of the Effect of Microbial Addition in Seed Germination and Seedling Growth of Cryptocarya densiflora L.	Arwan Sugiharto
10	11.50-12.00	Effect of Grain Moisture on Dehulling of Nymphaea pubescens Seed	Fatimah
	12.00-12.10	Discussion	

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PARALLEL SESSION: PLANT BIODIVERSITY B (PB_B)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Kusdania Handiyani, S. Hut

No.	Time	Title	Presenter
1	10.00-10.10	Carbon Stock Potential of Indonesian Local Fruit Trees, Some Collections of Purwodadi Botanic Garden	Titut Yulistyarini
2	10.10-10.20	Potential of Nipah (Nypa fruticans Wurmb.) and Utilization by Local People in Tabanio Village, Tanah Laut Regency, South Kalimantan	Anang Kadarsah
3	10.20-10.30	Conservation Threats of Pemphis acidula in the Tomini Bay Area, Gorontalo, Indonesia	Dewi Wahyuni K. Baderan
	10.30-10.40	Discussion	
4	10.40-10.50	Structure and Distribution of Palm Trees Species in the Plantation Forest Area of Gede Pangrango National Park-Bodogol Resort, West Java	Asep Sadili
5	10.50-11.00	Comparative Study of Antioxidant Effect from Extract and Fraction of Paku Atai Merah (Angiopteris ferox Coupel)	Andi Nur Aisyah
6	11.00-11.10	Microhabitat of Kasturi (Mangifera casturi Kosterm.) in Banjar Regency	M. Adam Malik
	11.10-11.20	Discussion	
7	11.20-11.30	Screening and Selection of an Elite Germplasm of Mucuna pruriens (L.) DC. Based on the L-DOPA Quantification, Biochemical Variations and Anti-Oxidant Studies	Rakesh B
8	11.30-11.40	Changes of Stomatal Distribution and Leaf Thickness in Response to Transpiration Rate in Six Dicotil Plant Species	Entin Daningsih
9	11.40-11.50	Preparation of Leaf Anatomy Slide Using Modification Protocols	Asriah Nurdini M
10	11.50-12.00	The Effect of Nanoparticles of <i>Piper crocatum</i> Leaves Ethanolic Extract on Liver and Insulin Receptor Expression of Diabetic Rat's Induced by Streptozotocin	Tri Wahyu Pangestiningsih
	12.00-12.10	Discussion	

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PARALLEL SESSION: PLANT BIODIVERSITY C (PB_C)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Dr. Eka Fatmawati Tihurua, S.Si., M.Si.

Opera	tor: Sapta Budia	rto	
No.	Time	Title	Presenter
1	10.00-10.10	Utilization of Galam Wood's (Malaleuca Leucadendron Linn.) Microcrystallin Cellulose as a Filler in the Fabrication of Edible Film	Dyera Forestryana
2	10.10-10.20	Biomimicry of Greater Club Rush (Scirpus grossus L.F) and Water Mimosa (Neptunia oleracea Lour): Barito River Basin Wetland Plants Inspiring Innovative Modular Design	Krisdianto
3	10.20-10.30	Trachea Features and Fiber Dimensions of Fast-Growing Tree: Case Study on 28 Branch Wood of Species From Eastern Indonesia	Asih Perwita Dewi
	10.30-10.40	Discussion	
4	10.40-10.50	The Effect of N-Butanol Fraction of Gaharu (Aquilaria microcarpa Baill.) Leaves on Blood Glucose and Liver Glycogen Levels in Alloxan-Induces Male Rats	Khoerul Anwar
5	10.50-11.00	Orchid Diversity in Lengguru Fold Belt on Limestone Karst, Kaimana Regency, West Papua, Indonesia: Conservasion Status and Endemism	Lina Susanti Juswara
6	11.00-11.10	Some Characteristic of Bryophytes at the Pine Urban Forest, Banjarbaru	Sasi Gendro Sari
	11.10-11.20	Discussion	
7	11.20-11.30	Size Doesn't Matter Shape Does: A Morphological Study of Pitcher Plant in Forest Distinct Forest Canopy Structures	Tri Surya Harapan
8	11.30-11.40	The Diversity of Smilacaceae in Java, Indonesia	Lulut Dwi Sulistyaningsih
9	11.40-11.50	Antioxidant Activity Profile of Extract and Fraction of Kersen (Muntingia calabura L.) Fruits by Different Method	Syamsu Nur
10	11.50-12.00	Notes on Leaf Anatomy: Additional Information for Indonesian Pandanus spp. (Pandanaceae)	Eka Fatmawati Tihurua
	12.00-12.10	Discussion	

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PARALLEL SESSION: MICROBE BIODIVERSITY A (MB_A)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

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Moderator: Frans Grovy Naibaho, S.Si., M.Si.

No.	Time	Title	Presenter
1	10.00 - 10.10	Diversity, Characterization and Effectiviness Phosphate Solubilizing Bacteria From the Soil and Rhizosphere on the Growth of Glycine max L. in Green House	Purwaningsih
2	10.10-10.20	Absorption of Dyes From Mixed Fungi by Cotton Fabric With Different Mordants and Dyeing pH	Suciatmih
3	10.20-10.30	Microbial Biodiversity in Shallot Planting in Peatlands Applied with Three Types of Botanical Pesticides	Salamiah
	10.30-10.40	Discussion	
4	10.40-10.50	Expression of Recombinant SARS-CoV-2 Papain-Like Protease (SARS-CoV-2 PLpro) in Escherichia coli RIPL.	Maria Ulfah
5	10.50-11.00	Antibacterial Activity of Infused Peel of Kaffir Lime, Manurun Banana, and Pineapple Against the Number of Staphylococcus aureus and Escherichia coli Colonies	Lia Yulia Budiarti
6	11.00-11.10	Screening of Indonesian Marine Bacteria and Their Potential for D-tagatose Production	Fina Amreta Laksm
	11.10-11.20	Discussion	
7	11.20-11.30	Biodegradation of Acetonitrile by Free and Immobilized Bacterial Cells of Corynebacterium sp. UBT9	Bambang Sunarko
8	11.30-11.40	Culturable Fungi From Tidal and Non-Tidal Swamplands in Indonesia	Ningsih Susilawati
9	11.40-11.50	Characterization of PGPR Isolated From Rhizospheric Soils of Various Plant and Its Effect on Growth of Radish (Raphanus sativus L.)	Dwi Agustiyani
10	11.50-12.00	Molecular Identification of Endophytic Fungi From Artemisia annua Mutant Based on Internal Transcribed Spacer (ITS) rDNA	Nani Radiastuti
	12.00-12.10	Discussion	

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PARALLEL SESSION: MICROBE BIODIVERSITY B (MB_B)

OCTOBER 24, 2021 | 10.00-12.00 Central Indonesian Time (UTC/GMT +8)

Moderator: Ika Oksi Susilawati, S.Si., M.Biotech.

Opera	tor: Rusyda Ulya		
No.	Time	Title	Presenter
1	10.00 - 10.10	Characterization of Plant Growth Promoting Rhyzobacteria (PGPR) Isolated From Water in Mangrove Ecosystem	Sri Widawati
2	10.10-10.20	Formulation Application of N Fixation and P Solubilizing Isolate From Two Type Soil Paddy Field on Growth and the Yield of Rice (Oryza sativa Linn.)	Ikhwani
3	10.20-10.30	Phytase Activity of Phytase Producing Bacteria Isolated From Mangrove Sediments	Suliasih
	10.30-10.40	Discussion	
4	10.40-10.50	Screening of L-Asparaginase Producing Bacterial Endophytes From Mangrove Rhizophora mucronato	Arin Nafisaturrahmah
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9	11.40-11.50	Microbial Activity Form Potential and Actual Acid Sulfate Soil	Erny Yuniarti
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Potency <u>The potency</u> of Endophytic Fungi Isolated from *Hippobroma longiflora* (L) G. Don as an antioxidant sources

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Abstract. Endophytic fungi are friendly microbes colonizing in plants and play an effective role in plant-environment interactions. They produce valuable secondary metabolites that both plants and human beings can benefit from such products. In this study, an antioxidant-producing endophytic fungi were screened and identified from the leaves of *Hippobroma longiflora* (L.) G.Don which is one of the traditional medicinal plants. The objective of this study to evaluate the antioxidant activity of ethyl acetate extracts of 6 endophytic fungi isolated from *Hippobroma longiflora* (L.) G.Don. The qualitative and quantitative antioxidant activity was screened by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH). Qualitatively 6 extracts from the endophytic fungi isolates (AOL₁, AOL₂, AOL₃, AOL₄, AOL₅, and AOL₆) from *Hippobroma longiflora* (L.) G. Don showed antioxidant activity, but quantitatively the extracts that showed very strong activity was extracts from AOL₁ isolate with IC₅₀ values of 28.50 μ g/mL. Based on phenotypic and molecular identification AOL₁ isolate identified as *Phyllosticta* sp and produce alkaloid, flavonoid, and terpenoid.

Keywords : endophytic fungi, Hippobroma longiflora, antioxidant, DPPH

1.Introduction

The increase in free radicals that cause various degenerative diseases cannot be avoided, but free radicals that enter the body can be reduced by taking preventive measures. Antioxidants are needed to counteract and protect the body from free radicals [1]. Antioxidants are compounds that are able to capture or reduce the negative effects of oxidants in the body. Antioxidants work by donating one electron to compounds that are oxidant so that the activity of oxidant compounds can be inhibited [2]. The antioxidant activity of a compound can be measured by its ability to reduce free radicals [3,4]. The free radical commonly used as a model for measuring antioxidant attenuation is DPPH (2,2-diphenyl-1-picrylhydrazyl) because it is fast, simple and easy to use [5].

Hippobroma longiflora (L.) G. Don is a type of medicinal plant found in tropical and subtropical areas, which belongs to the Campanulaceae family. *Hippobroma longiflora* has the potential as a producer of antioxidants. Research by Zarta [6] proved that *Hippobroma longiflora* contains secondary metabolites in the form of flavonoids, tannins, saponins, steroids, and alkaloids which have high antioxidant activity with an IC₅₀ value of 8.08 g/mL.

Endophytic fungi are fungi that interact with plant tissues and can produce the same secondary metabolites as their host [7]. The similarity of secondary metabolites is thought to be the result of genetic recombination between endophytic fungi and their hosts [8]. Endophytic fungi can be used to obtain more efficient bioactive compounds. Utilization of endophytic fungi is very beneficial, because it has a shorter life cycle [9].

The ability of endophytic fungi to produce secondary metabolites according to their host plants is a very large and reliable opportunity to produce secondary metabolites from endophytic microbes isolated from their host plants. According to Stierle *et al.*, [10], the use of endophytic microbes in producing active compounds has several advantages, including (1) faster production with uniform quality, (2) can be produced on a large scale, and (3) the possibility of obtaining new bioactive components by providing favorable conditions.

The objectives of this research were: 1. To obtain isolates of endophytic fungi from *Hippobroma longiflora* (L.) G. Don which <u>producing produces</u> antioxidant secondary metabolites, 2. To conduct in vitro tests to verify the antioxidant of the endophytic fungi from *Hippobroma longiflora* (L.) G. Don, 3. To identify endophytic fungal isolates from *Hippobroma longiflora* (L.) G.Don plants which have high potential in producing antioxidant.

2. Material and Methods

2.1. Sample collection

L

Sample collected from Palem Raya Village, Ogan Ilir Regency, North Indralaya District, South Sumatra. Geographically, it is located at coordinates 3°12'7.14"LS and 104°39'23.72"BT. The leaves that <u>are</u> collected must be in fresh and healthy condition, have no spots on the parts used.

2.2. Isolation and purification of endophytic fungi

Surface sterilization of *Hippobroma longiflora* (L.) G. Don leaf was carried out according to <u>the</u> method of Radji *et al.* [11]. Subsequently, sample was with 70% alcohol for 1 minute, dried and soaked in 1% NaOCl for 5 minutes, dried and was soaked n in 70% alcohol for 30 sec, then rinsed with steriled destilled water for 1 to 3 sec. Samples were cut to size 2 cm x 1 cm. Two pieces of sterile *Hippobroma longiflora* (L.) G. Don leaves with a size of 1x2 cm were aseptically placed on the surface of potato dextrose agar (PDA) medium which was added with chloramphenicol as an antibacterial in a petri dish and incubated at room temperature (28°C) until fungi were grow<u>n</u>. Each <u>colonies</u> <u>colony</u> with different morphological characteristics were purified into a new PDA medium, and incubated for 5-7 days.

2.3. Cultivation and extraction of secondary metabolites of endophytic fungi

A total of 10 pieces of agar plug with a 10 mm diameter from pure culture of fungi were put into a cultivation bottle containing 500 mL of Potato Dextrose Broth (PDB) medium, incubated at room temperature for \pm 30 days under static conditions. The change of the color of the medium indicating indicates the formation of secondary metabolites. The fungal biomass was filtered and dried, the medium was extracted with ethyl acetate solvent with a ratio between medium and solvent of 1: 1 and evaporated using a rotary evaporator to obtain a concentrated extract.

2.4. Qualitative test of antioxidant activity

Each extract from six isolates and ascorbic acid as a control were spotted on the TLC plate as much as $\pm 5 \ \mu$ L. To determine an antioxidant activity was conducted by spraying TLC plate with DPPH 0,1 mM. Isolates selection were was done with by comparing the closest color spots of the isolates to the yellow spot of ascorbic acid.

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2.5. Quantitative test of antioxidant activity by DPPH radical scavenging activity

The free radical scavenging activities of extracts were measured by using 1, 1- diphenyl-2-picryl-hydrazyl (DPPH). Quantitative test of antioxidant activity was carried out using the DPPH method [12]. DPPH solution was made by 5 mg of 0.05 mM DPPH and dissolved in 250 ml of methanol. Endophytic fungi extract was dissolved in dimethyl sulfoxide (DMSO) with a concentration of 1000 g/mL then the extract was diluted to 200; 100; 50; 25; 12.5; and 6.25 g/mL. A total of 0.2 ml of extract was added with 3.8 ml of 0.05 mM DPPH solution, homogenized and left for 30 minutes in a dark place, then the absorbance was measured using a spectrophotometer. Ascorbic acid was used as the positive control, and DMSO as negative control. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [(Abs control-Abs sample)/Abs control] \times 100 The extract concentration providing 50% inhibition (IC50) was calculated was obtained by interpolation from linear regression analysis.

2.6. Thin Layer Chromatography (TLC) analysis of endophytic fungi extracts

The endophytic fungi extracts were analyzed by thin layer chromatography using plate silica gel 60 F_{254} (Merck). The extract with ethyl acetate solvent was taken using a capillary tube and plotted on the TLC plate. Then the TLC plate was eluted into a chamber containing the eluent solvent in the form of ethyl acetate and n-hexane with a ratio of 2:1, until a chromatogram pattern was obtained. The chromatograph pattern formed was then sprayed with 10% H_2SO_4 and heated on a hot plate to see the color spots. From the color spots formed and then measured the migration distance of the extract and the migration distance of solvent to determine the Rf value [13].

2.7. Characterization and identification of endophytic fungi

Endophytic fungi that have the potential to produce the highest antioxidant compounds were characterized and identified phenotypically based on their macroscopic and microscopic morphology and molecularly. Macroscopic characterization was carried out by growing endophytic fungi isolates on czapex dox agar (CDA), malt extract agar (MEA), and potato dextrose agar (PDA) media in petri dishes, then incubated at room temperature (\pm 28°C) for approximately 5 days. Characteristics of fungal isolates observed included colony growth, colony diameter, colony color, and colony reverse color [14]. Microscopic characterization was carried out by making preparations using the Henrici's slide culture (HSC) method and using lactic acid preparation. Fungi were taken aseptically using a loop needle and inoculated on PDA medium which was dropped on a sterile glass slide, then incubated for approximately 1-2 days at room temperature. The microscopic morphological characters observed included cells (unicellular/multicellular), hyphae (septat or aseptat, dark pigmented or hyaline), reproduction (sexual/asexual), branching hyphae, and asexual spores (shape, color, surface, and diameter) and other characters that characterize the special character of a type of fungus [14, 15].

Molecular identification of endophytic fungi was carried out by **a**mplification of the fungal ITS region was performed using universal primer set ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4 5'TCCTCCGCTTATTGATATGC 3') [16]. The PCR products were sent to 1st BASE. The sequences were combined by Bioedit before analyzed using the BLAST algorithm (www.ncbi.nlm.nih.gov). The evolutionary tree for the dataset was carried out by Mega 7.

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3.Results and Discussion

3.1. Isolation and purification of endophytic fungi

Six isolates of endophytic fungi were obtained from *Hippobroma longiflora* leaves, namely AOL₁, AOL₂, AOL₃, AOL₄, AOL₅ and AOL₆. The macroscopic morphological characters of the six isolates of endophytic fungi obtained showed variation in form, size, and color of colonies (Figure 1).

3.2. Cultivation and extraction of secondary metabolites of endophytic fungi

The results of cultivation of each endophytic fungi isolates in 300 mL PDB medium for \pm 30 days obtained extract weights ranging from 0.042 to 0.261 grams and biomass weights ranging from 0.33 to 1.86 g (Figure 2). The highest ethyl acetate extract obtained from AOL₂ isolate extract (0.261 grams) and the highest of biomass obtained from AOL₄ isolate extract (1.86 grams). Fungal biomass is not directly related with the amount of extract. This is because between biomass and secondary metabolite extracts produced through different metabolism. According to Srikandace *et al.* [17], endophytic fungi produce secondary metabolites in the stationary phase, where the cells become old, the rate of reproduction decreases and some cells die due to the shrinking of nutrients in the medium. However, metabolism will continue which causes an abundance of secondary metabolite production in the medium.

Figure 2. Etyl acetate extract and biomass of endophytic fungi Hippobroma longiflora

3.3. Qualitative test of antioxidant activity

The results of the qualitative test of the antioxidant activity of endophytic fungi extract are presented in Fig 3. The presence of antioxidants is indicated by a color change from purple

to yellow when it is sprayed with DPPH. All extracts obtained from six endophytic fungi isolates qualitatively all showed antioxidant activity with varying intensity of color changes.

The spot results shown in Fig 3. show that all extracts have antioxidant activity, and there are still impurities in some isolates so that it is continued by elution of the extracts to separate compounds that have the potential as antioxidants.

Figure 3. TLC profile extract of endophytic fungi *H Longiflora* after being sprayed with DPPH. A. Spotted extract on TLC plate B. Extract eluted with ethyl acetat: n-hexane (2:1)1.AOL₅, 2.AOL₂, 3.AOL₄, 4.AOL₁, 5AOL₃, 6.AOL₆, 7.Ascorbic acid, 8.Etyl acetate

Yellow spot formed indicate the activity of antioxidant compounds. The ability of endophytic fungal extracts to donate hydrogen atoms to the DPPH solution which acts as a free radical to become a more stable compound, causing DPPH to be reduced and absorbance reduced, indicated by a change in the color of the DPPH solution from purple to whitish whitish-yellow. It is also explained by Prakash [18] that compounds containing antioxidants give a yellow color with a purple background on the silica plate.

3.4. Antioxidant activity of endophytic fungi extract

Antioxidant activity was indicated by the IC_{50} value, which is a parameter that indicates the effectiveness of a compound in inhibiting 50% of free radical activity. As a comparison, ascorbic acid was used as a control.

No.IsolateIC50(μg/mL)Antioxidant activity*)1.AOL1 28,50 Very strong2.AOL2168Weak3.AOL3385,29Very weak4.AOL464,09Strong5.AOL5115,15Moderate6.AOL6257,09Very weak7.Ascorbic acid 15,14 Very strong					
1. AOL1 28,50 Very strong 2. AOL2 168 Weak 3. AOL3 385,29 Very weak 4. AOL4 64,09 Strong 5. AOL5 115,15 Moderate 6. AOL6 257,09 Very weak 7. Ascorbic acid 15,14 Very strong	No.	Isolate	$IC50(\mu g/mL)$	Antioxidant activity*)	
2. AOL2 168 Weak 3. AOL3 385,29 Very weak 4. AOL4 64,09 Strong 5. AOL5 115,15 Moderate 6. AOL6 257,09 Very weak 7. Ascorbic acid 15,14 Very strong	1.	AOL1	28,50	Very strong	
3. AOL3 385,29 Very weak 4. AOL4 64,09 Strong 5. AOL5 115,15 Moderate 6. AOL6 257,09 Very weak 7. Ascorbic acid 15,14 Very strong	2.	AOL2	168	Weak	
4. AOL4 64,09 Strong 5. AOL5 115,15 Moderate 6. AOL6 257,09 Very weak 7. Ascorbic acid 15,14 Very strong	3.	AOL3	385,29	Very weak	
5. AOL5 115,15 Moderate 6. AOL6 257,09 Very weak 7. Ascorbic acid 15,14 Very strong	4.	AOL4	64,09	Strong	
6. AOL6 257,09 Very weak 7. Ascorbic acid 15,14 Very strong	5.	AOL5	115,15	Moderate	
7. Ascorbic acid 15,14 Very strong	6.	AOL6	257,09	Very weak	
	7.	Ascorbic acid	15,14	Very strong	

Table 1. Antioxidant activity of endophytic fungi extract

*) Criteria: IC50 >200 (very weak); IC50 150-200 (weak); IC50 100-150 (moderate); IC50 50-100 (strong); IC50 ≤50 (very strong) [19].

Extracts that have antioxidant activity are able to inhibit an oxidation reaction from free radicals. According to Karim *et al.* [20] (2015) antioxidant activity is the ability of an extract or

compound to inhibit the oxidation reaction which is expressed by the percentage of inhibition or the percentage of inhibition. Based on Table 1, the antioxidant activity of the AOL₁ extract was very strong because they had IC₅₀ values of 28.50 g/mL. The smaller the IC₅₀ value, the stronger the antioxidant activity of the compound. The difference in IC₅₀ value can be caused by the amount of antioxidants contained in the extract.

3.5. TLC of AOL1 endophytic fungi extract

Based on the TLC analysis of AOL1 extract presented in Figure 4, showed several different color pattern on the TLC plate. The color differences are affected by different types of compound inside each extract hence when separated by eluted TLC it will separated. The extract of AOL1 isolates contained alkaloids, flavonoid and terpenoid according to Harborne [21]. The secondary metabolite extract compound detected from the color that were formed on the plate. According to research by Normansyah et al. [22]. that the detection of compounds can be observed from the color of the stain formed. Alkaloids form orange or brick red color, brown tannins and yellow stains on the plate prove the presence of phenolic compounds. Phenolic compounds such as flavonoids are natural antioxidants [23]. The content of alkaloid and terpenoid compounds in AOL_1 isolate was able to act as a natural antioxidant. Reda [24] explained that the antioxidant activity of flavonoids is based on their ability to donate hydrogen atoms which can neutralize the toxic effects of free radicals. The phenolic hydroxyl groups of flavonoids have the ability to capture free radicals and their activity as metal chelators causes antioxidant activity in flavonoids. Yuhernita and Juniarti [25], explained that alkaloid compounds have the ability to efficiently stop free radical chain reactions by acting as hydroxy radical absorbers. The research of Graßmann [26], explained that terpenoids have been shown to have potential antioxidant activity and protective effects against oxidative stress in mitochondria, especially lipophilic terpenoids.

Figure 4. Chromatogram extract of AOL1 endophytic fungi isolate 1.Alcaloid, 2. Flavonoid, 3. Terpenoid

Table 2. TLC analysis and <i>Rf</i> of AOL ₁ extract from endophyitic fungi					
Isolate	Rf	Color	Active Compound group [21]		
	0,250	Brownish orange	Alkaloid		
AOL ₁	0,575	Yellow	Flavonoid		
-	0,700	Pink	Terpenoid		
-	0,875	Pink	Terpenoid		

3.6. Endophytic Fungus Characterization and Identification

1

Based on the results of macroscopic and microscopic characterization of endophytic fungi, isolates of AOL1 which were incubated at room temperature for 7 days on PDA medium had a colony diameter of 4.6 cm, the colonies were greyish green. Colonies in MEA medium had a diameter of 4.5 cm, the color of the colonies was greyish green. Colonies on CDA medium had a diameter of 4.3 cm and the color of the colonies was greyish green (Figure 6).

С Figure 5. Colony of AOL1 isolates on different media A.CDA, B. MEA, C.PDA, 1.Colony, 2. Reverse colony

2

Figure 6. Microscopic morphology of AOL1 endophytic fungi isolate

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Isolate	BLAST result	Accession	Identity (%)
		Number	• • •
	Phyllosticta rhizophorae isolate NCYUCC	MT360030.1	99.54
	19-0-352		
	Phyllosticta fallopiae MUCC 0113	NR_147316.1	99.84
AOL ₁	Guignardia musicola CBS 123405	NR_137716.1	99.84
	Guignardia alliacea isolate MUCC0014	AB454263.1	99.68
	Phyllosticta capitalensis CPC 18848	NR_144914.1	99.83
	Phyllosticta paracapitalensis CBS 141353	NR_153303.1	99.48

Table 3. BLAST analysis of ITS region AOL_1 fungi isolate

Figure 7. Phylogeny tree of AOL₁ fungal isolates together with the reference strain constructed based on the Neighbor-Joining algorithm. The numbers in each branch indicate the bootstrap. The scale indicates a substitution of 1 per 10 nucleotides in the ITS rDNA region sequence

Based on the BLAST results, it was found that AOL_1 isolates had similarities with members of the Phyllosticta genus with identity values above 99% (Table 3). This means that

the ITS rDNA region sequence of AOL_1 isolates has a homology of at least 99% with the fungal strain sequence in the same region in Genbank. The construction of the AOL_1 fungal isolate phylogeny tree with the reference strain (Figure 1) showed the same results as the BLAST results, namely the AOL1 strain was in the same cluster as the BLAST reference strain. However, in the cluster there were still several species so that the isolate AOL1 was identified as a member of the *Phyllosticta* genus. Further identification down to the species level can be done using other, more specific primers.

4. Conclusion

Based on the research qualitatively 6 extracts from the endophytic fungi isolate (AOL₁, AOL₂, AOL₃, AOL₄, AOL₅, and AOL₆) from *Hippobroma longiflora* (L.) G. Don showed antioxidant activity, but quantitatively the extracts that showed very strong activity was extracts from AOL₁ isolate with IC₅₀ values of 28.50 μ g/mL. Based on phenotypic and molecular identification AOL₁ isolate identified as *Phyllosticta* sp and produce alkaloid, flavonoid, and terpenoid.

5. AknowlegmentAcknowledgment

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Dengan hormat, Bersama ini saya kirimkan revisi artikel 67-128-1-RV rev 02122021 a.n. Hary Widjajanti.

Terima kasih. Hormat saya,

Hary Widjajanti

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