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## Preface

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## 2<sup>nd</sup> International Conference Earth Science and Energy (ICESE) 2020



## **2<sup>nd</sup> International Conference Earth Science and Energy (ICESE) 2020**

We are glad to introduce you the proceedings of the first 2nd International Conference Earth Science and Energy (ICESE) 2020. The 2<sup>nd</sup> ICESE 2020 addresses challenges and innovations the field of Food Science, Earth Science, Environmental Science and Environmental Issue. It also provides a premier interdisciplinary platform for researchers, educators and practitioners to present and discuss the most recent innovations, trends, and concerns as well as practical challenges encountered and solutions adopted in the fields of science, Energy, Food Science, Forest Science and environmental issue related.

As we may aware, the World Health Organization officially declared the novel coronavirus COVID-19 a pandemic. Governments around the world are now issuing restrictions on travel, gatherings, and meetings in an effort to limit and slow the spread of the virus. The health and safety of the author and reseacher community is our first priority and we are supporting these efforts. Therefore, the ICESE 2020 conference was held virtually on 11 November 2020.

The ICESE conference is hosted by PT. Kresna Acitya Nusantara Mediatama and co-hosted by Universitas Muhammadiyah Sidoarjo, Relawan Jurnal Indonesia. This year, we held this flexible online conference to gather experts and scholars around the globe with the aim to continue disseminating the latest advanced research in the field of Food Science, Earth Science, Environmental Science and Environmental Issue. The conference was held from Yogyakarta as the host of the event. The ICESE 2020 event is virtually implemented with a model that all invited speakers are given time to present their material for about 30-45 minutes each. It then followed by a question and answer by the participants with a direct questioning system, through chat forums and Q&A forums provided by the zoom application. Overall, the conference took 6 hours.

The number of participants who joined the zoom room was recorded around 271 participants. The authors or participants are came from 5 countries, namely Indonesia, Malaysia, Brunei Darussalam, Philippine and India. Indonesian Participants are come from 19 Provinces of 33 Provinces.

The committee of the conference are honored to have invited following renowned experts as our keynote speakers. Prof. Dato' Dr. Kamaruzzaman Sopian from Universiti Kebangsaan Malaysia; Assoc Prof Ir. Ts. Dr. Mohd Nazri Mohd Warip, P.ENG, CENG, CITP from Universiti Malaysia Perlis; Dr. Ir. Jamaaluddin, M.M from Universitas Muhammadiyah Sidoarjo.

We are glad to share with you that around 163 pre-registered authors are submitted their work in the conferences. However, its about 96 papers are selected and accepted for the conferences. All the papers have been through rigorous review by a panel of reviewers who provide critical comments and corrections, and have contributed substantially to the improvement of the quality of the papers to meet the requirements of International publication standard and IOP EES Scope.

We would like to express our sincere gratitude to the Chairman, the distinguished keynote speakers, as well as all the participants. We also want to thank the publisher for publishing the proceedings. May the readers could enjoy the gain some valuable knowledge from it. We are expecting more and more experts and scholars from all over the world to join this international event next year.

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# Table of contents

Volume 819

2021

◀ Previous issue    Next issue ▶

**2nd International Conference Earth Science And Energy 11 November 2020, Kuala Lumpur, Malaysia**

Accepted papers received: 02 July 2021

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Open all abstracts

## Preface

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Peer review declaration

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## Agriculture

**OPEN ACCESS** 012001

*Ganoderma boninense* control in palm oil plantations using *Trichoderma harzianum* in various Media

Aries Sukariawan, Eka Bobby Febrianto, Sakiah, Muhammad Ridho and Doli Karnando

+ Open abstract  View article  PDF

**OPEN ACCESS** 012002

Preparation and characterization of cellulose microcrystalline made from palm oil midrib

Pada Mulia Raja, Ika Ucha P. Rangkuti, M. Hendra Ginting, Giyanto and Wahyu F. Siregar

+ Open abstract  View article  PDF

**OPEN ACCESS** 012003

The study of soil biological and chemical properties on palm oil plant rhizosphere with and without palm oil mill effluent applications

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 Evaluation of the Value of the Defective and Taste of Arabica Coffee (*Coffea Arabica L*)  
 West Sumatera

Rince Alfia Fadri, K Kesuma Sayuti, Novizar Nazir and Irfan Suliansyah

[+ Open abstract](#) [View article](#) [PDF](#)

**OPEN ACCESS** 012005  
 Utilization of alternative ingredients as combination feed on the growth of catfish  
 (*Clarias sp.*)

RC Mukti, M Amin and Yulisman

[+ Open abstract](#) [View article](#) [PDF](#)

**OPEN ACCESS** 012006  
 Assessment on shallot farming development in North Padang Lawas Regency, North  
 Sumatra

Moral Abadi Girsang, Palmarum Nainggolan, Shabil Hidayat, Suheri Sitepu and Khadijah El Ramija

[+ Open abstract](#) [View article](#) [PDF](#)

## Agrotechnology

**OPEN ACCESS** 012007  
 Application of Trichoderma as an Alternative to the use of Sulfuric Acid Pesticides in the  
 Control of Diplodia Disease on Pomelo Citrus

Mei Silvia and Sutarman

[+ Open abstract](#) [View article](#) [PDF](#)

**OPEN ACCESS** 012008  
 The Effect of Trichoderma and Onion Extract on the Success of Grafting in Mango  
 Seedlings


Feroniza Berlian Sentosa, Sutarman and Intan Rahma Nurmallasari

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**OPEN ACCESS** 012009  
 Isolation and Performance Testing of *Bacillus subtilis* As Biological Agents to Control  
 the Diplodia Disease on Siam Citrus

Dinda Aprilia, Agus Miftakhurohmat and Sutarman

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Syahriati, UA Nur Fitriani and Muhammad Yusuf

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Physicochemical, nutritional, and color characteristic of Nori Snack from Snakehead Fish (*Ophiocephalus striatus*), *Eucheuma cottonii*, and *Gracilaria sp*

Muhammad Yusuf, UA Nur Fitriani and Syahriati

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## Animal Science

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012013

Reproductive characters of hampala fish (*hampala macrolepidota kuhl & Van Hasselt, 1823*), correlation with body length

Suhestri Suryaningsih, Sri Sukmaningrum, Adi Amurwanto and Aswi Andriasari Rofiqoh

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012014

Efficacy of application time of *Penicillium sp.* suspension on White Root Fungus (*Rigidoporus lignosus*) in Nutmeg (*Myristica fragrans*)

Chairudin, Agustinur and J Permadi

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012015

The correlation between mangrove ecosystem with shoreline change in Indramayu coast

Sofyan Hasanuddin Nur and Endang Hilmi

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Sugarcane bagasse for environmentally friendly super-absorbent polymer: synthesis methods and potential applications in oil industry  
Shabrina Sri Riswati, Rini Setiati, Sugiarto Kasmungin, Suryo Prakoso and Muhammad Taufiq Fathaddin  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012018  
Lithofacies Association of Fluvial Deposit, Walat Formation in Cibadak Area West Java  
S D Hafiz, H Firman, A Ramadhan and W T Koesmawardhani  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012019  
Geothermal Well Targeting in Consideration To Geological Structures of Mataloko Field, Flores  
Untung Sumotarto, Yudistian Yunis, Fajar Hendrasto, Kris Pudyastuti, Evan R. Sammuel, Dhany Rizky and Arthur G.P. Nayoan  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012020  
New Insight of Surface and Subsurface Sedimentology of Salodik Group, Banggai Basin  
Firman Herdiansyah, M Burhannudinnur, M Ali Jambak and Teddy Irano  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012021  
Petrophysical Analysis to Determine the Initial Oil Reserves in the AHP Field  
Apolonius Harda Putranta Sudi, Ratnayu Sitaresmi and Prayang Sunny Yulia  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012022  
Compatibility Test for Screening Surfactant Flooding  
M Dhafa, R Setiati, M T Malinda and A Anugrahadi  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012023  
The Opportunities and Constraints of Wind Energy  
A A S Siahaya, R Setiati and M T Malinda  
[+](#) [Open abstract](#) [View article](#) [PDF](#)
- 
- OPEN ACCESS** 012024  
Effect of Improvement of Temperature and Gas Amount on Nitrogen Flooding and out more,  
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M T Malinda, R Setiati, M A Azizi and Y F Simbolon



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Laboratory Study: The Effect of Clay Mineral in Sandstone on Resistivity of Rock

Ratnayu Sitaesmi, Rosidelly, Lia Mega and M. Taufiq Fathaddin

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012026

Drill Holes Spacing Analysis for Estimation and Classification of Coal Resources Based on Variogram and Kriging

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The Palu Earthquake Formed a New Geological Structure

A. Anugrahadi, U. Sumarto and T. T. Purwiyono

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012028

Solar Energy Hybrid Control System Design Photovoltaic Using Fuzzy Logic Controller

Ramadan Salem Ramadan

[+ Open abstract](#) [View article](#) [PDF](#)

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012029

Analysis of Overcurrent Safety in Miniature Circuit Breaker *AC (Alternating Current)* and *DC (Direct Current)* in Solar Power Generation Systems

Jamaaluddin Jamaaluddin, Indah Sulistiyowati, BWA. Reynanda and Izza Anshory

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012030

**The Application of Geostatistical Analysis on Radiometric Mapping Data to Recognized the Uranium and Thorium Anomaly in West Sulawesi, Indonesia.**

I Gde Sukadana, I Wayan Warmada, Agung Harijoko, Frederikus Dian Indrastomo and Heri Syaeful

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Causes, Dynamics, and Environmental Conflict Resolution Models in Coal-Fired Steam Power Plant Development Locations

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Illegal artisanal and small-scale mining practices: re-thinking the harm

Putu P. S. Agustina, Herdis Herdiansyah and Anggi A. Harahap

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Good Governance Role for a Sustainable Solid Waste Management in Rural Community

Ari Naldi, Herdis Herdiansyah and L G Saraswati Putri

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Climate Village Program for Climate Change Adaptation and Mitigation for Green Villages

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Sustainable Development on Indonesian Environmental Risk Assessment

Agastyo Djanardono Basoeki, Herdis Herdiansyah and Yuki M. Adhitya Wardhana

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Soybean production response: A study of Jambi's acreage response under policy program

Edison and Dharia Renate

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Contribution of Oil Palm Plantation to Household in Kolaka District of Southeast Sulawesi

Haji Saediman, Rahmayana Rahmayana, Yusna Indarsyih, Nuryamin Budi and Lukman Yunus

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012038

The Development of Communication Model for the Empowerment of Highly Poor Villages in Pangandaran Regency, West Java, Indonesia

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Managing the uncertainty during COVID-19 pandemic: Communicating disaster and food industry sustainability

Myra V. de Leon, Daniel Susilo, Teguh Dwi Putranto, Fadjar Kurnia Hartati and Roberto Rudolf T. Santos

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Elfrida Ratnawati

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012041

Social-Ecological Vulnerability and Livelihood Improvement Strategies of Landslide Victims in Pattalikang Village, Gowa Regency in National Security Perspective

Khairunnisa Adri, Siswo Hadi Sumantri, Sugeng Triutomo and Deffi Ayu Puspito Sari

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012042

Determination of priority locations for the implementation of rice farming insurance: a case study on disaster hazards in Cilacap regency

Unggul Pramukti, Suryanto and Evi Gravitaniani

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012043

Smallholder adoption of horticultural crops: the case of dragon fruit in Southeast Sulawesi

Haji Saediman, Ilham Saediman Mboe, Budiyanto Budiyanto, Sarinah Sarinah and Hidrawati Hidrawati

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012044

The government policy on the covid-19 handling viewed from environmental and biodiversity perspectives

Suwari Akhmaddhian, Toto Supartono, Erga Yuhandra, Haris Budiman and Diding Rahmat

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Fish diversity monitoring in Maninjau Lake, West Sumatra using the eDNA with the next generation sequencing (NGS) technique



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012046

Protecting Biodiversity: The Importance of Understanding the Role of Government and Society in Human-Urban Wildlife Interaction in Indonesia

Peni Puspitasari, Herdis Herdiansyah and Donna Asteria

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The effect of maggots lentera flies (*hermetia illucens*) growing media as the solution of using organic waste

Nina Herlina, Nurdin, Bambang Yudayana, Iing Nasihin and Ai Nurlaila

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Habitat characteristics and population of *leptophryne javanica* in Curug Cisurian, Kopi Bojong, and Ciinjuk in Gunung Ciremai National Park

Ika Karyaningsih, Nurdin, W Indra, Nina Herlina and Ai Nurlaila

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012049

Structure and similarities of Understorey Vegetation Communities in the mining area of PT. Indocement Tungal Prakarsa Tbk. Palimanan Unit, Cirebon

Iing Nasihin, Dede Kosasih, Nina Herlina and Ai Nurlaila

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Dynamics of dengue hemorrhagic fever incidence and climate as potential factors in Palembang 2013 – 2019

Krisna Delita, Nurhayati Damiri, Rico Januar Sitorus, Poeji Loekitowati Hariani, Fika Minata Wathan and H Annisa Tassia

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012051

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012052

## Quality of Fermented Purple Sweet Potato (*Ipomea batatas* var. *Ayamurasaki*) (Tapai) in Various Yeast Concentration and Steaming Time

R Azara, L Qur'aini, I A Saidi, L Hudi, S R Nurbaya and R U Budiandari

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012053

## Characteristics of Dry Carrot Noodle (*Daucus carota* L) with Proportion of Wheat Flour and White Oyster Mushroom Flour (*Pleurotus ostreatus*)

L Hudi, I A Saidi, R Azara and T R Pratiwi

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012054

## Determination of Level of Lead (Pb) and Cadmium (Cd) Migration on Bowl and Clear Plastic Cup Using Atomic Absorption Spectrophotometer

Hurip Budi Riyanti and Y Yeni

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## Optimization and Validation Methods for Assay of Acyclovir Cream Determination by High Performance Liquid Chromatography

Muhamad Zaenudin, Almawati Situmorang and S Supandi

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## Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

Ni Putu Ermi Hikmawanti, Tuti Wiyati, M. Abdul Muis, Farah Aisyah Nurfaizah and Windy Septiani

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012057

## Chemical Component of Kencur (*Kaemferia galanga* L.) Ethanolic Extract Using Gas Chromatography-Mass Spectrometry

Ni Putu Ermi Hikmawanti, Supandi, Lusi Putri Dwita and Yeni

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012058

## Effect of Glycerin as Plasticizer in Formulation of Grape Seed Oil (*Vitis vinifera* L.) Emulgel Peel-Off Mask

Fith Khaira Nursal, Nining and Aisyah Rahmani

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012059

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The Effects of Pineapple Extract Toothpaste (*Ananas comosus L.Merr*) on Saliva Flow Rate

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012061

The use of coconut water (*cocos nucifera l.*) as alternative media to substitute Sabouraud Dextrose Agar (SDA) for the growth of *aspergillus flavus*

Widhorini, Asep Ginanjar Arip and Astrini Wulandari

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012062

Diversity of insect pollinator on farmland near to mount Ciremai National Park

Ai Nurlaila, Ika Karyaningsih, Nina Herlina, Iing Nasihin and Bambang Yudayana

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012063

Vegetation structure and species composition of habitat types *Goniothalamus macrophyllus* (Blume) Hook.f. and Thomson in Lowland Forest, Kuningan Regency, West Java

Ilham Adhya, Yayan Hendrayana, Toto Supartono, Agus Yadi Ismail and Nurdin

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Activity of valerian catalase in Ultisol growing media by adding humic acid

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012065

*Siam Madu* citrus seeding in supporting the development of Sumatra citrus zone

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012066

A cross-sectional study of vitamin A, C and D and Iron daily intake among adolescents and its correlation with over nutrition status

M A M Putri, D Indarto and V Widyaningsih

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012067

**Biochemical content of Robusta coffees under fully-wash, honey, and natural processing methods**

Sri Wulandari, Makhmudun Ainuri and Anggoro Cahyo Sukartiko

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OPEN ACCESS

012068

**Characterization of GABA (gamma-aminobutyric acid) levels some fermented food in Indonesia**

Heny Herawati, Diana Nur Afifah, Eni Kusumanigtyas, Sri Usmiati, Agus S. Soemantri, Miskiyah, Elmi Kamsiati and Muchamad Bachtiar

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012069

**Differences in the effect of online nutrition education through lectures without and with online games on increasing knowledge of Nutrition, Iron, and vitamin C intake of young women**

N S O Lorenza, E S Sulaeman and V Widyaningsih

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012070

**Diversity and abundance of insect pollinator on *dimocarpus longan l.* in Sawitsari research station, Sleman, Yogyakarta**

Ultha Rifqy Riswanta, Nico Chandra Aditya, Anas Sobri and Sukirno Sukirno

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012071

**In silico development of new candidate of NADPH oxidase inhibitor for hypertension treatment**

Agung Budi Laksono, Ratna Kusumawati, Yuliana Heri Suselo and Dono Indarto

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012072

**Iron supplement for anemia developed from home industry waste of snake fruit Pondoh (Snake fruit *edulis Reinw.*)**

K Natasia, D Indarto, B Wasita, D Melati and I K Ristanti

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012073

**Study of proximate composition, antioxidant activity and sensory evaluation of cooked rice with addition of cherry (*Muntingia calabura*) leaf extract.**

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Innovation development information system for sales of traditional food processed product web based in the Cibinuang Village

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Forest Land Management System and Effect on The Presence Of Birds at Nusa Laut Island

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012078

Distribution and association of *Ficus* spp in the shrubs area of Gunung Ciremai National Park Indonesia

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012079

Use of faecal pellet count method in estimating population density of mammals in Gunung Ciremai National Park

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## Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

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# Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

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**Abstract.** *Cayratia trifolia* leaves contain flavonoids compound that can dissolve well in polar solvents. This study aimed to evaluate the total flavonoids content of polar extracts which extracted sequentially from *C. trifolia* leaves. The dried *C. trifolia* leaves were macerated continually using n-hexane, ethyl acetate, and ethanolic solvent. The total flavonoids content was determined using spectrophotometric methods with a  $\text{AlCl}_3$  reagent. TLC chromatogram of the extracts were evaluate using chloroform: acetone: formic acid (10:2:1) as a mobile phase and silica gel  $\text{F}_{254}$  as a stationary phase. The citroborate and ammonia vapour were used as detection spray reagents. The highest total flavonoids content ( $27.95 \pm 0.62$  mgQE/g) were found in 70% ethanol extract of *C. trifolia* leaves compared to ethyl acetate extract ( $17.98 \pm 0.89$  mgQE/g). The ethanolic extract is found six compound spots, while the ethyl acetate extract found four compound spots which were thought to be a flavonoid group. Thus, it can be concluded that the different polar extracting solvents are able to attract flavonoid compounds with different levels. The flavonoids in *C. trifolia* extract need to be separated to determine the types.

## 1. Introduction

In Indonesia, *C. trifolia* is known as galing or lakum. In English, this plant is called Fox grape [1]. The leaves of this plant are used by several people in Indonesia as medicine to cure headaches [2], boils, speed drying of the wound [3], muscle pains, and antidandruff [4]. The polar fraction (ethyl acetate and methanol) of galing leaves has been known to have antioxidant activity [5]. The ethanol crude extract of the galing plant has hepatoprotector properties [6].

Flavonoids are secondary metabolites of polyphenol derivatives with many benefits. Flavonoids function as strong antioxidants and also have the potential to be developed into a nutraceutical source [7]. The good solubility of flavonoids in polar solvents can be used as a basis for guiding the separation procedure for these compounds. Polar solvents that are good for the extraction of flavonoids include alcohol (ethanol, methanol, n-butanol, etc.), ethyl acetate, acetone, or water [8]. Selection of the right solvent will have an impact on the chemical content extracted, the extraction time, the composition of the extracted compound, the ease of handling the extract, and the effectiveness of the extract produced [9].

Plant extracts can be easily identified qualitatively for their flavonoid content using thin-layer chromatography (TLC). Compared to paper chromatography, TLC has a better ability in terms of separating phenolic derivatives, especially in extract samples. The TLC method is also quite



inexpensive and is able to identify several samples at the same time so that it is efficient in terms of using analysis time [10]. TLC can be used to provide an overview of the chromatogram pattern of an extract material. Meanwhile, quantification of total flavonoids is generally carried out by the colorimetric method using a spectrophotometer assay because it is considered simple and fast [10]. Measurement of total flavonoids from plant extracts that have been added with  $\text{AlCl}_3$  reagent was carried out in the range 410-423 nm [8]. In this study, the total flavonoid levels in *C. trifolia* leaf extract were sequentially extracted using organic solvents and the chromatogram pattern was determined using the TLC method.

## 2. Material and Methods

### Preparation of Sample

The *C. trifolia* leaves were collected from Tuban, East Java, Indonesia. The plant was identified in Conservation Research Center and Botanical Garden, Indonesian Institute of Sciences, Bogor, Indonesia. The *C. trifolia* fresh leaves were dried for a few days at indirect sunlight condition and were made into powder. The 1.5 Kg of dried leaves powder was extracted by cold maceration (2 times, 24 hours) respectively in *n*-hexane, ethyl acetate, and ethanol-water (70:30) according to their increasing strength of polarity. Each filtrate was evaporated using a vacuum rotary-evaporator N-1200 BS series (EYELA, Shanghai, China) at 40 °C. The percentage yield of *n*-hexane, ethyl acetate and ethanolic extracts of *C. trifolia* leaves were calculated [11].

### Determination of Total Flavonoids Content (TFC)

The determination of TFC follows the procedure on Chang et al., (2002) with modification [12]. Quercetin as standard. Each extract of *C. trifolia* leaves in methanol (at concentration 1000 ppm) is piped as much as 0.5 mL then 3 mL of methanol is added and 0.2 mL of  $\text{AlCl}_3$  (10%) reagent is added, 0.2 mL of sodium acetate (1M) and sufficient with aquadest up to 5 mL. The solution was incubated for 60 minutes at room temperature. The absorbance of the extract solution was measured with a UV-Vis Shimadzu UV-1601 Series (Kyoto, Japan) spectrophotometer at a wavelength of 428.5 nm. The absorbance obtained is plotted into the linear line equation. TFC is expressed in mg which is equivalent to quercetin per gram of extract. Each extract was tested for 5 repetitions and reported as mean  $\pm$  SD.

### Analysis of Sampel using Thin Layer Chromatography (TLC)

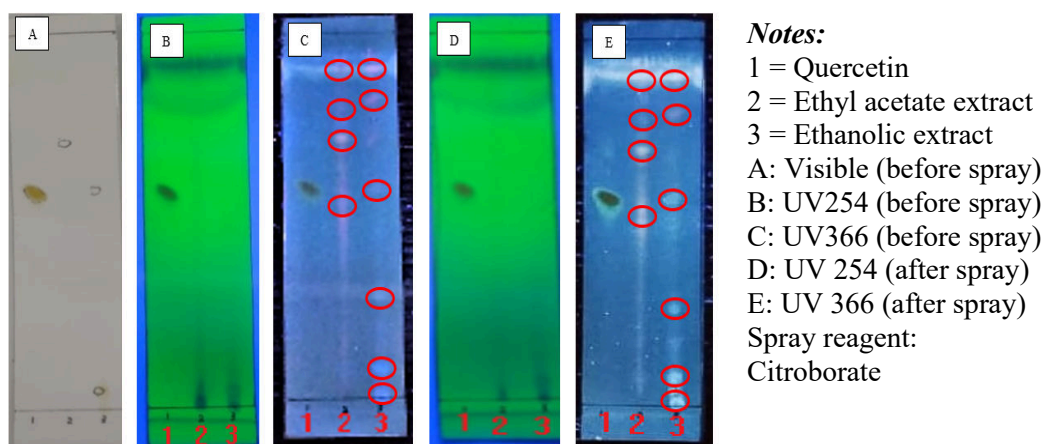
The profile chromatogram of flavonoids in each extract was analysis using TLC. Each extract was dissolved in methanol (at concentration 3%). Chloroform: acetone: formic acid (10:2:1) were used as a mobile phase. Silica gel F<sub>254</sub> as a stationary phase. The citroborate solution was used as detection spray reagents. The visualisation of spots was performed in visible and UV (UV box, Camag).

## 3. Results and Discussion

The percentage of yield of the *C. trifolia* sequentially extracts were 1.75% for ethyl acetate extract and 21.30% for ethanolic extract. This means that *C. trifolia* leaves are dominated with polar compounds, either phenolics or polar non-phenolic compounds (such as saponins, glycoside, sugar, etc.). The highest total flavonoids content ( $27.95 \pm 0.62$  mgQE/g) was found in ethanolic extract compared to ethyl acetate extract ( $17.98 \pm 0.89$  mgQE/g) of *C. trifolia* leaves. The ethanolic extract of *C. trifolia* leaves is found six compound spots, while the ethyl acetate extract found four compound spots which were thought to be a flavonoid group (**Figure 1**).

Flavonoids are structurally very diverse compounds with stable chemical properties in plants and are often used as chemotaxonomic markers. The TLC method is a method that is often used for rapid screening of flavonoids as pharmacological target compounds in plant extracts. Extraction of flavonoids with polar solvents such as ethyl acetate and alcohol usually begin with the removal of unwanted interfering compounds such as fat, chlorophyll using nonpolar solvents such as *n*-hexane

[13]. Ethanol polarity is caused by the presence of the free OH group, while ethyl acetate is caused by its presence of  $\pi$  electrons and lone-pair electrons present on O [14]. Flavonoid aglycones have a low molecular weight. Its aglycone form is often soluble in less polar solvents than ethanol. Meanwhile, flavonoid glycosides will be extracted more in polar solvents such as alcohol (methanol or ethanol), water, or a mixture of both. [15]. Based on the results obtained, it shows that the extract was extracted using ethanol solvent contains more varied flavonoids and more quantitatively than the extract extracted with ethyl acetate. Further separation accompanied by bioassays is necessary for the purpose of isolating flavonoid compounds that play an active role in a pharmacological activity.



**Figure 1.** TLC Chromatogram of the *C. trifolia* extracts are compared with quercetin as standard

#### 4. Conclusion

The different polar extracting solvents can attract flavonoid compounds with different levels. The flavonoids in *C. trifolia* extract need to be separated to determine the types.

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