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# **PROCEEDINGS BOOK**

The 2nd International Conference on Biology Education, Natural Science, and Technology (INCOBEST)

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July 06<sup>th</sup>, 2024 - Surakarta

INCOBE

DEPARTMENT OF BIOLOGY EDUCATION FACULTY OF TEACHER TRAINING AND EDUCATION UNIVERSITAS MUHAMMADIYAH SURAKARTA

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# **Proceedings Book**

# The 2<sup>nd</sup> International Conference on Biology Education, Natural Science, and Technology (INCOBEST)

"Biological Roles to Solve the Environmental Issues"

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**Proceedings Book of International Conference on Biology Education, Natural Science, and Technology (INCOBEST) 2024** *Emerging Issues on Natural Science, Environment, and Its Learning Innovation* 

Surakarta, October 29th, 2024

# Incobest 2024 Secretariat

Department Of Biology Education Faculty Of Teacher Training And Education Universitas Muhammadiyah Surakarta Email: incobest@ums.ac.id Website: incobest.ums.ac.id



FOREWORD

# FOREWORD

Alhamdulillah, with all gratitude for the presence of Allah SWT, who has bestowed His grace, guidance, and inayah so that the Biology Education Study Program, Faculty of Teaching Training and Education (FKIP), Universitas Muhammadiyah Surakarta (UMS) can organize The 2nd International Conference on Biology Education, Natural Science, and Technology (INCOBEST) 2024. The conference, with the theme "Biological Roles to Solve Environmental Issues", emphasizes biology's pivotal role in understanding and tackling environmental challenges such as climate change, pollution, and biodiversity loss. INCOBEST aims to foster collaboration, promote innovation, and inspire action in addressing environmental issues through biological solutions. It serves as a platform for interdisciplinary dialogue and concrete actions toward environmental sustainability. Key themes include environmental conservation, climate change mitigation, sustainable agriculture, renewable energy, pollution control, ecosystem restoration, biotechnology applications, education for sustainable development, and policy frameworks for environmental protection..

This conference aims to achieve the implementation of the Tri Dharma of Higher Education, encompassing education, research, and community service, and to realize academic freedom by introducing the Biology Education Study Program FKIP UMS as a part of the Institute of Education Personnel, that is capable of collaborating with various educational and non-educational agencies. Muhammadiyah Surakarta

The activities of this International Conference have been made possible by the assistance of an array of parties. Thus, on this occasion, appreciation is extended to:

1. Dean of FKIP UMS

2. All committees of INCOBEST 2024.

- 3. Reviewer team, both internal and external.
- 4. Participants and speakers.

5. Other parties that cannot be mentioned one by one.

Finally, we hope this International Conference will benefit all parties and improve education in Indonesia.

Surakarta, October 29th 2024

Committee



INTRODUCTION

# **INTRODUCTION**

Science and the environment are ever-changing. The world is experiencing one of the most rapid advancements in science and technology. The world has numerous innovations and advancements in various disciplines, including industry, information, and telecommunications, high technology in space, robot technology, and biotechnology and molecular advances. In the era of globalization, where science and technology are one of the indicators of a nation's progress in facing global competition, almost every nation in the world has raced to develop every aspect of their life based on science and technology as a result of the development and progress in these various fields.

John Naisbitt and Patricia Aburdene, in the book 'Megatrends 2000', predicted that one of the 21st-century megatrends is the shift from physical models and metaphors to biological models and metaphors to help us understand today's dilemmas and opportunities. The prediction of John Naisbitt and Patricia Aburdene is realistic. Isn't it that before entering the 21st century, advances in biology have been felt up to the extraordinary advances in modern biology? He continued, we will prepare further on the threshold of a significant era: Biotechnology. In the era of the 21st century, biotechnology, as previously predicted, will be as important as computers. Biotechnology will be booming; at least the first directions of biotechnology that have been developed are in the fields of agriculture and animal husbandry, the food industry, to the clothing and health industries. Biotechnology companies are racing with new drug discovery and development to reach more than 300 drug products and 200 vaccines for diseases worldwide, including cancer, Alzheimer's, heart disease, AIDS, arthritis, and various infectious diseases in developing countries.

The genetic manipulation of plants and animals is another biotechnology development that is advancing rapidly at present. Transgenic vegetation can be produced through genetic engineering. This is an innovation in the development of high-yielding, disease-resistant, and postharvest storage-resistant plants of superior quality.

The accelerated advancement of science and technology has an effect on education. In order to actualize the expectation that education will produce graduates who can compete in the workforce, numerous innovations must be developed. Curriculum modifications at various levels, including elementary, secondary, and tertiary education, seek to equip graduates with life-applicable skills.

The preparation of the Indonesian National Qualifications Framework, a competency alignment framework that can juxtapose, equalize, and integrate between the fields of education and job training as well as work experience in the context of providing recognition of work competence by the structure of work in various sectors, enables the alignment of graduates at various levels of education in Indonesia so that they can compete globally. Regarding Indonesia's national education and training system, the framework is a manifestation of the quality and identity of the Indonesian nation.

Educators (teachers and lecturers), as well as researchers and observers in these disciplines, must be aware of current issues in science, the environment, and learning innovation in order to adapt and continually expand their knowledge. Following the most recent developments in their field is hoped to be a catalyst for improving the quality of research and education in Indonesia.

In response to the extremely rapid development of science, the environment, and learning innovations, the Biology Education Study Program FKIP UMS has scheduled the second International Conference on Biology Education, Natural Science, and Technology (INCOBEST) 2024 as the continuation of the previous National Education and Science



# INTRODUCTION

Seminar (SNPBS) VII 2022 as a form of concern and commitment to the improvement of the quality of research and education in Indonesia. This is the second International Conference to continue SNPBS, which was implemented successfully between 2016 and 2022.

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International Conference on Biology Education, Natural Science, and Technology

V



COMMITEE

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			Dr. Paramitha Cahvani M. Dd
			Di. i arannuna Canyani, ivi. i u.



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# Embodied, Affective and Connected Science Education for A Sustainable World

Simone M. Blom, Ph.D.

Southern Cross University, Australia

	ABSTRACT
KEYWORDS:	Sustainability and environmental education are often taught through the Science
Sustainability education, Embodied learning,	discipline in the curriculum. The biological sciences accommodate much of the
	work students will do in learning about ecological, biological and sustainable
Human-nature	practices and systems of the living world. This work is important, however, it
Affective education	can focus too heavily on content without paying attention to the natural
njjeenve eureunon.	environment to enable students to experience embodied and affective responses.
	Learning about the human body with/as nature is and should be an essential aspect of science education.
	Learning about the human body is typically achieved through the biological
	sciences curriculum and is not considered in relationship to the natural world
	and the environmental crisis. In science education we have the opportunity to
	consider our embodied relationship with/as the natural world and how this
	fundamental relationship may shift thinking of the environmental crisis as
	happening 'out there'. This opportunity transforms our thinking from trying to
	fix the problem using exterior solutions to considering our responsibility as
	intrinsically connected human bodies.
© 2024 The Author(s). Published	This research presentation explores the affective turn to consider how our
by Biology Education Department, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta.	embodied relationship with nature can be activated as an educational apparatus
	to enhance sustainable practices. Sustainable solutions cannot be considered
This is an open access article under the CC BV NC license:	devoid of the human body that is made of the same biological matter that forms
https://creativecommons.org/license	the natural world. This presentation proposes an education that is embodied,
s/by-nc/4.0/.	affective and connected for a sustainable future world.



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# **Eco-Friendly Production of Biodegradable Plastics From Waste Biomass Using Bacteria**

# Assoc Prof. Nazia Jamil, Ph.D

Institute of Microbiology and Molecular Genetics, University of the Punjab, Quaid-i-Azam Campus, Lahore-54590, Pakistan.

	ABSTRACT				
KEYWORDS:	Environmental pollution has shifted interest towards the development of				
Bioplastics,	bioplastics, which offer the dual benefits of utilizing waste and enabling of				
Biopolymers,	effective production of biodegradable materials. Polyhydroxyalkanoates				
Polynyaroxyalkanoates, Renewable carbon	(PHAs) are a class of biodegradable plastics synthesized intracellularly by various bacteria. Inexpensive carbon substrates such as PET, wastewater,				
Biocompatibility.	agricultural waste, molasses, lauric acid, whey, cellulose, plant oils, and starch				
Medical implantations.	hydrolysates can serve as excellent feedstocks for PHA production, presenting significant economic advantages. PHAs have emerged as potential useful				
	materials for different applications owing to their unique properties. The				
	biocompatibility, desired surface modifications, wide range of physical and				
	chemical properties, cellular growth support, and attachment without				
	carcinogenic effects have enabled their use as in vivo implants. Microbial				
	production of PHAs also provides the opportunity to develop PHAs with more unique monomer compositions economically through metabolic engineering approaches. At present, it is generally established that the PHA monomer				
	composition and surface modifications influence cell responses. PHA synthesis				
	by bacteria does not require the use of acatalyst (used in the synthesis of other				
	polymers), which further promotes the biocompatibility of PHA-derived polymers.				
	We have stock of more than hundred bacterial stains which are able to produce				
	Biodegradable plastic. Biodegradable plastic was extracted from bacterial				
© 2024 The Author(s). Published by Biology Education Department, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. This is an open access article under	strains, which were isolated from different environments of Pakistan. All the				
	strains were analyzed for resistant markers. Extraction of PHA was done by				
	different methods. PCR base strategy was used to amplify <i>Pha</i> biosynthesis				
	operon. These studies are benefitting from the fact that the PHA polymer				
the CC BY-NC license: https://creativecommons.org/license	presents a great variety of characteristics in terms of its biodegradability,				
s/by-nc/4.0/.	elasticity, non-toxicity, biocompatibility, ability to function as nanoparticles,				
	and possibility for tailor-made physical-chemical properties.				



E-ISSN: 3026-5428

# **Climate Change Impacts on Coastal Ecosystems**

#### Vina Listiawati, Ph.D

Department of Biology Education, Universitas Muhammadiyah Surakarta, Indonesia

	ABSTRACT
KEYWORDS:	Coastal ecosystems such as seagrass beds and coral reefs are unique habitats at
Coastal ecosystems,	the interface between terrestrial and oceanic environments. Such ecosystems
Ocean warming and acidification,	are among the most productive and dynamic ecosystems, which are highly sensitive to climate change including ocean warming and acidification.
Calcifiers and macrophytes.	Calcifiers such as corals and sea urchins are suggested to be negatively affected by ocean warming and acidification. Meanwhile macrophytes such as
Climate change impacts.	seagrasses and seaweeds are suggested to be positively affected by ocean
	warming and acidification, as the increase in temperature and $CO_2$ concentration in seawater can potentially increase their photosynthetic rates and
	productivity. Here, some recent studies are introduced to explore how ocean
	warming and acidification affect calcifiers and macrophytes by conducting
	interaction studies. Ocean warming and acidification were negatively affected
	coral growth rate and sea urchin physiology, while photosynthesis and growth
	of seagrasses and seaweeds were particularly increased under ocean warming
	and acidification, thus potentially alleviating the negative effects of ocean
© 2024 The Author(s). Published	warming and acidification. However, they do not always provide habitat refugia
by Biology Education Department,	for calcifiers. Ocean warming and acidification are also suggested to modify the
Education, Universitas	trophic interactions in coastal ecosystems. The present studies showed that
Muhammadiyah Surakarta. This is an open access article under	ocean warming and acidification have the potential to shift the balance in
the CC BY-NC license:	coastal ecosystems and assessing the organism interactions is important to give
s/by-nc/4.0/.	a better understanding of the response of coastal ecosystems to climate change.



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# Analysis of Animal Structure Practicum Module Based Virtual Lab

#### Meti Maspupah\*, Nurul Hidayah

Biology Education Study Program, Faculty of Tarbiyah and Teacher Training, UIN Sunan Gunung Djati Bandung \*Corresponding Author. E-mail address: metimaspupah@uinsgd.ac.id

ABSTRACT
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KEYWORDS:	Practicum as part of the biology learning process plays an important role in				
Understanding the	developing students' mastery of material concepts. Limitations on the				
material, fish surgery,	conditions for the practicum implementation, making the practicum module as				
<i>practicum</i> a guide for activities experiencing more advanced development. The					
	this study was to find out how students responded to the virtual lab-based				
	practicum module in animal structure courses. The device used is a student				
response questionnaire consisting of seven aspects related to the					
based practicum module. The results suggest that 53.7% of student r					
	agree, and 37% strongly agree with the attachment and use of the virtual lab in				
© 2024 The Author(s). Published by Biology Education Department.	practicum. Additional components such as interactive modules that get				
Faculty of Teacher Training and	responses of 55.6% agree and 42.6% strongly agree are further considerations				
Muhammadiyah Surakarta.	in understanding student responses to practicum activities. The use of a virtual				
This is an open access article under the CC BY-NC license:	lab as a new development of the animal structure practicum module is				
https://creativecommons.org/license s/by-nc/4 0/	considered capable of developing and increasing students' understanding.				

# 1. INTRODUCTION

Practicum as an important part of the learning process which aims to train skills and assess the extent to which students understand the concept of material, actually needs to be implemented in every course. Practical orientation as a practical activity is not only always carried out in the laboratory with various kinds of laboratory equipment as it should be done in courses that contain content such as animal structures. The animal structure course is one of the courses which in its learning is integrated with practicum as many as fourteen meetings in one semester. Animal structure practicum activities are mandatory learning that must be carried out in the framework of implementing the theory that students have learned as well as a step in fulfilling one of the achievements of animal structure practicum learning, namely students are able to know, understand, analyze, distinguish and compare microscopically animal tissues.

Referring to the achievements of the animal structure practicum course as the implementation of theory, especially on the topic of tissue in animals, has its own obstacles in its application. Animal tissue as one of the topics of the animal structure practicum taught in semester three is an obstacle for students, especially when the practicum is carried out online during the Covid-19 pandemic. This is because the Covid-19 pandemic has changed all the arrangements for learning activities, especially the move from offline to online which was carried out for quite a long time for about two years ((Ni Luh Putu Ananda Saraswati, 2020). This is a different condition and this is the first time this has happened requires practicum activities to continue in the midst of completely restricted conditions, including practicum in the laboratory which is temporarily suspended. This is a challenge for educators to prepare all learning tools, especially practicum modules that are in accordance with conditions and needs (Kana Puspita, 2021) The use of a virtual lab in animal structure practicum during a pandemic is a new modification in the animal structure module, which is presented with two options for offline and online practicum methods

Virtual lab as a new practicum method that is chosen as a modification of the animal structure practicum module, can be accessed via the website from cellphones or laptops. Animal tissue material is considered quite difficult material, based on the values of student practicum reports. This is also based on animal tissue practicum which is quite abstract when students observe microscopic objects and requires sufficient accuracy and mastery of material concepts because the material concepts are abstract (Ummul Barokahhuda, 2021), so valid literature is needed such as histology atlases to validate the observation results. The use of the virtual lab practicum model is expected to be one of the opening ways that the learning process can be carried out anywhere in any situation and condition. One of them is practical activities that do not always have to be carried out in the lab. Thus, with these conditions, research was carried out on how students responded to the use of virtual labs in animal structure practicums carried out during a pandemic.

# 2. METHODS

The method used in the study entitled analysis of animal structure modules based on virtual labs uses a descriptive qualitative approach. Data was collected using a response questionnaire instrument made using a Google form which consisted of seven aspects of the statement with four possible answers in the form of strongly disagree, agree and strongly agree. The data population for this study were all Biology Education students at UIN Sunan Gunung Djati Bandung Class of 2020, totaling 3 classes. The samples taken were 54 students from a combination of the 3 classes, which are expected to be an illustration of the answers from the Biology Education student population Batch 2020 at UIN Sunan Gunung Djati Bandung.

The purpose of this study is to find out how students respond to the virtual lab-based practicum module. Student response data was analyzed descriptively by interpreting the percentage of each aspect of the statement in the response questionnaire.

# 3. RESULTS AND DISCUSSION

In this study, the results in the form of percentages for each aspect or statement indicators related to the animal structure module and virtual lab were analyzed descriptively one by one to be translated clearly and easily so that conclusions can be obtained that are easily understood by everyone. Eight aspects or statement indicators related to the virtual lab-based animal structure practicum module are presented as follows:

No	PRACTICUM MODULE INDICATORS	STUDENT RESPONSE		NSE	
		STS	TS	S	SS
1	Attachment to the Virtual Lab link in the module helps in the process of understanding the material	1.9 %	7.4 %	53.7 %	37%
2	Representative images with the contents of the manuscript (module)		5.6 %	57.4 %	37 %
3	The module is interactive with supporting attachments such as questions and learning support links		1.9 %	55.6 %	42.6 %
4	The module contains learning objectives			46.3 %	53.7 %
5	Load learning material packaged in a specific form/explanation		1.9 %	53.7 %	44.4 %
6	The module contains practice questions that can support students' understanding		1.9 %	50 %	48.1 %
7	The practical steps are clear and easy to understand		3.7 %	50 %	46.3 %

Tabel 1. Results of Student Responses to the Animal Structure Practicum	Module
Based Virtual Lab	

Information :

STS : Strongly Disagree

S : Agreed

TS : Disagree

#### SS : Totally Agree

In Table 1. There are seven statement indicators, where in indicator 1. the addition of a virtual lab link attachment in the form of a website in the modified animal structure module obtains a response of 53.7% of students agreeing, and 37% strongly agree that the virtual lab helps students in carrying out practicum animal tissue. In line with research that has been conducted by (Hermansyah, 2017) that the use of virtual labs helps in increasing students' understanding of concepts, while research that has been conducted by (Egidius Dewa, 2020) that virtual labs can increase interest in learning. There were only 4 students who disagreed, due to their lack of understanding in using the virtual lab website, so what they were looking for did not find the answer. The following is a virtual lab display in animal tissue practicum:

#### Langkah kerja

- Siapkan alat dan bahan
- 2. Ambil preparat yang telah disediakan
- 3. Ambil mikroskop dengan hati hati
- Letakan preparat dibawah mikroskop, atur pembesaran dimulai dari pembesaran terendah
- Amati preparat kemudian dokumentasikan gambar preparatnya. Pengamatan preparat dapat dibantu dengan penggunaan atlas histology
- 6. Setelah selesai, simpan alat bahan kembali ke tempatnya

#### Langkah Kerja Praktikum Virtual

1. Akses laboratorium virtual pada alamat web berikut ini

https://biologycorner.com/anatomy/histology/

- 2. Amati gambar setiap jenis jaringan epitel yang terdapat pada slide
- 3. Gambar ulang pada lembar kerja anda.

# Figure 1. Display of the Virtual Lab Link on the Module



II. <u>Histological Staining and Cell Structure</u>. Introduction to the structure of the cell and the methods used in histology to visualize cells and tissues.

# Figure 2. Display Virtual Lab Website

Lab Outlines for Tissues				
1	Epithelium			
Ш	Glands and Secretion			
Ш	Connective Tissue			
IV	Cartilage			
V	Bone			
VI	Blood			
VII	Muscle			
VIII	Nervous System			
IX	Cardiovascular System			
X	Lymphoid Tissue			
XI	Digestive System			
XII	Integument			
XIII	Respiratory System			
XIV	Urinary System			
xv	Male Reproductive System			
XVI	Female Reproductive System			
XVII	Endocrine System			

Figure 3. Histology of Animal Tissue in Virtual Lab

Indicator 2. Obtaining responses 57.4% of students agree and 37% strongly agree with the addition and appearance of material images in the module that are in harmony with the content or materials presented, so that there are no misunderstandings between narration and illustrations. The addition of images related to this material is part of the characteristics of the module itself where the clarity of the material is accompanied by an attractive design (Dinatha, 2019). Indicator 3. The interactive module obtained a fairly high response between agreeing with 55.6% and strongly agreeing as much as 42.6% so that the module is not only one-way communication, but there is reciprocity or feedback for students. Indicator 4. Only very agree and agree responses are obtained, so that the placement of learning objectives in the module becomes a convenience for students in carrying out practicums.

Indicator 5. Obtained results of 53.7% of students agreeing with a brief and clear description of the material that is aligned with the topic of the practicum to be carried out. This is also based on the condition of students who mostly skip reading the material because it is too much and only theory. So that the provision of specific material makes students' reading interest increase slightly, connected with indicator 6. regarding the existence of practice questions in the module which obtain a percentage of 50% agree and 48.1% strongly agree, so that the existence of practice questions becomes feedback for students after carrying out practicum which makes theory and practice well integrated. Indicator 7. as the last indicator, obtained a percentage of 50% agreeing and 46.3% strongly agreeing with the practicum steps which are written neatly and clearly, so there are very few misunderstandings between one anotherConclusions

Conclusions describe the answers of hypotheses and/or research objectives or scientific findings obtained. Conclusions do not contain a repeat of the results and discussion, but rather a summary of the findings as expected in the objectives or hypotheses. If necessary, at the end of the conclusion can also be written things that will be done related to further ideas from the research.

# 4. CONCLUSIONS

Based on the research that has been done by analyzing the results of student responses to the virtual lab-based animal structure practicum module, it can be concluded that the use of virtual labs in practicums, especially animal tissue material, has a fairly high influence in helping students

understand material and abstract concepts, and students provide excellent response in the use of the virtual lab-based animal structure practicum module, based on the dominance of agree and strongly agree responses in the questionnaire results.

# 5. ACKNOWLEDGMENTS

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# Quality of Biology Laboratory SMA Muhammadiyah 1 Simo Boyolali Academic Year 2023/2024

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#### **KEYWORDS:**

Laboratory **Biology Learning** Infrastructure Laboratory Laboratory Quality Practicum

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The laboratory is one of the facilities available at school as a support for learning biology. The fulfillment of facilities and infrastructure in the biology laboratory will affect the success of learning. Biology learning does not only include theory but also direct practical activities. Therefore, to support the success of practicum-based learning, a biology laboratory must have adequate facilities and infrastructure by the minimum standards listed in Permendiknas Number 24 of 2007. This research aims to determine the quality of the biology laboratory at SMA Muhammadiyah 1 Simo Boyolali in the academic year 2023/2024. This research uses a qualitative descriptive method with data collection techniques in the form of observation and interviews. The results of this study indicate that the quality of the laboratory at SMA Muhammadiyah 1 Simo Boyolali has good criteria with a percentage of 78,31%, where these results are still lacking in meeting the minimum standards listed in Permendiknas Number 24 of 2007. This research concludes that the quality of the biology laboratory is classified as good even though it has not met the minimum standards by Permendiknas Number 24 of 2007.

ABSTRACT

# 1. INTRODUCTION

The laboratory is a place where students can gain skills in demonstration, experimentation, and science development. In addition, laboratories have a very important role in science learning at school because, with the activities in the laboratory, students are expected to foster interest in laboratory activities (Munarti and Sutjihati 2018). Laboratories are very important for the advancement of teaching and the development of an increasingly complex curriculum. Laboratories can also affect the development of schools, colleges, and Islamic boarding schools. Each unit of educational institution must have a laboratory room to improve the quality of education. Therefore, an educational institution must optimize the use of laboratories to achieve the teaching and curriculum objectives (Harahap et al. 2017). Laboratories in schools help students learn scientific attitudes and knowledge and test theories with facts (Balqis et al. 2018).

A laboratory is a place where students can learn and develop process skills in practical activities directly in the laboratory through experiments and simple research activities (Laeli and Maryani 2020). However, the ability of students to utilize laboratory space is still considered lacking. To anticipate this, the biology laboratory must always be in a ready-to-use state, the infrastructure and media in it must be in good condition, and various administrations must work well (Nulngafan and Khoiri 2020). Therefore, the laboratory is an important component of classroom learning, especially biology. Learners must be present in the laboratory to get a direct understanding of how the theory they have previously learned. After the theory is obtained,

students can take part in practical activities in the laboratory as a support for the biology learning process (Aritawarni et al. 2023).

Biology is a science that continues to develop and can be understood through scientific processes in particular activities. This means that learning biology is not only mastering a collection of data in the form of natural diversity and living things but also a discovery process (Purnama et al. 2017). The skills to know and understand various phenomena that occur in nature are important in learning biology. So practical activities or experiments are an inseparable part of biology. Therefore, in improving students' ability to explore the surrounding nature naturally, biology learning must be emphasized on direct experience, because biology learning becomes less optimal if there is no real experience from students through practical activities (Mastika et al. 2014).

Biology learning includes practical activities both inside and outside the laboratory. The practicum must also be equipped with biological facilities and infrastructure that meet the minimum laboratory standards set out in Permendiknas Number 24 of 2007 (Siregar et al. 2023). According to Permendiknas Number 24 of 2007 which explains that the biology laboratory room functions as a place for the implementation of biological assessment activities both in theory and in practice which requires special and adequate equipment. The biology laboratory room must be able to meet at least one class of study, besides that it must have good lighting when carrying out object observations. The types of infrastructure that must be contained in the biology laboratory room are; (1) Building / Laboratory Space (2) Furniture, (3) Educational Equipment (4) Tools and Materials, (5) Educational Media, (6) Consumables, and (7) Other Equipment.

To ensure that biology laboratories in schools remain available and their facilities and infrastructure can be used for a long time, laboratories must be assisted with proper care and maintenance. Given that the laboratory is one of the learning methods where students can apply the theory or knowledge they learn directly through practicum, demonstration, and skills (Rifa'i et al. 2021). Therefore, it is important for an educational institution that has a laboratory space, especially biology, to maintain the quality of the laboratory so that it can be used as a learning support.

SMA Muhammadiyah 1 Simo is one of the oldest private schools in the Boyolali Regency. The facilities and infrastructure in the school are good enough to support learning including the biology laboratory. However, the biology laboratory has facilities and infrastructure that do not meet the minimum laboratory standards set out in Permendiknas Number 24 of 2007 such as the lack of practicum materials, tools that are not maintained and neatly arranged, and the absence of a laboratory assistant. This is the same as the results of Munarti and Sutjihati's (2018) research on five high schools (SMA PGRI 4, SMAN 7, SMAN 4, SMA PGRI, and SMA Al-Nur), which found that the facilities and infrastructure in these schools when observed in total still cannot be said to meet the standards as stated in the Permendiknas Number 24 of 2007. Then another study found that the biology laboratories of the four secondary schools in Pontianak City had standard B because five criteria were met, namely at least one rumble was accommodated in one room, adequate facilities, well-maintained and clean conditions, but SMAN 05 did not meet the standard room area, but in laboratory utilization, SMAN 05 more often carried out practicum than other high schools, while two high schools were in category E because they did not meet the existing criteria (Adilah et al. 2021).

Based on the description above regarding the importance of the completeness of facilities and infrastructure contained in the laboratory, the researchers are interested in examining the quality of the laboratory as a support for biology learning. This study aims to determine the quality of the biology laboratory of SMA Muhammadiyah 1 Simo in terms of laboratory facilities and infrastructure by the minimum standards set out in Permendiknas Number 24 of 2007.

# 2. MATERIALS AND METHODS

# 2.1. Types of Research

This research uses descriptive qualitative research where the method is a research method that describes the state of the object under study by the situation and conditions in the field and the data from this research is not in the form of numbers but in the form of value categorization (Sugiyono 2017).

# 2.2. Place and Time of Research

This research was conducted in the biology laboratory of SMA Muhammadiyah 1 Simo Boyolali at J1. Madu No.152, Ngreni, Simo, Simo, Boyolali, Central Java 57377. This research was conducted from January to May 2024.

# 2.3. Population, Sample, and Sampling

The population in this study were all those involved in using the biology laboratory of SMA Muhammadiyah 1 Simo. Samples taken in this study were biology laboratories, laboratory heads, and biology teachers. This study used a sampling technique, namely purposive sampling because this technique is by the research, namely by selecting samples based on groups or areas through certain considerations and criteria and is believed to represent all that will be analyzed.

# 2.4. Data and Data Source

# **Table 1.** Data and Data Sources

Data	Source	Methode	Instrument	
Laboratory room	Biology laboratory	Observation	Observation sheet	
Completeness facilities and infrastructure according to Permendiknas Number 24 Tahun 2007	Biology laboratory	Observation	Observation sheet	

# 2.5. Data Collection Techniques

# 2.5.1. Observation

Observations were made directly to the research location to collect data in the field, Observation data is in the form of an observation sheet instrument that contains a checklist (v) to obtain data from observations regarding the quality of the laboratory including the laboratory room and the completeness of laboratory infrastructure facilities that refer to Permendiknas Number 24 of 2007.

# 2.5.2. Interview

Interviews were used to complement laboratory data regarding the quality of facilities and infrastructure in supporting biology learning.

# 2.6. Research Procedure

This research was conducted through three stages, starting from the preparation stage to determine the sample, compile research instruments in the form of observation sheets of laboratory space and infrastructure, interview lists, and make a permit for research. The second stage is the implementation of research to collect the required data. The last stage is the analysis of research data.

# 2.7. Data Analysis Techniques

This research data analysis technique uses qualitative descriptive techniques with the formula:

$$Score = \frac{Skor \ perolehan}{Skor \ maksimal} \ge 100\%$$

Then the value is categorized as follows:

81 - 100 %: Excellent61 - 80 %: Good41 - 60 %: Enough21 - 40 %: Less0 - 20 %: Very Lacking(Agustina et al. 2019).

# 3. RESULTS AND DISCUSSION

Laboratories play an important role in curriculum development and more complex teaching. All biology laboratories must have facilities or tools that are by the standard infrastructure according to Permendiknas Number 24 of 2007. These tools can be used in classroom demonstrations, lab work, and observations in the school environment (Romadhoni and Saifuddin 2021). The quality of the laboratory itself is very important to support the practicum-based biology learning process. By optimizing the right facilities and infrastructure, students' understanding of concepts and learning outcomes will be more optimal. This will also affect the result of the learning objectives. In addition. The learning process using laboratories and practicum can improve and foster students' scientific attitudes (Sinangkling et al. 2022).

Variables	Aspects	Percentage (%)	Description
Biology laboratory facilities and infrastructure	Laboratory Room	87.5	Excellent
	Furniture	50	Enough
	Education Equipment	88.52	Excellent
	Education Media	100	Excellent
	Consumables	53.85	Enough
	Other Equipment	90	Excellent
Average		78.31%	Good

## **Table 2. Observation Result Data**

Based on Table 2. it is known that the quality of the biology laboratory of SMA Muhammadiyah 1 Simo has a good quality category which obtained an average of 78.31%, which means that the laboratory has not fulfilled 100% of the minimum standards listed in Permendiknas Number 24 of 2007, but this laboratory is sufficient to support biology learning activities. Biology laboratories must have adequate facilities so that practice-based learning such as practicum activities can run optimally (Lubis and Rizkika 2017). From the observation data that has been done, the biology laboratory room of SMA Muhammadiyah 1 Simo has very good criteria with a percentage of 87.5%. The biology laboratory is separate from other laboratory rooms, namely physics and chemistry laboratories, but the biology laboratory room at SMA Muhammadiyah 1 Simo is used as a classroom. According to the results of interviews with the head of the laboratory and biology teachers, due to limited space and land, this laboratory is used as a classroom XI-2. Therefore, this makes it difficult for teachers to do conditioning if there are classes that have practicum schedules. So the solution taken is to do practicum in each of these classes that have a practicum schedule. This is in line with the research of Lestari et al. (2017) that one of the obstacles experienced by teachers in several schools is that biology laboratories are converted into classrooms because of an imbalance between the number of students and available classrooms.

Laboratory environmental conditions also need to be considered, among others, with an adequate air circulation and ventilation system, and a room temperature that is maintained constant and safe from interference (Maharani and Sasi 2019). The biology laboratory room of SMA Muhammadiyah 1 Simo is equipped with good lighting and circulation so that students can read books and observe experimental objects. The storage room for tools and materials is located separately from the experimental room and already has a minimum standard area of 18 m<sup>2</sup>, but the storage room has a less clean and untidy condition. The laboratory room also has clean water located outside the laboratory room.

The condition of the facilities in the form of furniture in the biology laboratory of SMA Muhammadiyah 1 Simo has a percentage of 50% with enough category. The category shows that the furniture facilities of the laboratory have not met the minimum standards of Permendiknas Number 24 of 2007. Of the 7 aspects observed, only chairs, material cabinets, and tool cabinets are by the standards. The learner's table is a table that is generally located in the classroom, which is only for 2 students because it adapts to the condition of the room used as a classroom. This biology laboratory room does not have a preparation table, demonstration table, or sink either in the laboratory does not have furniture facilities that meet the standards, it will hurt the effectiveness of practicum-based laboratory learning (Sinangkling et al. 2022).

Furthermore, for the aspect of laboratory facilities in the form of educational equipment, the observation data obtained an average percentage of 88.52% with an excellent category which can be interpreted that the aspect of educational equipment is good in achieving the minimum standards of Permendiknas Number 24 of 2007. This aspect of educational equipment is divided into 2 parts, namely teaching aids and experimental tools and materials. Teaching aids used in practicum are used to improve students' psychomotor abilities through tools that can be seen, held,

assembled, or changed in shape (Dewi et al. 2021). The teaching aids have 25 aspects that are observed, among these 25 aspects, 4 aspects do not comply with the standard, namely mitosis preparations, meiosis preparations, plant anatomy preparations, and animal anatomy preparations. The components of the teaching aids are not by the minimum standards contained in Permendiknas Number 24 of 2007, this is because the preparations of mitosis, meiosis, plant anatomy, and animal anatomy should be 6 pieces/lab, but this laboratory only have 3 pieces/lab, besides that the condition of the preparations is also poorly maintained.

Another part of educational equipment facilities is experimental tools and materials. According to Permendiknas Number 24 of 2007, there are 36 aspects observed in the category of experimental tools and materials. Of these 36 aspects, 13 tools and materials are lacking in meeting the minimum standards, including monocular microscopes, binocular stereo microscopes, preparation boxes, stopwatches, three legs, stative rod devices, universal clamps, bossheads, gauze, thermometers, respirometers, squares, and experimental instructions. The tools and materials that have been mentioned are still lacking in meeting the minimum standards listed in Permendiknas Number 24 of 2007 due to the number that does not meet the standards and the lack of maintenance carried out by laboratory staff. This is also because the biology laboratory at SMA Muhammadiyah 1 Simo does not have a laboratory assistant. The results of interviews with the head of the laboratory and biology teachers stated that laboratory equipment and materials also do not have codes and specifications for their use, but only names. In addition, all preparations and implementation in practicum learning are carried out by biology subject teachers themselves because they do not yet have a laboratory assistant. Munarti and Sutjihati (2018), state that the management of laboratory equipment and materials is very important because it involves work safety in the laboratory. It is better if the tools and materials that will be used during the practicum are prepared in advance by the laboratory assistant. The absence of laboratory assistants is a challenge for teachers, because before practicum teachers must prepare everything themselves, including cleaning and checking. Laboratory assistants are very important in optimizing practicum activities, starting from the preparation of tools and materials to the evaluation after practicum activities are carried out.

Learning media in education are tools, methodologies, and techniques used as intermediaries in communication between teachers and students. Used to improve communication and interaction between teachers and students during the learning process at school (Umar 2014). The condition of educational media has a percentage of 100% with an excellent category. It can be interpreted that the educational media in the biology laboratory has met the minimum standards listed in Permendiknas Number 24 of 2007 having 1 media in the form of a blackboard with a minimum size of 90 cm x 200 cm. The blackboard already has a position that allows all students to see it.

Table 2 shows the observation results of consumables that obtained a enough category with a percentage of 53.85%. This shows that the quality of consumables in the biology laboratory of SMA Muhammadiyah I Simo still does not meet the minimum standards by Permendiknas Number 24 of 2007. There are 13 aspects of consumables in the form of 12 chemicals and filter paper where these materials are materials that run out quickly and are not durable. Of the 13 materials, there are only 4 materials that meet the standards, namely glucose, universal indicators, KOH, and vaseline. In this aspect, 5 materials are not available in the laboratory including acetocarmine, eosin, iodine, MnSO4, and NaOH. Meanwhile, other materials such as acetocarmine, HCL, ethanol, and filter paper do not meet the standards because the amount does not meet the minimum standards of Permendiknas Number 24 of 2007. In addition, some materials are not maintained and are not suitable for use. The results of the interview also mentioned that the materials in the laboratory of SMA Muhammadiyah 1 Simo are still lacking, so practicum activities cannot run smoothly. Therefore, in practicum-based learning only utilize materials around and within reach.

The last aspect observed was other equipment, where this aspect obtained a percentage of 90% with an excellent category. This aspect of other equipment includes 5 components, namely contact boxes, fire extinguishers, first aid equipment, trash cans, and wall clocks. Of the 5 components,

only the contact box does not meet the standards, this is because the number does not meet the minimum standards of Permendiknas Number 24 of 2007, namely 1 piece on each student table, 2 pieces on the demo table, and 2 pieces in the preparation room. Generally, every laboratory in the school already has electricity that supports it, but considering the importance of a laboratory to meet the minimum standards listed in Permendiknas Number 24 of 2007, it is hoped that the SMA Muhammadiyah 1 Simo laboratory can improve the lack of the required number of ratios. When carrying out activities in the laboratory to handle work accidents, therefore it is important to have first aid equipment in the laboratory to handle work accidents in the laboratory rooms must have work safety equipment, especially first aid kits because they are very important for laboratory work procedures and can be a place of first aid when technical errors or accidents occur.

Interviews were conducted with the head of the laboratory and biology teachers. There were ten questions covering the state of the biology laboratory space, storage of practicum tools and materials, laboratory management, and constraints in managing and preparing the laboratory for practicum activities. From the interview results, it was found that the biology laboratory is separated from other laboratories such as physics and chemistry laboratories. However, besides being used for practicum activities, this laboratory is used as a classroom and sometimes for meeting rooms due to limited space and location. The tools and materials in this laboratory have not supported 100%, but approximately 80% have supported practicum activities by the material to be carried out for practicum activities. This is by the research results obtained, namely the facilities and infrastructure in this laboratory 78.31% have supported activities in the laboratory. Laboratory tools and materials are arranged according to their groups to make it easier when they are to be used, This is in line with the opinion of Romadhoni and Saifuddin (2021) that to reduce the risk of damage, laboratory materials require special treatment based on their properties and characteristics. But there are no codes and specifications for how to use them. The head of the laboratory and biology teacher said that the practicum was carried out at least once a month according to the material that had been delivered. An inventory of tools and materials is carried out at the beginning of the semester, which is approximately twice a year. If there is damage to laboratory equipment then the manager will make a table or list of tools that are damaged, but if the damage to the tool is caused by students, then students must repace the tool. In preparing the biology laboratory for practicum activities, biology teachers first check whether the tools and materials whether adequate to carry out practicum by the material, then conduct conditioning of students. In managing the laboratory, there are obstacle faced by the head of the laboratory and biology teachers including laboratory space which is used as a classroom so that teachers have dissiculty in conditioning the class, lack of practicum materials that are by the material so that they only use materials that are around the school environment. The results of this interview also obtained information that the biology laboratory at SMA Muhammadiyah 1 Simo does not yet have a laboratory assistant so all activities in the laboratory are carried out by each biology teacher.

# 4. CONCLUSIONS

Based on the research that has been done, it can be concluded that the quality of the biology laboratory of SMA Muhammadiyah 1 Simo Boyolali in the 2023/2024 academic year has a good category with an average percentage of all aspects observed, namely 78,31%. This shows that the laboratory is good at supporting practicum activities even though it has not met the 100% standard stated in Permendiknas Number 24 of 2007. However, aspects of laboratory space, educational equipment, educational media, and other equipment already have an excellent category in fulfilling the quality of the laboratory to support practicum-based learning activities.

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# **Turtle Nesting Habitat Conditions on Pandan Island, Pieh Island Conservation Area, West Sumatera**

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# ABSTRACT

**KEYWORDS:** Green Sea Turtle Hawksbill Turtle Nesting habitat Olive Ridley Turtle Pieh Island Conservation Area

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Potential as a suitable habitat for turtles to settle and breed. The objective of this work is to assess the state of turtle nesting sites and evaluate the findings from turtle monitoring efforts in the Pieh Island Conservation Area. Observations were conducted directly from September 25th to September 30th, 2023. A quantitative method was employed. The materials that were utilized consist of green sea turtles (Chelonia mydas), hawksbill turtles (Eretmochelys imbricata), and olive ridley turtles (Lepidochelys olivaceae). The nesting habitat data encompasses information on the proximity of vegetation to the tidal limit, beach slope temperature and humidity levels, as well as the depth and quantity of eggs. Four stations serve as research sites. The distance of vegetation from the nests ranged from 7.6m to 13.4m, while the beach slope on Pandan Island varied between 3.62° and 6.19°. The temperature inside the nests ranged from 28°C to 29.1°C, with a humidity level of 77% to 88%. The nests had a depth of 50cm to 60cm. The results of this study show that Pandan Island Beach is a highly suitable site for turtle nesting.

# 1. INTRODUCTION

Turtles, as one of the endangered animal species, play an important role in aquatic ecosystems, and have high ecological and conservation value. West Sumatra Province has long been a landing site for turtles that roam Indonesian waters. Factors such as a long coastline, a marine environment rich in biodiversity, and a suitable climate make this region ideal for turtles to lay eggs and breed. These turtles often choose beaches in West Sumatra as nesting places because of their innate instinct to return to their birthplace (Damanhuri, 2007). All types of turtles, including those that live in Indonesian waters, have unique nesting areas. These biophysical characteristics can be viewed from sediment parameters, beach slope, tides, temperature and surrounding vegetation (Isdianto et al., 2022).

The Pieh Island Conservation Area is one of the turtle landing sites in West Sumatra. The beach in the Pieh Island Conservation Area has great potential as a landing and nesting place for turtles. According to Wicaksono et al (2022), The characteristics of a long and sandy coastline, as well as a marine environment rich in food resources are the main factors that make the beaches in this area ideal for nesting turtles. The Pieh Island Conservation Area consists of five small, uninhabited islands, namely Pieh Island, Air Island, Bando Island, Toran Island and Pandan Island. Currently, turtle conservation activities carried out on Bando Island and Pandan Island have succeeded in saving 146,477 turtle eggs, releasing 128,184 hatchlings, and maintaining a hatching success rate of 80.11% (LKKPN Pekanbaru, 2022). The types of turtles found on Pandan Island in order of dominance are the green sea turtle (*Chelonia mydas*), the hawksbill turtle (*Eretmochelys imbricata*), and the olive ridley turtle (*Lepidochelys olivacea*).

Until now, the threat to turtle conservation is still quite high, both due to natural and anthropogenic factors. Natural factors include coastal erosion, climate change which can damage turtle habitat and nesting locations (Hawkes, Broderick, Godfrey, & Godley, 2009), and the threat of predators, while human activity factors (Suryawan & Tehupeiory, 2023) including marine pollution, the accidental capture of turtles by fishing gear (by-catch), and the use of materials originating from turtles such as meat for traditional purposes (Tambunan, Wiryono, & Senoaji, 2021), eggs, and carapace (Firliansyah, Kusrini, & Sunkar, 2017; Juliono & Ridhwan, 2017).

The threat of turtle extinction can be overcome by managing turtles through several actions, namely protecting turtle habitats, population monitoring to identify trends and effectiveness of conservation efforts, education and public awareness about the importance of turtle protection (Jemarut, Webliana, & Sari, 2021; Juliono & Ridhwan, 2017), law enforcement against illegal practices, such as egg catching and turtle hunting (Tarigan, Syarifuddin, & Wati, 2020), controlling accidental catch by fishing equipment, and turtle egg conservation programs, including turtle breeding (Fitri, Rauzana, Yasmin, Suwarno, & Dharma, 2023; Komarudin & Fahrunnisa, 2023; Zikri, Putera, & Kusdarini, 2023). The aim of turtle management is to minimize the negative impact of threats to turtles and ensure their survival. Turtle management through data collection is very necessary to preserve the existence of turtles and minimize the threat of turtle extinction, especially in the Pieh Island Conservation Area. This research aims to record turtle data and determine the conditions of turtle nesting habitat on Pandan Island, Pieh Island Conservation Area.

# 2. MATERIALS AND METHODS

### 2.1. Location of the study

Pandan Island is included in the administrative area of Padang City, West Sumatra Province and is located at coordinates 00°51'77"-00°57'8.25" S-100°08'6'-100°08'23'E. This island has an area of 16.64 hectares with 14.8 hectares covered with vegetation and 0.8 hectares covered with sand (Siahaan, Thamrin, & Tanjung, 2020). The method used to determine the condition of turtle habitat in this research is the field observation method on 25-30 September 2023 on Pandan Island. The distribution of field data collection locations can be seen in Figure XX. The data observed and recorded includes data on the type of turtle landed; turtle morphometrics; size of turtle tracks, turtle landing location, vegetation around the nest, beach slope data, sand temperature and humidity, nest depth, number of turtle eggs, which are then processed quantitatively. According to Sugiyono (2007), this method is used in research that uses a lot of numbers starting from data collection, data interpretation, and the appearance of the results in the form of pictures, tables, graphs, or other displays.



Fig. 1. Location of Field Data Collection.

Figure 1 shows that the field data collection locations are divided into four stations based on the cardinal directions, namely east (East SK), south (South SK), west (West SK), and north (North SK). Taking data in the field around Pandan Island, four turtle nests were obtained between the east station (East SK) and the south station (South SK). Hanif, Damanhuri, & Suparno, (2022) stated that the majority of nesting turtle landings on Pandan Island were green turtles, with the highest landings being in the eastern and southern regions and having a dense coastal vegetation structure.

# 2.2. Material and tools

The material and tools used for this study are shown in table 1 below:

No	Material and Toold	Description
1.	Rollmeter	Measures the turtle's morphological size, turtle tracks,
		nest size, and the distance of the nest to the shoreline.
2.	Scale stick	Measure slope
3.	Waterpass	Measures the levelness of the meter
4.	L ruler	Measure the angles
5.	Flashlight	Lightning aid during monitoring
6.	GPS	Determines the turtle landing coordinates
7.	Plastic sample	Sample container for sand samples around nest
8.	Hygrometer	Measures humidity and temperature
9.	Camera	Documentation

# Table 1. Tool and material for the study.

# 2.3. Method

# 2.3.1. Nest temperature and humidity

Measuring the temperature and humidity of turtle nest sand using a hygrometer. Temperature and humidity measurements are carried out by placing a hygrometer pen in the sand where the eggs are found in the turtle nest. The temperature and humidity measurement results will appear on the hygrometer display.

### 2.3.2. Depth of the nest

Nest depth was measured when the eggs had been transferred into the bucket by pulling a tape measure straight from the inside to the surface of the nest. An illustration of measuring nest depth can be seen in figure 2.



Fig. 2. Measurement of Turtle Egg Nest Parameters.

# 2.3.3. Beach Slope

Beach slope measurements are made by measuring a straight line between the outermost vegetation and a scale stick on the beach. The measurement of the slope of the beach slope was carried out using the measurement stages according to Sulmartiwi, Tjahjaningsih, & Putera (2015) are as follows: First, install stakes on the highest coastline. Second, the rope is connected from the outermost vegetation to the beach when it is first wet by the waves (highest tide), so that it forms a 90° angle. Third, the angle is measured using a right-angled ruler. Next, the length of the rope is measured using a roll meter. Lastly, measure the height of the stick marked by the string. An illustration of the beach slope measurement process can be seen in figure 3 below.



Fig. 3. Beach Slope Measurement.

The results of this measurement are processed using the Mursalin (2017) elevation formula, namely:

$$\tan \tan a = \left(\frac{a}{b}\right) \text{ or Slope } (\%) = \left(\frac{a}{b}\right) x 100\%$$

a = The height of the stick up to the roll meter limit is tied at an angle of  $90^{\circ}$ .

# b = Length of roll meters

The results of processing the beach slope data are then classified into beach slope categories according to Darmawijaya (1997), namely:

Slopeness	Category
$3.00^{\circ} - 8.99^{\circ}$	Sloping
$9.00^{\circ} - 16.99^{\circ}$	Tilt
$17.00^{\circ} - 30.00^{\circ}$	Steep

# 3. RESULTS AND DISCUSSION

### *3.1. Turtles appearance*

The results of observations of turtles on Pandan Island on 25-30 September 2023 were types of green sea turtles (*Chelonia mydas*) that landed to lay eggs or landed without laying eggs.

No	Species	Fou	und Eggs		Size	Morphometric (cm)			
		Turtle	Eggs	t t	(cm)	LK	РК	FD	FB
1	Green Sea Turtle	-	✓	89	101	-	-	-	-
2	Green Sea Turtle	✓	✓	85	90	67	93	52	35
3	Green Sea Turtle	-	✓	73	99	-	-	-	-
4	Green Sea Turtle	~	✓	76	109	101	108	56	30
5	Green Sea Turtle	✓	-	-	118	-	-	-	-

During field data collection, the number of green sea turtles found on Pandan Island consisted of 4 nesting turtles and 1 non-laying turtle. The number of eggs found from each turtle egg nest ranged from 73-89, with trace sizes ranging from 90-118 cm. Meanwhile, the morphometrics of green turtles can be measured in two out of five turtle landings.

# 3.1.1. Nest temperature and humidity

Measurements of temperature and humidity in natural nests found during field observations showed that the temperature range for natural green turtle nests was 28 °C - 29.1 °C. The nest temperature conditions found were normal and optimal according to the statement by Harnino et al (2021) that the sand temperature range required for successful hatching of turtle eggs is 25-35°C with an optimal temperature of 29°C. Temperature affects the development of turtle eggs and prevents egg rot. Nest temperature conditions are also a parameter that influences the sex determination of turtle hatchling (Herrera et al., 2020). More male hatchlings are produced at incubation temperatures below 29 °C, whereas more female hatchlings are produced above 29 °C (Santoso, Hestirianoto, & Jaya, 2021). This shows that maintaining the temperature in the optimal temperature range can increase the success rate of egg hatching while modifying the nest temperature, which can be used as an alternative for controlled hatching of eggs to determine the sex ratio of hatchlings.

The humidity conditions of natural turtle nests found during observations on Pandan Island ranged between 77% - 84%, indicating normal conditions based on research by Anshary, Setyawati, & Yanti, (2014), which states that sand moisture is in the normal range of 69-95%. The

results of humidity observations on Pandan Island are slightly above the optimum humidity threshold for turtle hatching, namely 64% - 75% (Akbar, Luthfi, & Barmawi, 2020). Humidity can be caused by the change of seasons, which affects the intensity of rainfall. Nest humidity is an environmental parameter that influences the success of turtle egg hatching (Benni, Adi, & Kurniawan, 2017), which depends on the water content of the sand substrate, nest depth, rainfall, and the distance and slope of the nest to the beach (Sulmartiwi et al., 2015).

# 3.1.2. Depth of the nest

The depth of the nest, measured from 4 findings of turtle nesting nests, ranged from 50-60 cm, the optimal depth for eggs to hatch optimally (Sulmartiwi et al., 2015). Several factors, including the number of eggs and turtle morphology, can influence the difference in nest size. The number of eggs the turtle will lay in the nest will determine how deep and wide the nest will be. Apart from that, the length of the hind legs (flippers) also determines the size of the nest (Winarto & Azahra, 2022).

# 3.1.3. Beach Slope

The results of measuring the slope of the beach showed that the slope of the Pandan Island beach was around 3.63% - 6.19%, which was included in the gentle category, based on the classification of slope values by Darmawijaya (1990). The characteristic slope of Pandan Island is one of the supporting factors for turtles to carry out landing and nesting activities on Pandan Island. The slope category is supported by the statement of Benni et al., (2017) that the contour of the beach and the slope of the beach are essential factors in landing sea turtles on the coast. Anshary et al. (2014) added that the steeper the beach, the more difficult it will be for turtles to see objects in front of them, so the more significant the energy required for turtles to climb to the beach.

The characteristics of the beaches, which are relatively sloping, with white sand, and covered with various vegetation on Pandan Island, are relevant to the characteristics of the nesting habitat for green and hawksbill turtles. Research by Yakardinata, Nurifdiansyah, & Damanhuri (2014) (2014) stated that beaches that are suitable for laying eggs for Green Sea Turtles (*Chelonia mydas*) are beaches that are not too sloping, have fine sand, strong currents, and have stretches of coral covered with seaweed. The results of previous measurements in June 2023 by Wilker TWP Pieh officers found that the measured slope of the Pandan Island beach from the highest tide to the outermost vegetation was 2.12 °- 3.38 °. This difference is caused by changing currents and erosion of the shoreline (abrasion), which makes the slope of the beach on each beach not the same (Siahaan et al., 2020). Differences in beach slope occur due to the accumulation of sand buildup caused by the transfer of sand masses by wind and waves at high tide (Yustina, Suwondo, Arnentis, & Hendri, 2014)

# 4. CONCLUSIONS

The environmental characteristics and parameters of the turtle nesting habitat on Pandan Island are classified as ideal and suitable based on observations of the temperature range (28-29.1  $^{\circ}$ C), humidity (77-84%), beach slope (3.63-6.19%), beach width (760 -1,343 cm). There are coconut, ketapang, and waru vegetation types in which turtles like to lay eggs. The type of landed turtle found at the end of September 2023 on Pandan Island was the green turtle (Chelonia mydas) with a footprint size of 90-118 cm and dug an egg nest 50-60 cm deep.

#### 5. ACKNOWLEDGMENTS

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# Effect of Combination of Porang Tuber Glucomannan (Amorphophallus muelleri blume) with Moringa Leaf Extract on Red Blood Cell Count in Obese White Rats (Rattus norvegicus) Strain Wistar

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# ABSTRACT As times have changed, so have lifestyles that have adverse health effects,

including obesity. Obesity is known to potentially affect the number of red

**KEYWORDS:** Glucomannan Moringa Leaf **Obesity** Red Blood Cells White Mice

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blood cells in the body. Obesity can cause iron deficiency in children and adolescents, which can develop into anemia. The aim of this study was to determine the effect of obesity on the number of red blood cells and to investigate the effect of a combination of glucomannan and moringa leaf extract on the number of red blood cells in obese white Wistar strain rats. The research method used was a true experimental design in the form of a post-test only control group with three treatment groups: the control group, treatment group P1 and treatment group P2. The results of the calculations showed differences in the number of red blood cells in each treatment. In the control treatment, there were 7.16/L. In the P1 treatment, the RBCs were 3.24 million/L. In contrast, the P2 treatment yielded a result of 5.02 million red blood cells per liter. It can be concluded that the combination of Porang Tuber Glucomannan with Moringa Leaf Extract can reduce body weight and normalize the number of red blood cells

# 1. INTRODUCTION

Globalization has had a profound impact on the lifestyle of the majority of Indonesians in the contemporary era. It is indisputable that the use of technology and the prevalence of fast food have a discernible impact on health. One of the adverse consequences of an unhealthy lifestyle is obesity. In 2018, the prevalence of obesity among individuals aged 18 years and above in Indonesia was 21.8% (Widyawati et al., 2022). Meanwhile, data from the WHO (2022) indicate that 2.5 billion adults aged 18 years and over are overweight. In a study conducted by Lucas (2022), the influence of lifestyle on the development of obesity in adolescents was investigated. The findings indicated that lifestyle plays a role in the development of obesity, particularly the use of electronic devices and exercise. Moreover, research conducted by Hamalding (2019) also indicates a correlation between physical activity, fast food consumption, television viewing, and the use of electronic devices and weight gain. Individuals who are obese are at a significantly elevated risk of developing degenerative diseases such as diabetes (Selman, 2022). Additionally, a high body mass index (BMI) is strongly correlated with an increased risk of developing cardiovascular disease, liver and kidney dysfunction, and hypertension (Domingo-Ortí, 2021). Furthermore, obesity has been linked to alterations in the number of white blood cells and red blood cells in the body. It has also been observed to result in iron deficiency in children and adolescents, which can potentially lead to iron deficiency anemia (Rogozińska, 2021).

Kohsari's (2021) research on the relationship between red blood cell index, anemia, and obesityrelated diseases indicates that obese individuals tend to experience a decrease in the number of red blood cells. Syah's (2022) research, which examined the relationship between obesity and anemia in secondary school adolescents in Bekasi, revealed that 12.5% of obese adolescents suffered from anemia. In contrast, Jeong (2022) examined the relationship between body mass index (BMI) and various hematological parameters, including red blood cells. The findings of Jeong's study indicated that children with obesity and overweight exhibited a higher number of red blood cells than children with a normal weight. The objective of this study was to ascertain the impact of obesity on the number of red blood cells and to determine the effect of a combination of porang tuber glucomannan (*Amorphophallus muelleri* blume) with moringa leaf extract on the number of red blood cells in obese Wistar strain white rats.

# 2. MATERIALS AND METHODS

# 2.1. Preparation of samples

The research was initiated in September 2022 and concluded in May 2023. The research was conducted in multiple locations, including the biology education laboratory of Universitas Muhammadiyah Surakarta, CV Chemmix Pratama, the Laboratory of Animal Experiments of Universitas Sebelas Maret, and the laboratory of Universitas Setia Budi Surakarta. The research commenced with the preparation of porang flour using a combination of moringa leaves. Subsequently, the Wistar white rats were acclimated for a period of one week, after which the treatment group was fed a high-fat diet for a further two weeks. Moreover, glucomannan and moringa leaf extract preparations were manufactured and evaluated in comparison to each dose. The control group was provided with a standard diet, whereas the treatment group was given a high-fat diet with glucomannan and moringa leaf extract at 120 mg/kg and 80 mg/kg, respectively. The treatment group was administered a high-fat diet in conjunction with glucomannan and moringa leaf extract at a dosage of 50 mg/kgBB, as previously described by Nugraheni et al. (2014). This was modified by Nahar et al. (2016) to 50 mg/kgBB. The treatment was administered for a period of five weeks, after which blood samples were collected for the purpose of calculating the number of red blood cells. Blood samples were obtained via the orbital sinus, with a maximum volume of 2 cc, and were stored in a microtube devoid of anticoagulant. After a 30-minute period of standing at room temperature, the serum was separated by centrifugation at 1500 rpm for 15 minutes.

#### 2.2 Data Analizes

The methodology employed is a true experimental design arranged in a completely randomized design (CRD) in the form of a posttest-only control design. This design employs three treatment groups: a control group with normal feed, a treatment group receiving a high-fat diet supplemented with glucomannan and moringa leaf extract at a dose of 120 mg/kg body weight (BW), and a treatment group receiving a high-fat diet supplemented with glucomannan and moringa leaf extract at a dose of 50 mg/kg BW. The treatment group was administered a high-fat diet with glucomannan and moringa leaf extract at a dosage of 80 mg/kg BW, as previously described by Nugraheni et al. (2014). The control group was provided with a high-fat diet supplemented with glucomannan and moringa leaf extract at a dosage of 50 mg/kgBB, as previously described by Nahar et al. (2016, modified). The red blood cell count was determined using a hemocytometer, with the following formula:

Red Blood Cell Count:  $\frac{n}{v} x p$ 

In this formula, (n) represents the total number of cells counted, (v) is the volume of the sample multiplied by the number of boxes, and (p) is the sample dilution in the haemocytometer pipette. Subsequently, the results of the calculation were subjected to one-way analysis of variance
(ANOVA) on SPSS version 20, followed by Duncan's multiple range test (DMRT) to ascertain the level of difference between treatment groups.

## 3. RESULTS AND DISCUSSION

# 3.1. Results

The results of the calculations indicate that there are statistically significant differences in the number of red blood cells in each treatment group. In the control treatment, the number of red blood cells per liter was determined to be 7.16 million. In the P1 treatment (glucomannan dose ratio with Moringa Leaf Extract 50 mg/kgBB: 50 mg/kgBB), the number of red blood cells was found to be as high as 3.24 million/L. In contrast, the P2 treatment (comparison of glucomannan dose with Moringa Leaf Extract) yielded different results. The analysis using one-way analysis of variance (ANOVA) demonstrated a significant difference in the number of red blood cells between the 120 mg/kgBB and 80 mg/kgBB treatments. The former exhibited a higher number of red blood cells (5.02 million/L) than the latter (3.24 million/L). This discrepancy is demonstrated in Table 1.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30.819	2	15.410	1447.666	.000
Within Groups	.096	9	.011		
Total	30.915	11			

## **Table 1. ANOVA Analysis Results**

Note: P 0.05

As illustrated in the table above, the calculated significance level is 0.000, which is less than 0.05 (P < 0.05). This suggests a discrepancy in the number of red blood cells across the various treatments. Subsequently, a one-way analysis of variance was conducted, and Duncan's test was employed to ascertain the mean comparison between treatments.

	Erythrocyte Count						
Treatment group	N	Subset for alpha = 0.05					
I reatment group	IN —	1	2	3			
P1	4	3.2400					
P2	4		5.0200				
Control	4			7.1600			
Sig.		1.000	1.000	1.000			

Table 2. DMRT Test Result (Duncan N	Aultiple Range Tes	5 <b>t</b> )
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The results of the DMRT test indicate that there is a statistically significant difference in the average number of red blood cells in rats across the various treatments. The P1 treatment exhibited an average red blood cell count of 3.2 million per liter (/L), while the P2 treatment demonstrated an average of 5.02 million/L. In contrast, the control treatment exhibited an average of 7.16 million red blood cells per liter.

## 3.2 Discussion

Obesity is a disease that results from the accumulation of excessive adipose tissue (WHO, 2024). The etiology of obesity is multifactorial, encompassing dietary habits, sedentary lifestyle, psychological factors, genetic predisposition, and pharmacological agents (Sari, 2021). Obesity

can result in the onset of a number of diseases, including diabetes, insulin resistance, and hypertension. It is established that obesity affects blood cell parameters (Jeong, 2022). Moreover, obesity is linked to chronic inflammation, which can lead to iron deficiency and subsequent anemia (Al-Attar, 2020). The typical red blood cell count in rats is documented to fall within the range of 7.8 to 10.6 million per liter (O'Connell, 2015). However, the three treatments exhibited an average red blood cell count that was below the normal range for rats. This suggests that the three rats in question were anaemic, or lacked red blood cells. As stated by Harahap (2018), anemia is defined as a condition wherein the body lacks red blood cells or hemoglobin. Although all three treatments were diagnosed with anemia, the severity of the condition differed among them. The P1 treatment exhibited the most severe level of anemia, with a red blood cell count of only 3.24 million per liter of blood. The control group demonstrated the highest number of red blood cells, with a concentration of 7.16 million per liter of blood. The data suggests that increasing the dose of glucomannan and moringa extract results in a closer approximation of the normal red blood cell count. Moreover, obesity has been demonstrated to exert a considerable influence on the number of red blood cells. This conclusion was also reached by Abro et al. (2020), who identified a significant correlation between obesity and the number of red blood cells.

It is well documented that the prevalence of obesity is increasing. It is therefore imperative that alternative products be developed to help prevent further increases in obesity. In this context, porang tubers (*Amorphophallus muelleri* Blume) may prove a valuable addition to the diet, as they have been demonstrated to possess anti-diabetic properties. Porang (*Amorphophallus muelleri* Blume) is a member of the Araceae family, which is rich in glucomannan fiber, with concentrations reaching up to 70% (Aini et al., 2020). The glucomannan present in porang has been demonstrated to possess the capacity to reduce cholesterol and glucose levels in the blood, facilitate weight loss, influence intestinal activity, and exert an impact on the immune system (Nissa and Majid, 2016). Moreover, porang tubers are a rich source of carbohydrates, possess low caloric value, and contain a substantial amount of fiber, rendering them a suitable dietary option for individuals with obesity or diabetes (Widiyawati et al., 2023).

In addition to porang tubers, moringa leaves have been demonstrated to possess anti-obesity properties when employed as an alternative food ingredient. Moringa leaves (Moringa oleifera) are a highly nutritious plant and are often referred to as the "miracle tree" due to the fact that all parts of its body can be utilized. Moringa plants contain a variety of compounds that have been demonstrated to possess antitumor, antiepileptic, anti-inflammatory, cholesterol-lowering, antioxidant, and antidiabetic properties (Jusnita, 2019). It is evident that Moringa plants have the potential to act as anti-obesity ingredients. The results of the study conducted by Mewally et al. (2017) indicated that an ethanol extract of moringa plants was capable of significantly reducing the body weight of mice. In a study conducted by Alkhudhayri et al. (2021), the anti-obesity effect of moringa leaf extract (Moringa peregrina) was tested on rats induced by a high-fat diet. The results indicated an anti-obesity effect, although not statistically significant. Additionally, the antiobesity properties of moringa plants have been demonstrated to normalize the number of red blood cells affected by obesity. This finding is consistent with the results reported by Coulibaly et al. (2020), who examined the impact of moringa leaf powder (Moringa oleifera) on anaemic rats induced by obesity. The results demonstrated that the number of red blood cells returned to normal following the administration of moringa leaf powder.

The combination of porang tuber glucomannan with moringa leaf extract has been demonstrated to be highly efficacious in promoting weight loss, thereby preventing obesity and normalizing the number of red blood cells. These findings align with those of Wati and Setyaningsih (2024) and Anggitasari and Setyaningsih (2023), which indicate that the combination of moringa leaf extract and porang tuber glucomannan is highly efficacious in promoting weight loss and regulating the number of red blood cells.

# 4. CONCLUSIONS

The findings of the study suggest that the combination of porang tuber glucomannan with moringa leaf extract may result in a reduction in body weight and the normalization of red blood cell count. Moreover, the study has demonstrated that obesity can influence the number of red blood cells. The P2 treatment, which combines glucomannan (*Amorphophallus muelleri* Blume) with Moringa leaf extract (*Moringa oleifera*) at a ratio of 120 mg/kgBB:80 mg/kgBB, has been identified as the most effective in normalizing the number of red blood cells.

# 5. ACKNOWLEDGMENTS

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# In Cilico Study and Genetic Variation of Genus Rhododendron using DNA Barcode trnL-trnF Intergenic Spacer

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#### ABSTRACT **KEYWORDS:** Rhododendron is a flowering plant which is the largest genus in the Ericaceae DNA barcoding, family. Around 1,157 Rhododendron species have been assessed using the in cilico, IUCN Red List criteria, and 316 are categorized as endangered species. Apart Rhododendron, from maintaining species population numbers, conservation activities also need trnL-trnF to pay attention to genetic aspects through DNA barcoding techniques. This research uses molecular in-silico methods using sequence data from NCBI's GenBank. This research aims to carry out an inventory of Rhododendron types based on genetic aspects, analyzing genetic variations and kinship relationships in the Rhododendron Genus. The resulting phylogenetic tree is divided into three clades in the *Rhododendron* group and one outgroup. The phylogenetic © 2024 The Author(s). Published tree results show that the trnL-trnF DNA barcode can differentiate and identify by Biology Education Department, Faculty of Teacher Training and up to species level in the genus Rhododendron. The trnL-trnF DNA barcode Education, Universitas Muhammadiyah Surakarta. region of the Rhododendron genus is mostly conserved because it only has eight This is an open access article under the CC BY-NC license: genetic variations, namely T71C, G225C, T245C, T229A, T485C, A491G, https://creativecommons.org/license T705C, and A783G. The trnL-F gene is an informative chloroplast and can s/bv-nc/4.0/. show the relationship between types

# 1. INTRODUCTION

*Rhododendron* is a flowering plant, the largest genus in the Ericaceae family. The word Rhododendron comes from two syllables originating from Greek, namely Rhodos (rose) and dendron (tree), which means rose tree (Mellieve, 2002; Masnawati et al., 2017). The Rhododendron genus is a flowering plant of which more than 1,000 species have been recorded with an average height of 1.5 m, a habitus ranging from shrubs to small trees, terrestrial or epiphytic (Sleumer, 1966). The Rhododendron genus is often found in mountain forests at altitudes up to 4,000 m above sea level, but some are also found in lowland and mangrove environments. These plants can grow on grassy ground and rocks. The Rhododendron genus can grow well in acidic soil with a pH range of 4.5-5.5 (Bowers, 1960; Sekar & Srivastava 2010; Putri, 2011). Based on its economic and ecological aspects, the *Rhododendron* genus has crucial aesthetic value, namely its flower morphology, which is unique, beautiful, and attractive (Goetsch et al., 2005). In the Papua region, the presence of *Rhododendron* species is utilized in ecotourism activities, which can increase the use value of this genus for the economy of the surrounding community (Beljai et al., 2016; Putri & Warseno, 2020). Around 1,157 *Rhododendron* species have been assessed using the IUCN Red List criteria, and 316 are categorized as endangered species (Gibbs et al., 2011; Putri & Warseno, 2020). In the Malesia region, several *Rhododendron* species are potentially threatened with extinction due to habitat destruction. Differences in pollinators and flowering time are also contributing factors causing *Rhododendron* to become threatened (Singh & Gurung, 2009). The availability of very few seeds/saplings in nature has also caused many *Rhododendrons* to be threatened (Semwal & Purohit 1980).

There is a tendency for the population of the genus *Rhododendron* to be threatened globally, making conservation efforts for this plant important. The initial steps that can be taken to preserve Rhododendrons are exploration, characterization based on morphology and molecular, and grouping according to character (Hamid et al., 2017). Besides maintaining species population numbers, conservation activities must consider genetic aspects (Chika et al., 2024). Conservation activities provide various information, such as bioecology and taxonomy, including genetic information (Nurvanto & Solihin, 2006). This information is necessary to determine appropriate conservation methods (Saleky & Dailami, 2021). Molecular identification is a step in plant breeding efforts, conservation biology, and other aspects of plant science (Hanifa et al., 2021). DNA barcoding is a technique that uses one or several DNA regions with short sequences to identify a species (Hebert et al., 2003). DNA barcodes that are very often used for DNA barcoding analysis in plants are trnL-F (de Groot et al., 2011), ITS (Chen et al., 2011), rbcL (de Groot et al., 2011), matK (Techen et al., 2014), and trnH-psbA (Zhang et al., 2014). The trnL-trnF DNA barcode is an informative chloroplast gene and can show relationships between types. The trnL*trnF* genes are very suitable for identification up to the species level, uniparental, or detecting up to the hybrid level (Hoggard et al., 2004; Khaira et al., 2024). Research on the genus Rhododendron using the DNA barcoding approach has not been carried out much. Therefore, initial studies on the analysis of kinship relationships in the genus Rhododendron using the in silico-based DNA Barcode trnL-trnF Intergenic Spacer need to be carried out. This research aims to carry out an inventory of *Rhododendron* types based on genetic aspects, analyzing genetic variations and kinship relationships in the Rhododendron Genus.

## 2. MATERIALS AND METHODS

This research uses molecular in-silico methods using sequence data originating from GenBank NCBI (National Center for Biotechnology Information). The research steps are as follows:

#### 2.1. Rhododendron Sequence Selection on the NCBI website

Sequences were searched and selected on the NCBI website (<u>https://www.ncbi.nlm.nih.gov/</u>) by choosing the nucleotide category and writing the keywords "*Rhododendron*" *trnL-trnF*. Next, the sequences that have been obtained are subjected to a BLAST (Basic Local Alignment Search Tool) process, and several target sequences are selected by considering the appropriate sequence length, the highest query cover value, and the highest percent identity value. The outgroup sequence used is from the *Andromeda polifolia* species. All selected nucleotide sequences are then downloaded and saved in FASTA format.

#### 2.1.1. Analysis of Genetic Data of the Genus Rhododendron

Several sequences that have been downloaded in FASTA format are then analyzed in the MEGA 11 application (Tamura et al., 2021). The sequence was aligned using the ClustalW method. The alignment process is based on sequence homology to identify sequences with similarities. Sequence alignment is a way of arranging DNA sequences to identify the same or different regions in the nitrogen base sequence, which is a consequence of the evolutionary relationship between sequences from several related species (Wiltgen, 2019). Genetic variation analysis was performed using the MULTALIN website-based application (Multiple Sequence Alignment by Florence Corpet) via link http://multalin.toulouse.inra.fr/multalin/.

## 2.1.1.1. Phylogenetic Tree Reconstruction and Genetic Distances Analysis

Phylogenetic tree reconstruction was carried out using the MEGA 11 application (Tamura et al., 2021). The phylogenetic tree was reconstructed using the Maximum Likelihood method and the Tamura 3-parameter model with a bootstrap value of 1000x (Tamura et al., 1992). Next, genetic

distance analysis was carried out using the pairwise deletion method with the maximum composite likelihood model in the MEGA 11 application (Tamura et al., 2021).

# 3. RESULTS AND DISCUSSION

## 3.1. Description and Morphology of the Genus Rhododendron

*Rhododendron* is better known in Indonesia by the common name Azalea, and its growing location has different regional names. The distribution of *Rhododendrons* in Indonesia is as follows: Sumatra has 22 types, Java has seven kinds, Nusa Tenggara has three types, Kalimantan has 55 types, Sulawesi has 29 kinds, Maluku has nine types, and Papua has 113 types (Hiller & Pollard, 2010). This plant has aesthetic value because its flowers are colorful, have various shapes according to their type, and also have a distinctive aroma, so they have the potential to be used as ornamental plants. In mountainous areas with cold temperatures, the color of *Rhododendron* flowers becomes sharper and more attractive to the eye (Wawo et al., 2021). According to GBIF (2023), the classification of the genus *Rhododendron* is as follows:

Kingdom	: Plantae
Phylum	: Tracheophyta
Class	: Magnoliopsida
Order	: Ericales
Family	: Ericaceae
Genus	: Rhododendron L.

*Rhododendron* is a flowering plant that appears to be a shrub or small tree, terrestrial or epiphytic. It has the general character of brown scales under the leaves or on other organs. The stem often swells at the base. The leaves have stalks, long and spirally arranged, facing each other, sometimes alternately. Compound interest. Pansy flower; curved petals, tubular or bell-shaped corolla, asymmetrical; stamens consist of 5-10, stuck at the base of the flower corolla, and are often unbalanced; The anther stalk is long, and the anther head is upright, generally facing inward. The ovary is attached to the base of the flower. The fruit is a capsule that rises upwards and has several seeds. Seeds are numerous, small, and thin (Sleumer, 1966). *Rhododendrons* usually form in groups, with the flowers at the branches' ends. The flowers are usually single and colorful. Some have contrasting spots (flares) and have a scent (*R. dalhousiae*, *R. edgeworthii*, *R. johnstoneanum*, etc.) (Goetsch et al., 2005; Beljai et al., 2016). The morphology of several *Rhododendron* species is presented in Figure 1.



Figure 1. (A) *Rhododendron ponticum* subsp. ponticum. a : plant; b : leaf; c : bracts; d : bracteols; e : flower; f : pistil; g : stamen; h : capsule; i : nutlets. (Küçük et al., 2018).

(B) Floral morphology of Rhododendron. Type A: A1, R. griffithianum; A2, R. lindleyi; A3, R. maddenii; A4 R. dalhousiae var. rhabdotum; A5, R. edgeworthii. Type B: B1, R. lanatum; B2, R. ciliatum; B3, R. campylocarpum; B4, R. wallichii; B5, R. tsariense; B6, R. aeruginosum; B7, R. wightii. Type C, R. keysii. Type D, R. baileyi. Type E: E1, R. kesangiae; E2, R. hogsonii; E3, R. falconeri; E4, R. grande; E5, R. niveum. Type F: F1, R. argipeplum; F2, R. thomsonii; F3, R. barbatum; F4, R. cinnabarinum; F5, R. neriiflorum; F6, R. arboreum; F7, R. succothii. Type G, R. anthopogon. Type H: H1, R. setosum; H2, R. triflorum; H3, R. virgatum (Namgay & Sridith, 2021).

*Rhododendrons* are often found in cool weather areas (temperate climate), especially on mountain peaks 800-6000 m above sea level. However, it grows well at 3001-3500 m above sea level (Sekar & Srivastava, 2010). It can grow and adapt to lower altitudes and hot weather, but its development could be more optimal. Environmental factors such as temperature and humidity greatly influence the number of types of *Rhododendrons* that flower and bear fruit. *Rhododendrons* will grow well with good water and air circulation, sufficient water conditions, and no excess or lack of water (Kelley & Drain, 1994; Masnawati et al., 2017). The community has widely used several types of *Rhododendron* as medicinal and ornamental plants. In the Malesiana area, *Rhododendron* has been widely used as an ornamental plant and is an essential horticultural commodity, be it the original type or the hybridized one. Apart from that, some Southeast Asians use several types of *Rhododendron* as traditional medicines. According to several studies conducted in Indonesia, another potential of *Rhododendron* is as an antibacterial (*R. kanori* and *R. macgregoriae*) and producer of flavonoid compounds (*R. javanicum* and *R. macgregoriae*). *Rhododendron* mucronatum varieties are also helpful for relieving fever, gland stimulants, rheumatism, and coughs (Putri & Sudiatna, 2009; Beljai et al., 2016).

## 3.1.1. In cilico analysis of Rhododendron based on DNA Barcode trnL-trnF

A total of 13 *Rhododendron* nucleotide sequences and one outgroup based on the DNA barcode *trnL-trnF* underwent phylogenetic tree reconstruction. The outgroup used in this research is the *Andromeda polifolia* species, which is still in the same family as *Rhododendron*, namely the Ericaceae family. The outgroup aims to determine the primitive characters (plesiomorphs) and derivative characters (apomorphies) of the in-group group and to determine the starting point for the formation of a phylogenetic tree (Subari et al., 2021). A reconstruction of the phylogenetic tree of the genus *Rhododendron* is presented in Figure 2.



Figure 2. Reconstruction of the phylogenetic tree of the genus *Rhododendron* based on the *trnL-trnF* DNA barcode using the Maximum Likelihood method and the Tamura 3-parameter model with a bootstrap value of 1000x

Phylogenetic tree reconstruction was carried out using Maximum Likelihood and Tamura 3parameter models with a bootstrap value 1000x. This method and model are used based on the model program in the MEGA11 application, which recommends methods and models for reconstructing phylogenetic trees of all sequences (Tamura et al., 1992). The resulting phylogenetic tree is divided into three clades in the Rhododendron group and one outgroup. The in-group in clade 1 consists of R. exuberans, R. crassifolium, R. planecostatum, R. javanicum, R. praetervisum, and R. suaveolens. The group in clade 2 consists of R. rugosum, R. lowii, and R. buxifolium. The in group in clade 3 only consists of one species, R. orbiculatum. The outgroup is the species Andromeda polifolia. The phylogenetic tree also shows that R. crassifolium accession KP979069.1 groups with R. crassifolium accession KP979089.1 and R. buxifolium accession KP979085.1 and R. buxifolium accession KP979084.1 are also in the same clade and branch. This shows that the *trnL-trnF* DNA barcode can differentiate and identify up to species level in the genus Rhododendron. The figure's cladogram indicates that the Rhododendron species in the ingroup are grouped within each clade based on similarities in nucleotide sequence. The similarities and differences in characters between these species are used to determine their kinship relationships (Anafarida & Badruzsaufari, 2020).

Phylogenetic analysis aims to compile phylogenetic relationships which is generally depicted in a line that branches like a tree, called a phylogenetic tree (Irawan, 2013). A phylogenetic tree is a tree that shows the evolutionary line of different species, organisms, or genes from a common ancestor. Phylogeny helps know biological diversity, compile classifications, and explain phenomena during the evolutionary process (Baum, 2008). Maximum Likelihood is a characterbased statistical method that compares all sequences in an alignment to calculate the probability value for each tree (Yang & Rannala, 2012). This method considers all possible numbers of changes/mutations in the sequence for each tree. Therefore, it is suitable for reconstructing phylogenetic trees with few sequences. A phylogeny test was used as a bootstrap method that carried out repeated resampling to see the tree arrangement's validity level, with several replications of 1000x.

Analysis of genetic variation in the genus *Rhododendron* is presented in Figure 3. The length of the entire *Rhododendron trnL-trnF* sequence is 894 bp. Based on this image, it shows that the *trnL-trnF* DNA barcode region of the *Rhododendron* genus is mostly conserved because it only has eight genetic variations, namely T71C, G225C, T245C, T229A, T485C, A491G, T705C, and A783G. The *trnL-F* gene is an informative chloroplast and can show the relationship between types. This region is located in the large single-copy region of the chloroplast genome. This region consists of the *trnL* gene, an intron group, and the *trnL-trnF* intergenic spacer (Hao et al., 2009). The *trnL-F* gene is a sequence located in the *trnL* (UAA) 5'exon to *trnF* (GAA), which is then called *trnL-F* (Adjie et al., 2008; Khaira et al., 2024). Next, all *Rhododendron* sequences were characterized to determine the nucleotide composition of each sequence. The nucleotide composition of the genus *Rhododendron* using the *trnL-trnF* DNA barcode is presented in Figure 4.



Figure 3. Genetic variation of the *Rhododendron* genus based on the *trnL-trnF* DNA barcode analyzed using the MULTALIN (Multiple Sequence Alignment by Florence Corpet)

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			T(U)	С	А	G	Total
KP979054.1	Rhododendron	javanicum	30.6	18.5	35.9	15.0	894
KP979092.1	Rhododendron	planecostatum	30.6	18.6	35.9	14.9	894
KP979089.1	Rhododendron	crassifolium	30.6	18.6	35.9	14.9	894
KP979080.1	Rhododendron	exuberans	30.6	18.6	35.9	14.9	894
KP979069.1	Rhododendron	crassifolium	30.6	18.6	35.9	14.9	894
KP979022.1	Rhododendron	lowii	30.6	18.6	35.8	15.0	894
KP979083.1	Rhododendron	rugosum	30.8	18.5	35.8	15.0	894
KP979050.1	Rhododendron	orbiculatum	30.8	18.5	35.8	15.0	894
KP979085.1	Rhododendron	buxifolium	30.9	18.5	35.7	15.0	894
KP979062.1	Rhododendron	praetervisum	30.8	18.5	35.9	14.9	894
JF801624.1	Andromeda pol	lifolia	31.5	17.1	35.5	15.9	889
KP979084.1	Rhododendron	buxifolium	30.9	18.5	35.7	15.0	894
KP979032.1	Rhododendron	suaveolens	30.8	18.5	35.9	14.9	894
Avg.			30.8	18.4	35.8	15.0	893.

### Figure 4. Nucleotide composition of the genus Rhododendron using DNA barcode trnL-trnF

Based on Figure 4, the *Rhododendron* genus has a nucleotide composition with the highest percentage of thymine (T) and adenine (A), 30.8% thymine and 35.8% adenine. The higher the content of the A and T base pairs, the higher the melting point of DNA. This is because the A and T pairs are more stable and require more heat energy to decompose than the G and C pairs. The high percentage of AT content compared to GC content is influenced by the location of the amplified *trnl-F* gene, which has many nucleotide substitutions. The low percentage of CG content indicates that this species is more primitive, as seen from the average calculation results (Hapsari et al., 2015; Khaira et al., 2024).

#### 4. CONCLUSIONS

Based on this research, the phylogenetic results show that the *trnL-trnF* DNA barcode can differentiate and identify up to species level in the genus *Rhododendron*. The *trnL-trnF* DNA barcode region of the *Rhododendron* genus is mostly conserved because it only has eight genetic variations, namely T71C, G225C, T245C, T229A, T485C, A491G, T705C, and A783G. *Rhododendron* has a nucleotide composition with the highest percentage of thymine (T) and adenine (A), namely 30.8% thymine and 35.8% adenine.

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# Analysis of Non Specific Parameters of Simplicia Bloodleaf Plant (Iresine herbstii Hook) During Storage

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## **KEYWORDS:**

Iresine herbstii Hook Storage duration Quality of simplicia MFA

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# ABSTRACT

Bloodleaf plant, scientifically known as Iresine herbstii Hook, is an ornamental shrub with possible medicinal properties. Storage of simplicia is necessary to guarantee the accessibility of raw materials from I. herbstii. Prolonged storage of simplicia can impact its quality, including many non-specific factors. Indeterminate parameters suggest a relationship between the characteristics of Hook's I. herbstii simplicia during extended storage. This study seeks to ascertain the impact and correlation between the duration of storage of simplicia and the non-specific characteristics of I. herbstii simplicia. Moisture content, extractive value, and ash content were examined in samples with shelf lives of 0 month (IH0), 4 months (IH4), 10 months (IH10), 20 months (IH20), and 32 months (IH32). The acquired data were analyzed using DMRT (Duncan's Multiple Range Test) and MFA (Multiple Factor Analysis). The analysis results indicated that the storage duration impacted all parameters examined except acid-insoluble ash concentration. Prolonged storage of I. herbstii simplicia decreased the concentration of water-soluble and alcohol-soluble extractive value. At the same time, the water content and total ash content increased. The MFA analysis revealed a strong link between non-specific parameters and the duration of storage of I. herbstii simplicia, except for acid-insoluble ash concentration.

# 1. INTRODUCTION

The perennial plant *Iresine herbstii* Hook is a plant from the Amaranthaceae. Widespread in tropical and subtropical areas, including Indonesia (Jaafar and Jaafar, 2021). Indonesian people have used natural ingredients from plants as a form of treatment. One plant that has the potential to be used as traditional medicine is bloodleaf plant, but so far this plant is mostly known as an ornamental plant due to its vibrant red leaves. I. herbstii has alternative names such as chicken gizzard, beefsteak plant, and herbst's bloodleaf (Jaafar & Jaafar, 2021). I. herbstii originates from tropical South America and is believed to have been initially gathered in Brazil, it which be found in the tropical forest across many regions of India and tropical Asia (Flores-Olvera, Zumaya & Borsch, 2016). Bloodleaf plant possesses wound healing properties, exhibits low antioxidant activity, affects the central nervous system, demonstrates affinity for various cerebral receptors, and shows potential as an antiviral agent (Andleeb et al., 2020). Apart from being an ornamental plant, this is also a medicinal plant containing alkaloids, flavonoids, and anthocyanins (Iswoyo et al., 2023; Safrina and Joko Priyambodo, 2018; Asikin et al., 2014). People use the compounds in I. herbstii as repellant for waste remediation, as antibacterial agents, antiviral agents, and natural dyes (Efendi et al., 1970; Andleeb et al., 2020; Spórna-Kucab et al., 2020; Agustina et al., 2022; Kiran et al., 2022; Sai-Ut et al., 2023).

The increasing number of people adopting the Back to Nature lifestyle has influenced the increase in demand for raw materials for herbal medicines, but there are still problems in maintaining the sustainability of raw materials. Simplicia possesses the benefit of being capable of being preserved in a simple form for an extended period, which can impact the quality of simplicia due to the proliferation of microorganisms, leading to alterations in color, structure, scent, taste, and biological activity (Safrina & Supriadi, 2020). Hence, proper storage plays a crucial role in the processing of medicinal plants to safeguard the integrity of their chemical constituents (Nurapni et al., 2023). Sustaining an industry or business depends on the continuous supply of raw materials. Stock storage is crucial to maintain continuity when field production is inconsistent (Hossain et al., 2023). Despite sufficient supplies, various problems can disrupt raw material stability (Saptadi et al., 2023). Likewise, with the stock of *I. herbstii* as raw material for medicinal plants, requiring controlled environment conditions, strict inspection, regular maintenance, until appropriate storage systems to maintain quality (Ching et al., 2023; Tejesh and S. J., 2023; Li et al., 2022).

Non-specific parameters are important parameters in the quality of simplicia. These parameters include water content, ash value, and extractive value (Kementerian Kesehatan Republik Indonesia, 2000). Water content indicates the minimum amount of water contained in the extract when it is excessive during storage because of environmental conditions causing the growth of microbes and damage to the stability of the simplicia (Maciel & Steppe, 2017). The total ash content is used to describe the mineral content found on the inside and outside of the simplicia so that a high ash content can indicate that the simplicia contains many minerals during storage (Sari, Elya & Katrin, 2019). The extractive value shows the quality of the simplicia by knowing the content of the compounds extracted in the solvent used (Safrina, Herera & Kusumadewi, 2021). The opportunity to develop *I.herbstii* as a raw material for traditional medicine is still very large, so specific and accurate data is needed, one of which is the sustainability of raw material availability. Research on the effect of shelf life on extracted value, water content, and ash value can be used to determine the extent to which simplicia *I. herbstii* still has good quality for use after storage. This study aims to determine the effect of shelf life on non-specific *I. herbstii* simplicia parameters and the correlation between the two.

# 2. MATERIALS AND METHODS

# 2.1. Sample Preparation

The *I. herbstii* plants used as samples were grown at an elevation of around 1800 meters above sea level at the medicinal plant garden of Tlogodlingo Village in Tawangmangu District. Planting, maintaining, harvesting, and seeding are all part of I. herbstii farming. The samples of I. herbstii plants used in the study were obtained from the Tlogodlingo medicinal plant garden, approximately 1800 meters above sea level. The garden covers an area of 1200 m<sup>2</sup>, and the plants are spaced 30 cm x 30 cm apart. The application rate of fertilizer is 20 tonnes per hectare of manure. Flowers were collected as specimens when they were three months old after planting. During the harvesting phase, I. herbstii undergoes a sorting procedure to remove any impurities from the material. The samples were subsequently washed in a continuous flow of water until they were completely free from contaminants and then left to dry. The I. herbstii leaves undergo processing using a leaf chopper machine, yielding fragments measuring 8-10 cm long. The samples were then dehydrated in an oven maintained at around 43 °C for approximately 4-5 days until their water content decreased to 10% or below. The simplicia is contained within HDPE plastic packaging, with a precise thickness of 0.13 mm. Furthermore, each bottle is equipped with ten silica gel sachets. The packaging step entails employing the Hoover technique. The processed herbal materials are stored in the postharvest laboratory warehouse.

## 2.2. Storage of Research Samples

The *I. herbstii* simplicia plants are kept in the simplicia warehouse at a temperature ranging from 20 °C to 25 °C and a humidity level between 65% and 75%. Observations were conducted to assess the storage period of the simplicia, considering the water content and non-specific simplicia. The observed samples were of simplicia that were preserved for various durations: 0 months (IH1), 4 months (IH2), 9 months (IH3), 10 months (IH4), 20 months (IH5), and 32 months (IH6). Every storage period consists of three repetitions. The storage duration for *I. herbstii* simplicia is determined based on the stock inventory data in the Simplicia warehouse. The parameters observed include moisture content (MC), water soluble extractive (WSE), alcohol soluble extractive (ASE), total ash (TA), and acid insoluble ash (AIA).

## 2.3. Non-Specific Parameters

#### 2.3.1. Moisture content (MC)

Determination of water content was carried out using the gravimetric method using a Moisture Analyzer MFX-50. The temperature used to determine water content is 105 °C.

#### 2.3.2. Determination extractive value

## 2.3.2.1. Water soluble extractive (WSE)

Preparation of the test equipment includes preparing a porcelain cup, heating it to a temperature of 105 °C, placing it in a desiccator, and then cooling it. Next, weigh until the weight is constant and the results are recorded. Next is the preparation of the water-chloroform test material. Preparation of the water-chloroform test material is to take 2.5 mL of chloroform (CH<sub>3</sub>Cl) PA, add distilled water to 1000 mL in a 1000 mL beaker, and transfer it to a media dispenser bottle. The steps for determining the water-soluble essence content are carefully weighing 5 g of the medicinal plant simplicia powder, putting it in a laboratory bottle with a lid, and adding 100 mL of water chloroform. Shake using a shaker at 80 rpm for 6 hours and leave for 18 hours. Filter the entire filtrate, take 20 mL, put it in a porcelain cup, and evaporate the filtrate until dry. Heat the remainder at 105 °C, place in a desiccator, then weigh until constant weight.

## 2.3.2.2. Alcohol soluble extractive (ASE)

Preparation of the test equipment includes preparing a porcelain cup, heating it to a temperature of 105 °C then placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. Then, 96% ethanol was put into the container connected to the Finnpipette dispenser. The steps for determining the ethanol-soluble essence content are carefully weighing 5 g of the simplicia powder and putting it in a laboratory bottle with a lid. Moreover, adding 100 mL of 96% ethanol. Shake using a shaker at 80 rpm for 6 hours and leave for 18 hours. Filter the entire filtrate, take 20 mL, put it in a porcelain cup, and evaporate until dry. Heat at 105 °C until constant weight.

#### 2.3.3. Determination ash value

## 2.3.3.1. Total Ash (TA)

Preparation of the test equipment includes preparing a silicate crucible, heating it to a temperature of 800 °C, then placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. The procedure for determining the TA content is carried out carefully weighing 2 g of the simplicia powder, placing it in a silicate crucible, gently igniting it until the charcoal runs out, placing it in a desiccator, and weighing it until the weight is constant. If the

charcoal cannot be removed, add hot water and filter using ash-free filter paper. Then, filter the remainder in filter paper in the same crucible. Place in a desiccator and weigh until constant weight.

## 2.3.3.2. Acid insoluble ash (AIA)

Preparation of the test equipment includes preparing a silicate crucible, heating it to a temperature of 800 °C and placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. 226 mL HCl solution into a 1 L beaker, add distilled water to 1 L, and put it in a dilute HCl container. The procedure for determining the acid-insoluble ash content is to boil the ash obtained from determining the TA content with 25 mL of dilute HCl, collect the part that is not soluble in acid, filter it using ash-free filter paper, let it stand, put it in a desiccator, and weigh it until constant.

## 2.4. Data Analysis

Data analysis was carried out with DMRT and R-statistics software. Multiple factor analysis (MFA) summarizing and visualizing a complex data table in which individuals are described by several sets of variables (quantitative and/or qualitative) structured into groups. The effect of storage time for *I. herbstii* simplicia was used as the main factor.

## 3. RESULTS AND DISCUSSION

During the storage process of *I. herbstii* there was a decrease in WSE and ASE but there was an increase in the MC of TA, and AIA (**Table 1**). DMRT analysis showed that the length of storage of *I. herbstii* simplicia influenced MC, WSE, ASE, TA, but had no effect on AIA.

-	8									
Samples	MC		WSE		ASE		ТА		AIA	
IH0	$6.97\pm0.18$	а	$3.43\pm0.01$	d	$0.88\pm0.02$	d	$12.30\pm0.05$	а	$1.56 \pm 0.56$	a
IH4	$8.5\pm0.39$	b	$3.16\pm0.05$	c	$0.84\pm0.01$	c	$13.33\pm0.38$	b	$1.82 \pm 0.88$ a	a
IH10	$9.33 \pm 0.51$	c	$2.91 \pm 0.06$	b	$0.66\pm0.03$	b	$14.66\pm0.07$	c	$2.42 \pm 0.40$ a	a
IH20	$10.29\pm0.56$	d	$2.83\pm0.08$	а	$0.59\pm0.01$	а	$14.73\pm0.17$	c	$2.46 \pm 0.26$ a	a
IH32	$12.38\pm0.29$	e	$2.79\pm0.02$	а	$0.59\pm0.02$	а	$14.82\pm0.02$	с	$2.49 \pm 0.13$ a	a

 Table 1. Non-specific parameters of I. herbstü simplicia during storage simplicia during storage results

Remarks: The value is average value  $\pm$  deviation standard; n=3. The same alphabet on the same column shows no significant difference at 5%.

Changes in water content in simplicia *I. herbstii* are influenced by temperature and humidity during storage. Storage of *I. herbstii* simplicia for 32 months increased by 4.9%. WSE was reduced by 0.65% and ASE was reduced by 0.31%. Based on the research results, the value of the WSE in each treatment was higher when compared to the ASE, indicating that the constituent components are more polar in nature. The decrease in WSE in simplicia is caused by increased microbial activity due to an increase in water vapor during storage so that it can damage the compounds contained during storage (Safrina, Herera & Kusumadewi, 2021). WSE is higher than ASE because the content of most secondary metabolite compounds is polar in nature found in *I. herbstii* compared to semi-polar secondary metabolite compounds, so that these compounds will easily dissolve in water compared to alcohol (Febrianti et al., 2019). There was no significant decrease in WSE and ASE after storage of simplicia for 20 months because the content of soluble compounds is relatively stable against bacterial damage. AIA describes soil and sand contamination in the simplicia production process (Fatimawali, Kepel & Bodhi, 2020). Storage

duration did not have a significant effect on AIA because there was no increase in sand or soil content in the vacuum-packed simplisia in plastic containers.



Figure 1. Distribution of explained variance across dimensions of non-specific variables parameters of simplicia *Iresine herbstii* during storage.

A scree plot uses multiple-factor analysis to visually depict the relationship between dimensions and the proportion of explained variations (Fig. 1). The X-axis represents the quantity of dimensions considered in the investigation. Simultaneously, the Y-axis represents the proportion of the overall variability accounted for by each dimension in the four-dimensional graph. The first dimension represents 82.96% of the total, and the second dimension contributes 12.2%. Collectively, these two dimensions account for over 95% of the entire sum. The percentage of explained variance experiences a significant decrease from the first to the second dimension, and this loss continues, albeit at a slower pace, from the second dimension onwards. This trend indicates that most of the variation in the dataset can be accounted for by the initial dimension, with declining benefits from including other dimensions. The sharp decline following the initial dimension indicates that the first principal component holds considerably greater significance than the others in elucidating the variation within the sample of simplicia I. herbstii Hook during storage. The scree plot indicates the reduction of the data dimensionality from five principal components to the first two dimensions due to representing 95% variability of the majority of the information from all variables (Abdi, Williams & Valentin, 2013; Mewengkang, Mananohas & Komalig, 2022).



Figure 2. Results of multiple factor analysis-groups of variables non-specific parameters of simplicia *Iresine herbstii* Hook during storage (2a); Percentage contribution of the non-

# specific variable group Simplicia *I. herbstii* during storage in dimension 1 (2b); Percentage contribution of the non-specific variable group Simplicia *I. herbstii* during storage in dimension 2 (2c).

The results of MFA-group variables show a correlation between variables and dimensions (**Fig. 2a**) where the active variable group is shown in a plot located in dimension 1 (contribution of 82.96%) that is used in this study for the non-specific parameters since the high contribution value illustrates the strong correlation between simplified storage time and changes in the values of WSE, ASE, and MC (Abdi, Williams & Valentin, 2013). In this study, the more extended storage of simplicia (*I. herbstii*) can reduce the value of WSE, reduce the value of ASE, and increase the MC of Simplicia. The coordinates of these three groups of variables are almost the same, which means they make almost the same contribution to dimension 1 (**Fig. 2b**). The complementary variable group is shown in purple on the variable plot and is in dimension 2 (contribution 12.2%). The variable AIA is the variable that is most strongly correlated with dimension 2. In this study, AIA provided the most dominant contribution in dimension 2 (**Fig. 2c**)

The results of previous research explain that simplicia's MC is an essential component in its storage process. The stored medicinal plant simplicia must have a low MC because high MC will make it easier for fungi and mold to grow, which can damage the simplicial (Pusmarani et al., 2019). Other active parameters are WSE, and ASE. The WSE can describe the number of compounds contained in the simplicia that are polar or have the same polarity as water. The higher the value of the WSE of a simplicia, the expectation is that the chemical compounds dissolved in water will also be higher. The ASE describes the number of compounds that dissolve in alcohol. In terms of quality, the aim of determining the ASE is almost the same as determining the WSE (Husni, Ismed & Awaliana A, 2021).



Figure 3. Results of multiple factor quantitative analysis of non-specific variables parameters of simplicia *Iresine herbstii* during storage

The results of multiple factor analysis-quantitative variables (**Fig. 3**) show that three groups of variables group/cluster each other. The WSE and ASE are two variables grouped into 1. The MC of simplicia and TA of simplicia are grouped into 1, with the plots being close together. This shows that the WSE and ASE levels are correlated with the storage time of the simplicial *I. herbstii* (Lê, Josse & Husson, 2008). The longer *I. herbstii* simplicia is stored, the WSE and ASE levels will decrease. Meanwhile, *I. herbstii* simplicia, which is stored for a long time, will increase the MC and TA of the simplicia.



Figure 4. Individual factor map of non-specific variables parameters of simplicia *Iresine herbstii* during storage.

The graph (Fig. 4) illustrates the distribution of different data points over two main dimensions. The x-axis represents Dim 1, which explains a large percentage of the variance, exactly 82.96%, making it the primary axis of variation among the data points. Dim 2, which is represented on the y-axis, explains a lesser amount of the variation, exactly 12.28%. The data points are numerically labeled from 0 to 32 and are distributed randomly on the graph. Every point in the collection represents a distinct observation or case. The dashed vertical line at x=0symbolizes the mean or median of Dim 1. It separates the data into two groups based on their scores in relation to this center value. The dashed magenta line could perhaps indicate a trend line or a distinct threshold inside Dim 2, emphasizing a certain element or subdivision within the data. The AIA exhibits a clear trajectory, progressing from the lower left to the top right quadrant, indicating a robust correlation with both dimensions. The concentration of MC around the center indicates a moderate impact on both dimensions. The WSE and ASE are distributed unevenly, suggesting different levels of impact on the dimensions. The various factors contribute to the primary dimensions, likely drawn from a broader set of underlying data. The arrangement and distribution of the data points can facilitate the detection of patterns, correlations, or groups among the variables during storing *I. herbstii* simplicia.



Figure 5. Results of multiple factorial analysis-individual variable non-specific parameters of simplicia *Iresine herbstii* during storage

Individual multiple-factor analysis is an analysis that can group individuals with similar profiles who are close to one another (**Fig. 5**). Individuals analyzed in MFA had storage times ranging from 0 to 32 months. The results of the individual multiple factor map analysis show that storage duration 0 (SD\_0) and storage duration 4 (SD\_4) are in the first-dimension plot; this shows that fresh simplicia (0 months storage duration) and four months storage duration are the best storage time of *I. herbstii* simplicia (positive coordinates). This is in sharp contrast to the storage duration 32 (SD\_32) plot, which is closest to dimension two, which is a negative coordinate (Abdi, Williams & Valentin, 2013). Storing *I. herbstii* simplicia for 32 months is the worst storage method because it can reduce the quality of the product. This research shows that the longer the simplicia is stored, the higher the MC. This means that the longer the simplicia is stored, the worse its quality. Other parameters that indicate simplicia's quality is worsening after long storage are the decrease in WSE and the decrease in ASE (Sari, Elya & Basah, 2020).

## 4. CONCLUSIONS

The DMRT study revealed that the duration of storage of *I. herbstii* simplicia had a significant impact on MC, WSE, ASE, and TA, but did not affect AIA. The MFA analysis demonstrated a significant correlation between general parameters and the length of storage of *I. herbstii* simplicia, with the exception of the concentration of AIA. There is a positive correlation between the storage period of 0 month and 4 months on non-specific factors, indicating that the optimal storage period is up to 4 months.

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# Analysis of Non Specific Parameters of Simplicia Bloodleaf Plant (Iresine herbstii Hook) During Storage

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## **KEYWORDS:**

Iresine herbstii Hook Storage duration Quality of simplicia MFA

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# ABSTRACT

Bloodleaf plant, scientifically known as Iresine herbstii Hook, is an ornamental shrub with possible medicinal properties. Storage of simplicia is necessary to guarantee the accessibility of raw materials from I. herbstii. Prolonged storage of simplicia can impact its quality, including many non-specific factors. Indeterminate parameters suggest a relationship between the characteristics of Hook's I. herbstii simplicia during extended storage. This study seeks to ascertain the impact and correlation between the duration of storage of simplicia and the non-specific characteristics of I. herbstii simplicia. Moisture content, extractive value, and ash content were examined in samples with shelf lives of 0 month (IH0), 4 months (IH4), 10 months (IH10), 20 months (IH20), and 32 months (IH32). The acquired data were analyzed using DMRT (Duncan's Multiple Range Test) and MFA (Multiple Factor Analysis). The analysis results indicated that the storage duration impacted all parameters examined except acid-insoluble ash concentration. Prolonged storage of I. herbstii simplicia decreased the concentration of water-soluble and alcohol-soluble extractive value. At the same time, the water content and total ash content increased. The MFA analysis revealed a strong link between non-specific parameters and the duration of storage of I. herbstii simplicia, except for acid-insoluble ash concentration.

# 1. INTRODUCTION

The perennial plant *Iresine herbstii* Hook is a plant from the Amaranthaceae. Widespread in tropical and subtropical areas, including Indonesia (Jaafar and Jaafar, 2021). Indonesian people have used natural ingredients from plants as a form of treatment. One plant that has the potential to be used as traditional medicine is bloodleaf plant, but so far this plant is mostly known as an ornamental plant due to its vibrant red leaves. I. herbstii has alternative names such as chicken gizzard, beefsteak plant, and herbst's bloodleaf (Jaafar & Jaafar, 2021). I. herbstii originates from tropical South America and is believed to have been initially gathered in Brazil, it which be found in the tropical forest across many regions of India and tropical Asia (Flores-Olvera, Zumaya & Borsch, 2016). Bloodleaf plant possesses wound healing properties, exhibits low antioxidant activity, affects the central nervous system, demonstrates affinity for various cerebral receptors, and shows potential as an antiviral agent (Andleeb et al., 2020). Apart from being an ornamental plant, this is also a medicinal plant containing alkaloids, flavonoids, and anthocyanins (Iswoyo et al., 2023; Safrina and Joko Priyambodo, 2018; Asikin et al., 2014). People use the compounds in I. herbstii as repellant for waste remediation, as antibacterial agents, antiviral agents, and natural dyes (Efendi et al., 1970; Andleeb et al., 2020; Spórna-Kucab et al., 2020; Agustina et al., 2022; Kiran et al., 2022; Sai-Ut et al., 2023).

The increasing number of people adopting the Back to Nature lifestyle has influenced the increase in demand for raw materials for herbal medicines, but there are still problems in maintaining the sustainability of raw materials. Simplicia possesses the benefit of being capable of being preserved in a simple form for an extended period, which can impact the quality of simplicia due to the proliferation of microorganisms, leading to alterations in color, structure, scent, taste, and biological activity (Safrina & Supriadi, 2020). Hence, proper storage plays a crucial role in the processing of medicinal plants to safeguard the integrity of their chemical constituents (Nurapni et al., 2023). Sustaining an industry or business depends on the continuous supply of raw materials. Stock storage is crucial to maintain continuity when field production is inconsistent (Hossain et al., 2023). Despite sufficient supplies, various problems can disrupt raw material stability (Saptadi et al., 2023). Likewise, with the stock of *I. herbstii* as raw material for medicinal plants, requiring controlled environment conditions, strict inspection, regular maintenance, until appropriate storage systems to maintain quality (Ching et al., 2023; Tejesh and S. J., 2023; Li et al., 2022).

Non-specific parameters are important parameters in the quality of simplicia. These parameters include water content, ash value, and extractive value (Kementerian Kesehatan Republik Indonesia, 2000). Water content indicates the minimum amount of water contained in the extract when it is excessive during storage because of environmental conditions causing the growth of microbes and damage to the stability of the simplicia (Maciel & Steppe, 2017). The total ash content is used to describe the mineral content found on the inside and outside of the simplicia so that a high ash content can indicate that the simplicia contains many minerals during storage (Sari, Elya & Katrin, 2019). The extractive value shows the quality of the simplicia by knowing the content of the compounds extracted in the solvent used (Safrina, Herera & Kusumadewi, 2021). The opportunity to develop *I.herbstii* as a raw material for traditional medicine is still very large, so specific and accurate data is needed, one of which is the sustainability of raw material availability. Research on the effect of shelf life on extracted value, water content, and ash value can be used to determine the extent to which simplicia *I. herbstii* still has good quality for use after storage. This study aims to determine the effect of shelf life on non-specific *I. herbstii* simplicia parameters and the correlation between the two.

# 2. MATERIALS AND METHODS

# 2.1. Sample Preparation

The *I. herbstii* plants used as samples were grown at an elevation of around 1800 meters above sea level at the medicinal plant garden of Tlogodlingo Village in Tawangmangu District. Planting, maintaining, harvesting, and seeding are all part of I. herbstii farming. The samples of I. herbstii plants used in the study were obtained from the Tlogodlingo medicinal plant garden, approximately 1800 meters above sea level. The garden covers an area of 1200 m<sup>2</sup>, and the plants are spaced 30 cm x 30 cm apart. The application rate of fertilizer is 20 tonnes per hectare of manure. Flowers were collected as specimens when they were three months old after planting. During the harvesting phase, I. herbstii undergoes a sorting procedure to remove any impurities from the material. The samples were subsequently washed in a continuous flow of water until they were completely free from contaminants and then left to dry. The I. herbstii leaves undergo processing using a leaf chopper machine, yielding fragments measuring 8-10 cm long. The samples were then dehydrated in an oven maintained at around 43 °C for approximately 4-5 days until their water content decreased to 10% or below. The simplicia is contained within HDPE plastic packaging, with a precise thickness of 0.13 mm. Furthermore, each bottle is equipped with ten silica gel sachets. The packaging step entails employing the Hoover technique. The processed herbal materials are stored in the postharvest laboratory warehouse.

## 2.2. Storage of Research Samples

The *I. herbstii* simplicia plants are kept in the simplicia warehouse at a temperature ranging from 20 °C to 25 °C and a humidity level between 65% and 75%. Observations were conducted to assess the storage period of the simplicia, considering the water content and non-specific simplicia. The observed samples were of simplicia that were preserved for various durations: 0 months (IH1), 4 months (IH2), 9 months (IH3), 10 months (IH4), 20 months (IH5), and 32 months (IH6). Every storage period consists of three repetitions. The storage duration for *I. herbstii* simplicia is determined based on the stock inventory data in the Simplicia warehouse. The parameters observed include moisture content (MC), water soluble extractive (WSE), alcohol soluble extractive (ASE), total ash (TA), and acid insoluble ash (AIA).

## 2.3. Non-Specific Parameters

#### 2.3.1. Moisture content (MC)

Determination of water content was carried out using the gravimetric method using a Moisture Analyzer MFX-50. The temperature used to determine water content is 105 °C.

#### 2.3.2. Determination extractive value

## 2.3.2.1. Water soluble extractive (WSE)

Preparation of the test equipment includes preparing a porcelain cup, heating it to a temperature of 105 °C, placing it in a desiccator, and then cooling it. Next, weigh until the weight is constant and the results are recorded. Next is the preparation of the water-chloroform test material. Preparation of the water-chloroform test material is to take 2.5 mL of chloroform (CH<sub>3</sub>Cl) PA, add distilled water to 1000 mL in a 1000 mL beaker, and transfer it to a media dispenser bottle. The steps for determining the water-soluble essence content are carefully weighing 5 g of the medicinal plant simplicia powder, putting it in a laboratory bottle with a lid, and adding 100 mL of water chloroform. Shake using a shaker at 80 rpm for 6 hours and leave for 18 hours. Filter the entire filtrate, take 20 mL, put it in a porcelain cup, and evaporate the filtrate until dry. Heat the remainder at 105 °C, place in a desiccator, then weigh until constant weight.

## 2.3.2.2. Alcohol soluble extractive (ASE)

Preparation of the test equipment includes preparing a porcelain cup, heating it to a temperature of 105 °C then placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. Then, 96% ethanol was put into the container connected to the Finnpipette dispenser. The steps for determining the ethanol-soluble essence content are carefully weighing 5 g of the simplicia powder and putting it in a laboratory bottle with a lid. Moreover, adding 100 mL of 96% ethanol. Shake using a shaker at 80 rpm for 6 hours and leave for 18 hours. Filter the entire filtrate, take 20 mL, put it in a porcelain cup, and evaporate until dry. Heat at 105 °C until constant weight.

#### 2.3.3. Determination ash value

## 2.3.3.1. Total Ash (TA)

Preparation of the test equipment includes preparing a silicate crucible, heating it to a temperature of 800 °C, then placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. The procedure for determining the TA content is carried out carefully weighing 2 g of the simplicia powder, placing it in a silicate crucible, gently igniting it until the charcoal runs out, placing it in a desiccator, and weighing it until the weight is constant. If the

charcoal cannot be removed, add hot water and filter using ash-free filter paper. Then, filter the remainder in filter paper in the same crucible. Place in a desiccator and weigh until constant weight.

## 2.3.3.2. Acid insoluble ash (AIA)

Preparation of the test equipment includes preparing a silicate crucible, heating it to a temperature of 800 °C and placing it in a desiccator. Next, weigh until the weight is constant and the results are recorded. 226 mL HCl solution into a 1 L beaker, add distilled water to 1 L, and put it in a dilute HCl container. The procedure for determining the acid-insoluble ash content is to boil the ash obtained from determining the TA content with 25 mL of dilute HCl, collect the part that is not soluble in acid, filter it using ash-free filter paper, let it stand, put it in a desiccator, and weigh it until constant.

## 2.4. Data Analysis

Data analysis was carried out with DMRT and R-statistics software. Multiple factor analysis (MFA) summarizing and visualizing a complex data table in which individuals are described by several sets of variables (quantitative and/or qualitative) structured into groups. The effect of storage time for *I. herbstii* simplicia was used as the main factor.

## 3. RESULTS AND DISCUSSION

During the storage process of *I. herbstii* there was a decrease in WSE and ASE but there was an increase in the MC of TA, and AIA (**Table 1**). DMRT analysis showed that the length of storage of *I. herbstii* simplicia influenced MC, WSE, ASE, TA, but had no effect on AIA.

-	8									
Samples	MC		WSE		ASE		ТА		AIA	
IH0	$6.97\pm0.18$	а	$3.43\pm0.01$	d	$0.88\pm0.02$	d	$12.30\pm0.05$	а	$1.56 \pm 0.56$	a
IH4	$8.5\pm0.39$	b	$3.16\pm0.05$	c	$0.84\pm0.01$	c	$13.33\pm0.38$	b	$1.82 \pm 0.88$ a	a
IH10	$9.33 \pm 0.51$	c	$2.91 \pm 0.06$	b	$0.66\pm0.03$	b	$14.66\pm0.07$	c	$2.42 \pm 0.40$ a	a
IH20	$10.29\pm0.56$	d	$2.83\pm0.08$	а	$0.59\pm0.01$	а	$14.73\pm0.17$	c	$2.46 \pm 0.26$ a	a
IH32	$12.38\pm0.29$	e	$2.79\pm0.02$	а	$0.59\pm0.02$	а	$14.82\pm0.02$	с	$2.49 \pm 0.13$ a	a

 Table 1. Non-specific parameters of I. herbstü simplicia during storage simplicia during storage results

Remarks: The value is average value  $\pm$  deviation standard; n=3. The same alphabet on the same column shows no significant difference at 5%.

Changes in water content in simplicia *I. herbstii* are influenced by temperature and humidity during storage. Storage of *I. herbstii* simplicia for 32 months increased by 4.9%. WSE was reduced by 0.65% and ASE was reduced by 0.31%. Based on the research results, the value of the WSE in each treatment was higher when compared to the ASE, indicating that the constituent components are more polar in nature. The decrease in WSE in simplicia is caused by increased microbial activity due to an increase in water vapor during storage so that it can damage the compounds contained during storage (Safrina, Herera & Kusumadewi, 2021). WSE is higher than ASE because the content of most secondary metabolite compounds is polar in nature found in *I. herbstii* compared to semi-polar secondary metabolite compounds, so that these compounds will easily dissolve in water compared to alcohol (Febrianti et al., 2019). There was no significant decrease in WSE and ASE after storage of simplicia for 20 months because the content of soluble compounds is relatively stable against bacterial damage. AIA describes soil and sand contamination in the simplicia production process (Fatimawali, Kepel & Bodhi, 2020). Storage

duration did not have a significant effect on AIA because there was no increase in sand or soil content in the vacuum-packed simplisia in plastic containers.



Figure 1. Distribution of explained variance across dimensions of non-specific variables parameters of simplicia *Iresine herbstii* during storage.

A scree plot uses multiple-factor analysis to visually depict the relationship between dimensions and the proportion of explained variations (Fig. 1). The X-axis represents the quantity of dimensions considered in the investigation. Simultaneously, the Y-axis represents the proportion of the overall variability accounted for by each dimension in the four-dimensional graph. The first dimension represents 82.96% of the total, and the second dimension contributes 12.2%. Collectively, these two dimensions account for over 95% of the entire sum. The percentage of explained variance experiences a significant decrease from the first to the second dimension, and this loss continues, albeit at a slower pace, from the second dimension onwards. This trend indicates that most of the variation in the dataset can be accounted for by the initial dimension, with declining benefits from including other dimensions. The sharp decline following the initial dimension indicates that the first principal component holds considerably greater significance than the others in elucidating the variation within the sample of simplicia I. herbstii Hook during storage. The scree plot indicates the reduction of the data dimensionality from five principal components to the first two dimensions due to representing 95% variability of the majority of the information from all variables (Abdi, Williams & Valentin, 2013; Mewengkang, Mananohas & Komalig, 2022).



Figure 2. Results of multiple factor analysis-groups of variables non-specific parameters of simplicia *Iresine herbstii* Hook during storage (2a); Percentage contribution of the non-

# specific variable group Simplicia *I. herbstii* during storage in dimension 1 (2b); Percentage contribution of the non-specific variable group Simplicia *I. herbstii* during storage in dimension 2 (2c).

The results of MFA-group variables show a correlation between variables and dimensions (**Fig. 2a**) where the active variable group is shown in a plot located in dimension 1 (contribution of 82.96%) that is used in this study for the non-specific parameters since the high contribution value illustrates the strong correlation between simplified storage time and changes in the values of WSE, ASE, and MC (Abdi, Williams & Valentin, 2013). In this study, the more extended storage of simplicia (*I. herbstii*) can reduce the value of WSE, reduce the value of ASE, and increase the MC of Simplicia. The coordinates of these three groups of variables are almost the same, which means they make almost the same contribution to dimension 1 (**Fig. 2b**). The complementary variable group is shown in purple on the variable plot and is in dimension 2 (contribution 12.2%). The variable AIA is the variable that is most strongly correlated with dimension 2. In this study, AIA provided the most dominant contribution in dimension 2 (**Fig. 2c**)

The results of previous research explain that simplicia's MC is an essential component in its storage process. The stored medicinal plant simplicia must have a low MC because high MC will make it easier for fungi and mold to grow, which can damage the simplicial (Pusmarani et al., 2019). Other active parameters are WSE, and ASE. The WSE can describe the number of compounds contained in the simplicia that are polar or have the same polarity as water. The higher the value of the WSE of a simplicia, the expectation is that the chemical compounds dissolved in water will also be higher. The ASE describes the number of compounds that dissolve in alcohol. In terms of quality, the aim of determining the ASE is almost the same as determining the WSE (Husni, Ismed & Awaliana A, 2021).



Figure 3. Results of multiple factor quantitative analysis of non-specific variables parameters of simplicia *Iresine herbstii* during storage

The results of multiple factor analysis-quantitative variables (**Fig. 3**) show that three groups of variables group/cluster each other. The WSE and ASE are two variables grouped into 1. The MC of simplicia and TA of simplicia are grouped into 1, with the plots being close together. This shows that the WSE and ASE levels are correlated with the storage time of the simplicial *I. herbstii* (Lê, Josse & Husson, 2008). The longer *I. herbstii* simplicia is stored, the WSE and ASE levels will decrease. Meanwhile, *I. herbstii* simplicia, which is stored for a long time, will increase the MC and TA of the simplicia.



Figure 4. Individual factor map of non-specific variables parameters of simplicia *Iresine herbstii* during storage.

The graph (Fig. 4) illustrates the distribution of different data points over two main dimensions. The x-axis represents Dim 1, which explains a large percentage of the variance, exactly 82.96%, making it the primary axis of variation among the data points. Dim 2, which is represented on the y-axis, explains a lesser amount of the variation, exactly 12.28%. The data points are numerically labeled from 0 to 32 and are distributed randomly on the graph. Every point in the collection represents a distinct observation or case. The dashed vertical line at x=0symbolizes the mean or median of Dim 1. It separates the data into two groups based on their scores in relation to this center value. The dashed magenta line could perhaps indicate a trend line or a distinct threshold inside Dim 2, emphasizing a certain element or subdivision within the data. The AIA exhibits a clear trajectory, progressing from the lower left to the top right quadrant, indicating a robust correlation with both dimensions. The concentration of MC around the center indicates a moderate impact on both dimensions. The WSE and ASE are distributed unevenly, suggesting different levels of impact on the dimensions. The various factors contribute to the primary dimensions, likely drawn from a broader set of underlying data. The arrangement and distribution of the data points can facilitate the detection of patterns, correlations, or groups among the variables during storing *I. herbstii* simplicia.



Figure 5. Results of multiple factorial analysis-individual variable non-specific parameters of simplicia *Iresine herbstii* during storage

Individual multiple-factor analysis is an analysis that can group individuals with similar profiles who are close to one another (**Fig. 5**). Individuals analyzed in MFA had storage times ranging from 0 to 32 months. The results of the individual multiple factor map analysis show that storage duration 0 (SD\_0) and storage duration 4 (SD\_4) are in the first-dimension plot; this shows that fresh simplicia (0 months storage duration) and four months storage duration are the best storage time of *I. herbstii* simplicia (positive coordinates). This is in sharp contrast to the storage duration 32 (SD\_32) plot, which is closest to dimension two, which is a negative coordinate (Abdi, Williams & Valentin, 2013). Storing *I. herbstii* simplicia for 32 months is the worst storage method because it can reduce the quality of the product. This research shows that the longer the simplicia is stored, the higher the MC. This means that the longer the simplicia is stored, the worse its quality. Other parameters that indicate simplicia's quality is worsening after long storage are the decrease in WSE and the decrease in ASE (Sari, Elya & Basah, 2020).

## 4. CONCLUSIONS

The DMRT study revealed that the duration of storage of *I. herbstii* simplicia had a significant impact on MC, WSE, ASE, and TA, but did not affect AIA. The MFA analysis demonstrated a significant correlation between general parameters and the length of storage of *I. herbstii* simplicia, with the exception of the concentration of AIA. There is a positive correlation between the storage period of 0 month and 4 months on non-specific factors, indicating that the optimal storage period is up to 4 months.

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# **Biology Learning Result of X Grade Senior High School Students through the Implementation of PBL combined with Flipped Classroom**

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## ABSTRACT

**KEYWORDS:** Flipped classroom is a learning method that allows students to learn the material flipped classroom, in advance at home according to the assignments given by the teacher. The learning outcomes, intent of this study is to discover out whether the flipped classroom-based PBL problem based learning learning approach impacts biology learning outcomes in class X SMA Negeri 4 Surakarta. This study conducted quasi-experimental research, using a post-test only control group approach. The population comes from class X students of SMA Negeri 4 Surakarta with the sample comprised of two classes generated using the cluster random sampling method, that is, phase E3 as the experimental class and phase E1 as the control class. Assessments, survey questionnaires, and observes are some of the data gathering procedures applied. Data analysis employs statistical techniques that are both descriptive and inference-based. The research results show that 1) The average cognitive learning results in the experimental class were 76.61, whereas the control group had 63.02. 2) The © 2024 The Author(s). Published by Biology Education Department, average affective test scores in the experimental class were 72.73, alongside the Faculty of Teacher Training and Education, Universitas control class had 67.91. 3) The average results of psychomotor learning section Muhammadiyah Surakarta. in the experimental group had 67,36 along with the result from control group This is an open access article under the CC BY-NC license: had 61,11. In accordance with the research findings, it can be inferred that https://creativecommons.org/license flipped-based PBL learning classrooms influence biology learning outcomes in s/by-nc/4.0/. biotechnology material. Even though it has advantages, implementing flipped classroom learning can also potentially cause several obstacles.

# 1. INTRODUCTION

Learning is an assortment of change experienced by students, who are able to engage in inventive manners as a result of stimuli and reactions. Learning can also be referred to as a person's process or effort to achieve several kinds of skills, competencies, and attitudes (Salam, 2017). So, learning activities play a very important role in creating experiences that will later become the basis for students' social life. Learning is a way or a process or action that can change someone's learning; in other words, learning is a method that is deliberately carried out or designed so that students learn (Ziraluo, 2021). Learning in education includes many branches of science, including science, such as biology. Biology is the science of life (life science), the phenomena of life processes that interact directly in society (Rustaman, 2016).

The learning model is one of several aspects that determine the success of learning, both internally and externally. The framework for learning is a sequence of activities implemented by the teacher in charge with the aim of conveying lesson material. Learning models frequently emerge using the concepts of learning, psychological and sociological paradigms, systems evaluation, or various other concepts that support them (Khoerunnisa & Aqwal, 2020). Teachers can use various types of learning models in the learning process. Choosing creative and innovative learning models can increase student competence. According to (Fitri, 2022) a learning model is

an approach of instruction that is revealed from its inception to the finish of learning that becomes the frame for implementing an approach, method, strategy, and learning technique.

Problem-based Learning (PBL) is an educational model that is considered to promote student engagement. It is a learning system that relies on a problem faced by students during the process of gaining knowledge (Barokah, 2019). According to (Pramana, Jampel, & Pudjawan, 2020) Problem-based learning is a learning strategy that encourages the students to think critically when tackling current issues. This is related to problem-based learning, which focuses students on being able to solve problems around the students' learning environment.

The characteristics of PBL include three essential elements, namely the existence of a problem, student-centered learning, and learning in small groups (Hotimah, 2020). The goal of the problem-based learning (PBL) method of teaching is to enhance the problem-solving abilities of students and in helping them to gain various experiences and be able to change students behavior, strengthen critical thinking skills and the capacity to address any circumstance.

(Febrita & Harni, 2020). Research (Silvi, Witarsa, & Ananda, 2020) states that the advantages of the PBL approach are that students are encouraged to have the capacity to overcome issues in actual settings. Students can also expand their understanding through learning activities, learning is focused on problems, so that irrelevant information does not need to be reviewed by students.

. Besides the advantages of PBL, there are also several weaknesses of PBL based on (Eskris, 2021). PBL requires quite a long time in preparation for the course of study, and students are less motivated to study when they fail to comprehend the difficulties they're handling. Some students will also experience difficulties if they study independently. Then (Sinmas, Sundaygara, & Pranata, 2019) stated that one of the learning frameworks that can be effectively applied to complement the PBL learning approach is the Flipped Classroom method.

The Flipped Classroom is an educational strategy in which students examine learning materials at home prior class, directed by the teacher's assignments. Before discussing new subjects in class, the teacher provides assignments for students to familiarize themselves with the material utilizing a variety of learning materials (Agustini, 2021). In pursuance of (Maolidah, Ruhimat, & Dewi, 2017) this type of learning style makes the most of learning resources that students can access online as part of their learning in the classroom. Thus, the flipped teaching technique is an instructional strategy that employs a blended learning approach by reversing the conventional classroom dynamic and delivering educational content outside the traditional classroom setting. (Most are carried out online) (Susanti & Hamama Pitra, 2019).

The ultimate purpose of classroom learning activities is to produce outcomes that promote learning. Academic results may be enhanced by various deliberate attempts carried out systematically, resulting to beneficial changes that are then referred to as the learning process (Rusnawati, 2020). In research (Wardana & Sagoro, 2019) These results may be examined using tools such as learning outcome evaluation items; an improvement in learning outcomes can be demonstrated through comparing the mean results from learning to the percent of student learning fulfillment in pre-research and all subsequent cycles.

The teaching and learning process consists of cognitive, affective, and psychomotor aspects, which are used as guidelines for assessing student learning outcomes. These three factors will demonstrate students accomplishments while absorbing knowledge. According to Bloom (Sudjana, 2006) learning outcomes can be classified into three main groups: cognitive, affective, and psychomotor. The cognitive field encompasses mental (brain) behavior (Ismail M. I., 2021). As for opinion (Huliatunisa, 2022) in his book states that the cognitive area relates to memory or knowledge and intellectual abilities and skills in the cognitive area. There are six levels of mental functions, ranging from the lowest domain to the most complex dimension. The emotive domain essentially refers to beliefs and principles (Suryadi, 2020). Zainal (2014) states that the area of affect cannot be examined in the same way that the cognitive field can. This is because aptitudes measured in the domain of affect involve acquiring (paying attention), reacting, embracing,

coordinating, and valuing qualities. The psychomotor domain is pertain to the ability to respond once the students obtain specific learning encounters; this learning outcome is a continuation stage of affective learning outcomes, which only appear in students' tendencies to behave (Widodo, 2021).

This study was excuted in SMA Negeri 4 Surakarta. Based on preliminary research (Mahardika, 2021), learning at SMA Negeri 4 Surakarta emphasizes on mastering the contents, so the learning method applied is the lecture method. Student learning outcomes are not optimal because students' enthusiasm and urge to learn are insufficient. In order to enhance the quality of education, consider using a learning paradigm such as exploratory learning, problems-based instruction, or contextual education.

The main objective of this study is to discover how the PBL learning paradigm, in conjunction with a flipped classroom, affects biology learning results in class X at SMA Negeri 4 Surakarta.

# 2. MATERIALS AND METHODS

2.1. Type of Research and Research Design

## 2.1.1. Type of Research

This is quasi-experimental research. The study's dependent variable is Biology learning outcomes. Meanwhile, the independent variable is the learning model.

# 2.1.2. Research Design

The research design applied to this study is a post-test only control group. In this approach, both the experimental and control classes are submitted to a posttest, however only the group classified as experimental is treated, hence the design was arranged as follows:

## **Table 1 Posttest Only Control Group Design**

Class	Treatment	Post-test
E	Х	P1
K	Y	P1

# Information:

- E = Experimental Class
- K = Control Class
- P1 = Post-test
- X = The treatment uses a PBL learning model combined with Flipped Classroom

Y = Treatment without using the PBL learning model combined with Flipped Classroom

# 2.2. Place and Time of Research

## 2.2.1. Place of Research

The study was performed in class X of SMA Negeri 4 Surakarta throughout the 2023/2024 term of study.

# 2.2.2. Time of Research

The research was carried out in February to May 2024.

2.3. Population, Sample, and Sampling

# 2.3.1. Population

The population involved was X grade of SMA Negeri 4 Surakarta for the 2023-2024 academic year which consists of 11 classes, there are approximately 36 students in each of the classes, for a total of 396 students.

## 2.3.2. Sample

The study's sample consisted of students in E1 and E3 classes at SMA Negeri 4 Surakarta. Phase E1 is the control group, and Phase E3 is the experimental group.

# 2.3.3. Sampling

This study applied cluster random sampling. Cluster random sampling is a research tool of the source of large quantities of data. Cluster random sampling was adopted as a sampling strategy since the data gathered was regular and identical; samples were consequently selected at random.

Table 2. Research Sample for Class X SMA Negeri 4 Surakarta for the 2023/2024 Academic Year

No Class		Am	Total	
		Male	Female	
1	Phase E1	19	17	36
2	Phase E3	20	16	36
	Total	39	33	72

## 2.4. Data and Data Sources

## **Table 3 Data and Data Sources**

No	Data	Data Source	Data Collection Technique	Instrument
1	Cognitive learning outcomes	Student	Test	Question sheet
2	Affective learning outcomes	Student	Questionnaire	questionnaire sheet
3	Psychomotor learning outcomes	Student	Observation	observation sheet

# 2.5. Data Collection Techniques

Data about studies on the implementation of Problem-Based Learning with Flipped Classroom was collected through observation, questionnaires, and interviews.

# 2.6. Research Procedure

Researchers are required to carry out stages in carrying out research activities. These stages include preparation, implementation, and data processing.

# 2.7. Data Analysis Technique

In this study data was analyzed using both descriptive and inferential statistic. Specifically, data analysis of post-test outcomes from the experimental and control classes. The data was analyzed afterwards to determine the impact of the Problem-Based Learning model with Flipped Classroom on the biology learning outcomes of class X SMA Negeri 4 Surakarta.
## 2.7.1. Descriptive Statistical Analysis

A descriptive statistical approach is used to offer a fundamental overview of the features of student learning outcomes in the experimental and control groups. This data analysis can be calculated using SPSS Statistics 25; the data analysed is learning outcome data, which is first compared with the student learning outcome criteria as follows:

Lable I Categorization	usie i cutegorization of statemes ziology zearining outcomes				
Value Interval	Predicate	Information			
93-100	А	Very Good			
84-92	В	Good			
75-83	С	Enough			
67-74	D	Less			
$\leq 66$	E	Very Less			

Table 4	Categori	ization d	of Students'	Biology	Learning	Outcomes
I GOIC I	Cutty		n bruachto	DIVIUS	Louining	oucomes

Source: (Kemendikbud, 2017)

## 2.7.2. Quantitative Analysis

The analysis of quantitative data was conducted in two stages, namely, testing prerequisites for analysis and hypothesis testing. Normality and homogeneity tests are utilized in precondition assessments. Apply one-way ANOVA for assessing the hypothesis.

## 3. RESULTS AND DISCUSSION

#### 3.1.Results

The goal of this study is to examine whether PBL with flipped classrooms influences biology learning outcomes in class X at SMA Negeri 4 Surakarta in the school year 2023/2024. This research was performed during three meetings on Biotechnology material. In the experimental setting, treatment was distributed to execute the PBL with a flipped classroom model utilizing Google Classroom as a virtual educational platform and learning videos; in contrast, the control class used conventional methods of learning supported by PowerPoint media. The findings of the study can be explained as follows.

## 3.1.1. Cognitive Learning Outcomes

Details on cognitive learning outcomes were acquired from posttest results on Biotechnology material. A description of cognitive learning outcome data is presented in Tables 5 and 6.

# Table 5. Cognitive Learning Outcomes of Class 10 Students at SMA Negeri 4 Surakarta for the 2023/2024 Academic Year Through the Application of PBL with Flipped Classroom Learning Model

No.	Class	Ν	KKM	Ā	S	$S^2$	Xmax	Xmin
1.	Experimental	36	75	76.61	16.59	266.62	100	33
2.	Control	36	75	63.02	8.52	32.63	93	33

## **Table 6. Frequency of Cognitive Learning Outcomes**

Category	Class Experimental	Class Control
Very Good	55.55%	22.22%
Good	38.90%	52.78%
Enough	5.55%	25%
Not Enough	-	-

Based on Table 5, a normality test was performed. The normality test utilizes the Kolmogorov-Smirnov and Shapiro-Wilk tests (Table 7).

	Class	Kolmoş	gorov-Sn	nirnov <sup>a</sup>	Shapiro-Wilk		
	Chubb	Statistic	Df	Sig.	Statistic	df	Sig.
Learning	Class Experimental	.150	36	.040	.926	36	.019
Outcomes	Class Control	.097	36	.200*	.940	36	.052
		~ .					

#### **Table 7 Normality Test**

a. Lilliefors Significance Correction

Based on the normality test table previously, the df value (degrees of freedom) for the experimental and control classes is 36, indicating that the Shapiro-Wilk technique is adequate for addressing data normality in this study. Then, The output result is Sig. for the experimental category of 0.019 and Sig. for the control category of 0.052. Because the value of Sig. for both groups is higher (>) than 0.05, it may be maintained that the learning outcomes of the experimental and control classes are typically distributed as data.

The analysis prerequisite test is then continued with a homogeneity test (Table 8). Following to the findings of the homogeneity, the relevance value is 0.420 (more than 0.05), and the data provided is homogeneous and may be used for analysis of difference tests or ANOVA.

## **Table 8 Homogeneity Test**

		Levene Statistic	df1	df2	Sig.
Learning	Based on Mean	.659	1	70	.420
Outcomes	Based on Median	.636	1	70	.428
	Based on Median and with adjusted df	.636	1	69,990	.428
	Based on trimmed mean	.681	1	70	.412

Hypothesis testing was carried out using one-way ANOVA (Table 9).

## Table 9 One Way ANOVA

C Learning Outcomes						
_	Sum of Squares	Df	Mean Square	F	Sig.	
Between Groups	3321.125	1	3321.125	10.740	.002	
Within Groups	21645.528	70	309.222			
Total	24966.653	71				

Significant provisions (Sig.) < 0,05 according to the ANOVA table previously, the values p-value (Sig.) is 0,002 < 0,05. Thus, the test decision contradicts H0, which states that there is a substantial disparity in outcomes for learning between the control and experimental classes.

## 3.1.2. Affective Learning Outcomes

Questionnaires assessing students' creative thinking abilities provided data regarding affective learning outcomes. Tables 10 and 11 describe affective learning outcome data.

# Table 10. Affective Learning Outcomes of Class 10 Students at SMA Negeri 4 Surakarta for the 2023/2024 Academic Year Through the Application of PBL with Flipped Classroom Learning Model

			8					
No.	Class	Ν	KKM	Ā	S	$\mathbf{S}^2$	Xmax	Xmin
1.	Experimental	36	75	72.63	8.59	73.86	98.33	58.33
2.	Control	36	75	67.91	5.59	31.32	83.33	58.33

## **Table 11. Frequency of Affective Learning Outcomes**

Category	Class Experimental	Class Control
Very Good	33,33%	2,78%
Good	66,67%	97,22%
Enough	-	-
Not Enough	-	-

## 3.1.3. Psychomotor Learning Outcomes

Data on psychomotor learning outcomes were gathered through observations on attitude assessments. Tables 12 and 13 describe the psychomotor learning outcome data.

# Table 12. Psychomotor Learning Outcomes of Class 10 Students at SMA Negeri 4 Surakarta for the 2023/2024 Academic Year Through the Application of PBL with Flipped Classroom Learning Model

No.	Class	Ν	KKM	ā	S	$\mathbf{S}^2$	X <sub>max</sub>	X <sub>min</sub>
1.	Experimental	36	75	67.36	12.5	156.25	91.67	41.67
2.	Control	36	75	61.11	12.78	162.69	83.33	41.67

1 0	• 8	
Category	<b>Class Experimental</b>	Class Control
Very Good	33.33%	25%
Good	61.11%	63.89%
Enough	5.56%	11.11
Not Enough	-	-

## Table 13. Frequency of Psychomotor Learning Outcomes

## 3.2. Discussion

The deployment of the flipped classroom-based PBL learning technique has been explored in two classes: experimental and control. This study contains two variables: the independent variable and the dependent variable. The independent variable in the current research is the flipped classroom-based PBL learning approach, featuring the use of a web-based educational tool called Google Classroom. The dependent variable in this research inquiry is learning outcomes, which are assessed using cognitive, psychomotor, and affective measures. The findings and discussion of the study may be interpreted as follows.

## 3.2.1. Cognitive Learning Outcomes

Cognitive learning outcomes are a form of understanding that must be possessed by students who are actively participating in the learning programs, this will help students comprehend the learning content and improve their knowledge, resulting in improved cognitive learning achievements. Cognitive learning outcomes are an indicator of students' level of mastery about the subjects they have studied or their understanding of a particular topic in learning activities in the context of understanding or theory related to knowledge, and the enhancement of cognitive competencies that include recalling and recognizing of facts, operational patterns, and ideas in developing students' intellectual skills and competencies (Lestari & Irawati, 2020). Data on student learning outcomes in cognitive section for class 10 at SMA Negeri 4 Surakarta in year of study 2023-2024 were obtained from post-test results. Based on the previously mentioned analyzed data results, the experimental class had higher average on the cognitive learning outcomes compared to the control class; this can be seen in Table 5. The experimental class averaged grades is 76.61, following by the mean in the control class was 63.02. The gap between the average scores of the experimental class and the control class is quite significants, which is 13.59, so from this average value, the treatment of problem-based learning methods based on the flipped classroom influences the learning result afterwards. This aligns with previous research from (Putri, Masruhim, & Widiyowati, 2021) titled *The Effect of Flipped Classroom Model Towards Student's Learning Outcomes*. According to the subject of the buffer solution, this research results show that average points in the control class was 76.62 beside the experimental class was 76.62.

The frequency of cognitive learning outcomes can be categorized into four categories: excellent, good, enough, and not enough. In table 6, The occurences of cognitive learning outcomes observed in the first category, namely the outstanding category in the experimental class, has a percentage of 55.55%, and for the control group, the result show of 22.22%. The sound category of the control class has a higher number in 52.78%, while for the control class, it is 38.90%. The next category is simply an experimental class that only has a percentage of 5.55%, the control class has a 25% in this category. However in the last category, both the experimental and control sections had the same results that is 0%. Therefore, the experimental students get more of cognitive learning outcomes than of the control class.

Statistical test is required to assess if the differences between averages of the two classes is significant or not. Before determining the type of test to use, first off, all prerequisite tests are analyzed with normality and homogeneity tests. The normality test determines whether the data is regularly distributed or not. The Saphiro-Wilk test is suitable for normality testing. The criteria used is if value Sig. > 0.05, and the material is typically distributed. The normality test results obtained values Sig. The data is usually distributed in 0.019 for experimental classes and 0.052 for the control area. This result is in accordance with previous research by (Ismail S., 2022). It is known that the Sig value is 0.158 for the experimental group different from the control group that had 0.455, therefore because the Sig value for both groups > 0,05, it can be interpreted that the *Shapiro Wilk* normality test result has shown as normally distributed data.

Usmadi (2020), in his research, stated that if the data group maintains as a normal distribution, then the homogeneity test may be applied. Based on Table 7, the significance value (Sig) based on the mean for the learning outcome variable is 0.420. Since the value of Sig (0.420) is greater than 0.05, it can be concluded that the variance of learning outcome data in both the experimental class and the control class is homogeneous. This is align with previous research from (Wijayanto, Sukestiyarno, Wijayanti, & Pujiastuti, 2022), and the homogeneity test results obtained values Sig. =  $0.384 > \alpha = 0.05$ , which can be interpreted as meaning that the collected information comes from a population that shares the same/homogeneous variance. Prerequisite analysis result has shown that it can be informed that the data is usually distributed and the data is homogeneous, so it passess the parameter for further testing, thereby is the one-way ANOVA test. As mentioned from the table 8 before, the one-way ANOVA test result show the value Sig. 0.002 < 0.05. As this implies, it is possible to infer that the hypothesis was rejected, indicating that there are variations in learning outcomes between the comparison group and trial classes. These findings are in accordance with research by Nisya & Nindiasari (2023) result of the one-way ANOVA test has shown value Sig. 0.21 < 0.05. Then it could potentially presented that H0 is rejected, implying that there is a difference in the mean score of numeracy ability between courses given with the Flipped Classroom model, which has been integrated with Problem-based Learning, and classes delivered using the Problem-based Learning model. This research is reinforced by the results of descriptive statistics analysis data shown in Table 5, this is indicate that the average score of the experimental class which is 76.61 is higher than the control class that is 63.02.

In research, Rusnawati (2020) stated that students from SMKN 1 Negara have higher study outcomes when the flipped classroom learning model was implemented. The condition can happen because students are more impressed by being active in participating in learning, so students think it is easier to comprehends the course content provided through the flipped classroom learning model. This statement was confirmed by the opinion (Mirlanda et al., 2020) that optimizing learning activities in class by exchanging ideas and practicing allows students to train and hone their reasoning, which can be interpreted indirectly as students practicing making choices and drawing conclusions in a way that is appropriate and of course makes sense.

## 3.2.2. Affective Learning Outcomes

Affective learning outcomes assess student capabilities that relies on thoughts, sensations, and reactions rather than logical reasoning. This particular type of evaluation was directly related to attitudes among students and desires, encompassing self-assurance, dedication, courtesy, integrity, self-control, collaboration, and other distinctive features. it is understandable that affective assessment focuses on measuring learning outcomes, which is related to character cultivation so that the teacher could implement the evaluation finding to enhances the studying system and maximize an already good learning system (Akbar et al., 2021).

Data from affective student learning outputs were obtained from the survey answer on students' inventive thinking capacity. According to the results of descriptive data analysis, the mean score of effective learning outputs for the experimental class were more significants than the average scores from the control class; this result has shown in Table 8. The experimental class has average grades of 72.63, while the control section has an average value of 67.91. The difference of average scores between the two research classes were relatively small that is at 4,72; this is different from the achieved cognitive learning outcomes of 13.59. Next, the maximum score in the experimental class is 98.33, and the maximum value for the control class. For the lowest scores, both of the class have the same value, which is 58.83 for experimental and controls. This is in accordance with research Rizaldi's (2022) the fundamental overview data on students' affective learning outcomes, which could be mentioned as the Mean or average score obtained; there is an increase in the value in the class being tried (Experimental), mean gain of 77.93 and the control group is 70.45.

The amount of frequency of affective study outcomes is categorized into four categories: excellent, good, enough, and not enough. In Table 4.7, the total amounts of affective learning outcomes for the first category, namely perfect for the experimental section, has a percentage of 33.33%, however for the control class, it has a percentage of 2.78%. Move to the second category, namely good for the experimental class, has a percentage of 66,67%, following by the control class that has a percentage of 97.22%. The third category is enough, and the fourth category is not enough; both of the research classes have the same percentage, which is 0%. Therefore, the experimental class outperformed the control class in affective learning sections.

According to research (Pratiwi et al., 2017), Effective learning outcomes show positive values when implementing the flipped classroom learning model. This situation occurs because students feel confident about taking part in learning in class. After all, they already have the material for learning in advance. Besides that, the flipped classroom learning model also helps students to learn independently because it requires them to manage their own time. This can teach them to have an orderly attitude towards the rules and be committed to carrying out the tasks given by the teacher (Fatimah et al., 2022). In research, Fatimah (2022) states that when implementing the flipped classroom learning model, student learning initiative increases by 30%, which means students can carry out learning activities independently without influence from other people.

#### 3.2.3. Psychomotor Learning Outcomes

Psychomotor learning outcomes are aspects of learning outcomes related to the use of basic skills and physical movements. Psychomotor aspects play a vital role in human life. This ability is developed through learning and practice. The psychomotor domain is also related to skills (keterampilan) of a manual or motor nature (Neno, 2023). According to Sugiarti (2018), a student's psychomotor abilities are closely related to skill abilities, which can be seen through practical or experimental activities. It could be potentially inferred that the psychomotor domain is the domain connected to skills (keterampilan) or the capacity to respond when someone obtains or perceived a certain phase of learning (Fathurrahman, 2020).

Data on psychomotor student learning outcomes were obtained from the output of attitude assessment observations. Depend on the previous results of descriptive data analysis in Table 9, it shows that the average score of experimental class students is higher on this section; this is in accordance with cognitive learning outcomes and affective learning outcomes. Based from the result of experimental class, the average effective learning outcomes are 67.36, meanwhile the control class only has 61.11 as their average score. Hence, there is some distinction between the experimental and the control classes that is 6.25. Furthermore, the maximum score for psychomotor learning outcomes is also greater than the control class, with the maximum score in the experimental class being 91.67, but for the control class only has 83.33 points. For the minimum value, there are some traits in common between the experimental and the control classes, which is 41.67.

Table 9 also displays the frequency of psychomotor learning outcomes; in this table, there are four categories of evaluations result by psychomotor learning outcomes that can be mentioned as very good, good, enough, and not enough. The first category is outstanding; the experimental results obtained have a percentage of 33.33%, while for the control class, it has a percentage of 25%. The second category, which is good, obtained the percentage of the experimental class at 61,11% and for the control class at 63.89%. For the third category, it is enough to obtain the respective percentage of results for the both research classes, which are 5.56% in experimental and 11.11% for the control group. The last score from this category by both classes performed the same percentage, which is 0%. As a result of the study this learning outcome occur more frequently in the experimental class.

The control class demonstrade inferior outcome in learning from psychomotor domain than those of the experimental. This can be considering that the control class only uses non-PBL learning models. This metodhology demand students to merely write down and pay attention to the teacher's explanation, which results in students lacking skills in the learning process and finding it difficult to achieve optimal learning success. The non-PBL learning model emphasizes teacher-centered learning. Besides that, the psychomotor skills that appear in learning using this model do very little to develop students' psychomotor skills.

They are referring to research (Hamid & Effendi, 2019) that shows that the flipped classroom learning model influences the outputs of learning in students during the psychomotor domain. However, this is different from research findings (Rahma et al., 2023), where the flipped classroom learning model does not influence psychomotor learning outcomes. External factors or internal factors can cause this incident. Students' habits can influence internal student learning outcomes at home, in addition there is an abundance on student learning outcomes and their willingness to learning for the time ahead. Neighborhood environment, school, and family also have an impact on external influences.

## 4. CONCLUSIONS

The main findings of this research and statistical tests carried out can be informed that flipped classroom-based PBL learning influences biology learning outcomes in biotechnology material. This effect is caused by the PBL with Flipped Classroom learning process, which gives students more time to understand the lesson material, as well as the existence of online learning media, which allows students to learn independently. Typically, flipped classroom learning contains with both outside and inside activities in class. These two activities are equally important and must run well to obtain maximum results. Although it has more significant advantages, the application of flipped classroom learning may also additionally present some several obstacles. The teacher's role is vital in overcoming existing obstacles for the successful implementation of PBL with Flipped Classroom learning.

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# Gender Differences and Learning Outcomes Using the Ethno-PJBL Model

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#### ABSTRACT

<b>KEYWORDS:</b> PjBL,	Biology learning in the 21st century is a challenge for teaching staff in guiding students in developing their abilities in accordance with the 4Cs and will
Ethnoscience,	produce competent students. One learning model that integrates 4C is project-
Gender	based learning (PjBL) which is oriented to ethnoscience. The aim of this
	research is to determine gender differences in the implementation of ethno-
	PjBL learning. The research method is descriptive quantitative. Data analysis
	uses the Rasch model to analyze the suitability of the items to the model used
	and the t-test to determine the difference in cognitive scores of male and female
	students. The research results show that there are differences in learning
	outcomes between men and women. The average score for male is 68 and
	female is 73.5. The Levene Test results show $0.591 > 0.05$ , which indicates that
	the two gender groups have different learning outcomes. Male students
© 2024 The Author(s). Published by Biology Education Department	complete assignments more quickly, tend to focus on content and are less
Faculty of Teacher Training and	interested in ethnoscience material, but their learning outcomes are no better
Education, Universitas Muhammadiyah Surakarta.	than female students. Female students take longer to complete project
This is an open access article under the CC BY-NC license: https://creativecommons.org/license	assignments than male students. Female students are more interested in
	contextual learning presented through ethnoscience and structured design
s/by-nc/4.0/.	assignments through PjBL.

## 1. INTRODUCTION

21st century learning has characteristics which are usually referred to as the 4Cs which include creative thinking skills, critical thinking and problem solving, communicating and collaborating (Gunn & Hollingsworth, 2013). 21st century learning is a challenge for teaching staff in guiding students in developing their abilities in accordance with the 4Cs and will later produce competent students (Laal et al., 2012). One type of learning that is influenced by the 21st century is learning in the field of Biology. The demands of 21st century skills must be implemented in all fields, one of which is Biology learning (Fung et al., 2022).

Biology learning in the 21st century has experienced many significant developments along with advances in technology, new scientific discoveries, and changes in learning approaches or methods (Ligan & Tacadena, 2022). The learning model that is expected to help students have the ability to think creatively in the biology learning process is the Project-based Learning (PjBL) learning model (Elliott, 2020). The Project-Based Learning (PjBL) model is an innovative learning model which uses projects as a learning medium, the use of this project is expected to be able to actively involve students in learning and problem solving, students are also expected to be able to work in groups to produce useful products (Shuhailo & Derkach, 2021).

Project-based learning is easier for students to understand so that learning is linked to daily life according to the potential of their respective areas (Al Mulhim & Eldokhny, 2020). The potential possessed by each region includes natural resources, culture and local wisdom (Lavrillier & Gabyshev, 2021). There is a lot of Indonesian culture that can be applied in ethnoscience-based learning, especially in biology subjects or what is usually called bioethnoscience (Suciyati et al., 2021).

Ethnoscience is an activity of transforming original knowledge into scientific knowledge (Abonyi et al., 2014). Efforts to use the environment as a learning resource in science learning are by integrating culture into society, known as ethnoscience learning (Moodie, 2019). Ethnoscience is currently one of the things studied in Indonesia because it can make students aware of rediscovering local intellectual values and integrating them into the learning process (Zidny et al., 2021).

Tanaka (2023) has investigated the influence of gender on PBL, and concluded that gender influences the way PBL students approach project work. Afriana et al., (2016) describe women's tendency toward context orientation in engineering design tasks, and note that female learners. The research results of Ali Samsudin et al., (2018) states that several factors influence the success of PjBL learning, including gender. Female students tend to be interested in structured design tasks and tend to undertake a broader range of engineering problems than males. However, the investigation carried out by Muspiroh, (2020) actually showed different results. Muspiroh stated that there is no influence of gender on the implementation of learning using PjBL. Therefore, research needs to be carried out to determine the influence of gender on PjBL by integrating an ethnoscience approach.

## 2. MATERIALS AND METHODS

## 2.1. Research Design

This type of research is quantitative descriptive research. Descriptive research is research with the aim of testing a hypothesis by describing the actual situation of a variable. Meanwhile, quantitative is research with data presented in the form of numbers from the start of data collection until the results obtained.

#### 2.2. Population, sample and sampling

#### 2.2.1. Population

The research population is class IX students in the second semester at SMPN 3 Babakancikao, Purwakarta, West-Java, Indonesia, in the 2023/2024 academic year with a total of 66 students.

#### 2.2.2. Sample

The sample in this study was all students in class XI Science semester II, totaling 66 students.

#### 2.2.3. Sampling

The sampling method used is total sampling. total sampling is a sample selection technique carried out on all members of the population. In this research, samples were taken from the entire existing population.

#### 2.3. Data analysis

#### 2.3.1. Item Analysis Using Rasch Model

The approach taken in Rasch modeling is different. The main goal of Rasch modeling is to create a measurement scale with equal intervals. Because raw scores do not have interval properties, they are not used directly to provide an interpretation of student abilities. Rasch modeling jointly uses score data based on each person and score data per item. These two scores

are the basis for estimating the true score which shows the level of individual ability and the level of item difficulty.

#### 2.3.2. T-test

The t-test is an inferential statistic used to determine whether there is a significant difference between the means of two groups and their patterns of association. The t test was carried out using SPSS 16 software.

## 3. RESULTS AND DISCUSSION

#### 3.1. Item Analysis

Fit items mean that the item behaves consistently with what is expected by the model (Sumintono, 2018). If it is found that the questions do not fit, this is an indication that there are misconceptions among students regarding the questions. Some of the fit indices provided in Rasch analysis are Person Infit ZSTD, Person Outfit ZSTD, Person Infit MNSQ, Person Outfit MNSQ, Item Infit ZSTD, Item Outfit ZSTD, Item Infit MNSQ, and Item Outfit MNSQ (Boonee- et al., 2014).

Perso	on	66 II	NPUT	66 MEASU	RED	INFI	Т	OUTF	IT
	1	TOTAL	COUNT	MEASU	RE REALSE	IMNSQ	ZSTD	OMNSQ	ZSTD
MEAN		17.6	25.0	1.	04 .53	1.00	.1	1.00	.1
P.SD		2.3	. 0	-	61 .07	.17	.8	.58	.7
REAL	RMSE	.54	TRUE SD	.29	SEPARATION	.54 Pers	on REL	IABILITY	.22
Item		25 INPL	UT 25	MEASURE	 D	INF I	т	OUTF	IT
	1	OTAL	COUNT	MEASU	RE REALSE	IMNSQ	ZSTD	OMNSQ	ZSTD
MEAN		46.5	66.0		36 .47	1.00	.1	1.00	.1
		13.8	. 0	1.	74 .43	. 08	.9	.23	1.1
P.20									202.0000

Figure 1. Results of Item Analysis

The MNSQ value is always positive and moves from zero (0) to infinity ( $\infty$ ). In this case, the MNSQ value is used to monitor the suitability of the data to the model. The expected value is 1 (one). The mean square infit or outfit value is less than 1, indicating that the observed data has 22% less variation than predicted. The research results show that the z value is -1 and the standard deviation is 1.1, which means that the observed data is in accordance with the model. Meanwhile, a negative value indicates that there is not much variation in the answers. The responses were fairly regular and predictable.

The results of the analysis carried out obtained an MNSQ value of 1.00 and a ZSTD of -1, which means that the question items are good and can be used, even though the variation in answers is not too high. The level of suitability of this item is greatly influenced by the size of the sample. Errors in answer keys, many individuals who are careless in working on questions, and questions that have low differentiation power can reduce the appropriateness value of items (Bambang Sumintono. & Wahyu Widhiarso., 2013).

## 3.2. Gender Differences in Learning

Cognitive scores are obtained through a posttest in the form of multiple choice questions. These values are analyzed to determine the highest value, lowest value, mode, average, standard deviation, standard error for both types of gender. The results of the analysis are presented in table 1.

	Gender			
Cognitive	Male	Female		
Ν	36	32		
Max	68	92		
Min	48	52		
Mode	64	84		
Mean	68	73.5		
Standart deviasi	8.504901	8.9861		
Standart. Error Mean	1.438	1.614		

#### Table 1. Cognitive Learning Outcomes by Gender

Table 1 shows that the maximum value, minimum value, mode and mean of female students are better than male students. This value was obtained from the posttest questions in the form of 25 multiple choice questions which were completed at the end of the ethnoscience-based project learning. Even though the post-test results obtained show significant differences between the two gender groups, it is necessary to carry out a t test further to see the significance of the differences between the two. T test results are presented in table 2.

			I	ndepende	ent Samp	les Test			
	Levene for Equ Varia	e's Test iality of ances	ſ	_	t-test	for Equality	of Means		
	F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Co Interva Differ Lower	nfidence l of the rence Upper
Equal variances assumed	.291	.591	-2.399	66	.019	-5.167	2.154	-9.467	866
Equal variances not assumed			-2.390	64.014	.020	-5.167	2.161	-9.484	849

#### Table 2. T-test Results of Cognitive Scores Based on Gender

Based on the t-test that has been carried out, it is known that the Levene's Test results are 0.591 > 0.05, so the data used is Equal variances assumed data with a significance result of 0.019 < 0.05, which indicates that there is a significant difference between the cognitive scores of male and female students use the ethnoscience-based PjBL learning model. Through the t-test, it is known that the two gender groups show different learning outcomes.

The term gender is defined as a socio-cultural phenomenon, a social consciousness, and as a concept for analysis. Gender is a cultural concept used to differentiate the roles, behavior, mentality and emotional characteristics of men and women that develop in society. Gender in education is not differentiated. Every student, both male and female, has the same opportunities in the learning process. Male and female students have the same opportunity to achieve maximum cognitive achievement.

Based on the research that has been conducted, it appears that overall the cognitive scores of female students are higher than those of male students. Through ethnoscience-based project learning, female students obtained better results than male students, but showed different patterns. Male students tend to focus more on the core of the material being studied, regardless of whether there is assistance in the form of an ethnoscience approach or not. Because male students focus more on the material, they tend to be faster in completing project assignments. Although male students excel in terms of speed of completing tasks, they do not show the same results in terms of cognitive tests.



Figure 2. Ethno-PjBL learning patterns for male and female students

The use of ethnoscience-based project learning for female students has had a positive impact. Project-based learning builds collaboration patterns between students. The integration of ethnoscience has an impact on the material being studied to become more concrete. The process of transforming local science into modern science requires additional time. Through learning using this model, female students take longer to complete ethnoscience-based project assignments, but they show much better cognitive achievement results than male students.

Small group activities play an important role in project-based learning for cooperative decision making and problem solving among team members (Torres et al., 2019). Sometimes it is very easy for students to develop individual plans for a project, but it is necessary to determine the best solution through agreement and negotiation within the team. This will always be a challenge for lecturers (Guo et al., 2020). Collaborative learning is essential, and students can learn real-world skills naturally through project-based learning (Lin et al., 2021). Project-based learning is defined as a learning technique that allows students to carry out meaningful (Castro-Vargas et al., 2020). Project-based learning is a method where students learn problem solving through activities that involve in-depth work on assigned projects. Project-based learning is characterized by meaningful activities, learning, collaborative decision making, and problem solving through the use of digital videos, and changes in the role of lecturers (Huysken et al., 2019).



Figure 3. Interaction in PjBL and Ethnoscience Learning

Ethnoscience learning can transform teacher-centered learning into student-centered learning in the form of contextual and meaningful learning (Croce, 2019).Students can not only understand the material but also apply it in everyday life (Yustina et al., 2020).This makes students more interested in studying natural science and applying it to the living environment (Bohensky & Maru, 2011). The activities are ethnoscience-based, including relating material to the culture in the surrounding environment and context. The implementation of learning activities is adapted to local situations and always connects national knowledge with related topics. In other words, the application of an ethnoscience-based science learning approach only applies to certain material that can be linked to an ethnoscience approach (Zidny et al., 2020). Ethnoscience-based learning can be applied using student-centered learning methods, namely, methods of observation, questioning, classification, problem solving and drawing conclusions (Croce, 2020).

The use of the ethno-PjBL model in this research shows that most male students joke more or disturb their friends who are doing their assignments. Many male students are less motivated to participate in learning, and do not do the assignments or exercises given optimally. In general, female students are more diligent in studying and obey school rules. This is one of the factors that on average, female students have better academic achievement.

The above findings are confirmed by the results of Agustrin & Rindrayani (2016) research which states that female students tend to care more about studies and they work harder than male students. Boys are more interested in technology than girls because they are always exploring computers and playing video games and they rely on technology more than girls. There are definitely solutions that can keep both genders on the same academic level such as including more technology in learning and including more practical work in academics.

Several types of research have been conducted on the topic of comparison between the performance of male students and female students. Female students are found to do better in class than male students. Female students are claimed to have better learning skills compared to boys (Ali Samsudin et al., 2018).Female students (regardless of educational background) were found to be more active in academics than male students (Afriana et al., 2016). It is a fact that male students have a lot of knowledge about the technical world and their intelligence level is completely different from that of girls but still, the difference between the values is the same and still in place. Grades in the academic field (whether students come from one educational institution or some other educational stage) have created a typical pattern that female students are better than male students (Noviyana et al., 2016.).

## 4. CONCLUSIONS

The use of the ethnoscience-based PjBL model shows that female students achieve higher cognitive scores than male students. Female students tend to be actively involved in group projects even though they take longer to complete assignments. The integration of ethnoscience in learning provides contextual effects for female students. However, there are still many other factors (research limitations) that must be considered for further research such as students' psychological conditions, parental background, parental education, number of siblings, and ethnicity.

## 5. ACKNOWLEDGMENTS

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# E-Booklet Based Learning Media to Increase Motivation and Learning **Outcomes**

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#### **KEYWORDS:**

E-Booklet, Learning outcomes, Instructional media, Motivation to learning, Learning.

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Learning is an activity that involves educators and students. In implementing learning, teaching staff still carry out learning using conventional methods. Appropriate learning media will increase student motivation and learning outcomes. Instructional Media e-booklet is a media used to convey learning material in the form of summaries and attractive images on an electronic basis. The purpose of this research is to find out use of media-based learning e-booklet to foster motivation and learning outcomes for SMA Negeri 5 Metro City students. Research result in the experimental class the average value pretest namely 53.61 and the average value posttest namely 77.5. Meanwhile in the control class the average value pretest namely 60.83 and the average value posttest 66,11. Media based learning e-booklet can improve student learning outcomes based on the results of analysis.

ABSTRACT

## 1. INTRODUCTION

Learning is an activity carried out by teaching staff to convey material to students (Alti, 2022). The learning process is an interaction activity between teaching staff and students in order to achieve learning goals (Wulandari et al. 2023). Implementation of a learning process that goes well will greatly influence the assessment of student learning outcomes (Perwita and Indrawati 2020). The implementation of learning often encounters problems such as teachers' habits which are still comfortable with implementing conventional learning activities (Rahma et al. 2023). Learning has learning components which include learning objectives, teaching materials, learning evaluation, students, teaching staff, and learning media (Adisel et al. 2022).

Learning media is a tool to assist teachers in delivering learning material so that students have interest and interest in the learning material presented (Wulandari et al. 2023). A creative learning media will help create a pleasant learning atmosphere (Fadliya et al. 2022). There are various technology-based learning media that can be used to support the learning process(Firmadani 2020). One of the technology-based learning media is *e-booklet*.

*E-booklet* is a medium for conveying material in summary form and providing attractive images on an electronic basis (Yuliani 2021). Media e-booklet is a medium electronic learning or e-learning (Sopanda et al. 2023). Research (Prananda et al. 2022) shows the results of research that the use of media *e-booklet* proves that there is an increase in motivation and learning outcomes in students so that it is valid and suitable for use in every lesson. Media e-booklet it contains material, examples of questions and their solutions, exercises, quizzes and evaluations that can attract students' interest and desire to learn thereby increasing student motivation and learning outcomes (Sopanda et al. 2023).

*E-booklet* has the advantage that it can be used as an independent learning medium, provides easier understanding of the material provided, has a longer storage time and wider capacity, and is more *flexible* because it can be accessed via cellphone (Yuliani 2021). But along with the advantages, e-booklet It also has several disadvantages, namely that it has an unfavorable effect

on students' eye health and reduces students' writing skills (Putri et al. 2022). Therefore, the author feels that there is a need for innovative learning media that helps students to understand by combining writing and attractive graphic illustrations so that it can increase learning motivation and student learning outcomes.

This research aims to determine the use of media-based learning *e-booklet* to foster motivation and learning outcomes for SMA Negeri 5 Metro City students.

## 2. MATERIALS AND METHODS

#### 2.1. Types and Design of Research

The type of research used is *Quasi Experiment* is a type of research that uses all subjects in a study group to be given treatment, rather than using subjects taken at random(Abraham and Supriyati 2022). Study *Quasi experiment* This was done by selecting two groups of subjects that had no significant differences (Rahmawati et al. 2023). Then given *pretest* with the aim of knowing the initial conditions of the two groups. Different treatments were given between the two groups, the control group was not given treatment using learning media *e-booklet*, while the experimental group was given treatment using learning media *e-booklet*. After giving treatment to the experimental group, both groups were tested again by administering it *posttest* The purpose of giving questions *posttest* to determine whether or not there was an increase in motivation and learning outcomes in the two research groups at SMA Negeri 5 Metro City.

This research uses a research design *Nonequivalent Control Group Design*. Two existing groups were given *pretest*, then given treatment, and finally given *posttest* (Astuti et al. 2023). The design is written in the table as follows:

Table 1	. Research	Design	Table
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Class	Pretest	Treatment	Postest
Experimen	T1	Х	T2
Control	T1	-	T2

Note (s): T1 is an initial test before the use of media, T2 is the final test after media use, X is learning using learning media *e-booklet*, and - is learning without using learning media *e-booklet*.

## 2.2. Data Collection Techniques

The research was conducted at SMA Negeri 5 Metro City in September 2023 – May 2024. Using the technical sampling method *simple random sampling* namely simple sampling from members of a population at random, without paying attention to the strata contained in a population. Data collection techniques are written in the following table:

**Table 2. Data Tables and Data Sources** 

Data	Data source	Data Collection Techniques	Instrument
Motivation to learn	Student	Questionnaire	Questionnaire Sheet
Learning	Student	Handa	Sheet pretest
outcomes	Student	Hallus	and <i>posttest</i>

Data collection is divided into questionnaire data collection as a measurement of learning motivation and pretest posttest data as a measurement of learning outcomes. Structured questionnaire in the form of a list *checklist* questions related to student learning motivation. The number of questions in the questionnaire given was 25 questions using a 4,3,2,1 Likert scale with categories namely: SS (Strongly Agree), S (Agree), R (Doubtful), and TS (Disagree). With value percentage categories, namely:

Mark	Criteria
0-20	Verry less
21-40	Not good
41-60	Pretty good
61-80	Good
81-100	Very good
	(Tresnaningsih et al. 2019).

**Table 3. Questionnaire Assessment Category** 

Data collection on learning outcomes uses pretest and posttest on reproductive system material. The data obtained will then be analyzed using SPSS 25.0 through the Independent Sample T-Test. To carry out this test, you must first carry out prerequisite tests, namely the normality test and homogeneity test.

The assessment on the questionnaire is in the form of Likert scale criteria consisting of a score of 1-4. The questionnaire filled out by respondents will be analyzed and presented as 4,3,2,1 with categories namely: SS (Strongly Agree), S (Agree), R (Doubtful), and TS (Disagree). The validation percentage for each component is calculated using the formula (Sugiyono 2016):

$$P = \frac{\Sigma x}{N} \times 100\%$$

Note (s) : P is the percentage gain,  $\sum x$  is the number of scores for each selected criterion and N is the maximum number of scores.

Assessment of learning outcomes is assessed using tests, namely *pretest* and *posttest*. The test used is *multiple choice* 10 questions for *pretest* and *posttest*. To determine the score *multiple choice* What is obtained is by calculating the number of correct answers divided by the number of questions, written using the formula (Syaifuddin et al. 2022) :

$$Skor = \frac{B}{N} \times 100$$

Note (s) : B is the number of correct questions, and N is the number of questions.

#### 2.3. Research procedure

This research was carried out through preparatory stages starting from preparation, implementation and final stages. In the preparation stage, a request for permission was made from the relevant school, then the research instruments were prepared. During the implementation stage, gifts are made *pretest* reproductive system material in both class groups. Then the implementation of learning in the control group and experimental group. After that, the gift is given *posttest*. The final stage of the research was analysis of the data that had been obtained from the research that had been carried out.

#### 2.4. Data analysis technique

The data analysis technique used in this research is quantitative descriptive. The quantitative descriptive method is used because this method is carried out with the main aim of creating a picture or describing a situation objectively (Jayusman and Shavab 2020).

Data obtained from the results of student questionnaire responses were analyzed using quantitative data. The assessment on the questionnaire is in the form of Likert scale criteria consisting of a score of 1-4. The questionnaire filled out by respondents will be analyzed and

presented as 4,3,2,1 with categories namely: SS (Strongly Agree), S (Agree), R (Doubtful), and TS (Disagree). With the average scoring calculated using the formula (Arikunto et al. 2023) :

$$P=\frac{\sum x}{n}$$

Where P is the average scoring,  $\sum x$  is the number of answers per respondent for each item assessed, and n is the number of respondents.

Learning outcome data were analyzed using SPSS 25.0 with the Independent Sample T-Test. To carry out this test you must first carry out a normality test and a homogeneity test. The normality test is carried out with the aim of testing whether the data that will be used to predict a construct is normally distributed or not. The homogeneity test is used to find out whether the data used has the same variance. This test is carried out as a condition for carrying out a T-Test test where the data used for the Independent Sample T-Test test must be homogeneous to get reliable results. Then test the Independent Sample T-Test.

## 3. RESULTS AND DISCUSSION

#### 3.1. Motivation to learn

Student learning motivation is measured in general. Research measurements were carried out by giving a questionnaire sheet to each student. Therefore, the following analysis results were obtained:

Class		
Mean	91,2	72,96
Min	79	63,2
Max	104	83,2
Std Dev	5,518951	4,415161
Var	30,45882	19,49365

 Table 4. Results of Analysis of Learning Motivation Questionnaires in The Experimental

 Class

Table 5. Results of Analysis of Learning Motivation Questionnaires in The Control Class

Mean	88,83333	71,06667
Min	76	60,8
Max	102	81,6
Std.Dev	5,261722	4,209377
Var	27,68571	17,71886

From the results of the analysis, data on learning motivation between the two class groups, namely for the experimental class, obtained an average of 72.96, which means that learning motivation in the experimental class was in good criteria. For the control class, the results of the analysis of learning motivation were obtained with an average of 71.06, which means that learning motivation in the control class was also in good criteria. From the results of the analysis of the two groups, it can be concluded that both classes already have good learning motivation but it is still possible to improve further through the use of appropriate learning media. This is in accordance with research conducted by (Lince 2022) showing that the use of technology-based learning media in learning can increase students' learning motivation, which is measured by factors such as interest in learning, self-expectations, perceptions of the important value of learning, and self-confidence. in learning ability.

## 3.2. Learning outcomes

## 3.2.1. Statistical descriptive test results

Descriptive analysis of the pretest and posttest, namely:

Descriptive Statistics								
Ν	Min	Max	Mean	Std. Deviation	Var			
Pretest								
36	20.00	80.00	53.6111	13.55471	183.730			
Experimen								
Posstest								
36	60.00	100.00	77.5000	12.50714	153.429			
Experimen								
Valid N (listwise)								
36								

#### Table 6. Results of Descriptive Analysis of The Experimental Class

## Table 7. Control Class Descriptive Analysis Table

Descriptive Statistics							
N Min Max Mean Std. Deviation Var							
Pretest Control	36	10.00	90.00	60.8333	15.00000	225.000	
Posttest Control	36	10.00	90.00	66.1111	17.28381	298.730	
Valid N (listwise)	36						

From the table data, the results show that in the experimental class the average value is *pretest* namely 53.61 and the average value *posttest* namely 77.5. Meanwhile in the control class the average value *pretest* namely 60.83 and the average value *posttest* 66.11. Based on the data obtained, the value *pretest* both classes are lower compared to the grades *posttest*. This is because when it is given *pretest* students have not been given treatment while it is being given *posttest* students have been given treatment. It can be concluded that the provision of learning media is based on *e-booklet* can increase the average value of student learning outcomes.

Data on student learning outcomes are presented in figure 1 and figure 2.



Figure 1. Distribution of Pretest and Posttest Scores for Experimental Class

Figure 1 shows that the histogram has the highest data frequency for pretest scores at 50, totaling 12 students. Meanwhile, the highest posttest score was 80, amounting to 11 students. The histogram above does not show a normal curve, this data provides an interpretation of the curve showing that the data is not normal.



Figure 2. Distribution of Pretest and Posttest Scores for Control Class

Figure 2 shows that the histogram has the highest data frequency for pretest scores at 60, totaling 17 students. Meanwhile, the highest posttest score was 60 with 12 students. The histogram above does not show a normal curve, this data provides an interpretation of the curve showing that the data is not normal.

Results of Statistical Analysis of Student Learning Outcomes. In statistical analysis, prerequisite tests will be carried out, namely normality and homogeneity tests on the available data.

## 3.2.2. Normality test

The Normality Test is carried out with the aim of being a benchmark to determine whether the total data population is normally distributed or not. If the significance is  $\geq 0.05$  then it is declared normal, whereas if the significance is 0.05 then the distribution is declared normal. In this study, researchers used the Shapiro-Wilk test with the help of IMB SPSS Statistics 25.0 to test normality.

		Tests of 1	Normality				
	Kolm	ogorov-Smi	rnov <sup>a</sup>	Shapiro-Wilk			
	Statistic	df	Sig.	Statistic	Df	Sig.	
Pretest Experimen	.253	36	.195	.900	36	.330	
Posttest Experimen	.214	36	.200*	.858	36	.144	
Pretest Control	.160	36	.200*	.935	36	.591	
Posttest Control	.267	36	.140	.894	36	.294	

## Table 8. Normality Test

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Based on the above data obtained through the One Sample Kolmogorov Smirnov Test for the results of the pretest scores seen from the significance results in the experimental class of 0.195

 $\geq 0.05$  and in the control class of  $0.200 \geq 0.05$ , it can be concluded that the data distribution is normal. Then for the results of the posttest scores, it can be seen from the significance results in the experimental class of  $0.200 \geq 0.05$  and in the control class of  $0.140 \geq 0.05$ , it can be concluded that the data distribution is normal. If the pretest and posttest data for the experimental class and control class are normally distributed, then proceed with the homogeneity test.

## 3.2.3. Homogeneity Test

The Homogeneity Test was carried out to determine whether the experimental class and control class research data were homogeneous. The basis for making homogeneity test decisions is that if the significance is  $\geq 0.05$  then it is said that the data population is homogeneous, conversely if the significance is  $\leq 0.05$  then the variance of two or more data population groups is not homogeneous. In this study, researchers used IMB SPSS Statistics 25.0 to test homogeneity.

	8 · · · · · · · · · · · ·				
		Levene Statistic	df1	df2	Sig.
Posttest Exerimen &	Based on Mean	1.120	1	68	.294
Control	Based on Median	1.033	1	68	.313
	Based on Median and with adjusted df	1.033	1	63.419	.313
	Based on trimmed mean	1.290	1	68	.260

## Table 9. Test of Homogeneity of Variances Experimen and Control

From the results of the calculation of the output table for the homogeneity test of posttest scores for the experimental and control class groups, it can be seen that based on Based on Mean, the Sig value is  $0.294 \ge 0.05$ . So it can be concluded that the posttest score data for the control and experimental class groups are homogeneous.

After carrying out the prerequisite tests and the data is proven to be normally distributed, statistical analysis of the Independent Sample T-Test is then carried out with the following analysis results:

## Table 10. Independent T Test

Group Statistics						
	Posttest Measurement	Ν	Mean	Std. Deviation	Std. Error Mean	
Posttest Experimen & Control	Experimental Class	35	77.4286	12.68228	2.14370	
rostest Experiment & Control	Control Class	36	66.1111	17.28381	2.88064	

## **Table 11. Independent Samples Test**

		Levene for Equ Varia	's Test ality of ances	T-Test for Equality of Means						
		F	Sig.	t	df	Sig. (2- tailed	Mean Difference	Std. Error Differen	95% Co Interv Diffe	onfidence al of the erence
						)		ce	Lower	Upper
Posttest Experime	Equal variances assumed	1.284	.261	3.138	69	.002	11.31746	3.60618	4.12333	18.51159
n & Control	Equal variances not assumed			3.152	64.224	.002	11.31746	3.59075	4.14459	18.49033

Based on the T-Test test output table for 2 independent samples between the experimental and control groups above, it is known that the posttest score in the experimental class has an average of 77.42, while the posttest score for the control group has an average of 66.11. From the results of the t-test using the independent sample t-test, the significance value in the table was  $0.002 \le 0.05$ , so it can be concluded that there is a significant difference in posttest learning outcomes between the experimental group and the control group. So it can be concluded that there are differences in learning outcomes for classes given learning media *e-booklet* with classes that are not given learning media.

Based on the results of the analysis, there are differences between the learning outcomes of the control group and the experimental group. In the control group the average posttest score was 66.11. Meanwhile, for the experimental group, the average posttest score was 77.42. So the experimental group had a higher posttest average score compared to the control class posttest average score. From the results of the Independent Sample T-Test, the significance value is 0.002  $\leq 0.05$ . Based on this significance value, it proves that learning media is based *e-booklet* provide a significant influence on the growth of student learning outcomes. Research conducted (Rahmadiningrum and Wulandari 2023) states that the results of the research use *e-books* experienced a significant increase. Where the experimental class score was from 32.42 to 43.04 after being given treatment. Meanwhile in the control class from 29.00 to 39.57. So that learning media is based *e-booklet* provide a significant influence for the experimental class.

Use of learning media *e-booklet* has an influence on student learning outcomes on reproductive system material in the experimental class. Instructional Media *e-booklet* used in the hope of improving student learning outcomes. Research (Rahmatussyifa et al. 2024) shows that the results of research using e-booklets will improve student learning outcomes in terms of content test and practicality aspects. This is explained in research (Norazijah 2022) stated that the results of the practicality of the content showed that *e-booklet* what has been developed has the advantage of complete material content, there are indicators and learning objectives and the arrangement is based on a logical sequence. Content of *e-booklet* becomes easy to understand because of the words contained in it *e-booklet* is a language that is commonly used every day. So it is stated that the results of the research analysis are based on learning media *e-booklet* had a good influence on the experimental class compared to the control class. So that learning media is based *e-booklet* can be used in everyday learning.

#### 4. CONCLUSIONS

This research was conducted to determine media-based learning *e-booklet* can improve student learning outcomes and motivation. Therefore, learning media is based *e-booklet* can foster student learning motivation in two class groups, namely the experimental group with an average of 72.96 and the control group with an average of 71.06. Media based learning *e-booklet* can improve student learning outcomes in experimental classes based on the results of analysis in SPSS 25.0.

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# Potency of Ear Mushroom and Shrimp Head as Natural Flavoring on **Variations of Drying Temperature**

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# ABSTRACT

**KEYWORDS:** Ear mushroom, shrimp head, Natural flavoring, drying temperature protein, organoleptic quality.

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Natural flavoring is a flavoring made from edible mushrooms as a flavor enhancer food of powder or liquid form. Ear mushrooms and shrimp heads contain high protein and glutamic acid and can be used as natural flavoring and give additional savory taste of food . The objective of this research was to analyze the total protein content and organoleptic quality of natural flavoring components of ear mushrooms and shrimp heads at various drying temperatures. This research method used a completely randomized design (CRD) and two treatment components. The first component was the ratio of ear mushroom and shrimp heads: (25g:75g), (50g:50g), and (75g:25g). The second component was different drying temperatures of 40°C and 50°C. The research results showed that the highest content of total protein and the best organoleptic quality of natural flavoring in the K1T2 treatment (25g ear mushrooms + 75g shrimp heads with 50°C drying temperature) was 35.67% ie dark brown color, savory taste, delicious aroma, very softtexture, and liked.

## 1. INTRODUCTION

Natural flavoring is a natural flavoring made from edible mushrooms to enhance the taste of food in powder or liquid form. In general, the flavoring that is often used made from synthetic ingredients, called MSG (Monosodium Glutamate) added to the food of some Indonesian people and often has an adverse impact on health. According to Ningsih (2018), excessive and continuous use of MSG can cause stomach and sleep disorders, nausea, allergic reactions, trigger hypertension, asthma, cancer, diabetes, paralysis, decreased intelligence, and impaired spermatogenesis due to the formation of free radicals. Natural flavoring is a flavoring that can be created from basic ingredients including protein. These ingredients may consist of animal or vegetable protein. The use of natural ingredients can result in flavorings that are equally delicious as synthetic ones. The addition of spices can affect the taste, color and aroma of food. The spices can alter the taste and color of food. garlic, shallots, and white pepper offer a delightful aroma that can enhance the natural flavor (Widyastuti, 2015). According to Zhang (2013), protein from edible mushroom can enhance the taste food of ingredient can be used as a substitute for MSG is ear mushroom. Ear mushroom is dark brown, ear-like shape, form of irregular wavy sheets with an elastic and springy texture. The savory taste obtained from mushrooms has led to people's passion for consuming them in everyday life, one of which is ear mushrooms. The results of research by Bandara (2019) stated that the content of dried ear mushrooms included 79.9-93.2% carbohydrates, 6.5–13% crude protein, 9.9–17.9% sugar content, 0.48–4.5% crude fat, and 3.5–

12.5% fiber. Sekara (2015) states that ear mushroom contains benefits as an anti-tumor agent, low cholesterol, anticoagulant, antioxidant, immunomodulator and anti-microbial. Another primary ingredient that can be utilized as combination for producing natural flavoring is shrimp heads, which contain nutritious protein.. According to Rathore (2018) states that shrimp heads have a protein content of 43.12%. According to Bawinto (2015) shrimp heads and shells can be processed into stock that can give savory flavor for food processing. Next to that, being used as a flavor enhancer, shrimp heads and shells have benefits against free radicals ten times superior than natural product of fruit and vegetables due to its 'contain the antioxidant astaxanthin. The important factor of making natural flavorings is the process of reducing the water content therefore it can inhibit the growth of pathogen microbes. Lisa's (2015) research showed that drying temperature will affect the water content of ear mushrooms. The appropriate oven temperature will not damage the protein content and other important nutrients of food ingredients, because the protein will be denaturation at too high temperature

The objective of this research was to analyze the total protein content and organoleptic quality of natural flavoring components of ear mushrooms and shrimp heads at various drying temperatures.

## 2. MATERIALS AND METHODS

#### 2.1. Materials

The tools used in this research were blender, knife, digital scale, small bowl, medium sized steamer, frying pan, spatula, electric oven, small blender, gas stove, Kjeldahl flask, analytical balance, funnel, watch glass, and evaporating cup, 100 ml Erlenmeyer, 250 ml beaker glass, 100 ml measuring flask, 100 ml measuring cup, and dropper pipette volume 10 ml

The materials and spices used in this research were ear mushroom, shrimp heads, shallots, garlic, white pepper, tapioca flour, granulated sugar, distilled water, sulfuric acid, sodium hydroxide, selenium, selenium catalyst, cupric sulfate, ethanol, methyl red indicator, sodium sulfate, pp indicator, hydrochloric acid, concentrated nitric acid, and sodium tetra borate

#### 2.2. Research Procedure

The research process: 1.Preparation of ear mushrooms, shrimp heads, shallots, garlic, white pepper, brown sugar, tapioca flour and water; 2. Making natural flavorings : 1.Cut into small pieces and grind ear mushrooms as much as 75g (K1), 150g (K2), and 225g (K3) and shrimp heads as much as 225g (K1), 150g (K2), and 75g (K3) using a blender .2 Roast the shrimp head flour with bay leaf to reduce the strong smell. 3.Blend all the spices (for every 100 g of mixed ear mushrooms and shrimp heads) 15g garlic, 5g shallots, 2g white pepper, 4g salt and 5g palm sugar shrimp heads, ear mushrooms, garlic, shallots, white pepper, salt and palm sugar, tapioca flour); 4. Put the mixture of natural flavoring spices and ingredients in baking dish and steam for 15 minutes; 5.Dry the dough in an oven of 40°C and 50°C temperatures and drying time for 12 hours; 6.Smooth the dough from the oven and filter the natural flavoring into fine powder; (Widyastuti, 2015) 7. Natural flavoring was ready to be tested for protein content (the Kjeldahl method : the stage of destruction, destillation and titration (Munthe I, 2016) and organoleptic quality that were color, flavor, aroma texture, and acceptability)

#### 2.3. Research Design

This research method used a completely randomized design (CRD) and two treatment components. The first component was the ratio of ear mushroom and shrimp heads: (25g:75g),

(50g:50g), and (75g:25g). The second component was different drying temperatures: 40°C and 50°C with 3 replications of each treatment. The parameters were total protein content (the Kjeldahl method) and organoleptics quality (color, flavor, aroma and community accepantance)

# 3. RESULTS AND DISCUSSION

## 3.1. Results

Total protein level and Organoleptic Quality Of Natural Flavoring Component Of Ear Mushroom and Shrimp Heads in the following table:

Table 3.1. Total Protein level and Organoleptic Quality of Natural Flavoring Component

#### of Ear Mushrooms and Shrimp Heads at different of Drying Temperatures **Test Aspect** Treatments Protein (%) Community Color Flavor Aroma Texture acceptance K1T1 25.01 lesssavory less delicious quite like dakbrown very soft K2T1 22.51 darkbrown bit delicious very soft like savory K3T1 16.35\* darkbrown savory delicious very soft like 29.71\* K1T2 darkbrown delicious savory very soft like K2T2 24.34 darkbrown savory delicious very soft like K3T2 20.67 darkbrown savory delicious very soft like

Explanation :

\*: the lowest protein level

\*\* : the highest proteini level

K1T1 : 25g of ear mushroom + 75g of shrimp head, with 40°C drying temperature

K2T1 : 50g of ear mushroom + 50g of shrimp head with  $40^{\circ}$ C drying temperature

K3T1 : 75g of ear mushroom + 25g of shrimp head with 40°C drying temperature

K1T2 : 25g of ear mushroom + 75g of shrimp head with 50°C drying temperature

K2T2 : 50g of ear mushroom + 50g of shrimp head with 50°C drying temperature

K3T2 : 75g of ear mushroom + 25g of shrimp head with 50°C drying temperature

Table 3.1 showed the highest natural flavoring protein content of 29.71% in the K1T2 treatment(25 g of ear mushroom + 75 g of shrimp head at 50 °C drying temperature) and the lowest natural flavoring protein content in the K3T1 treatment (75 g of ear mushroom + 25 g of shrimp head at 40 °C drying temperature) was 16.35%. The color of all treatments of natural flavoring componentof ear mushrooms and shrimp heads was dark brown. There was also no difference texture of the natural flavoring across all treatments, it was very soft. The flavor of natural flavoring in the K1T1 treatment (25g of ear mushrooms + 75g of shrimp heads at 40°C drying temperature) was less savory, however the flavors of other samples were savory. The aroma of natural flavoring in treatments K1T1 (25g of ear mushrooms + 75g of shrimp heads at 40°C drying temperature ) andK2T2 (50g of ear mushrooms + 50g of shrimp heads at 40°C drying temperature ) was less delicious, while the aroma of other treatments were delicious. The accepted community of natural flavoring in the K1T1 (25g of ear mushrooms + 75g of shrimp heads at 40°C drying temperature ) was less delicious, while the aroma of other treatments were delicious. The accepted community of natural flavoring in the K1T1 (25g of ear mushrooms + 75g of shrimp heads at 40°C drying temperature ) was less delicious, while the aroma of other treatments were delicious. The accepted community of natural flavoring in the K1T1 (25g of ear mushrooms + 75g of shrimp heads at 40°C drying temperature) was quite like, however the panelists preferred the other treatments.

#### 3.2.Discussion

The composition of higher shrimp head and the higher temperature resulted the higher proteincontent. This was supported by research (Akbar, Riyadi, & Jaya, 2017) stated that different concetration of shrimp heads broth given showed significant differences of protein content in kemplang crackers. The addition concentration of shrimp head stock can increase the protein content with value between 9.89% - 16.30%. Beside that, the protein content of 100 g of shrimp heads was higher than ear mushrooms. According to Rathore (2018), shrimp heads have protein level of 43.12%, while according to Bandara (2019) ear mushrooms have protein content of 6.5-13%. Drying temperature also affects total protein content from the Kjeldahl method. This research showed that 50°C heating temperature and additional drying time of 15 minutes can increase crude protein content. This was due to the decrease of water content on dried material. This opinion is also strengthened research (Riansyah, Supriadi, & Nopianti, 2013) showed that total protein content of salted astringent fish showing total protein content at 50°C temperature (T1)was 21.73%, then 60°C temperature (T2) was 21.97% and also at 70°C temperature (T3) was 24.12%. The longer time and the higher temperature used for drying process, the higher protein content will be.

The color of all treatments was the same (dark brown). The dark brown color produced by all treatments was caused by ear mushroom composition. The drying process of natural flavoring usedoven was also one of the factors that influences color. According to (Bhattacharya, Bhakta, & Ghosh, 2011) the dark brown color produced can also be caused by a chemical reaction that causes a browning reaction. This was also stated by (Irawati, Warnoto, & Kususiyah, 2015) that the browning reaction of natural flavorings is non-enzymatic reaction in reducing sugars from carbohydrates with primary amino groups results in large evaporation of water. The formation of brown color is triggered by an oxidation reaction catalyzed by the enzyme phenol oxidase or polyphenol oxidase. These two enzymes can catalyze the oxidation reaction in phenol compounds causes the color change to brown.

The flavor of natural flavoring was also influenced the spices used during the making process. The spices addition of natural flavoring is intended to increase and improve taste (Wahidah, 2010). The savory taste can be caused the protein content hydrolyzed into amino acids (glutamic acid) which gives specific taste. The metal ion of food content can affect the food taste. Metal ions will be released from their complex bonds due to hydrolysis or degradation and will reduce the bitter taste of food (Winarno, 2004).

The aroma of natural flavoring was delicious except for the K1T1 treatment (25g of ear mushrooms + 75g of shrimp heads at 40°C drying temperature) the aroma was less delicious. The ratio addition of shrimp head extract created aroma liked panelists (Adawyah, Asyah, Puspitasari, & Candra, 2017). The aroma of the K1T1 was considered less delicious due to the higher shrimp head component than that of 75g ear mushroom, therefore the shrimp head aroma was considered more pungent and stronger. Next to that, the different aroma of the panelists influenced the assessment of the aroma of the natural flavoring. The strong aroma prevention of shrimp heads was anticipated by roasting them using bay leaf to reduce the unpleasant aroma. According to (Harismah & Chusniatun, 2016) the adding bay leaf of food will make the aroma delicious because of the distinctive smell of bay leaf. Bay leaf can also absorb excess CO2 and serve as an alternative medicine. According to (Atika & Handayani, 2019) the roasting (heat) treatment at the stage of making flavor powder causes the volatile compounds and other chemical contents of the shrimp shell to change chemical content such as evaporating, giving specific aroma of the shrimp.

The texture of natural flavoring components of ear mushroom and shrimp head were very softall treatments. The texture of natural flavoring was also influenced the water content of the ingredients used. Ear mushrooms and shrimp heads had low water content to making it easier to dry. According to (Winarno, 2002), the dough made during the oven process will affect the resulting texture of material. The too thick mixture of material will cause the heat to propagate less evenly and make the solid less homogeneous. The thinner the dough, the more even the heat will

propagate, and the resulting dough will have softer texture. According to (Dewita & Syahrul, 2014), processing food ingredients that contain carbohydrates, proteins and fats will affect the appearance (aroma, taste, mouthfeel, aftertaste and texture, consistency, softness, elasticity and crunchiness).

According to the panelists, the natural flavoring was most preferred at the K1T2 (25 g of ear mushrooms + 75g of shrimp heads at 50°C drying temperature). The natural flavoring was less liked at the K1T1 (25g ear mushrooms + 75g shrimp heads with 40°C drying temperature). According to panelists, the natural flavoring flavor were savory, delicious, very soft, as well ummami respectively. According to (Suhaenah & Nuryanti, 2017), the high level of liking and acceptance shows that the product is accepted panelists and can be used as natural flavoring product made from natural ingredients and reduces chemical-based food ingredients. The differences of age and tastes can be the factor results of organoleptic quality. The 20 panelists range age of 14 years t- 48 years. According to (Djohar, Timbowo, & Mentang, 2018) the age panelist influences the results of organoleptic quality because the person of 40 years old become slightly less sensitive, beside that each person's taste was different.

## 4. CONCLUSIONS

In the K1T2 (25 g of ear mushrooms + 75 g of shrimp heads at 50 °C drying temperature), the highest protein content of the natural flavoring was total of 29.67%. The best organoleptic quality of natural flavoring in K1T2 (25g ear mushrooms + 75g shrimp heads at 50°C drying temperature) was dark brown, savory taste, delicious aroma, very soft texture and liked.

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# **Biotechnology Application of Individual and Multienzyme in Feed, and Its Effect on Broiler Growth Performance**

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	ABSTRACT
KEYWORDS:	Bromelain as a single enzyme is widely used as a meat tenderizer; however this
Body weight,	can damage the meat cell's. The effect of adding multienzyme Phyprozyme HP
Broiler,	in feed is not yet known, so it needs to be investigated. This study aimed to
Bromelain,	evaluate the growth performance of broilers with the addition of single and
Carcass persentage,	multienzymes. This study used 42 Lohman MB 202 Grade Platinum broilers
Phyprozyme HP	aged 21 to 35 days, using 6 treatments and 4 replications. P0 as the control feed.
	Bromelin was added in feed at the dose of P1: 600 GDU/kg, P2: 1200 GDU/kg,
	P3: 1800 GDU/kg, P4: 2400 GDU/kg, and P5: 3000 GDU/kg. Phyprozyme HP
	was added at P1: 0.19 g/kg, P2: 0.26 g/kg, P3: 0.33 g/kg, P4: 0.40 g/kg, and P5:
	0.47 g/kg. Parameters measured included body weight, body weight gain, and
	carcass percentage. The results were analyzed using One-Way ANOVA. The
© 2024 The Author(s). Published by Biology Education Department,	addition of bromelain in feed decreased body weight, while the addition of
Faculty of Teacher Training and Education Universitas	multienzyme significantly improved broiler performance up to a dose of 0.33
Muhammadiyah Surakarta.	g/kg feed. The addition of bromelain decreased carcass percentage to 11.07%,
This is an open access article under the CC BY-NC license:	while the addition of multienzyme increased carcass percentage to 8.83%, but
https://creativecommons.org/license	decreased it to 7.68% at the highest dose.

# 1. INTRODUCTION

International Broiler production in the livestock industry plays an important role in providing the world's animal protein supply (Nkukwana 2019). Broilers are highly favored by consumers due to several advantages, such as a short harvest period, lower costs compared to native chickens, more tender meat, and ease of maintenance in tropical climates. However, there are some drawbacks in raising broilers, including the limited nutritional value of feed and the health status of the animals against diseases (Chuka 2014). Several studies have also indicated that broiler productivity is still low and needs to be increased to meet the market demand for broiler meat (Tavárez and de los Santos 2016). Because of this problem, currently research is still being carried out on efforts to improve broiler performance, one of which is using vegetable ingredients, high temperature processing and adding enzymes.

Efforts to improve the quality and cost of feed can be done by utilizing vegetable ingredients that are rich in protein and phosphate (especially grains). The use of vegetable raw materials is also widely available in nature because Indonesia is an agricultural country. Apart from that, the use of vegetable waste such as fine rice bran and soybean meal is also more beneficial for the environment and can be obtained at a cheaper price. The plant-based feed ingredients that have been used reach a presentation of 90% of the total feed formulation regardless of the region, however the high use of plant-based feed is also feared to result in an increase in anti-nutritional

compounds which interfere with the absorption of nutrients in the feed (Syed Fazal ur Rahim and Muhammad Abdullah Bin Masood 2022). Antinutrients in the form of phytate have quite a large negative effect on most animals, namely strong binding to minerals such as Fe, Zn, Ca, Mg, Mn, Cu, Se, and Ni, as well as protein and starch (Sahu et al. 2024). Therefore, enzyme supplementation in feed is one solution and is useful in increasing the nutritional value of feed (Kari et al. 2022). Enzymes are defined as proteins that facilitate certain chemical reactions (Lovelock et al. 2022). Enzymes work on a substrate specifically by catalyzing reactions to convert complex substances into substances that can be absorbed (Khan 2021). Several studies state that the use of enzymes in feed can help nutrient digestibility, maintain intestinal health, reduce the possibility of nutrient waste, and can be done conventionally, thereby saving costs (Mahmoud Alagawany et al. 2022).

The application of enzymes as biocatalysts plays a role in accelerating the occurrence of a reaction. Enzymes that are capable of hydrolyzing protein substrates in feed ingredients are protease enzymes, one of which is bromelain. The bromelain enzyme can hydrolyze proteins as complex protein compounds into amino acids as simpler compounds. The use of bromelain in the food and livestock sectors is to make meat more tender when consumed. However, several studies also state that adding bromelain to feed can cause cell damage in meat (Grassin and Coutel 2009). Enzyme research on broilers still needs further research to find the right dosage and its impact on broilers.

Several research results show that enzymes working together are more effective when compared to individual enzyme systems (Hwang and Lee 2019) (Ellis et al. 2019) (Dubey and Tripathi 2021). These enzymes are usually referred to as multi-enzyme systems. Several studies have shown that enzymes that work synergistically are more effective than those that work individually. These enzymes are usually referred to as multi-enzyme systems. Several enzymes commonly used in animal feed include protease, xylanase, hemicellulase and phytase. These enzymes are available in a multi-enzyme system, such as Phyprozyme HP, which is used to feed pigs, various poultry and fish (Mohammed and Budihargo 2020). The raw material components of these enzymes are derived from dried extracts obtained from the fermentation of fungi (*Aspergillus niger, Aspergillus oryzae*) and bacteria (*Bacillus subtilis, Escherichia coli*). To date, there has been no research into the application of this multi-enzyme system in broilers. Therefore, more research is needed to determine the appropriate dosage and its impact when adding this multi-enzyme system to broiler feed.

## 2. MATERIALS AND METHODS

## 2.1. Materials

Day-old broiler chicks (DOC) of 42 Lohman MB 202 Grade Platinum strain were weighed using digital scales. The weighed DOC were randomly divided into 6 groups, each consisting of 3 broiler chicks, with 4 replicates per group. This study was conducted using a litter-based cage system (with rice husk as the base). The cage system consisted of 24 compartments, corresponding to 6 treatments and 4 replications. The dividers for each compartment were made of bamboo. Each compartment measured 100 cm x 40 cm x 40 cm, thus meeting the minimum size requirements for cages.

## 2.2. Methods

The research was conducted at the Pekuncen broiler farming unit, Banyumas, Central Java, Indonesia. Proximate analysis of feed and carcasses was carried out at the Food Engineering Laboratory, Sebelas Maret University and the Biochemistry Laboratory, Faculty of Mathematics and Natural Sciences, Jendral Soedirman University. The research method used was a completely randomized design (CRD). Broiler chickens were reared from day 14 until harvest on day 35. Feed was given twice a day (at 07.00 and 17.00 WIB). The amount of feed given was adjusted to the age of the chickens, following broiler rearing management guidelines. Drinking water was provided ad libitum. This experiment used a uniform feed formulation and consisted of 6 different dose treatments: P0, P1, P2, P3, P4, and P5. For single bromelain enzyme, P0 was set as the control group without enzyme supplementation, while the dose variations were P1 with 600 GDU/kg feed, P2 with 1200 GDU/kg feed, P3 with 1800 GDU/kg feed, P4 with 2400 GDU/kg feed, and P5 with 3000 GDU/kg feed. In the Phyprozyme HP multi-enzyme system, P0 was the control group without enzyme supplementation, P1 was dosed with 0.19 g/kg feed, P2 was dosed with 0.26 g/kg feed, P3 was dosed with 0.33 g/kg feed, P4 was dosed with 0.40 g/kg feed, and P5 was dosed with 0.47 g/kg feed. The sampling method was carried out by purposive sampling where one chicken was taken to represent a group with normal and healthy growth conditions.

#### 2.3. Data analysis

Growth parameters were measured every seven days throughout the experimental period to assess broiler growth performance. Performance measurements included average body weight, weight gain, and feed conversion ratio. The average body weight of broilers was calculated using the following formula:

Average body weight = 
$$\frac{\text{Total body weight (g)}}{\text{Number of broilers}}$$

Weight gain was used as a criterion for growth and the ability of livestock to convert nutrients from feed. Chicken body weight was measured in grams using an electronic scale at the start of the experiment, every seven days during the experiment, and at the end of the experiment. Weight gain was calculated using the formula as follows:

Weight gain = 
$$\frac{(Wt-Wo)}{Wo} \ge 100\%$$

description:

Wo = Initial body weight at the start of the experiment (g)

Wt = Final body weight at the end of the experiment (g)

Carcass weight is defined as the weight of the chicken meat without feathers, feet, internal organs, neck, and head. Carcass weight determines the economic value of the meat. The carcass percentage was calculated using the following formula:

% Carcass = 
$$\frac{\text{Carcass weight (g)}}{\text{Live body weight (g)}} \times 100\%$$

Data were analyzed using One-Way Analysis of Variance (ANOVA) with a 95% confidence level to determine the effect of different doses of Phyprozyme HP supplementation. Statistical analysis was conducted using SPSS software (Version 22, IBM Corporation, New York, USA).

## 3. RESULTS AND DISCUSSION

## 3.1. Results

The research results on broiler growth performance using individual and multienzyme enzymes at different doses are detailed in Table 1. The highest final body weight was observed in treatment P1 with the addition of a multienzyme dose of 0.16g/kg feed, while the lowest final body

weight was recorded in treatment P3 with the addition of bromelain enzyme dose of 1800 GDU/kg feed.

Treatment	Body Weight (g)				
Treatment	Bromelin	Phyprozhyme HP			
PO	1655.86 ±114.06*	$1533.50 \pm 15.40$			
P1	$1641.09 \pm 127.02$	$1716.63 \pm 19.65*$			
P2	$1655.43 \pm 167.14$	$1689.75 \pm 6.91$			
P3	1417.42 ±159.49**	$1664.50 \pm 11.55$			
P4	$1562.57 \pm 215.21$	$1571.38 \pm 9.28$			
P5	$1462.57 \pm 125.34$	$1568.13 \pm 3.97 **$			

Table 1	Compariso	n of final bro	iler weights	using differe	ent types and	l doses of enzymes
I abit I.	Compariso	u ui iiiai di u	mei weignis	using uniter	спі туреб апі	i duses of enzymes.

Notes:

\* Highest final broiler body weight before harvesting

\*\* Lowest final broiler body weight before harvesting

The final body weight results of broilers using individual enzymes and multienzymes at different doses are detailed in Figure 1.



## Figure 1. Comparison of final body weight using different enzymes

The research findings on the weekly weight gain of broilers using individual and multienzyme enzymes at different doses are detailed in Table 2.

			Weight	Gain (g)			
Treatment	В	romelin (Da	ıy)	Phypr	Phyprozhyme HP (Day)		
	21	28	35	21	28	35	
PO	291.73	433.98	442.07	312.18	333.41	414.25	
P1	230.31	447.57	475.13	357.26	347.01	488.75	
P2	221.16	448.72	497.47	372.55	350.64	423.84	
P3	193.45	393.43	340.46	351.06	336.06	419.05	
P4	208.45	461.10	404.94	338.38	331.71	414.13	
P5	163.45	359.10	451.94	303.83	323.78	418.50	

Table 2. Comparison of broiler weight gain with different enzyme supplementation

Notes:

\* Highest broiler weight gain every week

\*\* Lowest final broiler weight gain every week

The research results in Table 2 indicate that the weight gain of broiler chickens increases with the addition of multienzyme doses ranging from 0.19 g/kg to 0.33 g/kg of feed and decreases with doses exceeding 0.33 g/kg of feed. The increase in chicken weight is a positive effect of enzyme supplementation in feed, while negative effects of enzyme supplementation may occur with high enzyme doses.

Carcass weight refers to the weight of chicken meat without feathers, feet, internal organs, neck, and head. Carcass weight is significant in the trading industry due to its economic value. The carcass percentage is calculated by comparing the carcass weight to the live weight of broilers. The carcass percentages of broilers using different enzyme supplementation are presented in Table 3.

Treatment	Carcass Weight (g)	Body Weight (g)	Carcass Percentage (%)
Enzim Bromelin	l		
TO	1220.00	1655.86	73.68*
<b>T1</b>	1076.67	1641.09	65.61
T2	1133.00	1655.43	68.44
Т3	1042.00	1417.42	73.51
<b>T4</b>	1127.34	1562.57	72.15
Т5	958.34	1462.57	65.52**
Enzim Phyprozy	yme HP		
TO	1018.25	1508.50	67.50
<b>T1</b>	1310.25	1716.63	76.33*
T2	1151.00	1689.75	68.12
Т3	1018.75	1564.50	65.12
<b>T4</b>	955.75	1496.38	63.87
Т5	878.25	1468.13	59.82**

Table 3. Comparison of broiler carcass percentage with different enzyme supplementation

Notes:

\* Highest carcass percentage every treatment

\*\* Lowest carcass percentage every treatment





#### 3.2. Discussion

Broilers are among the most extensively farmed livestock due to several advantages, such as rapid harvesting and suitability for cultivation in tropical environments like Indonesia. Broiler meat remains one of the largest contributors to animal protein worldwide. Research on the effects of various enzyme doses as a biotechnology application in broiler feed is still necessary to determine accurate dosages and their impacts. Previous studies have indicated that bromelain enzyme, when used singularly, is widely employed as a livestock feed supplement to produce tender broiler meat (Juárez et al. 2012) (Naveena, Kiran, and Mendiratta 2013). However, other research suggests that bromelain usage may result in cellular damage within the meat (Guevara and Daleo 2018)(Humayan Kabir et al. 2021). The use of Phyprozyme HP enzyme as a multienzyme has not been previously studied in broilers. Therefore, this study examines the effects
of both types of enzymes on broiler growth performance, considering final body weight, weight gain, and carcass percentage, which represents the economically valuable part of the broiler.

The research findings demonstrate that the addition of different enzyme doses, whether using individual or multienzymes, does not significantly affect broiler growth performance. Surprisingly, the addition of bromelain as a singular enzyme may decrease the formed carcass percentage by up to 11.07%. Some studies suggest that adding bromelain at specific doses to broiler feed can indeed impart tenderness to the meat and reduce fat content (Chandrasekaran 2015). However, it may decrease carcass percentage compared to the use of multienzymes and control treatments. Giving bromelain can also reduce liver lipid disorders, especially free cholesterol, total cholesterol, fatty acids, triglycerides and glycerol by 15-30% (Achilonu et al. 2018). The fatty acid content in chicken meat is also an indicator of meat quality. Bromelain has a function as an anti-obesity drug by inhibiting the process of adipose cell differentiation (Hasoon, Kadhim, and Rahmah 2022). The number and size of adipose cells determine the mass of fat tissue formed. High-fat diets cause reversible changes in the gut microbiota, but can further lead to inflammation, obesity and cancer if poor diets persist (Cândido et al. 2018). Foods high in glucose and fructose also cause increased endotoxin levels, inflammation, loss of microbial diversity, and increased intestinal permeability (Jamar, Ribeiro, and Pisani 2021).

The carcass portion of broilers comprises the parts of the chicken without feathers, blood, internal organs, head, neck, and claws. Carcass percentage can be influenced by several factors such as chicken genetics, feed quality, gender, slaughter age, and environment. In this case, carcass percentage is affected by the addition of enzymes compared to the control group. If the nutrition and digestibility of the feed entering the chicken's body decrease, the carcass weight will also decrease. The research results show that although bromelain may impart a more savory and tender taste to the meat, it can decrease the carcass percentage. This research outcome provides a distinct evaluation for stakeholders in the broiler carcass market. If traders prioritize taste, then bromelain can be a solution. However, if traders require a higher broiler carcass percentage, then bromelain cannot be used due to its resulting impact.

The research findings indicate that the supplementation of multienzymes in feed can help improve the growth performance of broiler chickens by increasing their weight gain at doses ranging from 0.19 g/kg<sup>-1</sup> to 0.33 g/kg<sup>-1</sup> of feed (P1, P2, and P3). The highest growth performance based on weight gain data is observed in chickens given an enzyme dose of 0.26 g/kg<sup>-1</sup> of feed (P2), while the lowest weight gain is observed in chickens given the highest enzyme dose compared to other enzyme dose treatments, namely treatment P5 with an enzyme dose of 0.47 g/kg<sup>-1</sup>. This suggests that multienzyme supplementation is beneficial for chicken growth but can also decrease chicken weight at high doses. Phyprozyme HP enzyme has a positive impact on broiler chicken growth (0.19-0.33 g/kg<sup>-1</sup> of feed), but it can have negative effects, such as weight loss, when supplemented with high doses (0.4-0.47 g/kg<sup>-1</sup> of feed). The research results show that the amount of feed consumed by chickens significantly affects broiler chicken growth performance, but it is not the sole determining factor for improving chicken growth performance. This is evidenced by the fact that the amount of feed consumed by chickens in the control treatment is higher than in all other treatments, yet the weekly weight gain of chickens in the control treatment is lower than that in P1, P2, and P3. Other factors affecting growth performance may include the presence of certain plant proteins in feed that are not well digested in the chicken's digestive tract (Oaisrani et al. 2015).

The growth performance response of broiler chickens based on the research findings demonstrates that the digestibility of plant materials can be tolerated up to a certain limit with the addition of multienzymes. This is evidenced by the addition of multienzyme doses up to 0.33 g/kg of feed, resulting in an increase in carcass percentage, while doses exceeding that can decrease carcass percentage by up to 7.68%. The research results also prove that the multi-enzyme system can enhance growth performance and protein digestibility better than adding enzymes individually. These findings are consistent with various previous studies (Shekarabi et al. 2022) (Lu et al. 2016)

(K.A. et al. 2011). The research results indicate that multienzymes, which can enhance growth performance better than individual enzymes, will also have negative impacts if excessive enzyme doses are used. Some research facts also show that broiler chicken growth performance may decline if the enzyme doses added to the feed are too high (Raza, Bashir, and Tabassum 2019) (M. Alagawany, Elnesr, and Farag 2018). Therefore, it can be concluded that enzyme concentration plays a crucial role in feed.

The addition of exogenous enzymes such as xylanase and hemicellulase in feed is highly recommended to increase the digestibility of NSP which initially cannot be digested properly (Morgan et al. 2022). Starch as a polysaccharide is important as an energy source in feed ingredients. However, there are several things that make starch indigestible in poultry digestion. First, the content of new feed energy sources can be increased if NSP which is found in significant amounts in some vegetable ingredients (soybean meal) can also be utilized (Aftab and Bedford 2018) (Nguyen, Bedford, and Morgan 2021). Apart from that, poultry, as a monogastric animal, cannot utilize this energy source because it does not have the enzymes used to digest complex NSP (Musigwa et al. 2021). One of the enzymes that contains xylanase and hemicellulase enzymes is Phprozyme HP. The Phprozyme HP enzyme acts as an anti-NSP enzyme whose role is to break down NSP into sugar and oligosaccharides so that the energy source used for growth can be increased. The use of several enzymes in feed can work in different locations and target different substrates so that it can increase the digestibility of feed nutrients in the gastrointestinal tract (Bedford 2018).

Research on the addition of Phyprozyme HP has so far only been carried out on tilapia (*Oreochromis niloticus*) (Mohammed and Budihargo 2020). The research results showed that there was a significant difference in the average final weight of tilapia due to the effect of adding Phyprozyme HP to the feed. Phyprozyme HP can be a supplement to tilapia fish feed without having a negative effect on tilapia carcass composition. The growth response parameters of *Oreochromis niloticus* increased at a dose of 0.05-0.4 g/kg feed, but could reduce fish weight at a high dose, namely at 0.47 g/kg feed. The results of this research are a very good start and can be developed again in the future because there has been no further research regarding Phyprozyme HP supplementation in animals, including broilers, which has shown the same results. In general, some plant-based feed ingredients used as feed ingredients contain anti-nutritional factors. These anti-nutritional factors play a negative role in the gastrointestinal tract and cause the digestibility of nutrients in feed to be disturbed. The Phyrozyme HP enzyme which contains several enzymes that act as anti-NSP can break down NSP so that the digestibility of nutrients in feed can increase. The Phyrozyme HP enzyme also plays a role in increasing the efficiency of the gastrointestinal tract and maintaining intestinal health in livestock.

The negative impact of Phyprozyme HP enzyme supplementation was found at high dose concentrations in the research results. This may be due to the excessive release of monosaccharides. The presence of excessive monosaccharides can lead to hyperglycemia (Arnone et al. 2022). Research on carbohydrate tolerance in experimental animals suggests that high concentrations of galactose in the blood can cause galactosemia, which cannot be tolerated in the bodies of broiler chickens (Haskovic et al. 2020) (Van Laar, Grootaert, and Van Camp 2021) (Md Benzamin et al. 2020). Prolonged galactosemia in chickens can lead to decreased growth performance and feed intake.

## 4. CONCLUSIONS

The research findings demonstrate that the addition of single enzyme bromelain and multienzyme Phyprozyme HP did not significantly differ in terms of broiler growth performance. The addition of bromelain in all treatments could lead to weight loss, while the addition of multienzyme could increase weight gain up to a dosage of 0.33g/kg of feed. Adding Phyprozyme HP at doses exceeding 0.33 g/kg of feed may decrease broiler weight. Bromelain addition could decrease carcass percentage by up to 11.07%, while multi-enzyme addition could increase carcass percentage by up to 8.83%, but could decrease carcass percentage by up to 7.68% at the highest dose.

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## Marchantiophyta Family Radulaceae in Batang Toru Forest, North Sumatra Indonesia

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ABSTRACT	

KEYWORDS:	The diversity of liverworts in North Sumatra is currently limited, highlighting
Batang Toru West Block,	the need for further investigations. Therefore, this study aims to collect and
Indonesia,	determine data on the liverworts of the Radulaceae family in the Batang Toru
Marchantiophyta,	forest of North Sumatra. This forest was selected because it has very high
Radulaceae, North	rainfall with suitable humidity for the growth of liverworts. The samples were
Sumaira	collected using an exploratory survey method, namely by collecting all the
	liverworts of the <i>Radulaceae</i> family along the Batang Toru forest hiking trails.
	The results showed 17 species of which three are new records for Sumatran
© 2024 The Author(s). Published by Biology Education Department,	liverworts namely <i>Radula iwatsukii</i> , <i>Radula kinabaluensis</i> and <i>Radula obscura</i> .
Faculty of Teacher Training and	The <i>Radulaceae</i> family was found as epiphytes on tree trunks and leaves. Based
Education, Universitas Muhammadiyah Surakarta.	on the number of specimen collections the most commonly found species was
This is an open access article under	Radula javanica. In addition. Radulaceae in the Batang Toru forest was spread
https://creativecommons.org/license	at altitudes between 800 and 1100 m as 1 and can be included in the Lewen
s/by-nc/4.0/.	at antitudes between 800 and 1100 m.a.s.1 and can be included in the Lower
	Montane Forest category.

## 1. INTRODUCTION

Liverworts are a diverse group of plants that can be found in a wide range of habits including lowland and upper montane forests (Gradstein, 2021) with an estimated 5000 species (Heinrichs et al. 2005). They live in moist, shaded environmental conditions, and can grow submerged in water or terrestrial habit (Kuglerová et al. 2016). One type of liverwort that lives in the environmental conditions described above is the Radulaceae family (Zartman et al. 2015).

The *Radulaceae* family is spread almost all over the world in areas with humid, tropical, or warm climates (Gradstein et al. 2001). They grow as epiphytes on tree trunks, branches, or on leaves (Promma & Chantanaorrapint 2015). Members of this family are characterized by rhizoids that arise from their lobules and lack ventral leaves (Yamada 1979).

Information about the distribution of Radulaceae in Indonesia is still limited although previous studies have been conducted in several regions, specifically in North Sumatra, and the results have not been published. Collection and information about leafy liverworts of the Radulaceae family in the Batang Toru Forest Block West Block, have also been reported to date. Therefore, this study aims to examine the Radulaceae species in the Batang Toru Forest Area West Block North Sumatra, for an inventory of the species.

## 2. MATERIALS AND METHODS

This study was conducted in the Batang Toru Forest Block, North Sumatra, Indonesia, which has an area of 133,841 hectares. This forest is divided into two regional blocks separated by the fracture of the Sumatran fault. The western block has an area of 78,891 hectares, while the eastern block has an area of 54,950 hectares. Furthermore, the location of Camp Mayang in the West Block is a 12,000-hectare Flora and Fauna monitoring station located between 49°93'31" East Longitude and 18°63'20" South Latitude. The tropical climate has high rainfall ranging from 4,500 to 5,000 mm per year, while temperatures at night can drop to 14°C. The highest temperature during the day is 31°C, and humidity ranges from 33% - 95% (YEL & WALHI 2013)

The samples were collected using an exploratory survey method by exploring the study area as has been done by Hussain *et al.* (2019). All samples obtained were photographed and collected using a sharp knife or pry tool from various substrates such as soil, tree trunks, rocks, weathered wood, and leaves. The specimens were collected dry and wet by my self. The dry collection was carried out by placing the specimens obtained from the field into paper envelopes. Meanwhile, the wet collection was performed by inserting the specimens into a plastic clip filled with wet wipes and labeled with a collection number (POCS 2020). The study location is shown in (Figure 1) below.

The collected specimens were identified using identification keys and species descriptions from various available literature specifically (Gradstein, 2011);(Renner et al., 2013);(Bakalin & Klimova 2020) and (Oliveira-Da-Silva et al. 2020) and other publications on Malesiana and Asian liverworts such as (So 2006); (Yamada 1979);(Yamada & Piippo 1989); (Majumdar *et al.* 2016); (Renner 2014a); (Söderström *et al.* 2016); (Promma & Chantanaorrapint 2015); and (Hedwigia & Oliveira 2020). Some of the important morphological characteristics include plant width, lateral leaf shape, base, sac, margin, apex, and trigon cell, as well as underleaves, and perianth. Identification was carried out at the Laboratory of Plant Taxonomy, Department of Biology, Universitas Sumatera Utara.



Figure 1. Map of Study location in Batang Toru Forest (Damanik et al., 2022)

## 3. RESULTS AND DISCUSSION

The results showed onegenus consisting of 17 species, including three new record types for Sumatra, namely *Radula iwatsukii*, *Radula kinabaluaensis*, and *Radula obscura*. Based on the distribution of altitude, the *Radulaceae* family in the West Block Batang Toru Forest area is in the lower montane forest category between 800-1300m as illustrated Figure 2.



Figure 2. Distribution of *Radulaceae* families based on altitude in Batang Toru Forest

Key Identification of the Radulaceae family in the Batang Toru Forest area

1.	a.	Branching with amentulose in each leaf axil	2
	b.	Branching without amentulose in each leaf axil	4
2.	a.	Amentulose is equivalent in length to the lobules	4. R. formosa
	b.	Amentulose is longer than the lobules	3
3.	a.	Leaves attachment is flat	5. R. iwatsukii*
	b.	Leaves attachment is flat to curved	17. R. yangii
4.	a.	The base of the dorsal leaf is flat	12. R. protensa
	b.	The base of the dorsal leaf is curved	5
5.	a.	Lobules with obtuse apex, trigon clearly defined	6. R.javanica
	b.	Lobules with subacute apex, trigone not clearly defined	6
6.	a.	Trunk branches irregular	
	b.	Trunk branches regular	7
7.	a.	The bases of the lobules are flat	1. R. anceps
	b.	The bases of the lobules are curved	
8.	a.	Lobules ovoid in shape, flat margins	3. R. campanigera
	b.	Lobules are rhombus-shaped, edges curved	9
9.	a.	The lobe ends are tapered	2. R.apiculata
	b.	The lobe ends are flat	
10.	a.	Lobule length 1/2 of the lobe, rectangular	8. R.meyeri
	b.	Lobule length 1/3 of the lobe, round in shape	
11.	a.	Lobules attachments tapered, blunt ends	13. R.retroflexa
	b.	Lobules attachments curved, rounded ends	
12.	a.	Lobules are rectangular.	11. R.oreopsis
	b.	Lobules are round resembling a crescent moon14. R. ret.	roflexa var fauciloba
13.	a.	Cell shape is square to round, the cell wall is thin	R.kinabaluensis*
	b.	Cell shape is round, the cell wall is thick	14
14.	a.	Cell surface is smooth, trigons are tight	10. R.obtusiloba
	b.	Cell surface is rough, trigons are sparse	
15.	a.	Cell wall is thick, trigon not clear	9. R.obscura*
	b.	Cell wall is thin, trigon clear	16
16.	a.	The edges of the leaves have gemma, smooth cell surface	16. R. tjibodensis
	b.	The edge of the leaf has no gemma, the cell surface is rough	15. <i>R</i> .
		Sumaterana	

\* New record for Sumatera

Description of the Radulaceae family in the Batang Toru Forest area

## 3.1. Radula anceps Sande. Lac (Figure 3)

Nederl. Kuidk. Arch. 3 :419 (1854)- *Radula acuta* Mitt. In Seeman, FI. Vitiensis: 410 (1871) The specimens were brownish-yellow in color with a width of 1.3-3 mm and radula-type branching. The leaves are closely arranged together, oval in shape, 0.68-0.9 mm long, and 0.41-0.5 mm wide. They also have a flat attachment, dorsal base, ventral base, and scalloped edges. The teeth range from short to long and pointed, measuring 2-5 cells long with a tapered tip. The cells are elongated and round with thin walls, smooth surface, as well as tight trigons. The lobules are rectangular in shape, and measure 1/3 of the lobes'length. They also have a flat base, attachments, edges, as well as pointed to a blunt tip.

**Specimen examined**: Sumatra: North Sumatra: Batang Toru forest, epixylous, June 13<sup>th</sup>, 2019 alt. 895 m asl. RD 314

**Distribution**: Indonesia (Sumatra, Java), Borneo, Philippines, Japan, Papua New Guinea, New Caledonia, Caroline (Yamada 1979).

**Habitat and Ecology**: Epiphylloussat an altitude of 895 m asl, humidity at 82% and temperature at 24,1°C.

## 3.2. Radula apiculata Sande. Lac (Figure 4)

Hedwigia 23: 150 (1884) - *Radula paucidens* Steph. Ex Castle, Rev. Bryol. Lichenol. 30:39 (1961)

The specimens have a brownish-yellow color with a width of 1-1.7 mm. The branches are not of radula type, while the leaves are closely arranged together, oval in shape, 0.5-0.68 mm long and 0.41-0.5 mm wide. They have a curved attachment, a tapered tip with a flat dorsal base. Both the dorsal and ventral edges are flattened and dentate, while the tip has one tooth rounded and pointed, 5 cells long. The cells are round to elongated with thin walls, and a smooth surface. Moreover, the trigon is quite tight, the lobule is quadrangular measuring one-fourth of the length of the lobe, with a curve base, flat attachment, obtuse dorsal edge, ventral edge covering the shaft, and a blunt tip.

**Specimen examined**: North Sumatra (Batang Toru forest) epiphyllous, June 25<sup>th</sup>, 2019 alt. 905 m asl. RD 2296.

**Distribution**: Indonesia (Sumatra), Thailand, Philippines, Borneo, Taiwan, Papua New Guinea, Samoa, Tahiti, Caroline (Yamada 1979).

**Habitat and Ecology**: Epiphytes on the leaves and stems are found at an altitude of 893 m asl, humidity at 85%, and a temperature of 26.1°C.

#### 3.3. Radula campanigera mont. (Figure 5)

Piippo, S. 1990. Annotated catalogue of Chinese Hepaticae and Anthocerotae. J. Hattori Bot. Lab. 68: 1–192.

Lee, G. E., S. R. Gradstein, E. Pesiu & N. Norhazrina Binti Nik Mohamed Kamil. 2022. An updated checklist of liverworts and hornworts of Malaysia. PhytoKeys 199: 29-111.

The specimens are characterized by a yellowish-green color and a width of 1.5-2 mm. Their branching pattern is irregular, while the leaves arrangement are close together with oval-shaped leaves measuring 0.75-0.98 mm long and 0.61-0.75 mm wide. They also have a flat attachment, dorsal base, curved ventral base, as well as a flat edge and rounded ends. The cells are rounded in shape with a thick cell wall, smooth cell surface, and a clear trigon with a bulging shape. Additionally, the lobules are ovate, measuring 1/3 of the lobes' length, with a flat base, attachment, edge, and rounded tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 17<sup>th</sup> until 24<sup>th</sup>, 2019 alt.752-1006 m asl, RD 277, 289, 296, 874, 1207, 1219, 1367, 1987,2003, 2118, 2164

**Distribution**: Malay Peninsula, Indonesia (Sumatra, Java, Kalimantan, Sulawesi), Taiwan, Thailand (Renner 2005); (Lukitasari 2018)

**Habitat and Ecology**: Epiphytes on tree trunks at an altitude of 752-1006 m asl, humidity at 72-85%, and temperature at 25.1-29.2°C.

#### 3.4. Radula formosa Meissn. (Figure 6)

In Gott. *et al.*, Synop. Hepat: 258 (1845).- *Jungermania formosa* Meissn. Sprengel in Linnaeus Syst. Veg. 4(2): 325 (1827).-*Radula pycnolejeunoides* Schifn., Nova Acta Acad Caes. Leop. Carol 60 (2): 247 (1893).-*Radula novae guineae* Steph., Spec, Hepat 4: 233 (1910).

The specimens have a brownish-yellow color, and a 1.2-4 mm width. They exhibit regular branching with small shoots in each leaf axil, and the length of small shoots is equivalent to that of the lobules. The leaf arrangement is close together, and the leaves are oval-shaped, measuring 0.53-0.9 mm long and 0.4-0.6 mm wide. Furthermore, they have rounded attachments, a flat tip with a curved dorsal base and flat ventral base, a flat edge, and rounded leaf tips. The cells are rounded in shape, have thin to thick walls, a smooth surface, as well as a dense trigon that is triangular in shape. The lobules are oblong, measuring 1/2 the length of the leaf, with a flat base, attachment, edge, and tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 17<sup>th</sup> until 24<sup>th</sup>, 2019 alt. 863-931 m asl, RD 186, 1225, 1664, 1674, 1746, 1775, 1882, 1990, 2015, 2134, 2169, 2184.

**Distribution**: Indonesia (Sumatra, Java), Borneo, Thailand, Malaya, Ceylon, Papua New Guinea, New Caledonia, Fiji, Tahiti, Japan, Philippines (Yamada 1979)

**Habitat and Ecology**: Epiphytes on weathered wood and leaves at an altitude of 863-931 m asl, humidity at 87-90% and temperature at 24.1-25.9°C.

## 3.5. Radula iwatsukii Yamada (Figure 7)

Yamada, kohsaku, and Sinikka Piippo. "Bryophyte Flora of the Huon Peninsula, Papua New Guinea. XXXII. Radula (*Radulaceae, Hepaticae*)." *Annales Botanici Fennici*, vol. 26, no. 4, 1989, pp. 349–87. *JSTOR*.

The specimens are brownish-yellow in color and have a width of 1.2-2 mm. They exhibit an amentulose branching pattern with small shoots in each leaf axil, and the length of small shoots is larger than that of leaf size. The leaf arrangement can be sparse or close together, and the leaves are ovoid in shape, measuring 1-1.6 mm long and 0.4-0.8 mm wide. They have rounded adherents, a curved dorsal base, a rounded ventral base, a flat edge and rounded tips. Furthermore, the cells are rounded in shape with thin to thick walls, a smooth surface, as well as a clear and bulging trigon. The lobules are oblong, measuring 1/2 length of the leaf with flat and bubbly, flat attachment, edge flat, and tip flat.

**Specimen examined**: North Sumatra (Batang Toru forest) epiphyllous, June 16<sup>th</sup>, 2019 alt. 891 m asl, RD 793

**Distribution**: Indonesia (New record for Sumatra), Borneo, Solomon Islands (Yamada 1979)

**Habitat and Ecology**: Epiphytes on leaves at an altitude of 908 m asl, humidity at 81%, and temperature at 20.5°C.

#### 3.6. Radula Javanica Gott. (Figure 8)

In Gott. *et al.*, Synop. Hepat: 257.1845. Yamada. J. Hatt. Bot. Lab. 45: 235. 1979. Yamada & Piippo. Ann. Bot. Fennici 26: 360. 1989.

The specimens range from green to yellowish in color with a width of 1.3-2.5 mm, and an irregular branching pattern. The leaf arrangement is close together with ovate-shaped leaves,

measuring 1.2-1.3 mm long and 0.8-0.9 mm wide. They also have curved attachments with a dorsal and ventral base, flat edge, and rounded ends. The cells are rectangular to elongated in shape, with thin cell walls, a smooth surface, and a fairly clear trigon. Additionally, the lobules are rhombus-shaped, and measure 1/3 - 1/2 the leaf length, with curved base, attachments, edges, and an almost pointed tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 17<sup>th</sup> until 25<sup>th</sup>, 2019 alt. 857-931 m asl, RD 737, 1041, 1043, 1055, 1095, 1099, 1158, 1206,1270, 1558, 1607, 1720, 1751, 1791, 1805, 1904, 1998, 2040, 2091, 2291.

**Distribution**: South America, Central America, Sri Lanka, India, Japan, Philippines, Malay Peninsula, Indonesia (Sumatra, Java, Kalimantan, Papua), Papua New Guinea, Thailand (Yamada 1979)

**Habitat and Ecology**: Epiphytes on tree trunks, weathered wood and leaves at an altitude of 861-931 m asl, humidity at 78-86% and temperature at 25.8-30.1°C.

#### 3.7. Radula kinabaluensis Yamada. (Figure 9)

Misc. Bryol. Lichenol 6:97 (1973)

The specimens range from green to yellowish in color with a width of 1.5–2.3 mm and an irregular branching pattern. The leaf arrangement overlaps, with elongated round-shaped leaves measuring 0.8-0.95 mm long and 0.4-0.5 mm wide. Furthermore, they have a curved attachment, flat tip, curved dorsal base, ventral base, flat edge, and rounded tip. The cell shape is square to round with a thin cell wall, smooth surface, and a clearly bulging trigon. The lobules are small, rectangular in shape, 2/3 of leaf length, with a curved base, attachment, edge, and an almost blunt tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 19<sup>th</sup> until 23<sup>th</sup>, 2019 alt. 802-891 m asl, RD 1588, 1600, 1638, 1878

**Distribution**: Indonesia (New record for Sumatra), Borneo (Yamada 1979); (Söderström *et al*, 2016).

**Habitat and Ecology**: Epiphytes on tree trunks, weathered wood and leaves at an altitude of 802-891 m asl, humidity at 78-90% and temperature at 27.1-29.5°C.

#### 3.8. Radula meyeri Steph. (Figure 10)

Hedwigia 27: 62. 1888. Yamada. J. Hatt. Bot. Lab. 45: 242. 1979.

The specimens are yellowish-green in color with a width of 1.2-1.5 mm and an irregular branching pattern. The leaves overlap and have an ovoid shape, measuring 0.5-0.7 mm long and 0.4-0.6 mm wide. Furthermore, they also have curved attachments, with a dorsal base, ventral base, flat edge, and rounded ends. The cell shape ranges from round to hexagon with a thick wall, smooth surface, and clear triangular trigon. The lobules are rectangular, and measure 1/2 the lobes' length, with a flat base, curved attachments, flat edges, and an almost pointed tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 19<sup>th</sup> until 23<sup>th</sup>, 2019 alt. 802-891 m asl, RD 655, 1267, 1268, 1572, 1575, 1729, 1801, 1820, 1888, 1918, 1995, 2186, 2247.

Distribution: Africa, Indonesia (Sumatra), Thailand (Yamada 1979; Lai et al. 2008).

**Habitat and Ecology**: Epiphytes on tree trunks, weathered wood and leaves at an altitude of 857-931 m asl, humidity at 78-91% and temperature at 20.1-26.9°C.

## 3.9. Radula obscura Mitt. (Figure 11)

Jour. Proc. Linn. Soc. London 5 : 107 (1861)

The specimens are yellowish-green in color with a width of 1.5-1.7 mm and an irregular branching pattern. The leaves are closely arranged together, have an elongated ovoid shape, and

measure 0.7-0.8 mm long and 0.5-0.6 mm wide. Furthermore, they have a flat attachment, curved dorsal base and ventral base, flat edge, and rounded tip. The cell shape ranges from round to elongated, with a thick cell wall, rough surface, and a sparse star-shaped trigon. The lobules are square in shape, and measure 1/3 the lobe length, with a blunt base, attachment, slightly curved edges, and a blunt tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 19<sup>th</sup>, 2019 alt.802-972 m asl, RD 1382, 1654

**Distribution**: Indonesia (New record for Sumatra), Borneo, Thailand, India, Ceylon, Nepal, Philippines, Taiwan, China (Yamada 1979); (Söderström *et al.* 2016; Shu *et al.* 2017).

**Habitat and Ecology**: Epiphytes on weathered wood at an altitude of 872-908 m asl, humidity at 78-87% and temperature at 21.2-29.1°C



Figure 3. Radula anceps, 4. Radula apiculate, 5. Radula campanigera, 6. Radula formosa, 7. Radula iwatsukii, 8. Radula javanica, 9. Radula kinabaluensis, 10. Radula meyeri. Habit of Radula (seen from the ventral side)

## 3.10. Radula obtusiloba Steph. (Figure 12)

Bull. Herb. Boiss. 5: 105 (1897). *Radula japonica* Gott. Var. Minor Amak., Jour. Hattori Bot Lab. 13:58 (1995).

The specimens range from yellow to brown in color with a width of 1.3-1.6 mm, and an irregular branching pattern. The leaves overlap, have an oval shape, and measure 0.7-0.9 mm long and 0.40-0.5 mm wide. Furthermore, they have a flat attachment to the stem, dorsal base, flat ventral base and edge, as well as a rounded tip. The cells are rectangular in shape, with thick walls, a smooth surface, and adjacent trigons which are triangular in shape. The lobules are small and rectangular, measure 1/3 the leaf length, with a flat base, attachment, edge, and blunt tip.

**Specimen examined**: North Sumatra (Batang Toru forest) epiphyllous, June 20<sup>th</sup>, 2019 alt. 802 m asl, RD 1587.

Distribution: Indonesia (Sumatra), Korea, Japan (Yamada, 1979); (Bakalin et al., 2022)

**Habitat and Ecology**: Epiphytes on tree trunks at an altitude of 802 m asl, humidity at 80% and temperature at 29.3°C.

#### 3.11. Radula oreopsis M.A.M.Renner. (Figure 13)

M.A.M. Renner 6270, V.C. Linis. E.A. Brown, 24 Maret 2012.

The specimens are yellowish-green in color and have a width of 1.6-2.1 mm with an irregular branching pattern. The leaves are arranged close together, ovoid in shape, as well as 0.8-1.2 mm long and 0.67-0.8 mm wide. Furthermore, they have a flat attachment, dorsal base, slightly curved, with a flat edge and rounded ends. The cell shape ranges from round to elongated, with thick cell walls, rough surface, as well as sparse and triangular trigon. The lobules are asymmetric rhombic, 1/3-1/2 of leaf length, with a tapered base, curved attachment, flat edge slightly curved, and a pointed tip.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 12<sup>rd</sup> until 22<sup>rd</sup>, 2019 alt. 857-931 m asl, RD 266, 1046, 1054, 1815, 1873, 1996, 1997.

**Distribution** : Indonesia (Sumatra), Australia (Renner 2014b)

**Habitat and Ecology**: Epiphytes on tree trunks and rotting wood at an altitude of 863-1035 m asl, humidity at 82-87% and temperature at 28.1-29.6°C.

## 3.12. Radula protensa Lindenb. (Figure 14)

In Meissner, Bot. Zeit. 6: 462 (1848)

The specimens are yellow to brownish in color and have a width of 0.6-3 mm with an irregular branching pattern. The leaves are tightly arranged laterally, overlapping and folding at the tips. They have a flat attachment, are oval-elongated, 0.3-0.5 mm long, and 0.3-0.4 mm wide, with a flat dorsal base, edge, and rounded tip. The cells are hexagonal in shape with thick walls, a smooth surface, tight trigon, and a triangular shape. The lobules are ovate-shaped, measuring 1/8 the length of the leaf, with a flat base, curved flat edge, rounded tip, and curved attachment. However, generative organs are absent.

**Specimen examined**: North Sumatra (Batang Toru forest) epiphyllous, June 17<sup>th</sup>, 2019 alt.1050 m asl, RD 1094

**Distribution**: Indonesia (Sumatra, Java), Borneo, India, Philippines, China, Taiwan, New Guinea (Williston 1912; Yamada 1979).

**Habitat and Ecology**: Epiphytes on leaves at an altitude of 1050 m asl, at 80% humidity and temperature at 25.4°C.

## 3.13. Radula retroflexa Tayl. (Figure 15)

London J. Bot. 5: 378. 1846. Yamada. J. Hatt. Bot. Lab. 45: 281. 1979. Yamada & Piippo. Ann. Bot. Fennici 26: 383. 1989.

The specimens are yellowish-green in color, with a width of 1.3-1.6 mm, and exhibit an irregular branching pattern. The leaves are arranged closely together, resembling kidney shapes measuring 0.3-0.4 mm long and 0.3-0.6 mm wide. Furthermore, they have a tapered attachment, with a dorsal base that is fully curved, resembling a crescent moon, and a ventral base that is half curved. The edges are hairy, with long and pointed hairs, and rounded ends. The cell shape is isodiametric with thick walls, rough surface, a tight trigon, and a triangular shape. The lobules are oblong, measuring 1/2 the length of the leaf, with a tapering base, attachment, a flat and curved edge on the ventral side, and a blunt tip.

**Specimen examined**: North Sumatra (Batang Toru forest) epiphyllous, June 19<sup>th</sup>, 2019 alt.893 m asl, RD 1468

**Distribution**: Indonesia (Sumatra, Java), Australia, Samoa, Papua New Guinea, New Caledonia, Marquesas, Philippines, Taiwan, Japan (Yamada 1979; So 2006)

**Habitat and Ecology**: Epiphytes on leaves at an altitude of 893 m asl at 82% humidity and temperature at 26.5°C.

## 3.14. Radula retroflexa var fauciloba (Steph.) Yamada (Figure 16)

*Radula fauciloba* Steph., Spec. Hepat.4:188 (1910).-*Radula weymouthiana* Steph., Spec. Hepat. 4: 190 (1910).-*Radula helmsiana* Steph., Spec. Hepat.4:123 (1910).-*Radula lunalatiloba* Horik, Jour. Hiroshima Univ. Scr. B, div. 2, 2: 226 (1934).

The specimens exhibit a green to yellowish color, with a width of 2-2.5 mm and a regular branching pattern. The leaves are arranged closely together, with ovoid shapes, measuring 1-1.1 mm long and 0.9-1 mm wide. They also have curved attachments, a dorsal base, a flat edge, and tips that range from rounded to blunt. Furthermore, the cell shape is elongated, with thin walls, a smooth surface, and a tight trigon. The lobules are crescent moon-shaped, measuring 1/3 of the length of the leaf, with curved bases, attachments, and edges, and a relatively blunt tip, with sinuous edges almost forming a sinus.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 19<sup>th</sup> until 22<sup>rd</sup>, 2019 alt. 863-931 m asl, RD 1258, 1269, 1304, 1534, 1567, 1603, 1610, 1764, 1975, 1993.

**Distribution**: Japan, Taiwan, Philippines, Thailand, Malaysia, Indonesia (Sumatra, Java, Borneo), Hawaii, Tasmania, New Zealand (Yamada 1979; So 2006)

**Habitat and Ecology**: Epiphytes on tree trunks at an altitude of 863-931 m asl, humidity at 82-83% and temperature at 22.3-29.5°C.

## 3.15. Radula Sumatrana Steph. (Figure 17)

Spec. Hepat. 4: 204. 1910. Yamada. J. Hatt. Bot. Lab. 45: 250. 1979.

The specimens are yellowish-green in color and have a width of 2-2.6 mm with an irregular branching pattern. The leaf arrangement varies from sparse to dense at the tip stature. Furthermore, they have an ovoid shape, 1.15 - 1.3mm long and 0.82 - 0.9 mm wide, with a flat to slightly curved attachment, a curved dorsal base, a flat ventral base, a flat edge, and rounded tips. The cells are round in shape, with thick walls, a rough surface, and tight triangular trigones. The lobules are quadrangular, measuring 1/2 the length of the leaf, with a curved bases, attachments, flat edges, and blunt tips.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 20<sup>th</sup> until 25<sup>th</sup>, 2019 alt. 802-911 m asl, RD 1605,1763, 2245

**Distribution**: Indonesia (Sumatra, Java, Kalimantan), India, Thailand, Malaysia (Yamada 1979; Lai *et al*, 2008; Singh *et al*, 2016)

**Habitat and Ecology**: Epiphytes on tree trunks and leaves at an altitude of 802-911 m asl, humidity at 82-85% and temperature at 26.2-26.1°C.

## 3.16. Radula tjibodensis Goebel. (Figure 18)

Nova Acta Acad. Caes. Leop. Carol. 60 (2): 249. 1893. Yamada. J. Hatt.Bot. Lab. 45: 294. 1979

The specimens exhibit yellowish-green color with a width of 1.2-1.5 mm and irregular branching. The leaves are closely arranged together, ovate-shaped, 0.63-0.8 mm long and 0.5-0.6 mm wide. They also have curved attachments, a dorsal base, a flat ventral base and edges. The tip are rounded and have gemma cups. Furthermore, the cells are hexagonal in shape with thick walls, a smooth surface, and tight and triangular trigones. The lobules are rectangular, measuring <sup>1</sup>/<sub>4</sub> of the leaf length, with flat bases, attachments, edges, and tips forming a bulge.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 13<sup>th</sup> until 24<sup>th</sup>, 2019 alt. 825-928 m asl, RD 321, 800, 1432, 1435, 2018

**Distribution**: India, Taiwan, Philippines, Thailand, Vietnam, Brunei, Singapore, Malaysia, Indonesia (Sumatra, Java, Kalimantan, Papua), Papua New Guinea, Samoa (Yamada, 1979; Gradstein, 1997; So, 2006; Lai *et al.*, 2008).

**Habitat and Ecology**: Epiphytes on tree trunks and leaves at an altitude of 825-928 m asl, humidity at 82-86% and temperature at 20.1-26.5°C.

#### 3.17. Radula yangii Yamada, nom. Nov (Figure 19)

*Radula pinnulata* Yang, Taiwania 7:35-39 (1960), non Mitt. In. Seeman, Flora Vitiensis: 410 (1871)

The specimens are brownish-yellow in color and have a width of 1.1-2.1 mm. The branching pattern is amentulose, with shoots in each leaf axil. The length of the shoot can sometimes be as small as a lobe or large as the leaves. They have an elongated oval shape, 1-1.8 mm long and 0.8-1 mm wide, with a flat attachment, a curved dorsal base, a rounded ventral base, flat edges, and rounded tips. Moreover, the cell shape ranges from rectangular to elongated rectangle, with thin walls, a smooth surface, and tight trigons. The lobules are oblong, measuring 1/2 the length of the leaf, with flat bases, attachments, edges, and tips.

**Specimens examined**: North Sumatra (Batang Toru forest) epiphyllous, June 16<sup>th</sup> until 22<sup>rd</sup>, 2019 alt. 877-937 m asl, RD 784, 786, 788, 1820, 2017, 2075

**Distribution**: Indonesia (Sumatra), Borneo, Thailand, Taiwan, Malaysia, Ceylon (Yamada 1979; Promma & Chantanaorrapint 2015)

**Habitat and Ecology**: Epiphytes on tree trunks, weathered wood and leaves at an altitude of 877-937 m asl, humidity at 80-86% and temperature at 21.6-27.4°C.



Figure 11. Radula obscura, 12. Radula obtusiloba, 13. Radula oreopsis, 14. Radula protensa, 15. Radula retroflexsa, 16. Radula retroflexsa var fauciloba, 17. Radula sumatrana, 18. Radula tjibodensis. 19. Radula yangii. Habit of Radula (seen from the ventral side)

## 4. CONCLUSIONS

This study, conducted in the Batang Toru Forest Area, West Block, North Sumatra, discovered 17 species of the Radulaceae family, of which three are new records for Sumatran

liverworts, namely R. iwatsukii, R. kinabaluensis, and R. obscura. They were found as epiphytes on tree trunks and leaves. Based on the number of specimen collections, the most commonly found species is Radula javanica. Additionally, Radulaceae in the Batang Toru forest are distributed at altitudes between 800 and 1100 meters above sea level, hence they are included in the Lower Montane Forest category.

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## Implementation of Indigenous Knowledge-Laden Leaflets on Student Learning Outcomes on Global Warming Materials

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## ABSTRACT

KEYWORDS:	This study aims to examine the Implementation of Indigenous Knowledge-
Leaflet,	Laden Leaflets on Student Learning Outcomes on Global Warming Material.
Indigenous Knowledge,	This study used a quasi-experimental method with a Nonequivalent control
Learning Outcomes, Global Warming	group design. This study used classes XA, XD, XE and XF, each class
Giobai warming.	consisting of 36 students. In the class there is a selection of criteria, for class
	XA with high class criteria, class XD with medium class criteria, class XE with
	low class criteria, and class XF as a control class (Biology Teacher document).
	The research instrument is in the form of cognitive, affective, and psychomotor
	results. Data analysis of learning outcomes using one way anova test with
© 2024 The Author(s). Published	normality test and homogeneity test. The results of the calculation of learning
	outcomes with one way anova test obtained a significance value (2-tailed) of
by Biology Education Department, Faculty of Teacher Training and	(0.000) < (0.050) then the null hypothesis (H0) is rejected. And then the further
Education, Universitas Muhammadiyah Surakarta. This is an open access article under the CC BY-NC license: https://creativecommons.org/license	test obtained a significance probability value of $(0.917) > (0.050)$ , therefore
	(H0) is accepted. This means that the treatment has an effect on student learning
	outcomes and the results of further tests have no effect on the learning outcomes
s/by-nc/4.0/.	of experimental classes (high, medium, and low clas)

## 1. INTRODUCTION

Biology learning is part of natural science, where biology is basically not a difficult science to learn, learning biology means learning about yourself and the environment (Harefa et al. 2022). The important task of learning biology is to develop the ability to think creatively, critically and innovatively, so that students are able to face the increasing development of science and technology in this era (Setiawan 2019). When studying biology, problems that arise during learning include the ability to identify problems by asking the teacher, students find it difficult to express their opinions. In addition, it is difficult for students to actively participate in learning due to poor classroom conditions. For example, students pay less attention to learning through conversation, so that the teacher's teaching strategy does not get feedback from (Hariyatmi et al. 2020). Students are not able to develop their thinking skills, instead students memorize information or memorize material and collect various information that can be remembered. Therefore, students are unable to connect what has been learned (Gita Lestari et al. 2020). Therefore, the application of models in biology learning can be the right choice to develop students' thinking skills, and it is good to learn biology with the help of learning models so that it helps deliver learning well (Agnesa and Rahmadana 2022).

Learning models can be called plans or models that can be used to plan, design materials and guide learning in the classroom or elsewhere. The learning model can be used as a selection model, meaning that teachers can choose the right and effective learning model to achieve their educational goals (Mirdad, 2020). Therefore, there are different learning models, namely constructivism model, inquiry-based learning model, problem solving model, problem-based learning model, cooperative learning model, project-based learning model, case-based learning model, flipped classroom model. Master learning model, simulation-based model and gamification-based model. The success of teachers in implementing learning is supported by several things, namely the teacher's ability to manage and implement learning models that are in accordance with the characteristics of the material (Fakhrizal and Hasanah 2021).

The problem-solving learning model (PS) is a learning approach that presents a problem as a context in which students can learn critical thinking and problem-solving skills as well as obtain important information and concepts from the learning material. Problem solving is the study of solving real-world problems in a scientific, rational, and systematic way (Ahmad 2023). In problem solving the process mainly lies within the learner which can be seen as a process where the learner finds a combination of rules that he has learned first which he uses to solve new problems (Suhadah 2023). Problem solving learning aims to foster students' abilities by using the widest possible thinking to the maximum of their capacity. To train students' thinking skills, students will generally use the principles of rational thinking as a reference to gain understanding in answering questions and problems (Diva and Purwaningrum 2023). For this reason, this learning model was chosen as one of the solutions to develop students' problem solving skills by using learning media (Zendrato et al. 2022).

Learning media is useful to support learning activities so that it makes the learning environment more lively, innovative and creates a learning spirit in students. Even the material presented is clear and easy for students to understand so that in the end it improves learning outcomes (Rosanaya and Fitrayati 2021). Leaflets are printed or graphic media that can be used as learning materials, information sources such as pages that are equipped with images to generate more interest in readers than seeing them (Wahyuni et al. 2022). Leaflets as teaching aids can make it easier for teachers to convey subject matter to students. Magazines have advantages and disadvantages. The advantage is being able to visualize messages, information and concepts and present images that make it easier for students to explain the material. The disadvantages are that making brochures requires language and illustration skills, the process of making magazines is quite long and easy to tear (Yusandika et al. 2018).

Biology learning leaflets can be used as an alternative to interesting learning materials so that students are not bored, and have a positive effect on student learning outcomes, and the content is a summary of the subject matter (Sari et al. 2021). The leaflet contains a summary of educational material collected from various learning sources, both books and the internet, which is compiled into a leaflet (Darsad 2020). The nature of biological material usually involves understanding various aspects of life, including the structure and function of organisms, ecosystems, genetics, evolution, and more. Biology course materials may include concepts such as cells, metabolism, reproduction, adaptation, and interactions between organisms. In addition, biology teaching materials often include a scientific approach, the use of research methods and an understanding of environmental impacts (Puspitasari and Purbosari 2021).

Current biology teaching materials that include indigenous knowlegde include environmental protection. Teachers can use the context of local wisdom in teaching materials to make it easier for students to acquire their knowledge (Ilhami et al. 2021). Local wisdom or indigenous knowledge (IK) is local local knowledge owned by a certain community or culture, which is formed over a long period of time as a result of processes and mutual relationships between the community and its environment, and becomes a cultural norm. then expressed in the form of mythology, rituals, art and other livelihood activities of indigenous peoples (Priyambodo et al. 2023). Therefore, biology teaching materials now have provisions to integrate local wisdom in the classroom, especially in biology subjects, and require teachers to identify local wisdom in their area that can be used for learning (Muchsin et al., 2023).

The application of local wisdom in biology education to support value conservation and natural resource conservation through school biology education in the form of mantenan. Through the creative hands of biology teachers, local wisdom integrated in student learning can make children literate, ie. students not only understand biology material, but use their knowledge and skills to solve everyday life problems that come from local wisdom (Alimah 2019). To realize local wisdom material, knowledge related to biodiversity, ecosystems and global warming dominates the Sasaki tribe on Lombok Island (Muchsin et al., 2023). And for the implementation of the learning environment itself, there is a local wisdom-based brochure, where this media is compiled based on the needs of teachers and students regarding plant learning materials (Melati et al. 2020).

The learning material chosen in this study is solutions to overcome global warming. The selection of this material is very important to be understood and applied by students because it is closely related to environmental problems that can cause many causes and impacts of global warming. the surrounding environment (Wahdaniyah et al. 2024). The global warming solution material is material that contains problems related to the students' environment (local wisdom) is believed to be able to build science concepts and will apply them in problem solving to reduce the effects of global warming so that it will also have an impact on students' learning outcomes (Putra et al. 2023). From the previous statement, it can be concluded that if learning uses a model such as problem solving, learning media such as brochures should also be used so that students can get more information, are interested in learning, increase activity and improve student learning outcomes (Panjaitan et al., 2021). However, in reality, there are still many teachers who only explain the material without any intermediary in delivering the material in the form of educational media, so that students do not understand the teaching given by the teacher (Abdullah, 2017).

According to the results of observations of biology teachers at Sman 1 Gondang, they have not implemented problem solving learning aided by leaflets containing indigenous knowledge. In sman 1 gondang itself, the learning media still often uses power point with a lecture delivery method conducted by the teacher. Therefore, based on the description above, a study will be conducted entitled implementation of indigenous knowledge-laden leaflets on student learning outcomes on global warming materials. Which aims at Implementation of Indigenous Knowledge-Laden Leaflets on Student Learning Outcomes on Global Warming Materials.

## 2. MATERIALS AND METHODS

## 2.1. Types and Research Design

The research design used in this study was non-equivalent control group (Hotman et al. 2018). In this design, the experimental and control groups were not randomly selected because the selection was determined by the teachers of SMAN 1 Gondang itself. this study involved 4 classes, namely for the experimental class had 3 classes and for the control only 1 class.

Class	Pretest	Treatment	Posttest
High ability class	O <sub>1 Pretest</sub>	P <sub>1 Leaflet</sub>	O <sub>5 Posttest</sub>
Medium ability class	O <sub>2 Pretest</sub>	P <sub>2 Leaflet</sub>	O <sub>6 Posttest</sub>
Low ability class	O <sub>3 Pretest</sub>	P <sub>3 Leaflet</sub>	O7 Posttest
Control	O <sub>4 Pretest</sub>	$P_{4 \text{ Non Leaflet}}$	O <sub>8 Posttest</sub>

Table	1.	Noneo	uivalent	control	group
	_				<b>B</b> <sup>-</sup> • • • •

O<sub>1</sub> : Pretest for experimental groups that have not been given treatment

P<sub>1</sub>: Leaflet-assisted PS model that contains IK

 $O_5$ : Pretest for the control group that has not been

given treatment

O<sub>2</sub> : Posttest for the experimental group that has been given treatment

P<sub>2</sub>: Leaflet-assisted PS model that contains IK

O<sub>6</sub>: Posttest for the experimental group that has been given treatment

O<sub>3</sub> : Pretest for experimental groups that have not been given treatment

P<sub>3</sub>: Leaflet-assisted PS model that contains IK

 $O_7$ : Pretest for the control group that has not been given treatment

O<sub>4</sub> : Pretest for the experimental group that has not been given treatment

P<sub>4</sub>: Giving the PS model without the help of leaflets containing IK

O<sub>8</sub> : Pretest for the control group that has not been given treatment

## 2.2. Population, sample, and research sampling

In this study, the population was all students in class X SMA N 1 Gondang even semester of the 2023/2024 academic year consisting of 10 classes, each class consisting of 36 students. The samples used in this study were students in classes XA, XD, XE and XF, each class consisting of 36 students. In the class there is a selection of criteria, for class XA with high class criteria, class XD with medium class criteria, class XE with low class criteria, and class XF as a control class (Biology Teacher document). In this study, purposive sampling was used because it can select samples that are specifically relevant in the population and can provide deep insights related to the objectives of the study.

## Table 2. Data and Data source

Data	Source	Method	Instrument
Cognitive outcomes	Class student X	Pre-test dan	Cognitive outcome measurement sheet through
Cognitive outcomes	Class student A	post-test	multiple choice questions
Affective outcomes	Class student X	Observation	Affective outcome measurement sheet
Psychomotor outcomes	Class student X	Presentasi	result Presentation
r sychomotor outcomes	Class studelit A	report	result resentation

## 2.3. Research Procedures

The research begins with observations of the school location regarding the number of students, the learning schedule (to determine the learning material taught in this study), then Determination of the class to be used by discussing the class X biology teacher. After that, the preparation of the global warming teaching module and its devices (global warming teaching materials, learning media in the form of leaflets containing Indigenous knowladge, cognitive assessment instruments (pretest-postest), Affective (attitude), Psychomotor (skills) Problem Solving learning and consulted to the supervisor, learning scenarios in accordance with the Problem Solving learning model. The implementation of learning begins with teaching in the classroom using teaching media in the form of leaflets containing Indigenous Knowledge in experimental classes and conventionally in control classes and conducting pretests. Then deliver the material to be taught to experimental and control class students. After finishing delivering the material, students can immediately discuss in groups and make LKPD assignments that are presented. Furthermore, experimental and control class students take a post test. then close the learning activities and collect the data that has been obtained. The next stage is to analyze the data with data analysis techniques for learning outcomes in this study using a prerequisite test consisting of normality test and homogeneity test, then hypothesis testing using One Way Anova and then further tests are carried out, namely LCD (Least Significant Different).



Fig. 1 Research Procedures.

## 3. RESULTS AND DISCUSSION

## 3.1. Result

This study is to examine the implementation of PS assisted by leaflets containing Indigenous Knowledge on the learning outcomes of Class X even semester students of SMA N 1 Gondang TA 2023/2024, on the material of solutions to overcome global warming. This research is an experimental research using leaflets and control without using leaflets. For the experiment, 3 classes were used with the criteria of good class XE, medium class XA, weak class XD, and for the control class XF (Biology Teacher document).

Based on table 3 above, it can be seen that the average value on the pretest and posttest cognitive assessment that gets the biggest value is class XD (low ability) with a pretest of (73.6) and a posttest of (81.4). Meanwhile, for the pretest and postest cognitive assessment, the smallest score was in class XF (control) with a pretest of (62.3) and a posttest of (72.12). In the affective assessment, the largest get an average value in class XF (control) of (86) and the smallest assessment gets an average value in class XD (low ability) of (79.7). And in the psychomotor assessment, the largest get an average value in class XE (high ability) of (87.2) and the smallest assessment gets an average value in class XD (low ability) of (73.2).

# Table 3 Data on The Results of The Implementation of Indigenous Knowledge-Laden Leaflets on Student Learning Outcomes on Global Warming Materials

		Hasil	Belajar		
Data		Cogi	nitive	A ffalations	
		Pretest	Posttest	Allektive	Psychomotor
Class XE	Max	90	90	100	100
(Kemampuan	Min	40	70	66,6	66,6
Tinggi)	Mean	69,12±9,32	79,7±4,03	85,2±10,3	87,2±10
Class XA	Max	90	100	100	100
(Kemampuan	Min	45	45	55,5	55,5
Sedang)	Mean	70,14±7,51	79,12±7,18	83,8±11	84,8±9,6
	Max	90	90	100	100

	M	55	(5	<i></i>	44.4
Class XD	Min	22	65	55,5	44,4
(Kemampuan	Mean	73.6±4.56	81.4±3.73	79.7±10.3	73.2±13.8
Rendah)			- , - ,		
Kelas XF	Max	85	85	100	100
Konrol	Min	25	50	66,6	55,5
Komor	Mean	62,3±6,65	72,12±4,51	86±10,5	79,5±7,6

To determine the effect of the implementation of PS assisted by leaflets containing indigenous knowledge on the learning outcomes of class X students of SMA N 1 Gondang in the 2023/2024 academic year on the material of solutions to overcome global warming, hypothesis testing was carried out with One Way Anova. Before hypothesis testing, the data obtained must be prerequisite tested first using the normality test and homogeneity test.



Figure 2 Analysis of the Average results of learning outcomes on cognitive, affective, and psychomotor aspects

## 3.1.1. Normality Prerequisite Test

The normality test serves to determine whether the data obtained is normally or abnormally distributed. The normality test in this study used the Kolmogorov-Simirnov test, the results of which are presented in table 4.

 Table 4. Normality Test of Implementation of Indigenous Knowledge-Laden Leaflets on

 Student Learning Outcomes on Global Warming Materials.

Crosse	Kolmog	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
Group	Statistic	df	Sig.	Statistic	df	Sig.	
PreTest Eks1	.076	32	$.200^{*}$	.984	32	.902	
PosTest Eks1	.137	32	.130	.951	32	.155	
PreTest Eks2	.051	32	$.200^{*}$	.987	32	.964	
PostTest Eks2	.062	32	$.200^{*}$	.990	32	.987	
PreTest Eks3	.089	32	$.200^{*}$	.961	32	.292	
PosTest Eks3	.129	32	.189	.957	32	.233	
PreControl	.109	32	$.200^{*}$	.946	32	.108	
PosControl	.108	32	$.200^{*}$	.971	32	.522	

\*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Based on table 4 above, the data on the learning outcomes of students in the pretest and posttest of leaflet treatment 1 obtained a significance value of (0.200) and (0.130), in leaflet treatment 2 obtained the same significance value (0.200), and in leaflet treatment 3 obtained a significance value of (0.200) and (0189), while in the pretest and posttest control class treatment obtained the same significance value (0.200). The significance value of all treatments is greater than the significance setting (0.05), so it can be concluded that the data above is included in normal data.

## 3.1.2. Homogeneity Prerequisite Test

The homogeneity test serves to determine the variants of several populations are the same or not. For this reason, the calculation results are presented in table 5.

Table 5. H	lomogeneity	Test of Impl	ementation of	of Indigenous	Knowledge-Laden	Leaflets on
5	Student Lear	ning Outcor	nes on Globa	l Warming M	laterials.	

Test of Homogeneity of Variance								
Levene df1 df2 S Statistic								
	Based on Mean	1.237	1	264	.267			
Learning	Based on Median	.518	1	264	.472			
outcomes	Based on Median and with adjusted df	.518	1	263.958	.472			
	Based on trimmed mean	.982	1	264	.323			

Based on table 5 above, it can be seen that in the homogeneity test of the four classes, the significance value (0.267) > (0.050) is obtained, it can be concluded that the data on learning outcomes based on teaching methods have the same variance or can be called this data homogeneous.

## 3.1.3. Test One Way Anova

Hypothesis testing in this study uses the One Way Anova test, the requirements of this test are that the data is normally distributed and homogeneous. From the data that has been tested, it shows that the data is normally distributed and homogeneous, so the next stage of data can be analyzed using the One Way Anova test. The data from the calculation of the hypothesis test is presented in table 6.

# Table 6. Hypothesis Test Results of Implementation of Indigenous Knowledge-LadenLeaflets on Student Learning Outcomes on Global Warming Materials

ANOVA					
Learning Outcomes					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3788.123	3	1262.708	8.386	.000
Within Groups	39448.719	262	150.568		
Total	43236.842	265			

Table 6 shows that the mean of the above categories obtained a significance value (2-tail) (0.000) < (0.050), so the null hypothesis (H0) is rejected. Therefore, it is concluded that there is an influence on the average learning outcomes taught with the help of leaflets 1, 2, 3 and the control class. So this treatment has an effect on student learning outcomes.

To determine which treatment affects which class (research class), the LSD (Least Significant Different) further test was conducted (Table 7).

Multiple Comparisons							
Dependent Variable: Learning Outcomes							
LSD							
95% Confidence						nfidence	
(I) Kalag		Mean Difference (I-J)	Std. Error	Sig.	Interval		
(1) Kelas	(J) Kelas				Lower	Upper	
					Bound	Bound	
	Kelas Leaflet2	221	2.104	.917	-4.36	3.92	
Kelas Leaflet1	Kelas Leaflet3	-3.088	2.137	.150	-7.30	1.12	
	Kelas Kontrol	$7.215^{*}$	2.120	.001	3.04	11.39	
	Kelas Leaflet1	.221	2.104	.917	-3.92	4.36	
Kelas Leaflet2	Kelas Leaflet3	-2.868	2.137	.181	-7.08	1.34	
	Kelas Kontrol	7.435*	2.120	.001	3.26	11.61	
	Kelas Leaflet1	3.088	2.137	.150	-1.12	7.30	
Kelas Leaflet3	Kelas Leaflet2	2.868	2.137	.181	-1.34	7.08	
	Kelas Kontrol	$10.303^{*}$	2.153	.000	6.06	14.54	
	Kelas Leaflet1	-7.215*	2.120	.001	-11.39	-3.04	
Kelas Kontrol	Kelas Leaflet2	-7.435*	2.120	.001	-11.61	-3.26	
	Kelas Leaflet3	-10.303*	2.153	.000	-14.54	-6.06	

Table 7. Advanced Test Results of Implementation of Indigenous Knowledge-Laden Leaflets
on Student Learning Outcomes on Global Warming Materials

\*. The mean difference is significant at the 0.05 level.

Table 7 shows that the significance probability value is (0.917) > (0.050), therefore (H0) is accepted. So the conclusion is that there is no effect of treatment on the learning outcomes of the experimental class and the implementation of PS assisted by leaflets containing indigenous knowledge has an effect on the learning outcomes of class x students of SMA N 1 Gondang in the 2023/2024 academic year on the material Solutions to overcome global warming.

## 3.2. Discussion

Based on the results of the anova test of cognitive assessment, it was found that giving leaflet treatment containing IK had an effect on student learning outcomes. For this reason, learning using leaflet media can affect student learning outcomes and as a teacher can be helped in delivering material to students so that it can be understood easily. In line with the opinion of (Basir & Rohmawati, 2023) Learning using leaflet media indirectly facilitates students to sort and improve mastery of the material that has been learned, so the function of the leaflet can help speed up the process of receiving messages to be conveyed and help students receive the material simply and encourage them to be active in learning. This is also in line with (Antika et al., 2023) that students feel that learning with the help of leaflet learning media is easy, interesting, fun, profitable, useful, and makes them more active in learning. And also in line with (Yusandika et al. 2018) that leaflets are able to visualize messages, information and concepts and present images that make it easier for students to explain the material.

Leaflet learning is also able to make students interested in the material in the leaflet because it is not only the content of the reading material in the leaflet but also presented with an attractive design in the form of pictures and the latest news about the material Solutions to overcome global warming. The content in the leaflet is also clear in the form of important points of material that students will learn such as understanding, cases of global warming, the impact of global warming solutions, and of course indigenous knowledge associated with the material Solutions to overcome global warming which is a new learning or still rarely applied to leaflets. This is in line with the opinion of (Wahyuni, 2022) that leaflets are in the form of sheet-shaped information sources, equipped with pictures so that readers are more interested in seeing them and the use of leaflets can make learning more interesting, innovative, and most importantly, the learning outcomes of students. This is also in line with (Winarso & Yuliyanti, 2017) Leaflet learning media are usually simpler and portable. This shows that leaflet media is more effective than other printing products because of its attractive and simple design. The presence of leaflet learning media is believed to make students easier and faster to understand the messages conveyed by the teacher.

Problem solving learning aided by leaflets also affects student learning outcomes because the material for solutions to overcome global warming is teaching material that relates problems that require solutions, therefore the PS learning model is suitable. This is in line with (Wiria & Alberida, 2023) In the problem solving learning model, it is necessary to understand how to see the solution to the problems it faces, for this reason, during the cognitive process, it is important to understand the problem, collect reliable facts to solve the problem, look for the right answers and identify problems, and this is described as an action that teachers must take in class because it can help students solve problems that arise during learning. This is also in line with (Lestari, 2023) The use of problem solving learning models in the classroom helps students become more focused and creative, and also makes it easier for teachers to explain the material. Utilizing this model as one of the main strategies to answer students' needs for new material so that learning is not boring. And also in line with (Diva and Purwaningrum 2023) that learning with Problem solving fosters students' ability to use the widest possible thinking with the maximum of their capacity to capture.

Learners in this study are not only required to understand the material but also in the material studied there is indigenous knowledge or local wisdom that students must understand and relate to learning. According to (Muchsin et al. 2023) oppenions Indigenous Knowledge is a form of knowledge based on beliefs, understanding and perceptions of the community related to habits that serve as guidelines for behavior in relation to the ecological and systemic environment. For this reason, indigenous knowledge can be related to the material on solutions to global warming, pretest, postest, and LKPD questions that must be understood and done by students. In the answers to the pretest questions, the average student's answers were wrong and some were missed. Then after the leaflet was distributed and learning was carried out, students began to understand the indigenous knowledge associated with the material, students worked on the LKPD and also the postest results were good, while in the control class there were still students who were confused about indigenous knowledge associated with the material and had to be given understanding and examples repeatedly until students understood.

The application of leaflet learning media whose material contains indigenous knowledge this time is a new thing for students by looking for examples of solutions to overcome global warming in LKPD and for the overall LKPD results make students active to think critically in answering all activities in LKPD. Indigenous knowledge is associated with the material so that students know about local wisdom that can be taken as learning that can be applied and preserved. In line with (Dewi, 2021) opinion Indigenous knowledge-based learning media can be used to increase children's creativity (content), because the more advanced the times, the higher the level of global competition that will be faced in the future. The purpose of this success is to increase educators' awareness and attention to research and development of local cultural education (indigenous knowledge) based on local potential. This is also in line with (Hadi & Dazrullisa, 2018) Integration of local wisdom values in teaching materials can be done by adjusting the local wisdom values analyzed with the learning materials presented in the teaching materials.

In the affective or attitudinal assessment itself, the results vary greatly for each student, for the attitude assessment, 3 indicators are taken from the profile of Pancasila students, namely faith, fear of God and noble character, mutual cooperation, and critical reasoning. In the results of the highest average value of attitude assessment obtained in the control class and for the lowest attitude assessment obtained in the low-criteria class. This shows that the use of leaflet media has nothing to do with attitude assessment because attitude assessment is taken based on the innate attitude that each student has from the beginning of learning to the end of learning. This is in line with (Nurholis et al., 2022) the teacher's attitude/affective assessment uses the observation method by direct observation of the teacher himself, observing students in everyday life in the school environment. In line also with (Novitasari & Wardani, 2020)Attitude assessment is an assessment that focuses on student attitudes, so it requires a rubric that is made based on observations and during learning activities. And in line with (Erni 2022) An important form of attitude assessment carried out by teachers to determine the character or behavior of students both inside and outside the classroom, socially and emotionally.

In the psychomotor assessment, 3 assessment indicators are also taken, namely mastery of material, cooperation, and delivery/performance, the results of which are the average value of each class are different in experimental and control classes. For this reason, in this psychomotor assessment, the highest average value is obtained in the high ability class and the lowest average value is obtained in the low ability class. This shows that the use of leaflets has something to do with psychomotor assessment because psychomotor assessment is taken when students form groups to discuss working on LKPD with the help of leaflets containing teaching material and then the results of the discussion are presented. This is in line with (Veronika Sitepu et al., 2022) Psychomotor assessment refers to the skills and abilities that students learn after learning, product and process oriented assessment, and the form of psychomotor assessment of students is a test that directly observes their behavior. In line with (Ulfah and Arifudin 2021) The psychomotor aspect is related to physical activity and also from skills that are directly related to the learning experienced by students. And in line with (Rizqiani and Wijayanti 2022) Skills assessment in the form of an assessment rubric that contains a number of assessment techniques such as practice, projects, products, portfolios and other techniques.

Based on the results of the research that has been conducted, it can be concluded that the implementation of leaflet media containing indigenous knowledge makes students' learning outcomes better in doing assignments and discussing in groups. Students are also more active in class learning activities, the material associated with indigenous knowledge can be understood, and are able to develop their critical thinking skills on the material of solutions to overcome global warming. But based on the LSD further test of cognitive assessment, it was shown that there was no effect of the treatment of using leaflets containing IK on the learning outcomes of the experimental class for high, medium, and low classes, which means that it could not show differences in learning outcomes in high, medium, and low classes.

## 4. CONCLUSIONS

Based on the research and discussion, it can be concluded that: the implementation of Problem solving assisted by leaflets containing indigenous knowledge has an effect on the learning outcomes of class X students of SMA N 1 Gondang in the 2023/2024 academic year on the material of solutions to overcome global warming, but does not make a difference in high, medium, and low classes.

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## The Effect of Auxin and Cytokinin Hormones on the Growth of Robusta **Coffee (Coffea Canephora) Seedlings in the Grafting Propagation Method of Plagiotrop Rootstock Cuttings**

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	KEYWORDS:	Robusta coffee is a type of coffee that can be cultivated vegetatively. Grafting
	Auxin, Cytokinin, Robusta Coffee, Vegetatif Propagation.	cuttings technique is one of the propagarion techniques commonly used in
		Indonesia. The use of auxin and cytokinin hormones can be used in the coffee
		seedling phase to support the growth of coffee plants. The puspose of this study
		was to determine the effect of auxin and cytokinin hormones on the growth of
		robusta coffee seedling in the cuttings grafting method. The method use was a
		factorial completely randomized design with 2 factors. Factor 1 is the
		concentration of auxin hormones consisting of 3 levels, namely $A_1$ (0 ppm), $A_2$
		(50 ppm), and $A_3$ (100 ppm). Factor 2 consentration of cytokinin hormone
		which consists of 3 levels namely $S_1$ (0 ppm), $S_2$ (50 ppm), and $S_3$ (100 ppm).
		The result were analysed using ANOVA and further test using Duncan Multiple
		Ranage Test (DMRT). The parameters observed were the percentage of cuttings
© F E N T	© 2024 The Author(s). Published by Biology Education Department, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. This is an open access article under	success, number of leaves, number of shoots, shoot height, shoot diameter,
		chlorophyll content. The result showed that the provision of 50 ppm auxin and
		50 ppm cytokinin had the best trend in the variable number of leaves, shoot
		diameter and shoot height.
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## 1. INTRODUCTION

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Robusta coffee is a type of coffee that is widely cultivated in Indonesia. Based on the aspect of cultivation, coffee commodities are dominated by smallholder plantations of more than 90% while the remaining less than 10% comes from private or state plantations (Rahardjo, 2021). In 2020 coffee production amounted to 762,38 thousand tonnes rising to 786,19 thousand tonnes in 2021 or an increase of 3,12 percent, and in 2022 coffee production fell to 774,96 thousand tonnes or a decrease of 1,43 percent (Statistik Kopi Indonesia, 2021). Factors causing the decline in coffee production include the age of coffee trees exceeding productive age, the spacing of coffee trees that are not in accordance with the provisions of the Ministry of Agriculture, and the use of inputs in the form of coffee trees that are still hereditary from the 1980s (Septiani & Kawuryan, 2021). One way to increase the production of coffee cultivation is to use superior planting material (Pujaningrum & Simanjuntak, 2020).

Robusta coffee seedlings cannot be propagated generatively because Robusta coffee is crosspollinated, so the provision of Robusta coffee seedlings must be done clonally or vegetatively, one of which is by grafting cuttings. Grafting cuttings is relatively faster than grafting cuttings, because the root initiation process in the cutting process coincides with the recovery process of the grafting

process (Irlando, 2020). However, because of this, optimal conditions are needed to support the growth of coffee seedlings during the nursery period so that the growth of coffee seedlings is not hampered.

According to Irlando (2020), growth stimulating substances are also called hormones that are indispensable for plants, especially in the seedling phase. Growth hormones can also be used to stimulate root growth to grow more, larger root volume, and sturdier so that coffee plants will not collapse easily. Auxin is one of the plant hormones that can regulate many physiological processes such as growth, cell division, cell differentiation, and protein synthesis (Tamba et al., 2019). Auxin is a growth hormone whose activation in tissues is related to the balance between synthesis and loss of auxin due to transport and metabolism (Rodyady et all., 2024). Meanwhile, cytokinins are adenine-derived compounds and play a role in the regulation of cell division and morphogenesis. Cytokinins are used to stimulate the formation of buds, influence cell metabolism, and stimulate dormant cells and their main activity is to encourage cell division (Karjadi and Buchory, 2008). The purpose of this study was to determine the effect of auxin and cytokinin hormones on the growth of robusta coffee seedlings in the grafting method.

## 2. MATERIALS AND METHODS

This study was conducted in January – June 2024 in Jenggawah sub-district, Jember, East Java. the planting material used was clone BP 308 as rootstock and BP 939 as entres. The preparation of entries for grafting was carried out by practitioners who are accustomed to grafting with a high percentage of success. The preparation of entries for grafting was carried out by practitioners who were accustomed to grafting with a high percentage of success, thus reducing human error in each experimental unit. The spliced planting material will then be planted on the planting media that has been provided.

After completing the process of making a 100 ppm stock solution of auxin and cytokinin hormones, then the stock solution is stored in a closed bottle. Next, the application is carried out on the seedlings of robusta coffee cuttings by spraying as much as 20 ml for each plant using a sprayer. the application is given foliarly at the bottom of the leaves with an application interval of 2 weeks once for 2 months.

The method use was a factorial completely randomized design with 2 factors. Factor 1 is the concentration of auxin hormones consisting of 3 leveLS, namely A<sub>1</sub> (0 ppm), A<sub>2</sub> (50 ppm), and A<sub>3</sub> (100 ppm). Factor 2 consentration of cytokinin hormone which consists of 3 levels namely S<sub>1</sub> (0 ppm), S<sub>2</sub> (50 ppm), and S<sub>3</sub> (100 ppm). The result were analysed using ANOVA and further test using Duncan Multiple Ranage Test (DMRT). The parameters observed were the percentage of cuttings success, number of leaves, number of shoots, shoot height, shoot diameter, chlorophyll content.

## 3. RESULTS AND DISCUSSION

The results of observations on the percentage of success of cuttings show that the grafting of cuttings carried out is 100% successful, marked by no dead or withered coffee plants. This is because the process of grafting cuttings is carried out by practitioners who are experienced in grafting cuttings. thus minimising failure in the process of grafting cuttings. Coffee propagation by grafting cuttings is done by connecting the entres with the rootstock first, then at about the same time the bottom of the rootstock is cut diagonally and then planted in polybags (Tanwir, 2018).

The treatment of 100 ppm auxin and 0 ppm stockinine showed the highest average value (24.50 leaves). The interaction of auxin and cytokinin hormones in affecting the number of leaves of cuttings grafted seedlings is due to the formation of leaves determined by the number and size of cells in the plant. Auxin can develop cell size by affecting osmotic pressure (Sa'adah et all.,

2021). The increase in water absorption is caused by the softening of the cell wall due to the action of auxin. This eventually causes the cell to expand. Auxin will increase plant growth by affecting the plasma flow of cells and making nutrient absorption more effective. Exogenous auxin hormone application will help activate the endogenous auxin hormone in the plant so that leaf formation can take place better. Plants that are given the application of the hormone auxin will give a rapid growth response, especially in the number of leaves because the hormone auxin plays a role in plant cell division.



Figure 1. Number of leaves, Means followed by the same letter show no significant difference in the DMRT test at the 5% level

Observations of stem diameter were made by measuring the increase in diameter length (mm) of the upper stem. this is because, in the process of grafting cuttings that are expected to grow and develop is the upper stem. so, if a branch grows at the bottom of the stem, the branch is immediately cut to ensure the growth of the desired shoot. The results showed that the treatment combination 50 ppm auxin hormone treatment and 50 ppm cytokinin ( $A_2S_2$ ) gave the best results with an average value of 4.43 mm (Figure 2).

Exogenous hormones given to plants will help the work of auxins in plants. Auxin is used for cell division and root differentiation. Cytokinin is a growth regulator that is used to stimulate adventitious budding (Yusnita, 2003). Auxin can spur the development of vessel tissue and encourage cell division in the cambium of the vessel so as to support the growth of stem diameter. Auxin hormones and cytokinin hormones that work together in the process of growth of cells in the cambium or often referred to as secondary growth are very influential on shoot diameter parameters (Arimarsetiowati and Ardiyani (2012). the results of observations on the shoot diameter parameter showed significantly different results. the highest results were obtained from a combination of 50 ppm auxin hormone treatment and 50 ppm cytokinin.



Figure 2. Shoot diameter, Means followed by the same letter show no significant difference in the DMRT test at the 5% level

In plants there is a process of cell elongation is strongly influenced by the hormone auxin either synthesised by the plant itself (endogen) or given to the plant in the form of growth regulators (exogen) (Novianti, 2015). Plants that have undergone the absorption process of auxin and cytokinin will work together to activate food reserve energy and increase cell division, cell elongation, and cell differentiation which ultimately forms the shoot elongation process. One of the functions of cytokinin in plant growth is to assist meristem tissues in the formation of buds (Lukikariati, 1996). Cytokinin plays a role in regulating cell enlargement, organ formation, and the development of shoots and bud eyes (Harjadi 2009). The observation results showed that there was a significant difference based on the height of the shoots (Figure 3) from the treatment of auxin and cytokinin hormones. From these data, it was obtained that the treatment combination of auxin 50 ppm and cytokinin 50 ppm (A<sub>2</sub>S<sub>2</sub>) showed optimal results and was also efficient and because the results of this study were in accordance with (Rosyady et al.,2023) that, the application of IAA with a concentration of 50 ppm increased the growth shoot length. This is because the process of shoot height growth in plants is influenced by auxins that can stimulate shoot formation in plants and cytokinins play a role in stimulating the growth of adventitious shoots.



Figure 3. Shoot height, Means followed by the same letter show no significant difference in the DMRT test at the 5% level.

## 4. CONCLUSIONS

The results showed that the treatment of 50 ppm auxin and 50 ppm cytokinin gave the best number of shoots and shoot height. while the administration of 100 ppm auxin and 0 ppm cytokinin gave the best results on the variable number of leaves.

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## Implementation of Indigenous Knowledge Teaching Materials in Biology Learning Quasi-Experimental Study on Environmental Change Material

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#### **KEYWORDS:**

Biology Environmental Change Indigenous Knowledge **Teaching Materials** 

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The purpose of this study was to determine the biology learning outcomes of SMA Negeri 1 Teras class X students using teaching materials containing indigenous knowledge in the 2023/2024 school year on environmental change material. This type of quantitative research with a Quasi Experiment design, this form of Quasi Experiment uses Nonequivalent Control Group Design. This research was conducted in two classes, namely the control class and the experimental class. In this study, an initial test (pretest) was conducted before treatment and a final test (posttest) was conducted after treatment. A total of 72 students were sampled in this study. The research instrument was a test of cognitive, affective, and psychomotor learning outcomes. Analysis of learning outcomes data using normality test, homogeneity test and independent sample t-test test. The results of the calculation using the independent sample t-test test obtained a significance value (2-tailed) of 0.033 < 0.050, so the null hypothesis (H0) is rejected. It can be concluded that the use of teaching materials containing Indigenous Knowledge is better and proven to have an effect on the learning outcomes of Biology class X students of SMA Negeri 1 Teras in the 2023/2024 school year on environmental change material.

ABSTRACT

## 1. INTRODUCTION

Studying biology means studying the natural environment, biology is related to the discovery and understanding of nature systematically, and biology is not only a process of searching and collecting knowledge in the form of facts and concepts, but also a process of discovery (Harefa, Lase & Zega, 2022). Biological science is based on actual experience (empirical), and is characterized as a science that includes problem-solving techniques of interest to biology, the object of research in biology is a concrete object, can be captured by the five senses, based on real experience, and has systematic steps (Ikhtiara, et al, 2022). The culmination in the learning process is the acquisition of learning outcomes.

Learning outcomes in the world of education are the results of student measurements after going through a learning process (Khairani, 2019). There are three areas of learning outcomes, namely cognitive, affective and psychomotor. The cognitive domain includes changes that occur in cognition as a result of learning activities, starting from stimulation, receiving material, to storing material in the brain. The affective domain is the value associated with student behavior. After students master the basic learning level, the psychomotor domain learning outcomes are ranked from lowest to highest (Nabillah & Abadi, 2019). Therefore, educators should pay attention to biology learning activities by including them in teaching materials.

Teaching materials are arranged to build a learning environment for students, teachers and students should organize and use materials systematically in learning, teachers should use materials to support the learning process, materials should be designed in accordance with classroom regulations (Nurvasana & Desiningrum, 2020). Teaching materials are systematically organized and designed in accordance with the curriculum for the purpose of student self-study, the role of the teacher in designing or assembling materials greatly determines the success of learning with materials and the learning process (Magdalena et al., 2020). The material consists of three core components, namely the main component, supplementary component, and learning outcomes assessment component. The main part contains the main information or topic that students want to convey or need to master. This complementary component can be in the form of information/topics to add insight to students, and an evaluation component to evaluate the results of the material that has been created (Manurung, Haloho & Napitu, 2023). Teachers and students must be active in carrying out learning, and teachers must be able to lead the class well and apply learning strategies that are aligned with learning objectives, on the other hand students are given the task of processing results and seeking information to communicate results, in the form of ideas, after making observations, problems in teacher performance in carrying out learning are identified, students are given the opportunity by the teacher to identify problems by asking questions (Hariyatmi., Septiety, D. D., Wijayanti, R., 2020). Teachers must provide alternative learning methods that are suitable for overcoming these difficulties, one of which is by applying a problemsolving learning model.

The problem solving model is a teaching method in which students are presented with a problem and asked to solve it, this model requires the ability to identify cause and effect, observe problems, look for relationships between various data collected, and draw conclusions from the results of problem solving, problem-based learning methods are chosen and used as a solution to improve students' skills and activities in learning biology related to environmental change material. (Musyafa, 2022). The problem-solving model is not just a teaching method, but also a way of thinking. Because other methods can also be used when solving problems, from finding data to drawing conclusions (Handayani, 2019). The solution that researchers found was to apply the problem-solving learning model to biology topics.

Environmental change materials included in the education module include environmental damage and pollution. The learning activities include: 1) Identification of fctors causing environmental damage and analysis of the impact of environmental changes, 2) Identify various types of pollution that occur in the environment and analyze solutions that can be given to deal with environmental changes. Environmental change was chosen as the material for module development, because various human activities cause an imbalance in the ecosystem. Environmental problems are also an obstacle in learning the culture of protecting the environment, but the number of environmental change modules is still very small (Muttaqin, Fatirul, & Hartono, 2019). One curriculum approach that is gaining traction is incorporating indigenous knowledge into learning.

Indigenous Knowledge is knowledge based on the recognition of community beliefs, understandings, and customs that guide actions related to ecological and systemic environments (Muchsin, Sriyati, & Sabayar, 2023). Indigenous knowledge raises questions about the suitability and relevance of curriculum materials in a global and contemporary context (Jessen et al., 2022), one of the teaching materials by adding local wisdom to learning, students will be able to research and scientifically investigate existing local wisdom, so that it will increase their awareness of environmental protection and conservation along with the science learning materials they receive (Maharia, Yorida, & Malpaun, 2018). Indigenous scientific knowledge in the form of local wisdom needs to be studied and socialized, one way to apply local wisdom is in school learning, local wisdom can be used as a learning resource for teachers and students, and local traditions can be used as a local wisdom-based learning resource (Rikizaputra et al., 2022).

Integration of Indigenous Knowledge in biology learning allows students to explore and train their thinking skills and get used to doing problem solving activities based on local wisdom, learning biology with local wisdom-based problem solving activities activates student knowledge, analyzing problems that occur in the surrounding area, including local wisdom, as a learning experience. Thus, critical and creative thinking skills can be introduced through biology learning
with local wisdom-based problem solving activities (Alimah, 2019). Selection of teaching materials based on Indigenous Knowledge, students can respond to a variety of local wisdom, this makes students more critical and easy to apply what they have learned to their environment, teaching materials that involve learning are adapted to the environmental conditions of students, making learning more meaningful and supporting students (Masihu, 2021).

## 2. MATERIALS AND METHODS

## 2.1. Type of research

This type of research is quantitative with a pseudo-experimental research design (Quasi Experiment Design), the form of Quasi Experiment Design this research uses Nonequivalent Control Group Design. This research was conducted in two classes, namely class X J (control) and class X D (experimental). In this study, an initial test (pretest) was conducted before treatment and a final test (posttest) was conducted after treatment. In this study, there are two research variables, namely the independent variable and the dependent variable, the independent variable in the study is learning to implement teaching materials that contain indigeous knowledge. The dependent variable is student learning indigeous knowledge.

## Table 1. Nonequivalent control group design

Class	Pretest	Treatment	Postest
Experiment (X D)	P1	Х	P2
Control (X J)	P3	-	P4

Note :

P1 : Pretest in the experimental group that has not been given treatment

X : Learning by using environmental change teaching materials that contain indigenous knowledge

P2 : Posttest in the experimental group that has not been given treatment

P3 : Posttest in the control group that has been given treatment

P4 : Posttest in the control group that has been given treatment.

## 2.2. Population, Sample and Sampling

All students of class X SMA Negeri 1 Teras even semester of the 2023/2024 school year consisting of 10 classes became the population in this study, each class consisting of 36 students. The sample consists of experimental class students (X D) and control class (X J). The sampling used was purposive sampling technique.

## Table 2. Data

Data	Data Sources	Data Retrieval Technique	Instrument
Cognitive learning outcomes	Class student X	Pretest and posttest	Multiple choice question sheet
Affective learning outcomes	Class student X	Attitude observation	Attitude assessment sheet
Psychomotor learning	Class student V	Presentation	Presentation assessment sheet
outcomes	Class student A	Discussion result	discussion result

## 2.3. Research Procedure

The research began with determining the research site, namely SMA Negeri 1 Teras, then determining the class using the population purposive sampling technique, namely the sample of class X.D as an experimental class using teaching materials containing indigenous knowledge, and class X.J as a control class.



**Figure 1. Research Procedure** 

Make an agreement with the teacher regarding the material that will be delivered in class X.D and X.J, namely environmental change material. After that, make learning tools that will be used, including Teaching Modules on environmental change materials, teaching materials for environmental changes, LKPD for environmental changes, making pretest and post-test questions on environmental changes and their rubrics, cognitive assessment instrument sheets (pretestpostest), Affective (attitude), Psychomotor (skills). The implementation of learning begins with conducting learning activities at school in accordance with the Teaching Module and its devices that have been made, providing teaching materials containing Indigenous Knowledge in the experimental class, then taking cognitive data with a pretest in the form of a test Affective data (discipline, responsibility and mutual cooperation) is taken by observing students during learning, psychomotor data (presentation and LKPD discussion results) is taken when presenting the results of the discussion. Cognitive, affective, and psychomotor data were tabulated for further analysis using the independent sample t test. Furthermore, completing learning activities and collecting data obtained. Data obtained from SMA Negeri 1 Teras were analyzed using SPSS 25. Before analyzing the data, prerequisite tests were conducted. The test carried out as a prerequisite is the normality and homogeneity test, if the data obtained is normal and homogeneous, then the next step is the independent samples t-test.

## 3. RESULTS AND DISCUSSION

3.1. Result

Hapsari, Melati Arum & Hariyatmi (2024)

The purpose of this study was to determine the biology learning outcomes of SMA Negeri 1 Teras class X students using teaching materials containing indigenous knowledge in the 2023/2024 school year on environmental change material. This study involved an experimental class using teaching materials containing indigenous knowledge and a control class without using teaching materials containing indigenous knowledge at SMA Negeri 1 Teras.

Data	EKSPERIMENT		CON	FROL
	PRETEST	POSTEST	PRETEST	POSTEST
COGNITIVE				
Maximum	90	90	90	90
Minimum	40	40	40	40
Mean±sd	63,89±13,58	71,11±12,82	64,72±14,24	65±14,04
AFFECTIVE				
Discipline		91	8	6
Responsibility		93	84	
Mutual cooperation		88	82	
Mean	90,6		84	
PSYCHOMOTOR				
Presentation		81	7	2
Discussion results (LKPD)	99		90	
Mean		90	81	

# Table 2. Data on the Implementation of Indigenous Knowledge Teaching Materials in Biology Learning Quasi-Experimental Study on Environmental Change Material

Based on Table 2, the experimental class knowledge value is 71.11, while the control class is 65, meaning that the experimental class value is higher than the control class. The value of student activeness during the learning process in the experimental class was 100, while the control class was 90, meaning that the value of the experimental class was higher than the control class, the value of student learning process skills in the experimental class was 90, in the control class was 81, this shows that the learning outcomes of the experimental class were higher than the control class.



Figure 2. Analysis of biology learning outcomes

3.1.1. Normality Test

In this study, normality was tested using the Kolmogorov-Smirnov test. This test determines whether the data obtained is normally distributed or not. The calculation results are:

Table 3. Normality Test of the Implementation of Indigenous Knowledge Te	eaching Materials
in Biology Learning Quasi-Experimental Study on Environmental	<b>Change Material</b>

Treatment	Significance	Description
Pretest Exsperiment	0,051	Normal data
Postest Experiment	0,052	Normal data
Pretest Control	0,055	Normal data
Postest Control	0,076	Normal data

Based on table 3. on the Kolmogorof-Smirnov test, the Experiment class pretest data shows a significance value of 0.051 > 0.050, meaning that the data is normal, for data on the Experiment Postest shows a significance value of 0.052 > 0.050, meaning that the data is normal, while in the control class pretest with a significance value of 0.055 > 0.050, meaning that the data is normal, and for the control class postest shows a significance value of 0.076 > 0.05, meaning that the data is normal. It can be concluded that both classes have normally distributed data and the data is greater than the significance determination (0.050).

## 3.1.2. Homogeneity Test

This test is used to determine whether two populations have the same variation, the calculation results are as follows :

Table 4. Homogeneity Test of Implementation of Indigenous Knowledge T	eaching Materials
in Biology Learning Quasi-Experimental Study on Environmenta	l Change Material

Homogeneity Test	Levene Statistic	df1	df2	Sign	Description
Biology Learning Outcomes	0,179	1	70	0,674	Homogeneous data

Based on table 4. the homogeneity test of the experimental and control classes shows a significance value of 0.674 > 0.050, meaning that the population varies equally or homogeneously. It can be concluded that the population has the same or homogeneous variants that are normally distributed and the data is greater than the significance setting (0.050).

## 3.1.3. Independent Sample T Test

This test is used to test hypotheses about two or more populations. In this case, each sample group is independent. That is, the two populations are not related to each other. the following calculation results:

Table 5	. Independent Sa	mple T Test In	plementation of In	digenous Know	wledge Teaching
	Materials in B	Biology Learnin	g Quasi-Experime	ntal Study on	Environmental
	Change Materia	ดไ			

Class	Number	Mean	Sig. (2	t-	Description
	of students		tailed)	count	
Control	36	63.47	0,033	-2,173	H0 rejected
Eksperiment	36	68,19	0,033	-	H0 rejected
				2,173	

Based on Table 5, the t-test of biology learning data in the control and experimental classes amounted to 36 students each. The average value of control class students is 63.47, while the experimental class is 68.19, it can be concluded that there is a difference in the average learning outcomes of biology students in the control class and experimental class. The 2 tailed significance value in the T Test table is 0.033 <0.50, it is concluded that H0 is rejected, meaning that there is a significant difference between the learning outcomes of experimental class students who are given teaching materials containing indigenous knowledge and control classes without teaching materials containing indigenous knowledge. To find out whether the two populations have the same variation, here are the calculation results :

#### 3.2. Discussion

Teaching materials are learning resources that are systematically arranged and prepared in accordance with the curriculum so that students can learn independently, it can be understood that the role of the teacher in designing teaching materials greatly determines the success of the teaching and learning process by using these teaching materials (Magdalena et al., 2020), and this is supported by research (Kusuma, Faizah, Nur, Elmustian & Hermandra, 2023), educational materials are included in an important part of the learning process, one of which is the part of educational material that contains learning messages both specific and general that can be used for learning purposes. According to research (Yurinda, Ulia & Yustiana, 2021), teaching materials for teachers play an important role in conveying knowledge, and teachers can help students think more actively and expand their knowledge. Teaching materials in the form of companion books should provide a very diverse learning resource for teachers and students.

According to Pesurnay (2018), local wisdom is a form of knowledge based on community beliefs and perceptions about customs that are used as guidelines for behavior related to ecological and systemic environments. This is in accordance with Listia's (2020) field study, that wisdom can be in the form of beliefs in a community about something that can be learned by the younger generation, customs that can be used as a guide for behavior related to the ecological and systemic environment celebrated, and moral values, if related to moral values, lessons can be learned from legends and myths in the community.

Teaching materials that contain indigenous knowledge, namely cultural values that live and develop in society, students can easily solve the problems presented, this is in line with Riza's research (2022) which says that indigenous knowledge-based learning materials contain topics based on the curriculum that incorporate values developed in society, indigenous knowledge-based teaching materials contain information and content that students can learn, and contain noble values that allow students to internalize good values, the availability of indigenous knowledge-based learning materials causes learning and learning outcomes to be more effective, This is in accordance with the research of Tanjung, Daulay, and Ghafari (2018), stated that students who study descriptive text about Labuhan Batu which is the local wisdom of the West Bira region of North Sumatra have higher learning outcomes, students can achieve higher learning outcomes than students who use ordinary textbooks, students also understand the true meaning of learning because it is familiar to them in everyday life.

The environmental change material included in the Class X SMA education module includes environmental damage and pollution material, the learning activities carried out include: 1) Identify the factors that cause environmental damage and analyze the impact of environmental changes, 2) Identifying various types of pollution that occur in the environment and analyzing solutions that can be provided to deal with environmental changes, by integrating teaching materials that contain local wisdom into Javanese traditions, namely the Ruwat Bumi tradition in Guci Village, the Ruwat Jolotundro tradition in Mojokerto, and the Merti tradition in Kemetul Village, Semarang, these traditions are developed through respect for nature's role in protecting. This tradition contributes to maintaining the balance of ecosystems and natural beauty which is an inseparable part of identity, through environmental preservation and recognition of human relationships with the environment (Aminuddin, 2023).

Learning in experimental classes based on indigenous knowledge developed in this study improves student learning outcomes based on the results of the t test, that there is a significant difference between the learning outcomes of experimental classes given teaching materials containing indigenous knowledge and control classes without teaching materials containing indigenous knowledge, and these learning outcomes are proven to be very effective in improving student learning outcomes, this is in line with Azizah's research (2022) that indigenous knowledge-based teaching materials to improve student learning outcomes are very suitable to be used as a basis for education because they are able to foster caring and responsible personalities. This also helps in student character education and is very effective in improving student learning outcomes.

#### 4. CONCLUSIONS

The results showed that there were differences in student learning outcomes in Biology subjects with the use of teaching materials containing indigenous knowledge and control learning without using teaching materials containing indigenous knowledge, meaning that the use of indigenous knowledge-based teaching materials proved to be better and effective on biology learning outcomes in the material of environmental changes in class X SMA Negeri 1 Teras in the 2023/2024 school year, the use of indigenous knowledge-based teaching materials is superior.

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## **Optimization of Broiler Feed Conversion Ratio by Addition of Probiotics EM-4 and Herbal**

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	ABSTRACT
KEYWORDS:	Effective and efficient broiler management is necessary. The smaller the feed
broiler chicken (Gallus	conversion ratio value, the more effective and efficient chicken maintenance.
doemesticus),	Efforts to reduce feed conversion include reducing feed consumption by adding
feed conversion ratio,	EM-4 probiotics and herbal spices. The purpose of this study was to determine
herbs,	the effect of the addition of EM-4 probiotics and herbal spices on the feed
probiotics EM-4	conversion ratio of broiler chickens (Gallus doemesticus). Experiments were
	conducted using a sample of 20 broiler chickens aged 14 days, with treatments
	P0H0 (control), P0H1 (6g herbs), P1H0 (0.6% probiotic EM-4), P1H1 (6%
	Probiotic EM-4 + 6g Herbs) which were divided into four treatment groups with
	5 replicates. The results showed that the treatment with 0.6% EM-4 probiotics
@ 2024 The Author(a) Published	+ 6 g herbal spices had the lowest feed conversion. Then the effect of probiotics
by Biology Education Department,	and herbal spices on broiler feed conversion rate was analyzed by Anova test,
Faculty of Teacher Training and Education, Universitas	followed by Duncan test. It can be concluded that the addition of 0.6% EM-4
Muhammadiyah Surakarta.	probiotics and 0.6% herbs is the most optimal feed ratio. These findings can
the CC BY-NC license:	help farmers, entrepreneurs or the general public who use EM-4 and herbs that
https://creativecommons.org/license s/by-nc/4 0/	can optimize their income by reducing the feed conversion ratio of broiler
	chickens.

## 1. INTRODUCTION

Broiler farms in Indonesia are increasing in number as public awareness of the importance of nutrition derived from broilers increases (Rasyaf, 2008)). According to data from the National Food Agency (Bapanas), in 2023 the average Indonesian consumes 7.46 kg/capita/year of broiler meat, an increase of 4.3% compared to 2022 (Ahdiat, 2024). Rapid growth in broilers is not only caused by genetic factors but also by external factors, such as effective and efficient maintenance (Samadi et al., 2020) Feed can be suppressed for effective and efficient maintenance without adversely affecting the growth and performance of broilers (Nadiya Listyasari, Soeharsono, 2022)

Feed can be a single or mixed food material, processed or unprocessed, which is given to animals for survival, production, and breeding (Daud, 2006). According to (Primacitra, Dhika Yonika, Osfar Sjofjan, 2014) ways can be done to increase feed efficiency without adversely affecting productivity, namely increasing enzymatic digestion in the poultry digestive tract, namely by utilizing probiotics.

Probiotics as a future product are used as an alternative to antibiotics and as a growth promoter containing microorganisms that support animal growth and health (Gunawan; Bagus Harianto, 2011). Probiotics play a role in limiting or killing pests and diseases, and can reduce the level of damage to livestock tissues (Izzaha et al, 2019). The use of probiotics (Feliatra, 2018) added to chicken feed can improve digestion and accelerate chicken growth, and if consumed in sufficient quantities causes increased absorption of food substances (Widiawati, Mariani Jesika, Muharlien Muharlien, 2018). In the digestive tract, probiotics can stimulate the growth and activity of certain bacteria and improve host (Wahyuni, 2019). Effective microorganism (EM-4) is a mixed culture of microorganisms that are beneficial for plant and livestock growth used as an inoculant to increase the diversity and population of microorganisms (Palenga *et al.*, 2018), which is able to optimize the utilization of food substances because the bacteria contained therein can digest cellulose, starch, sugar, protein, and fat (Yadnya *et. al.*, 2014).

Feed consumption comes from the amount of feed consumed by livestock which functions to fulfill the basic life and production of these livestock (Arifin, 2014) and (Rahmawati *et. al.*, 2023). Various ways are done to increase the efficiency of poultry feed use, one of which is feed additives added to animal feed (Widodo, 2017). Feed additives derived from herbal plants can replace the use of antibiotics by preventing disease in poultry so as to avoid antibiotic residues and bacterial resistance (Aidah, 2021). Probiotic natural additives can increase feed efficiency, produce healthy broiler meat, low fat but high protein criteria, and can suppress the growth of pathogenic bacteria in the digestive (Harumdewi et al., 2018). According to (Sulistyoningsih; Anas Dzakiy; Atip Nurwahyunani, 2014) herbs that can be added to chicken feed include turmeric, sambiloto, temulawak, and ginger.

Turmeric is useful as a hepatoprotector, antioxidant (curcuminoids), antibacterial, antiinflammatory (essential oil), and anticancer (Supomo, 2018) and (Noor Anisah Pujianti; Achmad Jaelani; Neni Widaningsih, 2013). Turmeric added in broiler rations can improve growth and feed efficiency and as an alternative to the use of antibiotics combined with other herbal plants to get more effective results (Pertiwi; R. Murwani; T. Yudiar, 2017). Sambiloto leaves contain andrografolid which helps increase antibody production and stimulates phagocyte cells to digest foreign microorganisms and contains flavonoids that function as growth hormones in livestock and enzyme inhibitors by forming complexes with proteins (Novia Rahayu; Nurul Frasiska, 2019). Ginger is an herb for the treatment and prevention of various (Masoud Adibmoradi, Bahman Navidshad, Jamal Seifdavati, 2006). The essential oil content in ginger helps the work of digestive enzymes so that the feed rate increases along with the growth rate which causes meat production to also increase (Ucop Haroen; Agus Budiansyah, 2018). Curcumin in ginger can improve the work of digestive organs, stimulate pancreatic sap containing amylase, lipase, and protease enzymes and even has pharmacological activities such as antibacterial, anti-inflammatory, hepatoprotector, antioxidant, immunomodulator, antihypertensive, anticancer, neuroprotector, nephroprotector, and anticoagulant. The addition of ginger extract causes higher chicken weight compared to broiler chickens without ginger (Novia Sri Hapsari; Dian Wahyu Harjanti; Anis Muktiani, 2018) and (G F Zhang 1, Z B Yang, Y Wang, W R Yang, S Z Jiang, 2009). Temulawak as an herb contains active substances xanthorrizol, curcuminoids, essential oils, proteins, fats, cellulose and minerals (Anggraini et. al., 2019) Giving herbs in feed can kill bacteria in the digestive tract of poultry (Atika Rahmah, Nyoman Suthama, 2013) & (Mustika et. al., 2022).

The amount of feed consumed by a chicken within a certain period of time to achieve optimal shape and weight is the feed conversion ratio (Supomo, 2020). The feed conversion ratio of chickens is determined from the ratio between the amount of feed used and the amount of weight gain of broilers produced (Andriyanto, 2015) and the feed conversion ratio of chickens is said to be good when the value is getting smaller (Suyasa, I.N; Parwati, 2018) and (Ridwan; Widodo; Mukti, 2020). According to (Azizah, N. K., Sarmanu, S., Utomo, B., Sabdoningrum, E. K., Lokapirnasari, 2020) the provision of lactic acid bacteria probiotics in broiler feed can increase body weight gain by reducing feed conversion values.

Based on this description, research has been conducted on the addition of EM-4 probiotics and herbs (a mixture of ginger, turmeric, temulawak, and sambiloto) to the feed conversion ratio of broiler chickens (*Gallus domesticus*). The aim is to optimize the effect of the addition of EM-4 probiotics and herbs on the feed conversion ratio of broiler chickens so that it is useful to help farmers, entrepreneurs or the public and observers regarding the use of EM-4 probiotics and herbs in chicken feed to optimize income by reducing the conversion ratio of chicken feed.

## 2. MATERIALS AND METHODS

Experiments were conducted with a completely randomized design (CRD) design, using 20 ayambroiler Strain Rose 308 weighing  $\pm$  3.8 kg obtained from PT Super Unggas Jaya, 14 days old.

The research was conducted at HM Farm Candirejo Village, Wonokerso, Kedawung, Sragen. The research design used a complete randomized design (CRD), using four groups with five replicates. The feed used was GM-1 from PT CJ Cheil Jedang Indonesia, EM-4 probiotics from PT Songgolangit Persada, and herbs obtained from Pasar Bunder Sragen. The addition of herbs was 6g and the addition of EM-4 was 0.6% (Astuti et al., 2015).

Pureeing a mixture of herbs from ginger, turmeric, temulawak and sambiloto each as much as (1.5g) finely ground and then dried to get 6g of herbs to be mixed in the feed according to the treatment.

The experiment used a completely randomized design (CRD) with each group of 5 chickens (Table 1).

Т	able	1.	Research	design
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	H0 (no herbs)	H1 (with herbs)
P0 (without probiotic EM-4)	$P_0H_0$	$P_0H_1$
P1 (with probiotic EM-4)	$P_1H_0$	$P_1H_1$

Notes:

 $P_0H_0$  = Feeding without the addition of EM-4 probiotics and herbs (ginger, turmeric, Curcuma domestica (temulawak), dan sambiloto)

 $P_0H_1 = Feed + 6g Herbs$ 

 $P_1H_0 = Feed + 0.6\%$  Probiotic EM-4

 $P_1H_1 = Feed + 0.6\%$  Probiotic EM-4 + 6g Herbs

The treatment was conducted on broiler chickens aged 14 days with the treatment of finisher feed. The addition of EM-4 probiotics as much as 0.6% (Astuti et al., 2015) done by spraying on the feed evenly, then fermented anaerobically.

## 2.1. Preparation Stage

Acclimation of DOC Strain Rose 308 was carried out for a week in a sterilized cage, equipped with 500 ml of brown sugar drinking water per drinking container and given a 65 watt warming lamp (fig.1), on the 4th day ND (Newcastle Disease) vaccine was carried out through eye drops (fig.2). The feed was GM-1 feed and drinking water for 14 days.



Fig. 1. Broodstock Cage



Fig. 2. Providing Vaccine ND (*Newcastle Disease*)



Fig. 3. Herbal Mashing

Herbs are made from a mixture of Ginger, Turmeric, Temulawak, and Sambiloto which are mashed in the same ratio (fig. 3.) then dried and after drying mixed in the feed according to the treatment (6g). Probiotic EM-4 was sprayed on the feed evenly according to the treatment.

## 2.2. Implementation Stage

After 14 days of acclimation, the chickens were placed in the cage according to the treatment (4 cages, @ 5 chickens), feeding was given until day 35. The treatment began when the chickens were 14 days old, starting to be fed the treatment with the addition of probiotics and herbs according to the design until harvest (day 35).

The parameters in this study were the feed conversion ratio of chickens including the weight gain of chickens every week and the amount of feed consumption every week. Measurement of chicken weight was carried out by weighing chickens on days 22, 28, and 35, as well as measuring the remaining feed consumption on days 22, 28, and 35. The feed conversion ratio was calculated based on the amount of feed during maintenance divided by the total weight of harvested chickens

Data on chicken weight and remaining feed consumption (feed conversion ratio) were analyzed statistically using Analysis of Variant (ANOVA). This was used to analyze whether there was an effect on the conversion ratio of broiler feed after the addition of probiotics and herbs. Then to find out which one is more significant continued with the Duncan Multiple Range Test (DMRT). The research flow is presented in Fig. 4.



Providing Vaccine ND

## Figure 4: Research flow chart.

## 3. RESULTS AND DISCUSSION

Based on the rearing and feeding treatment with different probiotics until day 35 and the observations that have been made, the data in table 2 are obtained.

Table 2. Mean feed conversion ratio of broiler	chickens (Gallus domesticus) with the addition
of EM-4 Probiotics and Herbs	

Mean ± SD		
$2.01^{a} \pm 0.23$		
$1.74^b\pm0.03$		
$1.63^{b} \pm 0.23$		
$1.42^{c} \pm 0.18$		

Notes: Different letters in the same column indicate significant differences (p<0.05)

Table 2 shows the feed conversion ratio of the chickens after 35 days of treatment, which showed different feed conversion ratios ranging from 2.01 to 1.42. The highest ratio was in the control (2.01) and the lowest in the treatment of feeding with the addition of Probiotic EM-4 and Herbal. Table 2 shows that the lowest feed conversion ratio was observed in treatment P1H1 (Feed + Probiotic EM-4 0.6% + Herbs 6g).

To determine whether the treatment of the addition of Probiotic EM-4 and herbs to the feed conversion ratio, the Anova test was conducted. Before the Anova test, Kolmogrov-Smirnov and Shapiro-Wilk normality tests were carried out at the  $\alpha = 5\%$  level, the data were said to be normal if the value Sign. > 0.05, while the data is said to be abnormal if the value Sign. < 0,05. The results of the normality test of feed conversion ratio in broiler chickens (*Gallus domesticus*) through SPSS calculations (Table 3).

Table 3.	Normality test results of	of broiler feed conversion ratio	data (Gallus domesticus) wit	th
	the addition of EM-4	probiotics and herbs.		

	Treatment -	Kolmogrov-Smirnov <sup>a</sup>			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Feed Conversion Ratio	$P_0H_0$	.4.07	5	.007	.684	5	.007
	$P_0H_1$	.217	5	$.200^{*}$	.897	5	.394
	$P_1H_0$	.251	5	$.200^{*}$	.868	5	.257
	$\mathbf{P}_{1}\mathbf{H}_{1}$	.317	5	.112	.749	5	.029

Table 3. shows that all treatments showed an  $\alpha$  value of more than 5% (P>0.05), which means that the distribution of feed conversion ratio data in broilers has a normal distribution and qualifies for Anova testing, then a homogeneity test is carried out at the  $\alpha = 5\%$  level to determine parametric or nonparametric analysis (Table 4).

Table 4.	Homogeneity test results of broiler feed conversion ratio data (Gallus domesticus)
	with the addition of EM-4 probiotics and herbs

Lavene Statistic	df1	df2	Sig.
3.197	3	16	.052

From table 4. it can be concluded that the Sig. value is more than 5% (P>0.05), which means that the distribution of feed conversion ratio data comes from a population that has the same variance (homogeneous), so the data meets both requirements for the Anova test (table 5).

(Out	(Guines universitents) with the utuation of problotics Envi 1 und herbs.							
	Sum of Squares	df	Mean Square	F	Sig.			
Beetween Groups	.909	3	.303	13.30	.000			
Within Groups	.365	16	.023					
Total	1.274	19						

 Table 5. Anova test results of data distribution of feed conversion ratio of broiler chickens

 (Gallus domesticus) with the addition of probiotics EM-4 and herbs.

The results of the Anova test (table 5.) treatment of the addition of EM-4 probiotics and herbs to the feed conversion ratio in broiler chickens (*Gallus domesticus*) showed that the Sig value. Feed Conversion Ratio  $< \alpha (0.00) < 0.05$ ) then H0 is rejected. This means that the addition of EM-4 probiotics and herbs has an effect on the feed conversion ratio in broiler chickens. In addition to the Sig. value, it can be seen from the F hit. If F hit>F table 0.5 then it can be stated that the treatment given is not significantly different. F tablo.5 obtained is 3.44 (F table). So it can be concluded that F hit> Ftab (13.30>3.44), the provision of EM-4 probiotics and herbs gives the results of a significant effect on the feed conversion ratio of broiler chickens, and to find out which treatment has the most effect, further tests are carried out, using the Duncan Test (Table 6.).

 Table 6. Duncan test results of broiler feed conversion ratio data with the addition of probiotics EM-4 and herbs

Treatment	N	Subset for alpha = 0.05			
1 reatment		1	2	3	
$P_0H_0$	5			2.01ª	
$P_0H_1$	5		1.74 <sup>b</sup>		
$P_1H_0$	5		1.63 <sup>b</sup>		
$P_1H_1$	5	1.42 <sup>C</sup>			
Sig.		1.00	.28	1.00	

Averages for groups in homogeneous subsets are shown \*using the harmonic mean of a sample with size = 5.00

Based on table 6. it can be seen that the lowest chicken feed conversion ratio is  $P_1H_1$  (1.42) and the highest feed conversion ratio is  $P_0H_0$  (2.01). In addition, it can be seen that there are differences in the treatment of each group of broilers, namely the  $P_0H_0$  treatment group has no effect on the feed conversion ratio of broilers because it is located in the largest subset, the  $P_0H_1$  and  $P_1H_0$  treatment groups have the same effect on the feed conversion ratio of broilers, which means that the  $P_0H_1$  and  $P_1H_0$  treatments have the same significant effect because they are located in the same column, while the  $P_1H_1$  treatment group (addition of probiotics and herbs) has the most effect on the feed conversion ratio of broilers because it is located in the smallest subset. Thus, the addition of EM-4 probiotics and herbal spices ( $P_1H_1$ : Feed + EM-4 0.6% + Herbal 6g) to the feed conversion ratio in broiler chickens has an effect on the feed conversion ratio of broiler chickens.

Feed conversion ratio is the result of the comparison of the amount of feed consumption with the resulting weight gain (Lestari D., Rukmiasih R., Suryati T., 2017). The lower the feed conversion rate means the better feed quality to produce body weight, in other words, the higher the feed conversion ratio value means the more wasteful use of (Ridwan; Widodo; Mukti, 2020). Larger chickens will eat more to maintain body weight, 80% of protein is used to maintain body weight and 20% for growth so that feed efficiency will be reduced and if the feed conversion value is far above the maximum number, then the maintenance can be said to be ineffective and inefficient. Similarly, according to (Rahmawati, 2023) that the feed utilization ratio represents the

efficiency of feed utilization, the smaller the feed equivalent value, the greater the feed equivalent value.

One environmental factor that negatively impacts chicken productivity is the tendency towards high temperatures and high humidity. Such conditions exacerbate microbial growth and spoilage, which together weaken livestock immunity. Although the use of antibiotics has been shown to improve health and productivity, their use in medicine has recently been restricted due to concerns that the use of antibiotics can cause resistance to the target bacteria (Wiryawan, 2023). Some research findings suggest that the use of edible and mild ingredients such as herbal medicine (Wiryawan, 2023) can improve poultry health (Ananda et al., 2023). The addition of probiotic cultures to chicken feed has a positive impact on growth and feed utilization efficiency (A. P. Kusuma, 2014). This is in accordance with the results of research by (Hanum Shofura; Suminto; Diana Chilmawati, 2018) that the addition of probiotics to chickens at a dose of 0.4 is able to produce feed efficiency and a small feed conversion ratio of chickens. Probiotics produce a balance of bacteria that are antagonistic to pathogens, so that the digestive tract of chickens is better at digesting and absorbing nutrients in feed. Similarly, according to Saili et al. (2019) that feed containing herbal probiotics and mangrove shell extract has a significant effect on feed consumption and daily weight gain, but has no effect on feed conversion of native chickens.

Giving herbs as feed additives to broiler chickens can increase feed efficiency and animal health. This is in accordance with research (Hariyati Y., Soeparjono S., Setiyono S., 2020); (Moghaddam et al, 2021), and (Laswi Irmayanti, Jaetun Rasyid, Muhammad Nur, 2021) which states that the addition of herbs containing andrographolide and flavonoids can increase lymphocyte proliferation and increase antibodies. The same thing in (Noor Anisah Pujianti; Achmad Jaelani; Neni Widaningsih, 2013) that the addition of herbs in chicken rations has a significant effect on protein digestibility, herbs containing curcumin can stimulate bile to secrete bile and stimulate the pancreas which contains amylase, lipase, and protease which are useful for increasing the digestion of feed ingredients such as carbohydrates, fats, and proteins. Supported by (Suwarta, 2021)which explains that giving turmeric flour and sambiloto leaf powder in sufficient quantities can show good feed conversion achievements, low feed conversion values are the best values. Curcumin is widely known as a yellow pigment with a very broad role, including antioxidant prevention, anti-inflammatory, and insect control.

Similarly, it was reported (Boki, 2020) that the addition of EM-4 probiotics in fermented rations can increase feed digestibility so that it affects the daily weight gain of broiler chickens. The results of research by (Subekti E., 2015) on duck-type livestock given the addition of probiotics and herbs, showed an increase in feed utilization efficiency so that the feed conversion value decreased. Even supported by (Ananda et al., 2023) that EM-4, a probiotic containing 90 types of lactic acid bacteria (Lactobacillus sp.) photosynthetic bacteria (Rhodopseudomonas sp.), and yeast (Saccharomycess sp.) makes livestock healthy and useful for keeping healthy. Maintaining microbial balance in the gastrointestinal tract of broiler chickens by reducing animal stress, increasing appetite, and increasing the number of positive microbial populations. This can have beneficial effects and reduce the emergence of pathogenic microorganisms. Therefore, EM-4 probiotics as a growth stimulant can increase body weight, decrease feed conversion, and reduce the formation of abdominal fat that can affect carcass quality in broilers. Probiotics that are relatively affordable and easy to find on the market are EM4. The microorganisms contained in EM4 are fermentative and synthetic and consist of five groups of microorganisms: lactobacilli, fermentation bacteria, yeasts, photosynthetic bacteria, and actinobacteria (Rahmawati et. al., 2023).

In this study conducted at HM Farm Sragen, using broiler chickens reared from day old and started to be treated at the age of 14 days. Maintenance in the first week to the third week of each group did not show significant differences in feed consumption and weight gain. In the fourth and fifth weeks of weight gain and feed consumption there were significant differences.

The most influential treatment is the addition of probiotics and herbs at a dose of 0.6% and 6g respectively which can increase feed consumption and body weight gain, this is due to the role of beneficial microorganisms in the digestive tract, namely the effectiveness of feed consumption, so that the absorption of nutrients takes place perfectly. Probiotics and herbs can significantly increase fiber digestibility, increase body weight so that feed conversion becomes more efficient. This is in accordance with the research of (Sjofjan et al., 2020) the provision of herbs and probiotics can minimize the activity of pathogenic bacteria in the digestive system of broiler chickens.

Rahmawati et. al., (2023) stated that turmeric (herbal) is one type of phytobiotic that can increase broiler productivity and increase meat production, curcumin which is one of the active ingredients in turmeric which has broad spectrum antibacterial activity, can fight various pathogenic and non-pathogenic bacteria and has antiviral and antitumor effects. This statement describes turmeric as an effective antibiotic alternative, turmeric contains essential oils consisting of vitamin C (45-55%), zingiberene (25%), sesquiterpene ketones, turmeron (6%), borneol, chiniel, phellandrene, and sabinene (6%) and minerals Fe, P and Ca.

Based on the research that has been done, probiotics and herbs affect the feed conversion ratio in broiler chickens (Gallus domesticus). This is in accordance with (Nadiya Listyasari, Soeharsono, 2022) that the use of a combination of herbs and probiotics is effective for increasing the body weight of chickens because it causes a lower feed conversion ratio, which means a higher level of effective and efficient feed used.

#### 4. CONCLUSIONS

Based on the research that has been done, it can be concluded that the addition of 0.6% EM-4 probiotics and 0.6% herbs affects the feed conversion ratio in broiler chickens (Gallus domesticus). These findings can help farmers, entrepreneurs or the general public who use EM-4 and herbs that can optimize their income by reducing the feed conversion ratio of broiler chickens

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# **Analysis of Biology Education Students' Learning Outcomes in Environmental Biology Practicum Plankton Identification Material**

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#### ABSTRACT

KEYWORDS:	Practicum is an activity that supports students to interact directly with
Environmental Biology,	real material. One of the practical activities is observing objects so they
Learning outcome, Cognitive	are easy to learn. This research aims to determine the implementation of
Psychomotor.	environmental biology practicum materials on plankton identification
	for biology education students at Muhammadiyah University of
	Surakarta. This type of research is descriptive qualitative. The sample
	for this research was 42 first semester students at the Biology Education
	Study Program Faculty of Teacher Training and Education
	Study Hograni, Lacuty of Teacher Hanning and Education,
	Muhammadiyah University of Surakarta. Data collection techniques for
	cognitive learning outcomes were carried out using pretest and posttest,
© 2024 The Author(s) Published	as well as observation questionnaires regarding student activities in
by Biology Education Department,	practice as a result of psychomotor learning. Data analysis uses
Education, Universitas	descriptive statistics. The research results show that cognitive learning
Muhammadiyah Surakarta. This is an open access article under the CC BY-NC license:	outcomes seen from the average test results have differences, namely,
	the pretest average is 58.5 and the posttest average is 86.3. Psychomotor
s/by-nc/4.0/.	learning results were 78.8 with good criteria.

## **1. INTRODUCTION**

Education is defined as business humans used for grow as well as develop talent as well as the potential it has since born Good physical nor spiritual in accordance with the values that exist within life society and culture (Pristiwanti et al., 2022). Education in schools tall make generation This as figure role model from teaching previous generation. Until Now This is education No have limitation For explain the meaning of education in a way complete Because its complex nature like the target that is man. Its nature is complex That often called knowledge education. Knowledge education is continuation from education. Knowledge education more relate with theory priority education thinking scientific (Rahman et al., 2022). Education and science education own linkages in meaning practice as well as theoretical. So, in the process of life man both of them each other collaborating, for one is knowledge Biology.

Biology is one The science studied is very broad about creature life and life. In learning biology No free from role from lecturer as educator and motivator for students, meanwhile student is object active learning in do activity invention in obtain knowledge his knowledge (Aryani & Nugroho, 2022). Biology is very close connection with life everyday, then from That as lecturer as educator must collaborate between knowledge knowledge with life daily when implementation of the learning process going on, so will obtained meaningful learning. Knowledge Biology is closely related with practice (Mahrawi et al., 2022).

Practice Biology is closely related fiber with Skills in knowledge knowledge like observation, creation questions, communication, as well as use tools and materials can explained as required capabilities in carry out the scientific process. Method practice can increase skills and results Study (Putri et al., 2021). Practicum is very good for develop and improve skills and results Study Because student can Study direct through practical, so they can find Alone facts and concepts (Purnamasari, 2020). Activity practicum also has a number of goals, for one is for hone skills required by students, provide chance for apply knowledge and skills in a way direct in practice. One of them is in practicum biology environment (Hamidah, 2022).

Biology environment is courses that discuss various draft about biology as well as environment (Chan & Budiono, 2021). Biology one 's environment branch knowledge studying biology interaction organism with environment they as well as impact activity man to ecosystem . Ecosystem waters own abundant diversity , for one is plankton diversity . Subject biology the environment also learns regarding plankton that can made as bioindicators water pollution through activity practice . student can identify structure and types of deep plankton activity the .

Learning outcomes is acquisition from the learning process student in accordance with objective teaching (ends are being attained). Objective teaching become results Study potential that will achieved by students through activity learning (Sapitri , 2020). Learning outcomes cognitive is behavior that occurs covers the area of cognition . Learning outcomes cognitive can interpreted as change behavior in scope encompassing cognition a number of aspect cognitive domain abilities . Learning outcomes cognitive be measured aim For get accurate information about aspects ability in the cognitive domain (Qomariah & Sutama, 2022). Learning outcomes cognitive refers to achievement or results achieved by someone in realm cognitive , that is related aspects with thinking , knowledge , and abilities intellectual . Learning outcomes cognitive covers various type skills and knowledge that can be measured and assessed.

#### 2. MATERIALS AND METHODS

#### 2.1. Research design

The design of this research is a pre-experimental one group pretest posttest design. The research design according to Sugiyono 2008 is as follows:

Table 1.	Research	design	one	group	pretest	posttest	design

Pretest	Treatment	Posttest
01	Х	O2

Information :

01	:	Test initial	(pretest)	before	treatment	given
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- O2 : Test final (posttest) after treatment given
- X : Treatment to group experiment that is with apply practice For identify plankton

#### 2.1.1. Population and Sample

Population in study This is first semester students taking eye studying practice biology environment totaling 42 students . Sample in research This is Senua There were 42 first semester students in the Biology Education Study Program , Faculty Teacher Training and Education , Muhammadiyah University of Surakarta.

#### 2.1.1.1. Instruments

Test questions are used For get mark results Study cognitive with method administering the test. Test given before practice carried out (pretest) and after implementation practicum (posttest). Observation and documentation sheet used at the time activity practice taking place For get mark results Study psychomotor . Observation sheet For results Study psychomotor includes ( observation , filing question , using tools and materials , and communicating ).

#### 2.1.1.2. Data Collection Techniques

Analysis of result data Study cognitive.

Analysis of result data Study cognitive done with analysis quantitative which includes two stages, namely First testing precondition with normality and homogeneity tests, secondly next with hypothesis testing using paired sample t-test.

Analysis of result data Study psychomotor

Analysis of result data Study psychomotor done in a way descriptive from results observation. According to (Putri et al., 2021) observation data obtained analyzed with use formula as following :

 $Persentase \% = \frac{Total \ score \ obtained}{Maksimum \ score} \times 100\%$ 

From the results the can categorized as into the criteria in table 2.

 Table 2. Percentage category psychomotor

Percentage (%)	Category
86-100	Very good
76-85	Good
60-75	Enough
55-59	Not enough
≤ 54	Very less

#### 3. RESULTS AND DISCUSSION

3.1. Learning outcomes cognitive students in practicum biology environment material plankton identification

The research implementation involved 42 students as samples. Data on students' cognitive learning outcomes were obtained from the average of the pretest and posttest in the plankton identification practicum. A description of the cognitive learning outcome data is presented in table 2 below:

8	8	<b>L</b>
Description	Pretest	posttest
Amount sample	42	42
Lowest value	10	30
The highest score	60	80
Mean	58.5	86.3
Standard Diviation	12.33	14.91

Table 3. Learning outcomes cognitive student in practicum material plankton identification

Based on table 2. Analysis results show that results Study students on the pretest was 0 for minimum value and 60 for mark maximum with an average pretest score of 58.5. Whereas results Study students on the posttest was 60 for minimum score and a score of 90 for mark maximum with the average of 86.3.

Learning outcomes cognitive student furthermore tested hypothesis . Before testing the hypothesis so preceded with normality and homogeneity tests . The test results show that the data is normally distributed and homogeneous Because mark significance more from 0.05. Hypothesis

testing done with paired sample t-test. Hypothesis test results done For know difference in average values before practicum and after practice . Hypothesis test results shown in table 3 below :

Test category	Mean	Significance Value
Pretest	58.5	0.007
Posttest	86.3	0.007

Table 4.	Results	of the	paired	sample	t-test
			<b>P</b>	r	

Based on Table 3. Shows that mark significance 0.007 < 0.05 then there is difference between the average values pretest with the average value posttest. That matter means There is significant difference between results Study cognitive student before do practicum and after practice. So that results study This show that implementation practice biology environment on the material identification of influential plankton to results Study cognitive student.

Research result This show that implementation practice make student more understand March plankton identification. With do observation direct on student plankton can identify in a way concrete types of plankton. That matter in line with (Ikhsan , 2020), which states that with method practice student more understand material Because faced direct with things related concrete with material studied.

Implementation practice is possible activities teach student For think critical and analytical so that can build broad and creative insights Power more detailed knowledge. Activity practicum is very necessary done in learn required materials studied directly by students. Especially regarding material plankton identification, student must do practice plankton observations directly in the laboratory. Practice according to (Winangun, 2021) it is activity Study teaching carried out by participants educate with do experiment in face in a way direct material to be studied it. Therefore That practicum is very helpful student in build knowledge and understanding to material plankton identification.

# 3.1.1. Learning outcomes psychomotor students in practicum biology environment material plankton identification

Implementation observation For evaluation psychomotor use four indicators, namely Observation, filing question, using tools and materials and communicating. Result of analysis of result data Study psychomotor served in table 5 as following:

No	Indicator Skills observed	Percentage	Category
1.	Observation	89 %	Very good
2.	Submit Question	76 %	Good
3.	Using tools and materials	67 %	Enough
4.	Communicate	87 %	Very good

**Table 5. Learning Results Psychomotor Student** 

Learning outcomes practicum in the realm psychomotor shows the category average Good . Based on Table . 5 indicators observation show highest percentage with mark by 89%, value percentage indicator observation , incl in very good category . Based on study This is an indicator Skills observation student can use ability sense For gather fact in something object observation . Indicator observation seen when student use microscope to obtain data regarding Observation of plankton found in water samples . Observations made student the For get fact related material lessons learned , such as observation students on the structure of plankton and zooplankton who can observed from shape , color and size found in water samples during activities practice the . According to (Anwar, 2021), stated that observing ( observation ) can used For find a number results measurements in practice biochemistry with Good . On research show that Skills submit question student increase interest in answer and submit question, student tend interested with convey results discussion in a way clear, precise and effective (Farikhatun Nikmah et al., 2023). On activities practice material bioindicators Water pollution can also occur bring up Skills form submit question with percentage 76% category Good. Activity This seen moment student submit question during observations, they also ask to One each other about what are they learn and discover moment observation taking place. Activity submit question become form liveliness students at activities practice Because of the 42 students who submitted question as many as 40 students.

Next skill is use materials and tools with percentage results 67% category Enough. That matter seen at the moment student use tools and materials in accordance with its function for example like moment student use a dropper to take water samples during activities practice. Although thereby indicator use tools and materials in research This Not yet maximum because part student Not yet understand principles base about tools and materials practicum that will be used. According to (Eliyart & Rahayu, 2021), in the his research state that use tools and materials practicum that is not in accordance with instruction will influence results practice. Students feel it too Afraid when will use tools and materials practice Because Afraid make error nor damage tool.

Skills The next thing that appears in the practicum is communicate . Communicate is No only seen from delivery in a way verbally, so in Skills observed communication is How ability student communicate results test in form graphics and shapes report (Rahayu, 2020). Percentage achievement Skills communicate namely 87% in the very good category. There is activity communicate This seen from ability students who can communicate results his observations through activity explain results practicum, discuss results, and presenting results practice so that happen exchange information, science and knowledge between student in accordance with material studied. Indicator communicate shown to students can read results practice. Besides that, so that students can also do it explain and explain in a way systematic and clear results from something practice.

#### 4. CONCLUSIONS

Conclusions Based on results study implementation practice influential significant to results Study cognitive and psychomotor student . Average results Study student after implementation practice more tall before implementation practice . Learning outcomes psychomotor of 4 indicators average with criteria Good.

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