JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 01 – 10 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

The urgency of developing augmented reality-based biology learning media on genetic substance material

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Article Info

Article History:
Received 11 November 2023
Revised 19 March 2024
Accepted 27 March 2024
Published 30 April 2024

Keywords:
Learning media
Augmented reality
Biology learning
Genetic substance

ABSTRACT

The implementation of biology learning in the field encounters numerous challenges. Specifically on genetic substance material, students experience learning difficulties due to abstract, interdisciplinary concepts and difficulties in understanding textbooks. This problem can be overcome one way by using learning media. Under current learning demands, technology-based media is appropriate to use. Augmented Reality (AR) based media is one option. The accessibility of augmented reality-based media remains limited, especially for genetic substance material, despite the necessity for media capable of visualizing its complex and abstract characteristics. The results of the needs analysis for teachers and students also show that AR-based media is needed in genetic substance learning. The use of AR media is very possible because of the support of adequate infrastructure. Teachers and students also expressed their willingness to use AR media in learning. Based on the research results, it can be concluded that AR-based biology learning media on Genetic Substance material is urgent to be developed. This media will be able to enrich the variety of biology learning media and become one of the solutions to overcome problems in biology learning, especially genetic substance material.

Citation: Azrai, E.P., Dewahrani, Y.R., Suryanda, A., Rini, D.S., & Hamam, Z. (2024). The urgency of developing augmented reality-based biology learning media on genetic substance material. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 01-10. DOI: https://doi.org/10.31932/jpbio.v9i1.2950

INTRODUCTION

The practical application of biology learning still experiences numerous challenges. Students often perceive biology as a challenging subject due to its extensive scope and numerous complex concepts that need to be understood (Awidi & Paynter, 2018; Erbas & Demirer, 2019; Mulyanto et al., 2018), concepts are abstract and interrelated (Chrzanowski et al., 2018). One of the

materials discussed in Biology learning is Genetic Substance. In this material, students also experience learning difficulties.

The reasons given by students regarding the difficulties in learning genetic substance material were due to abstract, interdisciplinary concepts and difficulties in understanding textbooks (Çimer, 2012). Concepts that are considered difficult by students in genetic substance material are the differences between DNA and RNA, transcription and translation stages, and the genetic code (Suhermiati, 2015; Wulandari et al., 2020). Research (Hala et al., 2018) shows that as many as 47.62% of students do not understand the concept and 26.67% of students experience misconceptions about genetic substance material.

Problems in biology learning, especially in genetic material, certainly need to be resolved. One solution that can be implemented is the use of learning media. Learning media is a means or message channel that contains sources of information in learning material that can be conveyed to data recipients (Munasti & Suryadi, 2022). Utilizing educational media can help maintain students' engagement during learning activities, catering to diverse learning styles (Ritakumari, 2019)

In response to modern learning requirements, technology integration has become essential, leading to the adoption of technology-based educational media. Augmented reality (AR) stands out as a promising medium for learning applications. AR technology seamlessly merges virtual 3D objects into real-world environments in real time (Azuma, 1997). Utilizing AR technology offers students the opportunity to visualize intricate scientific phenomena typically imperceptible to the naked eye, such as chemical reactions and biological processes within the human body (Klopfer & Squire, 2008). AR-based learning media also holds the advantage of enhancing students' cognitive, affective, and psychomotor skills, facilitating deeper comprehension of abstract and complex concepts. (Mantasia & Jaya, 2016), provides more realistic interactions, and can increase student interest and motivation (Irfansyah, 2017; Kamelia, 2015). Learning enriched with innovative technology can explain abstract and complex genetic substance concepts, such as the structure of genetic material and the process of gene expression (Rini et al., 2022). In order, to explain this abstractness and complexity, teachers can use AR as a technology-based learning medium.

Based on the description above, it is necessary to examine the urgency of developing AR-based biology learning media on Genetic Substance Material. This media will later become one of the solutions to overcome problems in biology learning, especially genetic substance material.

RESEARCH METHODS

Research Design

This research is a qualitative descriptive research using literature review and survey techniques. Qualitative research data is collected through interviews to collect visual, virtual, or digital data. Data were analyzed by coding and interpreting conversations, narratives, and discourse analysis (Flick, 2022). Qualitative descriptive research aims to objectively elucidate the subjects and objects under study, systematically describing typical occurrences and factual observations (Zellatifanny & Mudjiyanto, 2018). Qualitative research has two main objectives, the first is to describe and explore and the second objective is to describe and explain (Siyoto & Sodik, 2015).

Population and Samples

The subjects involved in this research were biology teachers and students of Al-Azhar Islamic High School 22 Cikarang. The number of respondents involved was 37 people. Determination of samples using sampling techniques based on objectives. In qualitative research, sampling is very appropriate if it is based on the research objectives or problems, and uses the researchers' considerations, in order, to obtain the accuracy and adequacy of the information needed by the objectives or problems being studied (Patton, 1990) Samples based on this concept can range from n = 1 to n = 40 or more (MacMillan & Schumacher, 2001).

Instruments

The instrument used to collect data was a biology teacher interview guide. The aspects asked during the interview are outlined in the instrument grid in Table 1.

Table 1. Teacher needs analysis interview guide framework

No.	Indicator	Question	Item	Amount
1.	Curriculum	Curriculum used	1	1
		Biology KKM value	2	1
2.	Biology learning characteristics	Student's difficulties in understanding genetic substance material	3, 4	2
		Indicators of competency achievement that students must achieve in the material	5	1
3.	Learning Activities	Learning methods used	6, 7	2
		Learning obstacles	8	1
4.	Learning media needs	Learning media used	9, 10	2
		Required learning media	11	1
5.	Views on augmented reality-based learning media on genetic substance material	Use of technology in learning	12	1
		Teachers' opinions on augmented reality-based learning media on genetic substance material	13, 14	2
		Willingness to use augmented reality-based learning media on genetic substance material	15	1
Total				15

The next instrument is a student needs analysis questionnaire distributed via Google Forms. The aspects responded to by students are contained in the instrument grid in Table 2.

Table 2. Student needs analysis instrument grid

No.	Indicator	Question	Item	Amount
1.	Views on genetic substance material	Difficulty in understanding genetic substance material	1, 2	2
		Alternatives overcome the difficulty of understanding genetic substance material	3	1
2.	Views on learning media	Learning media used	4, 5, 6	3
		Required learning media	7	1
3.	Views on augmented reality-based learning media on genetic substance material	Students' opinions regarding augmented reality-based learning media on genetic substance material	8, 9, 10, 11, 12	5
		Willingness to use augmented reality-based learning media on genetic substance material	13	1
Total				13

Procedures

The research began with a literature review related to the development of AR-based media in biology learning. AR media is also studied in learning genetic material. To explore more specifically the importance of developing and utilizing AR-based media on genetic substance material, interviews were conducted with biology teachers. To find out students' responses and needs, a needs analysis of students was also conducted by distributing a needs analysis questionnaire.

Data Analysis

The data obtained during the research was analyzed using descriptive statistics. The data analysis steps follow data analysis techniques (Miles & Huberman, 1994). Data from the research literature is presented in descriptive form. The results of interviews with teachers are described based on the aspects asked. Student needs analysis data is also presented in narrative form. The presentation of analysis data is equipped with graphs and charts.

RESULTS

Literature Review Results

The results of a review of articles conducted (Saputra et al., 2023) found that the research topics that were least conducted were learning media in the form of applications, artificial intelligence, Augmented Reality, and virtual environments. Based on searches using Publish or Perish (Google Scholar and Scopus) spanning the years 2017-2023, only a few articles were found that discussed AR-based learning media on genetic substance material. The first article is about research into the development of AR application marker methods using DNA transcription material. When the object is displayed, the DNA transcription process and the components involved in this process are visible, such as DNA helicase and gyrase (Diki et al., 2022). Next, researchers (Nuraini et al., 2019) developed an AR application for the marker method using DNA structure material.

Research on the use of AR-based media in teaching biology and other material includes research (Haryanto et al., 2017) on the application of augmented reality as a learning medium for cell division material. The implementation of augmented reality in Biology Subjects for the introduction of human sensory organs using the marker method was researched by (Lestari et al., 2018).

The impact of using AR-based media in biology learning has also been stated in several studies. The effect of applying AR in biology learning on academic achievement and motivation was researched by (Demircioglu et al., 2022; Kul & Berber, 2022; Omurtak et al., 2022).

There are quite a lot of articles related to the development and use of augmented reality-based media in other subjects. For example, in learning physics, chemistry, and mathematics. In chemistry learning (Abdinejad et al., 2021) developed a simple and cost-effective markerless AR tool for teaching chemistry. Research conducted (Qingtang et al., 2022) in chemistry learning regarding the effects of using AR on increasing knowledge, learning motivation, and students' perceptions of technology. The effectiveness of using the Augmented Reality Module in Geometry Learning on mathematics achievement among elementary school students was researched by (Yusra et al., 2023)

In social learning, several articles were also found that discussed the use of AR. The impact of using AR in microeconomics courses was researched by (Ali et al., 2023). Development of Augmented Reality-Based Educational Media for Interior and Exterior Design (Wahyudi et al., 2017).

Needs Analysis Results

. Based on an interview with one of the high school biology teachers, it was revealed that students found the genetic substance material challenging due to the extensive memorization required for numerous terms. Additionally, factors such as a tendency to guess answers, lack of thoroughness, and reluctance to seek clarification even when concepts were not fully understood were identified among the students. The teaching methods employed by teachers include lectures and group presentations, supplemented with PowerPoint presentations, learning videos, and direct explanations on the blackboard. While teachers believe these methods and media should enhance learning effectiveness, they find the current media less engaging, and there are persistent factors among students hindering effective learning. The teacher advocates for innovative, engaging

learning media with superior visualization compared to current teaching materials. Additionally, the teacher expresses enthusiasm for the adoption of AR-based learning media, especially for genetic substance material, believing it would greatly enhance the learning experience.

The results of the needs analysis for students revealed that 78.38% of students considered genetic substance material to be difficult material. Concepts that are considered difficult by students are the concept of the protein synthesis process (62.16%), the concept of RNA (40.54%), and genetic material (43.24%). An illustration of the percentage of students who experience difficulties with each concept can be seen in Figure 1.

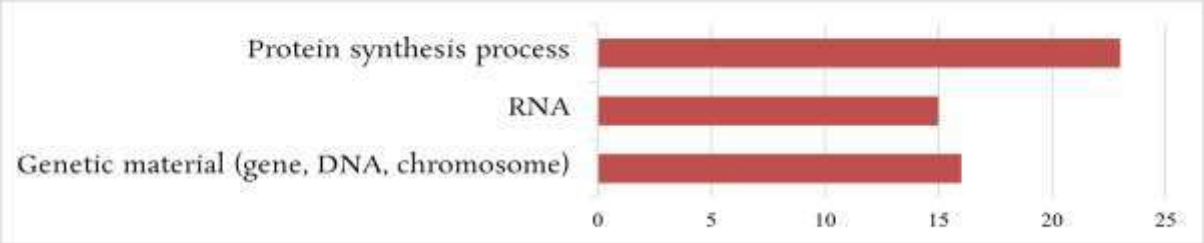

Figure 1. Difficult concepts in genetic substance material

Throughout their learning experience, students have utilized various media, including smartphones, tablets, laptops, posters, teaching aids, and PPT. A comparative description of the use of these media can be seen in Figure 2.

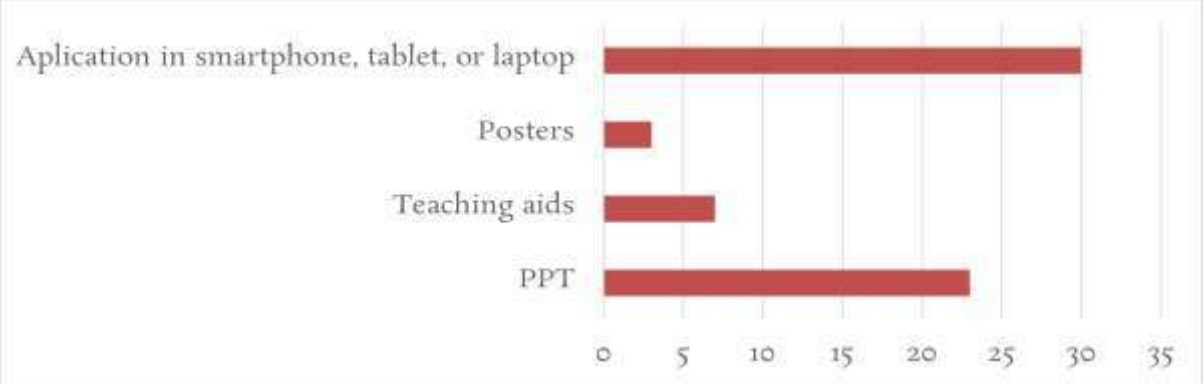

Figure 2. Media commonly used in biology learning

Based on Figure 2, it can be seen that the use of smartphones is quite high, followed by the use of PPT media. When queried about their preferred learning media, the responses from students can be seen in Figure 3.

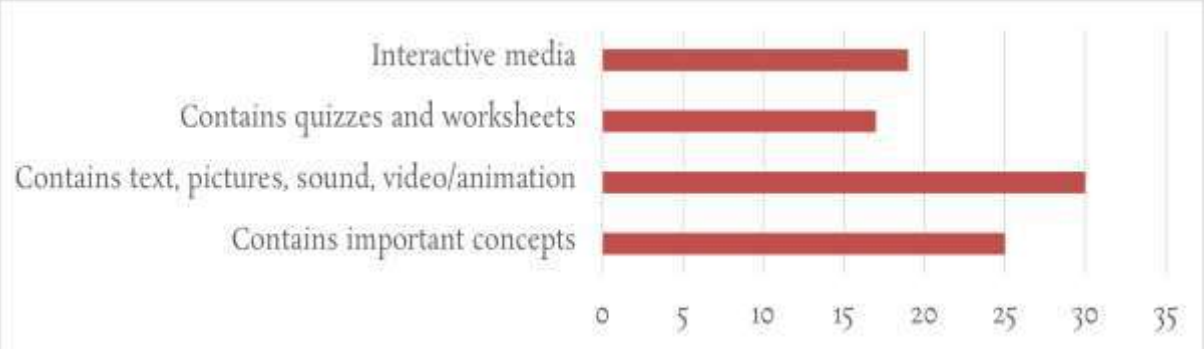

Figure 3. Media needed by students

Based on Figure 3, it can be seen that the media most needed by students is media that contains text, images, audio, and video/animation. Regarding AR technology, 53% of surveyed students demonstrated prior awareness, with 52.63% of those respondents having utilized it. Additionally, when questioned about their receptiveness to augmented reality-based learning media for genetic substance material, 59.46% expressed willingness to incorporate this technology into their learning.

DISCUSSION

Based on the results of the article search, it turns out that there is a limited number of augmented reality-based media currently in development focusing on genetic substance material. While numerous studies exist on the development and impact of augmented reality (AR) media in physics, chemistry, and mathematics learning, comparatively fewer studies are available in biology learning, particularly regarding genetic substance material. This gap presents an opportunity for the development of AR-based media tailored to genetic substance material. Given the abstract nature of genetic substance concepts, visual support becomes imperative. The inherent characteristics of AR, facilitating the visualization of abstract concepts, can significantly aid students in understanding genetic substance material. AR applications transform abstract concepts into tangible entities, thereby fostering deeper comprehension and enhancing the overall learning experience (Omurtak et al., 2022). The use of AR is very informative and interesting (Rizki & Riau, 2019)

The development of augmented reality-based media holds immense potential in meeting the current demands of learning. Learning and technology must complement each other (Leshchenko et al., 2021). Employing AR technology can facilitate students in constructing concept maps and support their conceptual understanding (Çakıroğlu et al., 2022). AR technology can improve students' cognitive and affective skills and increase their ability to understand abstract and complex things (Mantasia & Jaya, 2016). AR technology can improve students' cognitive and affective skills and increase their ability to understand abstract and complex things (Schmidthaler et al., 2021)

The importance of developing this media is also supported by the results of the needs analysis that has been conducted. Needs analysis reveals problems, needs, and development potential (Qamariah & Nurhadi, 2021). The need for this media was expressed by the teacher. According to the teacher, interesting media is needed. Learning media designed and created by teachers will inspire students, fostering enthusiasm and encouraging attentive engagement with the lesson material (Akrim, 2018). Media also makes learning more effective and increases student motivation (Sabbah et al., 2023; Simanjuntak et al., 2021). The use of AR media can improve learning outcomes and student learning completeness (Rejekiningsih et al., 2023)

During the interview, the teacher expressed a desire for innovative and engaging learning media with enhanced visualization, which could be achieved through AR-based technology. The unique capabilities of AR media enable the visualization of structures in three dimensions, fulfilling this expectation (Abdinejad et al., 2021). The use of AR-based media in science learning has a positive impact on understanding concepts (Ropawandi et al., 2022) and achieving learning outcomes(Jdaitawi et al., 2022). AR-based technology enables open access to materials and improves the organization of modern library media spaces (Horban et al., 2023).

Specifically for learning genetic substance material, the results of the needs analysis show that students think genetic substance material is difficult material. This difficulty is due to the abstract and interdisciplinary characteristics of genetic material (Çimer, 2012). Concepts that students consider difficult are the concept of the protein synthesis process (62.16%), the concept of RNA (40.54%), and genetic material (43.24%). These results are in line with research (Fitriana et al., 2022) which concluded that students had difficulty learning genetic material (chromosomes, genes,

DNA, and RNA) as well as foreign concepts and terms contained in the process of cell division and inheritance.

The development of AR-based media certainly requires supporting facilities for its application in the field. The results of the needs analysis show that the facilities available at school support the application of this media in learning. Facility support determines the smoothness of the learning process. Apart from supporting facilities, the willingness and readiness of teachers and students to use AR-based media are also needed (Arthur-nyarko, 2020; Bernacki et al., 2019). The results of the needs analysis found that teachers expressed their willingness to use AR-based media and expressed a great need for this media.

The device support that students have is also very adequate for using technology-based media. The devices that most students own, such as smartphones, have great potential for developing innovative technology-based learning media (Rejekiningsih et al., 2023).

Integrating technology in current learning conditions is a necessity. Teachers need to integrate technology into their learning design. The integration of this technology is aimed at improving the quality of the learning process and achieving learning outcomes. AR technology is a form of technology that teachers can integrate into learning.

CONCLUSION

Augmented Reality-based biology learning media on Genetic Substance Material is very urgent to be developed based on the trend of using AR-based media in the world of education and the positive benefits of this media on learning processes and outcomes. In biology learning, especially genetic substance material, the availability of AR-based media remains limited, highlighting the significance of addressing this gap. Teachers and students also expressed their need for technology-based media, especially AR-based media. Explanation of abstract and complicated material can be facilitated through the utilization of AR-based media. Based on these reasons, AR-based media on genetic substance material is urgent to be developed. The development of AR-based media will be able to enrich the learning media used for biology learning, especially genetic substance material.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 11 – 20 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Improving problem-solving ability and collaboration skills of ecosystem material through STEM integrated project-based learning

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Article Info

Article History:
Received 15 November 2023
Revised 19 February 2024
Accepted 07 April 2024
Published 30 April 2024

Keywords:
Project Based Learning
STEM
Problem Solving Ability
Collaboration Skills

ABSTRACT

The learning process at Cipasung Islamic High School is still teacher-centered so the formation of 21st century skills in students is still neglected. The purpose of this study was to determine the effect of the STEM-integrated project-based learning model on the problem-solving ability and collaboration skills of students on ecosystem material. This research was conducted in May 2023. The research method used is an experimental method with a Quasi Experiment design and the matching-only posttest-only control group design form. The research population was the X MIPA class of Cipasung Islamic High School in the 2022/2023 school year. The sample was taken using a purposive sampling technique and obtained X MIPA 1 class as the control class and X MIPA 4 as the experimental class. The research instruments used in this study consisted of 3 validated instruments, namely the problem-solving ability test instrument, the CSAT (Collaboration Self-Assessment Tool) questionnaire, and the collaboration skills observation sheet. The data analysis technique used is One way ANOVA test. Based on the results of the analysis, it can be concluded that there is an effect of STEM integrated project-based learning model on problem-solving ability and collaboration skills of students on ecosystem material in Class X MIPA SMA Islam Cipasung.

Citation: Ningrum, T. L., Wahidin, & Diella, D. (2024). Improving Problem Solving Ability and Collaboration Skills of Ecosystem Material through STEM Integrated project-based learning. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 11-20. DOI: https://doi.org/10.31932/jpbio.v9i1.2962

INTRODUCTION

21st-century skills are important skills that must be mastered by every individual to face challenges in the 21st century. One of the 21st-century skills that are important for students to

have is problem-solving ability (Fitriana & Supahar, 2019) and collaboration skills (Alfaeni et al., 2022). Problem-solving ability is the basic process of identifying problems, considering options, and making informed choices (Supiandi & Julung, 2016). Collaboration skills are one of the skills that invite students to actively contribute in working together and interacting during learning so that learning will be easier to understand (Junita & Wardani, 2020).

Unfortunately, the results of the report from the Program for International Student Assessment (PISA) show that students' problem-solving skills in Indonesia are still relatively low when compared to other countries. (Ilmi, 2019). Based on various empirical findings in the research of Ayu et al. (2018) indicate low critical thinking and problem-solving skills, communication, and collaboration skills in school-age and working-age children. Ofstedal & Dahlberg (2009) also stated that the education field was very slow in realizing the need to teach collaboration skills to students. Collaboration skills are needed by students because these skills are more than just working together, including active listening skills, responding with respect, expressing ideas clearly through various ways of communicating, and using these skills to reach consensus and compromise (Sahrir, 2019).

Based on the results of interviews with Biology Teacher class X, the teacher has never measured and taught the problem-solving ability and collaboration skills of students. During the learning process, the teacher only explains the material and students listen to the explanation from the teacher so that from the learning process there is no process of forming students' basic skills, especially problem-solving ability and collaboration skills. Therefore, problem-solving skills are very important for students to have (Fitriana & Supahar, 2019). This is indicated by the development of learning models that demand the improvement of problem-solving skills (Fitriana & Supahar, 2019). Likewise, collaboration skills are one of the important aspects of lifelong learning (Sari et al., 2021).

Science learning including Biology in the 2013 curriculum has provided a reference in the selection of learning models that are by the scientific approach. These learning models include project-based learning (PjBL), problem-based learning (PBL), and discovery learning(Afriana et al., 2016). Project Based Learning (PjBL) is one of the learning models suggested in the implementation of the 2013 curriculum to be applied in learning (Budiharti et al., 2016). Project-based learning is a learning model that is relevant to the demands of the 21st century (Parno et al., 2020). PjBL is a student-centered learning model and provides meaningful learning experiences for students. Students' learning experience or concept acquisition is built based on the final product produced in learning. The characteristics of the project-based learning model according to Kemendikbud (2014) include the existence of problems posed to students and students being collaboratively responsible for solving problems. Based on these characteristics, there is a connection between project-based learning problem-solving ability, and collaboration skills.

In addition to project-based learning, learning today needs to follow the trends of the globalization era, one of which is by integrating science, technology, engineering, and mathematics known as STEM. Projects in PjBL are related to the fields of Science, Technology, Engineering, and Mathematics (Parno et al., 2020). STEM is a learning approach that connects four fields, namely science, technology, engineering, and mathematics into a holistic unit (Jauhariyyah et al., 2017). The STEM approach to learning is expected to provide meaningful learning for students through the systematic integration of knowledge, concepts, and skills. The project approach in STEM is based on constructivist theory which is proven to improve student achievement in higher-order cognitive tasks, such as scientific processes and problem-solving (Laboy-Rush, n.d.). The STEM approach aims for learners to have the skills to identify problems (Permanasari dalam Utomo et al., 2020) and develop collaboration skills (Winarni et.al. dalam Utomo et al., 2020). This shows that STEM is related to problem-solving and collaboration skills.

Research on project-based learning (PjBL) integrated with STEM has been carried out but is still rare in Biology learning. This study integrates STEM with a project-based learning model to be applied to one of the Biology materials, namely ecosystems. The results of research by Parno et al. (2020) revealed that the use of a project-based learning model integrated with STEM can improve problem-solving skills and get a positive response from students to the implementation of the model. In addition, STEM in PjBL also provides challenges and motivation for students because it trains students to think critically, and analytically, and improve higher-order thinking skills (Capraro et al., 2013).

Ecosystem material is one of the materials taught in class X SMA where it discusses biotic and abiotic components and interactions between their components. This material is very supportive in the application of the STEM-integrated project-based learning model where students are required to make a project that is integrated with science, technology, engineering, and mathematics to train students' problem-solving skills and collaboration skills.

Based on the background description above, the author aims to research the effect of STEM-integrated project-based learning model on problem-solving ability and collaboration skills of learners on ecosystem material (experimental study in class X SMA Islam Cipasung academic year 2022/2023)". This research is expected to be useful for improving the quality of education in Indonesia, especially in improving students' problem-solving abilities and collaboration skills.

RESEARCH METHODS

Research Design

The research method used is a quasi-experimental design with the form of a matching-only posttest-only control group design. The matching-only posttest-only control group design can be seen in Table 1.

Table 1. The Matching-Only Posttest-Only Control Group Design

Treatment group	M	X	O
Control Group	M	C	O

Description:

M : Classes that have been determined as control/experiment classes
X : Experimental class with STEM-integrated project-based learning model treatment
C : Control class with discovery learning model treatment
O : Post-test

Population and Samples

The population in this study were all X MIPA classes of Cipasung Islamic High School in the 2022/2023 school year consisting of six classes. The sample of this study used 2 classes. The sampling technique used in this study was purposive sampling. The samples in this study were X MIPA 1 class totaling 34 people and X MIPA 4 class totaling 34 people. The reason for selecting these samples is because class X MIPA 1 and class X MIPA 4 have an average score that is not much different and also based on consideration with the Xth grade Biology teacher. Class X MIPA 1 was the control class using the discovery learning model and class X MIPA 4 was the experimental class using the STEM-integrated project-based learning model.

Instruments

The research instruments used in this study were a problem-solving ability test, CSAT(collaboration self-assessment tool), and collaboration skills observation sheet. The test instrument was used to measure students' problem-solving ability. The test form is an essay question that contains 5 indicators of problem-solving ability. The CSAT questionnaire is given to

measure students' collaboration skills which consists of 11 indicators. Then the collaboration skills observation sheet contains 4 indicators. The test instruments used have previously passed the validity test and reliability test. The questionnaire instrument is the result of the adoption of Ofstedal & Dahlberg (2009)) research which was then translated into Indonesian. The results of construct validity conducted by expert validity show that the instrument is suitable for use. After construct validity, content validity was carried out on the test instrument using SPSS 26 software. Based on the results of the validity test on 20 questions, 9 questions were declared invalid and 11 questions were considered invalid. However, there was 1 question that was modified by changing the sentence structure of the question. The purpose of this modification is to complete the use of the instrument because this instrument is in the form of a question package that must contain 5 indicators. So, the number of questions used as a test instrument is 10. The result of the reliability test on 10 questions is 0.90. These results indicate that the instrument has a very high level of reliability. The grid of the problem-solving ability test instrument can be seen in Table 2.

Table 2. Grids of Problem-Solving Ability Instruments

No.	Indicator	Question number	Number of question
1	Defining the problem	1 and 6	2
2	Diagnosing the problem	2 and 7	2
3	Formulate alternative strategies	3 and 8	2
4	Determine and implement preferred strategies	4 and 9	2
5	Evaluate the success of the strategy	5 and 10	2
	Total		**10**

The grid of the collaboration skills questionnaire instrument(Collaboration Self-Assessment Tool) can be seen in Table 3.

Table 3. Collaboration Skills Questionnaire Instrument Grid

No.	Indicator	Item Number
1	Contribution	1
2	Motivation/participation	2
3	Quality of work	3
4	Time management	4
5	Team support	5
6	Preparedness	6
7	Problem solving	7
8	Team dynamics	8
9	Interaction with others	9
10	Role flexibility	10
11	Reflection	11

The grid instrument of the collaboration skills observation sheet (Collaboration Self-Assessment Tool) can be seen in Table 4.

Table 4. Collaboration Skills Observation Sheet Instrument Grid

No	Collaboration Skills Indicator	Observation Item
1	Productive work	1,2,3
2	Showing respect	4,5,6
3	Compromise	7,8,9
4	Sharing responsibility	10,11,12

Procedures

The research procedure consists of three stages, namely preparation, implementation and data processing. In the preparation or pre-research stage, researchers made observations to the school that would be used for research, made instruments, and tested research instruments. At the implementation stage, researchers collected data obtained from the posttest. Learning activities used the discovery learning model for the control class and the STEM-integrated project-based learning model for the experimental class. learning was carried out for 3 meetings on ecosystem material. The last stage is data processing, researchers process data obtained from the field and analyze all research results.

Data Analysis

The data analysis technique uses descriptive statistical analysis to find the average of each class. Then the prerequisite analysis test was carried out, namely, the normality test using the Kolmogorov-Smirnov test and the variance homogeneity test using the Levene test. Hypothesis testing was carried out using the one-way ANOVA test. All data were analyzed using IBM SPSS software version 26.

RESULTS

The post-test was given to students after being given treatment in each class using validated instruments. The research data first went through the prerequisite test stage of analysis, namely normality and homogeneity. The normality test results have a significance value of 0.05, meaning that all data comes from a normally distributed population. Furthermore, the homogeneity test shows that all data have homogeneous variances, so the hypothesis test used in this study is a parametric statistical test using the One-Way ANOVA test. The results of the One-Way ANOVA test analysis can be seen in Table 5.

Table 5. One-Way ANOVA Hypothesis Test Results

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Problem-Solving Ability	Between Groups	612.000	1	612.000	60.659	.000
	Within Groups	665.882	66	10.089		
	Total	1277.882	67			
Collaboration skills	Between Groups	124.471	1	124.471	4.449	.039
	Within Groups	1846.471	66	27.977		
	Total	1970.941	67			

Then to see the effect simultaneously (together), a simultaneous test was conducted with the following results.

Table 6. Simultaneous Hypothesis Testing Results

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	612,295	2	306,148	29,898	0,000[b]
	Residual	665,587	65	10,240		
	Total	1277,882	67			

a. Dependent Variable : KPM
b. Predictors : (Constant), Class , Collaboration

The average score of each indicator of problem solving ability in the control and experimental classes can be seen in Figure 1.

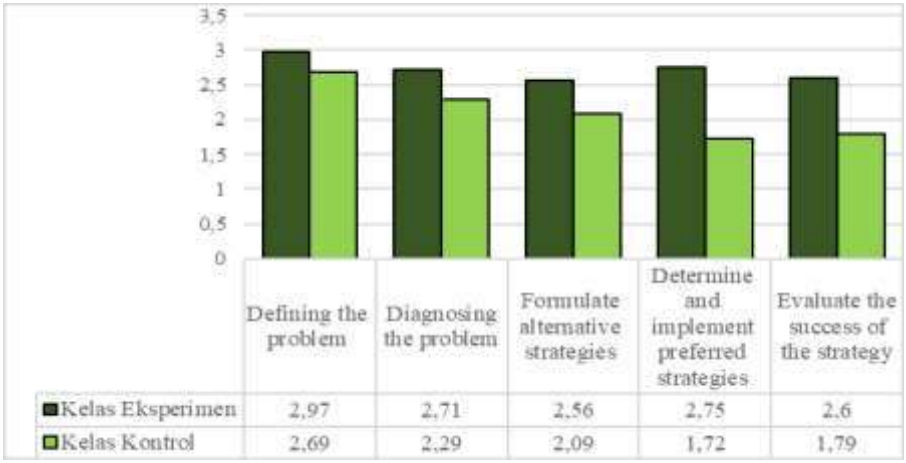

Figure 1. Comparison Diagram of Posttest Average Score of each Indicator of Problem-Solving Ability in Experimental and Control Classes

The acquisition of collaboration skills scores for each indicator in the control and experimental classes can be seen in Figure 2.

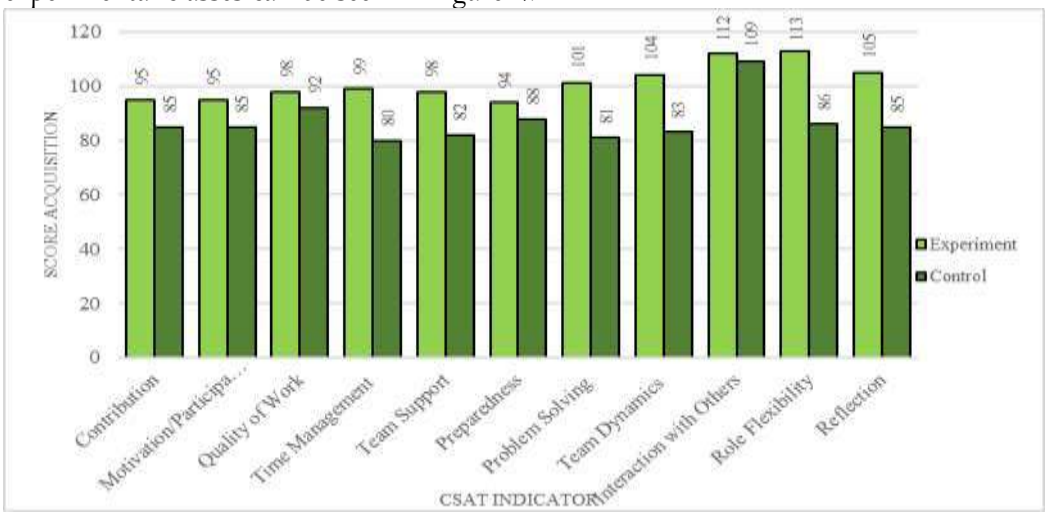

Figure 2. Comparison Diagram of CSAT Score Acquisition for Each Indicator of Collaboration Skills in Experimental and Control Classes

The measurement of collaboration skills is supported by observations that have been made by observers and the results obtained as in Table 7.

Table 7. Observation Results of Collaboration Skills

Class	Indicator	Score	Criteria
Control	Productive work	78,42	Exemplary
	Showing respect	89,21	Exemplary
	Compromise	58,27	Proficient
	Sharing responsibility	46,08	Basic
Experiment	Productive work	88,89	Exemplary
	Showing respect	91,83	Exemplary
	Compromise	65,69	Proficient
	Sharing responsibility	58,82	Proficient

DISCUSSION

STEM-integrated project-based learning or STEM-PJBL is an approach that directs students to explore ill-defined problems that integrate STEM in a limited environment (Samsudin et al., 2020). The results prove that STEM-integrated project-based learning affects problem-solving ability and collaboration skills. Based on the results of hypothesis testing using the one-way ANOVA test in Table 5, where partially the STEM-integrated project-based learning model affects problem-solving ability with a significance value of 0.000 and affects collaboration skills with a significance value of 0.039. Then simultaneously obtained a significance value of 0.000 while the value of $\alpha = 0.05$ (0.000 0.05) which means H0 is rejected and Hq is accepted (table 6). This means that simultaneously there is an effect of using project-based learning integrated with STEM on the problem-solving ability and collaboration skills of students on ecosystem material in class X MIPA SMA Islam Cipasung in the 2022/2023 school year. This shows that the use of STEM-integrated project-based learning models has a positive influence on improving problem-solving ability and collaboration skills. In line with the research of Megawati et al. (2023) which shows that PjBL-STEM-based learning is effective for improving 21st-century skills including developing students' collaboration skills by providing opportunities for students to work together in teams through problem-solving in the given project.

Improving students' problem-solving ability and collaboration skills can occur due to several factors, one of which is the use of the STEM-integrated PjBL model. Learning with STEM-integrated PjBL invites students to do meaningful learning in understanding a concept and exploring through a project activity (Jauhariyyah et al., 2017). STEM-PjBL gives students experience in solving real problems with practicum activities so that it can increase effectiveness, and meaningful learning, and support future careers (Tseng et al., 2013).

The problem-solving ability of students is measured using a test instrument in the form of a description of 10 questions consisting of 5 indicators. The average score of each indicator of the problem-solving ability of the control and experimental classes contained in Figure 1 shows that the average of each indicator in the experimental class tends to have a higher value than the control class. This high score is because the learning stages in the STEM-integrated project-based learning model are in line with the problem-solving ability indicators where students are presented with a problem that they need to study (reflection stage in the STEM approach) first to understand the context of the problem and then formulate a solution in the form of a product that they will make. In addition, in PjBL learning integrated with STEM, there is also a research and discovery approach where students will research the problems presented so that students can identify problems including their causes. This is in line with the opinion of Supiandi & Julung (2016) where project-based learning has learning characteristics in the form of proposing problems that can train students in problem-solving habits that will affect students' higher-order abilities, for example getting students used to thinking creatively by exploring and expressing ideas, as well as identifying problem-solving that can be applied to solve given problems. Anugrah et al. (2020) added that learning is done by giving problems that are relevant to students' lives where students are asked to actively think so that they can solve the problems given.

Collaboration skills are the ability to participate in any activity to foster relationships with others, respect relationships and teamwork to achieve the same goal (Rahmawati et al., 2019). Collaboration skills in this study use the CSAT(Collaboration Self-Assessment Tool) questionnaire which consists of 11 indicators. Each indicator consists of 4 statements, so this questionnaire totals 44 statements. The score is then categorized into 3 criteria, namely emerging, developing, and established (Ofstedal & Dahlberg, 2009). Based on the research results, the "emerging" criteria consisted of 6 respondents from the experimental class and 8 people from the control class. The criteria for "developing" in the experimental class were 19 people, while the

control class was 24 people. Then for the "built" criteria, there were 9 people from the experimental class and 2 people from the control class.

The acquisition of experimental and control class CSAT scores for each indicator contained in Figure 2 shows that the scores of all experimental class indicators are higher than the acquisition of control class scores where it can be seen that the highest score for the experimental class is in the flexibility indicator, while the lowest score is in the preparation indicator. The flexibility indicator score is 113 which is the highest score. This high score is because the experimental class learning using the STEM-integrated project-based learning model emphasizes the collaboration process in making projects to stimulate students to adapt flexibly to lead their groups and as members (Ofstedal & Dahlberg, 2009). Meanwhile, the preparation indicator score was 94. The low preparation score can be caused by learners not being accustomed to doing assignments that are project making so learners are not used to preparing the things needed in making projects. Ofstedal & Dahlberg (2009) suggest that the preparation referred to here is the readiness of students to work when they come to class. In this case, students in the experimental class are not accustomed to preparing in advance for the needs of working in groups.

In addition to the CSAT questionnaire, the assessment of collaboration skills is also measured by the collaboration skills observation sheet. Referring to the results of the observation of students' collaboration skills in the control class contained in Table 7, the highest score is on the indicator "showing respect" with a score of 89.21 which is included in the exemplary criteria. The lowest score is on the "sharing responsibility" indicator with a score of 46.08 which is included in the basic criteria. Similar to the experimental class, the highest score for the experimental class on the indicator "showing respect" with a score of 91.83 including exemplary criteria, and for the lowest score there is also an indicator "sharing responsibility" with a score of 58.82 which includes proficient criteria. The indicator of showing respect can be seen in group members who are openly willing to listen to and respect the opinions of their fellow members during discussions. As for the indicator of sharing responsibility, some group members have not completed the tasks that are their part. The attitude of collaboration is very important to be familiarized to students so that students have good academic performance to solve problems in project work (Hidayanti et al., 2020).

CONCLUSION

Based on the results of research, data processing, and hypothesis testing, it is proven that there is an effect of the STEM-integrated project-based learning model on the problem-solving ability and collaboration skills of students on ecosystem material in class X MIPA SMA Islam Cipasung in the 2022/2023 school year. The conclusion of this study states that the STEM-integrated project-based learning model is effectively used in learning biology, especially on ecosystem material to improve students' problem-solving ability and collaboration skills.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 21 – 35 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

In silico analysis: Activity of active compounds in passiflora foetida to diabetes

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Article Info

Article History:
Received 23 November 2023
Revised 12 February 2024
Accepted 01 April 2024
Published 30 April 2024

Keywords:
In silico
Passiflora foetida L.
Diabetes

ABSTRACT

Plants contain good compounds that are simple to complex including medicinal plants. Herbs are all plant species that are around settlements, cultivated, wild plants that are known to have medicinal properties. An example is the rambusa plant (*Passiflora foetida* L.). Rambusa leaves are efficacious in relieving fever, insomnia, colds, headaches, diabetes, and asthma. The purpose of this study is to find out physicochemical & pharmacokinetic predictions, toxicity levels, and the affinity of active compounds and a comparison of toxicity levels and affinity of rambusa plants or *Passiflora foetida* L. with metformin. The method used in this study is qualitative descriptive. *Molecular docking* with *in silico approach* between active compound *Passiflora foetida* L. with target protein *Alpha-glucosidase* subunit B to inhibit blood sugar levels (diabetes). The compound that has the best pharmacokinetic profile compared to metformin is *Passifloricin A. 5-Hydroxy-7,4'-dimethoxyflavone, Deidaclin, Linamarin, Volkenin, (1S,4S)-Tetraphyllin B (S)-Tetraphyllin A* and *Passifloricin A* compounds are relatively safer, not mutagen and not toxic to the liver, except *Passifloricin A* is toxic to the liver, because the compound has a safer level of toxicity compared to the comparison medicine, metformin.

Citation: Soendoess, K., Hernawati, D., & Putra, R.R. (2024). In silico analysis: Activity of active compounds in passiflora foetida to diabetes. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 21-35. DOI: https://doi.org/10.31932/jpbio.v9i1.2974

INTRODUCTION

Each plant contains good compounds that are simple to complex, including medicinal plants. The richness of this content gives a plant a lot of potential to prevent and treat a disease, especially degenerative diseases. Degenerative diseases are caused due to reduced antioxidant ability to neutralize increased free radical activity in the body that causes cell damage (Mulyani et al., 2022).

Herbs are all plant species that are around settlements, cultivated, or wild growth that are known and believed to have medicinal properties (Lingkubi et al., 2015). Herbs are believed by traditional people to cure diseases, both minor and severe diseases. As well herbs, namely turmeric to cure minor diseases such as ulcers, as for cancer, namely antanan plants. The plant parts used can come from leaves, fruits, seeds, bulbs, stems, roots, and rhizomes.

Figure 1. Morphology of parts of the vine: (a) roots, (b) stems, (c) leaves, (d) flowers, (e) fruits; (f) seeds (Source: Personal Documentation)

Rambusa (*Passiflora foetida* L.) is one type of plant that is found creeping on other plants. This plant is usually found in watery areas such as swamps and rivers (Lim, 2016). Rambusa has anti-inflammatory, antitumor, anticancer, anti-hepatotoxicity, and antimicrobial activity (Nagulapati et al., 2021). According to Yepes et al., (2021), rambusa leaves are efficacious in relieving fever, insomnia, colds, headaches, and asthma.

Passiflora foetida L. is a family *of Passifloraceae* native to South America, which has spread to tropical regions around the world, including Thailand. The leaves of this plant are also used as a folk remedy for the treatment of hysteria, fever, ear infections, emmenagogue, asthma, insomnia, and skin diseases. among them are Pseudomonasputida, Vibriochlerae, Shigellaflexnerian, and Streptococcus pyogenes. Further research conducted by Mohanasun from etal. did not show the active compound content in a crude extract of *Passiflora foetida*. The main phytochemical constituents *Passiflora foetida* have several active constituents such as hydrocyanic acid, flavonoids, harmful alkaloids, passifloricin, polyketides, a-pyron, and vitexin. Vitexin is reported to have antioxidant, anti-inflammatory, anti-thyroid, anti-arteriosclerotic, antihypertensive, and antihepatotoxic properties. Vitex levels in different plant extracts have been determined by various techniques, including spectroscopic and chromatographic methods. High-performance thin-layer chromatography (HPTLC), coupled with densitometry (Shuayprom et al., 2016).

According to Fatimah (2015), Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or the body cannot use the insulin produced effectively. This causes the concentration of glucose in the blood to be high (hyperglycemia). The combination of genetic factors and environmental factors can be the etiology of diabetes. Genetic factors are inherited from parents and inherited, while environmental factors are factors influenced by lifestyle. In addition, factors for the occurrence of diabetes, namely age ($\geq$ 40 years), obesity, heredity (genetic), and smoking habits have a higher risk of diabetes (Arisma et al., 2017). Along with lifestyle changes that tend to be less healthy, the prevalence of diabetes is getting higher. Indonesia

was ranked 7th as the country with the highest number of diabetics in 2019 (Ministry of Health of the Republic of Indonesia, 2020).

Diabetes mellitus affects the quality of human resources and will have an impact on increasing health costs. Therefore, all communities actively participate in efforts to overcome and prevent diabetes mellitus. This diabetes mellitus can also cause diabetic retinopathy (DR) disease is an important microvascular complication that is very specific for diabetes mellitus (DM), from these complications to permanent vision loss or even blindness (Wang et al., 2023).

According to the World Health Organization (WHO) in 2030 there will be an increase in the population affected by Diabetes Mellitus by at least 366 million people. While the results of a survey conducted by WHO, Indonesia is included in the 4 highest countries whose population suffers from DM as well as China, the US, and India (Utomo et al., 2020). While WHO predicts around 21.3 million Indonesians will be at risk of diabetes by 2030 (Arisma et al., 2017). Meanwhile, during this pandemic, diabetes mellitus is in the second position as the most comorbid for COVID-19 patients in Indonesia, reaching 33.6% (Wahyuni et al., 2022).

The use of traditional medicine for keeping health and disease disorders is still needed and developed, especially with the high cost of treatment and the price of medicines. The use of traditional medicine is studied by the community through ethnobotany (Wulansari, 2020). According to Khaerati et al., (2015) the results of their research tested the effectiveness of rambusa leaf extract (*Passiflora foetida* L.) can reduce blood sugar levels in mice with the dose used 750mg / kg body weight which is most effective as an antidiabetic.

Current technological advances cause the initial procedure of testing acetic acid compounds for blood sugar controllers in the body, it is necessary to predict in advance to see the performance of compounds by modeling chemical structures through an in silico approach (Hairunnisa, 2019). The technique used in this in silico approach is reverse docking which is a technique to analyze the potential of a compound against target proteins in the human body (Issa et al., 2019).

For this reason, the purpose of this study is to find out: 1) physicochemical and pharmacokinetic predictions *of Passiflora foetida* L. compounds to inhibit sugar in the blood; 2) the level of toxicity of the active compound *Passiflora foetida* L. in silico; 3) the affinity of the active compound *Passiflora foetida* L. to alpha-glucosidase receptors on blood sugar in silico; 4) comparison of the level of toxicity and affinity of the active compound *Passiflora foetida* L. with metformin comparison medicine in silico; and 5) The novelty of the research conducted is molecular docking using Biovia Studio Discovery 2021 software and Autodock Tools 1.5.7 to analyze compounds found in rambusa plants (*Passiflora foetida* L.).

RESEARCH METHODS

Research Design

This research is research with a qualitative descriptive method, namely the data collected in the form of words or images so that it does not emphasize numbers and emphasizes more processes than products (Sugiyono, 2016). The object of this study is plants that have the potential as antidiabetic candidates. These plants contain several active compounds that can inhibit blood sugar levels. molecular docking with an in silico approach between the active compound *Passiflora foetida* L. with the target protein Alpha-glucosidase subunit B to inhibit blood sugar levels (diabetes).

Instruments

The tools used in this study are hardware in the form of an Asus Vivobook Laptop with model specifications named S14, Intel AMD Ryzen 5 3500U CPU, 8 GB RAM, 512 GB SSD Storage, 14.0" IPS LCD Full HD Display, and 4 Cell Battery. The *software* used is the Windows

11 Home SL operating system, MGLTools 1.5.7 which is equipped with Autodock Tools 1.5.7, the Biovia Discovery Studio Visualizer 2021 application, Protein Data Bank (https://rcsb.org/) to download 7KBJ receptors, PubChem (https://pubchem.ncbi.nlm.nih.gov/) to download the active compound *Passiflora foetida* L. (ligand), KNApSAck (http://www.knapsackfamily.com/knapsack_core/top.php), to see the active compound content in *Passiflora foetida* L., pkCSM (pkCSM (unimelb.edu.au)), for pharmacokinetics and toxicity tests and ProTox online tools (https://tox-new.charite.de/protox_II/) for toxicity tests. The ingredients used are 7 active compounds in *Passiflora foetida* L

Procedures

The research procedure starts from the stage of identifying problems for medicine of diabetes from rambusa plant compounds (*Passiflora foetida* L.), then conducting literature studies related to diabetes and *Passiflora foetida* L. plants, then determining the purpose of the study, then collecting data in the form of names of active compounds found in *Passiflora foetida* plants. from the KNApSack website, then downloading the 3-dimensional conformation of active compounds obtained from the PubChem database, then downloading the *docking* target macromolecules obtained from the protein data bank (GDP) provider site, then processing data through the Discovery Studio Visualizer 2021 software, AutoDock Tools 1.5.7, the pkCSM website to predict pharmacokinetics and physicochemistry, the ProTox website to predict toxicity from Compounds are used, and finally make analyzing results from data processing.

Predict physicochemistry of active compounds in *Passiflora foetida* L. using five parameters (*Lipinski rule of five*) including molecular mass weight, logarithm of partisioctanol/water coefficient (Log P), hydrogen bond donor (HBD), Hydrogen bond Acceptor (HBA), and violation. As for pharmacokinetic prediction using ADME indicators (*Absorbtion, Distribution, Metabolism,* and *Excretion*). How to see the toxicity of the active compound *Passiflora foetida* L. can be seen from the LD50 value, *ames toxicity*, *hepatotoxicity,* and toxicity class using *the protox online tool* website and pkCSM, where each parameter has a maximum value limit.

How to see the affinity value between the active compound and *the Alpha-glucosidase* receptor is by taking data from autodock tools 1.5.7 and biovia discovery studio visualizer 2021 software. The software is used for docking via command prompt, and getting the affinity binding value, rmsd u.b, rmsd lb. From this value, it can be seen that the more negative the value of the affinity binding, the better the ligand is to bind to the receptor.

Comparing toxicity parameters such as LD_{50} the greater the value, the better the compound can be tolerated by the human body, ames toxicity if the positive results show that the compound is mutagenic and can act as a carcinogen, hepatoxicity to find out the potential of medicines that can induce damage to the liver, toxicity class if the higher the value then the compound is not toxic. While affinity is seen from ligands that have the most negative affinity value, if the active compounds contained in research plants and comparison medicines have the most negative affinity value, then the compound can be said to be the best ligand to bind to the receptor.

Data Analysis

Data analysis uses qualitative descriptive analysis techniques. Qualitative descriptive analysis was carried out by predicting physicochemistry using five parameters (Lipinski Rule of Five), namely, consisting of molecular mass weight (BM) < 500, logarithm of partisioctanol/water coefficient (Log P) < 5, hydrogen bond donor (HBD) < 5, hydrogen bond acceptor (HBA) < 10, and violation < 2 (Sharma et al., 2023). This prediction uses the help of an online website, Swiss-ADME prediction. Pharmacokinetic predictions were analyzed using ADME (Absorbtion, Distribution, Metabolism, and Excretion) indicators through the pkCSM online website. The

toxicity prediction by looking at the LD50 value, ames toxicity, hepatotoxicity, and toxicity class using the protox online tool and pkCSM online website. Meanwhile, affinity energy prediction is carried out by molecular docking using Autodock Tools 1.5.7 software and Biovia Discovery Studio Visualizer 2021.

RESULTS

Based on the results of this study, there are several results, namely from prediction of physicochemical properties, prediction of pharmacokinetic properties, prediction of toxicity properties, molecular docking, ligand, and amino acid interactions.

Prediction of physicochemical properties

Prediction of physicochemical properties by looking at the parameters of Lipinski's rule of five with the help of pkCSM online tool. The following are the results of the prediction of the physicochemical properties of the ligands of the test compounds in Table 1.

Table 1. Results of prediction of physicochemical properties of compounds using pkCSM Online Tool.

Compound Name	Parameters of Lipinski's Rule of Five				Application of Lipinski's Rule of Five
	BM (g/mol)	Log P	HBA	HBD	
5-Hydroxy-7,4'-dimethoxyflavone	562,524	-0,5092	13	7	Yes, 3 errors
Deidaclin	271.269	-1.58472	7	4	Yes, 0 error
Linamarin	247.247	-1.89492	7	4	Yes, 0 error
Volkenin	287.268	-2.61392	8	5	Yes, 1 error
(1S,4S)-Tetraphyllin B	287,268	-2,61392	8	5	Yes, 1 error
(S)-Tetraphyllin A	271,269	-1.58472	7	4	Yes, 0 error
Passifloricin A	454.692	5,9825	5	3	Yes, 0 error
Metformin (control)	129.167	-1.24383	1	3	Yes, 0 error

Description of the Maximum Limit of the Law of Five Lipinski:
BM : Molecular Weight < 500;
Log P: Partition coefficient < 5;
HBA : Hydrogen Bond Acceptor < 10; and
HBD : Hydrogen Bond Donor < 5.

After testing, physicochemical predictions showed that some compounds did not meet Lipinski's rule of five, namely 5-Hydroxy-7,4'-dimethoxyflavone, volkenin, and (1S,4S)-Tetraphyllin B. The results of this study also showed that there are five compounds that meet Lipinski's rule of five, and one of these compounds is metformin (control) compound.

Prediction of Pharmacokinetic Properties

Prediction of pharmacokinetic properties of test compounds is carried out by looking at the Absorption, Distribution, Metabolism, and Excretion (ADME) parameters using the help of the pkCSM online tool website. The results of the prediction of pharmacokinetic properties can be seen in Table 2.

Table 2. Prediction of Pharmacokinetic Properties of Test Compounds

Compound Name	Absorpsi		Distribution			Metabolism		excretion	
	Intestinal Absorption (%)	Skin permeability (Log kp)	Distribution Volume (Log L/kg)	BBB Permeability (Log BB)	Fraction Unbound (FU)	CYP2 D6 Inhibitor	CYP3 A4 Inhibitor	Renal OCT1 2	Total Clearance (log ml/min/kg)
5-Hydroxy-7,4'-dimethoxyflavone	34,969	-2,735	-0.254	-1.764	0.248	No	No	No	0.225
Deidaclin	45,894	-3,246	-0.104	-0.865	0.8	No	No	No	1.46
Linamarin	40,86	-3,246	-0.214	-0.888	0.828	No	No	No	1.422
Volkenin	38,743	-2,988	-0.002	-0.956	0.808	No	No	No	1.465
(1S,4S)-Tetraphyllin B	38,743	-2,988	-0,002	-0,956	0,808	No	No	No	1.465
(S)-Tetraphyllin A	45,894	-3,246	-0.104	-0.865	0.8	No	No	No	1,46
Passifloricin A	91,043	-2,754	-0.362	-1.106	0.064	No	No	No	1.874
Metformin (control)	59,401	-2,735	-0,232	-0,946	0,811	No	No	No	0,1

It can be seen in Table 2. Passifloricin A compounds have a good percentage of intestinal absorption with a value of 91.043%, and all compounds have good skin permeability values, Deidaclin, Volkenin, (1S,4S)-Tetraphyllin B and (S)-Tetraphyllin A compounds have VDss log values of -0.104; -0.002; -0.002 and -0.104. The compounds 5-Hydroxy-7,4'-dimethoxyflavone, and Passifloricin A have log BB values of <-1 which are -1.764 and -1.106 so it is said that they cannot be evenly distributed, the six active compounds of *Passiflora foetida* L. have lower unbound fraction values than the control compounds. All active compounds of *Passiflora foetida* L. also have no inhibition on the CYP isoform and do not affect OCT2 substrates.

Prediction of Toxicity Properties

This toxicity prediction uses LD_{50} parameters, toxicity class, ames toxicity, and hepatotoxicity. The predicted toxicity results of the test compound and metformin comparison compound can be seen in Table 3. Where there is only one active compound *Passiflora foetida* L. that is toxic to the liver, namely the compound Passifloricin A. From the results of this study, there are also class 6 with 1 compound, class 5 with 1 compound, and class 4 with 5 active compounds *Passiflora foetida* L.

Table 3. Results of prediction of toxicity properties.

No.	Compound Name	Toxicity			
		LD 50 (m/kg)*	*Ames Toxicity***	*Hepatotoxicity***	Toxicity Class*
1	5-Hydroxy-7,4'-dimethoxyflavone	3.919	Yes	No	5
2	Deidaclin	2.000	No	No	4
3	Linamarin	29.700	No	No	6
4	Volkenin	2.000	No	No	4
5	(1S,4S)-Tetraphyllin B	2.000	No	No	4
6	(S)-Tetraphyllin A	2.000	No	No	4
7	Passifloricin A	1.890	No	Yes	4
8	Metformin (control)	680	Yes	No	4

Note:
(*) Using the ProTox Online Tool (**) Using pkCSM Online Tool

Molecular docking ligand of test compound with protein Alpha-glucosidase Sub-unit B

The results of molecular docking in the form of binding affinity and the values of RMSD L.B and RMSD U.B can be seen in Table 4.

Table 4. Results of *molecular docking* of test compounds and comparison medicines at 7KBJ receptors.

No.	Ligand	Conformation	*Binding Affinity* (kcal/mol)	*dist. from best mode*	
				rmsd Lb.	rmsd u.b.
1	5-Hydroxy-7,4'-dimethoxyflavone	1	-10,4	0,000	0,000
		2	-10,4	10,636	14,995
		3	-10,2	11,390	15,004
		4	-9,8	3,079	8,957
		5	-9,7	24,919	27,861
		6	-9,7	2,090	2,586
		7	-9,6	30,128	33,328
		8	-9,5	14,592	17,983
		9	-9,5	25,651	29,517
2	Deidaclin	1	-7,4	0,000	0,000
		2	-7,4	15,370	17,935
		3	-7,2	2,252	3,763
		4	-7,1	14,951	17,254
		5	-7,1	14,424	16,332
		6	-7,1	13,641	15,209
		7	-7,0	16,541	19,232
		8	-6,8	13,491	14,812
		9	-6,8	17,541	19,529
3	Linamarin	1	-7,7	0,000	0,000
		2	-7,0	1,611	3,981
		3	-6,7	2,213	5,369
		4	-6,4	22,695	24,656
		5	-6,4	35,500	37,413
		6	-6,3	2,489	5,103
		7	-6,3	12,989	14,172
		8	-6,3	21,547	23,434
		9	-6,3	22,099	23,990
4	Volkenin	1	-7,5	0,000	0,000
		2	-7,4	13,020	15,602
		3	-7,3	13,100	16,271
		4	-7,2	14,421	16,294
		5	-7,2	15,246	16,279
		6	-7,2	22,304	23,890
		7	-7,2	2,271	4,162
		8	-7,2	2,568	4,138
		9	-7,1	2,318	5,828
5	(1S,4S)-Tetraphyllin B	1	-8,3	0,000	0,000
		2	-8,2	15,848	19,140
		3	-7,9	27,005	28,287

No.	Ligand	Conformation	*Binding Affinity* (kcal/mol)	*dist. from best mode*	
				rmsd Lb.	rmsd u.b.
5	(1S,4S)-Tetraphyllin B	4	-7,8	2,278	3,526
		5	-7,7	31,342	33,373
		6	-7,7	2,737	3,493
		7	-7,7	2,964	3,871
		8	-7,6	2,628	3,587
		9	-7,6	16,850	20,358
6	(S)-Tetraphyllin A	1	-7,3	0,000	0,000
		2	-7,3	18,149	21,253
		3	-7,3	32,194	34,743
		4	-7,1	17,458	19,573
		5	-7,0	14,359	16,897
		6	-6,9	24,641	25,644
		7	-6,9	5,306	7,328
		8	-6,8	27,527	29,248
		9	-6,8	20,116	22,186
7	Passifloricin A	1	-9,1	0,000	0,000
		2	-8,9	29,198	34,401
		3	-8,8	16,844	22,597
		4	-8,7	14,916	19,449
		5	-8,6	31,452	33,941
		6	-8,6	35,766	38,850
		7	-8,6	23,502	26,350
		8	-8,4	36,947	41,362
		9	-8,4	28,471	33,869
8	Metformin (control)	1	-5,3	0,000	0,000
		2	-4,8	36,756	37,920
		3	-4,7	1,431	2,269
		4	-4,7	19,682	20,816
		5	-4,6	39,837	40,991
		6	-4,4	38,479	39,837
		7	-4,4	38,745	39.386
		8	-4,4	20,221	21,167
		9	-4,3	21,260	22,593

The results are shown in the data in Table 4. All active compounds of *Passiflora foetida* L. have greater values of binding affinity than control compounds and have lower bound rmsd and upper bound rmsd values with valid poses on ligands of -9.7 kcal/mol.

Ligand and Amino Acid Interactions

The results of the amino acid interaction of the test compound with the alpha-glucosidase receptor (7KBJ) are shown in Table 5.

Table 5. Results of amino acid interactions of test compounds on 7KBJ receptors.

No.	Compound Name	Amino Acid Interactions
1	5-Hydroxy-7,4'-dimethoxyflavone	Conventional Hydrogen Bond: TRP C463, PHE C468, GLY C498
		Van der Waals Bond: PRO C471, GLN C470, PRO C469, THR C462, TRP A523, TYR C499, ARG C500, ASP A496, VAL A495, SER A497, PRO A526, SER A528, GLU C553
		Hydrocarbon Bond: ASP C456
		Alkyl Hydrophobic Bond: PRO C465, LEU C472
		Unfavorable donor-donor Bond: LYS A494
2	Deidaclin	Conventional Hydrogen Bond: SO C41023*, ARG A705, ARG A426, ARG C422
		Van der Waals Bond: LEU C701, ARG C426, ASN C424, GLY C704, ASN A424, TYR A425, ASP A430
		Alkyl Hydrophobic Bond: ARG C705
3	Linamarin	Conventional Hydrogen Bond: THR C703
		Van der Waals Bond: ARG C705, LEU C701, TRP C423, SO C41023*, ASN A424, ARG A422, ARG A705, TYR A425
		Alkyl Hydrophobic Bond: LEU A701, ARG C426, ARG A426
		Unfavorable donor-donor Bond: ARG C422*, ASN C424
		Unfavorable acceptor-acceptor Bond: ARG C422, ASN C424
4	Volkenin	Conventional Hydrogen Bond: GLY C498, PHE C461, TRP C463
		Van der Waals Bond: ASP A496, TYR C499, ARG C500, THR C462, PRO C465, ASP C456, ARG C459, ARG A459, LYS A494, ASP A496
		Hydrocarbon Bond: GLU C553
5	(1S,4S)-Tetraphyllin B	Conventional Hydrogen Bond: ASP C456, GLY C498, PRO C469
		Van der Waals Bond: ARG A459, ARG C459, LYS A494, THR C462, PRO C465, TRP C463, TRP A523, PRO C471, GLN C470, GLU C553, MET C546, ARG C500, TYR C499, VAL C501
6	(S)-Tetraphyllin A	Conventional Hydrogen Bond:
		Van der Waals Bond: VAL A576, ASP H305, PHE H307, ARG A624, MET A565, PHE A673, PHE A674, TRP A423, HIS A700, TRP A525
		Hydrophobic bonds of pi-alkyl: PHE A571
		Unfavorable acceptor-acceptor Bond: ASP A640

No.	Compound Name	Amino Acid Interactions
7	Passifloricin A	Conventional Hydrogen Bond: THR A466
		Van der Waals Bond: GLN A436, LYS A481, LEU C701, ARG A467, TRP C423*, HIS C700, PHE C674, TRP C525, PHE C571, VAL C576, ASP J305, SO B41803, GLN A436, LYS A481
		Alkyl Hydrophobic Bond: HIS A477, LEU A432, ALA A429
		Hydrophobic bonds of pi-alkyl: HIS A477, LEUS A432, ALA A429
		Pi-Anion Bond: PHE J307
8	Metformin (control)	Conventional Hydrogen Bond: LEU C701, ASN C424
		Van der Waals Bond: TRP C423, ARG A426, THR C703
		Attractive Charge Bond: SO C41023
		Unfavorable donor-donor Bond: ARG C705, ARG C426, ARG C422
		Unfavorable positive-positive Bond: ARG C705, ARG C426, ARG C422

(*): Same Amino Acid residue as Metformin control.

From Table 5. It can be seen that compounds that have the same bond, namely Linamarin in the ARG C422 protein and Passifloricin A in the TRP C423 protein.

DISCUSSION

The predicted results of physicochemical tests in Table 1. show that 6 active compounds in *Passiflora foetida* L. meet the lipinski's rule of five, namely having a molecular weight of <500 and the number of H bonds of acceptors (HBA) <10, all active compounds of *Passiflora foetida* L. meet the Log P value of <5, and 4 active compounds whose number of donor H bonds (HBD) <5 (Fakih &; Dewi, 2020). This means that it is predicted to be easily absorbed, has good permeability, and has good oral bioavailability. Based on the lipinski's rule of five and the results of analysis, almost all active compounds of *Passiflora foetida* L. have the potential to be medicine.

The compound that has the greatest molecular weight is 5-Hydroxy-7,4'-dimethoxyflavone. That is, the compound is quite difficult to penetrate through biological membranes. The greater the molecular weight, the more difficult it is to penetrate through biological membranes. Medicines with a molecular weight greater than 500 have a large molecular size so it is quite difficult to penetrate through biological membranes (Ruswanto, 2015). Other compounds have a molecular weight smaller than 500 so they are said to be able to be penetrated through biological membranes.

Log P value is related to lipophility or hydrophobicity, namely the ability of chemical composition to be soluble in fats, oils, lipids and nonpolar solvents (Ruswanto, 2015). Compounds that have a Log P value greater than metformin are 5-Hydroxy-7,4'-dimethoxyflavone and Passifloricin A. meaning that the test compound is easier to penetrate biological membranes so that it easily binds to receptors than metformin.

The compound 5-hydroxy-7,4'-dimethoxyflavone has HBA values of 13 and HBD 7, in addition, volkenin and (1S,4S)-Tetraphyllin B has HBD values of 5. The value of hydrogen bond acceptor and hydrogen bond donor has a relationship with the biological activity of a medicine molecule. Changes that can affect the biological activity of compounds caused by hydrogen bonds

are the chemical-physical properties of compounds, namely boiling point, melting point, solubility in water, ability to form chelate, and also similarity (Ruswanto, 2015).

See Table 2. Passifloricin A compound with a percentage of intestinal absorption sequentially, namely, 91.043%. The compound has an intestinal absorption value of more than 80% and not less than 30% which indicates the compound has good absorption. Compounds can be said to have good absorption if the intestinal absorption value is > 80% and is said to be less good if the intestinal absorption value is < 30% (Chander et al., 2017).

A compound is said to have low skin permeability if it has a log Kp value of > -2.5 (Pires et al., 2015). All compounds have a log value of Kp > -2.5. This means that all compounds have good skin permeability. Medicinal materials that have good skin permeability can be used to advance consumer products in developing new medicines by trans-dermal administration (Pires et al., 2015).

The distribution parameters are carried out by looking at the VDss value, blood-brain barrier, and fraction unbound. The higher the VDss value, the more medicine is distributed into tissues rather than plasma. The compound is said to have a low VDss value if the VDss log value is < -0.15 and high if the VDss log value is > 0.45 (Pires et al., 2015). The compounds Deidaclin, Volkenin, (1S,4S)-Tetraphyllin B and (S)-Tetraphyllin A respectively have a VDss log value of -0.104; -0,002; -0.002 and -0.104. This means that the compound has a value greater than -0.15 and less than 0.45 so it is said to be evenly distributed to provide the same concentration as in blood plasma. While other compounds are not able to provide the same concentration in blood plasma because the VDss value is less than -0.15.

The human brain is protected by exogenous compounds by the blood-brain barrier. The ability of a medicine to enter the brain is an important parameter to consider to help reduce side effects and brain toxicity. The compound is said to be able to penetrate the blood-brain barrier well if it has a log BB value of > 0.3 and is said to be unable to be well distributed if it has a log BB value of < -1 (Pires et al., 2015). The compounds 5-hydroxy-7,4'-dimethoxyflavone, and Passifloricin A have log BB values of <-1 which are -1.764 and -1.106 so it is said to be unevenly distributed. While other compounds are predicted to be able to be distributed evenly so that they can provide the same concentration in blood plasma.

Most medicines in plasma will exist in equilibrium between unbound states or bound to serum proteins. The efficacy of the medicine is influenced by the extent to which it binds to proteins in the blood (unbound value). The more that is bound, the less efficient it will be in crossing cell membranes or diffusing (Pires et al., 2015). The results said the six active compounds *Passiflora foetida* L. has a lower unbound value than metformin, because if the greater the unbound value of a compound, the more it will bind to plasma proteins, so that the six active compounds *Passiflora foetida* L. are efficient in crossing cell membranes or diffusing.

Metabolic profiles were analyzed using the presence or absence of inhibition in cytochrome P450, especially in CYP2D6 and CYP3A4 isoforms. Cytochrome P450 is an important detoxification enzyme in the body, mainly found in the liver. This cytochrome is capable of oxidizing xenobiotics to provide its excretion facility. Some medicines are inactivated by cytochrome P450 and some can be activated by P450. This enzyme inhibitor is similar to grape fruit juice which can affect medicine metabolism and is contraindicated (Pires et al., 2015). From the results of the prediction above, all compounds in *Passiflora foetida* L. do not have inhibition in cytochrome P450 isoform CYP2D6 and isoform CYP3A4.

In predicting the excretion process is carried out with constant parameters. Total Clearance (CLtot) and Renal Organic Cation Transporter 2 (OCT2). CLtot is a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance (excretion through the kidneys). CLtot is related to bioavailability and it is very important to determine the dosing rate to achieve

steady-state concentrations (Pires et al., 2015). From this CLtot value can be predicted the speed of a compound to excrete. All compounds have higher total clearance values than metformin. OCT2 is a renal uptake transporter that plays an important role in the disposition and clearance of endogenous medicines and compounds. OCT2 substrates potentially exert adverse interactions when administered together with OCT2 inhibitors (Pires et al., 2015). All compounds do not affect OCT2 substrates so it is predicted that they are not OCT2 substrates.

The results of the study in Table 3 with toxicity parameters, namely Ames Toxicity, is a widely used method to assess the mutagenic potential of a compound using bacteria. Positive results indicate that the compound is mutagenic so that it can act as a carcinogen (Pires et al., 2015). Based on the results of the prediction above, all test compounds are not mutagen in silico. Hepatotoxicity test is a test to determine the presence or absence of potential medicines that can induce damage to the liver (Pires et al., 2015). From the results of the prediction above, it can be seen that six active compounds in *Passiflora foetida* L. and metformin comparison medicines are not toxic to the liver, except Passifloricin A is toxic to the liver.

Compounds in class 4 (Deidaclin, Volkenin, (1S,4S)-Tetraphyllin B, (S)-Tetraphyllin A, and Passifloricin A), class 5 (5-Hydroxy-7,4'-dimethoxyflavone) and class 6 (Linamarin) are relatively safer, not mutagen and not toxic to liver, except for Passifloricin A compounds which are toxic to liver. This means that the compound has a safer level of toxicity compared to the comparison medicine, metformin.

Binding affinity is a measure of a medicine's ability to bind to a specified receptor. The lower the value, the higher the affinity between receptors and ligands, and vice versa, the higher the value, the lower the affinity between receptors (Saputri et al., 2016). The ligands of the test compounds that have better binding affinity values than metformin are 5-Hydroxy-7,4'-dimethoxyflavone, Deidaclin, Linamarin, Volkenin, (1S,4S)-Tetraphyllin B, (S)-Tetraphyllin A and Passifloricin A with sequential binding affinity values of -10.4 kcal/mol, -7.4 kcal/mol, -7.7 kcal/mol, -7.5 kcal/mol, -8.3 kcal/mol, 7.3kcal/mol and 9.1 kcal/mol. Meanwhile, the binding affinity value of metformin is -5.3 kcal/mol. The best ligands are those ligands that have the most negative binding affinity value (Yahmin et al., 2019).

There are two RMSD values, namely, lower bound RMSD (RMSD lb.), and upper bound RMSD (RMSD ub.). The RMSD value for the conformational alignment of the structure that is still acceptable is < 3 but it is optimal if it is < 2, if the closer to 0, the better the alignment value (Listyani &; Herowati, 2018). Based on this theory, it was found that not all ligands with interaction poses between these receptors are said to be valid. Ligands that interact well in valid poses are found in ligands of the test compounds 5-Hydroxy-7,4'-dimethoxyflavone, Deidaclin, Linamarin, and (1S,4S)-Tetraphyllin B by -9.7 kcal/mol, -7.2 kcal/mol, -7.0 kcal/mol, and -7.8 kcal/mol, respectively. While the valid pose on the ligand of the comparison medicine metformin was obtained at -4.7 kcal / mol. From these results, it can be predicted that the test compound 5-Hydroxy-7,4'-dimethoxyflavone has a better ability than metformin to inhibit the enzyme Alpha-glucosidase to the 7KBJ receptor.

The interaction of amino acid residues seen in Table 5 is the same as the comparison compound or native ligand and can be said to have the same biological activity ability as the comparison compound or native ligand (Prasetiawati et al., 2021). Test compounds that have the same bond with comparison medicines, namely metformin on ARG C422 protein is Linamarin, and TRP C423 protein on Passifloricin A so it is predicted that both compounds have the same mechanism of action as metformin. The two compounds together with metformin form interactions with unfavorable donor-donor bonds and van der Waals bonds. Active compounds can be said to have strong bonds with target receptors if they have strong bonds through hydrogen

bonds and can bond on the active side with one of the same amino acid residues (Wibisono &; Martino, 2023).

CONCLUSION

Based on research, almost all active compounds *Passiflora foetida* L. meet Lipinski's rule of five it is said to be a candidate medicine. The compound that has the best pharmacokinetic profile compared to metformin is Passifloricin A. 5-Hydroxy-7,4'-dimethoxyflavone, Deidaclin, Linamarin, Volkenin, (1S,4S)-Tetraphyllin B (S)-Tetraphyllin A and Passifloricin A are relatively safer, not mutagen and not toxic to hepar, unless Passifloricin A is toxic to liver, because the compound has a safer level of toxicity compared to comparison medicines, namely metformin. Test compounds that have better binding affinity values than Metformin are 5-Hydroxy-7,4'-dimethoxyflavone, Deidaclin, Linamarin, Volkenin, (1S,4S)-Tetraphyllin B (S)-Tetraphyllin A and Passifloricin A with consecutive binding affinity values of -10.4 kcal/mol, -7.4 kcal/mol, -7.7 kcal/mol, -7.5 kcal/mol, -8.3 kcal/mol, -7.3 kcal/mol and -9.1 kcal/mol. The most valid test compound compared to metformin in certain poses is 5-Hydroxy-7,4'-dimethoxyflavone with binding affinity -9.7 kcal/mol, RMSD value l.b 2.090 and RMSD u.b 2.586. In contrast to metformin which has a binding affinity of -4.7 with RMSD l.b 1.431 and RMSD u.b 2.269. Further research is needed on the potential of the active compound *Passiflora foetida* L. as an antidiabetic candidate in inhibiting blood sugar levels in vitro or in vivo.

ACKNOWLEDGMENT

The author expresses gratitude to Allah SWT, who has provided convenience in completing this research. The author would like to thank both parents, family, and friends who have provided support in the form of prayers and motivation. The author also thanks advisors, namely Dr. Diana Hernawati, M.Pd., and Rinaldi Rizal Putra, S.Pd., M.Sc., who also provided direction and guidance during the implementation of this research.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 36 – 44 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

The use of alternative coloring variations to mitotic observations of shallots using the squash method

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Article Info

Article History:
Received 10 December 2023
Revised 13 March 2024
Accepted 07 April 2024
Published 30 April 2024

Keywords:
Mitotic cleavage
Onions
Alternative coloring

ABSTRACT

Practicum cannot be carried out due to limited facilities and infrastructure such as dyes for observation of mitotic cell chromosomes in onions. Alternative dyes that can be obtained from nature can be explored. The purpose of this study was to obtain preparation of the mitotic division process in various onion root cells using alternative dyes using dyes from dragon fruit peel and turi flower. In obtaining the right preparation using the squash method. Data were obtained using observation sheets that observed each variation of the material. The instrument used in this study is a valid observation sheet. The results showed that the coloring of the preparation using dragon fruit skin and turi flowers was successful and clear coloring results were obtained. Coloring in practicum activities using turi flowers is better than dragon fruit coloring. Natural dyes from dragon fruit peel and turi flower can be used as alternative dyes in the squash method to observe onion root mitosis, replacing synthetic dyes.

Citation: Tirtasari, N.L., Saputri, L.H., Dewi, N.K., & Siswanti. (2024). The use of alternative coloring variations to mitotic observations of shallots using the squash method. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 36-44. DOI: https://doi.org/10.31932/jpbio.v9i1.3053

INTRODUCTION

Observation preparations for practicum are often needed to support Biology learning. Quality preparations require good staining to sharpen and clarify the picture of tissues and cells so that they are easily observed under a microscope. In order, for learning and practicum activities to be realized and run well, diligence, skills, and supporting infrastructure in the laboratory and equipment are needed. One of them is making observations in the form of preparations or specimens in the form of fresh preparations or specimens of both animals and plants and preserved specimens of both plant and animal anatomy However, many Biology lessons cannot be carried out due to limited facilities and infrastructure, especially in coloring preparations in practicum material for observing mitotic cell chromosomes in onions (Latifa, 2015).

The material in the observation of mitotic cell chromosomes in onions is a concrete activity that can be understood by observing cell division directly. Practicum activities with direct

observation can train skills and improve understanding. Mitosis is a cell division in which daughter cells have the same properties as the parent cell. The phases in mitosis consist of prophase, metaphase, anaphase, and telophase (McIntosh, 2016). The main material for making mitotic preparations is cells that carry out mitotic division. Cells that undergo division are found in the parts of plants that are actively undergoing growth (meristematis) most easily found at the tip of the root (Perilli et al., 2012). A commonly used method of making mitotic preparations is by squash. The squash method is a method of obtaining a preparation by tissue squeezing a piece or an organism as a whole so that a thin preparation can be observed on a microscope (Yang, 2009).

Previous research related to onion mitotic index studies for making learning media for mitotic preparations concluded that IM A. sativum, A. cepa and A. fistulosum were different even though in one genus IM A. sativum the highest occurred at 09.00 WIB with a value of 11.410%; The highest IM A. cepa occurred at 12.00 WIB with a value of 11.326%; while the highest IM A. fistulosum occurred at 06.00 WIB with a value of 12.617%. Squash mitotic preparation media about the mitotic index of Allium root tip meristem with hematoxylin dye, suitable for use as a learning medium for observing cell mitotic division (Abidin et al., 2014).

The problem that is often encountered in mitotic observations in onions is the difficulty of adding coloring to clarify the appearance or appearance of onion cells and also get the right time during the mitotic division process in onion cells. Widely used dyes are synthetic dyes with certain dyes that can be attached to tissues. Usually, the dye is safranin which gives the preparation its red color. Safrantiin dye is preferred because it is practical and its coloring properties are stable and diverse (Aryadini et al., 2003). Virgianti & Luciana, 2017 state that in addition to being used as food and textile dyes, natural dyes from natural materials can also be used as dyes in the process of staining microorganisms.

However, artificial dyes have drawbacks that when used long-term have carcinogenic properties. Waste from artificial dyes will also have an impact on the environment, therefore environmentally friendly alternative dyes are needed. The limitations of the use of dyes can be anticipated with natural alternative dyes from plant extracts. Natural dye pigments are safer to use even though the level of stability to heat, light, and acidity is erratic. Natural dyes that have not been widely explored for use as dyes for onion root mitotic preparations are extracts of dragon fruit peel and turi flower. Therefore, this study aims to explore the extraction of dragon fruit peel and turi flower, as well as its use as a dye in the observation of mitotic preparations of onions, garlic, onions, and leeks. The novelty of this study is the use of dyes from dragon fruit peel extract and turi flower flowers by considering the soaking process.

RESEARCH METHODS

Research Design

Observation of the results of the study was carried out descriptively, namely by observing the results of coloring using dragon fruit skin and turi flowers as coloring materials using observation sheets. The coloring results are then compared with the control. Parameters observed include clarity and color contrast.

Population and Samples

The population and samples used in this study were dragon fruit skin waste, turi flowers, and onion variations. Tools and materials used FAA, onion bulbs, garlic bulbs, onion bulbs, onion bulbs, dragon fruit, turi flowers, glacial vinegar acid and hydrochloric acid, analytical balances, microscopes with digital cameras connected to computers/laptops, Erlenmeyer flasks, beakers, measuring flasks, measuring cups, drip pipettes, glass objects and glass decks, Petri dishes, falcon bottle, funnel, electric bath, pH meter or pH indicator, stirring rod, filter paper, mortar and pestle, vacuum pump, blender.

Instruments

Data collection was carried out by making observations using observation sheets from onion preparation. The observations were photographed and then validated by experts based on their expertise.

Procedures

The method used in this study began with the extraction of dragon fruit skin and turi flowers using maceration methods. Furthermore, the extraction of natural dyes is used to color cell mitotic observation preparations in onion roots. Some of the stages carried out in this study include

1. Preliminary stage

At this stage, the tools and materials used in the study are prepared which include root growth on onion bulbs, garlic, onions, and leeks in beakers for a period of ± one week.

2. Extraction stage of dragon fruit peel and turi flower

Next, dragon fruit skin and turi flowers are extracted by cutting the ingredients and then crushing them until smooth with a blender or pestle die. Then, in the mortar, a little water is added and then the extract is filtered with filter paper. Then 10% (w/v) of citric acid is added to the filtrate obtained.

3. Trial phase as a natural dye for preparations

Sprouted onion roots are cut at the ends by 3-5 cm. Then soak into the FAA solution for 15-20 minutes. The soaked onion roots are then rinsed using aquades three times. Onion roots are soaked in natural dyes that have been prepared, namely extracts of dragon fruit peel and turi flower. Each marinade is heated in a water bath to a boil and the dye is firmly absorbed into the onion roots.

4. Observation stage

At the observation stage, microscopes and equipment are prepared along with onion root preparations on glass objects and covered with glass decks. The onion root cells are then observed using a microscope.

Data Analysis

Data analysis in this study is descriptive qualitative to see the quality of preparate as staining mitotic preparations. Data is collected by observing objects under a microscope and then photographed. The indicator used to determine the quality of the preparation consists of color contrast and preparate clarity. The criteria for preparation clarity and color contrast are in Table 1.

Table 1. Criteria of clarity and contrast of preparat

Aspects	Criterion	Indicator	Score
Clarity	Very clear	Fulfilled when parts of the tissue can be distinguished very clearly When the division stage includes prophase, metaphase, anaphase, telophase.	3
	Clear	Fulfilled if the tissue parts can be clearly distinguished when the division stage includes prophase, metaphase.	2
	Not clear	Fulfilled when parts of the tissue can be distinguished very clearly When the division stage includes prophase.	1
Contrast	Highly contrast	Fulfilled if the dye is only firmly bound to certain parts (does not color all tissues).	3
	Contrast	Fulfilled if the dye is only bound to a certain part (does not color all tissues).	2
	Lowly contrast	If the dye is bound to all tissues (dyeing the tissue).	1

The preparation clarity and contrast criteria that have been assessed by experts are then calculated using the percentage technique. The assessment criteria can be seen in Table 2.

Tabel 2. Kriteria penilaian preparat

Percentage (%)	Criterion
$33{,}3 \leq Percentage \leq 46{,}64$	Bad
$46{,}64 < Percentage \leq\leq 59{,}98$	Not Good
$59{,}98 < Percentage \leq \leq 73{,}32$	Good enough
$73{,}32 < Percentage \leq \leq 86{,}66$	Good
$86{,}66 < Percentage \leq \leq 100$	Excellent

RESULTS

The pH and absorbance value of red dragon fruit peel extract and red turi flower crown can be seen in Table 3.

Table 3. pH and absorbance value of red dragon fruit peel extract and red turi flower crown

Types of extracts	pH		Wavelength	Absorbance value
	first	last		
Red dragon fruit skin	5.0	2.0	399	0.570
			425	0.584
			452	0.654
			539	1.997
Red turi flower crown	5.0	2.0	381	2.586
			405	1.097
			533	1.388

The quality results of the preparation using coloring preparations of red dragon fruit peel extract and red turi flower crown can be seen in Table 4.

Table 4. The quality results of the preparation using coloring preparations of red dragon fruit peel extract and red turi flower crown.

Dye	Criterion
Dragon fruit skin	Good
Red turi flower crown extract	Excellent

DISCUSSION

The extraction of natural dyes derived from the skin of red dragon fruit and red turi flowers has been successfully carried out by optimizing the soaking process. The skin of the red dragon fruit (Hylocereus polyrhizus) and the crown of the red turi flower (Sesbania grandiflora L.) have anthocyanin color pigments that are classified as flavonoid compounds and have strong color characters and are soluble in water. Anthocyanins in the skin of red dragon fruit and red turi flowers have the potential to be natural dyes for food and in this study can be used as an alternative to synthetic dyes that are safer for health (Anam, 2016).

The extraction of red dragon fruit peel and red turi flower produces a strong red dye derived from anthocyanin color pigments that can be applied to the coloring of onion root squash preparations. Extraction can be interpreted as a way of pulling out or separating a material from its mixture, generally by using solvents. The nature of anthocyanin pigments is greatly influenced by

pH, where anthocyanin extract if added base then the pigment will turn green, while if reacted with acidic compounds, the extract will turn red. In the extraction process, citric acid is added which is acidic in nature so that a strong dark red pigment is obtained. The solubility of pigments in water solvents and citric acid has a compatibility between solutes and solvents (like dissolve-like). This solvent affects the pH, absorbance, color intensity, content, and yield of extracted pigments. The color intensity values of dragon fruit peel extract and red turi flower are shown in Table 5.

Table 5. The intensity of color and degree of redness of red dragon fruit peel extract and red turi flower crown

Types of extracts	Color intensity	Degree of redness
Red dragon fruit skin	+ +	Red is strong enough
Red turi flower crown	+ + +	Strong red

Based on qualitative observational analysis, visually it is known that there is a difference in the intensity of the red color obtained from the skin of the red dragon fruit and the crown of the red turi flower. For red dragon fruit peel extract, the redness level is quite strong while the color intensity is ++, while the red turi flower crown extract has a strong redness level and +++ color intensity. These results show that the type of plant organ chosen has different pigment levels and affects the extraction results. The results of research on the extraction of red turi flowers using aqueous solvents: citric acid: ethanol produced extracts containing anthocyanin pigments and the solvent affects pH, absorbance, color intensity, and yield (Saati, 2008). The pH and absorbance measurement results of red dragon fruit peel extract and red turi flower are shown in Table 6.

Table 6. pH and absorbance value of red dragon fruit peel extract and red turi flower crown

Types of extracts	pH		Panjang gelombang	Absorbance value
	first	end		
Red dragon fruit skin	5.0	2.0	399	0.570
			425	0.584
			452	0.654
			539	1.997
Red turi flower crown	5.0	2.0	381	2.586
			405	1.097
			533	1.388

The results of the extraction of red dragon fruit peel and red turi flower crown show a deep red color, a characteristic of anthocyanin pigments (Figure 1). The addition of citric acid, the color of anthocyanin pigments that react with acidic compounds shows a strong red color. Anthocyanin properties are strongly influenced by pH and the results of pH measurements on dragon fruit peel extract and red turi flower crown extract after the addition of citric acid show pH 2.0.

Figure (a) represents the appearance of dragon fruit skin before extraction. Dragon fruit peel is used as a base material for dye preparations. Figure (b) shows the appearance of turi flowers before the extraction process. The extracted dragon fruit peel seen in Figure (c) has an initial pH of 5.9 then citric acid pH is added to 2.0. Turi flowers that have gone through the extraction process seen in Figure (d) have an initial pH of 5.0 and then given citric acid pH of 2.0.

The highest absorbance value of red dragon fruit peel extract was found at a wavelength of 1,997 nm, while for the crown of red turi flowers, the highest absorbance value was obtained at a wavelength of 1,388 nm. From these data, the intensity and pH value of pigments affect pigment absorbance. The higher the pH value, the smaller the absorbance. Anthocyanin pigments are more

stable under acidic conditions and their absorbance value depends on the concentration in the extract. In this case, the concentration of anthocyanin pigments in red turi flower crown extract is higher than that of red dragon fruit peel extract. Anthocyanins are a group of pigments that are red to blue in color that are present in plants including in fruits, flowers, roots, and leaves. From the absorbance data, it is proven that anthocyanins from the skin of the red dragon fruit and the crown of the red turi flower contain pelargonidin and cyanidin which are shown the maximum absorbance wavelength of the spectrum at 500-550 nm. The type of anthocyanin read at the 525 nm wavelength is pelargonidin, while the one read at the 535 nm wavelength is cyanidin (Simanjuntak & Sinaga, 2014).

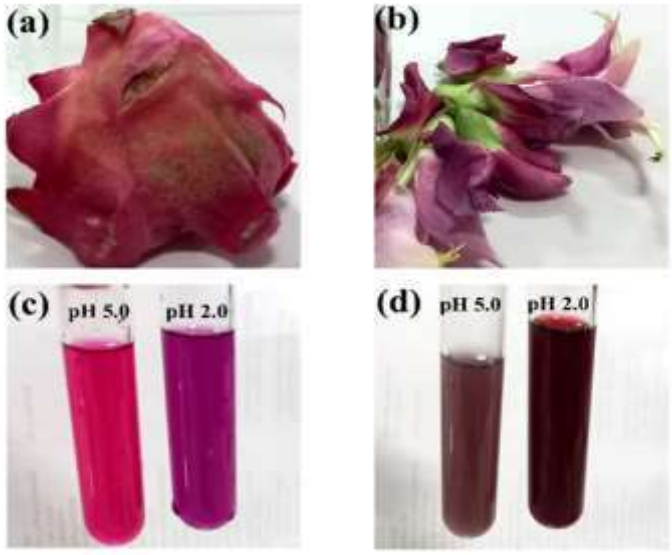

Figure 1. Presentation of red dragon fruit skin (a) and natural dyes from the extraction of red dragon fruit peel (c), red turi flower crown (b), and natural dyes from the extraction of red turi flower crown (d).

The results of red dragon fruit peel extract and red turi flower crown were then applied to the preparation process of onion cell mitotic preparations by the squash method. The results of preparation of preparations and observations of preparations by staining red dragon fruit peel extract are presented in Figure 2a-d and red turi flower crown extract in Figure 2e-h.

This research proves that the use of natural dyes that have the same function as safranin dyes can be done with a simple extraction method. In some plants, natural dyes have several color pigments such as chlorophyll, carotenoids, tannins, and anthocyanins. Natural coloring substances obtained from plants have a great affinity for cellulose and plant fibers. In addition, natural dyes can be absorbed by cell nuclei that contain chromosomes, where chromosomes themselves can absorb color. The behavior of chromosomes at the time of cell division was successfully described as related to the process of mitotic division. Chromosomes are stainable bodies based on the concept of anatomical morphology and chromatin thread arrangement. Chromosomes in the nucleus can absorb dyes well so that they look contrasting with other parts of the cell (Aziz, 2019; Sharma, 2020).

Natural dyes used instead of synthetic dyes in preparations have received evidence from several studies that have been conducted. Roselle petal extract can clarify the picture of the epidermis, parenchyma, cambium, and xylem tissue in large red chili plant section preparations (Bisri, 2014). Research on the use of natural dyes using red dragon fruit skin and telang flower extract has been carried out on chicken femoral bone rubbing preparations (Wahyuni, 2015; Susliana & Wahyuni, 2019).

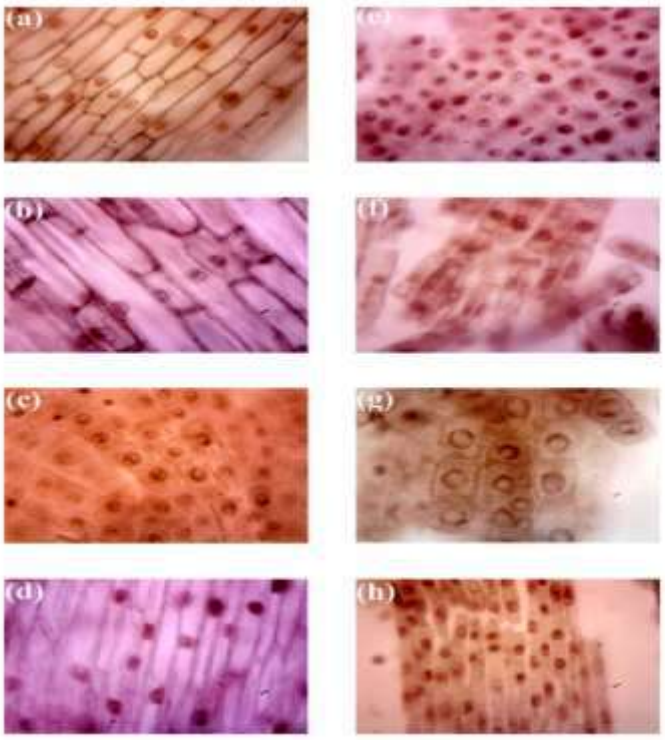

Figure 2. Results of observations of cell mitotic preparations (magnification 400×) by staining red dragon fruit peel extract on onion root preparations (a), garlic (b), onion (c), and onion (d); and coloring of red turi flowers on shallots (e), garlic (f), onions (g), and shallots (h) at the time of cleavage at 09.00 WIB for onions, garlic, and onions, and at 06.00 WIB for shallots.

Making microscopic preparations is an art that requires accuracy, accuracy, high ability, and is supported by the individual interests of the perpetrators. One of the methods of making microscopic preparations is the mitotic division of onion cells. Usually, the sample used is onion. In this study, onions, garlic, onions, and leeks were used as samples for making preparations by the squash method. (I. N. Wahyuni & Sabban (2022) in his research stated that in the skin of dragon fruit, there are two almi dyes, namely anthocyanin, and betacyanin. Betasianin merupakan zat warna yang berperan memberikan warna merah dan merupakan golongan betalain yang berpotensi menjadi pewarna alami (Said et al., 2021) Anthocyanin dyes are more stable at low Ph, therefore the solvent used is citric acid (Muslim & Rosidah, 2023). Citric acid solution used as a solvent functions as a control negative and dye ((Hidayah et al., 2014). In addition, according to other studies, anthocyanin chemicals in dragon fruit peel extract can effectively color soursop fruit preparations (Permatasari & Suriani, 2022).

The importance of research on the use of natural dyes, in this case, uses anthocyanin pigments from the skin of red dragon fruit (Hylocereus polyrhizus) and red turi flower crown (Sesbania grandiflora L.) to answer the development of science, especially in observing cell division and utilizing dragon fruit skin waste and red turi flower crown. The product of coloring preparations using red dragon fruit skin and red turi flowers can be used as a simple Biology learning medium. Biology learning activities are a process that requires student activities, such as learning process activities in practicum at school that use microscopic preparations of plant cells and tissues can be assisted by natural coloring that is easy to prepare and make by teachers and students. This is to reduce costs and limitations that exist in schools because the price of preparations that are quite expensive is also hampered by the high cost of synthetic dyes.

CONCLUSION

Natural dyes from the skin of the red dragon fruit and the crown of the red turi flower were successfully extracted by maceration techniques. Furthermore, the dye can be absorbed by chromosomes and facilitates the coloring of the cell nucleus at the time of mitotic cell division in root preparations of onions, garlic, onions, and leeks. This natural dye can be used as an alternative to synthetic dyes, making it easier to learn Biology practicum in high schools that have limited access to synthetic dyes.

ACKNOWLEDGMENT

We would like to thank Semarang State University for providing costs in conducting research activities through DPA funding in 2023. We would also like to thank the research members who completed this research to get the right dye preparation formula.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 45 – 57 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Exploration of mosses in urban-bogor habitats: Species identification, diversity and uniformity

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Article Info

Article History:
Received 15 January 2024
Revised 13 March 2024
Accepted 29 March 2024
Published 30 April 2024

Keywords:
Bryophyta
Diversity
Pajajaran Street
Urban

ABSTRACT

This research focuses on the exploration of moss in the Bogor urban habitat, to explore the identification of moss types, measuring the level of diversity, and assessing the level of uniformity in the context of the urban environment. Data collection at the research location was divided into 3 stations with different environmental parameters. The method used in this research was an exploratory survey using the belt transect technique. The sampling method is purposive sampling with the help of a quadratic plot. The results of this research found 20 moss species identified on the green belt of Jalan Pajajaran, Bogor with a diversity index for the three stations respectively, namely 2.45; 2.02; and 1.78 which is classified into the medium category, and the uniformity index for the three stations respectively is 0.835; 0.814; 0.811 which is classified as high. These results show that the moss found can live by adapting to environmental factors in urban areas such as fluctuating temperature, humidity, and light intensity.

Citation: Ristanto, R.H., Rini, D.S., Marthasari, A.D., Safitri, F.A., Sulistiyanto, T.Q., & Putri, A.C. (2024). Exploration of mosses in urban-bogor habitats: Species identification, diversity and uniformity. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 45-57. DOI: https://doi.org/10.31932/jpbio.v9i1.3182

INTRODUCTION

Bogor City is one of the most active cities in Indonesia. Based on the results of the 2021 Global Traffic Scorecard study, Bogor City is ranked 5th nationally and 821st in the world in terms of congestion level. One of the main roads in Bogor City which is most famous for its density of activity is Jalan Pajajaran. Jalan Pajajaran is known as a "primary arterial" road in Bogor City which has buildings with high activity (Wulanraini & Jaya, 2008; Jaya, 2021). The activities of a city will greatly impact the environmental quality of that city which can cause environmental problems, such as air pollution. One way that can be done to reduce the negative impact of high city activity is by opening urban Green Open Space (GOS) (Ali et al ., 2021; Isrowati et al., 2023).

Green Open Space (GOS) is an elongated area or path that can be used more openly as a place for natural or deliberately planted plants to grow. Urban GOS is an area of open space in an

urban area that is usually covered with plants and vegetation so that it can become an urban habitat that has ecological, socio-cultural, and economic functions. GOS as an urban habitat has a function in the ecological field, namely, it can reduce air pollution, improve air quality, improve groundwater quality, prevent flooding, and reduce city temperatures (Hendriani, 2016; Isrowati et al., 2023).

Forms of urban open space that have ecological functions include green belts, urban green belts, urban forests, botanical gardens, river borders, etc. (Hendriani, 2016; Ali et al., 2021). The form of green open space applied on Jalan Pajajaran is a green belt on the side of the road which functions as one of the public facilities of Bogor City with seating, trees, ornamental plants, and rocks. Plants that can live in this habitat include yard plants, trees, ferns, and moss (Bryophyta) (Viasari & Osly, 2017; Putrika et al., 2017).

One of the plants found on the Jalan Pajajaran green belt is the bryophyte group. Mosses can be classified into a group of lower plants with a transitional form from plants that have thallus to cormus plants (Maharani et al., 2017; Endang et al., 2020; Eman et al ., 2022). Moss can be identified by looking at several morphological characteristics of its body. As representatives of lower plants, mosses have a very important role in the ecosystem as markers of biodiversity and indicators of environmental health. Through morphological analysis, the adaptation and response of moss to environmental changes, such as air pollution and climate change, can be identified. In addition, a deeper understanding of the morphological characteristics of mosses also supports biodiversity mapping, making it an important tool in natural resource conservation efforts. Moss has the morphological characteristic of having a thallus, where this moss is a plant that cannot be differentiated between roots and stems, and does not have true leaves (Rahman & Pujiastuti, 2018; Endang et al., 2020).

Moss plants can be found in places that tend to be humid, so most of them are easy to find in tropical rainforests. Moss grows on a medium which is usually called a substrate. There are various kinds of substrates where moss grows, which are tree areas, river banks, new surfaces, and even the surface layer of soil. Apart from being a medium for attaching moss, the substrate functions as a medium for absorbing nutrients. The abundant substrate in an area can be one of the factors that can determine the diversity and uniformity of moss types. Not only that, the diversity and uniformity of moss types in an area can be determined by microclimatic conditions such as light intensity, air humidity, environmental temperature, and available vegetation (Ristanto et al., 2021; Eman et al ., 2022)

The nature of being very dependent on nature makes moss a plant that is sensitive to environmental changes, one of which is an indicator of air pollution. When air quality in the environment decreases, some moss species disappear due to increasing concentrations of pollutants in the air. The abilities possessed by moss can indicate that the diversity of moss in an area can be a bioindicator of air quality in that area. Environmental factors are related to the diversity of moss in tropical urban areas such as the Jakarta area which is characterized by adaptation and diversity of moss in the polluted category compared to highland areas (Putrika et al., 2023). Moss can indicate air pollution, especially pollutant gases caused by motorized vehicles. The pollutants produced will fly freely in the air which can inhibit the growth of moss (Wijayanti, 2013; Isrowati et al., 2023).

Analysis of moss diversity in urban areas is needed to determine the ability of moss to live in urban areas. There is still minimal discussion regarding moss in urban areas, especially in the city of Bogor. Similar research was conducted by Isrowati et al., (2023) regarding moss in green open spaces carried out at the Udayana GOS with the results of the discovery of 5 species of moss. Another research was conducted by Fastansidan & Wulansari (2021) regarding moss in the urban area of Bogor at the Cibinong Ecopark with the results finding 30 identified species. This shows that the level of moss diversity is caused by the influence of environmental factors in the area.

Based on the background that has been described, studies on mosses in urban habitats need to be carried out. This research aims to explore moss in urban habitats in Bogor with a focus on identifying the types of moss that exist, measuring the level of moss diversity, and evaluating the level of uniformity of moss distribution in the Bogor urban area. It is hoped that this will contribute to increasing understanding of moss diversity and the impact of high urban activity on the environment and plants. The research location for the Pajajaran Street green belt in Bogor was chosen because of its status as one of the GOS in Bogor City which is expected to be able to represent well the condition of the urban habitat in the city.

RESEARCH METHODS

Research Design

This research was conducted on November 11 2023 along the green belt of Jalan Pajajaran Bogor as shown in Figure 1.

Figure 1. Research Location in Green Open Space Jalan Pajajaran, Bogor

The location for the green lane on Jalan Pajajaran Bogor was chosen using purposive sampling because Jalan Pajajaran is the busiest highway in Bogor City because it is the city center and center of trade and services (Jaya, 2021). Meanwhile, the green belt is a damp area on Jalan Pajajaran which supports the growth of moss in urban habitats. The length of the observation route is around 503 meters with a path width of around 5 meters. The research method applied in this research is an exploratory survey method, which involves collecting and exploring information on populations and samples that have been determined through exploration of the research environment. Data collection was carried out by making direct observations of the moss along the green belt, with a focus on identifying the morphology and distribution of the moss substrate. To support the analysis, environmental parameters such as air humidity, light intensity, and temperature were measured.

Population and Samples

Sampling was carried out using a purposive sampling technique, which involves selecting samples with certain considerations, such as the number of mosses on each plot and factors that have the potential to influence diversity, such as humidity, temperature, and light intensity. The sampling technique in this research was carried out using a belt transect at several predetermined observation points along the green route of Jalan Pajajaran, Bogor. Moss sampling was carried out randomly to cover variations that might occur at the research location. Apart from that, data collection also used quadratic plots measuring 1 m x 1 m which were distributed at three research stations. Determining the division of research stations is based on differences in environmental parameter conditions in the form of humidity and light intensity which causes differences in characteristics at each station.

Instruments

Tools and materials used in the research include: Global Positioning System (GPS), hygrometer, luxmeter, camera, stationery, rope, plastic sampling, brown envelope, cutter, 70% alcohol, and moss identification book.

Procedures

The research began with selecting a location along the green belt of Jalan Pajajaran, Bogor, where observation points were determined based on environmental variations. Three station points were determined with each track length of around 167 m. Square plots measuring 1 m x 1 m were created which were spread out at three stations along the observation route with details of 1 plot for each meter so that there were 167 plots at each station as seen in the illustration in Figure 2.

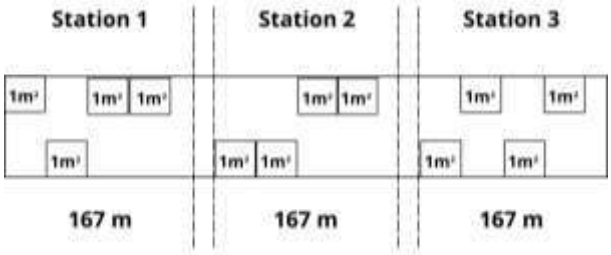

Figure 2. Illustration of technic sampling

Determination of plot placement is carried out with the following considerations: the presence of the type of moss and the amount of moss at the plot placement location. After the plot placement is complete, morphological identification is carried out and the type of moss found is determined. The types of moss present in the plots were documented and environmental parameters, such as air humidity, light intensity, and temperature, were measured during observations. Some of the mosses found were sampled by taking samples from the substrate and then used as a dry herbarium for further identification.

Data Analysis

In this study, the data obtained was analyzed descriptively qualitatively by looking at the morphological characteristics of each type of moss found during the research. Apart from that, the data was also analyzed descriptively quantitatively using the Shannon Wiener Diversity Index formula and the Uniformity Index formula. Shannon Wiener Species Diversity Index, calculated using the formula:

$$H' = -\Sigma pi\ In\ pi \text{ where, } pi = ni/N$$

Diversity index formula with the information that H' represents the diversity index; pi as the ratio between the number of individuals and the total number of individuals; ln for natural logarithm; ni states the number of individuals of each type; and N for total individuals. Meanwhile, the Species Uniformity Index is calculated using the formula:

$$E = H'/\ln S$$

The description of the species uniformity formula consists of E representing the species uniformity index, H' as the species diversity index; S for several types; and ln for natural logarithms. In Table 1, the criteria or categories for calculating species diversity and species uniformity are listed. Data analysis techniques include all the analysis needed to address the problem or research aims.

Table 1. Categorization of Species Diversity and Uniformity Index

	Index Value	Category
Diversity	H' < 1	low diversity
	1< H' <3	moderate diversity
	H' > 3	high diversity
Uniformity	0 < E ≤ 0.4	little uniformity, depressed community
	0.4 < E ≤ 0.6	moderate uniformity, unstable community
	0.6 < E ≤ 1	high uniformity, stable community

RESULTS

The exploration and observation of bryophytes on Jalan Pajajaran found a total of 20 identified species that spread across three observation stations. The division of observation stations is based on the results of different environmental parameters. Table 2 has found 19 identified moss species that are distributed in different substrates. Environmental factors at the station I have a temperature of 32°C with a humidity level of 61% and a measured light intensity of 1013 lx.

Table 2. Species of Bryophytes in Station I

No.	Species	Number found	Substrate
1.	*Fissidens perpusillus*	20	soil and stone
2.	*Fissidens taxifolius*	2	Stone
3.	*Fissidens zollingeri*	1	Stone
4.	*Fissidens obtusifolius*	3	Stone
5.	*Hypnum cupressiforme*	18	Wood
6.	*Mnium hornum*	17	Soil
7.	*Mnium stellare*	8	Soil
8.	*Marchantia emarginata*	3	Stone
9.	*Marchantia polymorpha*	3	soil and stone
10.	*Fontinalis antipyretica*	9	Stone
11.	*Serpoleskea confervoides*	11	soil and stone
12.	*Octoblepharum albidum*	2	Wood
13.	*Leucobryum sanctum*	2	Wood
14.	*Lejeunea sp.*	9	Stone
15.	*Neckera complanate*	2	Wood
16.	*Funaria hygrometrica*	1	Stone
17.	*Sematophyllum tristiculum*	2	Stone
18.	*Myrothecium microcarpum*	1	Soil
19.	*Pogonatum cirratum*	30	Stone
	Total	**144**	

Based on table 3 has found 12 moss species identified from station II. *Fissidens taxifolius* is the moss species that dominates station II. The temperature in station II was recorded at 32°C with a humidity level of 58%. The light intensity of station II is 3537 lx. These parameters influence the discovery of moss type.

Table 3. Species of Bryophytes in Station II

No.	Species	Number found	Substrate
1.	*Sematophyllum tristiculum*	4	Stone
2.	*Fissidens zollingeri*	3	Soil
3.	*Fissidens taxifolius*	38	Soil
4.	*Fissidens obtusifolius*	18	Soil
5.	*Fissidens perpusillus*	10	soil and stone
6.	*Octoblepharum albidum*	4	Wood
7.	*Serpoleskea confervoides*	12	Stone
8.	*Mnium hornum*	2	Soil
9.	*Marchantia emarginata*	4	Stone
10.	*Marchantia polymorpha*	6	soil and stone
11.	*Funaria hygrometrica*	2	Stone
12.	*Neckera complanata*	3	Wood
	Total	**106**	

Station III presented in table 4 data found 9 identified species. The environmental factors in station III have temperature 32℃ with humidity of 53%. The light intensity at station III is higher than the other station about 4975 lx.

Table 4. Species of Bryophytes in Station III

No.	Species	Number found	Substrate
1.	*Serpoleskea confervoides*	15	Stone
2.	*Pogonatum cirratum*	2	Stone
3.	*Fissidens perpusillus*	12	Soil
4.	*Mnium hornum*	10	Soil
5.	*Neckera complanata*	2	Wood
6.	*Marchantia emarginata*	4	Stone
7.	*Fontinalis antipyretica*	4	Stone
8.	*Myrothecium microcarpum*	2	Soil
9.	*Amblystegium serpens*	1	Wood
	Total	52	

In Table 5, the results of calculating the diversity index (H') and uniformity (E) of bryophytes at the three stations on Jalan Pajajaran are presented. Calculation of diversity index values uses the Shannon-Wiener index formula.

Table 5. Diversity Index Value (H') and Uniformity Index (E) of Bryophytes in Urban-Bogor

Station	Number of Species	Diversity Index (H')	Uniformity Index (E)
I	144	2,4592	0,8352
II	106	2,0233	0,8142
III	52	1,7830	0,8115

The moss diversity and uniformity index in Station I was higher than in Station II and Station III. The diversity of moss in the Green Open Space of Jalan Pajajaran has medium diversity criteria. The results of the moss uniformity index showed high uniformity criteria with a stable community distribution at each observation station.

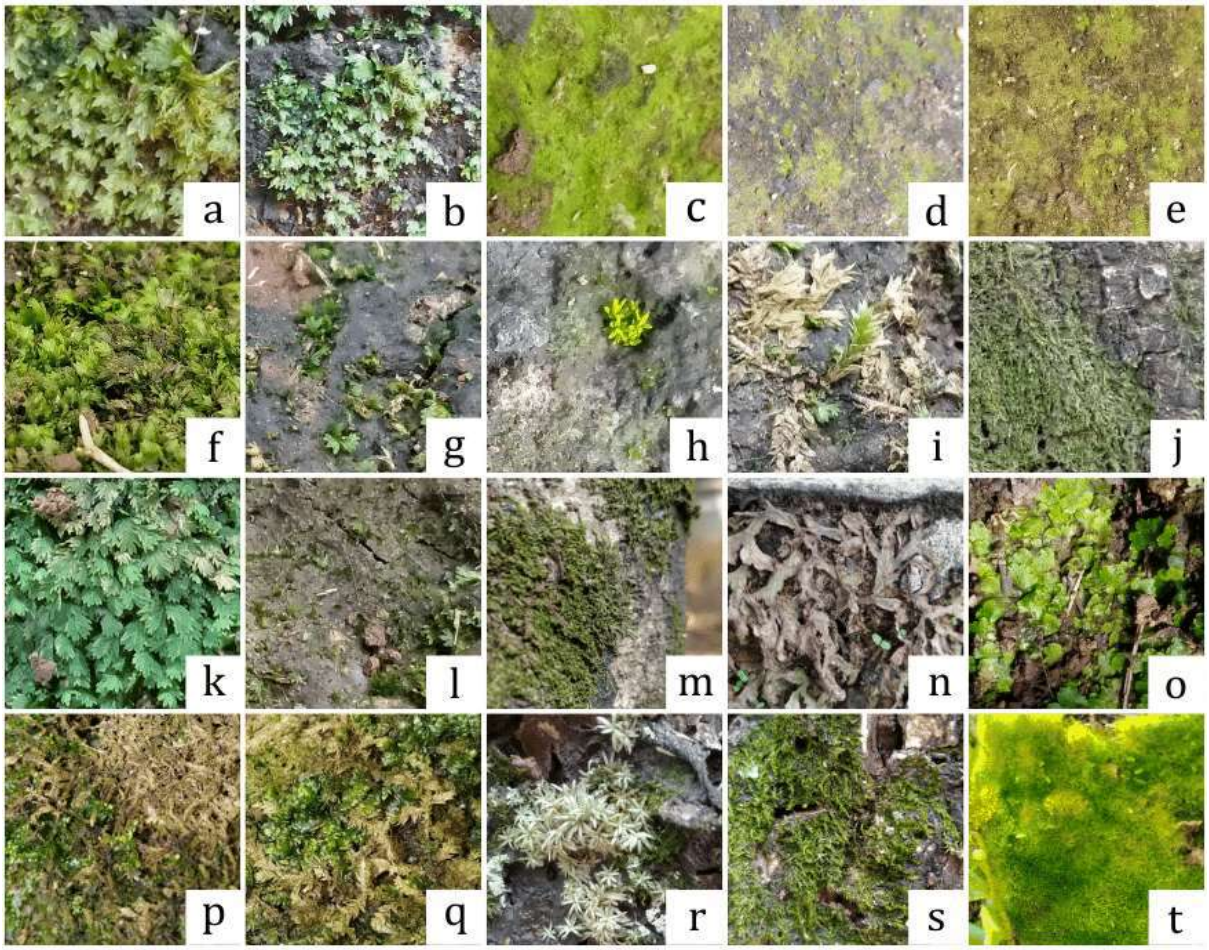

Figure 3. Species of Bryophytes in Urban-Bogor Region. (a) *Fissidens perpusillus*, (b) *Serpoleskea confervoides*, (c) *Pogonatum cirratum*, (d) *Lejeuna* sp., (e) *Sematophyllum tristiculum*, (f) *Fissidens taxifolius*, (g) *Fissidens obtusifolius*, (h) *Funaria hygrometrica*, (i) *Myrothecium microcarpum*, (j) *Neckera complanata*, (k) *Mnium hornum*, (l) *Fissidens zollingeri*, (m) *Hypnum cupressiforme*, (n) *Marchantia emarginata*, (o) *Marchantia polymorpha*, (p) *Mnium stellare*, (q) *Fontinalis antipyretica*, (r) *Octoblepharum albidum*, (s) *Leucobryum sanctum*, (t) *Amblystegium serpens*.

DISCUSSION

Characteristic of Canopy in Observation Station

The research uses data collection with stations divided into three at the research site. Station points are based on physical environmental characteristics, especially the amount of shade at the research location. The green route on Jalan Pajajaran with a length of approximately 503 meters has different shades along the road. This research using the belt transect technique divides three stations with each station being 167 meters long. The plants found in the green belt have various heights and widths in terms of tree height and shade area. Station I was observed to have very shady tree cover; several large and tall trees were found on the edge of the green route. The presence of tall buildings near Station I also hinders sunlight from shining towards Station I. This results in less light intensity coming in under the shade and station I being shadier. Station II has several tall

and large trees quite far away. The shade of the trees at station II is quite far apart and there are gaps for light to enter. This results in the light intensity at station II being greater than station I. In contrast to stations I and II, the light intensity entering station III is less due to the shade of trees and is not as extensive as the other two stations. The trees are quite far apart and there are parts of the green belt without being touched by tree shade.

Differences in shade in an area can cause differences in the physical environmental characteristics of that area. Shade has the function of lowering the air temperature, because the intensity of light hitting the area becomes lower, thereby increasing the humidity in the area. Other research also states that shade can function to optimize lighting for shaded plants so that the plants will not experience damage or death due to excess light. Therefore, it can be said that shade is the optimal way to regulate sunlight intensity, optimal light intensity will influence the productivity of a plant (Anni, 2013; Manullang, 2021; Sakiroh, 2021).

The optimal light intensity for several types of plants is different. Light intensity can affect temperature and humidity. Several plants can live optimally under direct sunlight, some plants grow and require shade or lower light intensity in the growth process, one of which is moss. Most plants can live in damp places, so if a place has a shade with low light intensity, that place will have higher humidity and will be an optimal place for moss to grow. So you can find more types of moss in places with more shade (Wati, 2016; Suryani, 2023).

Morphology Identification of Moss Species

The number of moss species found and identified on the green belt on Jalan Pajajaran Bogor consists of 20 species as depicted in Figure 3. (a) *Fissidens perpusillus*, this moss was found at all three stations a total of 42 discoveries. This indicates that this moss is one of the moss species that dominates in the area. *Fissidens perpusillus* is found in abundance, mostly attached to soil and rock substrates. The characteristics of moss when found are that it has a wide, segmented thallus, light yellowish green in color. (b) *Serpoleskea confervoides*, this moss was found at all three stations a total of 38 discoveries. This indicates that this moss is one of the species of moss that is quite abundant in the area. *Serpoleskea confervoides* isfound to be slightly dark green in color and attached to soil and rock substrates. The characteristic of moss when it is found is that it has a fairly large and dense thallus, the thallus is faintly serrated at the end. (c) *Pogonatum cirratum,* was found at two stations, namely station 1 and station 3, a total of 32 discoveries. This indicates that this moss species dominates in this area. *Pogonatum cirratum* is characterized by a very dense and smooth upright thallus that is bright yellowish green in color. (d) *Lejeuna* sp., this moss was found only at station 1 with a total of 9 discoveries. This moss is attached to the rock substrate. *Lejeuna* sp. It is characterized by a thallus that looks smooth and thin and has a greenish, slightly yellowish color. (e) *Sematophyllum tristiculum*, found at stations 1 and 2 for a total of 6 discoveries. This moss is found attached to rock substrates. *Sematophyllum tristiculum* is found with a slightly dry thallus that is yellow to greenish yellow in color.

Next, (f) *Fissidens taxifolius*, this moss was found at stations 1 and 2 a total of 40 number species. This shows that this moss is one of the mosses that dominates in this area. *Fissidens taxifolius* attaches to rock and soil substrates and is characterized by a thick, jagged-edged thallus that is fresh to dark green in color. (g) *Fissidens obtusifolius*, found at stations 1 and 2 for a total of 21 number mosses. This moss is found attached to the soil substrate. *Fissidens obtusifolius* is characterized by its thallus having a long and wide diameter, the tip of the thallus being serrated with a dark green to fresh green color. (h) *Funaria hygrometrica*, this moss was found at stations 1 and 2 a total of 3 discoveries. This shows that this species is relatively few in this area. This moss is found attached to rock substrates. *Funaria hygrometrica* is characterized by a long thallus in clusters of bright yellowish green color. (i) *Myrothecium microcarpum*, at stations 1 and 3 has found 3 number mosses. This moss attaches to the soil substrate. *Myrothecium microcarpum* is

one of the few mosses in this area. This moss is characterized by a long thallus that is stacked and has a pointed, yellowish green tip. (j) *Neckera complanata*, this moss was found at stations 1 and 3 a total of 4 number mosses. This moss attaches to the wood substrate. *Neckera complanata* is found characterized by its long, densely packed, slightly dry thallus which is pale dark green in color.

Furthermore, (k) *Mnium hornum*, this moss was found at the three stations a total of 29 discoveries. This moss attaches to the soil substrate. *Mnium hornum* is characterized by an erect thallus that spreads densely in dark green with a tapered tip. (l) *Fissidens zollingeri*, found at stations 1 and 2 with a total of 4 mosses. This moss attaches to soil and rocks. *Fissidens zollingeri* is characterized by its thallus being rather wide and the serrated tip is pale yellowish green. (m) *Hypnum cupressiforme*, found at station 1 18 discoveries. This moss attaches to the wood substrate. *Hypnum cupressiforme* has the characteristic that its thallus is long, small, and dense, dark green in color. (n) *Marchantia emarginata*, found at stations 1 and 2 with a total of 7 number mosses. This moss is found on stone substrates. *Marchantia emarginata* has the characteristic that its thallus spreads to form long and wide sheets with a brownish color when dry. (o) *Marchantia polymorpha*, found at stations 1 and 2 a total of 9 mosses. This moss is found attached to soil and rock substrates. *Marchantia polymorpha* is characterized by a bright yellowish green thallus in the form of a rather wide sheet and rounded edges.

Next, (p) *Mnium stellare*, was found at station 1 with a total of 8 discoveries. This moss is attached to the soil substrate. *Mnium stellare* is characterized by its slender and long thallus which is dark green and slightly brownish. (q) *Fontinalis antipyretica*, found at stations 1 and 3 a total of 13 discoveries. This moss is found attached to rock substrates. *Fontinalis antipyretica* is characterized by an elongated, dense, and rather wide thallus that is green and partly brownish. (r) *Octoblepharum albidum*, found at stations 1 and 2 for a total of 6 mosses. This moss attaches to the wood substrate. This moss is characterized by a star-shaped thallus that is pale green and whitish in color. (s) *Leucobryum sanctum*, found at station 1 with a total of 2 discoveries. This moss is attached to the wood substrate. This moss is characterized by its small, long, spreading thallus that is green to dark green. (t) *Amblystegium serpens*, this moss was found at station 3 discoveries. This moss is attached to a wood substrate which is characterized by its small, dense, and smooth thallus, fresh green to yellowish green in color.

All mosses found during the research were in the gametophyte phase, which is the dominant phase that occurs in mosses. In general, the morphological characteristics of urban moss include a body size that is not too large. Some moss was also found in a condition that was starting to turn black, dry, and pale in color. This occurs due to adaptation to environmental factors in urban areas in their growth and development.

Bryophytes Diversity

Based on the research results in Table 5, it can be seen that the moss diversity index (H') value along the Jalan Pajajaran green belt at all stations ranges between 1 and 3 which is included in the medium category. The research stations with the highest to lowest Diversity Index values were station I (2.4592), station II (2.0233), and station III (1.7830), respectively. This is influenced by differences in environmental factors such as temperature, light intensity, and air humidity at each station (So seen & Kurnia, 2021; Ristanto et al., 2021; Endang et al. 2020). Moss grows optimally at temperatures of 15-25°C, but this plant can adapt to live at temperatures of 40-50°C and will grow optimally at a humidity of more than 50%, which is around 85%-98% (Putri et al., 2019; Maharani et al., 2017). Mosses have different levels of adaptation and propagation phases, this is influenced by the level of intensity of sunlight they receive (So seen & Kurnia, 2021; Putrika, 2017).

Bryophytes Uniformity

The research results show that the value of the moss type uniformity index (E) at the three stations is around 0.8, where this value is classified as high (Table 5). The research stations with the highest to lowest Species Uniformity Index respectively are Station I (0.8352), Station II (0.8142), and Station III (0.8115). A high species uniformity index can reflect the dominance of species that are more resistant to environmental stress and able to adapt to rapid changes in urban conditions (Sujetoviene & Galinyte, 2016).

Species that can tolerate environmental stressors, such as air pollution and temperature fluctuations, have a competitive advantage in surviving and thriving in dynamic urban environments (Bhatt et al., 2022). High uniformity may indicate that some moss species dominate urban landscapes and can withstand environmental challenges better than other species. This phenomenon is thought to be related to the specific adaptive properties of some types of moss to survive in the face of rapidly changing environmental conditions (Grdovic & Stevanovic, 2006).

Relation of Species Identification, Diversity, and Uniformity of Bryophytes

Identification of moss types is the initial stage in characterizing moss communities along the green belt of Jalan Pajajaran, Bogor. This process involves identifying and documenting the various moss species found in the region. This variable is the basis for measuring moss diversity, which includes the number of species in the urban habitat (Bhatt et al., 2022). Moss diversity reflects the variety and abundance of species in a community (Fojcik & Stebel, 2014). Meanwhile, moss uniformity refers to how evenly distributed the number of individuals of the various species is (Julinova & Beckovsky, 2019).

The relationship between these variables is that the higher the identification of moss types, the more accurate our understanding of the diversity of species at the research location. This identification forms the basis for measuring moss diversity, which can reveal the level of variation and complexity of moss ecosystems in urban habitats. Meanwhile, the uniformity of moss can provide further insight into the extent to which certain species dominate or distribute themselves evenly throughout the region.

Research Limitations

In this research there are several limitations, especially related to the coverage of the urban area along Jalan Pajajaran, Bogor (with coordinate points between -6,595, 106,804 and -6,600, 106,805). Although this area was chosen because of its relevance to the urban habitat context, this research does not fully represent the complexity of the urban environment in Bogor City. These regional limitations may affect the generalization of research findings regarding the condition of mosses in urban habitats in general.

CONCLUSION

Green Open Space (RTH) as an urban habitat has an ecological function to reduce environmental problems in cities with the presence of plants that grow with human help and grow by themselves due to environmental factors. One of the plants that can adapt to urban environments, especially in green belts, is moss. The presence of moss can be used as an environmental indicator due to the diversity of moss types and the uniformity of types in urban areas. The results of research on Jalan Pajajaran, Bogor found that there were 20 species of moss consisting of leaf moss and liverworts. The diversity index of the three observation stations in sequence is 2.45; 2.02 ; 1.78 so it is classified as medium criteria. The uniformity index obtained at three observation stations was 0.8, which is included in the criteria for high uniformity and an even distribution of moss types along the green belt. Moss in urban areas adapts to environmental

factors such as temperature, humidity, and light intensity so that the morphological characteristics of the moss found will adapt to these factors. Based on this research, it is necessary to carry out further research related to a wider coverage of the research area so that it is more representative of the overall condition of moss in urban Bogor habitats. Apart from that, further research can be carried out regarding the comparison of the morphological characteristics of moss in urban habitats with moss in habitats where it is more likely that the moss will grow optimally, such as humid highland areas in the mountains. This will provide a deeper understanding of the influence of environmental impacts on moss growth in an area.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 58 – 65 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Moringa oleifera gum effect on blood sugar levels and rat pancreas histology induced by Streptozotocin

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Article Info

Article History:
Received 17 January 2024
Revised 14 March 2024
Accepted 26 March 2024
Published 30 April 2024

Keywords:
Gum *Moringa oleifera,*
Streptozotocin
Diabetic
Pancreas

ABSTRACT

Moringa gum is a product that people can use as an anti-hyperglycemic agent. This study aimed to determine the effectiveness of Moringa gum in reducing blood sugar levels and improving the histology structure of the rat pancreas induced by streptozotocin (STZ). This study used 21 male rats divided into 3 groups including control, diabetes (STZ), and diabetes (STZ + 3% Moringa gum). STZ treatment at a single dose (45 mg/kg BW) was given intraperitoneally, while Moringa gum was given through drinking water for 2 weeks. Blood sugar levels were measured on days 0, 7th, 14th, 21st, and 28th. The results showed that STZ administration induced diabetes in rats with blood sugar levels of 487 mg/dL on the 14th day. Moringa gum administration was able to reduce blood sugar levels on days 21st and 28th respectively to 306 and 234 mg/dl. The histology structure of the pancreas of rats treated with gum also showed improvement. This study concludes that Moringa gum is effective in lowering blood sugar levels in rats even though it still does not show normal blood sugar levels, and can repair STZ-induced damage to the histology structure of the rat pancreas.

Citation: Utami, E.T., Fajariyah, S., Lelono, A., & Nihayah, H. (2024). Moringa oleifera gum effect on blood sugar levels and rat pancreas histology induced by Streptozotocin). *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 58-65. DOI: https://doi.org/10.31932/jpbio.v9i1.3184

INTRODUCTION

Glucose is a simple carbohydrate that can be absorbed in the bloodstream. Blood sugar levels are closely related to diabetes mellitus. Diabetes mellitus is a metabolic disorder characterized by increased blood sugar levels above normal (hyperglycemia) due to impaired regulation of insulin levels in the body (Hidayaturrohmah et al., 2020). Diabetes mellitus can be caused by two factors, the first is insulin deficiency so that the body is unable to control blood sugar and the second is insulin resistance, the condition of the body's cells being insensitive to insulin secreted by the pancreas (Mc Clung et al., 2004).

As a research model, rats were induced by *streptozotocin* (STZ). STZ is used to induce diabetes in experimental animals because they can maintain the diabetic condition (Eleazu *et al.*,

2013). STZ has a half-glucose structure that can bind into *glucose transporter 2* (GLUT2) so that it can enter the β pancreas cell and cause a hyperglycemic response (Szkudelski, 2001). STZ causes an increase *in reactive oxygen species* (ROS) which can result in increased oxidative stress, especially in pancreatic β cells. Pancreatic β cells experience degeneration so that insulin secretion decreases increasing blood sugar levels because the body's cells cannot absorb glucose optimally (Lai, 2018; Hamdan et al., 2011; Husni et al., 2016). Blood sugar levels in diabetes sufferers can be controlled by taking medication that is generally consumed continuously (Trevor et al., 2009). However, in the long term, sufferers can experience several side effects, which has prompted a lot of research to be carried out regarding the use of natural ingredients to regulate blood sugar levels in diabetes sufferers (Wang et al., 2013).

One of plant that can be used as an alternative to stabilize blood sugar levels in diabetes mellitus sufferers is the Moringa plant *(Moringa oleifera)*. Many studies have been carried out regarding the efficacy of leaf, stem, fruit, and seed extracts of *M. oleifera* as a treatment ingredient for diabetes mellitus sufferers (Vargas-Sanches et al, 2019). However, but its potential has not been studied for the gum part. Research on the effect of gum on rat blood sugar levels has been carried out on Arabic gum by Nagar (2017) and Wiyono et al. (2021), but has never been studied on *Moringa* gum.

In general, *gum* contains the main chemical compounds such as *D-galactose, L-arabinose, L-rhamnose, D-glucuronic, and 4-0- methyl-D-glucmic acid* (Mohammad, 2015). With this content, the gum is included in high-fiber foods which can play a role in inhibiting glucose absorption in the small intestine, so it is hoped that it can reduce hyperinsulinemia (Glover *et al.*, 2009; Nasir et al., 2014). Apart from polysaccharides, there are also several secondary metabolite compounds in gum. During this time the use of *Moringa* gum. It is only known as a source of nutrition which is also used as a traditional medicine, so the potential of *M. oleifera* gum still needs to be studied specifically as lowering blood sugar levels in diabetes mellitus sufferers.

RESEARCH METHODS

Research Design

This research used a Completely Randomized Design (CRD). The test animals used were 21 male rat (*Rattus norvegicus* L)Wistar strain. Induction of diabetes is carried out by injecting a single dose of STZ (45 mg/kg body weight) and then the blood sugar level is measured when the blood sugar level has reached ≥ 165 mg/dL followed by the administration of 3% *Moringa* gum. Mice were divided into 3 groups and 7 replications, with the following doses:

Group 1: Rats were not induced *STZ* and without *Moringa* gum
Group 2: Rats were induced STZ without *Moringa* gum.
Group 3: Induced mice *STZ* and given a gum *Moringa* gum.

Observations were made on blood sugar levels and pancreatic histology. Blood sugar level was analyzed statistically using T-test and analysis of variance, further conducted using DMRT. The pancreas histology was analyzed descriptively.

Population and Samples

This research used male Wistar strain rats aged 8-10 weeks with a body weight of 150-200 grams 8 weeks in healthy condition. Rat was kept in cages with feed and drink was given ad libitum. Rat were grouped according to the treatment and repetition carried out in Completely Randomized Design.

Instruments

The research instrument includes cages, syringes, glucocheck and strips, surgical tools, dissecting boards, ovens, rotary evaporators, automatic stainers, microscopes, and optilabs.

Procedures

This research was begun by rat acclimatization, STZ dan Moringa gum treatment, blood sugar level measurements, pancreas histology preparations, observation and data analysis. Blood sugar levels are measured on 0,7th, 14th, 21st, and 28th day. The research procedures include all the steps carried out during the research described in Figure 1.

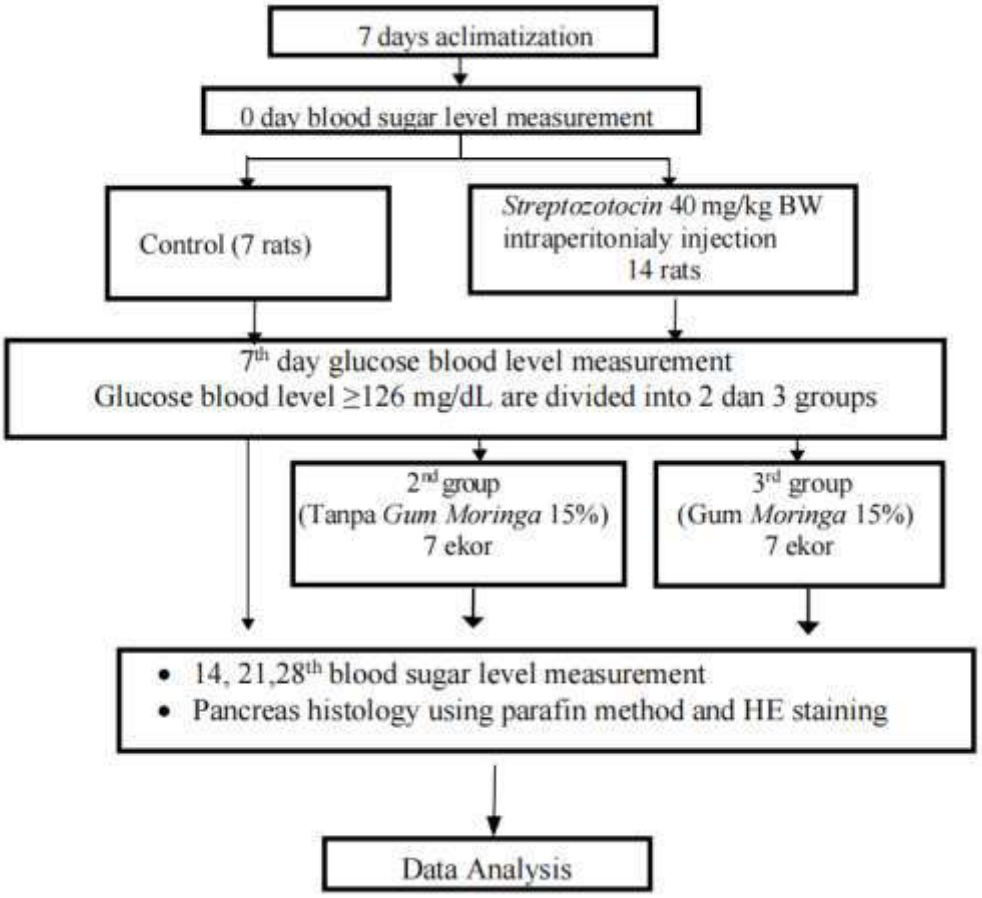


Figure 1. The flow diagram of research implementation

Data Analysis

Observation data were analyzed statistically using T-test to know the effect of STZ injection on blood sugar levels. Afterward continued by analysis of variance (F test) to know the effect of Moringa gum treatment and further tests were conducted using Duncan's multiple range test (DMRT).

RESULTS

In this study, STZ induction was carried out to increase rat blood sugar levels constantly and become diabetic. Blood sugar levels were measured on day 0 and then blood sugar levels were measured again on day 7th after STZ induction. Data on the results of measuring blood sugar levels can be seen in Table 1. The results of measuring the mean ± SD of blood sugar levels on days 14th, 21st, and 28th can be seen in Table 2. The results of observations of the histological structure of the rat pancreas can be seen in Figure 1.

Table 1. Average blood sugar levels of mice on day 0 and day 7th after STZ administration

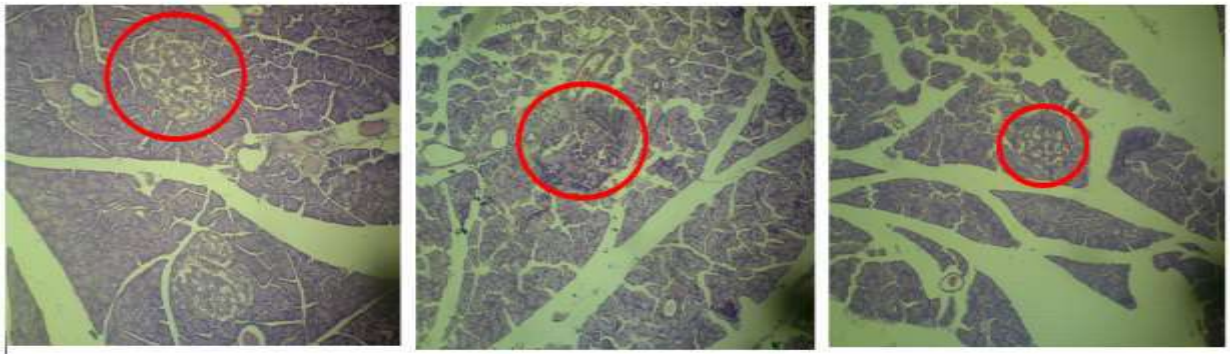
Treatment	Blood sugar level (x±SD)(mg/dL)	
	Day 0	Day 7th
Control	105±15,59[a]	111±14,11[a]
STZ	108±13,17[a]	437±46,22[b]

Numbers followed by different letters in the same column are significantly different based on the DMRT test at the level ($p<0.05$).

Table 2. Mean ± SD blood sugar levels of hyperglycemic mice after administration of gum *Moringa* 3% for 2 weeks on days 14th, 21st, and 28th

Group	Blood sugar level (mg/dL) ($\bar{x}$±SD)			
	Day 7th	Day 14th	Day 21st	Day 28th
Control	111±14[a]	125±4,47[a]	128,42±7,45[a]	114±8,40[a]
STZ	437±46,22[b]	487±66,01[b]	479,42±46,31[b]	420,28±63,24[b]
STZ + *Gum Moringa* 3%	364±19,23[b]	479,14±66,29[b]	306,42±39,47[a]	234,14±29,19[a]

Numbers followed by different letters indicate significant differences based on the DMRT test at the level ($p<0.05$).



(a) (b) (c)

a. Pancreas histology in control group
b. Pancreas histology in STZ-treated group
c. Pancreas histology in STZ followed by 3% *Moringa Gum* treatment group
The red circles showed the structure of the Langerhans islands
Figure 2. Histology of the rat pancreas (10x10 magnificent)

DISCUSSION

Table 1 showed that the average blood sugar levels before STZ administration (day 0) in the control group and the STZ group were not significantly different, namely within the normal range: 113±12 mg/dL and 107±10 mg/dL respectively. Normal blood sugar levels in rats range between 50-135 mg/dL (Hidayaturohmah et al., 2020). Meanwhile, hyperglycemic blood sugar levels in mice are shown to the blood sugar levels of more than 200 mg/dL (Vasukeshetti et al.,

2016). Measurement of blood sugar levels on day 7th in the control group showed levels of 111 ± 14 mg/dL, which is still within the range of normal blood sugar levels, and the group of rats injected with STZ showed increasing in blood sugar levels more than 387 ± 37 mg/dL so that classified into the diabetes group.

In this case, it was shown that STZ induction was able to increase blood sugar levels in rats which indicated diabetes. STZ increased *nitric oxide* (NO) synthesis resulting in the degeneration of β cells pancreas (Eleazu *et al.,* 2013). *Nitric oxide* (NO) is included in *reactive oxygen species* (ROS) which can increase oxidative stress in β cells pancreas so it can reduce the ability of the pancreas to secrete insulin (Lai, 2018; Hamdan *et al.*, 2011). Decreased insulin secretion causes blood sugar levels in the body to increase, thought to be caused by pancreatic β cells undergoing apoptosis (Husni *et al.*, 2016). From these results, the treatment was continued by gum administration at a dose of 3% for two weeks.

The results of measuring the mean ± SD of blood sugar levels on days 14th, 21st, and 28th after gum administration can be seen in Table 2. On the 14th day, the blood sugar level of mice with STZ treatment and STZ mice given gum *Moringa* was 3% increased compared to day 7 th. Blood sugar levels in the two groups did not show any significant differences. This is caused by *Moringa* gum first given on the 14th day. On the 21st day, there was a decrease in blood sugar levels in the diabetic rats that were given 3% *Moringa* gum, while diabetic mice that were not given 3% *Moringa* gum showed blood sugar levels that remained high, despite a slight decrease. There is a significant difference between the two groups. Likewise, on the 28th day, there was a decrease in blood sugar levels in both groups of diabetic rats treated with STZ and STZ with gum. However, the decrease in blood sugar levels in rats treated with gum was more than in rats treated only with STZ. This showed that 3% *Moringa* gum can act as an antihyperglycemic compound even though the sugar levels of diabetic rats are still high (the mice are still diabetic).

Decreased blood sugar levels in the group of mice given *Moringa* gum were thought to be due to *Moringa* gum being an *undigestable polysa*ccharide. *Moringa* gum contains salts, and polysaccharides including L-arabinose, D-galactose, L-Rhamnose, and D-Glucoronic acid. These polysaccharides have long chains and are highly branched with various types of branching. Colon bacteria ferment *Moringa* gum slowly until it produces *short-chain fatty acid* (SCFA) (Srinivasan *et al.,* 2005). The results of fermentation in the form of SCFA are divided into *acetate* (C2), *propionate* (C3), and *butyrate* (C4) (Cook & Selin, 1998). *Butyrate* stimulates increased secretion of *glucagon-like peptide-1* (GLP-1) by L cells so that GLUT 4 expression increases (Besten et al., 2013; Penacarrillo *et al.*, 2001). GLUT 4 acts as a glucose transporter so that it can enter skeletal muscle cells. GLUT 4 expression increases due to GLP-1 activation *phosphoinostide3-kinase* (PI3K) in skeletal muscle cells so that glucose entering the skeletal muscle cells also increases (Green *et al.*, 2012)] Thus, blood sugar levels decrease. The decrease in blood sugar levels in mice treated with Gum still did not show normal blood levels. This is because the gum dose given was not high enough (only 3%). Previous research results showed that giving gum Arabic at a dose of 15% was able to reduce the sugar levels of STZ-induced diabetic rats (Wiyono et al., 2021).

The results of observations of the histology structure of the rat pancreas can be seen in Figure 2. The histology structure of the pancreas of control mice shows that the islets of Langerhans have a large size and a compact cell structure. Meanwhile, in rats treated with STZ, the pancreatic islets of Langerhans appeared to have smaller structures and damaged structures. The damage experienced is thought to be due to the influence of STZ administration which causes necrosis on β pancreas cell. Moringa gum administration appears to be able to regenerate this damage. It can be seen in Figure 2 that the pancreatic islets of Langerhans in mice treated with gum*Moringa* The 3% appears more compact, although its size does not yet resemble the control. The results of observations of the histology structure of the pancreas showed that in the pancreas

of rats treated with STZ, there was damage to the islets of Langerhans, which was characterized by necrosis of the islets of Langerhans cells and the introduction of pancreatic acinar between the cells of the islets of Langerhans. Research shows that STZ is cytotoxic through its indirect role as a Nitric Oxide (NO) donor which can cause DNA damage in pancreatic β-cells in mice. NO molecules are formed when STZ undergoes metabolism in cells. Apart from NO, STZ can also cause the formation of ROS which can also cause DNA fragmentation and other cell damage (El Nagar, 2017).

Compared with controls, the cells of the islets of Langerhans appeared to be arranged compactly and regularly and there were no cells experiencing necrosis. In 3% *Moringa gum* treatment group showed that the islets of Langerhans structure improved so that the structure was more compact than in the STZ treatment (Figure 2). In this case, it is allegedly because *Moringa* gum has antioxidant activity that can react with free radical compounds (ROS) so that it can reduce damage to the structure of the pancreatic islets of Langerhans in diabetic mice. This is by research which states that *Moringa* gum contains phytochemical compounds including alkaloids, saponins, and oils (Annaamalai *et al.,* 2017). Saponin acts as an antioxidant by reducing superoxide through the formation of hydroperoxide so that it can prevent lipid damage (lipid peroxidation) (Hasan *et al.,* 2022). Meanwhile, alkaloids can act as antioxidants by donating H atoms to stabilize free radicals (Widiastuti *et al.,* 2021). In this way, it can prevent lipid peroxidation which can cause damage to the β cells of the islets of Langerhans in the rat pancreas.

CONCLUSION

The conclusion of this research is, that giving gum*Moringa* was able to reduce blood sugar levels in diabetic mice due to STZ induction, however, this reduction in sugar levels did not indicate normal blood sugar levels. Giving *Moringa* gum also showed an improved effect on the histology structure of the pancreas.

ACKNOWLEDGMENT

Thanks are expressed to LP2M Jember University as the research funder through the KeRis Di-Mas Research Grant based on Assignment Agreement Letter Number: 4152/UN25.3.1/LT/2022 Dated 18 July 2022

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 66 – 73 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Antibacterial activity in vitro investigation of eucheuma cottonii extract from aru islands against pathogenic bacteria

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Article Info

Article History:
Received 02 February 2024
Revised 14 March 2024
Accepted 16 April 2024
Published 30 April 2024

Keywords:
Clear Zone
Escherichia coli
Inhibition
Red Algae
Staphylococcus aureus

ABSTRACT

Red algae *Eucheuma cottonii* is one of the potential marine biodiversity that provides added value in the pharmaceutical and cosmetic fields and is known to have antibacterial compounds. Research focusing on the potential of *E. cottonii* as an antibacterial is still limited, particularly when it comes to the Aru Islands. This study aimed to measure the antibacterial activity of *E. cottonii* against pathogenic bacteria including *E. coli* and *S. aereus* at several concentrations and to determine the best concentration for inhibiting these two pathogenic bacteria. The study was true experimental laboratory research with a post-test-only controlled group design. Antibacterial tests using the disc diffusion method and phytochemical tests were carried out. This study revealed that *E. cottonii* could be able to inhibit *S. aureus* ranging from 0.27±0.03 – 2.1±0.14 at a concentration of 50%-100%, and 0.17±0.05 – 0.45 ± 0.03 against *E. coli* at a concentration of 80-100%. Meanwhile, saponins and flavonoids were two bioactive compounds found through phytochemical testing. The study concluded that the n-hexane extract of *E. cottonii* was able to inhibit the growth of *E. coli* and *S. aureus,* even with a weak inhibition category. This study recommends that improvements are needed in the *E. cottonii* extraction process to maximize the antibacterial properties of *E. cottonii*.

Citation: Yunita, M., Warella, J.C., Astuty, E., Ohiwal, M., & Alimudi, S. (2024). Antibacterial activity in vitro investigation of eucheuma cottonii extract from aru islands against pathogenic bacteria. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 66-73. DOI: https://doi.org/10.31932/jpbio.v9i1.3241

INTRODUCTION

Indonesia is a maritime country with 70% of its territory consisting of seas or oceans. The ocean is a habitat for various organisms, both plants and aquatic animals (Andriani et al., 2015).

Because they are exposed to such a wide range of marine conditions—including variations in salinity, temperature, chlorophyll content, and water quality—marine organisms can synthesize novel chemicals. Even though marine areas constitute ¾ of the Earth, they are still one of the underutilized biological resources. One of the potential biological resources from Indonesian marine waters is macroalgae with various types. The distribution of macroalgae is found in almost all Indonesian waters (Andriani et al., 2015).

Macroalgae, known to the community as seaweed, is one of the potential marine biodiversity that provides added value in the pharmaceutical and cosmetic fields, including the hepatoprotective effect (Wardani et al., 2017) and antioxidant activity so it is widely used as an additional ingredient in making facial creams or sunscreens (Nurjanah et al., 2019). Research shows that marine algae have many uses in various fields, such as nutraceuticals, pharmaceuticals, especially cosmeceuticals (Syad et al., 2013; Andriani et al., 2016; Teo et al., 2020). However, the use of macroalgae in the pharmaceutical sector is still limited, while the potential of macroalgae in Indonesia, especially Maluku, as an archipelagic country is very large to be developed as a raw material for medicine. Several researchers have studied the potential of macroalgae on Maluku Island, but it is limited to Seram Island, namely Kotania Bay, West Seram (Arfah & Patti et al., 2014), and East Seram waters (Rugebregt et al., 2021). However, this research focused more on describing biodiversity and conservation efforts.

Research focusing on the potential of macroalgae as antibacterial is still limited, particularly when it comes to the Aru Islands. Indeed, macroalgae, particularly red algae (*Eucheuma cottonii*), show promise for development as antibacterial agents. Research conducted by Julyasih et al., (2021) on ethanol extracts of red algae and green algae from Bali waters found that the highest antibacterial activity was respectively obtained by *E. cottonii* (11.1 mm), *Caulerpa* spp. (10.1 mm), *Gracilaria* spp. (6.0 mm), *E. spinosum* (4.2 mm). In the other investigation, it was revealed that the antibacterial activity of *E. cottonii* from Sumenep, Madura against *Escherichia coli* was 0.33 and there was no inhibition zone against *Staphylococcus aureus* (Andriani et al., 2015) Therefore, the present study tried to investigate the antibacterial activity in vitro against *S. aureus* and *E. coli* using *E. cottonii* extract from the Aru Islands on the growth of *E. coli* and *S. aureus* as representatives of gram-positive and negative bacteria. Apart from that, there is no specific study regarding any type of macroalgae in the waters of the Aru Islands, which is the reason for carrying out this research. The study was aimed to measure the antibacterial activity of the red algae *E. cottonii* against pathogenic bacteria including *E. coli* and *S. aereus* at several concentrations and to determine the best concentration for inhibiting these two pathogenic bacteria.

RESEARCH METHODS

Research Design

This study is a true experimental laboratory research with a post-test-only controlled group design. Post-test-only controlled group design is a design that uses two groups, where one group is used for the experiment (which is given treatment) and the other one is used as a control group (which is not given treatment). The study was conducted in July – September 2023. Sampling was carried out in the waters of Jabulenga Village, Aru Islands, Maluku with coordinates 5°46'52.82"S - 134°21'8.69"E. Meanwhile, laboratory tests will be carried out at FKIP Biology, Pattimura University, Ambon. The study site is presented in Figure 1. Determination of *E. cottonii* was carried out at the Department of Marine Science, Faculty of Fisheries and Marine Sciences.

Population and Samples

The population used in this study is the whole red algae *E. cottonii* which can be collected from the waters of Jabulenga Village, Aru Islands. Meanwhile, the sample used in this research was

n-Hexana *E. cottonii* extract which was the independent variable. Meanwhile, *E. coli* and *S. aureus* are the dependent variables.

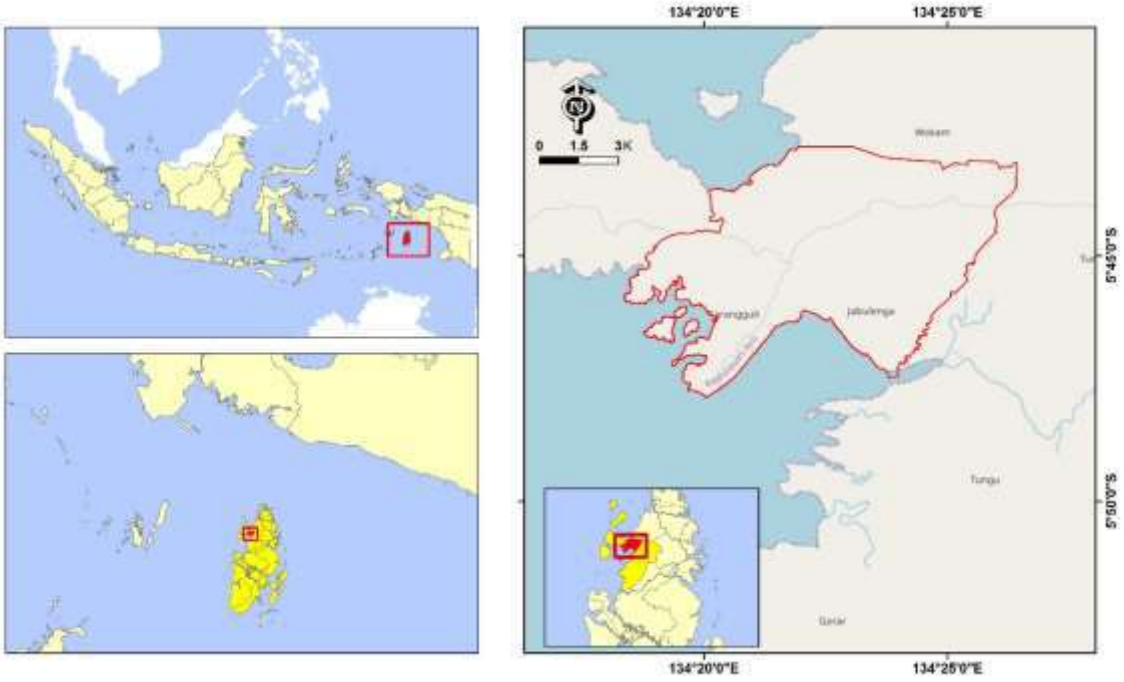

Figure 1. Sampling site of *Eucheuma cottonii*

Instruments

The following equipment was used in this study: sterile gauze, 100 ml measuring cups, 250 ml and 500 ml Erlenmeyer, bunsen lamps, micropipettes, magnetic stirrers, spatulas, autoclaves, oven, analytical balances, vortex, petric dishes, ose needles, paper disks, tweezers, label paper, cotton, and thread. Meanwhile, *E. cottonii* extract, pure cultures of pathogenic bacteria (*E. coli* and *S. aureus*), n-hexane solvent, Nutrient Agar (NA), disc paper, 70% and 96% alcohol, 5% sodium hypochlorite solution, distilled water as the negative control, and ampicillin as the positive control were the materials used for this investigation.

Procedures

Sample preparation was performed as follows: Red algae (*Eucheuma cottonii*) obtained from the waters of Jabulenga Village, Aru Islands were collected in zip plastic. Wet sorting was carried out by cleaning *E. cottonii* from adhering impurities and then washing thoroughly with running water. The *E. cottonii* sample was then doused with 5% sodium hypochlorite solution for surface sterilization. The samples were then stored in a cool box to be taken to the laboratory for further tests (Andriani et al., 2015).

The extraction process was performed by air drying *E. cottonii* samples for ± 4 days. The dried sample was cut into pieces and then ground using a blender until it became dry simplicia powder. A total of 50 g of simplicia powder was weighed and put into an Erlenmeyer. Maceration was carried out by soaking simplicia powder in n-hexane solvent in a ratio of 1:3 for 2x24 hours. Soaking functions to draw out the organic compounds contained in simplicia. The solution was filtered using ordinary filter paper and concentrated with an evaporator until a thick extract was formed. This extract was diluted and made into serial concentrations, namely 10%, 30%, 50%, 80%, and 100% (Teo et al., 2020).

Pathogenic bacterial culture preparation was carried out by subculturing *Escherichia coli* and *Staphylococcus aureus* obtained from the Maluku Province Health and Equipment Calibration

Laboratory in new Nutrient Agar media. The isolates were incubated at room temperature for 24 hours. The bacterial colonies that appeared were then transferred to a slant agar medium to be used as test isolates (Yunita et al., 2016; Yunita et al., 2022).

The antibacterial test was carried out using the Kirby Bauer disk diffusion method by testing the n-hexane extract of *E. cottonii* with serial concentrations against the growth of *E. coli* and *S. aureus*. Pathogenic bacteria are streaked or swabbed on Nutrient Agar media. Furthermore, 6 mm sterile paper disk that had been soaked in 200 µl of *E. cottonii* extract was placed on top of the pathogenic bacterial culture. The Petri dishes were wrapped in plastic wrap and stored in an incubator at 37^{0}C for 1-3 days. The positive control was tested using the antibiotic Amphicilin while the negative control was tested using sterile distilled water. Inhibition of the growth of pathogenic bacteria was visible as a clear zone around the paper disc. The size of the clear zone was an indication of the sensitivity of pathogenic bacteria to antibacterial compounds. The inhibition zone was calculated by subtracting the diameter of the inhibition zone obtained from the diameter of the paper disc, which was 6 mm. The diameter of the inhibition zone was measured using a caliper and the treatment was repeated 2 times (Kiriwenno et al., 2020).

Qualitative standard screening through phytochemical tests was performed to determine the presence of secondary metabolites, including tannins, terpenoids, alkaloids, phenolics, flavonoids, and saponin, in the crude extract of *E. cottonii*. The presence/positive reaction (+) and absence/negative reaction (-) of phytochemical were used to express the qualitative results (Yunita et al., 2023).

Data Analysis

Data were analyzed descriptively and presented with tabulations and figures. All data are expressed as the mean and calculated using Ms. Excel. Standard deviation was calculated between extract concentrations. Data processing was performed by measuring the diameter of the inhibitory zone of the independent variables (*E. cottonii* extract) on the growth of *E. coli* and *S. aureus*. The inhibitory zone results were then divided into four categories based on their size: extremely strong (>20 mm), strong (11-20 mm), moderate (6-10 mm), and weak (<5 mm) (Davis & Stout, 1971).

RESULTS

Pathogenic bacteria (*E. coli* and *S. aureus*) from the Maluku Province Health and Equipment Calibration Laboratory were subculturing into new NA media to initiate the study stage. The pathogenic bacterial isolates utilized in this study are displayed in Figure 1.

Figure 1. Isolate *S. aureus* (left) and *E. coli* (right) used in this study

The growth of pathogenic bacteria was shown to be inhibited by the n-hexane extract from *E. cottonii*, with varied inhibition zones, according to antibacterial testing. The inhibition zone formed against *S. aureus* ranged from 0.27±0.03 – 2.1 ± 0.14 at a concentration of 50%-100%, and 0.17±0.05 – 0.45±0.03 against *E. coli* at a concentration of 80-100%. Meanwhile, a very large zone of inhibition was formed in the control treatment using ampicillin, namely 19±0.41 for *S. aureus* and 21±0.00 for *E. coli*. The results of measuring the inhibition zone of *E. cottonii* on the growth of the two pathogenic bacteria can be seen in Table 1.

Table 1. Results of measuring the inhibition zone of *E. cottonii* extract on the growth of *S. aureus* and *E. coli*

Extract Concentration of *Eucheuma cottonii*	Inhibitory response (mm)			
	S. aureus	Category	*E. coli*	Category
10%	00±00	-	00±00	-
30%	00±00	-	00±00	-
50%	0.27±0.03	Weak	00±00	-
80%	1.25±0.35	Weak	0.17±0.05	-
100%	2.1±0.14	Weak	0.45±0.03	-
K+	19±0.41	Strong	21±00 mm	Very Strong
K-	-	-	-	-

When comparing the effect of the extract on the growth of the two pathogenic bacteria, it was known that the n-hexane extract could inhibit the growth of *S. aureus* better than *E. coli*. The *E. cottonii* extract was able to inhibit the growth *of S. aureus* at a concentration of 50%. Meanwhile, *in E. coli*, a very small inhibition zone was only formed at a concentration of 80%. A comparative visualization of antibacterial activity against the two pathogenic bacteria is presented in Figure 3.

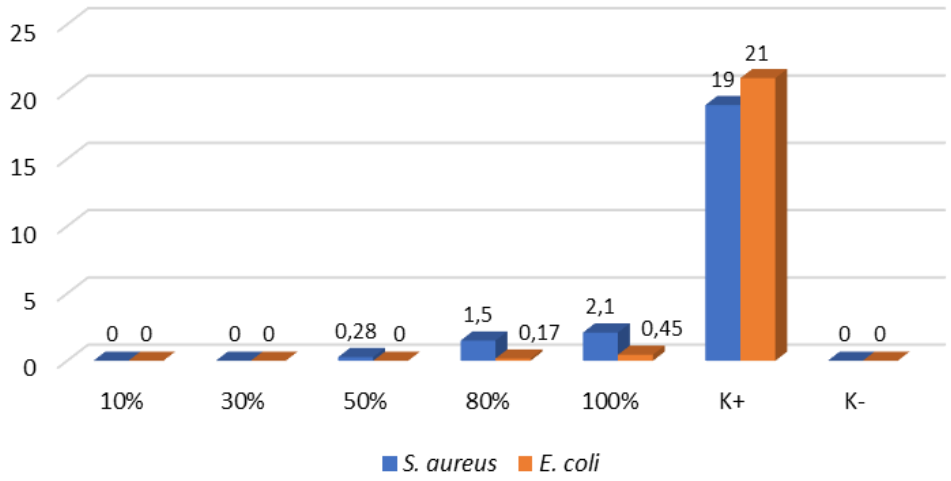

Figure 3. A comparison of antibacterial activity against pathogenic bacteria

The results of the phytochemical test were carried out to see the content of bioactive compounds contained in the hexane extract of *E. cotonii*. In the seven bioactive compounds tested, it is known that the n-hexane extract of *E. cottonii* contains flavonoids, phenolics, and saponins. In detail, the results of the phytochemical tests are presented in Table 2.

Table 2. Phytochemical test results of *E. cottonii* extract

No	Parameter	Indicator	Result
1	Alkaloid	Yellow	-
2	Flavonoid	Greenish yellow	+
3	Terpene	Red ring	-
4	Steroid	Yellow ring	-
5	Phenolic	Blue	-
6	Saponin	*Foam*	+
7	Tannin	Orange	-

DISCUSSION

Plant extraction is a process of separating active plant materials or secondary metabolites including alkaloids, flavonoids, terpenes, saponins, steroids, saponin, and glycosides from inert or inactive materials using an appropriate solvent and standard extraction procedure (Bhuiya et al., 2020). In this study, we extracted the red algae *E. cottonii* using n-hexane as a solvent. N-hexane is a solvent utilized in plant extraction because of its characteristics, which include easy recovery, non-polarity, low latent heat of vaporization (330 kJ/kg), and strong solvent selectivity. N-Hexane is quite good for the extraction process because of its low boiling point and high evaporation rate. However, the antibacterial activity of *E. cottonii* extract shown in this research was still in the weak category due to the small clear zone visible around the paper disc (Agboke & Attama, 2016). A clear zone that forms around the disc paper is an indication that the *E. cottonii* extract is inhibiting the growth of *E. coli* and *S. aureus*. Variations in the concentration of bioactive antibacterial chemicals within the inhibition zone as well as the rate that which the antibacterial material diffuses into the agar medium can account for variations in its size. The sensitivity of antibacterial growth, the interaction between the active ingredient and the medium, and the incubation temperature are other parameters that are thought to affect the formation of the inhibitory zone (Yusvantika, 2021).

In this study, flavonoids and saponins were known to be present in the *E. cottonii* hexane extract, out of the five components examined in the phytochemical test. This is consistent with the research findings of Fahrul et al. (2021), which also indicated that the red algae *E. cottonii* obtained from Karimun Regency, Riau province, solely contained flavonoids, saponins, and phenolic compounds. The flavonoids found in *E. cottonii* have antibacterial properties because of their ability to block the formation of nucleic acids, energy metabolism, and cytoplasmic membrane function. The antibacterial activity can be considered an effective indicator for *E. cottonii* to synthesize bioactive secondary metabolites. Another compound found in this study is saponin. Saponin is commonly found in plant extract (Agboke & Attama, 2021). High polarity, instability in both chemical and thermal processes, lack of volatility, and low concentrations are typical characteristics of saponins. Therefore, even when non-polar solvents such as n-hexane were used in plant extraction, saponins produced the highest yields (Majinda, 2012).

On the other hand, the phytochemical test findings of *E. cottonii* in this study did not reveal the presence of alkaloids or other more complex chemicals. Fahrul (2021) also found no alkaloids in the phytochemical test results, confirming the same findings. Teo et al., (2020) state that alkaloids are mostly found in higher plants (Angiosperms), especially in dicotyledonous plants. Many kinds and quantities of phytochemical substances that are found in plants can be impacted by environmental factors. Increased production and accumulation of secondary chemicals inside the plant can result from several conditions, including increased sunlight exposure, nutrient-deficient soil, pest infestation, and drought-induced stress (Andriani et al., 2015).

In summary, our study illustrates that the *E. cottonii* extract has potential as a natural antibacterial source, particularly when it comes to inhibit the growth of *E. coli.* This study provides preliminary findings that point to the need for more antibacterial research to enhance, optimize, and identify the bioactive compounds for possible use in complementary and alternative medicine to treat a range of diseases, especially those caused by *E. coli.* These results will also be helpful to others as a guide for the development of medicinal products

CONCLUSION

The study concludes that the n-hexane extract of the red algae *Eucheuma cottonii* was able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* with a weak inhibition category. While phytochemical test indicates that n-hexane extract of E. cottonii contained flavonoids, phenolics, and saponins. This study implies that improvements are needed in the *E. cottonii* extraction process to maximize the antibacterial properties of *E. cottonii*, such as using different solvents that have polar properties.

ACKNOWLEDGMENT

We sincerely thank the Faculty of Medicine, Pattimura University, which has funded this research through PNBP funds with contract number: 13/UN1366/SK/2023. We also thank the staff of the basic biology laboratory of FKIP Biology, Pattimura University.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 74 – 84 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Comparison effectiveness of e-booklets and videos based on SESD on students' analytical thinking skills

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Article Info

Article History:
Received 09 February 2024
Revised 14 March 2024
Accepted 30 March 2024
Published 30 April 2024

Keywords:
Thinking Analysis
E-booklet Media
Video Media
SESD

ABSTRACT

Analytical thinking skills are essential in science learning because they involve investigating a concept in everyday life, which is a product, fact, principle, and theory, and applying scientific methods as a problem-solving process. However, the analytical thinking skills possessed by junior high school students still need to improve. So, using media is one solution to improving analytical thinking skills. This research compares the effectiveness of SESD-based e-booklet media and SESD-based videos on analytical thinking skills. The method used includes quantitative comparative, which compares the effect of using e-booklets and SESD-based videos and tests the effectiveness of both media. The data analysis used was the Wilcoxon Test and effect size Cohen's d. The results of this study explain that video media and e-booklets influence analytical thinking skills. Still, SESD-based video media is superior to SESD-based e-booklet media in improving students' analytical thinking skills. The e-booklet and video media based on Science Education for Sustainable Development (SESD) is a learning media that integrates sustainable development education (ESD) into science learning, which contains the values of sustainable development for the future. So, the conclusion is that SESD-based video media is more effective than SESD-based e-booklet media.

Citation: Utomo, P.W., & Muna, I.A. (2024). Comparison effectiveness of e-booklets and videos based on SESD on students' analytical thinking skills. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 74-84. DOI: https://doi.org/10.31932/jpbio.v9i1.3318

INTRODUCTION

Analytical thinking skills are one of the essential higher-level thinking skills students possess. Students with good analytical thinking skills can help them understand complex material and solve every problem in the learning process (Ilma, Hamdani, & Lailiyah, 2017; Suryanda, Azrai, & Wari, 2018). *Analytical thinking* is a thinking ability that emphasizes students ' ability to solve problems

through the right solution. So that in the problem-solving process, students can describe, separate, and solve problems with the right solution (Yuwono, Sunarno, & Aminah, 2020; Zulfa & Rosyidah, 2020). Analytical thinking, in general, has three indicators, namely distinguishing, organizing, and connecting. (1) Distinguishing is the ability to group or classify into specific parts. (2) Organizing is a way to arrange and arrange certain parts to form order in a problem. (3) Connecting is linking one thing with another that still has a connection (Astriani, Susilo, Suwono, & Lukiati, 2018; Fitriani, Fadly, Fauziah, 2021). Analytical thinking is closely related to science learning.

Analytical thinking skills are needed in science learning because they involve investigating a concept in everyday life (Hasyim, 2018). *Science learning* is a scientific discipline emphasizing scientific methods directly related to analytical thinking skills. According to Risamasu (2016), science learning is a product of facts, principles, and theories, as well as the application of scientific methods used as a problem-solving process. The correlation between analytical thinking skills and science learning includes (1) the scientific research process; science learning often conducts practicum-based learning, where practicum collects data obtained systematically and adjusts the results to the theory. (2) decision making: science learning makes many decisions based on scientific facts. (3) the ability to find solutions to a problem; science learning requires analytical skills to identify problems and design solutions in the form of strategies that are appropriate to the need to be able to solve problems. Analytical thinking is crucial to help students develop a deeper understanding of scientific concepts by making good decisions based on scientific facts by science learning. So analytical thinking is a crucial ability to maximize learning in the classroom, especially in science learning (Ilma et al., 2017; Suryanda et al., 2018). However, analytical thinking skills in junior high school students still need to improve. According to Setiawaty et al. (2019), junior high school students' analytical thinking ability is still in the low category. In addition, Ilma et al. (2017) facts in the field show that the results of analytical thinking skills possessed by students still need to be higher. This is because students' ability to think analytically is less trained when in the learning process in class.

Based on interviews conducted with seventh-grade science teachers, students' analytical thinking skills are still in the low category, especially in class VII. One of the causes of this condition is the low motivation of students to discuss with the teacher. In addition, students also have difficulty in linking learning materials with everyday life. This causes teachers to face challenges in stimulating students to develop their analytical thinking skills. In addition, when the teacher tried to explain the material, most students ignored the explanation given. Instead, students prefer to be busy with their activities. This situation certainly adds to the complexity of the teacher's challenge in improving students' analytical thinking skills.

According to the opinion Annisa, Dwiastuti, & Fatmawati (2016), in the learning process in class, students rarely ask questions. Students prefer to answer questions from the teacher briefly, and not a few teachers also answer questions given to students. The average questions students give still range between C1 and C2 and are only dominated by sure students. Another factor that causes this is that most exam questions teachers give tend to be between C1-C3 and less to train students in solving C4 analysis-type questions. So Indonesian students still need to improve their ability in analytical thinking. Analytical thinking skills are essential for students. Students with good analytical thinking skills can help them understand complex material and solve every problem in the learning process. Thus, analytical thinking is a crucial ability to maximize learning in the classroom (Ilma et al., 2017; Suryanda et al., 2018). Therefore, educators are responsible for assisting students in improving analytical thinking skills; one of the efforts that can be made is to explore and utilize learning technology in science learning.

Nowadays, the development of technology has increased significantly. These developments affect all aspects of life, especially in education. One of the uses of technology in education is the innovative use of learning media to maximize more varied learning (Purnasari & Sadewo, 2020). Learning media is a tool or learning resource that aims to convey information effectively and efficiently in learning activities to achieve learning goals (Zahwa & Syafi'i, 2022; Ulia, 2018). In addition, Yusmanto (2018) suggests that learning media is a tool to make it easier for teachers to convey information. According to other studies, learning media is a tool used to improve the quality of education. This media makes learning more effective and efficient (Nasaruddin, 2018; Santanapurba & Hidayanti, 2018). Currently, the science learning media used by most teachers and students are books that tend to contain long readings, few pictures, and the colors displayed are less attractive, so students are less interested in reading the book (Rostikawati, Susanto, & Rahayu, 2019; Hanifah, Afrikani, & Yani, 2020). So, it is necessary to utilize other learning media to attract students' interest in learning. Some of these media that can be utilized are e-booklet media and videos based on Science Education for Sustainable Development (SESD).

Science Education for Sustainable Development (SESD)-based e-booklet and video media are learning media that include sustainable development in science learning. According to Eilks (2015), the SESD approach is an approach that integrates sustainable development education (ESD) in science learning. ESD is a sustainable development prepared for the future (Indrati & Hariadi, 2016). In addition, according to UNESCO, ESD is an approach based on the principles underlying sustainability and ideals so that it can produce good quality education and encourage sustainable human resources (Perkasa & Aznam, 2016).

SESD-based e-booklets and video learning media are essential to efficiently and innovatively conveying scientific information. The utilization of learning media is needed to attract students' attention so that teachers and students can achieve learning objectives and have a meaningful learning experience. (Baharizqi, Iskandar, & Kurniawan, 2023; Nurrita, 2018). According to Gultom, Retnowati, & Yani (2022); (Ginting, et al. (2022), e-booklets and video learning media can improve critical thinking ability in students from moderate to high levels. Critical thinking skills are closely related to analytical thinking. According to Anugraheni (2019), critical thinking is analyzing, connecting, and creating all aspects of a given problem. In addition, according to Nuryanti, Zubaidah, & Diantoro (2028), someone with critical thinking skills can analyze and evaluate the information obtained.

At this time, there are no studies that discuss the comparison of SESD-based e-booklet media and SESD-based video media on students' analytical thinking skills, such as research by Putri (2022) which discusses the comparison of the effectiveness of e-booklet media and animated videos on the knowledge and attitudes of adolescents related to anemia of adolescent girls at Kosgoro High School in Bogor City. In addition, research by Baliasti, A'yun, & Hidayati (2022) discusses the effectiveness of education using video and e-booklet media on the level of knowledge about dental and oral emergencies during the COVID-19 pandemic in high school students.Therefore, researchers raised the title with the theme of comparison of the use of SESD-based e-booklet media and SESD-based videos on biodiversity material to find out which media has a better influence and effectiveness on students' analytical thinking skills.

RESEARCH METHODS

Research Design

This research is included in quantitative comparative research where the data obtained are processed using statistical methods and comparing the results obtained to determine the difference in the results of a variable from two different groups. This research is intended to compare the

effect of using e-booklets and video media based on Science Education for Sustainable Development on analytical thinking ability. Figure 1. represents the research design.

A = O_a	X_1	O_b
B = O_c	X_2	O_d

Figure 1. Research Design

Note:

A : e-booklet group
B : video group
O_a : pre-test e-booklet group
O_b : post-test e-booklet group
O_c : pre-test video group
O_d : post-test video group
X_1 : SESD-based e-booklet media treatment
X_2 : SESD-based video media treatment

Population and Samples

This research was conducted at SMPN 1 Sawoo, Ponorogo Regency. The subjects of this study were all seventh-grade students at SMPN 1 Sawoo, totaling 154 students. The sample used amounted to 92 students. Then, the sample was divided into two groups: the e-booklet and video groups. In the sampling process, the researcher used a simple random sampling technique where the sample was taken randomly without regard to the strata in the population. This technique is used because the subjects in the population are homogeneous, so the selected sample can represent the population.

Instruments

This research instrument is an essay test consisting of a pre-test and post-test, each with 5 analysis-based questions. The instrument used has undergone a validation test conducted by two validators and obtained very valid results. After that, validity and reliability tests were conducted on 30 respondents outside the sample. The reliability test results for 10 questions obtained a result of 0.745; this value is higher than the Cronbach Alpha value (0.745> 0.6), so the instrument is reliable. The essay test instrument can be seen in Table 1. and Table 2.

Table 1. Biodiversity Pre-Test

No.	Indicators	Questions
1	Connecting	What is the relationship between littering behavior and biodiversity preservation?
2	Connecting	The Javan tiger (Panthera tigris sondaica) is a subspecies of tiger that lives on the island of Java. The Javan tiger was declared extinct in the 1980s. Based on your understanding, why did the Javanese tiger become extinct?
3	Organizing	Humans have an essential role in maintaining biodiversity; bad human behavior, such as littering and illegal logging, hurts biodiversity, causing scarcity and extinction of a species. Vice versa, good human behavior has a positive contribution to the preservation of biodiversity. What are some excellent human

		behaviors that affect the preservation of biodiversity?
4	Distinguishing	Conservation can be divided into two types: in situ and ex situ. Both types of conservation have the same goal of keeping biodiversity sustainable. But in its application, in-situ and ex-situ conservation have differences. What is the difference between in-situ and ex-situ conservation?
5	Distinguishing	In an area, residents found a tiger trapped in a pig trap made by an irresponsible person. The tiger suffered severe injuries to its legs and other parts of its body. So, with this condition, the tiger will not survive if released directly into the forest. Based on this problem, what conservation is appropriate for the tiger's condition? Why is this the case?

Table 2. Biodiversity Post-Test

No.	Indicators	Questions
1	Connecting	Take a look at this Figure 2. The picture is a picture of air pollution caused by motorized vehicles.  **Figure 2.** Pollution Caused by Motorized Vehicles. How is the picture related to the preservation of biodiversity?
2	Organizing	One of the factors that can affect biodiversity is climate change. Climate change is caused by global warming triggered by air pollution; one example comes from power plants burning fossil fuels, such as coal, oil, and natural gas. Based on this, what can be done to anticipate climate change so that biodiversity is maintained?
3	Connecting	In an area, it is known that the people there have a unique belief in sacred forests. The community believes that their ancestors inhabited the forest, so there are several rules or taboos from the sacred forest, namely not being allowed to go in and out without permission from the tribal leader and take a plant or hunt in the forest. The forest is only opened twice a year for spiritual activities of the local community. Based on this narrative, how can this behavior affect the preservation of biodiversity?
4	Distinguishing	In an area, residents found a tiger trapped in a pig trap made by an irresponsible person. The tiger suffered severe injuries to its legs and several other body parts. So, with this condition, the tiger will not survive if released directly into the forest. Based on this problem, what conservation is appropriate for the tiger's condition?
5	Distinguishing	The Ibu Kota Nusantara (IKN) is a city built by applying the environmentally friendly or green city concept. Unlike other

	cities, which were built without applying green city. What is the difference between cities that use the concept of green city and cities that do not apply the concept of green city to preserving biodiversity?

Procedures

The research procedure was carried out through 3 stages namely, (1) The preparation stage, namely by compiling research instruments and conducting instrument validation tests. (2) The second stage is to collect data by conducting a pre-test followed by giving treatment to each group and then carrying out the post-test. (3) The next stage is to analyze the data obtained from the pre-test and post-test using IBM SPSS Statistic 22 software to compare the results obtained from each group.

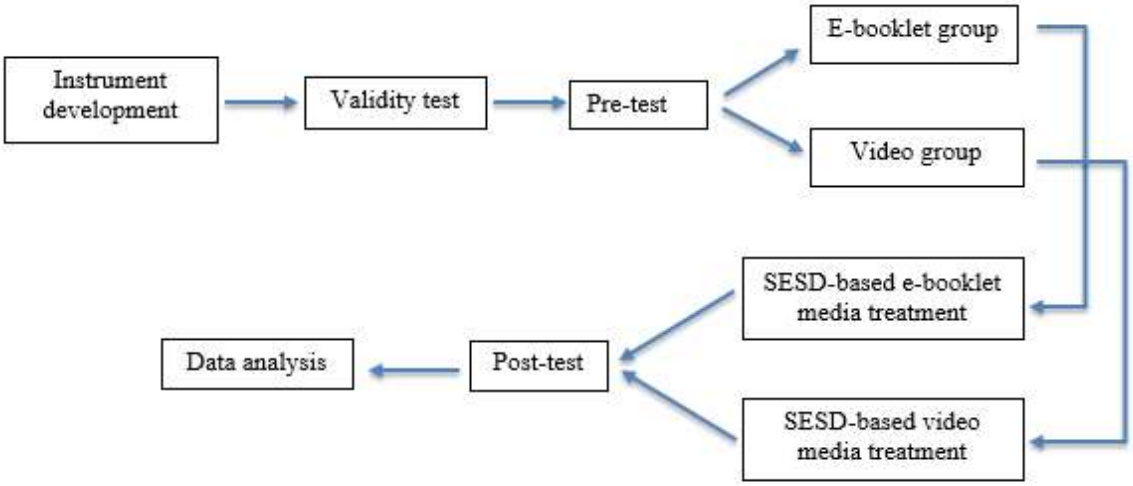

Figure 3. Research Procedures

Data Analysis

The data analysis used is a prerequisite test, namely the normality test and homogeneity test. If the prerequisite test meets the predetermined criteria, proceed with the hypothesis test, namely the paired sample t-test. However, suppose the prerequisite test needs to meet the criteria. In that case, the hypothesis test uses the Wilcoxon test, to determine the effect of the two media on students' analytical thinking skills. In addition, data analysis also uses the effect size Cohen's d, which is used to determine the effectiveness of using SESD-based e-booklets and video media.

Table 3. Categories Effect Size Cohen's d Distribution

Effect Size (ES)	Interpretation
0,00 ≤ ES < 0,20	Very low
0,21 ≤ ES < 0,50	Low
0,51 ≤ ES < 1,00	Moderate
1,00 < ES	Large

(Cohen's in Utami & Roektiningkroem, 2018)

RESULTS

This research examines and compares how SESD-based video and e-booklet media improve students' analytical thinking abilities by providing the same learning to both experimental groups.

Students who were taught using e-booklet media in the pre-test had a Sig. The value is 0.000, smaller than the α value (0.000 < 0.05), thus indicating that the data is not normally distributed. On the other hand, in the post-test normality test, the e-booklet obtained a value of 0.099, thus indicating a normal distribution because it was more significant than the α value (0.099 > 0.05). Furthermore, the students who instructed via video got Sig, especially in the pre-test. 0.022. It is not normally distributed because the value is smaller than the α value (0.022 < 0.05). The video media post-test findings also showed that the results were not normally distributed with Sig. The value is 0.030, smaller than the α value (0.030 < 0.05). The prerequisite test results do not meet the criteria, so the Wilcoxon Test is used as a substitute for the Paired Sample T-Test in non-parametric tests to determine the comparison of the increase in the utilization of SESD-based e-booklets and SESD-based videos on students' analytical thinking skills on biodiversity material.

Table 4. Normality Test Results

Media	Shapiro-Wilk		
	Statistic	df	Sig.
Pre-test e-booklet	.812	46	.000
Post-test e-booklet	.958	46	.099
Pre-test video	.941	46	.022
Post-test video	.945	46	.030

The Wilcoxon test results in Table 5. show that the Video and E-booklet groups obtained an Asymp. Sig value of 0.000, where the Asymp. sig value is smaller than the α value (e-booklet = 0.000 < 0.05 and video = 0.000 < 0.05). So, comparing the use of SESD-based e-booklets and SESD-based videos both experience a significant increase in students' analytical thinking skills.

Table 5. Wilcoxon Non-Parametrik Test Results

	Post-test – pre-test (e-booklet)	Post-test – pre-test (Video)
Asysmp. Sig (2-tailed)	.000	.000

A comparison of the improvement in the use of SESD-based e-booklets and SESD-based videos in detail can be seen in Table 6. The average post-test score on e-booklet media is 59.11, more significant than the average pre-test on e-booklet media, which is 42.20, with the highest score on the post-test score of 94. In addition, on video media, the average post-test score was 59.30, more significant than the average pre-test, which was 41.28, with the highest score on the post-test score of 88.

Table 6. Descriptive Statistics Wilcoxon Test

	N	Mean	Std. Deviation	Min	Max
Pre-test e-booklet	46	42.20	11.181	24	94
Pre-test Video	46	41.28	9.162	24	65
Post-test e-booklet	46	59.11	17.240	29	94
Post-test video	46	59.30	16.153	29	88

Based on table 6. shows an increase in the average score of both media groups. Students with video learning media experienced a higher increase than those using e-booklet media. However, the

average difference between video and e-booklet media is insignificant. From this, video media is slightly better than e-booklet media in improving analytical thinking skills.

Effect size Cohen's d test is used to determine how effective SESD-based e-booklet and video media use is in improving analytical thinking skills. Effect size Cohen's d values for e-booklet and video media are 1.07 and 1.17, respectively. So, SESD-based e-booklet media and SESD-based video media fall into the "large" category. Based on this, students who learn using SESD-based video media are slightly superior to those who use SESD-based e-booklet media.

Table 7. Effect size Cohen's d Results

Media	Effect Size	Category
SESD-based e-booklet	1,07	Large
SESD-based video	1,17	Large

DISCUSSION

Considering the outcomes of the Wilcoxon test in Table 5. Comparison the use of SESD-based e-booklet media and SESD-based video media both experienced an increase in analytical thinking skills, so it can be said that the research conducted here is by what was done by (Gultom et al., 2022; Ginting et al., 2022) that e-booklet and video learning media can enhance critical thinking ability in students from middle to high levels. Critical thinking ability has a strong relationship with analytical thinking ability. According to Anugraheni (2019), critical thinking is analyzing, connecting, and creating all aspects of a given problem. In addition, according to Nuryanti et al. (2028), someone with critical thinking skills can analyze and evaluate the information obtained. However, both media significantly affect analytical thinking skills, based on Table 6. and Table 7. SESD-based video media is more effective than SESD-based e-booklet media in improving analytical thinking skills.

E-booklets are learning media that present material content in a concise manner and are combined with various images to attract student interest and assist students in broadening their horizons, which is reachable through modern gadgets like computers, tablets, and smartphones (Darlen et al., 2015; Asyhari & Diani, 2017). The utilization of reading materials must be tailored to relevant information as well as the needs and abilities of students to motivate students and improve their analytical thinking skills. Meanwhile, learning videos are audio-visual media that produce dynamic and exciting impressions and can channel messages through thoughts, feelings, and attention that are useful in learning (Yudianto, 2017; Amir, 2016). Video media has quite a lot of interest as it can be accessed quickly and can help students obtain information without a teacher's assistance. (Hasiru, Badu, & Uno, 2021; Arsyad in Yudianto, 2017).

Science Education for Sustainable Development (SESD)-based e-booklet and video media are learning media that contain sustainable development values for the future. SESD integrates sustainable development education (ESD) into science learning. The Science Education for Sustainable Development approach emphasizes the importance of comprehending and protecting the environment, such as climate change, pollution, biodiversity, and natural resource management.

In addition, the SESD approach also emphasizes the importance of understanding social and developing knowledge and skills to prepare for future challenges. Through the SESD approach during the learning process, students are taught to understand the impact of human activities on the environment and how science can solve problems that arise from human actions. In addition, in the classroom learning process, students are also taught about the importance of maintaining economic and social sustainability in sustainable development and helping students develop responsible attitudes towards society and the environment. Students are taught to respect cultural

and social values, respect and maintain biodiversity, and actively participate in environmental conservation efforts in sustainable development (Eilks, 2015).

CONCLUSION

Based on this research, SESD-based video learning media and SESD-based e-booklets are learning media that affect students' analytical thinking skills. This research reveals that the use of video media is superior to the use of e-booklet media in improving analytical thinking skills. Science Education for Sustainable Development (SESD)-based e-booklet and video media are learning media that contain sustainable development values for the future. Video media and SESD-based e-booklet media can provide maximum results if adjusted to the needs of students at the student's ability level. Based on the results of this discussion, several suggestions are proposed which are expected to have usefulness in this study. For further researchers, this research can be used as a reference in studying more broadly by connecting to other variables to obtain more valuable results.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 85 – 97 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Expert response to the development of interactive video as teaching media on cell material

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Article Info

Article History:
Received 24 February 2024
Revised 28 March 2024
Accepted 09 April 2024
Published 30 April 2024

Keywords:
Learning media, interactive learning video
4D

ABSTRACT

This research originated from the lack of utilization of learning media by educators in learning biology in high school which has the potential to affect student interest in learning. This study aims to produce learning media in the form of a valid interactive video. This research refers to the research and development approach by applying the 4D development model, which consists of defining, designing, developing, and disseminating stages. The focus of this research is one of the development stages of interactive learning videos, involving the assessment of expert validators who assess three main aspects, namely the presentation of material, video quality, and language used. Data analysis was conducted using descriptive statistics. Based on the data analysis, the validity level of the developed interactive learning video reached a score of 4.6. The score indicates that the video was declared into the "valid" category. In this study, four discussion videos discussed cell material. The conclusion from the results of the data analysis shows that the interactive learning videos developed on cell concepts meet the valid criteria.

Citation: Meiriska, A., Adnan, & Junda, M. (2024). Expert response to the development of interactive video as teaching media on cell material. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 85-97. DOI: https://doi.org/10.31932/jpbio.v9i1.3384

INTRODUCTION

In this modern era, technology plays an important role in the learning process. Appropriate utilization of technology can increase the effectiveness of learning. In addition, the use of technology can also increase learners' knowledge about the development of technology itself (Channy & Wibawanto, 2015). In line with that, the Ministry of Education has implemented and developed Information and Communication Technology (ICT) in education, as stated by Adnan and his colleagues (Adnan et al., 2014).

ICT-based learning opens new opportunities in presenting various learning materials interestingly and interactively. Technology-based learning media, such as learning videos,

multimedia presentations, educational games, and online learning platforms, have become popular choices in the modern education process. The diversity of these learning media provides flexibility for learners to learn according to their learning styles. In addition, the use of ICT-based learning media also allows collaboration and discussion between learners, both in a classroom environment and virtually. Learners can interact with fellow learners and teachers online, share thoughts, ask questions, and give each other feedback. This creates a more dynamic learning atmosphere, builds social skills, and develops critical thinking.

Based on the results of a questionnaire distributed to twenty biology teachers in South Sulawesi, it was revealed that in teaching and learning activities, both theory and practice, teachers predominantly use printed teaching materials in the form of package books and sometimes interspersed with the provision of material through Powerpoint media. For some special materials, additional teaching materials are used in the form of learning videos sourced from YouTube. This is also supported by the results of questionnaire answers from several students of Senior High School 9 Makassar, who stated that some biology materials are difficult to understand because the media used by teachers are printed books with language that is sometimes less understood. In addition, students also argue that it would be better if learning media could be packaged more interestingly so that students are interested in reading and understanding it. Learning videos are considered good to use in the learning process, but there is a drawback video teaching materials tend to make students more passive.

Interactive learning comes as an effective solution in stimulating students to always want and have an interest in learning. In the process, students are required to respond to the material presented by the educator. The media can be used as a forum for students to respond and interact with each other. Many media can be utilized to provide interactive learning. In learning Biology, it is necessary to use learning media that can simultaneously integrate text, images, sound, and video so that learning biology becomes more interesting, effective, and efficient (Rizki, 2017)

According to Yudianto (2017), video is an electronic media that can combine audio and visual technology to produce a dynamic and interesting show. Through video media, learning can be easily accessed and used and can reach a wide and interesting audience. According to R. Rahardjo et al (2012), the use of video as a learning media will increase the concentration and memory of students in learning. Video can collect information entry in humans by 94% through vision and hearing, which can strengthen learners' memories by more than 50% of what is seen and heard in the video.

Based on the existing problems, interactive learning videos can be a good solution for students to understand the material and increase their interest in learning independently. For educators, interactive learning videos can also be used as an efficient way to deliver learning materials thoroughly. Interactive learning videos integrated with the Edpuzzle learning application can also be a solution for educators to monitor the extent to which students understand the material presented. This is supported by research conducted by Sirri & Lestari (2020) which states that the use of Edpuzzle and WhatsApp groups can improve learning outcomes and student interest in mathematics.

Based on the conditions and potential that exist in schools, both students who need interesting teaching materials and teachers who still have difficulty motivating students to learn, researchers are interested in conducting product development and research with the title "Development of Interactive Learning Videos on Cell Material for Class XI Senior High School." The cell material was chosen because it has a breadth and depth that can be classified as microscopic material. The discussion of cells cannot be seen directly with the human eye. Several factors can cause a low understanding of the cell concept, such as the complexity and abstraction of the cell concept, the lack of interactivity in the learning process, and students' difficulties in

understanding foreign languages or related Latin. This finding is in line with research conducted by Dinarni et al (2021) which shows that the concept of cells tends to be conceptual and often causes misconceptions, especially in the sub-concept of cell structure and function. This is due to the abstract nature of the concept which confuses students' understanding. Similarly, according to Rahman et al (2018), sub-concepts related to animal and plant cell organelles are considered abstract because they cannot be observed directly, which results in difficulties for students in imagining their structure and body shape.

By using interactive learning videos, it is expected that students can more easily understand cell material. This video will integrate text, images, sound, and video to make learning more interesting, effective, and efficient. In addition, through the use of the Edpuzzle application, students can actively interact with learning materials, and educators can monitor the extent to which students understand the material.

With the development of this interactive learning video, it is expected that learning biology at the Senior High School level can be more interesting, effective, and efficient. Students will be more motivated to learn and have a better understanding of cell material. Interactive learning can also help students in developing social skills and critical thinking. By utilizing technology in learning, we can create a better learning environment and support the development of learners in this modern era.

RESEARCH METHODS

Research Design

The type of research used in this study is research and development (R&D). The product development model used refers to the 4D model by Thiagarajan. This is relevant to the goals of researchers who want to produce valid products, in this case in the form of interactive learning video media on cell material for class XI Senior High School.

Population and Samples

This study involved two experienced validators who evaluated the validity of interactive learning videos. The data collected included both quantitative and qualitative information. Quantitative data was gathered through the assessment of module feasibility, covering aspects such as material presentation, video quality, and language proficiency. Additionally, practical feedback was collected from both lecturers and students. Qualitative data, on the other hand, stemmed from interviews and observations with lecturers, as well as notes on suggestions for product enhancement provided by the validators.

Instruments

The research instrument developed was a product validation questionnaire. The validation questionnaire aims to obtain assessments and suggestions from expert validators on interactive learning videos that have been made by researchers. The video validation questionnaire was developed by researchers by looking at several aspects, namely aspects of material presentation, video quality, and language use. The validation questionnaire is equipped with 5 answer choices, namely very good (5), good (4), sufficient (3), less good (2), and not good (1). Before use, the research instruments that have been developed are first validated by expert validators.

Procedures

The development of interactive learning videos on cell material begins with the defining stage, which consists of front-end analysis, learner analysis, concept analysis, task analysis, and formulation of learning objectives. The next stage is the design of interactive learning video media

on cell material. This stage includes the preparation of material, preparation of video scripts and storyboards, selection of video materials, and editing and mixing processes tailored to cell material. The interactive learning video developed then goes through the development stage by going through the validity test process. Media assessment was carried out by two expert validators using an interactive learning video assessment instrument that had been validated and declared suitable for use. The validity test of research instruments and products is carried out to ensure that the research instruments used and the products developed are truly feasible/valid based on the assessment of expert validators. After the research instruments and products are shown to the validator and declared valid, the data obtained is then analyzed.

Data Analysis

The data analysis technique used to process the research data is descriptive statistics. This analysis is used to process data obtained in the form of analysis of research questionnaire criteria scores using a Likert scale (1 to 5). According to Sudjana (2007), to determine the validity assessment by the validator, the formula is used:

$$\text{VSA} = \frac{\sum Validator\ Score}{\sum items}$$

Keterangan:

VSA	: Validation Score for each Aspect
∑ validator score	: The sum of the scores given by both validators
∑ items	: Number of items assessed for each aspect

RESULTS

This research produces products in the form of interactive learning videos on cell material that are valid. The development of this video refers to the 4D design which consists of four stages, namely the defining (define), designing (design), developing (develop), and disseminating (disseminate) stages. In the four stages of the 4D development design, three stages were carried out to produce a valid product. The results obtained at the stage of developing interactive learning videos on cell material are described as follows.

Results of Defining Stage (Define)

The development of interactive learning videos on cell material begins with the defining stage, which consists of front-end analysis, learner analysis, concept analysis, task analysis, and formulation of learning objectives. Front-end and learner analysis is the stage of collecting information by providing questionnaires to teachers and students to find out the conditions and problems experienced in the learning process, especially in biology subjects. the high needs of teachers and students for teaching media in the form of videos on cell material that are truly by the learning objectives to be achieved. The teacher's need for video teaching media is related to the substance of microscopic cell material so visualization is needed that makes it easier for students to understand the material. The use of video media is considered by teachers to be suitable for providing understanding to students, but there are shortcomings, namely, students tend to be passive when taught with video teaching media. So, teaching media is needed in the form of learning videos that can also increase student activity. In this case, researchers provide a solution by developing interactive learning videos on cell material.

After knowing the problems and outcomes to be developed, the concept analysis stage is carried out to analyze the sub-materials and indicators used in the video. The analysis is based on the core competencies (KI) and basic competencies (KD) listed in the 2013 curriculum (K13). At this stage of the analysis, the development of material that will be used in the video refers to the

syllabus of Senior High School biology subjects in the 2013 curriculum that applies and also looks at the needs of students in the learning process.

At the task analysis stage is done by mapping the materials based on the results of concept analysis. The results of this analysis become some of the main material, thus forming several learning videos according to the material. In each video material, several questions are inserted as a reference for teachers in assessing students' understanding. The materials that will be included in the interactive learning video are Cell Introduction, Cell Size and Shape, Prokaryotic Cell Structure, and Eukaryotic Cell Structure.

Goal analysis becomes a reference in the development of interactive learning videos. The activities carried out in the analysis of objectives are reviewing the Basic Competencies and Competency Achievement Indicators that have been developed. The formulation of learning objectives on cell material was developed by the 2013 Curriculum. The selected learning objectives are then formulated by containing ABCD components (Audience, Behavior, Condition, and Degree).

Results of the Design Stage (Design)

The design stage is the video design stage which includes the preparation of material, preparation of video scripts (scripts) and storyboards (storyboards), collection of video materials, to the editing and mixing stages. Based on the grouping of learning objectives that have been carried out, four sub-points of cell material are collected. The four materials are Cell Introduction, Cell Size and Shape, Prokaryotic Cell Structure, and Eukaryotic Cell Structure. Each material will be used as the subject matter in the interactive learning video developed so that the resulting product totals 4 (four) videos.

The preparation of video scripts and storyboards is adjusted to the learning components in general which consist of apperception, material provision, assignments, and closing. Storyboard snippets of interactive learning videos can be seen in Table 1.

Table 1. Overview of interactive learning video storyboard design

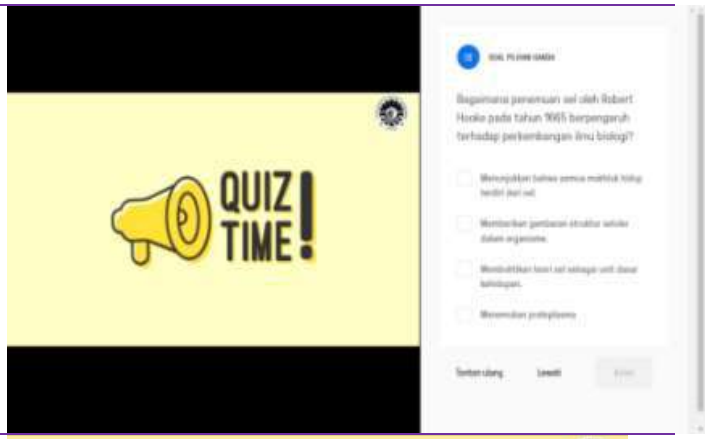
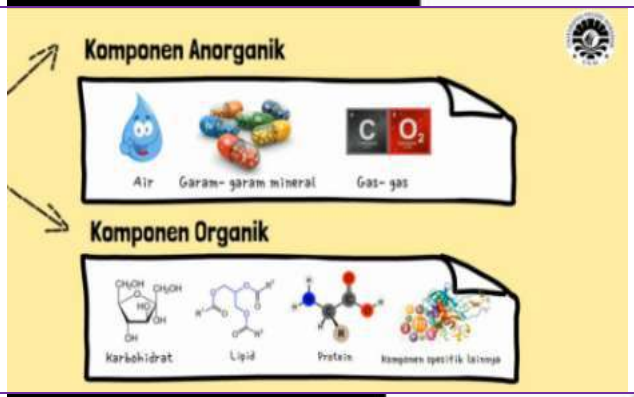
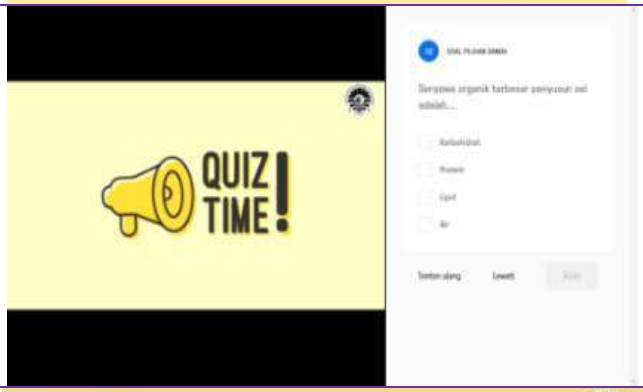
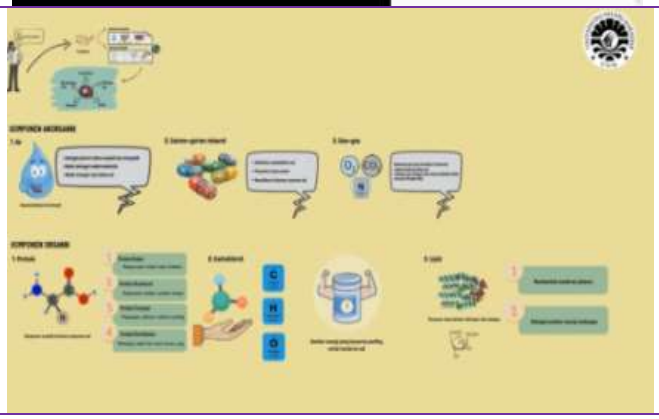
Video Component	Description
Apperception	Activities that lure learners into learning activities
Intro	Description of video title and subject matter
Opening	-
Material Description	Contains the main discussion or learning material. Learning materials are packaged by combining various supporting elements that are interesting and by the material. Some scenes in the explanation of the material are also presented with a typical whiteboard animation, which is a video display that illustrates the narrative with explanations as if using a whiteboard directly.
Interactive Scene	Scenes that display evaluation questions or comment columns that can be directly responded to by students. This interactive scene will appear as an interlude at the transition of the subject matter.
Advanced Material	Is the follow-up material after the interactive scene?
Interactive Scene	Follow-up questions or comments based on the material that has been presented previously.
Closing	contains the conclusion of the main discussion in the interactive video. At the end of the video, there is also an interactive scene that becomes a closing evaluation of the video.

Results of the Development Stage (Develop)

The development of interactive videos is based on the results of defining and designing that have been illustrated through storyboards. The interactive learning video developed contains 4 cell submaterials, namely video 1 cell introduction, video 2 cell size and shape, video 3 prokaryotic cell structure, and video 4 eukaryotic cell structure. The description of some components of the developed interactive learning video can be seen in Table 2.

Table 2. Description of interactive learning video specifications

Component	View
Apperception	
Intro	
Opening	
Description of material	

Component	View
Interactive Scene	
Advanced Material	
Interactive Scene	
Closing	

The results of the interactive video development were then validated by two expert validators. Expert validators assessed the product from various aspects and provided suggestions as

revision material for the improvement of interactive videos. The suggestions from the validators on the development of this interactive video are contained in Table 3.

Table 3. Corrections and suggestions from expert validators

No.	Suggestion
1	Provide a pause at each turn of the discussion. Learners need time to internalize knowledge before getting the next knowledge.
2	Pay attention to the suitability of the text that appears with the narration. Some are not yet appropriate.
3	The articulation of some words needs to be clarified, especially in scientific words, some still sound unclear.
4	The mention of the term "Pili" should be read as "Fili".
5	Provide an opening narration before entering the question at each transition of material to the evaluation question.
6	Pay attention to the duration of the video
7	Replace some animations and images that are considered unclear, and eliminate some video scenes that are considered less supportive of the material.

Suggestions from validators are used as a basis for making product improvements. After that, the assessment from the expert validator is analyzed to obtain validation data as a reference that the product developed has met the valid criteria and is suitable for use in the learning process. The results of the analysis of the validity of interactive learning videos based on aspects of content feasibility can be seen in Table 4.

Table 4. Interactive learning video validation data based on content feasibility aspects

No.	Indicator	Value	Posted on
1.	Suitability of material with KD 3.1 (2013 Curriculum)	4,8	Valid
2.	Suitability of material with learning objectives	5	Very Valid
3.	Clarity of material presentation	4,7	Valid
4.	Systematic presentation of material	4,8	Valid
5.	The material presented does not contain misconceptions	4,2	Valid
6.	The suitability of the animation / image used with the material	4,8	Valid
	Average	4,7	Valid

Based on the analysis in Table 4, the average value of the final product validation is 4.7 which means valid. The validation results show that the developed product has met the assessment criteria in the video quality aspect. Furthermore, the results of the analysis of the validity of interactive learning videos on cell material based on video quality aspects can be seen in Table 5.

Table 5. Interactive learning video validation data based on video quality aspects

No.	Indicator	Nilai	Posted on
1.	The visual quality of the video	4,6	Valid
2.	Clarity of animation/image display	4,1	Valid
3.	Clarity of audio in the video	4,6	Valid
4.	Audio compatibility with the text displayed (audio	4,2	Valid

	mixing quality)		
5.	The intonation of the narrator in the video	4,8	Valid
6.	The clarity of the narrator's articulation in delivering the material	4,5	Valid
7.	The suitability of the dominance of the narrator and background music in the video	4,2	Valid
8.	Suitability of video duration to the learning process	4,7	Valid
9.	The suitability of the text displayed with the narration	4,8	Valid
10.	Lighting Quality	4,7	Valid
11.	Readability of text on video	4,1	Valid
	Average	4,5	Valid

Based on the analysis in Table 5, the average value obtained in the validation of the final product validation is 4.5 which means valid. The results of this validation, indicate that the product that has been developed has met the assessment criteria on the video quality aspect. Furthermore, the results of the analysis of the validity of interactive learning videos on cell material based on language aspects can be seen in Table 6.

Table 6. Interactive learning video validation data based on language aspects

No	Indicators	Value	Posted on
1.	Use of Indonesian language by Refined Spelling (EYD)	4,5	Valid
2.	The language used is simple, easy to understand, and communicative	4,5	Valid
	Average	4,5	Valid

Based on the analysis in Table 6, the average value obtained in the validation of the final product validation resulted in a value of 4.5 which means valid. The results of this validation indicate that the product that has been developed has met the assessment criteria in the language aspect. Furthermore, the accumulated results of the validity of interactive learning videos can be seen in Table 7.

Table 7. Final results of product validation

No.	Assessment Aspect	Average	Note
1.	Presentation of Material	4,7	Valid
2.	Video Quality	4,7	Valid
3.	Language	4,5	Valid
	Average	4,6	Valid

Based on the analysis in Table 7, the total average value of the validity of interactive learning videos on cell material is Va = 4.6, which means that it falls into the valid category ($4 \leq Va \leq 5$) and interactive learning video products on cell material are feasible to use.

DISCUSSION

The development of interactive learning videos on cell structure and function material for class XI Senior High School Equivalent refers to the 4D model which consists of four stages, namely the defining (define), designing (design), developing (development), and disseminating (disseminate) stages. As for the dissemination stage, it was not carried out due to time constraints.

The product resulting from this development is an interactive learning video of valid cell structure and function material.

The video developed is an interactive learning video on cell structure and function material that is tailored to the characteristics of students, learning objectives, and learning environments that allow videos to be used in the learning process. The interactivity of the learning video is expected to increase students' activities in learning. This interactive learning video is also packaged with an attractive appearance to increase students' interest in learning.

The development of interactive learning videos on cell structure and function material begins with the defining stage, which consists of front-end analysis, learner analysis, concept analysis, task analysis, and formulation of learning objectives. Front-end and learner analysis is the stage of collecting information by providing questionnaires to teachers and students to find out the conditions and problems experienced in the learning process, especially in biology subjects. Questionnaires were given to 20 biology teachers in South Sulawesi and 52 students of class XI Senior High School 9 Makassar.

The results of the analysis of teacher and learner questionnaires show the high need of teachers and learners for teaching media in the form of videos on the material of the structure and function of cells that are truly in accordance with the learning objectives to be achieved. The teacher's need for video teaching media is related to the substance of microscopic cell material so that visualization is needed that makes it easier for students to understand the material. This is in line with Alexander et al (2018) statement that audio-visual media can make students understand better, be independent, active, and increase students' interest in learning in the learning process. The use of video media is considered by teachers to be suitable for providing understanding to students, but there are shortcomings, namely students tend to be passive when taught with video teaching media. So that teaching media is needed in the form of learning videos that can also increase student activity. In this case, researchers provide a solution by developing interactive learning videos on cell structure and function material.

After knowing the problems and outcomes to be developed, the stages of concept analysis, tasks, and formulation of learning objectives are carried out to analyze the sub-materials and indicators used in the video. The analysis is based on the core competencies (KI) and basic competencies (KD) listed in the 2013 curriculum (K13). At this stage of the analysis, the development of material to be used in the video refers to the syllabus of Senior high school biology subjects in the 2013 curriculum that applies and also looks at the needs of students in the learning process.

The next stage is the design of interactive learning video media on cell structure and function. This stage includes the preparation of material, preparation of video scripts and storyboards, selection of video materials, and editing and mixing processes tailored to the material of cell structure and function. After all the processes are completed, the initial product (prototype) of an interactive learning video of cell structure and function material is produced. Video design is made relevant to the learning process, where the content of the video begins with apperception activities, providing material, providing evaluation questions, and closing. As stated by Riyana (2007), video media development goes through several plans such as preparing the devices that will be used, and making a video framework that includes determining the introduction, opening show, introduction, video content, and closing.

The interactive learning video that was developed then went through the development stage by going through the validity test process. The validity test is carried out to determine the feasibility of the product that has been developed. Media assessment was carried out by two expert validators using an interactive learning video assessment instrument that had been validated and declared suitable for use before. The validation assessment consisted of nineteen questions that

were representative of three aspects of the assessment, namely aspects of material presentation, video quality, and language.

The validity test was assessed by two expert validators who assessed various aspects and the examination was carried out in stages. The interactive video developed has been revised several times based on suggestions from the validators. The first validation by the validator stated that the product could be used with minor revisions so improvements needed to be made to the product that had been developed. The validator then provided some improvement suggestions to improve the material in the video script. There are still some concepts that are not correct. Researchers are also advised to refer to more relevant and reliable sources to avoid misconceptions. In addition, the validator also suggested paying attention to the compatibility between the text that appears and the audio that is heard.

Another suggestion given by the validator was to provide a longer pause at each turn of the discussion. It was explained that learners need time to internalize the information received before moving on to the next information. Poor pause settings will have an impact on the level of understanding of learners. In addition, there are still some words whose articulation sounds less clear so it is necessary to improve the mention, especially in the mention of scientific terms. The validator also suggested giving preliminary instructions before giving interlude questions on the material so that students are not surprised when the interlude questions appear when the video is shown. Furthermore, the validator suggested replacing some animations and images that were considered unclear, as well as eliminating some video scenes that were considered less supportive of the material.

The interactive learning video that had been revised based on the suggestions and corrections from the validators was then assessed by the validators using an assessment sheet consisting of several aspects of assessment divided by several aspects, namely presentation of material, video quality, and language. The average value for the material presentation aspect is 4.7, the video quality aspect is 4.5, and the language aspect gets an average value of 4.5. Based on the validity level category, the value is categorized as valid and suitable for use in supporting more interesting and interactive learning. The interactive learning video developed by the researcher is declared valid with a validity value of 4.6 which is in the interval $4 \leq Va \leq 5$ which is assessed from various aspects and has met the requirements based on the assessment by validator one and validator two. So that the interactive learning video of cell structure and function material is declared feasible to be used as teaching media for class XI Senior High School students.

CONCLUSION

The resulting product from this development research is a valid interactive learning video, tailored to students' characteristics, learning objectives, and environments, aiming to enhance engagement through interactivity and appealing presentation. Beginning with front-end and learner analysis, followed by concept and task analysis, and formulation of learning objectives aligned with the 2013 curriculum, the content addresses syllabus requirements and student needs. In the design stage, material preparation, scripting, storyboarding, selection of video elements, and editing ensure relevance to the learning process. After validation by expert validators and subsequent revisions addressing script accuracy, articulation clarity, pacing, and visual clarity, the video achieves high scores in material presentation, video quality, and language, indicating its suitability for classroom use with a validity value of 4.6. Thus, the interactive learning video offers an engaging and effective teaching medium for Class XI Senior High School students.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 98 – 110 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Development booklet 3-dimensional image-based digital as learning media on virus materials for senior high school

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Article Info

Article History:
Received 28 February 2024
Revised 29 March 2024
Accepted 04 April 2024
Published 30 April 2024

Keywords:
Booklet Digital
3D Images
Learning Media
Viruses

ABSTRACT

Learning media is something that is used to increase student's interest in learning activities. Limited learning media makes biology material difficult for students to understand. Virus material is a type of biological material that has a lot of material and images that are difficult to understand. So, learning media needed that is concise and has attractive images in the form of booklet 3-dimensional image-based digital as a learning medium for viral material for Senior High School. This research aims to test the feasibility and practicality booklet 3-dimensional image-based digital as a learning medium for virus material at Kartika High School 1-5 Padang. This research is a development using Thiagarajan's 4-D development model. Based on the research results obtained as follows: material expert validation results 90.15% in the very good category, media experts 94.69% in the very good category, teacher practicality test results 95.83% in the very good category, student practicality test 89.10% in the very good category. Can be concluded that booklet digital material developed on viral material is feasible and capable of increasing student interest.

Citation: Sakinah, S., Arsih, F., Syamsurizal, & Rahmatika, H. (2024). Development booklet 3-dimensional image-based digital as learning media on virus materials for senior high school. *JPBIC (Jurnal Pendidikan Biologi), 9*(1), 98-110. DOI: https://doi.org/10.31932/jpbio.v9i1.3394

INTRODUCTION

Human needs are currently made easier by the existence of technology, technological advances are developing rapidly, and all information from mass media such as cellphones, radio, television, and others has become completely digital (Setiawan, 2017). Technological advances influence all aspects of human life, especially in the field of education (Maritsa et al., 2021). In the education system, there is knowledge that is important for students to obtain as much as possible so that it can be realized in real life and be useful for society and its surroundings. The implementation of the education system, apart from being for the future, is also prioritized for teenagers who are progressing to the stage of maturity (Ihsan, 2003).

The education system has a learning process that involves educators and students. The learning process is carried out so that there is a change in students' attitudes and behavior, this happens if there is interaction between teachers and students or between other students (Aunurrahman, 2012). During the learning process, media is needed that contains information about the material to be taught. According to Intika (2018) to support learning, media is used as an intermediary containing various learning resources for teachers and students.

Learning media is anything useful for conveying important things from the author so that students' feelings, thoughts, attention, and interests can be stimulated so that the learning process occurs (Sadiman, Arief S et al., 2014). Designing learning media must pay attention to the students' character (Novianti & Syamsurizal, 2021). Learning media can increase students' sense of responsibility and control learning as well as take students' long-term perspective regarding their learning (Hasan et al., 2021).

Learning media that suit students' needs will provide good output, including changes in students' attitudes. Using media appropriately when delivering material will achieve good learning results. Learning media in the digital era means students must have creative ideas. Educators are required to be able to use modern learning media. Several studies have suggested the positive influence of using media as part of overall learning activities or the main way learning takes place in class (Hasan et al., 2020). The use of interesting learning media accompanied by pictures and narratives is what students want. The learning media offered by researchers is in the form of a booklet digital with 3-dimensional images.

A booklet is a small book that has approximately five pages and no more than 48 pages outside the cover (Satmoko & Astuti, 2006). Booklet innovated with technology to be booklet digital which can be accessed as needed with Android or other device. Booklet digital is equipped with images that match the material presented. Design development on Booklet Digital begins with arranging materials and designing designs using the Canva application. Images are designed using web monstermash. zone which will then be merged into the content booklet digital and entered into the Heyzine flipbook website so that the display becomes Flipbook.

Biology is a subject in high school that is often considered difficult to understand because it does not explain theories with clear pictures, such as the excretory system, digestion, respiration, body defense, regulation, and reproduction (Alfiraida, 2018). In studying biology, learning media has not been used for a long time. Sources of information for learning are only obtained from textbooks and the delivery of material from teachers in class. Kartika High School 1-5 Padang currently only applies the independent curriculum in class X. Learning with the independent curriculum refers to the use of technology.

Based on the results of an interview with biology teachers. The learning resources used are only textbooks from the library, which are limited in number and cannot be taken by students to read and study again when they are not at school, while the learning methods used by teachers are lectures and discussions so that students feel bored and lack focus in learning biology.

Based on the results of filling out the preliminary study questionnaire by 28 students in class This causes biology learning activities to not run effectively. Even though in the independent curriculum, students are given the convenience of accessing various information from the internet. However, this does not allow students to get information about biological material clearly and correctly. Therefore, students must be able to choose which sources of information to use as learning references. The lack of clarity in the material on the internet makes students confused and doubtful.

Based on the results of material analysis through student questionnaires, it was found that virus material was the most difficult material to learn in class X E5 by 75%. According to students, the material about viruses is quite difficult to understand because the biology books do not show

many interesting pictures and are only in black and white so they are not clear and do not increase students' motivation to learn biology and understand the material about the virus in a more complex way. Based on the existing problems, the result was that there was no availability of interactive and creative learning media so learning resources on biological material, especially viruses, were limited.

Students find it difficult to understand the material and learning objectives are not achieved. So research was carried out regarding the development of Booklet 3-Dimensional Image-based Digital as a Learning Media on Virus Material for Senior High School. This research aims to test the feasibility and practicality of digital-based 3-dimensional image booklets as a learning medium for viral material at Kartika High School 1-5 Padang. The use of media learning with the latest innovations can act as a means to share and obtain information easily, with ease of access booklet because it was innovated with the development of technology that refers to android makes it easier for students to understand the material through pictures and concise explanations as well as the unique design of booklet digital which creates attraction and fosters students' interest and motivation to study biology.

RESEARCH METHODS

Research Design

This research is development research that uses the 4D development model. This model consists of 4 stages, namely analysis, design, development, and dissemination. The research aims to produce a 3-dimensional digital booklet product based on virus material which is used as a learning medium for students in class X E5 at Kartika High School 1-5 Padang. The design stages for developing a digital booklet based on 3-dimensional images as a learning medium for virus material for high school using the 4-D Models research procedure can be seen in Figure I.

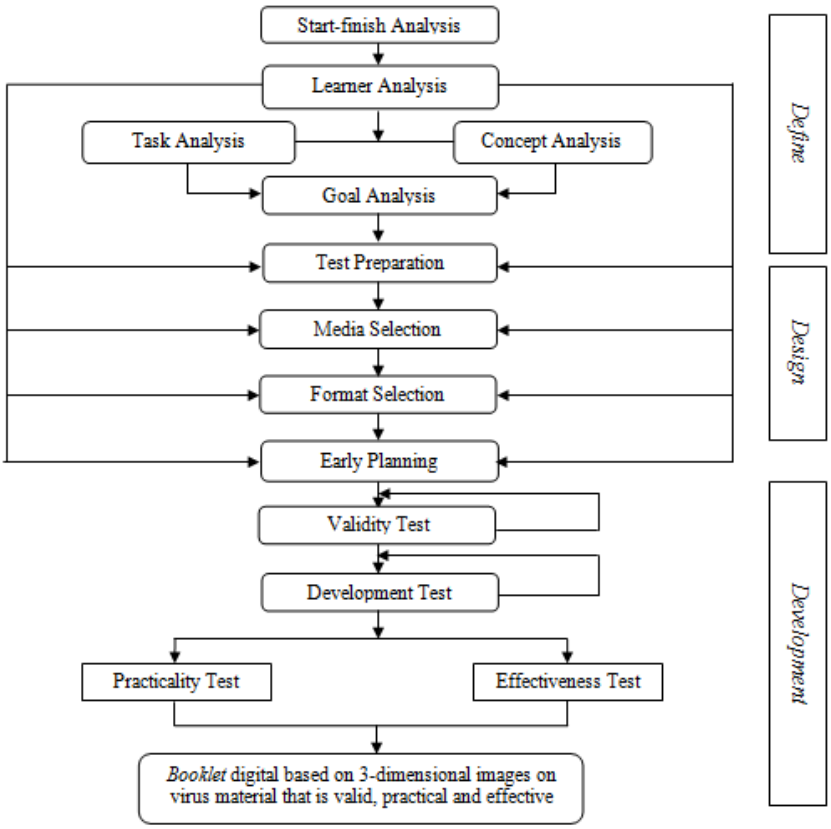


Figure I. Development Research Design Booklet 3-Dimensional Image-Based Digital Using 4D-Models

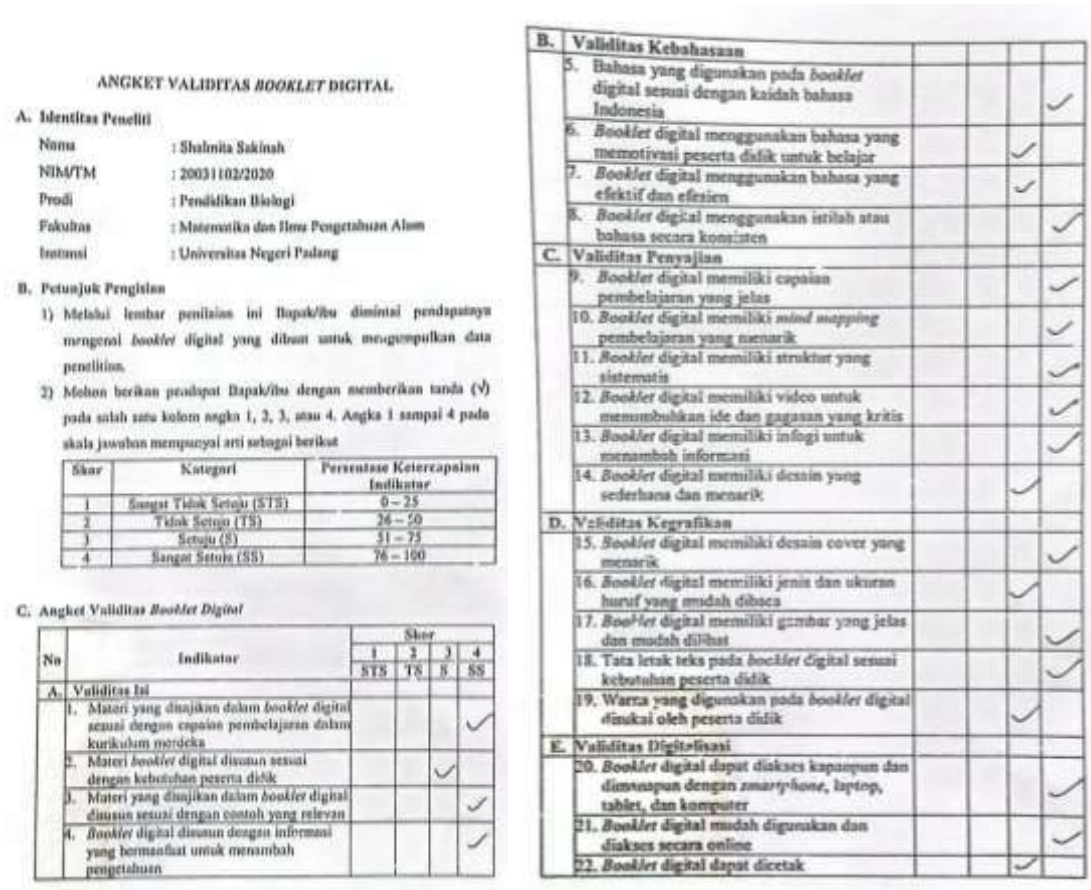
The development model begins with an explanation explaining the learning outcomes, teaching materials and student attitudes that have been analyzed. Next, design by formulating learning objectives as a material design containing the questions contained in booklet digital so that a product is obtained that has been prepared in outline with instruments as a tool to test the validity and practicality of a product. The final step is to develop with an application prepared such as Canva. After that, the product will be validated by material and media expert validators as well as class X high school teachers as users. The next step is to improve the booklet according to criticism or suggestions from validators and users.

Population and Samples

The research subjects were (1) two biology lecturers at the Faculty of Mathematics and Natural Sciences as validators (2) one biology teacher at Kartika High School 1-5 Padang as validator (3) twenty eight class X students at Kartika High School 1-5 Padang as a subject of practicality. The object of the research is a digital booklet based on 3-dimensional images as a learning medium for virus material for class X Senior High School

Instruments

The research instruments used to collect data in research are validation sheets and practicality sheets. The validity test of the digital booklet was carried out by 3 biology lecturers from Universitas Negeri Padang, 1 biology teacher at Senior High School of 2 Sikakap and 2 biology teachers at Pertiwi High School 1 Padang. Practicality instrument booklet digital was tested on biology teachers and students of class X at Kartika High School 1-5 Padang. The validity sheet can be seen in Figure 2.



ANGKET VALIDITAS *BOOKLET* DIGITAL

A. Identitas Peneliti

Nama	: Shalmita Sakinah
NIM/TM	: 20031102/2020
Prodi	: Pendidikan Biologi
Fakultas	: Matematika dan Ilmu Pengetahuan Alam
Institusi	: Universitas Negeri Padang

B. Petunjuk Pengisian

1) Melalui lembar penilaian ini Bapak/Ibu diminta pendapatnya mengenai *booklet* digital yang dibuat untuk mengumpulkan data penelitian.
2) Mohon berikan pendapat Bapak/Ibu dengan memberikan tanda (√) pada salah satu kolom angka 1, 2, 3, atau 4. Angka 1 sampai 4 pada skala jawaban mempunyai arti sebagai berikut

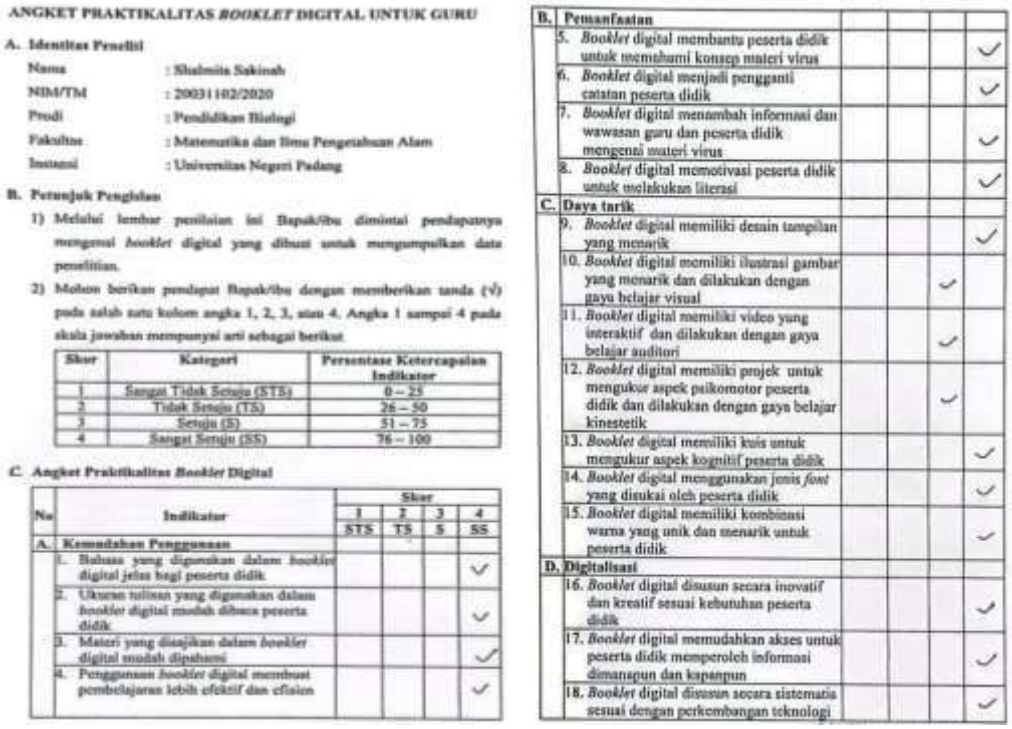
Skor	Kategori	Persentase Ketercapaian Indikator
1	Sangat Tidak Setuju (STS)	0 – 25
2	Tidak Setuju (TS)	26 – 50
3	Setuju (S)	51 – 75
4	Sangat Setuju (SS)	76 – 100

C. Angket Validitas *Booklet Digital*

No	Indikator	Skor 1 STS	2 TS	3 S	4 SS
A.	Validitas Isi				
1.	Materi yang disajikan dalam *booklet* digital sesuai dengan capaian pembelajaran dalam kurikulum merdeka				✓
2.	Materi *booklet* digital disusun sesuai dengan kebutuhan peserta didik			✓	
3.	Materi yang disajikan dalam *booklet* digital disusun sesuai dengan contoh yang relevan				✓
4.	*Booklet* digital disusun dengan informasi yang bermanfaat untuk menambah pengetahuan				✓
B.	Validitas Kebahasaan				
5.	Bahasa yang digunakan pada *booklet* digital sesuai dengan kaidah bahasa Indonesia				✓
6.	*Booklet* digital menggunakan bahasa yang memotivasi peserta didik untuk belajar			✓	
7.	*Booklet* digital menggunakan bahasa yang efektif dan efesien			✓	
8.	*Booklet* digital menggunakan istilah atau bahasa secara konsisten				✓
C.	Validitas Penyajian				
9.	*Booklet* digital memiliki capaian pembelajaran yang jelas				✓
10.	*Booklet* digital memiliki *mind mapping* pembelajaran yang menarik				✓
11.	*Booklet* digital memiliki struktur yang sistematis				✓
12.	*Booklet* digital memiliki video untuk memunculkan ide dan gagasan yang kritis				✓
13.	*Booklet* digital memiliki infogi untuk menambah informasi				✓
14.	*Booklet* digital memiliki desain yang sederhana dan menarik			✓	
D.	Validitas Kegrafikan				
15.	*Booklet* digital memiliki desain cover yang menarik				✓
16.	*Booklet* digital memiliki jenis dan ukuran huruf yang mudah dibaca			✓	
17.	*Booklet* digital memiliki gambar yang jelas dan mudah dilihat				✓
18.	Tata letak teks pada *booklet* digital sesuai kebutuhan peserta didik				✓
19.	Warna yang digunakan pada *booklet* digital disukai oleh peserta didik			✓	
E.	Validitas Digitalisasi				
20.	*Booklet* digital dapat diakses kapanpun dan dimanapun dengan *smartphone*, laptop, tablet, dan komputer				✓
21.	*Booklet* digital mudah digunakan dan diakses secara online				✓
22.	*Booklet* digital dapat dicetak			✓	

Figure 2. The validity sheets

The validity sheet consists of five aspects consisting of content, language, presentation, graphics, and digitalization. each aspect has an indicator with the highest score being 4 and the lowest score being 1. The practicality sheet can be seen in Figure 3.



ANGKET PRAKTIKALITAS *BOOKLET* DIGITAL UNTUK GURU

A. Identitas Peneliti

Nama : Shalmita Sakinah
NIM/TM : 20031102/2020
Prodi : Pendidikan Biologi
Fakultas : Matematika dan Ilmu Pengetahuan Alam
Instansi : Universitas Negeri Padang

B. Petunjuk Pengisian

1) Melalui lembar penilaian ini Bapak/Ibu dimintai pendapatnya mengenai *booklet* digital yang dibuat untuk mengumpulkan data penelitian.
2) Mohon berikan pendapat Bapak/Ibu dengan memberikan tanda (√) pada salah satu kolom angka 1, 2, 3, atau 4. Angka 1 sampai 4 pada skala jawaban mempunyai arti sebagai berikut

Skor	Kategori	Persentase Ketercapaian Indikator
1	Sangat Tidak Setuju (STS)	0 – 25
2	Tidak Setuju (TS)	26 – 50
3	Setuju (S)	51 – 75
4	Sangat Setuju (SS)	76 – 100

C. Angket Praktikalitas *Booklet* Digital

No	Indikator	Skor 1 STS	2 TS	3 S	4 SS
A.	**Kemudahan Penggunaan**				
1.	Bahasa yang digunakan dalam *booklet* digital jelas bagi peserta didik				√
2.	Ukuran tulisan yang digunakan dalam *booklet* digital mudah dibaca peserta didik				√
3.	Materi yang disajikan dalam *booklet* digital mudah dipahami				√
4.	Penggunaan *booklet* digital membuat pembelajaran lebih efektif dan efisien				√
B.	**Pemanfaatan**				
5.	*Booklet* digital membantu peserta didik untuk memahami konsep materi virus				√
6.	*Booklet* digital menjadi pengganti catatan peserta didik				√
7.	*Booklet* digital menambah informasi dan wawasan guru dan peserta didik mengenai materi virus				√
8.	*Booklet* digital memotivasi peserta didik untuk melakukan literasi				√
C.	**Daya tarik**				
9.	*Booklet* digital memiliki desain tampilan yang menarik				√
10.	*Booklet* digital memiliki ilustrasi gambar yang menarik dan dilakukan dengan gaya belajar visual			√	
11.	*Booklet* digital memiliki video yang interaktif dan dilakukan dengan gaya belajar auditori			√	
12.	*Booklet* digital memiliki projek untuk mengukur aspek psikomotor peserta didik dan dilakukan dengan gaya belajar kinestetik			√	
13.	*Booklet* digital memiliki kuis untuk mengukur aspek kognitif peserta didik				√
14.	*Booklet* digital menggunakan jenis *font* yang disukai oleh peserta didik				√
15.	*Booklet* digital memiliki kombinasi warna yang unik dan menarik untuk peserta didik				√
D.	**Digitalisasi**				
16.	*Booklet* digital disusun secara inovatif dan kreatif sesuai kebutuhan peserta didik				√
17.	*Booklet* digital memudahkan akses untuk peserta didik memperoleh informasi dimanapun dan kapanpun				√
18.	*Booklet* digital disusun secara sistematis sesuai dengan perkembangan teknologi				√

Figure 3. The practicality sheet

The practicality sheet consists of four aspects consisting of ease of use, utilization, attractiveness and digitalization. Each aspect has an indicator with the highest score being 4 and the lowest score being 1.

Procedures

Digital Booklets will be developed using the 4D development model. The 4D development model includes 4 stages, namely define, design, develop, and disseminate. Research was carried out until the development stage due to limited time, energy and costs.

1. Define

At this stage, the activities carried out are determining and defining learning requirements. This stage is called needs analysis. Each product requires different analysis. As for defining learning requirements, it can be done in 5 stages as follows:

a) Start-finish analysis
Start-finish analysis or Front-End Analysis carried out to determine the problems in biology learning at school.

b) Learner Analysis
Learner Analysis this was done to look at cognitive aspects and find out the extent of students' development during biology learning. At the analysis stage, the results of student interviews and questionnaires will be obtained, these results will be used as a reference for development booklet 3-dimensional image-based digital as a learning medium. At this stage the author can find out the attitudes and behavior of students during biology learning, including things that students do as well as students' relationships with peers or teachers during the learning process in class X Kartika High School 1-5 Padang.

c) Task Analysis

Task Analysis aims to determine the content of the material that will be achieved in biology learning. At the task analysis stage, it can be done by analyzing the learning outcomes and learning objectives in the independent curriculum so that you will get clearer and more detailed material so that it can be included in the booklet digital to develop.

d) Concept Analysis
Concept Analysis aims to determine the concepts contained in the booklet digital based on 3-dimensional images which will be developed as a learning medium for students. This stage can be carried out by systematically analyzing the concepts studied in the virus material to make it easier for students to understand the material.

e) Specifiying Instructional analysis
Specifiying Instructional analysis aims to find out the learning objectives that will be achieved during biology learning. The learning objectives are guided by the learning outcomes in the independent curriculum and are included in booklet 3-dimensional image-based digital as a learning medium.

2. Design

The design stage is carried out to prepare or design digital booklet learning media based on 3-dimensional images through several stages as follows:

a) Media Selection
Media selection carried out by referring to the results of student analysis, task analysis, concept analysis, and learning objectives. So it is generated booklet 3-dimensional image-based digital media that will be used as a biology learning medium for class X phase E virus material.

b) Format Selection
Format selection carried out in accordance with the learning media booklet digital. The components contained include the title, study instructions, competencies to be achieved, assignments, supporting information, work stages, and assessment for students.

c) Early Planning
Early planning or initial design done with the Canva application which includes cover, foreword, table of contents, instructions for use, learning outcomes, learning objectives, explanation of material and assignments in the form of projects. The evaluation section includes project evaluation, quiz, infogi (Information about Biology), and glossary.

3. Development

The development stage is carried out to develop the product under study. The development consists of 2 stages including validation and practicality tests.

a) Validity test
The validity test is carried out by validators with a validity questionnaire which aims to determine the level of validity of the media being developed.

b) Practicality Test
The practicality test was carried out by two biology subject teachers in class X E5 and 32 students in class X E5 using a practicality questionnaire. The purpose of conducting a practicality test is to determine the level of practicality of the media being developed.

Data Analysis

The data analysis used is qualitative analysis and quantitative analysis. The use of qualitative analysis is used at the definition stage define and design which will be presented in the form of a description. Quantitative data analysis is used at the development stage through validity tests and practicality tests. The data analysis used is validity and practicality analysis. Determine the validity value with the following formula:

$$\text{Validity} = \frac{\text{Total score}}{\text{Max score}} \text{ x100\%}$$

Table 1. Assessment of the value of validity (Sarinami et al., 2022).

Score range	Category
81% - 100%	Very valid
61% - 80%	Valid
41% - 60%	Fairly valid
≥21% - 40%	Invalid

Booklet The 3-dimensional image-based digital device developed is said to be valid if it has a validity value of ≥61% and is said to be invalid if it only reaches a value of ≤40%, then revisions will be made to the booklet digital and re-tested for validity. Determine the practicality value using the following formula:

$$\text{Practicality} = \frac{\text{Total score}}{\text{Max score}} \text{ x100\%}$$

Table 2. Assessment of the value of practicality (Khairul et al., 2019).

Score range	Category
81% - 100%	Very practical
61% - 80%	Practical
41% - 60%	Quite practical
≥21% - 40%	Not practical

Booklet The 3-dimensional image-based digital device being developed is said to be practical if it has a practicality value of ≥61% and is said to be impractical if it only reaches a value of ≤40%. If the value is in the impractical category then revisions are carried out booklet digital and re-tested for practicality.

RESULTS

Based on the research that has been carried out, qualitative and quantitative data was obtained by material and media experts. The results of validation tests by material experts and media experts can be seen in Table 3.

Table 3. Material expert validation test results for digital booklet quality

Aspect	Validator			Percentage (%)	Criteria
	1	2	3		
Contents	15	13	16	91,66	Very valid
Language	14	12	14	83,33	Very valid
Presentation	23	20	23	91,66	Very valid
Graphics	18	18	20	93,33	Very valid
Digitalization	11	9	12	88,88	Very valid
Total value		238		90,15	Very valid

Based on the assessment of material experts, the percentage score obtained by the three material experts was 90.15%, based on the validation results of the material experts booklet digital is included in the very valid category.

Table 4. Media expert validation test results for digital booklet quality

Aspect	Validator			Percentage (%)	Criteria
	1	2	3		
Contents	14	16	16	95,83	Very valid
Language	14	16	15	93,75	Very valid
Presentation	21	24	22	93,05	Very valid
Graphics	17	20	20	95	Very valid
Digitalization	11	12	12	97,22	Very valid
Total value		250		94,69	Very valid

Based on the assessment of media experts in Table 4, the percentage score obtained by three media experts is 94.69%, based on the validation results of media experts, booklet digital is included in the very valid category. So the conclusion is obtained that the booklet the digital product developed is suitable for use with very valid criteria by comments and suggestions from validators. Based on the research that has been carried out, the results obtained are that the booklet digital has passed the validation stage with minor improvements. Improvements were made according to suggestions and input from material experts and media experts including italicizing foreign words on each page, including learning outcomes and objectives on one page. Next, provide pictures of each type of disease caused by viruses and their sources.

Part quiz more enlarged and includes some information in the infogi and a glossary containing matters related to virus material. This is done so that booklets can be used as learning media or tools that have instructional material in an environment that can stimulate students to learn (Arsyad, 2014). According to research (Adiko, 2019) and (Putra & Milenia, 2021), through effective learning media, students can be stimulated to remember the material and increase motivation. Input from media experts is change cover which includes the name of the author, supervisor and validator from the lecturer, cover slightly changed the image and included a virus image, the image must be clear with a reference, the writing was corrected to be neater, the use of scientific names was italicized, the position of the reference text and the image number were changed, changed mind mapping by including a little explanation and hyperlink to go straight to the page you want to read, include all references to the image in the bibliography and delete the biography page. Several illustrations of revisions according to suggestions and input from material experts and media experts can be seen in Figure 4.

Figure 4. View of the digital booklet cover before revision and after revision

The booklet cover display is created in a creative way that contains a lot of pictures, usage booklet it is considered capable of increasing children's understanding of material regarding inspiring and interesting learning (Putri, 2020). Booklet making online access easier and use in learning more creative and innovative (Gustaning, 2014). Expected with deep 3 dimensional images booklet digital will be a characteristic of digital booklets that attract students' interest in reading difficult material (Irmanto, 2018).

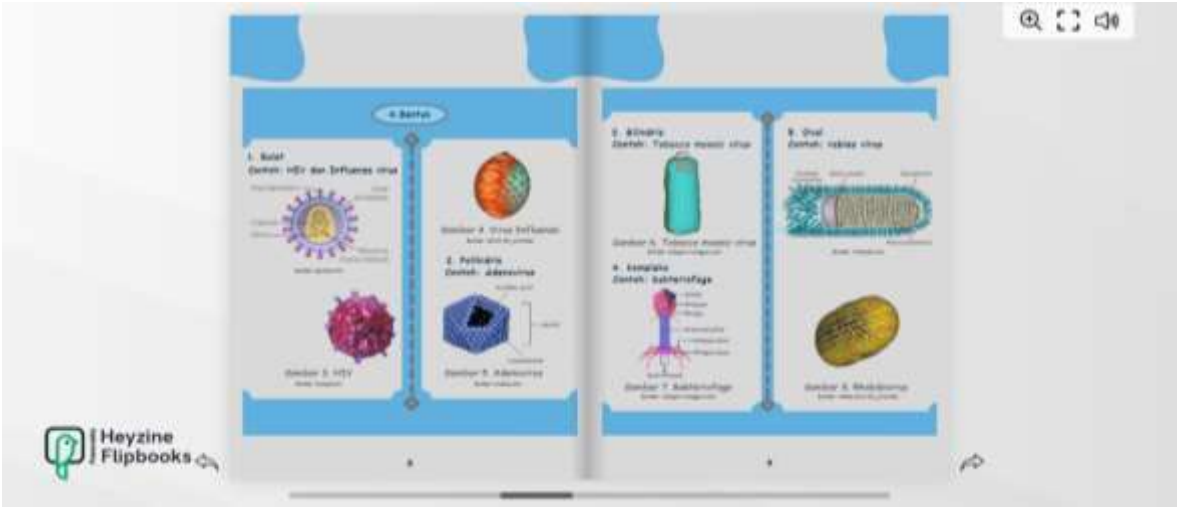


Figure 5. Display 3-dimensional images on digital booklets

Several images of virus in 3-dimensional form on Figure 5 namely HIV, Influenza virus, Adenovirus, Tobacco mosaic virus, Bacteriophage and Rabies virus. 3 dimensional images. Three-dimensional media is a work of fine art that has width, length and height or volume and occupies space (Rondhi & Anton, 2002). Three-dimensional media is a visual aid that has length, width and height and can be seen from various points of view (Sudjana, 2014).

Several characteristics of three-dimensional media are that it is practical to use and does not require much processing, the presentation of material is integrated or easy for students to understand, its use involves students, the process of delivering material is carried out simultaneously and overcomes space, time and the senses (Asrotun, 2014). So it is hoped that 3-dimensional images can become a characteristic of digital booklets to attract students' interest in studying the biology of virus material.

Based on the validity value booklet this digital test can then be tested on class X E5 students at SMA Kartika 1-5 Padang. Trials Booklet Digital aims to determine the response of teachers and students to a booklet developed digitally. The trial was carried out by two biology subject teachers and class X E5 students at Kartika High School 1-5 Padang.

The trial was carried out on two biology subject teachers by giving a booklet digitally via a link that has been sent to each teacher's number, after the teacher has read the content booklet digitally, the teacher fills in the practicality assessment sheet that has been given.

Table 5. Teacher practicality test results on digital booklets

Aspect	Teacher		Percentage (%)	Criteria
	1	2		
Ease of Use	16	16	100	Very practical
Utilization	16	14	93,75	Very practical
Attractiveness	25	27	92,85	Very practical
Digitalization	12	12	100	Very practical
Total value	138		95,83	Very practical

Based on the teacher's practicality assessment on Table 5, the percentage score obtained by two teachers is 95.83%, then booklet digital is included in the very practical category so booklet the digital technology developed can be used for class X phase E students on virus material at Kartika High School 1-5 Padang.

Next, a trial phase was carried out on 32 students in class X E5 at Kartika High School 1-5 Padang. During the trial, respondents were asked to look at the content booklet through the link which was sent via the class X E5 biology WhatsApp group. Respondents were given 15 minutes to look at the display and read the booklet digitally. Then respondents can open the video inside the booklet digitally. After 15 minutes respondents were asked to answer the quiz. Then the respondents filled out an assessment sheet in the form of a practicality questionnaire for the students that had been given.

The resulting data is quantitative, namely, the value of students' responses regarding the booklet digitally, and qualitative data is in the form of student comments on the booklet developed digitally. Several aspects assessed in the student practicality questionnaire include ease of use, utilization, attractiveness, and digitalization. Test results data can be seen in Table 6.

Table 6. Results of student responses to aspects of digital booklets

Aspect	Value	Max value	Percentage (%)	Category
Ease of Use	460	512	89,84	Very good
Utilization	450	512	87,89	Very good
Attractiveness	803	896	89,62	Very good
Digitalization	340	384	88,54	Very good
Total value	2053	2304	89,10	Very good

Based on the data above, it can be seen that students' responses in every aspect are in the very good category. The ease of use aspect was 89.84%, the utilization aspect was 87.89%, the attractiveness aspect was 89.62%, and the digitalization aspect was 88.54%. This indicates that every aspect has met the requirements to become booklet practical digital. So it can be concluded that the product booklet the digital technology developed is very practical for students to use as a learning medium.

The final stage is to revise the product, through the results of product trials to obtain assessments and responses from teachers and students that the product developed is good and suitable for use in learning activities. Suggestions from teachers to be able to upload products to social media can be concluded that a booklet digital has been developed to produce the final product.

DISCUSSION

This research is a type of research and development that aims to determine the feasibility of Booklet Digital as a learning medium for viruses material in Senior High School. The results of this research are a booklet digital which is used as a learning medium. This research was carried out using the 4D development model. The 4D model is a development model used to develop various types of learning media (Arkadiantika et al., 2020). Research only reaches the development stage to determine the feasibility booklet developed digitally. Steps for developing a learning media booklet digital begins with the beginning-to-end analysis stage. The learning media development process begins with a preliminary study stage, namely by conducting a needs analysis. Needs analysis has a role in connecting teachers, students, teaching materials, and teaching procedures well (Aflah & Rahmani, 2018). Needs analysis includes observations and interviews at Kartika High School 1-5 Padang to obtain the information needed by researchers. Then an analysis is carried out on the

students so that students' problems in understanding biology material can be identified. Therefore, learning media are needed that can increase students' interest and interest in learning.

Booklet digital as a learning medium is expected to increase students' interest in the material presented because it has a simple and attractive appearance. Interesting and informative learning media can arouse students' interest in learning (Hanifah et al., 2020). Booklet Digital is equipped with images and videos that can provide explanations that are easier to understand for students. There are many pictures included in the learning media so that the presentation of the material is more concise (Darlen et al., 2015). Through the observations that have been made, a literature study is needed to find solutions to existing problems booklet digital can be used as a learning medium as expected through relevant references. Based on interviews conducted with one of the biology subject teachers, it is known that students are still less interested in reading, and there is a lack of learning media at school. Therefore it is necessary to explain about booklet digital to students and teachers, that booklet digital as a learning medium can be the right solution to increase students' reading interest.

The next stage is to collect information through references that are relevant to the material that will be developed in the media booklet digital, namely class X phase E virus material. Booklet digital contains material sourced from previously used educational printed books and various articles from journals via Google Scholar, as well as several other supporting books. The next stage is to design the product, at this stage, an initial design is carried out to prepare digital booklet material that is appropriate to the virus material. Things must be done at the design stage, namely choosing learning media sources, choosing the format, and initial design (Hanifah et al., 2020). Next is making the design booklet digital, The booklet digital was created using the Canva application after which it was entered into Heyzine flipbook which is found in Canva until the display is shaped flipbook and accessed online at the link provided. A flipbook is a collection of sheets of paper such as an album or calendar that measures 21 x 28 cm (Rahmawati, 2018). Booklet digital will automatically open each page according to the user's wishes.

Booklet digital has the advantage of being more practical and easy to access and can be used anywhere. This agrees with Riyan (2021) one of the advantages of Android-based learning media is that it can be accessed anywhere and at any time. Making the process booklet digital is adjusted with suggestions and input from material experts and media experts. Therefore after the booklet digital, then the next stage is carried out, namely validation. Expert validation data uses validation sheets from various aspects such as appropriateness of content or material, language, and presentation (Hanifah et al., 2020). Based on the results of this research, the booklet digital has met the validity criteria of 94.69% in the very valid category. So the results obtained were that the learning media in the form of digital booklets was in the very good category for students to use during learning.

The next stage is design revision, based on the results of product validation, it still requires improvements according to the validator's suggestions. Suggestions given by material experts include: booklet It's good, only some of the writing needs to be corrected, the pictures made clearer, and some examples need to be added. Suggestions given by media experts are: cover the Independent Curriculum logo and supervisor's name included, the writing is tidier, the writing is improved, the use of colors brighter and the numbering is clear. Next, product trials are carried out, and products that have been revised by lecturers and teachers are then tested in the learning process. This trial aims to determine various shortcomings, weaknesses, or errors in the product (Mahyuddin et al., 2018). The trial was carried out by two biology teachers and class X E5 students at Kartika High School 1-5 Padang. The trial by two biology teachers obtained a percentage result of 95.83%, while the trial by 32 students obtained a percentage result of 89.10%.

CONCLUSION

Based on the results of research and development of digital booklets based on 3-dimensional images as learning media on virus material for Senior High School, it was concluded that this research produced digital booklets that were suitable and meet the criteria based on material and media expert assessments. The material expert test results were 90.15% and media expert test results were 94.69% with the category very valid and worthy of development. Practicality booklet digital based on an assessment by two biology subject teachers which had a percentage of 95.83% in the very practical category. The students' responses to booklet digital in the X E5 class is very good with a percentage of 89.10%. Every aspect is in the very good category. Therefore, it is hoped that it can be followed up and tested in learning to find out the expected positive impact and influence of the digital booklets on students' motivation and interest when studying.

ACKNOWLEDGMENT

Thank you Dr. Fitri Arsih, S.Si,. M.Pd, Mr Dr. Syamsurizal. M.Biomed and Mrs. Helsa Rahmatika, S.Pd., M.Pd who have helped me during the research process, thank you to the class X E5 students of Kartika High School 1-5 Padang who have contributed to the implementation of the research.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 111 – 122 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

Design of an e-encyclopedia of the order of odonata from sumatra as student teaching material

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Article Info

Article History:
Received 28 February 2024
Revised 29 March 2024
Accepted 06 April 2024
Published 30 April 2024

Keywords:
Design
E-Encyclopedia
Order Odonata
Teaching materials

ABSTRACT

This research discusses the design of the E-Encyclopedia of the Order of Odonata as teaching material for junior high school students. This research aims to introduce how to design an E-encyclopedia of the Order of Odonata from Sumatra which can be used as teaching material for junior high school students, especially children aged (13-15 years) in the Sumatra region. The urgency is that junior high school children become familiar with the various types of the Odonata Order from Sumatra so that the identity of the Odonata Order is maintained and maintained. This research is design research consisting of three phases, namely Preliminary research, Prototyping phase, and assessment phase. The design of the E-Encyclopedia of the Odonata Order from Sumatra is an effort to develop media in studying various types of the Odonata Order from Sumatra whose development is viewed from language, material, and media. Through the development activities carried out, an E-Encyclopedia Product of the Order of Odonata from Sumatra was obtained as suitable teaching material.

Citation: Siregar, N.S., Dimenta, R.H., & Sari, N.F. (2024). Design of an e-encyclopedia of the order of odonata from sumatra as student teaching material. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 111-122. DOI: https://doi.org/10.31932/jpbio.v9i1.3397

INTRODUCTION

Along with advances in science and technology today, there are many changes in the mindset of the people of a nation toward a more advanced one. Likewise, learning activities are increasingly varied and related to increasingly advanced science and technology. We can see this with the increasing social and cultural shifts in society as a result of advances in technology and information (Siregar et al., 2020). Biology is one of the subjects discussed at junior high school level whose material is abstract so it requires additional teaching materials or media to accompany textbooks. E-encyclopedias were chosen as a product of this balance because encyclopedias are identical to images, and seem lighter, more interesting, and more concise (Pardosi et al., 2023). Biology subjects require visualization such as pictures or videos that can show very large living creatures or even microscopic living creatures, and also to see natural phenomena that are not found directly in

the classroom. In order, to achieve maximum success in biology learning, learning concepts can be developed by current times, namely with the concept of appropriate teaching materials for students.

The reality in the field is that learning activities have not been fully implemented as expected. Students in active learning should find information related to the material being studied (Ahmad et al., 2022). In an interview conducted by researchers with one of the junior high school teachers in Kolata Labuhan Batu, information was obtained that in teaching biology, teachers tend to use conventional approaches. Where in this case learning is carried out by the teacher explaining the existing material using the lecture method. Next, the teacher gives practice questions and the teacher directs students to memorize the important concepts or material being studied. And it was also conveyed that the teaching materials used in learning were less varied so students' interest in learning tended to be low.

The learning and teaching process requires sufficient teaching materials to be used as a means of interaction between students and teachers and learning to acquire knowledge, skills, and positive values. The role of teaching materials in the learning process is a series of activities to realize student competence (Risti et al., 2017; Sabri et al., 2023). Various teaching materials currently being implemented include E-encyclopedias, Textbooks, Teaching Modules, and LKPD, which can present various meanings, explanations, and questions without being supported by images or photos packaged in print or online media. Interesting learning resources can increase students' understanding so that learning resources can be in the form of an encyclopedia. Encyclopedias can also be used to increase general cognitive knowledge in students (Arifah et al., 2017). Encyclopedias contain content in the form of information on knowledge terms which are not only explained in the form of definitions but also examples. Encyclopedias contain a collection of information that makes knowledge richer. Not only in the form of writing, the encyclopedia contains various images to make the encyclopedia seem interesting. Completeness of the material and several examples of images used as learning media for students (Rosyida et al., 2016). The use of encyclopedias in learning allows students to learn according to their abilities. Students can also find out how far their level of understanding is regarding the material that has been presented (Ismail et al., 2023).

Encyclopedia is taken from "encyclopedia" from Greek which means a complete circle of teaching. Interesting learning resources can increase students' understanding so that learning resources can be in the form of an encyclopedia (Solihah et al., 2022a). Encyclopedias are several writings containing explanations that store information comprehensively and quickly to be understood and understood about all branches of science, generally printed in the form of book summaries depending on the amount of material included (Marantika et al., 2023). An encyclopedia is a reference collection with basic and complete information about science (Safitri & Dewi, 2021). Encyclopedias are very popular reference books (Purbosari, 2016). According to Suwarno, an encyclopedia is a list of subjects accompanied by information about definitions, background, and bibliographic data arranged alphabetically and systematically (Nurhatmi et al., 2015). Encyclopedias contain various kinds of objects accompanied by comprehensive and complete descriptions and information relating to the objects discussed (Prihartanta, 2015). There is a difference between a book and an encyclopedia, namely that an encyclopedia contains a more detailed explanation, alphabetical and systematic in its arrangement. According to (Kemendikbud, 2017) e-modules are independent learning materials that are arranged systematically and presented in electronic format. The advantage of e-modules compared to printed modules is that they are interactive, contain images and animations, and are equipped with formative tests/quizzes which are expected to be able to provide immediate automatic feedback (Permatasari et al., 2017). This e-encyclopedia teaching material is suitable for developing biology subjects that require in-depth

visualization because it can display photos or animated images which can make it easier for students to understand the material.

The material developed in the encyclopedia is material about the order Odonata. One order of insects that has a fairly high terrestrial habitat and diversity and also has a beneficial role for the environment is the Odonata (Rahmawati et al., 2023). Dragonflies are flying insects that prefer habitats close to water sources (Solihah et al., 2022b). Dragonflies (Odonata) have a role in biological control because dragonflies consume insects that are detrimental to humans and livestock, such as mosquitoes, flies, and rice pests (Dwita et al., 2022). Because this can foster a sense of love for the surrounding environment. This is very important because children at the junior high school level can learn the most fundamental things in life, including living creatures found in the area where they live. In introducing the adonata order, utilizing an online media approach in the form of an e-encyclopedia is the solution.

Various previous studies have conducted studies on the Odonata order and have provided useful results for improving the learning process. The results of this research reveal that e-encyclopedias need to be published according to certain regions so that people have basic information in line with development and progress and the increasing need for information (Prihartanta, 2015). Furthermore, an inventory of Odonata in the Roro Kuning Bajulan Loceret Nganjuk waterfall area provided research results that showed that there were three families, namely Calopterygidae, Coenagrionande, and Rhinocypha (Prameswari & Sulistyowati, 2016; Rahmawati et al., n.d.). Furthermore, there has been various research into the development of the Encyclopedia of the Odonata to produce teaching material products that are suitable for teaching biology to students (As'ad et al., 2020; Cahyanti & Ibrahim, 2018; Dawam, 2021; Solihah et al., 2022a). From the previous description, there has been no research that examines the inventory of the order of Odonata from Sumatra, especially if it is discussed for teaching junior high school students. Therefore, the author is interested in researching the Design of the E-Encyclopedia of the Order of Odonata from Sumatra as Teaching Material for Junior High School Students.

RESEARCH METHODS

Research Design

This type of research is Design Research, which is a type of research in which the researcher designs learning materials (such as learning activities and learning trajectories) for a particular topic and also builds a theory about the learning process for that topic. The design research method is a systematic and flexible method for improving the quality of learning in the classroom (Muslimin et al., 2020). In this case, the design research aims to formulate, understand, and develop an e-encyclopedia of the order Odonata from Sumatra. The population of this study is the Odonata order from the Sumatran region. Using the purposive sampling method (Sugiyono, 2016), where for reasons of needing new knowledge about the Odonata order in the Sumatra region for junior high school students in North Sumatra, the sample chosen was the Odonata order in the North Sumatra region. Thus, the research subject is the design of the North Sumatran Odonata order and the object is the encyclopedia of the Odonata order from North Sumatra.

Design research consists of three phases, namely preliminary design, experimental design, and retrospective analysis (Cobb et al in Mulyana, 2008). The explanation of the three phases is:

I. Preliminary Design

Analysis in this case involves analysis and needs assessment carried out to determine the gap between actual and ideal conditions. Where in this research material, biology curriculum, student characteristics, analysis of student analysis work plans, lesson material analysis, task analysis, and learning objective analysis were carried out. The needs assessment was carried out using observation and interview methods. Observations and interviews were carried out to identify products that suit students, learning objectives, core competencies, basic competencies, and learning materials that will

be discussed in the learning media that will be developed. Next, Front-End Analysis is carried out to collect techniques that can be used as solutions to existing gaps. The Front-End Analysis carried out includes an analysis of the approach used in developing textbooks related to the principles, characteristics, and syntax used in the learning approach used. Furthermore, the book developed must refer to developing students' creative thinking abilities. The design stage is the stage of designing the product to be made. The design stage starts with making a research schedule plan, designing research instruments which include language expert assessment sheets, material expert assessment sheets, media expert assessment sheets, and designing an E-Encyclopedia of the Order of Odonata from Sumatra as teaching material for junior high school students. The E-Encyclopedia Order Odonata research product is designed for junior high school students.

2. Prototyping phase

This stage involves a design process in which the design of the product to be made is carried out. The design stage starts with making a research schedule plan, designing research instruments which include language expert assessment sheets, material expert assessment sheets, media expert assessment sheets, and designing an E-Encyclopedia of the Order of Odonata from Sumatra as teaching material for junior high school students. The E-Encyclopedia Order Odonata research product is designed for junior high school students.

3. Assessment phase

Assessment phase dilaksanakan untuk memperoleh produk berupa E-Ensiklopedia Order Odonata which can be applied to teach junior high school students. This activity is through product validation by material experts and experts in contextual learning approaches. The validation carried out was reviewed from the aspects of language, material, and media. The product obtained in this stage is an e-encyclopedia of the order of adonata which has passed expert validation. If the validator provides suggestions for improvement, improvements will be made so that the product developed meets the validity criteria.

Instruments

The research instrument involved a product validation sheet. The E-Encyclopedia of the Odonata Order validation sheet. The instrument is designed to obtain assessments and suggestions for improvements to the products being developed. Product assessment and development is focused on 3 aspects which include language, material, and media. Product assessment is through a questionnaire containing statements that are responded to using a Ricard scale with the options "Not Suitable" with a score of 1, "Not Suitable" with a score of 2, "Quite Suitable" with a score of 3, "Suitable" with a score of 4, "Very Suitable" Appropriate" with a score of 5 (Hunaepi et al., 2016). Next, questions are asked regarding the advantages, disadvantages, eligibility status, and suggestions for improvement of each existing validation sheet according to the aspects being validated.

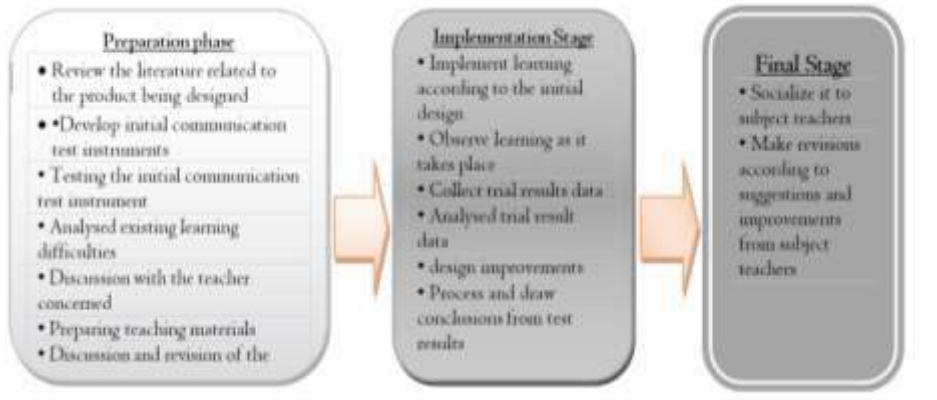

Figure 1. Research Procedure Chart

Procedures

This research procedure was carried out in three stages, namely the preparation stage, implementation stage, and final stage. The detail regarding these three stages are shown in Figure 1.

Data Analysis

Data analysis in this research uses quantitative descriptive methods. The data obtained based on the validator's assessment of each aspect will determine the average value. Then a categorization is carried out based on the average value obtained to interpret the quality of the product being developed. The criteria for interpreting the quality of the designed research product can be seen in Table 1.

Table 1. Interpretation of validator assessment score achievements

Achievement Score	Interpretation
$1 \leq nilai \leq 1{,}5$	Very bad
$1{,}5 < nilai \leq 2{,}5$	bad
$2{,}5 < nilai \leq 3{,}5$	Enough
$3{,}5 < nilai \leq 4{,}5$	Good
$4{,}5 < nilai \leq 5$	Very good

Source: Modification (Ahmad, Siregar, & Siregar, 2018)

RESULTS

Based on the previously determined development model for the Design of the E-Encyclopedia of the Order of Odonata from Sumatra as Teaching Material for Junior High School Students, the development was carried out in stages which included the Preliminary Design, Prototyping phase, Assessment phase (Plomp, 2010).

Preliminary Design defines the need for e-encyclopedia teaching materials as focusing on its use on junior high school students. Development activities aim to develop an E-encyclopedia of the order adonata. Analyze concepts/material by identifying, detailing, and systematically arranging relevant concepts/material to be taught by the initial final analysis. The material involved is related to the Classification of, therefore the author wants to discuss the Morphology of Dragonflies, the Behavior of Dragonflies, the Life Cycle of Dragonflies, the Habitat of Dragonflies, Benefits of Dragonflies, Classification of Dragonflies. Next, an analysis is carried out to analyze the gap between actual and ideal conditions. In this case, it was found that junior high school students were not fully interested in biology lessons, especially the order adonata material. And the teaching materials that support learning activities are not yet varied. The teaching materials used in learning activities should be varied, involving print and electronic media. Print media can be in the form of printed books, printed modules, printed encyclopedias, and others. Furthermore, online or electronic media can be in the form of e-modules, e-encyclopedias, e-books, and others. In this case, innovation is needed in teaching materials in electronic media in the form of e-encyclopedias on Ordo Adonata material.

The prototyping phase is carried out to design the e-encyclopedia product that will be developed. In this stage, the E-encyclopedia product is designed by the provisions found in the definition and analysis stage. This stage produces an initial draft product of the E-encyclopedia. The E-encyclopedia design is presented with an attractive appearance involving concepts, theories, and images that support students' understanding of the existing material presented. Apart from designing the initial draft product, a validation sheet instrument was also designed which included a language expert validation sheet, a media expert validation sheet, and a material expert validation sheet. The validation sheet is designed to contain statements that will be responded to on a Ricard

scale with the options of TS (Not Appropriate) with a score of 1, TS (Not Appropriate) with a score of 2, TS (Quite Appropriate) with a score of 3, TS (Suitable) with a score of 4 , TS (Very Suitable) with a score of 5 (Ahmad, Siregar, Siregar, et al., 2018). Next, questions are asked regarding the advantages, disadvantages, eligibility status, and suggestions for improvement of each existing validation sheet according to the aspects being validated.

The assessment phase is carried out to obtain the final product from the e-Encyclopedia product being developed. This activity was carried out through validation and revision activities in terms of the language, material, and media aspects contained in the design of the e-Encyclopedia of the order of adonata that was developed. This development involved 6 validators who have competence in various predetermined aspects. The validators involved consist of Lecturers, Teachers, and Practitioners who have competence in developing e-Encyclopedia designs.

The development of the language aspect was carried out by 2 validators who have competence in scientific language (Biology). Validation was carried out on aspects of flexibility with 3 items, interactiveness with 2 items, language rules with 3 items, and language accuracy with 3 items. The average value of the assessment results of the two validators is as in Table 2.

Table 2. Validation Assessment Through Language Aspects

No.	Language Aspects of Assessment	Validator		Average value
		1	2	
1	Straightforward	4,67	4,00	4,33
2	Interactive	4,50	4,00	4,25
3	Language Rules	5,00	4,00	4,50
4	Language Accuracy	4,67	4,00	4,33
	Total Average Value	4,71	4,00	4,35

Based on Table 2, it can be seen that the four aspects developed achieved good criteria. Furthermore, from the total average, assessment achievements are also obtained in the good category. Thus, the product assessment achievement in terms of the language aspect is that it meets the effective criteria. The comments from the linguist validator were that the product developed was generally straightforward to understand. Furthermore, there were shortcomings where foreign spelling should be written in italics. The comments and suggestions provided are used as a reference to improve the research products being developed. In language, the validator states that the ordo adonata e-Encyclopedia product being developed is feasible.

The development of the language aspect was carried out by 2 validators who have competence in scientific language (Biology). Validation was carried out on aspects of material coverage with 6 items, up-to-date material with 2 items, and e-Encyclopedia Presentation with 5 items. The average value of the assessment results of the two validators is as in Table 3.

Table 3. Validation Assessment Through Material Aspects

No.	Aspects (Material) of Assessment	Validator		Average value
		1	2	
1	Material Coverage	3,83	4,17	4,00
2	Update of Material	4,50	4,50	4,50
3	Presentation of e-Encyclopedia	4,40	4,20	4,30
	Total Average Value	4,24	4,29	4,27

Based on Table 3, it can be seen that the three aspects developed obtained good criteria. Furthermore, from the total average, an assessment achievement of 4.27 was also obtained which

was in the good category. Thus, the product assessment achievement in terms of the material aspect is that it meets the effective criteria. The comments from language expert validators are that the product developed has presented images accompanied by explanatory sentences that are easy to understand. Furthermore, comments were found regarding the weaknesses of the product, namely that there were some incomplete discussions of material, and there were writings about species that were not appropriate. This comment is used as a reference to improve the research product being developed so that the assessment of material aspects shows a proper assessment. Materially, the validator stated that the e-Encyclopedia product of the order adonata being developed was feasible.

The development of the language aspect was carried out by 2 validators who have competence in scientific language (Biology). Validation was carried out on the material aspects of the e-Encyclopedia with 1 item, the size of the e-Encyclopedia with 2 items, and the e-Encyclopedia cover design with 4 items. Design the contents of the e-Encyclopedia with 9 items. The average value of the assessment results of the two validators is as in Table 4.

Table 4. Validation Assessment Through Media Aspects

No	Aspects (Media) of Assessment	Validator		Average value
		1	2	
1	Book Material	4,00	4,00	4,00
2	The size of the e-Encyclopedia book	5,00	4,00	4,50
3	E-Encyclopedia cover design	4,25	4,25	4,25
4	E-Encyclopedia Content Design	4,56	4,33	4,44
	Total Average Value	4,45	4,15	4,30

Based on Table 4, it can be seen that the four aspects developed obtained good criteria. Furthermore, from the total average, an assessment achievement of 4.30 was also obtained which was in the good category. Thus, the product assessment achievement in terms of the media aspect is that it meets the effective criteria. The comments from media expert validators were that the product developed had presented images and presented material in language that was easy to understand and by the material studied by junior high school students. Furthermore, comments were found regarding the weakness of the product, namely that no other images were found that were similar to the adonata order. The comments given by media experts are that other insect models related to the order adonata should be presented. These comments are used as consideration to improve the research product being developed so that the assessment of material aspects shows a proper assessment. In the media, the validator stated that the e-Encyclopedia product of the order of adonata being developed was feasible.

DISCUSSION

Based on six validators' assessments of the e-Encyclopedia product being developed, a product has been obtained that is suitable for use as teaching material for junior high school students on ordo adonata material. The six validators involved in the validation process are competent experts in their respective fields. The six people consisted of practitioners and teachers who were relevant to their activities. The review/assessment of the encyclopedia is carried out from 3 aspects which include language, material, and media. Viewed from a language perspective, it involves 4 aspects, namely: 1) Straightforwardness as measured by indicators of suitability of sentences with book language rules, suitability of sentences with punctuation, and ease of sentences; 2) Interactiveness as measured by indicators of suitability and effectiveness of e-Encyclopedia e-Encyclopedia material, the accuracy of terms and material in the field of biology; 3) Language rules as measured by indicators of conformity to standard language rules, attractiveness of letters, regular

placement of letters; 4) Language accuracy as measured by indicators of language relevance, ease of language, use of terms. The validator's assessment of the language aspect obtained a total average of 4.35. Thus the e-Encyclopedia design is in the good category.

The results of this research are in line with the findings of (Sabri et al., 2023) in developing teaching materials in terms of the validity of the language aspect with the indicator aspects of grammatical correctness, appropriateness of sentences, encouraging learning, simplicity of sentence structure, questions that do not contain double meanings and clarity of instructions obtained. assessment of language aspects according to valid criteria with high interpretation. Furthermore, the development of the Android-based Kingdom Plantae Mini Encyclopedia for Class

Reviewing the material aspect involves 3 aspects that are assessed. The three aspects are 1) Material Coverage which is measured through the indicators Completeness of e-Encyclopedia Material, Breadth of E-Encyclopedia Material, Depth of e-Encyclopedia Material, Suitability and Effectiveness of E-Encyclopedia Material, Suitability of terms and material in the field of biology, Classification Accuracy; 2) Latest Material as measured by the Faqualization indicator of Material Benefits of e-Encyclopedia for Readers; 3) Presentation of the e-Encyclopedia as measured by indicators of element placement, consistent layout based on patterns, image quality, image placement, use of images, presentation of image captions. Through the assessment of 2 validators on the material aspect, a total average score of 4.27 was obtained. This acquisition value is in the good category.

The results of this research are in line with the findings of Sabri et al., (2023) in developing teaching materials in terms of the validity of the content or material contained in the teaching material products being developed which are in the valid category with high interpretation. The results of research by Azizah et al., (2021) show that the e-encyclopedia developed obtained an average material expert validation score of 85.41%, which means that the product developed is suitable for use as a supplement to teaching materials in class X MIPA 3. Research Solihah et al., (2022) regarding the preparation of an encyclopedia based on the diversity of dragonflies (Odonata) in the Teleng Ngawi Waterfall area as a learning resource for Class has met the eligibility criteria. The development of the Android-based Kingdom Plantae Mini Encyclopedia for Class

Next, 2 validators assessed the media aspects, assessing 4 media aspects consisting of 1) Book Material as measured by the e-encyclopedia Book Print Quality indicator; 2) The size of the e-encyclopedia book as measured by the indicator of the suitability of the size contained in the media, suitability of the size to the material contained in the media; 3) e-Encyclopedia cover design as measured by indicators, cover image, composition, and size of layout elements, title color, use of font; 4) e-Encyclopedia Content Design as measured by indicators: Consistent placement of layout elements based on patterns, clear separation between paragraphs, title and page numbers, image quality, image placement, use of variations in letters (bold, italic, underline, small, etc.), Width of Text Arrangement, Use of Color Composition in Content Material, Overall Media Appearance. From the assessment obtained from the validator, a score of 4.30 was obtained, which gives the interpretation that the research product in terms of the media aspect is in a good category.

The results of research by Azizah et al., (2021) show that the e-encyclopedia development that was developed obtained an average media expert validation score of 84.57%, which means that the teaching material product developed in terms of the media aspect is worthy of being used as teaching material for teaching high school students. Research by Solihah et al., (2022) on the preparation of an encyclopedia based on the diversity of dragonflies (Odonata) as a learning resource for biology subjects in Class meets the criteria of being very feasible. Subsequent research by Marinda et al., (2023) in the development of encyclopedia learning media for science subjects showed that the feasibility of the learning media with an average score of 0.87 was in the high

category, which means that the product being developed is valid. The results of the validity of the E-Encyclopedia of the Order of Odonata from Sumatra through the three aspects developed can be seen in Figure 2.

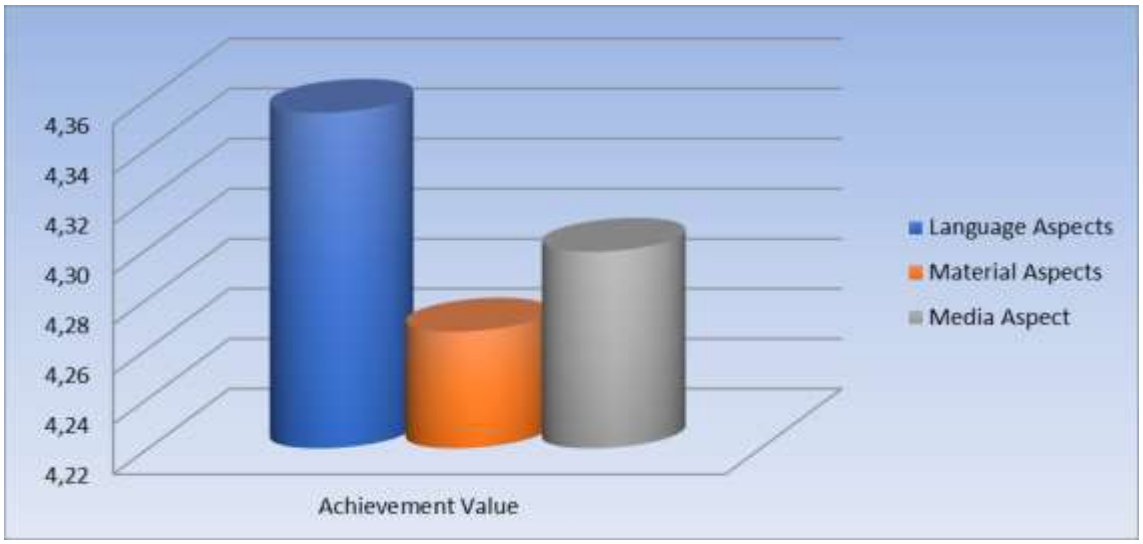

Figure 2. Graphic of Validation Results Assessment Achievement

By paying attention to the three assessment aspects which include language, material and media, these three aspects are in the good category which means that the design of the E-Encyclopedia of the Order of Odonata from Sumatra as Teaching Material for Junior High School Students is good and suitable for use for teaching biology in the material. order of adonata junior high school students.

The results of this research are in line with several previous studies in the development of E-Encyclopedias, namely research conducted by (Cahyanti & Ibrahim, 2018) which revealed that the insect encyclopedia as a learning resource for high school class X is suitable for use in teaching biology lessons to high school students. Likewise, with research conducted by (As'ad et al., 2020) through the development of an insect encyclopedia on organic and inorganic cocoa (theobroma cacao) plantations in Cermo village, Kare subdistrict, Madiun district, an encyclopedia product was obtained that is suitable for use as teaching material for students. Research conducted by (Dawam, 2021) revealed that through the development of an encyclopedia of dragonfly diversity in the Muria mountain area as a biology learning resource for class media experts at 70%; biology teachers at 84%; and the student response was 87.6%, which means that the dragonfly diversity encyclopedia developed has qualities that are suitable for use as a biology learning resource. Furthermore, research conducted by Solihah et al., (2022) through the preparation of an encyclopedia based on the diversity of dragonflies (odonata) in the Teleng Ngawi waterfall area as a class x learning resource, obtained a design for a dragonfly encyclopedia based on its diversity. Furthermore, the results of data analysis Ismail et al., (2022) from media validity tests on e-encyclopedias assessed by expert validators can be seen in Table 3. The total average (validity value) of media validity is all in the range $4 \leq Va < 5$, that is, it is included in the valid category, the movement system E-encyclopedia media as a learning resource for class XI that was developed is good/valid.

CONCLUSION

The conclusions from this research are: 1) The resulting e-Encyclopedia product is a good teaching material for the Order of Adonata that is suitable for use as teaching material for junior high school students; 2) This e-Encyclopedia product provides material on Morphology, Behavior,

Life Cycle, Habitat, Benefits, Classification of the order adonata which has passed the Design Research stage; 3) This e-Encyclopedia product also makes it easier for junior high school students and the general public to learn about the adonata order. Furthermore, based on the conclusions that have been described, the following can be recommended: 1) Students can use e-Encyclopedia as an alternative teaching material in studying the order adonata; 2) To parents or teachers in junior high schools to direct students to open applications that are useful and appropriate to the students' learning material; 3) This e-Encyclopedia can be easily distributed by utilizing the internet via online media. As an implication of this research, in the future it is hoped that teachers, students and the general public will be able to use e-encyclopedias in learning activities, especially in biology lessons regarding the odonata order, and other researchers will need to carry out broader studies on other animal orders and conduct field trials to test the practicality and effectiveness of the designs that have been discovered.

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JPBIO (Jurnal Pendidikan Biologi)
Vol. 9, No. 1, April 2024, 123 – 132 //e-ISSN 2540-802X
http://jurnal.stkippersada.ac.id/jurnal/index.php/JBIO/index

The Phytochemical constituents and biological activities of sungkai (Peronema canescens Jack) leaves hydroethanolic extracts

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Article Info

Article History:
Received 28 March 2024
Revised 04 April 2024
Accepted 07 April 2024
Published 30 April 2024

Keywords:
Antioxidant activity
Peronema canescens
Flavonoid
Phenolic
DNA protection

ABSTRACT

Peronema canescens, *Jack* commonly known as "sungkai," has been traditionally used as a herbal medicine for various health conditions. This study aimed to explore the bioactive compound of the *P. canescens* leaves hydroethanolic extract, along with assessing its antioxidant and antimutagenic properties. Liquid chromatography-mass spectrometry (LC-MS) was utilized for phytochemical analysis of the hydroethanolic extracts, while antioxidant activity was evaluated through the DPPH radical scavenging method. Quantification of total phenolic and flavonoid content was achieved via colorimetric analysis. Furthermore, the DNA protection activity was assessed using plasmid pBR322 subjected to free radical treatment. The primary bioactive compounds identified in the *P. canescens* hydroethanolic extracts belonged to the alkaloid and flavonoid groups. The antioxidant activity of *P.canescens* leaves hydroethanolic extracts showed an IC50 value of 0.02±0.00 µg/mL. Additionally, the total flavonoid and phenolic content were measured at 33,769±3,626 µg QE/mL and 638,924±6,683 µg GAE/mL, respectively. Notably, *P.canescens* exhibited significant potential in mitigating DNA damage. In conclusion, the *P. canescens* leaves hydroethanolic extracts demonstrate promising attributes as a herbal medicine, highlighting notable antioxidant and antimutagenic effects.

Citation: Rahayu, I., Sudrajat, S.E., Sancnia, Puspasari, M., & Timotius, K.H. (2024). Phytochemical constituents and biological activities of Sungkai (*Peronema canescens Jack)* leaves hydroethanolic extracts. *JPBIO (Jurnal Pendidikan Biologi), 9*(1), 123-132. DOI: https://doi.org/10.31932/jpbio.v9i1.3398

INTRODUCTION

Peronema canescens, Jack commonly known as sungkai or jati sabrang, is a wild plant from the *Verbenaceae* family. Sungkai is a significant export commodity, particularly in Sumatra and Kalimantan. This botanical species is utilized extensively in herbal medicine, with its entire plant being employed either through crushing or brewing for a range of medicinal purposes such as mouthwash, anti-parasitic treatment, relief for coughs and colds, among others (Brata and Wasih, 2021). Sungkai has been subject to analysis of its active constituents. This examination revealed flavonoids, alkaloids, steroids, phenolics, peronemin, sitosterol, isopropanol, phytol, diterpenoids, tannins, and saponins in Sungkai leaves. Notably, the compounds identified in Sungkai leaves exhibit primary activity as antioxidant and antibacterial (Ahmad & Ibrahim 2015).

Antioxidants are substances capable of counteracting the effects of free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS). The antioxidants present in sungkai leaves aid in counteracting and reducing the impact of free radicals, thus protecting biological molecules against oxidative stress (Nimse & Pal 2015). Redox homeostasis denotes the equilibrium encompassing the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), along with their neutralization by antioxidants. This phenomenon interconnects with fundamental cellular processes, where oxidative stress ensues from an imbalance between pro-oxidants and antioxidant entities (Shadfar et al. 2023).

Oxidative stress can potentially trigger DNA damage and impair DNA repair mechanisms. Oxidative stress disrupts numerous cellular functions, notably those responsible for preserving DNA integrity. The accumulation of DNA damage may contribute to an increased mutation rate and can induce alterations in gene expression, ultimately leading to disruptions in cellular metabolism (Włodarczyk and Nowicka 2019). DNA damage was involved in the initiation of carcinogenesis and the development of degenerative diseases, such as obesity, diabetes mellitus, atherosclerosis, and cardiovascular disease. Notably, age-related neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease, increasingly manifest DNA damage and deficiencies in DNA repair mechanisms (Izzotti 2002; Shadfar et al. 2023).

Several studies have been conducted on phytochemical analysis, antioxidant activity, and immunomodulatory effects (Ahmad and Ibrahim 2015; Dillasamola et al. 2021), yet no investigation has been conducted into their potential in protecting DNA from damage. Consequently, this research aims to evaluate the active compounds present in polar extracts, their antioxidant activity, and DNA protection capabilities.

RESEARCH METHODS

Research Design

This research was a laboratory experimental study. LCMS was employed to analyze active compound content. Antioxidant activity was quantified relative to the existence of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). DNA protection activity was assessed using plasmid pBR322 and treated with the free radical.

Population and Samples

Peronema canescens Jack (local name Sungkai) was taken from Palangkaraya, Kalimantan. One of the authors identified the plant utilizing the determination keys outlined by Maxwell (1978).

Instruments

The LC-MS/MS analysis was conducted using the Waters LCMS/MS-QTOF system, utilizing the Tof MSE operational mode. A CI8 column was utilized for this analysis. A

spectrophotometer was used to evaluate antioxidant activity, whereas an Electrophoresis apparatus was employed to assess DNA protection activity.

Procedures

1. Plant extraction

Ten grams of sungkai powder underwent maceration with 80% ethanol in 300 mL of distilled water for a duration of 24 hours. The filtrate obtained from this process was subsequently evaporated, yielding a pellet. This pellet was then employed for phytochemical analysis, evaluation of antioxidant activity, and conducting a DNA protection assay.

2. LC-MS analysis

The bioactive constituents present in Sungkai hydroethanolic leaf extract were analyzed using LCMS/MS-QTQF (Waters), utilizing TOF MSE as the operational mode. The chromatographic separation was conducted using a C18 column, with the mobile phase consisting of 0.1% formic acid in acetonitrile and 0.1% formic acid in distilled water. Initially, 5% (w/v) samples in methanol were prepared and homogenized for 30 minutes. Subsequently, 10 µL of the filtrate were injected into the system. The UNIFI software was utilized for the screening process to identify active constituents in the samples (Rahayu and Timotius 2022).

3. Total Phenolic Content

The total phenolic content was measured utilizing the Folin-Ciocalteau assay, with gallic acid as the standard. Specifically, 0.5 mL of the sample was combined with 2.5 mL of 10% Folin-Ciocalteau reagent. Incubation was done for 10 minutes. Then, 2.5 mL of 75 g/L Na2CO3 was added to the sample-reagent mixture. This mixture was left to incubate for 2 hours at room temperature. Subsequently, absorbance measurement was performed at 765 nm. The total phenolic content was determined by quantifying it as gallic acid equivalent (GAE) utilizing a standard curve and subsequently expressed as mg GAE /mL (Shukla et al. 2016).

4. Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride colorimetric method. A standard curve was prepared by dissolving 50 mg of quercetin in 1 mL ethanol 95%, and subsequent dilution. The diluted standard solution (0.5 mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% AlCl3.6H2O, 0.1 mL of 1M sodium acetate, and 2.8 mL of water. Following a 30-minute incubation period at room temperature, the absorbance of the solution was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer. The acquired results were interpreted directly against the quercetin standard curve and expressed as mg EQ/mL (Chia-Chi et al. 2002).

5. Antioxidant Activity with DPPH Radical Scavenging

A volume of 500 µL of various concentrations of hydroethanolic extract was reacted to 150 mM DPPH in absolute methanol, followed by an incubation process in the dark at room temperature for 30 minutes. An antioxidant standard, butylated hydroxytoluene (BHT), was employed as a reference substance. The absorbance of the mixture solution was measured at 517 nm (Singleton et al. 1999). The % free radical inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - \text{Abs Sample}}{\text{Abs control}} \text{ x } 100\%$$

6. DNA Protection Activity Assay

The DNA model utilized in this study was the pBR322 plasmid. The plasmid DNA pBR322 was treated with the OH^- radical generated from the Fenton reaction. The conversion of plasmid DNA pBR322 from its supercoiled conformation to open-circular and linear forms was utilized as an indicator of DNA damage (Jeong et al. 2009). The reaction mixture (15 µL)

consisted of 5 µL of phosphate-buffered saline (PBS, 10 mM, pH 7.4), 1 µL of plasmid DNA (0.5 µg), 5 µL of the sample, 2 µL of 1 mM FeSO4, and 2 µL of 1 mM H_2O_2. This mixture was then incubated at 37 °C for 30 minutes. Following the incubation period, 2 µL of loading dye (Geneaid) was added to stop the reaction. Subsequently, the solution mixture was subjected to electrophoresis on a 0.85% agarose gel supplemented with 3 µL of florosafe (Rahayu & Timotius, 2022).

Data Analysis

The information acquired from LCMS comprised active compounds, which were then sorted into specific categories according to their corresponding compound groups. Descriptive data analysis was employed to provide a thorough summary of the data, abstaining from extensive statistical inferences. Linear regression was utilized to assess total phenolic content, total flavonoid content, and antioxidant activity. The analysis of DNA protection was conducted descriptively.

RESULTS

Liquid chromatography-mass spectrometry (LC-MS) is an advanced analytical method that integrates the separation functionalities of liquid chromatography with the detection and characterization capabilities offered by mass spectrometry (Naczk and Shahidi, 2004). Two dominant compounds were identified in the hydroethanolic extract of Sungkai leaves: alkaloids and flavonoids. One alkaloid compound, betaine, was detected, while a total of 10 flavonoid compounds were identified, such as genkwanin, yuankanin, luteolin, sophorabioside, aceosidine, undulatoside A, and the derivatives of dimethoxyflavone, kaempferol, quercetin, and chromone (Table 1).

Table 1. LCMS Phytochemical analysis

No	Identified compound	Ionization mode	RT	MZ	Molecular formula	Response
		Alkaloid				
1	Betaine	Positif	0,58	118 229 365	C5H11NO2	13.860
		Flavonoid				
2	3,5,6-Trihydroxy-4',7-Dimethoxyflavone	Positif	15,09	270 331 463	C17H14O7	432.454
3	3,6,7- Trimethylquerce-tagetin	Positif	15,82	361 431 759	C18H16O8	45.879
4	6-Methoxy-2-[2-(4'-methoxyphenyl)ethyl] chromone	Positif	16,48	327 367 711	C20H22O4	208.719
5	Genkwanin	Positif	11,06	242 285 469	C16H12O5	197.619
6	Kaempferol 3-O-α-L-rhamnopyranosyl-(1-->2)-β-D-glucuronopyranoside	Positif	12,46	301 317 617	C27H28O16	22.009

No	Identified compound	Ionization mode	RT	MZ	Molecular formula	Response
7	Kaempferol-3-gentiobioside	Positif	11,23	317 611 633	C27H30O16	154.508
8	Luteolin-7,4'-di-O-β-D-glucopyranoside	Positif	11,41	317 611 633	C27H30O16	264.955
9	Quercetin-3-Oglucuronide 6″-methylester	Positif	11,17	315 493 625	C22H20O13	53.008
10	Yuankanin	Positif	10,46	285 579 601	C27H30O14	338.011
11	Sophorabioside	Positif	9,26	163 285 647	C27H30O14	68.836
12	Jaceosidin	Negatif	15,37	199 299 329	C17H14O7	130.995
13	Undulatoside A	Negatif	5,6	178 191 353	C16H18O9	10.103

The compounds constitute a group of potential antioxidants. This is demonstrated by the total phenolic and flavonoid contents and its ability to scavenge DPPH free radicals (Table 2). The research findings indicate that the antioxidant activity of Sungkai leaves hydroethanolic extract is stronger than that of BHT. This outcome is supported by its total phenolic and flavonoid contents. Phenolics and Flavonoids are commonly known for their antioxidant properties.

Table 2. Antioxidant activity of Sungkai leaves hydroethanolic extract

Sample	IC_{50} (µg/mL)	Total Flavonoids Content (µgQE/mL)	Total Phenolic Content (µgGAE/mL)
Sungkai	0,02±0,00	33,76± 3,62	638,92±6,68
BHT	6,21±0,21	-	-

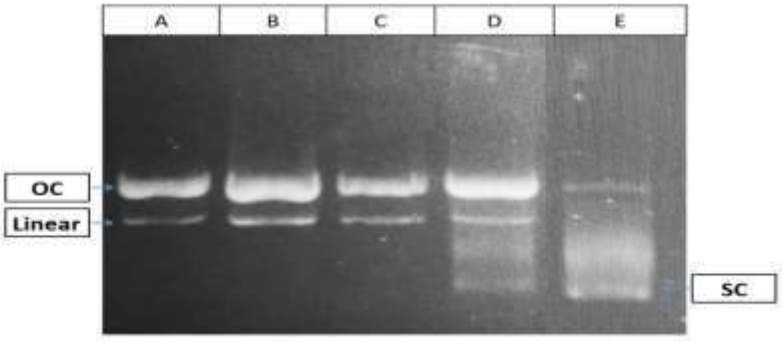

Figure 1. DNA protection activity; The changes in the topological structure of DNA plasmid pBR322 in various concentrations.

We also evaluated its antioxidant capability in protecting DNA from free radicals. In this study, we employed the plasmid DNA pBR322 as a model. The plasmid DNA was subjected to OH^- free radicals generated through the Fenton reaction. The results are evident from the changes in the plasmid DNA conformation (Figure 1). There is a difference in conformation between the normal plasmid DNA and those treated with free radicals. Administration of the sungkai leaves hydroethanolic extract restored DNA supercoil at 22,24 mg/mL. The results indicate that the antioxidant compounds of sungkai leave hydroethanolic extract have the potential to protect the DNA from free radicals.

Table 3. Antimutagenic analysis

Code	Treatment
A	Plasmid + H_2O_2 + Fe_2SO_4
B	5,56 mg/mL Hydroethanol + Plasmid + H_2O_2 + Fe_2SO_4
C	11,12 mg/mL Hydroethanol + Plasmid + H_2O_2 + Fe_2SO_4
D	22,24 mg/mL Hydroethanol + Plasmid + H_2O_2 + Fe_2SO_4
E	Non treated Plasmid

DISCUSSION

This research indicates that the flavonoid group comprises the predominant active compounds found in the hydroethanolic extract of Sungkai leaves, while alkaloids were present in lesser quantities. Flavonoids and alkaloids are two classes of organic compounds commonly found in plants, each with distinct chemical structures and biological properties. Alkaloids are a diverse set of compounds originating from amino acids, displaying diverse biological activities, and can be synthesized by a range of organisms, including bacteria, fungi, and plants (Boratyński et al. 2019; Liu et al. 2019).

Alkaloids primarily exert immunomodulatory effects by regulating cytokines such as IL-6, IL-12, IL-1α, TNF-α, IL-1β, and IL-10 (Liu et al. 2019). Betaine, also known as trimethylglycine, is a naturally occurring compound. It is derived from glycine by adding three methyl groups (Dobrijević et al. 2023). One of its primary functions is to modulate the levels of homocysteine in the bloodstream (McRae 2013). Homocysteine is associated with an increased risk of cardiovascular diseases, strokes, and various health issues when found in elevated concentrations. Betaine aids in the conversion of homocysteine into other beneficial compounds, thereby contributing to the maintenance of optimal levels of this amino acid in the bloodstream (Arumugam et al. 2021; Truit et al. 2021).

The flavonoid group identified in this study consists of luteolin, sophorabioside, jaceosidine, and derivatives of dimethoxyflavone, genkwanin, kaempferol, quercetin, and chromone. Flavonoids, which are polyphenolic compounds, significantly protect plant cells from microorganisms, insects, and UV radiation (Harborne & William, 2000). Flavonoids are synthesized via the phenylpropanoid metabolic pathway, forming diverse structural patterns within flavonoid subgroups. These structural differences account for the variations in the biological effects of these compounds on organisms (Santiago *et al.* 2021).

Flavonoids exhibit antioxidant, anti-inflammatory, and immunomodulatory properties (Serafini et al. 2010). Sophorabioside and undulatoside A are flavonoid glycosides. Sophorabioside has been reported to exhibit estrogenic activity, promote osteoblast proliferation, and inhibit IL-5 activity (Min et al. 1999; El Halawany et al. 2010; Xu et al. 2009). Sophorobioside is an anti-inflammatory agent by suppressing NF-κB signaling (Lee et al. 2013). Undulatoside A, recognized as a chromone derivative, is acknowledged for its anti-inflammatory, antimicrobial, and

immunomodulatory properties (Koz et al. 2009; Pereira et al. 2020; Yu et al. 2017). In silico studies have indicated its potential as an antiviral agent (Rahayu et al. 2022). Jaceosidin is a natural methylated trihydroxyflavone and has demonstrated antihyperglycemic, antioxidant, and anti-inflammatory characteristics (Han et al. 2018). Genkwanin is a methoxyflavone with antioxidant, anti-inflammatory, neuroprotective, anticancer, antidiabetic, and antiviral activities (El Menyiy et al. 2023). Luteolin, 3′,4′,5,7-tetrahydroxyflavone, demonstrates various biological effects, including anti-inflammatory, antiallergic, and anticancer properties (Lin et al. 2008). Kaempferol also suppresses the proliferation of cancer cells, hampers angiogenesis, and triggers apoptosis in cancer cells (Chen & Chen 2013). Quercetin is one of the flavonoid groups recognized for its anti-inflammatory, antihypertensive, vasodilatory, anti-obesity, antihypercholesterolemic, and antiatherosclerotic properties (David et al. 2016).

In this study, we also quantified the total phenolic and flavonoid content and its ability to scavenge the activity of the DPPH free radical (Table 2). The analysis revealed that the concentrations of flavonoids and phenolics were 33,769±3,626 µgGAE/mL and 638,924±6,683 µgQE/mL, respectively. The IC50 of antioxidant activity was 0,02±0,00 µg/mL, stronger than BHT (IC50 6,21±0,21 µg/mL). Sungkai leaves hydroethanolic extracts contain relatively high levels of total flavonoids and phenolics and exhibit potent antioxidant activity. Flavonoids and phenolics play a crucial role in antioxidant activity, wherein flavonoids are categorized within the phenolic compound group and exhibit a direct correlation with antioxidant effectiveness (Panche *et al.* 2016). Phenolic compounds contribute to antioxidant efficacy owing to their inherent structural characteristics (Munteanu & Apetrei 2021). This contribution manifests through various mechanisms, including reduction processes, capture of free radicals, chelation of metals, quenching of singlet oxygen, and donating electrons (Phaniendra et al. 2014). The findings highlight a direct association between antioxidant activity and the levels of total phenolics and flavonoids. The higher the levels of flavonoids and phenolics, the stronger the antioxidant activity of Sungkai leaves hydroethanolic extracts.

We assessed the antioxidant potency through its activity in protecting DNA from damage caused by free radicals. Free radicals are known to induce DNA damage, resulting in mutagenesis. It is closely associated with the onset of diseases (Izzotti, 2002; Shadfar et al. 2023). In this study, the DNA model utilized was plasmid pBR322. The hydroxyl radicals (OH-) generated through the Fenton reaction are responsible for instigating damage to the DNA strand, thereby causing a conversion from its typical supercoiled (SC) structure to an open circular (OC) conformation. Figure I demonstrates the potency of the Sungkai leaves hydroethanolic extract in protecting DNA. When DNA damage occurs, the regular conformation of the plasmid changes. The typical conformation of plasmid DNA consists of three forms: Open Circular (OC), Linear (L), and Supercoiled (SC) (Figure 1). DNA damage becomes apparent when there is a high intensity of OC and the absence of SC form. The research findings indicate that at a concentration of 22.24 mg/mL, the hydroethanolic extract of Sungkai leaves can restore the typical conformation of plasmid DNA (Figure 1).

DNA damage is recognized as a crucial determinant in cancer development. When DNA undergoes damage, it generates abnormal nucleotides, disrupting one or both DNA strands. The interruption of a DNA strand precipitates various intricate issues, including mutations and genome instability (Alhmoud et al. 2020). Consequently, when DNA sustains damage, genotoxic agents interfere with the covalent bonds between nucleotides, impeding normal replication and transcription processes within the genome (Cannan & Pederson, 2016). Although the DNA repair mechanism can rectify damaged DNA, its efficacy is not absolute and comprehensive. The outcomes of this repair mechanism may give rise to additional complications, such as chromosome aberrations and mutations that impede cellular functions (Sáez, 2018). Defects in genes can

manifest in oncogenes, tumor suppressor genes, and genes governing the cell cycle, which prove detrimental to cellular defense and proliferation. Such DNA damage is commonly referred to as carcinogenic (Basu, 2018).

Conversely, DNA damage contributes to the aging process through cumulative accumulation over time. At the cellular level, the ability to maintain balance diminishes as cells become unable to rectify protein distortions stemming from DNA damage. Consequently, this can disrupt the normal functioning of cells, thereby predisposing individuals to various diseases, including Alzheimer's. In Alzheimer's patients, cellular dysfunction arises due to the inability to produce adequately folded proteins, leading to the formation of toxic protein aggregates. Individuals with diminished DNA damage repair capabilities face an elevated risk of developing cancer as they age (Luu & Palczewski, 2018).

Therefore, it appears that the antioxidant compounds found in the Sungkai leaves' hydroethanolic extract may protect DNA from damage. Their activity is evident through their ability to scavenge free radicals and reduce damage effects. The hydroethanolic extract of Sungkai leaves has the potential to safeguard genomic stability, thus reducing mutations and the development of diseases.

CONCLUSION

In this study, we discovered that the hydroethanolic extracts of Sungkai leaves contain two main categories of compounds: alkaloids and flavonoids. The antioxidant compounds exhibit higher activity than BHT and demonstrate protective activity against DNA damage caused by free radicals. Further research is needed to investigate the activity of active compounds in preventing the progression of degenerative diseases.

ACKNOWLEDGMENT

We are grateful to the Research and Community Institute of Universitas Kristen Krida Wacana (LPPM-UKRIDA) for funding this research.

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