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Analysing the Bioactive Compound of Earthworm Pheretima Javanica Extract by Using Gas Chromatography Mass Spectrum

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Research Based Learning (RBL) is a learning method that uses contextual learning, authentic learning, problem solving, cooperative learning, hands on and minds on learning and inquiry learning. The STEM approach in learning is expected to provide meaning to students through the systematic integration of research-based knowledge, concepts and skills. STEM design begin with the identification of social problems. The social issue of typhoid fever cases caused by Salmonella enterica Typhi bacteria with a high mortality rate in the world is a public health problem. The existence of Pheretima javanica which is abundant in the environment can be utilized by conducting antibacterial activity testing and analysis of bioactive compounds by students on the form of the extract. This research is a development research carried out with the aim of developing a product and also a qualitative descriptive research which serves to provide an overview of the object. The steps in carrying out these tests were identified as part of learning that hone creative thinking skills. The results of the class experimental research showed that the N-Gain score was 0.8, which is in the high category. These results show that the application of developing problem-based learning tools with a STEM approach is effective. Learning outcomes contribute to the effort to find alternative drugs that are effective and do not cause resistance. In addition, students also produce scientific articles as learning products. This paper has implications for STEM integrated research-based learning.

Keywords: RBL, STEM, learning, effective, learning outcome, research based learning

INTRODUCTION

Characteristics in Natural Science learning is learning that is systematically structured through data collection by experiments based on facts, concepts, principles to work

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procedures related to the explanation of a symptom (Umar & Yusuf, 2011). Therefore, the development of research-based learning is very appropriate to be developed in science learning.

The principle of how to teach research-based learning is that in developing mastery, students must acquire skills, practice components to integrate them, and know when to apply what they have learned. Goal-directed practice coupled with targeted feedback improves the quality of student learning (Ambrose et al., 2010). Research-based learning is learning based on a research approach as a step in the process, that the learning process takes place as a combination of the characteristics of research action so as to create meaningful learning (Firmadani, 2017). Research on the development of research-based learning tools was conducted by Zhu and Singh C (2012) who developed research-based learning tools to help students build better knowledge structures about quantum measurement which concluded that this learning was effective in increasing students' understanding of quantum measurement.

The development of the times in the era of globalization, in addition to research-based learning, learning today needs to follow one of them by integrating Science, Technology, Engineering, and Mathematics (STEM) (Kocabas et al., 2020). STEM is a discipline that is closely related to one another. Implementation of STEM Integrated Project Based Learning to Improve Student's Science Literacy in terms of Gender was carried out by Afriana and her friends that there are significant differences in science attitudes between male and female students and in the conclusion of the study. The STEM approach to learning is expected to produce meaningful learning for students through the systematic integration of research-based knowledge, concepts and skills. Morison in Afriana states that some of the benefits of the STEM approach make students better able to solve problems, innovators, inventors, independent, logical thinkers, and technological literacy. STEM activity design begins with identification of social problems. Looking for antibacterial protein as an alternative medicine in cases of typhoid fever caused by *Salmonella enterica* Typhi bacteria in Indonesia has been posted as a public problem that must be resolved in this study (Crispin, 2019).

The social problem that becomes the background for finding antibacterial proteins in earthworm extracts in active protein isolation is typhoid fever cases which are still a public health problem with as many as 22 million cases per year in the world and causing 216,000–600,000 deaths (WHO, 2011; Brockett, et al., 2020). The results of case studies at major hospitals in Indonesia indicate an increasing trend in the number of typhoid cases from year to year with an average of 500 / 100,000 patients and an estimated death of 0.6–5%. Prevention by the government in the form of vaccination is inefficient and contradictory. Treatment with antibiotics still causes relapse and resistance (Deparytment of Health, 2006). So it is necessary to find efforts to find prevention and prevention that are natural in nature, including finding active protein ethanol extract 70% of earthworm *Pheretima javanica* which has an inhibitory power against the growth of *Salmonella enterica* Typhi as a cause of typhoid fever.

The university has the main task of organizing higher education and providing education based on Indonesian culture in a scientific way which includes education,

research, and community service to develop abilities and improve the quality of life and dignity of Indonesian people in an effort to realize national goals (Marbun et al., 2020).

As the reason for using earthworms in the development of learning because of its abundant availability in Indonesia. The genus *Pheretima* which is included in the Megascolecidae family is an earthworm that is found relatively the most in Indonesia (Gily, et al., 2020; Forster and Leder. 2021). Earthworm medicine has been used in various cities. Its use as a typhoid fever medicine in various ways, starting from boiling then drinking and some fried or crushed mixed with chayote or turmeric and then drink it. According to Boman in Waluyo (2004) antibacterial protein is recognized as the largest compound against self-protection with ease of production speed. Their wide specifications and lack of toxic effects on organisms are the reasons for the use of earthworms as antibacterials. besides that, it is also safer because its chemical components do not cause toxic effects for humans when consumed (Waluyo, 2004).

METHOD

This research was carried out in the Biology Education Laboratory of the Faculty of Teacher Training and Education, Jember University and Jember Polytechnic Bio-Science, to produce the development of teaching materials that will be included in the learning steps. This research is a combination research (mix methods), a research method that combines or combines two methods, quantitative methods with qualitative methods. These two methods are used simultaneously in a research activity. This research procedure consists of two studies, namely development research and experimental testing. The research began with research in the laboratory to produce teaching materials, then continued with R & D research to produce learning tools.

Sample Collection and Preparation

Identification by looking at the characteristics of the organs of the body by looking at the number of segments, the location of the clitellum in the segment, body color, the number of seta and the location of the seta, the shape of the mouth and the shape of the body. Identification using the reference book identification Gates (1948) and Edwards & Lofty (1972). The healthy and mature earthworms *P. javanica* that have been identified are then cleaned with distilled water and weighed as much as 1 kg for drying. Dried earthworms using an oven at 55°C for 1 hour to obtain dry samples. The dry sample is then crushed and used in the extraction process.

Extraction of Earthworm

Earthworm extract is a concentrated preparation obtained by extracting the active substances in earthworms using a suitable solvent. it is weighed as much as 114 grams then extracted with ethanol solvent 70%. Extraction was carried out by means of worms in an oven until they reached constant dryness, a mixture of earthworms with 1: 3 solvent in a blender, then macerated by soaking in solvent for 24 hours in a shaker in a place protected by light. After that it is filtered and the results of the filtration are evaporated using a rotary evaporator to remove the remaining solvent, so that a thick extract will be obtained.

Antibacterial Analysis

The antibacterial test of earthworm extract by the agar well diffusion method. The media is in the form of agar that has solidified in a petri dish that has previously been inoculated with *Salmonella enterica* Typhi. Each petri dish was made one well using pipe molding to be filled with extract. The concentration of the protein fraction tested was 1000 ppm. Petri dishes were incubated in an incubator for 24 hours at 37°C. The petri dish was then observed and the clear zone formed was measured. The clear zones indicate inhibition of *S.* Typhi by the earthworm extract.

Experimental Research Methods in Classroom Learning

Quantitative research with experimental research on classroom learning. Experimental research was carried out by 5th semester students of the Biology study program at the Indonesian Open University. The research subjects consisted of one class with 19 students as respondents. Research subjects were selected using a purposive sampling method, namely focusing on the suitability of the subject to the required categories according to the teaching materials used. The students selected were students taking biotechnology courses.

Data collection instruments for assessing the effectiveness of learning tools are carried out using assessments in the form of learning outcome tests. The learning outcomes test was carried out twice, namely a test before learning (pretest) and a test after learning (posttest). Then the learning results were analyzed using the n-Gain test using Microsoft Excel to determine the level of effectiveness of developing research-based learning tools using a STEM approach. The n-Gain category value scale uses three scales, including G>0.7, which is in the high category, 0.3<G<0.7, which is in the medium category, and G<0.3, which is in the low category. The following is a scheme for research design in class.

Table 1
Scheme of research design in the classroom

Experimental class	T_1	X_1	T_3

Information: T_1 : Pretest; X_1 : Experiment; T_3: Posttest

In developing the steps for STEM integrated research-based learning activities, several studies were carried out to produce material developed from theory. Besides that, it also comes from laboratory and field research. The aim is to test the antibacterial activity of the earthworm *Pheretima javanica* which begins with the identification and extraction process of earthworms.

After conducting the experimental test, it was continued with the development to design STEM integrated research-based learning steps in student learning activities. The syntactic chart of the stages of implementing RBL can be seen in Figure 1.

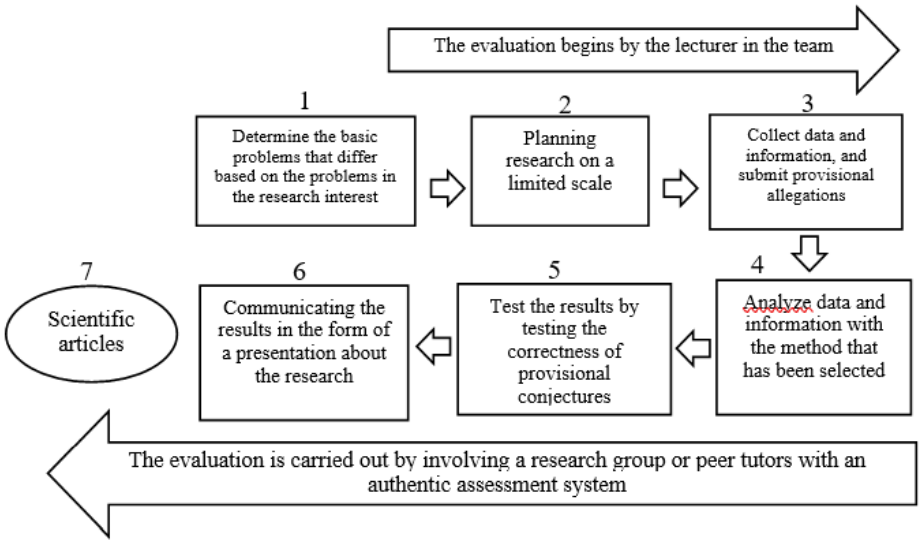

Figure 1
The syntactic chart of the stages

FINDINGS AND DISCUSSION

Experimental Test Development of STEM Activity Teaching Materials

In developing the steps of integrated STEM research-based learning activities, several studies were carried out to produce material developed from theory and laboratory and field research. The aim is to test the antibacterial activity of the earthworm *Pheretima javanica* which begins with the process of identification and extraction of earthworms. Test steps for antibacterial activity can be seen in Figure 2.

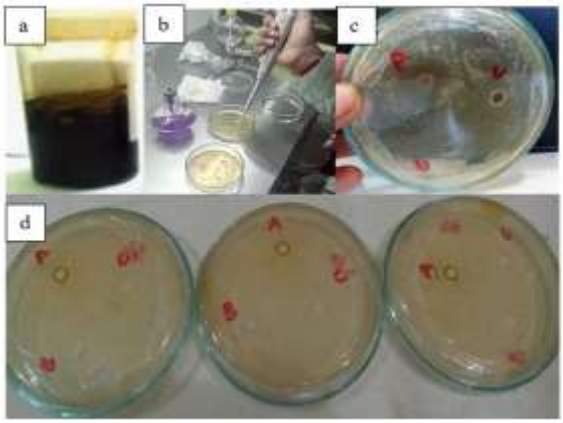

Figure 2
The steps of antibacterial activity test

Figure 2 shows the steps of antibacterial activity test of earthworm extract. Earthworm extract was obtained by extraction with materation method. The resulting extract is brown and in the form of a paste (a). The extract was further tested for antibacterial activity by the well method (b). The bacterial culture used was *Salmonella enterica* Typhi. The results of the antibacterial activity test were indicated by the presence of an inhibition zone around the well filled with extract samples (c). The inhibition zone in the form of a clear area indicates the presence of inhibitory activity against bacterial growth. The results of the test with different treatments compared to the diameter of the formed inhibition zone (d). These research results are used as student learning material.

Experimental test to produce teaching materials in conducting antibacterial activity test using earthworm extract which has been extracted using 70% ethanol solution. The results of the antibacterial activity test showed that there was an inhibition zone on the agar medium grown with *Salmonella enterica* Typhi bacteria. The observation data in Table 2 shows the results of three repetitions of the antibacterial activity test against *S.* Typhi bacteria. The average inhibition zone for *P. javanica* extract was 18.3 mm while chloramphenicol was 28.3 mm. The distilled water as a control negative, so have no zone of inhibition. The zone of inhibition for chloramphenicol is larger because chloramphenicol is a positive control, which is an antibiotic for the treatment of infections caused by bacteria. This shows that earthworm extract can inhibit the growth of bacteria that cause typhoid fever.

Table 2
Diameter of the inhibition zone of *P. javanica* extract against *Salmonella enterica* Typhi

Repetition	Inhibition zone diameter (mm)		
	Pheretima javanica extract	Chloramphenicol	Distilled water
1	15	35	-
2	20	25	-
3	20	25	-

Results of classroom experimental research

The results of the classroom experimental research in this study were in the form of pre-test and post-test learning outcomes and pre-test and post-test N-Gain results were obtained to determine the level of effectiveness of the learning tools developed.

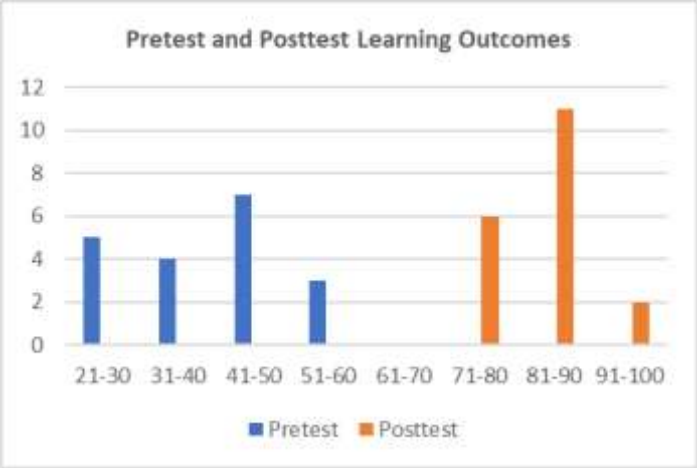

Figure 3
Pretest and posttest learning result scores

Based on Figure 3, the pretest and posttest learning results show significant differences in scores. The pre-test scores obtained ranged from 21 to 60, but the post-test scores obtained ranged from 71 to 100. This significant difference in scores shows that there is an influence of implementing the development of research-based learning tools with a STEM approach. The level of effectiveness in implementing the development of these tools will be described in Table 1 as follows.

Table 3
N-Gain pretest and posttest results

Number of Students	Average Score		Difference	*N-Gain*	Category
	Pretest	Posttest			
19	42.63	85.53	42.89	0.8	High

Based on Table 3, the pretest mean was 42.63 and the posttest score was 85.53 with a difference between pretest and posttest of 42.89. The N-Gain results obtained an average score of 0.8 in the high category. The results of N-Gain are that the application of developing problem-based learning tools using a STEM approach is very effective**.**

Framework of RBL in STEM

The framework for integrating research-based learning models with the STEM approach can improve students' creative thinking skills in solving problems by using *Pheretima javanica* extract as an alternative medicine in cases of typoid fever. The framework of RBL in STEM education can be seen in Figure 4.

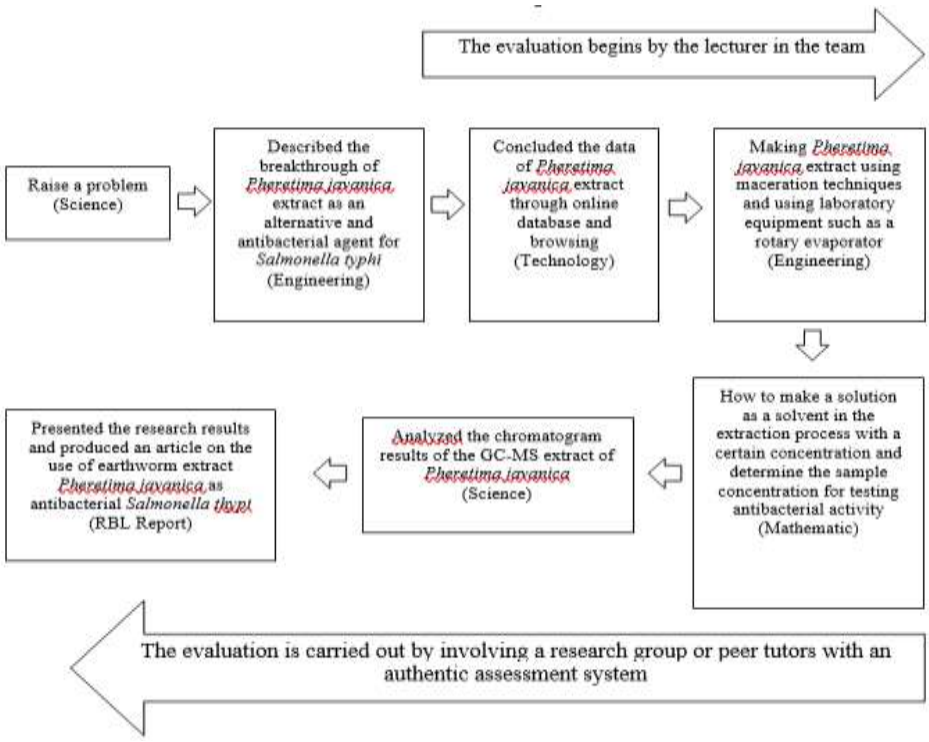

Figure 4
The framework of RBL in STEM education

Learning Outcome

Students can demonstrate the making of earthworm extracts, carry out antibacterial activity tests and analyze the results of GCMS. STEM integrated research-based learning activities carried out can improve students' creative and innovative thinking skills. Students are able to be creative in coming up with new ideas on making earthworm extracts from ideas that have long been seen as innovations. Creative and innovative will always go hand in hand. Creativity is the ability to generate new ideas by combining, changing, or re-applying existing ideas. Generating new ideas from existing ideas is seen as innovation.

Researcher Role

In implementing STEM integrated research-based learning in the process, students are accompanied by researchers in the Biology Education study program class. Students will learn from identifying earthworms, extracting earthworms, testing antibacterial activity to analyzing GCMS results on earthworm extracts. Extraction of *Pheretima javanica* was carried out using ethanol solvent with a concentration of 70%. Then the extract was tested for antibacterial activity by looking at the inhibition zone in the culture area of *Salmonella enterica* Typhi.

STEM integrated-based learning is problem-based learning that places scientific inquiry and the application of mathematics with the context of technological design to solve problems (Sanders, 2009). Students will gain an understanding of Science, Mathematics, Engineering and Computer (Technology) in the concepts of Science and Produce products using the skills required in the field (Ulfa, 2019). STEM Education subjects in learning activities are as follows:

Science - Students are expected to be able: (1) Understanding the potential of *Pheretima javanica* as an antibacterial in typoid fever. (2) Develop an understanding of earthworm characteristics. (3) Understanding how to make *Pheretima javanica* extract. (4) Develop the function of *Pheretima javanica* extract as an alternative treatment that is not resistant.

Technology – Students are expected to be able: (1) Using an online site (website) to find information about typoid fever problems. (2) Using an online site (website) to find the advantages of earthworms, especially *Pheretima javanica* species. (3) Using a computer application (software) to pick up the signal from the GCMS machine and read it in the form of a chromatogram. (4) Using an online database to analyze GCMS data.

Engineering – Students are expected to be able: (1) Understanding the steps of *Pheretima javanica* extraction. (2) Understanding how the rotary evaporator works. (3) Tested the antibacterial activity of *Pheretima javanica* extract. (3) Understanding how the GCMS engine works. (4) Analyzing data from GCMS.

Mathematic – Students are expected to be able: (1) Understanding how to make ethanol solution with a certain concentration. (2) Determining the concentration of *Pheretima javanica* extract for antibacterial activity test.

RBL Activites On *Pheretima javanica* STEM Education

STEM integrated RBL learning activities describe the stages of learning in the classroom. Students actively carry out research to find data based on research proposals designed by lecturers by paying attention to previous research on making earthworm extracts and testing antibacterial activity as well as analyzing bioactive compounds contained in earthworm extracts. Based on research results so that earthworm extract can be used as an alternative medicine in cases of typoid fever. The stages of learning activities can be seen in the following table:

Table 4
The first stage of STEM integrated RBL learning activities

Stage	Activities
Contract assessment and raise problems related to research material	1. Lecturers and Students pose problems that stem from previous research problems. 2. Lecturer with students plan the research. 3. Giving reference (initial knowledge) in the form of research journal. 4. Giving an explanation of the journal. 5. Discussions with students about the preparation of the research, contract assessment and the final results will be obtained in performing research. 6. Observing the students during the discussion and provide an explanation when there are questions. 7. Evaluating the results of discussions and contract appraisal.

Table 4 shows stage 1 learning activities RBL discusses the problem identification of problems that exist in society. The social problems discussed are related to cases of typhoid fever which is a public health problem. The lecturer also informs the journal about this issue. Furthermore, the lecturers and students designed the research and determined the next steps to be taken. Explanation of the learning assessment contract and the follow-up of the final results of learning in RBL learning are also discussed at this stage.

Table 5
The second stage of STEM integrated RBL learning activities

Stage	Activities
Earthworm identification Developing a breakthrough about earthworm extracts with initial activities: Identification of earthworms	1. Lecturers and Students pose problems that stem from previous research problems. 2. Lecturer with students plan the research. 3. Giving reference (initial knowledge) in the form of research journal. 4. Giving an explanation of the journal. 5. Observing the students in conducting research to identify earthworms. 6. Observing the students while discussing and providing explanations when there are questions about the characteristics of *Pheretima javanica*. 7. Evaluating research results.

Table 5 shows the RBL Learning Activity in the second stage discusses plans to develop the planned breakthroughs. The initial activities in the research step began by carrying out learning to identify earthworms. At this stage students find worms by using the identification key to find *Pheretima javanica* with the characteristics that are in the key of determination. Creative and innovative thinking ability of students at this stage will find other worm species with observations. Students will also innovate how to dry the earthworms that have been found before the extraction process is carried out at a later stage.

Table 6
The third stage of STEM integrated RBL learning activities

Stage	Activities
Extraction of *Pheretima javanica*: Make a solution and determine the concentration of the solution in the extraction process (Mathematic), Earthworm extraction using a rotary evaporator (Engineering)	1. Lecturers and Students pose problems that stem from previous research problems. 2. Lecturer with students plan the research 3. Giving reference (initial knowledge) in the form of research journal on how to make ethanol solution with certain concentrations. 4. Giving an explanation of the journal. 5. Observing and guiding students in determining the concentration of an ethanol solution. 6. Observing and guiding students in the extraction process using a rotary evaporator. 7. Observing the students while discussing and providing explanations when there are questions about the extraction process. 8. Evaluating research results.

Table 6 shows the RBL learning activity in the third stage discusses the process of extracting earthworms. The ability to understand the steps in the process of making extracts that have long stages is needed in this lesson. One of the important steps in this

study is to make an ethanol solution and determine a certain concentration according to the worm powder obtained. The student's ability to innovate can be maximally developed at this stage. An understanding of how the rotary evaporator works to produce earthworm extracts is an engineering subject in the STEM learning approach.

Table 7
The fourth stage of STEM integrated RBL learning activities

Stage	Activities
Antibacterial activity test: Determine the concentration of earthworm extract and measure the inhibition zone on the media growth of *Salmonella enterica* Typhi (Mathematic)	1. Lecturers and Students pose problems that stem from previous research problems. 2. Lecturer with students plan the research of antibacterial activities test. 3. Giving reference (initial knowledge) in the form of research journal on how to make a medium for growing bacteria and determine the concentration of earthworm extract for antibacterial activity test. 4. Giving an explanation of the journal. 5. Observing students when doing research on antibacterial activity tests. 6. Observing the students while discussing and providing explanations when there are questions about the steps and the results of the antibacterial activity test by looking at the inhibition zone on the growth of *Salmonella enterica* Typhi. 7. Evaluating research results. 8. Students communicate the results of the antibacterial activity test.

Table 7 shows the RBL learning activity in stage four discusses material on the subject of Mathematics in STEM learning. In the antibacterial activity test stage, students are expected to be able to carry out research on the ability of earthworm extracts to inhibit bacterial growth. Creative and innovative thinking ability of students in this study can be seen when determining the concentration of earthworm extracts that will be used to test their ability to inhibit bacterial growth. The stages of calculating the zone of inhibition in bacterial growth and determining the concentration of the extract are Mathematical subjects in the STEM approach.

Table 8
The fifth stage of STEM integrated RBL learning activies

Stage	Activities
Analysis of bioactive compounds by GCMS (SCIENCE) Understand how computer applications (software) work in producing chromatograms (Technology)	1. Lecturers and Students pose problems that stem from previous research problems. 2. Lecturer with students plan the research of analysis bioactive compound antibacterial. 3. Giving reference (initial knowledge) in the form of research journal on the workings of the machine GCMS. 4. Giving an explanation of the journal. 5. Observing students when doing research on the analysis of bioactive compounds in earthworm extracts using GCMS. 6. Observing the students while discussing and providing explanations when there are questions about the steps to analysis of GCMS results. 7. Evaluating research results. 8. Students communicate research results in the form of a chromatogram.

Table 8 shows the RBL learning activity in the fifth stage discussed GCMS analysis to determine the bioactive compounds contained in earthworm extracts. The analysis of bioactive compounds at this stage provides knowledge to students about how the GCMS machine works, which is the engineering subject of the STEM approach. In addition, the subject of Technology is also at this stage, because the signals from the GCMS machine will be read through the application on the computer. The results that are read on the computer produce a chromatogram that describes the bioactive compounds contained in the extract.

Table 9
The sixth stage of STEM integrated RBL learning activities

Stage	Activities
Analysis of a chromatogram and GCMS result data with an online database (Technology)	1. Lecturers and Students pose problems that stem from previous research problems. 2. Giving reference (initial knowledge) in the form of research journal that analyzes GCMS results. 3. Giving an explanation of the journal. 4. Guiding students in analysis GCMS data using an online data base. 5. Observing the students while discussing and providing explanations when there are questions about the steps to analyze data. 6. Evaluating the results of reserach. 7. Students communicate the analysis results.

Table 9 shows RBL learning activities with the STEM approach at this stage, students analyze the GCMS chromatogram results obtained from the learning process in the previous stage. The steps in the analysis are carried out using an online data base that provides information about the characteristics of bioactive compounds. This learning is the subject of technology in the STEM learning approach. The ability of students to think creatively and innovatively will appear in finding and determining online databases to get the right data (Widana, 2020; Tohara et al., 2021). Literature review to find the profile of antibacterial bioactive compounds through references requires the ability to think creatively and innovatively by students.

The application of RBL with a STEM approach provides several direct experiences for students, including: can increase students' experience in solving problems that are directly related to problems in everyday life, increase students' knowledge of practical work in the laboratory to find something abstract to become real, increase creative skills students by finding answers to existing problems, and indirectly training students in scientific research.

Resaerch-Based Learning activities will also appear in the final results of the lesson. Students are expected to produce scientific articles from the learning process. Content from scientific articles can be generated from creative and innovative thinking skills that arise during the learning process. The ideas contained in the resulting article are based on previous research.

CONCLUSION

Research-based learning is very appropriate to be developed by integrating it with the STEM approach. Learning outcomes are student mastery in acquiring knowledge, skills and products in the form of scientific articles. Learning activities of antibacterial activity test and analysis of bioactive compounds using GCMS describe learning with a STEM approach. STEM subjects in the form of science, technology, engineering and mathematics are reflected in learning activities. STEM integrated research-based learning can enhance the creative and innovative thinking ability of students to be able to resolve the social problems in the environment. The results of the class experimental research showed that the N-Gain score was 0.8, which is in the high category.

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