

Antioxidant activity screening of *Centella asiatica* as a biology learning innovation

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Abstract

The integration of medicinal plant antioxidant research into biology laboratory learning remains limited. Therefore, this study evaluated the effectiveness of a phytochemical screening and antioxidant activity testing practicum using *Centella asiatica* leaf extract. This study aimed to analyze the effectiveness of a phytochemical screening and antioxidant activity testing practicum using *Centella asiatica* leaf extract in improving biology students' learning outcomes, laboratory skills, and learning motivation. The study employed a mixed-method approach with a pre-experimental one-group pretest-posttest design involving 30 second-semester students who had completed a basic pharmaceutical biology course. The practicum included preparation of plant materials, phytochemical screening, antioxidant activity testing using the DPPH method, and evaluation of learning outcomes. Data were collected through pretest and posttest instruments, laboratory skill observation sheets, and student response questionnaires. Quantitative data were analyzed using a paired sample t-test, while qualitative data were analyzed descriptively. The results showed that the practicum effectively improved students' learning outcomes, laboratory skills, and learning motivation. Students directly observed phytochemical reactions and antioxidant activity, which enhanced their understanding of antioxidant concepts and free radical mechanisms. The average pretest score increased from 62.4 to 84.6, with a significant difference ($p < 0.05$). Laboratory skills were categorized as good (83%), while student responses were categorized as very good (>85%). Therefore, this practicum can be recommended as an innovative and contextual learning strategy in biology education.

Abstrak. Integrasi penelitian antioksidan tanaman obat ke dalam pembelajaran laboratorium biologi masih terbatas. Oleh karena itu, penelitian ini mengevaluasi efektivitas praktikum skrining fitokimia dan uji aktivitas antioksidan menggunakan ekstrak daun *Centella asiatica*. Penelitian ini bertujuan untuk menganalisis efektivitas praktikum skrining fitokimia dan uji aktivitas antioksidan menggunakan ekstrak daun *Centella asiatica* dalam meningkatkan hasil belajar, keterampilan laboratorium, dan motivasi belajar mahasiswa biologi. Penelitian menggunakan pendekatan mixed method dengan desain pra-eksperimental one-group pretest-posttest yang melibatkan 30 mahasiswa semester dua yang telah menempuh mata kuliah Biologi Farmasi Dasar. Kegiatan praktikum meliputi preparasi bahan tanaman, skrining fitokimia, uji aktivitas antioksidan menggunakan metode DPPH, serta evaluasi hasil pembelajaran. Data dikumpulkan melalui instrumen pretest dan posttest, lembar observasi keterampilan laboratorium, serta angket respon mahasiswa. Data kuantitatif dianalisis menggunakan uji paired sample t-test, sedangkan data kualitatif dianalisis secara deskriptif. Hasil penelitian menunjukkan bahwa praktikum yang dilaksanakan efektif meningkatkan hasil belajar, keterampilan laboratorium, dan motivasi belajar mahasiswa. Mahasiswa dapat mengamati secara langsung reaksi fitokimia dan aktivitas antioksidan sehingga pemahaman terhadap konsep antioksidan dan mekanisme radikal bebas menjadi lebih baik. Rata-rata nilai pretest meningkat dari 62,4 menjadi 84,6 pada posttest dengan perbedaan yang signifikan ($p < 0,05$). Keterampilan laboratorium mahasiswa berada pada kategori baik (83%), sedangkan respon mahasiswa termasuk kategori sangat baik (>85%). Oleh karena itu, praktikum ini dapat direkomendasikan sebagai strategi pembelajaran biologi yang inovatif dan kontekstual.

A. Introduction

Biology learning in higher education plays an important role in developing students' conceptual understanding as well as their scientific skills. In modern science education, learning is not only focused on mastering theoretical concepts but also on developing science process skills, critical thinking abilities, and laboratory skills through experimental activities (Artayasa et al., 2021). Laboratory practicums enable students to connect theory with directly observed biological phenomena, thereby enhancing both conceptual understanding and scientific skills (Padmiswari et al., 2026).

In recent decades, issues related to free radicals and oxidative stress have become global concerns in the fields of biology, pharmacy, and health. Free radicals are unstable molecules with unpaired electrons, making them highly reactive and capable of causing damage to biomolecules such as lipids, proteins, and DNA (Kurnia et al., 2025). An imbalance between free radical production and the body's antioxidant defense system can lead to oxidative stress, which contributes to various degenerative diseases, including cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders (Hambali et al., 2024). Therefore, research on natural antioxidant sources continues to develop in an effort to identify bioactive compounds capable of effectively neutralizing free radicals.

Medicinal plants are an important source of natural antioxidant compounds. These plants contain various secondary metabolites such as flavonoids, phenolics, alkaloids, and terpenoids, which exhibit biological activities as antioxidants (Gutiérrez-García et al., 2024). The utilization of medicinal plants in biology learning can provide contextual learning experiences while introducing students to the potential of local biological resources. Integrating medicinal plant research into practicum activities can help students understand biochemical and cellular metabolism concepts in a more applicable manner (Padmiswari et al., 2026).

One of the medicinal plants widely used in various Asian countries is *Centella asiatica*. This plant is known to contain various bioactive compounds with antioxidant potential, making it relevant as a contextual learning material in biology and pharmaceutical education (Padmiswari et al., 2025). The antioxidant compounds contained in *Centella asiatica* can neutralize free radicals and are commonly analyzed using the DPPH method, which is simple, easy to observe, and suitable for laboratory practicum activities (Rashid et al., 2023). In addition, recent studies have reported that *Centella asiatica* contains several active metabolites associated with antioxidant activity, supporting its potential to be utilized as an innovative practicum material in biology learning (Silvia et al., 2024).

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is one of the most commonly used techniques in antioxidant research due to its relatively simple and rapid procedure and its easily observable color change. This method is based on the ability of antioxidants to reduce the purple DPPH radical into a yellow reduced form, allowing changes in absorbance to be measured using a spectrophotometer (Ratnawaty et al., 2024). These characteristics make the method highly suitable for biology practicum activities, as students can directly observe reaction changes while learning the scientific principles of antioxidant analysis.

However, biology learning in higher education is still often dominated by lecture-based methods, resulting in limited opportunities for students to gain hands-on experience in conducting scientific experiments (Artayasa et al., 2021). Preliminary observations among pharmacy students indicate that many students experience difficulties in understanding the concept of free radicals and the mechanisms of antioxidant action in an applied context. Laboratory activities are generally still demonstrative in nature and do not sufficiently involve exploratory, research-based scientific activities.

Previous studies have shown that practicum-based learning can improve students' learning outcomes and scientific skills. However, several studies and preliminary observations indicate that biology learning in higher education is still frequently dominated by lecture-based instruction, resulting in limited opportunities for students to engage in hands-on scientific investigations. Consequently, students often experience difficulties in understanding abstract biological concepts, interpreting experimental data, and applying scientific reasoning in real situations. Practicum-based learning provides direct learning experiences that enable students to observe phenomena, conduct experiments, collect data, and draw evidence-based conclusions. Through active involvement in laboratory activities, students can construct their own understanding while simultaneously developing scientific process skills, critical thinking, and problem-solving abilities. Previous research has reported that students who participate in contextual and inquiry-based practicum activities demonstrate higher conceptual understanding, improved laboratory competence, and greater learning motivation compared to those who receive predominantly theoretical instruction (Restudila et al., 2025). However, most of these studies still rely on conventional laboratory chemistry experiments and have not widely utilized local medicinal plant research as a medium for biology learning.

Based on these conditions, there is a gap between the advancement of research on antioxidant activity of medicinal plants and its application in biology learning in higher education. Previous studies

have largely focused on the pharmacological aspects of medicinal plants, while their use as learning media to improve students' scientific skills remains limited. Therefore, a learning approach that integrates simple research activities with laboratory practicums is needed to make learning more contextual and applicable.

This study offers an innovative approach through a practicum activity on antioxidant activity screening of *Centella asiatica* using the DPPH method as a biology learning medium. Preliminary observations and interviews conducted with biology students revealed that most students experienced difficulties in understanding free radical concepts, antioxidant mechanisms, and laboratory data analysis because learning activities were still dominated by theoretical explanations and demonstrative practicum. Students also expressed the need for more contextual and hands-on laboratory activities that allow them to directly observe biological phenomena and actively participate in scientific investigations. Therefore, this practicum was designed to provide a more meaningful learning experience by integrating concepts of free radicals, antioxidant mechanisms, and laboratory analysis techniques. Through direct experimental activities, students are expected not only to understand theoretical concepts but also to develop scientific process skills, conduct experiments independently, and analyze research data systematically.

Based on the above description, this study aims to develop and implement a practicum-based biology learning activity on antioxidant activity screening of *Centella asiatica* using the DPPH method as a contextual learning medium for pharmacy students. In addition, this study aims to evaluate the effectiveness of the developed practicum activity in improving students' learning outcomes, laboratory skills, and learning motivation. This study supports previous research on the antioxidant potential of medicinal plants while expanding its application into innovative and contextual biology learning in higher education.

B. Material and method

Research design

This study employed a mixed-method approach using a pre-experimental one-group pretest-posttest design. The research was conducted to evaluate the effectiveness of a practicum activity on phytochemical screening and antioxidant activity testing of *Centella asiatica* using the DPPH method in improving students' learning outcomes, laboratory skills, and learning motivation. In this design, students were given a pre-test before participating in the practicum activities and a post-test after the practicum to determine the improvement in conceptual understanding resulting from the learning intervention.

Population and sample

The population of this study consisted of second-semester students of the Pharmacy Study Program at the Institute of Technology and Health Bali during the even semester of the 2025 academic year. The sample consisted of 30 students selected using purposive sampling because they had completed a basic pharmaceutical biology course and possessed prior knowledge related to plant secondary metabolites and basic laboratory techniques. The participants comprised 20 female students (66.7%) and 10 male students (33.3%), with ages ranging from 19 to 21 years. These demographic characteristics indicate that the participants represented the typical age and academic background of second-semester pharmacy students enrolled in biology-related laboratory courses.

Research procedure and data collection

The research procedure consisted of several stages, including preliminary study, preparation of practicum materials, implementation of laboratory activities, and evaluation of learning outcomes. Preliminary observations and interviews were conducted to identify students' learning difficulties related to antioxidant concepts and laboratory analysis. The practicum materials were then prepared using *Centella asiatica* leaves collected from the Denpasar area, Bali. Fresh leaves were washed, air-dried, and ground into simplicia powder. Extraction was performed using the maceration method with 70% ethanol. A total of 200 g of simplicia powder was soaked in 1 L of ethanol for three days with periodic stirring every 12 hours. The filtrate was filtered and evaporated using a rotary evaporator to obtain a concentrated extract.

The antioxidant activity test was conducted using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The DPPH solution was prepared at a concentration of 0.1 mM in methanol, while the extract was prepared in several concentration variations. The extract solution was mixed with DPPH solution and incubated for 30 minutes in dark conditions at room temperature. Absorbance was measured using a UV-Vis spectrophotometer at 517 nm. The decrease in purple color intensity indicated antioxidant activity. The percentage of inhibition and IC₅₀ value were calculated to determine antioxidant capacity.

Data were collected using three instruments: cognitive tests, laboratory observation sheets, and student response questionnaires. The cognitive test consisted of 15 validated multiple-choice questions administered before and after the practicum as pre-test and post-test. Content validity was established through expert judgment by biology education and pharmaceutical biology lecturers, while instrument reliability was confirmed through a pilot test prior to data collection. Observation sheets were used to assess students' laboratory skills, including procedural accuracy, equipment handling, laboratory safety,

teamwork, and interpretation of experimental results. Student responses toward the practicum activities were collected using a 5-point Likert scale questionnaire covering learning motivation, learning satisfaction, and relevance of the daily life practicum.

Data analysis techniques

Quantitative data obtained from pre-test and post-test scores were analyzed using a paired sample t-test with SPSS software to determine the significance of learning improvement after the practicum intervention (Padmiswari et al., 2026). Observational data regarding laboratory skills were analyzed descriptively by calculating the average score of each indicator and categorizing the results into very good, good, fair, and poor categories. Questionnaire data were analyzed quantitatively by calculating the percentage of responses in each category and interpreted descriptively to describe students' perceptions of the antioxidant activity screening practicum using *Centella asiatica*.

C. Results and discussion

Antioxidant activity test results

The results of the antioxidant activity test on the ethanol extract of *Centella asiatica* leaves indicated its ability to scavenge free radicals, as evidenced by the color change of the DPPH solution from purple to pale yellow. This color change reflects the reduction reaction of free radicals by antioxidant compounds present in the extract. The data of antioxidant activity are presented in Table 1 and Table 2.

Based on Table 1, an increase in extract concentration resulted in a significant increase in the percentage of inhibition. This indicates a strong dose-response relationship, where higher concentrations correspond to greater antioxidant activity. The IC₅₀ value of approximately 45.6 µg/mL categorizes the extract as a strong antioxidant. These findings are consistent with previous studies reporting high antioxidant activity of *Centella asiatica* using the DPPH method.

Table 1 Antioxidant activity of *Centella asiatica* ethanol extract

Concentration (µg/mL)	Control absorbance	Sample absorbance	% inhibition
10	0.700	0.574	18
20	0.700	0.476	32
40	0.700	0.315	55
60	0.700	0.210	70
80	0.700	0.126	82
100	0.700	0.063	91

Table 2 Visual observation of antioxidant activity test

Concentration	Color change	Clarity
Low	Purple → pale	3
Medium	Purple → yellow	4
High	Purple → bright yellow	5

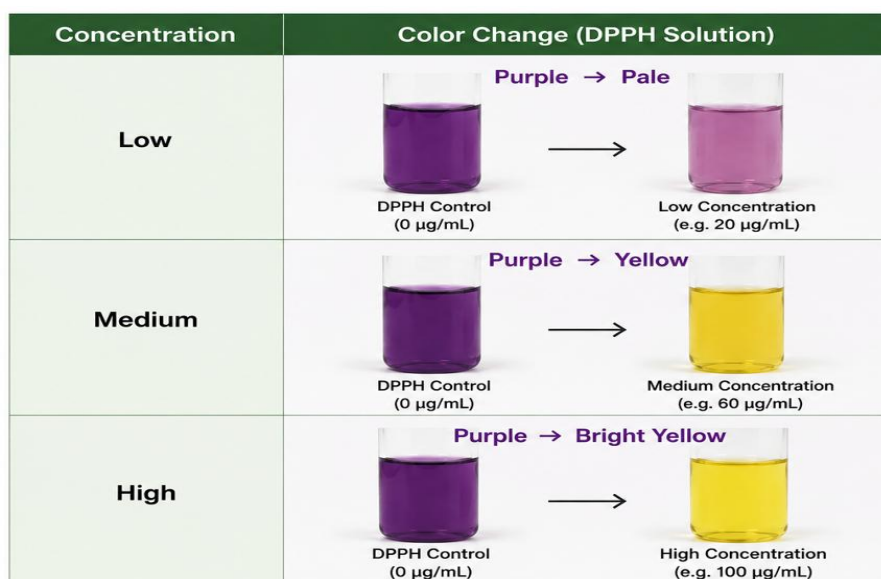


Figure 1 Visual observation of antioxidant activity test

Visual description of antioxidant test

Table 2 presents the visual observations of the color change in the DPPH solution after the addition of *Centella asiatica* extract. The clarity of the color change was assessed using a scale of 1–5, where 1 indicates very unclear and 5 indicates very clear. Table 2 shows that the clarity of color change increased with higher extract concentrations. At high concentrations, the color change was very clear and easily observed by all students. This indicates that the DPPH method is highly effective as a visual learning medium due to its observable and easily understood results.

Pre-test and post-test results

Table 3 presents the comparison of students' pre-test and post-test scores to evaluate the effectiveness of the antioxidant screening practicum on learning outcomes. The paired sample t-test analysis revealed a significant improvement in students' learning

outcomes after participating in the practicum. The higher post-test scores indicate that the antioxidant screening practicum effectively enhanced students' conceptual understanding.

Laboratory skills assessment

Table 4 shows the average laboratory skills of students during the practicum. Students demonstrated good laboratory skills with an overall average of 83%. The highest score was observed in laboratory safety, while result interpretation remained relatively lower, indicating the need for further improvement.

Student questionnaire results

Table 5 presents students' responses to the antioxidant screening practicum. Overall, students showed positive responses, indicating that contextual experiment-based learning enhances both motivation and conceptual understanding.

Table 3 Comparison of pre-test and post-test scores (n = 30)

Parameter	Pre-test (Mean ± SD)	Post-test (Mean ± SD)	p-value
Learning outcomes	62.4 ± 8.1	84.6 ± 6.7	0.001*

Note: p < 0.05 indicates significant difference

Table 4 Students' laboratory skills

Skill aspect	Average (%)	Category
Procedural accuracy	82	Good
Equipment usage	80	Good
Laboratory safety	90	Very good
Teamwork	85	Very good
Result interpretation	78	Fair
Overall average	83	Good

Table 5 Student responses to practicum

Indicator	Positive response (%)	Category
Helps understanding concepts	88	Very good
Increases learning motivation	85	Very good
Relevant to daily life	87	Very good

The results indicate that the ethanol extract of *Centella asiatica* possesses significant antioxidant activity, as demonstrated by the color change in the DPPH solution and the IC₅₀ value within the moderate to strong category. This suggests the presence of bioactive compounds such as flavonoids and phenolics that play a role in scavenging free radicals. These findings are consistent with previous studies reporting that *Centella asiatica* contains significant antioxidant compounds with potential applications in pharmacy and health (Padmiswari et al., 2025). The increasing percentage of inhibition with higher extract concentrations indicates a strong dose-response relationship. This suggests that higher concentrations enhance the ability to reduce free radicals, confirming the presence of natural antioxidant compounds (Kandasamy et al., 2023). The antioxidant activity observed is closely related to the presence of

secondary metabolites such as flavonoids and phenolic compounds, which function by donating electrons or hydrogen atoms to stabilize free radicals (Rashid et al., 2023). The IC₅₀ value obtained in this study further confirms the antioxidant strength of the extract. This value is widely used as a key indicator in determining antioxidant capacity, and the results align with previous studies using the DPPH method (Silvia et al., 2024).

The DPPH method proved effective due to its simplicity and clear visual output. The observable color change from purple to yellow provides a direct indication of antioxidant activity, making it highly suitable for educational purposes (Ratnawaty et al., 2025). This visual clarity enhances students' conceptual understanding of antioxidant mechanisms. The color change of the DPPH solution from purple to yellow at various concentrations of *Centella asiatica*

extract indicates antioxidant activity through a free radical reduction mechanism. Higher extract concentrations resulted in clearer color changes, reflecting the increased ability of bioactive compounds to donate electrons or hydrogen atoms to stabilize DPPH radicals. This visual clarity supports the quantitative findings, namely the increased percentage of inhibition, thereby providing consistent evidence that the extract possesses significant antioxidant activity (Artati et al., 2024). Compounds such as flavonoids and phenolics are known to play an important role in this process due to the presence of hydroxyl groups that can directly interact with free radicals. From an educational perspective, the clarity of the color change in the DPPH test provides high pedagogical value, as students can directly observe the chemical phenomena occurring during the reaction. The distinct visual changes at higher concentrations help students better understand abstract concepts related to antioxidant mechanisms, making learning more concrete and meaningful. Furthermore, variations in clarity at different concentrations train students' observational and interpretative skills in analyzing experimental data. This demonstrates that the DPPH method is not only effective as a scientific analytical tool but also as a learning medium that enhances students' science process skills and conceptual understanding simultaneously (Amalina et al., 2024).

The improvement in pre-test and post-test scores indicates that antioxidant-based practicum is effective in enhancing students' conceptual understanding. The statistical test results showing a significant difference ($p = 0.001$) suggest that the practicum activities made a substantial contribution to the improvement of learning outcomes. This finding is consistent with previous studies stating that practicum-based learning can enhance students' conceptual understanding and scientific thinking skills. It also aligns with research indicating that hands-on practicum and experimental learning improve students' understanding of biochemistry and analytical abilities (Li et al., 2020). This improvement in learning outcomes can be explained through a constructivist approach, where students build their own understanding through direct experimental experiences. Therefore, learning becomes more meaningful and memorable (Wahyuningsih, 2021).

In addition to the cognitive aspect, students' laboratory skills also showed improvement. Students were able to follow experimental procedures properly, use laboratory equipment accurately, and demonstrate a high level of awareness regarding laboratory safety. However, the ability to interpret results still needs to be improved through more intensive guidance in experimental data analysis. Students performed practicum procedures well, used equipment appropriately, and complied with laboratory safety standards. This indicates that practicum activities

make a significant contribution to the development of psychomotor skills (Herditiya et al., 2023; Sam et al., 2023; Jakubčínová et al., 2024). The high score in laboratory safety reflects students' strong awareness of the importance of safety in the laboratory, which is a crucial aspect of science education, particularly in pharmacy and biology. Nevertheless, students' ability to interpret results was categorized as moderate, indicating that data analysis skills still need to be strengthened through more intensive guidance and practice in interpreting experimental results (Susanti et al., 2022; Damayanti & Antari et al., 2025).

Students' responses to the practicum activities were highly positive. They perceived that the practicum helped them understand concepts more concretely, increased their learning motivation, and was relevant to everyday life. This indicates that contextual practicum-based learning can enhance student engagement in the learning process (Sudarmin et al., 2023; Fadilah et al., 2024; Resti et al., 2024; Syahirah et al., 2025). The use of local plants such as *Centella asiatica* as practicum materials provides added value in contextual learning. Students are able to relate learning materials to real-life situations, thereby increasing the relevance of learning (Susanti et al., 2021). The integration of biology, chemistry, and pharmacy concepts in this practicum demonstrates that the learning process is multidisciplinary, which is essential in preparing students to face real-world challenges that require interdisciplinary thinking skills.

Overall, the antioxidant activity screening practicum using *Centella asiatica* provides not only scientific benefits but also high pedagogical value. This learning approach is capable of simultaneously integrating cognitive, psychomotor, and affective domains. Therefore, practicum-based learning utilizing local medicinal plants can be recommended as an innovative strategy in biology education at the higher education level.

D. Conclusion

This study demonstrates that phytochemical screening and antioxidant activity testing practicum using *Centella asiatica* leaf extract are effective in improving students' learning outcomes, laboratory skills, and scientific attitudes. The results indicate that the extract contains secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids, and exhibits strong antioxidant activity, as evidenced by increasing inhibition values with higher concentrations. The significant improvement in pretest and posttest scores confirms that practicum-based learning has a positive impact on students' conceptual understanding. Furthermore, the use of local natural resources as learning media enhances students' motivation and the relevance of learning to real-life contexts, making it a recommended innovative strategy in biology education.

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