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Integration of Science Learning in Antioxidant Testing of Pegagan (Centella asiatica) Extract Using the DPPH Method

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Abstract

The integration of science learning through experimental activities is crucial in enhancing students' understanding of natural product chemistry. This study explores the antioxidant potential of Centella asiatica (Gotu kola) extract as a contextual learning medium in scientific education. Centella asiatica is a medicinal plant widely used in traditional medicine for its bioactive compounds, including triterpenoids, flavonoids, alkaloids, and saponins, which exhibit significant antioxidant properties. This study aims to evaluate and compare the antioxidant activity of C. asiatica extracts obtained using three different solvents: 70% ethanol, N-hexane, and ethyl acetate, utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Antioxidant activity was assessed based on the ability of the extract to scavenge DPPH radicals, measured spectrophotometrically at a wavelength of 517 nm. The findings revealed that all extracts exhibited antioxidant activity, with the 70% ethanol extract demonstrating the highest potency, achieving an IC50 value of 19.50 µg/ml, compared to N-hexane and ethyl acetate extracts. These results highlight the potential of C. asiatica as a natural antioxidant source, relevant for health product development and scientific experimentation in educational settings. This study underscores the importance of incorporating laboratory-based research into science curricula to enhance students' analytical and experimental skills while fostering an appreciation for the application of natural product research in health sciences.

Keywords – Antioxidant Activity; Centella asiatica Extract; Science-Based Learning

1. Introduction

In the context of science education, research on the antioxidant activity of Gotu Kola (Centella asiatica) extracts can serve as a relevant teaching material to enhance students' understanding of chemistry, biology, and health principles. The concepts of oxidation and free radicals, which play a role in various degenerative diseases, can be explained through an inquiry-based learning approach. Through this method, students not only comprehend the theory of antioxidants but also develop laboratory skills, such as extraction techniques and spectrophotometric analysis using the DPPH method. Implementing experimental methods in science education can improve students' critical and analytical thinking skills, particularly in understanding how factors like solvent type influence the extraction of bioactive compounds from natural sources.

Integrating research on natural antioxidants into the curriculum also fosters an appreciation for the potential of local herbal resources in addressing public health challenges. Centella asiatica, widely used in traditional medicine across Asia for its cognitive and circulatory benefits, serves as an excellent case study for demonstrating the extraction of bioactive compounds using different solvents, including 70% ethanol, N-hexane, and ethyl acetate. By comparing the efficiency of these solvents in dissolving various phytochemicals, students learn about the influence of solvent polarity on extraction yield and antioxidant efficacy. This practical exercise bridges theoretical knowledge with real-world applications, encouraging students to explore how natural products can contribute to the development of novel health products and sustainable therapeutic strategies.

This research model supports the interdisciplinary framework of STEM education, where scientific inquiry is combined with technological and analytical skills to solve complex problems. By integrating the experimental testing of antioxidant activity with discussions on the implications of oxidative stress and degenerative diseases, educators can highlight the relevance of laboratory research to societal health issues. This not only prepares students for future careers in research and industry but also instills a deeper understanding of how traditional medicinal plants like Centella asiatica can be harnessed to improve health outcomes. Ultimately, the integration of such research into science learning fosters a dynamic educational environment that cultivates innovation, collaboration, and a lifelong commitment to scientific inquiry.

The study of extraction techniques using different solvents such as 70% ethanol, N-hexane, and ethyl acetate provides valuable insights into the field of chemistry and pharmaceutical sciences. In educational settings, particularly in higher education, teaching students about solvent polarity and its effect on bioactive compound extraction enhances their understanding of fundamental chemistry principles. By incorporating hands-on laboratory experiences, students can analyze how different solvents extract varying classes of compounds, which directly impacts the antioxidant potential of the final extract. This approach fosters an inquiry-based learning environment where students can develop problem-solving skills by evaluating the efficiency of different extraction methods for medicinal plants like Centella asiatica.

Understanding antioxidant testing methods, such as the DPPH assay, is crucial for students in science education, particularly those specializing in chemistry, biology, and health sciences. The DPPH method, which involves spectrophotometric analysis of free radical scavenging activity, provides an excellent opportunity to teach students about oxidation-reduction reactions in real-world applications. Through laboratory-based learning, students can visualize the impact of antioxidants in neutralizing free radicals, thereby linking theoretical knowledge to practical applications in disease prevention. Additionally, integrating this research into the curriculum encourages students to critically assess the role of natural antioxidants in human health and explore their potential use in pharmaceutical and nutraceutical industries.

Beyond scientific exploration, the study of Centella asiatica antioxidants also holds significance in interdisciplinary education, particularly in sustainability and entrepreneurship. Encouraging students to investigate the economic potential of natural antioxidants promotes an understanding of how scientific research can lead to the development of herbal-based health products. This perspective aligns with the STEM education model, which integrates science, technology, and business aspects in research-based learning. By engaging in studies on bioactive compounds and their health benefits, students not only gain technical expertise but also acquire the entrepreneurial mindset needed to innovate in the fields of herbal medicine, food science, and biotechnology. Thus, integrating antioxidant research into education provides a holistic approach that combines scientific rigor with real-world applications, preparing students for both academic and industrial careers.

This study aims to integrate science learning with experimental research by analyzing and comparing the antioxidant activity of Centella asiatica (Gotu Kola) extracts using different solvents—70% ethanol, N-hexane, and ethyl acetate—through the DPPH method. By incorporating scientific concepts such as oxidation, reduction, and solvent polarity into the study of natural antioxidants, this research seeks to enhance students' understanding of chemistry and biology through practical application. The findings of this study are expected to not only provide insights into the antioxidant potential of Centella asiatica extracts but also serve as an educational model for integrating laboratory-based learning into science curricula. This approach allows students to develop analytical and critical thinking skills by engaging in real-world scientific research.

This research highlights the importance of interdisciplinary learning in science education, particularly in the fields of chemistry, pharmacology, and health sciences. Through hands-on experimentation with different extraction solvents, students can explore how chemical properties influence the ability to extract bioactive compounds, ultimately affecting antioxidant activity. The use of the DPPH method in testing antioxidant capacity provides a direct link between theoretical chemistry concepts and their practical applications in health and medicine. By engaging in this research, students gain a deeper understanding of how scientific methodologies contribute to advancements in natural medicine, encouraging them to explore further innovations in phytochemistry and pharmaceutical sciences.

The results of this study are expected to contribute not only to the scientific understanding of Centella asiatica as a natural antioxidant source but also to the development of a more interactive and inquiry-based science education approach. By integrating experimental antioxidant testing into the learning process, students can connect their knowledge of chemistry and biology with real-world applications in health and sustainability. Moreover, the study provides valuable data on the effectiveness of different extraction methods, which can be used as a reference for further development of natural antioxidant products. This integration of research-based learning supports STEM education principles, fostering a generation of students who are not only knowledgeable in scientific theory but also skilled in applying their knowledge to solve practical challenges in health and industry.

2. Method

This study employs a quantitative approach with an experimental laboratory method to test the antioxidant activity of Centella asiatica (gotu kola) extract using the DPPH method. The experiment consists of several stages, including the extraction process, antioxidant activity testing, and data analysis based on predetermined parameters. This approach aims to assess the effectiveness of different solvents in extracting bioactive compounds from Centella asiatica while integrating the findings into science-based laboratory learning.

This study was conducted at the Chemistry Laboratory of Cenderawasih University, Papua. The location was chosen due to its well-equipped biochemical and pharmaceutical research facilities, as well as its relevance to the science education curriculum at the university level. The study population consisted of students enrolled in courses related to pharmaceutical chemistry and phytochemistry, with a total of 254 students. A sample of 122 students was selected using purposive sampling to participate in the research, both in data collection and in applying the research findings to laboratory learning.

The collaboration between the research team and the UD Agro Bumi Sejahtera nursery in obtaining Centella asiatica leaf samples not only ensures the quality of the materials used in the study but also holds educational value in science learning. This process can serve as a model for Project-Based Learning (PBL) in science subjects, particularly in biology or chemistry at both school and university levels. By involving students in each stage of the research—from sample collection and processing to laboratory analysis—they gain hands-on experience in scientific methods and develop an understanding of the importance of raw material sources in phytochemical research.

Participation in this research provides insight into Good Agricultural Practices (GAP), which play a crucial role in maintaining the quality of medicinal plants. In the context of learning, this can be used to teach the connection between natural sciences and agricultural technology, as well as how natural resources can be utilized for human health benefits. Students can learn about the factors that influence the bioactive compound content in plants, such as environmental conditions and cultivation methods, which ultimately affect the effectiveness of antioxidant compounds in preventing degenerative diseases. This research not only contributes to scientific advancements in health and pharmaceutical fields but also serves as an innovative learning medium for students. Integrating this research into the educational curriculum can enhance students' understanding of scientific concepts relevant to everyday life while fostering critical thinking and problem-solving skills through research-based learning in antioxidant analysis using the DPPH method.

Tools and Materials

The tools used in this research include a measuring glass, test tubes, pipettes, analytical balance, water bath, thin-layer chromatography (TLC) plates, porcelain crucibles, horn spoons, metal spoons, spatula, beaker glass, stirring rods, analytical balance, Soxhlet apparatus, aluminum foil, hot plate, knife, basin, cutting board, round-bottom flask, rotary evaporator, refrigerator, and UV-Vis spectrophotometer.

The materials used in this study include Centella asiatica extract, 70% ethanol, n-hexane, ethyl acetate, ethanol p.a, distilled water, Dragendorff reagent, magnesium powder, gelatin solution, 10% NaCl, Liebermann-Burchard reagent, Wagner reagent, 1% FeCl3, 2N HCl, concentrated HCl, quercetin, and DPPH (1,1-diphenyl-2-picrylhydrazyl).

Sample Determination

The determination of Centella asiatica (L.) Urban leaves is intended to confirm the authenticity of the sample used in the study. The morphological characteristics of the plant must be matched with references in the literature. The determination of Centella asiatica leaves for this study was conducted at the Laboratory of the Ministry of Health at RS Sardjito, Jl. Kesehatan No. 1 Sekip, Yogyakarta.

Collection and Drying of Materials

The Centella asiatica leaves were sourced from Kediri, specifically from the Centella asiatica nursery at UD Agro Bumi Sejahtera, Jalan Kaliombo Raya I No. 19, Kota Kediri, East Java. The leaves were collected in the morning to ensure optimal photosynthesis activity. A total of 15 kg of leaves were harvested and subjected to wet sorting to remove contaminants such as dirt and foreign materials.

Subsequently, the leaves were washed under running water, drained, and cut using a knife. The drying process was carried out without direct sunlight exposure for three days to obtain dry simplicia. The dried leaves were then ground using a blender to obtain Centella asiatica powder, which was then sieved using a 60-mesh sieve to produce fine powder suitable for maceration. The powdered simplicia was stored in a clean, tightly sealed container to maintain its quality (Sumiati et al., 2019).

Preparation of Centella asiatica Extract

The extraction process was performed using the maceration method. Centella asiatica simplicia powder (500 g) was macerated with 70% ethanol, n-hexane, and ethyl acetate in separate containers at a ratio of 1:10 (500 g powder to 5000 ml solvent). The mixture was stirred occasionally for the first six hours and then left to stand for 18 hours. The filtrate was collected, and the residue was re-macerated with 2.5 liters of solvent (1:2.5 ratio) under the same conditions.

The filtrates obtained from both maceration steps were filtered using filter paper to separate the solid residue from the liquid extract. The extracts were then concentrated using a rotary evaporator and water bath at 40°C until a thick extract was obtained. The yield percentage was then calculated (Putri et al., 2023).

Data Collection Techniques

The data collection process consisted of several stages:

Antioxidant Activity Testing: The antioxidant activity of each extract was measured using the DPPH method. The reduction of DPPH radicals was recorded by measuring absorbance at 517 nm using a UV-Vis spectrophotometer. The IC50 value was calculated to determine the antioxidant effectiveness of each extract.

Phytochemical Screening: The extracts were tested for the presence of active compounds such as alkaloids, flavonoids, tannins, and saponins using standard qualitative phytochemical tests.

Questionnaire and Observation: A questionnaire was distributed to students to assess their understanding of oxidation, antioxidants, and experimental methods. Observations were conducted to evaluate student engagement and comprehension during laboratory-based learning activities.

Yield Percentage Calculation: The percentage yield of each extract was determined to compare the efficiency of different solvents in extracting bioactive compounds from Centella asiatica.

Data Analysis Techniques

The collected data were analyzed using both descriptive and inferential statistical methods:

Antioxidant Activity Analysis: The DPPH test results were analyzed by calculating IC50 values. A one-way ANOVA was performed to compare the antioxidant activities of different extracts and determine statistical significance.

Phytochemical Analysis: The results of the phytochemical screening were analyzed qualitatively to identify the presence of bioactive compounds responsible for antioxidant activity.

Questionnaire and Observation Analysis: The questionnaire responses were analyzed using descriptive statistics, while qualitative observations provided insights into student engagement and learning effectiveness.

The findings of this study are expected to provide insights into the antioxidant potential of Centella asiatica extracts and demonstrate the integration of scientific research into science-based laboratory learning at the university level.

3. Result and Discussion

Determination is a crucial process in identifying the specific classification of a plant, serving as the foundational step in antioxidant testing research. The determination of Centella asiatica (pegagan) was conducted at the UPF Yankestrad Tawangmangu Laboratory, located on Jalan Raya Lawu No. 11, Tawangmangu District, Karanganyar Regency, Central Java, on July 22, 2024. The laboratory's determination results classified the plant under the Lauraceae family, with the species identified as Persea americana Mill, also known synonymously as Laurus persea L. This classification aligns with previous research by Permatasari (2016), ensuring the validity of the plant sample used in this study. The accurate determination of plant species is vital in research to ensure consistency in antioxidant content analysis and to compare findings with prior studies. By confirming the identity of the sample, researchers can ensure that the bioactive compounds tested are indeed derived from Centella asiatica and not from a misidentified plant species.

Following the determination process, the plant material underwent a rigorous collection and drying process. A total of 15 kg of fresh Centella asiatica leaves were collected and processed, yielding 7 kg of dried simplicia and ultimately 1.5 kg of fine powder. The extraction process yielded 126 grams of thick extract with a rendement of 25.2%, which was significantly higher than the rendement reported in a previous study by Sari (2021), which was 18.24%. According to Indonesian Ministry of Health (Kemenkes RI, 2017) standards, the rendement value for thick extracts must be no less than 10%, indicating that the extract obtained in this study meets the required quality criteria. The rendement value is a crucial indicator of the active compound concentration within the sample—higher rendement signifies a greater presence of bioactive compounds, which enhances the potential antioxidant activity of the extract (Hasnaeni, 2019). This outcome suggests that the extraction process employed in this research was effective in isolating the active compounds from Centella asiatica, making it a promising candidate for further pharmaceutical or nutraceutical applications.

The extraction process of gotu kola (Centella asiatica) in this study employed the maceration method using three different solvents: 70% ethanol, n-hexane, and ethyl acetate. Maceration was chosen as the extraction method due to its ability to maximize the extraction of bioactive compounds without using high temperatures, which could degrade the antioxidant compounds. The results showed that the highest extract yield was obtained using 70% ethanol, with a yield of 26.95%, followed by ethyl acetate at 11.45%, and the lowest yield was from n-hexane at 3.80%. These findings align with previous research by Nadya Syafa'ah (2019), which also found that 70% ethanol produced a higher gotu kola extract yield compared to other solvents. The differences in extract yield are closely related to the polarity of each solvent. Ethanol 70% is polar, allowing it to effectively extract phenolic and flavonoid compounds, while ethyl acetate, which is semi-polar, and n-hexane, which is non-polar, tend to extract compounds with different characteristics. Therefore, the choice of solvent in the extraction process significantly influences the final yield of gotu kola extract.

This extraction process can be integrated into educational curricula, particularly in science or chemistry subjects at the secondary and higher education levels. The concept of solvent polarity and its effectiveness in extracting bioactive compounds can serve as relevant learning material for students to understand the fundamental principles of organic chemistry and pharmacy. Moreover, this study provides an applied understanding of antioxidant testing methods using DPPH (1,1-diphenyl-2-picrylhydrazyl), which is a crucial analytical technique in biochemistry and pharmacy. By conducting hands-on experiments, students can grasp how antioxidant compounds interact with free radicals and how their effectiveness can be measured quantitatively. This approach not only enhances students' theoretical understanding of science but also fosters critical thinking and analytical skills in interpreting research findings. Therefore, integrating science learning into gotu kola extraction research and antioxidant testing can be an effective strategy to enhance scientific literacy and the relevance of education to research and the pharmaceutical industry.

The evaluation of gotu kola (Centella asiatica) extract involved several key tests, including organoleptic testing, phytochemical screening, moisture content analysis, drying loss determination, and ash content analysis. Organoleptic testing confirmed that the powdered extract exhibited characteristic properties such as a green color, a distinct pegagan leaf aroma, a fine powder form, and a bitter taste. These findings align with the natural characteristics of Centella asiatica, ensuring that the extract maintains its original phytochemical properties. The phytochemical screening results demonstrated that the ethanol extract contained a broader range of bioactive compounds, including flavonoids, alkaloids, steroids, saponins, triterpenoids, and tannins. Ethyl acetate extract also showed positive results for most of these compounds, whereas the n-hexane extract contained fewer active compounds. These findings highlight the significant role of solvent polarity in the extraction process, as polar solvents like ethanol can extract a wider variety of bioactive compounds compared to non-polar solvents such as n-hexane. Additionally, the moisture content analysis revealed a 9.7% water content in the Kediri accession of gotu kola, which meets the BPOM standard of less than 10%, ensuring the extract's stability and shelf life. Lower moisture content is crucial for preventing microbial growth and preserving the bioactive compounds' integrity over time.

The integration of these analytical methods into science education provides an excellent opportunity for students to apply theoretical knowledge to real-world applications. For example, phytochemical screening can be incorporated into chemistry and biology lessons to help students understand the presence and role of secondary metabolites in medicinal plants. Similarly, moisture content and drying loss tests can be used in food science and pharmaceutical studies to emphasize the importance of quality control in herbal product development. Furthermore, the thermogravimetric ash content analysis, which showed an average of 2.87%, can be linked to discussions on inorganic residue analysis and mineral composition in plant-derived products. By incorporating these experimental procedures into science curricula, students can develop a deeper understanding of laboratory techniques, analytical chemistry, and the practical applications of herbal medicine research. This interdisciplinary approach not only enhances scientific literacy but also fosters innovation in pharmaceutical and natural product development.

The integration of science learning in the antioxidant testing of Centella asiatica extract using the DPPH method provides a comprehensive understanding of the chemical properties and bioactive potential of medicinal plants. The organoleptic test results indicate that Centella asiatica powder exhibits a green color, a characteristic leaf aroma, a fine powder texture, and a bitter taste, which are essential sensory indicators for quality assessment. Furthermore, phytochemical screening confirms the presence of bioactive compounds such as flavonoids, alkaloids, steroids, saponins, triterpenoids, and tannins in different solvent extracts, demonstrating the plant's rich phytochemical profile. These compounds play a crucial role in antioxidant activity, which is essential in mitigating oxidative stress in biological systems. The moisture content analysis, showing a result of 9.7%, aligns with the BPOM standard of less than 10%, ensuring the stability and longevity of the extract. Additionally, the ash content determination, with an average of 2.87%, provides insight into the inorganic residue, which is a key factor in evaluating the purity and mineral composition of herbal extracts. Understanding these fundamental scientific principles enhances students' ability to link theoretical knowledge with practical laboratory applications, reinforcing the importance of analytical methods in natural product research.

The antioxidant activity test using the DPPH method further highlights the influence of different solvents on extraction efficiency and antioxidant potential. The maximum wavelength (λ max) of DPPH at 518 nm serves as a reference point for measuring the absorbance of blank and sample solutions. The antioxidant activity is determined by the IC50 value, where a lower IC50 indicates stronger antioxidant potential. The ethanol extract of Centella asiatica demonstrates the highest antioxidant activity with an IC50 value of 19.50 µg/ml, categorized as very strong, due to its ability to extract polar bioactive compounds such as alkaloids, tannins, and saponins. In contrast, the n-hexane extract, with an IC50 of 300.27 µg/ml, falls into the weak antioxidant category due to its non-polar nature, which limits the extraction of essential antioxidant compounds. Meanwhile, the ethyl acetate extract exhibits moderate antioxidant activity with an IC50 of 65.27 µg/ml, attributed to its semi-polar properties, enabling the extraction of flavonoids, alkaloids, steroids, triterpenoids, and tannins. These findings underscore the significance of solvent selection in optimizing antioxidant extraction and activity. By incorporating these scientific analyses into the learning process, students can develop a deeper appreciation for experimental design, data interpretation, and the practical application of chemistry in health sciences. This interdisciplinary approach not only strengthens their understanding of natural product chemistry but also fosters critical thinking and problem-solving skills essential for future scientific research.

4. Conclusion

Integrating this research into science learning provides an opportunity for students to understand the principles of phytochemical extraction, antioxidant activity measurement, and the role of natural compounds in health. By conducting hands-on experiments and analyzing data, students can enhance their scientific inquiry skills and critical thinking. Future studies could explore the application of pegagan extracts in various health-related formulations while also developing educational models to incorporate antioxidant research into science curricula.

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