JPPIPA 12(5) (2026)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Phytochemical and Antioxidant Screening in Zuriat Fruit to Improve Spermatozoa Quality: Skill Development in Biotechnology Practical Learning

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Received: March 02, 2026
Revised: May 11, 2026
Accepted: May 25, 2026
Published: May 31, 2026

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DOI: 10.29303/jppipa.v12i5.14714

Open Access

Abstract: Research-based practical learning is essential in biotechnology education to develop laboratory competence and apply theoretical knowledge through scientific investigation. This study aimed to integrate phytochemical screening, antioxidant activity testing, and sperm quality analysis of Hyphaene thebaica (zuriat) fruit into a biotechnology practical and evaluate its effectiveness in improving pharmacy students' learning outcomes and laboratory competence. A mixed-methods, single-group pre-experimental pre-test–post-test design was conducted involving fifth-semester Pharmacy students at the Institute of Technology and Health Bali. The practical included phytochemical screening, antioxidant activity assessment using the DPPH method, and evaluation of sperm count, motility, morphology, and viability. Quantitative data were analyzed using paired-sample t-tests, while qualitative and observational data were analyzed descriptively. Phytochemical screening identified alkaloids, flavonoids, saponins, tannins, phenolics, steroids, triterpenoids, and terpenoids, whereas quinones were absent. The extract exhibited strong antioxidant activity (IC_{50} = 55.49 mg/L) and improved sperm count, motility, viability, and normal morphology compared with the control group. Students' learning outcomes increased significantly from 65 ± 3.5 to 85 ± 4.2 ($p = 0.001$), with laboratory skills rated good to very good across all assessed domains. These findings demonstrate that research-based biotechnology practicals using zuriat fruit effectively enhance students' cognitive achievement, laboratory competence, and understanding of herbal bioactive compounds.

Keywords: Biotechnology practical learning; Phytochemical screening; Skill development; Spermatozoa quality; Zuriat fruit

Introduction

Modern biotechnology plays a crucial role in the development of health sciences, particularly through the exploration of biological resources to discover bioactive compounds that have the potential to improve the quality of human life. One strategic learning activity in biotechnology education is research-based practicums. This approach not only provides students with hands-on experience in mastering laboratory techniques but also fosters critical, analytical, and creative thinking skills in solving biological problems. Phytochemical screening is a fundamental method in biotechnology for identifying secondary metabolites in plants. Metabolites such as flavonoids, alkaloids, phenolics, tannins, and saponins are known to possess various important biological activities, including antioxidant activity. Antioxidant activity is significant because it protects cells from oxidative stress, which can damage cellular structure and function, including sperm cells (Działo et al., 2016; Molyneux, 2004).

As a tropical country, Indonesia has the potential to cultivate plants originating from Asian countries. One

How to Cite:
Antari, N. W. S., & Agustini, N. K. T. (2026). Phytochemical and Antioxidant Screening in Zuriat Fruit to Improve Spermatozoa Quality: Skill Development in Biotechnology Practical Learning. *Jurnal Penelitian Pendidikan IPA*, *12*(5), 1039–1046. https://doi.org/10.29303/jppipa.v12i5.14714

example is the cultivation of the Zuriat plant (*Hyphaene thebaica*), commonly known as the Doum fruit. This plant originates from Egypt, which is known for its hot climate. The utilization of the Zuriat plant has begun in Indonesia, the part of the plant that is widely used and believed to have benefits is the fruit (Taha et al., 2020). Zuriat or Doum fruit has the potential as a natural antioxidant. According to research conducted by Antari & Damayanti (2025) Zuriat fruit contains antioxidants, antimicrobials and antihypertensives. Zuriat or Doum fruit has various active compounds. Some polyphenol flavonoid compounds from zuriat are myricetin, kaempherol, quercetin, and myricitrin. Phenol compounds are ellagic acid, gallic acid, caffeic acid, and ferulic acid (Damayanti et al., 2022; Uyi Ewansiha et al., 2021). Zuriat fruit also contains protein, carbohydrates, fat, amino acids, and various minerals such as Mg, Fe, Na, K, S, and Cu. Zuriat fruit is known as an herbal plant that is traditionally used to increase fertility (Antari & Damayanti, 2025). Phytochemicals, such as flavonoids and phenolics, have the potential to act as antioxidants, protecting cells against oxidative stress. In the male reproductive system, oxidative stress can reduce sperm quality, including motility, morphology, and viability (Melinawati et al., 2023; Santi et al., 2020). Although previous studies have demonstrated the phytochemical composition, antioxidant activity, and pharmacological potential of *Hyphaene thebaica*, these investigations have primarily focused on its biological or medicinal properties. To date, limited studies have integrated these findings into research-based biotechnology practical learning, particularly by combining phytochemical screening, antioxidant activity assessment, and sperm quality analysis within a single educational framework. Consequently, evidence regarding the effectiveness of such an integrated practical in improving students' cognitive achievement and laboratory competence remains scarce. The novelty of this study lies in the integration of three complementary components—phytochemical screening, DPPH-based antioxidant analysis, and sperm quality evaluation—into a single research-based biotechnology practical. Unlike conventional laboratory practicals that generally emphasize isolated analytical techniques, this approach enables students to connect phytochemical characteristics with biological functions and reproductive health outcomes while simultaneously developing laboratory, analytical, and scientific reasoning skills. Biotechnology is a compulsory course for undergraduate students in the Clinical and Community Pharmacy program at the Bali Institute of Health (ITEKES) in the fifth semester, with a total of 3 credits. The course includes both theory and practical work. The learning process encompasses not only theoretical understanding but also detailed analytical techniques, including extraction, phytochemical screening, and the application of herbal plants.

This integrated practical is important because it bridges fundamental biotechnology concepts with real-world biomedical applications. By investigating the relationship between phytochemical constituents, antioxidant activity, and sperm quality, students gain a comprehensive understanding of natural product research from extraction and biochemical analysis to biological evaluation. Such authentic research-based learning is expected to strengthen higher-order thinking, laboratory competence, and research skills that are essential for future pharmacists and biotechnology professionals. Therefore, this study aimed to develop and evaluate a research-based biotechnology practical integrating phytochemical screening, antioxidant activity testing, and sperm quality analysis of Hyphaene thebaica fruit extract. Specifically, the study evaluated the phytochemical profile and antioxidant activity of the extract, its effect on sperm quality, and the effectiveness of the integrated practical in improving pharmacy students' learning outcomes and laboratory competence.

Method

This study employed a mixed-methods, single-group pre-experimental design using a pre-test–post-test approach. This design was selected to determine the effectiveness of the phytochemical and antioxidant screening laboratory practical on zuriat fruit in improving students' understanding by measuring learning outcomes before and after the practical session. The participants were fifth-semester Pharmacy students at Institute of Technology and Health Bali who were enrolled in the Biotechnology Practical course.

Phytochemical Screening of Zuriat Fruit Extract (*Hyphaene thebaica*) Phytochemical screening was conducted to identify the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. The sample used in this screening was the extract of Zuriat fruit.

Alkaloid Identification

Two millilitres of the sample extract were placed into a test tube, followed by the addition of 5 mL of 2 N HCl. The mixture was heated, cooled, and divided into three separate test tubes. Three drops of specific reagents were added to each tube. A white or yellow precipitate after the addition of Mayer's reagent indicated a positive result for alkaloids. A brown precipitate following Wagner's reagent indicated the presence of alkaloids. An orange precipitate after the addition of Dragendorff's reagent also confirmed alkaloids (Godlewska et al., 2022; Peiris et al., 2023).

Flavonoid Identification

Two millilitres of extract were boiled for five minutes, followed by the addition of a small amount of magnesium powder and 1 mL of concentrated HCl. The formation of red, yellow, or orange colour indicated the presence of flavonoids (Aboshora et al., 2014; Peiris et al., 2023).

Saponin Identification

Two millilitres of extract were placed in a test tube, and pre-heated distilled water was added. The mixture was vigorously shaken for 10 seconds. The formation of foam measuring 1–10 cm in height, which remained stable for 10 minutes and did not disappear after the addition of one drop of 2 N HCl, indicated the presence of saponins (Dahiru & Nadro, 2022; Taha et al., 2020).

Tannin Identification

Two millilitres of extract solution were mixed with 3–5 drops of 10% ferric (III) chloride solution. The formation of a dark blue or greenish-black colour indicated the presence of tannins (Kurniawan & Wardany, 2021).

Terpenoid and Steroid Identification

Two millilitres of extract were mixed with 10 drops of glacial acetic acid (CH_3COOH) and two drops of sulphuric acid (H_2SO_4). The solution was gently shaken and left to stand for several minutes. A blue or green colour indicated the presence of steroids, whereas a red or purple colour indicated terpenoids (Dahiru & Nadro, 2022; Godlewska et al., 2022).

Antioxidant Activity Test (DPPH Method)

Antioxidant activity was assessed using the DPPH method with a UV–Vis spectrophotometer (Shimadzu UV-2700). The test was conducted to determine the antioxidant activity of Zuriat fruit extract. The procedure included preparation of the extract solution, preparation of 50 mM DPPH solution, preparation of vitamin C solution as a positive control, and antioxidant testing using the DPPH assay (Działo et al., 2016; Munteanu & Apetrei, 2021).

Observation of Spermatozoa Quality

Spermatozoa Count

Spermatozoa were collected using an erythrocyte pipette up to the 0.5 mark and diluted with diluent solution up to the 101 mark, followed by shaking for 15–20 minutes. A drop of the mixture was placed on an improved Neubauer haemocytometer chamber and observed under a microscope using a 10× objective lens. Counting was performed using a stopwatch and manual counter (Costa et al., 2024; Ge et al., 2023; McVay & Cooke, 2006).

Spermatozoa Motility

One drop of spermatozoa suspension was placed on the improved Neubauer chamber. Motility was observed under a microscope at 10× magnification. Movement distance was recorded every 10 seconds using a stopwatch. Motility speed was calculated by dividing the distance travelled by the recorded time. Observations were repeated three times, counting 100 spermatozoa cells. The observed criteria included immotile sperm, slow movement, circular movement, and progressive linear movement (Costa et al., 2024; Ge et al., 2023; McVay & Cooke, 2006).

Spermatozoa Morphology

One drop of sperm suspension was smeared onto a glass slide and fixed with 96% ethanol for five minutes. One drop of 1% eosin stain was added. Observations were conducted under a light microscope at 40× magnification. Normal morphology was defined as having an intact head and neck with a straight tail. Abnormal morphology included small head, absent head, enlarged head, double head, coiled tail, broken tail, or double tail (Ayad et al., 2021; Nowicka-Bauer & Nixon, 2020).

Spermatozoa Viability

One drop of sperm suspension was mixed with one drop of 1% eosin stain. Dead spermatozoa absorbed the stain and appeared red, whereas live spermatozoa did not absorb the stain and remained colourless. Observations were conducted under a light microscope at 40× magnification (McVay & Cooke, 2006; Nowicka-Bauer & Nixon, 2020).

Instruments and Data Collection

Quantitative data were obtained from students' pre-test and post-test scores, while qualitative data were collected through open-ended questionnaire responses, interviews, and laboratory observations. These data provided insights into student engagement, laboratory competence, and skill development. Laboratory skills were assessed across five domains: preparation and planning skills, including the preparation of tools and materials, understanding standard operating procedures (SOPs), and calculating material requirements; basic laboratory skills, which focused on understanding and implementing SOPs; experimental skills, encompassing accuracy in extraction, observation, and testing procedures; data processing skills, involving the analysis of phytochemical screening results, antioxidant activity, and spermatozoa quality; and safety and teamwork, which evaluated the appropriate use of personal protective equipment, laboratory tidiness, collaboration, and responsibility throughout the experimental process. Each domain was assessed using a four-point scale and validated through observational field notes and video recordings of student activities. Students also submitted individual laboratory reports containing procedural steps, observations, and reflections, which were evaluated based on completeness, accuracy, and data analysis skills.

The combined data provided comprehensive insights into student engagement and laboratory competence, strengthening the evidence that biotechnology practical learning can enhance students' skills in herbal material development.

Scoring Criteria:
1 = Poor
2 = Fair
3 = Good
4 = Very Good

Data Analysis

Quantitative data from pre-test and post-test scores were analyzed using a paired-sample t-test with SPSS software to determine the significance of improvement in learning outcomes. Observational laboratory skill data were analyzed descriptively by calculating the mean score for each indicator and classifying the results into very good, good, fair, and poor categories. Questionnaire data were analyzed quantitatively by calculating the percentage of responses in each category, followed by descriptive interpretation to provide an overview of students' perceptions of the Zuriat fruit phytochemical screening practical.

Result and Discussion

The phytochemical screening results of Zuriat Fruit extract (*Hyphaene thebaica*) demonstrated the presence of secondary metabolites. The findings indicate that Zuriat fruit contains bioactive compounds belonging to several classes of secondary metabolites. The detailed results of the phytochemical analysis are presented in Table 1.

Phytochemical identification constitutes a preliminary step in determining the presence of secondary metabolites and bioactive compounds in plants. The qualitative analysis (Table 1) demonstrated that the extract of Zuriat Fruit contained several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and terpenoids. However, quinones were not detected, as indicated by the absence of colour change during the NaOH test.

The presence of phenolic compounds and flavonoids is particularly significant in relation to spermatozoa quality. Flavonoids possess hydroxyl functional groups capable of donating hydrogen atoms to stabilise free radicals, thereby exerting antioxidant effects. This mechanism is essential in protecting spermatozoa from oxidative stress, which can impair motility, morphology, and viability.

Table 1. Phytochemical Screening Results of Zuriat Fruit (Hyphaene thebaica)

Compound Class	Positive Indicator	Interpretation
Alkaloids	White/yellow-orange precipitate	Positive (+)
Flavonoids	Red/orange–purplish colour	Positive (+)
Saponins	Stable foam ≥10 minutes	Positive (+)
Tannins	Black/greenish-blue colour	Positive (+)
Phenolics	Green/dark purple colour	Positive (+)
Steroids	Green–purplish colour	Positive (+)
Triterpenoids	Red/orange colour	Positive (+)
Terpenoids	Reddish-brown ring	Positive (+)
Quinones	Bright red colour	Negative (–)

Within the context of biotechnology practical learning, this stage provided students with hands-on experience in maceration extraction techniques and qualitative colourimetric analysis, both of which are fundamental competencies in natural product bioprospecting and herbal material development.

Antioxidant Activity Test Using the DPPH Method

Table 2. IC_{50} Value of Zuriat Fruit Extract

Sample	IC_{50} Value (mg/L)
Zuriat Fruit Extract	55.49

The antioxidant activity test using the DPPH method demonstrated that the IC_{50} value of Zuriat fruit extract was 55.49 mg/L. Based on commonly accepted antioxidant activity classifications, an IC_{50} value between 50–100 mg/L is categorised as strong antioxidant activity.

This result indicates that the extract possesses considerable free radical scavenging capacity, which may be attributed to the presence of phenolic and flavonoid compounds identified in the phytochemical screening (Damayanti et al., 2022). The strong antioxidant activity supports the potential role of Zuriat fruit extract in protecting spermatozoa from oxidative stress-induced damage (Dahiru & Nadro, 2022). Therefore, the IC_{50} value obtained in this study confirms that Zuriat fruit extract possesses strong free radical scavenging activity (Shi et al., 2022).

The underlying mechanism of the DPPH assay involves the donation of electrons or hydrogen atoms from bioactive compounds present in Zuriat fruit to the purple DPPH radical, converting it into the non-radical form DPPH-H, which appears yellow. The degree of colour change, measured spectrophotometrically, reflects the antioxidant capacity of the extract.

From a biotechnological learning perspective, this analysis provides students with practical experience in quantitative data processing using UV–Vis spectrophotometry, including linear regression analysis to determine IC_{50} values. Such activities strengthen students' competencies in analytical techniques and data interpretation within natural product research.

Doum is widely consumed as a traditional beverage in Egypt and is known to be rich in polyphenolic compounds (Xiang et al., 2023). Several studies have reported that doum fruit extracts contain high levels of flavonoids and phenolic compounds and exhibit antioxidant and antibacterial activities, thereby mitigating oxidative stress and preventing diseases associated with pathogenic bacteria (Aboshora et al., 2014; Kolla et al., 2021; Uyi Ewansiha et al., 2021). Plant phenolic compounds are recognised as highly effective free radical scavengers. Their antioxidant activity is associated with the presence of hydroxyl functional groups attached to aromatic rings, conjugated double bonds, and the overall annular molecular structure (Działo et al., 2016).

Furthermore, antioxidant activity increases with concentration, suggesting that the consumption of doum fruit may provide multiple health benefits due to its antioxidant and antimicrobial properties.

Table 3. Observation Results of Spermatozoa Quality Following Treatment with Zuriat Fruit Extractw

Variable	Observation Parameter	Result
Sperm Count	Number per microscopic field	With: Sperm count >100 Without: Sperm count <100
Motility	Progressive forward movement per second	With: Progressive forward movement >100 Without: Progressive forward movement <100
Viability	Red stain absorption / no stain (colourless)	With: Red-stained sperm <100; colourless sperm >100 Without: Red-stained sperm >100; colourless sperm
Morphology	Normal / Abnormal	With : Normal morphology >100; abnormal <100 Without : Normal morphology <100; abnormal >100

Based on the findings presented in Table 3, an improvement in spermatozoa quality was observed following administration of Zuriat Fruit extract. The improvement was reflected in increased sperm count, enhanced motility, improved viability, and a higher proportion of normal morphology compared with the control group.

The phytochemical screening results demonstrated that the extract tested positive for alkaloids and flavonoids (Taha et al., 2020). Flavonoids are well recognised for their antioxidant properties, as they inhibit oxidative reactions by scavenging free radicals and stabilising highly reactive molecules (Mutha et al., 2021). This mechanism plays a crucial role in preventing oxidative stress-induced reductions in spermatozoa count in male mice (House Mouse) (Kowalczyk, 2022).

Antioxidants function by neutralising or stabilising free radicals through electron donation, thereby interrupting the chain reactions involved in radical formation that may lead to cellular damage (Feoktistova et al., 2021). By inhibiting oxidative damage to cell membranes, antioxidants help maintain the integrity of hormonal signalling pathways involved in spermatogenesis. Consequently, hormone–receptor interactions remain optimal, supporting enhanced spermatogenic processes and resulting in increased spermatozoa production (Santi et al., 2020; Winarti et al., 2021).

The present findings are consistent with the study conducted by Antari & Damayanti, (2025). (2025), which reported that Hyphaene thebaica extract significantly reduced sperm DNA fragmentation. This protective effect is strongly associated with its high antioxidant activity. Furthermore, Damayanti et al., (2022) confirmed that the DPPH assay of Zuriat fruit demonstrated strong free radical inhibition, primarily attributed to its flavonoid content.

Overall, these results reinforce the hypothesis that the antioxidant capacity of Zuriat fruit extract contributes to improved spermatozoa quality through the mitigation of oxidative stress.

Table 4. Pre-Test and Post-Test Results of Students (n = 30)

Parameter	Learning outcome
Pre-Test (Mean ± SD)	65 ± 3.5
Post-Test (Mean ± SD)	85 ± 4.2
p-value	0.001

Note: p < 0.05 indicates a statistically significant difference.

The comparison of pre-test and post-test scores demonstrates a significant improvement in students' learning outcomes following the biotechnology practical training, particularly in laboratory utilisation, equipment handling, material preparation, and the application of herbal materials in biotechnology experiments. The paired-sample analysis ($p = 0.001$)

confirms that the research-based practical approach significantly enhanced students' cognitive achievement.

Table 5. Laboratory Skill Assessment Results

Skill Domain	Mean Score	Category
Preparation & Planning	3.45	Good
Basic Laboratory Skills	3.60	Very Good
Experimental Skills	3.30	Good
Data Processing	3.15	Good
Safety & Teamwork	3.75	Very Good

Overall, students' laboratory skills were categorised as Good to Very Good. The highest score was achieved in the Safety and Teamwork domain (3.75), indicating strong professional responsibility and awareness of laboratory safety standards (Maknun et al., 2020).

The Preparation and Planning domain (mean score 3.45; Good category) indicates that students demonstrated adequate competence in organising experimental requirements independently (Djannah et al., 2020). Nevertheless, further improvement may be achieved through strengthened technical instruction and increased practical exposure (Pujiastutik, 2017).

In the Basic Laboratory Skills domain, the very good score (3.60) reflects a high level of compliance with laboratory SOPs and effective translation of theoretical knowledge into practice (Byrd et al., 2019). This foundational competence is essential for conducting more complex experimental procedures.

Experimental Skills (3.30; Good) suggest that while students performed extraction and antioxidant testing with reasonable precision, further practice is required to enhance accuracy and efficiency in microscopic observations (Pujiastutik, 2017; Wardani & Djukri, 2019). Data Processing (3.15; Good) revealed that students were capable of interpreting phytochemical and antioxidant results; however, advanced statistical analysis remains an area requiring reinforcement. Greater emphasis on statistical software application and analytical interpretation is recommended to foster independent research capability.

The integration of spermatozoa observation into this practical served as a bridge between phytochemical theory and medical biotechnology application. Through sperm viability and motility testing following administration of Zuriat Fruit extract, students not only examined antioxidant effects in vitro but also developed quantitative microscopy and cellular evaluation skills, which are fundamental competencies in reproductive biotechnology.

This study therefore functioned not only as scientific research into the bioactivity of Zuriat fruit but also as a structured model for psychomotor and cognitive skill development in biotechnology education, including:

1. Sample Preparation Skills – Application of proper simplisia preparation and extraction techniques to maintain bioactive compound stability.
2. Bioassay Skills – Implementation of the DPPH method, fostering precision in micropipetting and optical instrument handling.
3. Critical Thinking – Integration of chemical data (phytochemical content) with biological outcomes (sperm quality parameters), a key competency in medical biotechnology.

Conclusion

This study concludes that the implementation of research based practical learning using Zuriat Fruit (*Hyphaene* thebaica) effectively enhanced students' competencies, both cognitively and in laboratory skills. Statistically significant improvement was observed in learning outcomes following the intervention. Biologically, Zuriat fruit extract demonstrated strong antioxidant activity (IC_{50} = 55.49 ppm), attributable to its rich phytochemical composition. The extract contributed to improved spermatozoa quality reflected in increased count, motility, viability, and normal morphology through mechanisms associated with oxidative stress inhibition. Overall, this research highlights the dual contribution of Zuriat fruit based biotechnology practical learning: advancing scientific understanding of herbal bioactivity while simultaneously strengthening higher education competency development in biotechnology.

Acknowledgments

The authors would like to express their sincere gratitude to the Department of Pharmacy, Institute of Technology and Health Bali, for providing laboratory facilities and academic support throughout the research process. Appreciation is also extended to the fifth-semester Pharmacy students who actively participated in the biotechnology practical sessions and contributed to the data collection process. The authors are grateful to the laboratory assistants and technical staff for their support in ensuring that all experimental procedures were conducted in accordance with established laboratory standards.

Author Contributions

Conceptualization, N.W.S.A. and N.K.T.A.; methodology, N.W.S.A.; formal analysis, N.W.S.A.; investigation, N.W.S.A.; writin original draft preparation, N.W.S.A.; writin review and editing, N.K.T.A.; supervision, N.K.T.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflicts of Interest
The authors declare no conflict of interest.

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