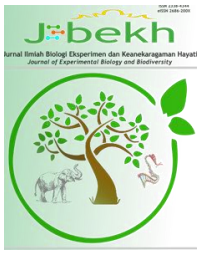
ISSN 2338-4344
eISSN 2686-200X

OPEN ACCESS

Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati (J-BEKH)

Volume 11, Issue 1, June 2024

Article History
Received: June 3, 2024
Accepted: June 21, 2024



Potential of Hibiscus (*Hibiscus rosa-sinensis* L.) Extract as a Substitute for Eosin in Hematoxylin-Eosin Histological Staining

Potensi Ekstrak Kembang Sepatu (*Hibiscus rosa-sinensis* L.) sebagai Pengganti Eosin pada Pewarnaan Histologi Hematoksilin-Eosin

Fitri Nadifah[1*], Yuliana Prasetyaningsih[1], Nurlaili Farida Muhajir[1], Sri Murtiningrum[1,2], Clara Shinta D.P Ohoiwutun[1], Elisabet Della Puspita[1]

1. Medical Laboratory Technology Department, STIKES Guna Bangsa Yogyakarta

2. RSPAU dr. S. Hardjolukito Yogyakarta

*Corresponding author: fitri@gunabangsa.ac.id ; 089605367007

Abstrak

Pewarnaan adalah salah satu tahap terpenting dalam pembuatan preparat jaringan. Jaringan yang diwarnai dengan baik mempermudah pengamatan struktur dan morfologi mikroskopis. Hematoxylin-Eosin pewarna yang paling umum digunakan untuk pewarnaan jaringan. Eosin terdaftar sebagai larutan kimia bersifat sintetik dan diketahui bersifat karsinogenik. Pemilihan pewarna alternatif dari bahan alami penting untuk meminimalisir masalah kesehatan dan pencemaran lingkungan. Kembang sepatu (*Hibiscus rosa-sinensis L.*) mengandung pigmen antosianin yang berpotensi sebagai pewarna alternatif pengganti eosin. Penelitian ini bertujuan untuk mengetahui potensi ekstrak kembang sepatu. sebagai pengganti eosin pada pewarnaan histologi. Sampel penelitian ini adalah preparat jaringan hepar mencit (*Mus musculus*) yang belum diwarnai. Jaringan diwarnai dengan ekstrak kembang sepatu dan eosin sebagai kontrol. Pengamatan dilakukan dengan memberikan penilaian kualitas jaringan yang telah diwarnai. Hasil penilaian mikroskopis menunjukkan bahwa jaringan hepar yang diwarnai ekstrak konsentrasi 60% dan 80% kurang baik dibandingkan eosin. Namun demikian, karena jaringan dapat terwarnai menjadi kemerahan, maka ekstrak kembang sepatu berpotensi untuk dikembangkan lebih lanjut sebagai pengganti eosin. Penggunaan metode ekstraksi yang lebih tepat diharapkan dapat menghasilkan zat warna antosianin yang utuh sehingga dapat mewarnai dengan maksimal.

Abstract

Staining is an important stage in preparing microscopic preparations so that the histological structure can be observed clearly. Hematoxylin-Eosin is the most widely used dye. Unfortunately, eosin is a synthetic chemical compound that is carcinogenic. To reduce the health and environmental impacts of eosin compounds, it is important to look for alternative dyes from natural ingredients. Hibiscus (Hibiscus rosa-sinensis L.) is a plant known to contain anthocyanins which can be used as eosin substitutes. This study aims to determine the potential of hibiscus extract as a substitute for eosin in histological staining. In this study, unstained liver tissue preparations from mice (Mus musculus) were used. Hibiscus extract dye was compared with eosin as a control. The color quality of the tissue preparations was used as an experimental parameter. The results of the microscopic assessment showed that liver tissue stained with Hibiscus extract at concentrations of 60% and 80% was less good than eosin. However, because tissue can stain reddish, hibiscus extract has the potential to be used as an eosin substitute. The right extraction method may be able to produce intact anthocyanin so that it can color the tissue optimally.

Keywords: *eosin, extract, hibiscus flower, anthocyanin*

Kata kunci: *eosin, ekstrak, kembang sepatu, antosianin*

How to Cite: Nadifah, F., Prasetyaningsih, Y., Muhajir, N.F., Murtiningrum, S., Ohoiwutun, C.S.D.P., Puspita, E.D. (2024). Potential of Hibiscus Flower (*Hibiscus rosa-sinensis* L.) Extract as a Substitute for Eosin in The Histological Staining Hematoxylin-Eosin. *Jurnal Ilmiah Biologi Eksperimen Dan Keanekaragaman Hayati (J-BEKH)*, *9*(2), 57-62.

INTRODUCTION

In the tissue preparation process, staining is one of the most important stages. The dye commonly used for histopathological examination is hematoxylin-eosin (HE). Hematoxylin-eosin staining using eosin reagent has both advantages and disadvantages. The advantages of eosin are that the color differences are clearly visible in cytoplasmic staining, it is stable and does not fade easily. However, the price of reagents is relatively expensive, contains carcinogenic chemicals, is flammable and difficult to decompose in soil and water [1].

Eosin is listed as a carcinogenic chemical solution in IARC class-3. Apart from causing cancer, if exposed for a long time, eosin can also cause irritation of the digestive tract, cyanosis and skin irritation [2]. Therefore, to overcome the impact of using synthetic dyes is to use alternative dyes from natural ingredients that are more environmentally friendly and do not harm health. Hibiscus plant (*Hibiscus rosa-sinensis* L.) is widely distributed throughout Indonesia. Its red-colored petals are known to contain hibiscetine, flavonoids, tartaric acid, cyclopropenoids, and anthocyanin pigments [3].

The anthocyanin content in the Hisbiscus flower has the potential to be used as an alternative to eosin in tissue staining. Anthocyanins in hibiscus flowers have properties that are similar to fluorescence in eosin which gives a pink color to the cytoplasm [4]. Suggestions to use Hibiscus flowers as natural dye because they are easy to find, more affordable, and do not contain hazardous chemicals. Based on a number of studies, hibiscus extract is not only used to stain tissue, but also to stain worm eggs [5].

Previous studies indicated that diff-quick staining using hibiscus flower extract can be used as a substitute for eosin. At the concentration of 0.5 g/mL + 1% HCl and the soaking time of 30 minutes the cells were not stained well, because the color of nucleous and cytoplasm was not clearly contrasted. However, at a concentration of 0.7 g/mL without the addition of 1% HCl and a soaking time of 15 minutes the color of cell nucleus and cytoplasm are clearly visible [6].

The aim of this research was to determine the potential of hibiscus flower extract as an alternative dye to eosin in tissue staining. What concentration of extract can produce the best staining when compared to Hematoxylin-Eosin. In this research, hibiscus flower extract will be used with concentrations of 60% and 80% with the addition of 1% HCl and a soaking time of 20 minutes. The staining results used in this study were divided into three levels, namely good, poor and poor quality using scores according to the assessment criteria [7].

METHODS

Plant samples

This research was conducted in May 2023 at the Clinical Laboratory of STIKES Guna Bangsa Yogyakarta. The hibiscus plant flowers used in this study were obtained from Gamping District, Sleman Regency, Yogyakarta. The petals were separated from the other parts, washed with distilled water and then dried using an oven at 40℃ for 18 hours [8]. The dried petals are ground into powder used further for extraction [9].

Extraction

Extraction was carried out using the maceration method. 300 grams of ground petals was added with 3000 mL of 96% ethanol, then stirred and left at room temperature for 24 hours and then filtered (repeated 3 times). The filtrate formed was then evaporated with a vacuum rotary

evaporator at a temperature of 50°C for 15 minutes and the flower extract was obtained [8].

Phytochemistry Test

Identification of anthocyanin compounds was carried out by qualitative phytochemical tests. Two drops of hibiscus flower extract were added with 2 drops of 10% NaOH so that a color change occurred. Furthermore, 2 drops of concentrated HCl were added so that the color turned red again [8].

Tissue staining

The slides were immersed in xylene for 30 minutes and in alcohol with decreasing concentrations: 96%, 85%, and 70% for 10 minutes, 5 minutes, and 1 minute, respectively. They stained in eosin dye (for the control group) or extract (for the treatment group) for 30 seconds and then washed with running water. The slides immersed again in 96% alcohol and in xylene for 2 and 10 minutes, respectively, with 2 replications each. After dried, they sealed with entelan. Microscopic observations are carried out to determine the staining results.

Data Analysis

Evaluation of the results of staining with eosin dye (control group) and hibiscus extract (treatment group) was carried out under a microscope. The results were expressed in three categories: good quality, poor and poor by two assessors. The results of this quality assessment are then converted into a score of 3 to 1 [7].

RESULTS AND DISCUSSION

The phytochemical test results showed a color change to red after the addition of HCl solution which indicated the presence of anthocyanin in the extract. Based on the staining quality assessment (Table 1), flower extracts with concentrations of 60% and 80% produced poor staining quality, where the bright color in the cytoplasm was not too clear. When compared to the control, the color clearness was still lacking.

Table 1. Results of the staining quality assessment

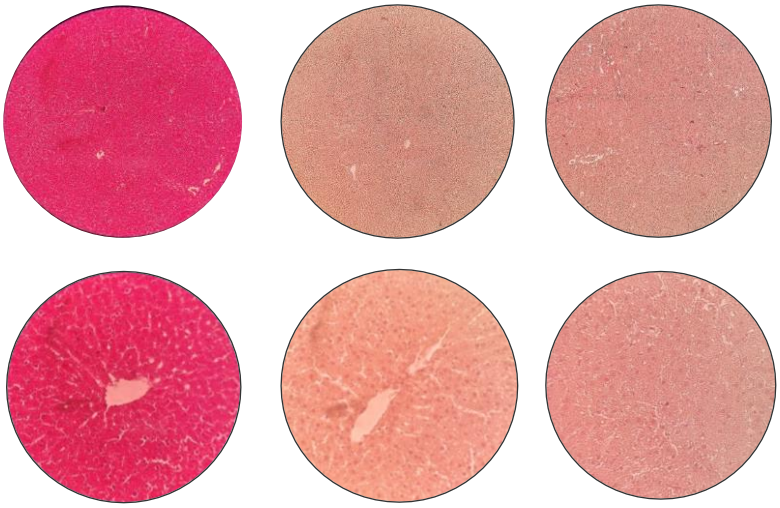
Treatments	Assessment	Quality	Score
Control	The light purple color of the cytoplasm is clear	Good	3
60% extract	The light purple color of the cytoplasm is less clear	Poor	2
80% extract	The light purple color of the cytoplasm is less clear	Poor	2

Hibiscus flowers extract has an anthocyanin content of 48.260mg/25g in spectrophotometric readings at λ 528 nm [3]. The results of this study indicate that both 60% and 80% hibiscus extracts have not been able to color cells well. This is likely because the anthocyanins in the extract have changed. It is known that anthocyanins are compounds that have low stability [10]. Anthocyanin stability is influenced by factors such as pH, light, temperature, copigmentation, sulfite, ascorbic acid, the presence of oxygen, and enzymes [4]. We consider that the lack of quantitative anthocyanin content data become the weakness of this study.

Although it produces less good coloring compared to the control, hibiscus extract is still able to color the cytoplasm pink (Table

2). Thus, the hibiscus extract used contains anthocyanins that work like eosin. It binds to acidophilic cell components (cytoplasm) so that it can be colored pink. Further research needs to use better methods to obtain intact anthocyanins. In addition, special reagents are needed as extract mixtures to obtain better staining results. It was reported that ultrasonic-assisted hibiscus extraction produced the most anthocyanins (179.32 mg/l) compared to microwave-assisted extraction (155.45 mg/l) and conventional extraction (100.88 mg/l) [9].

Table 2. Comparison of staining results with hibiscus flower extract and eosin

Control	60% extract	80% extract
		

*Magnification 100x (top) and 400x (bottom)

The results of this study are in line with previous studies showing that hibiscus flower extract as an alternative dyes at a concentration of 0.5 g/mL added with 1% HCl and a soaking time of 30 minutes, the cells are not stained well and the contrast between the cytoplasm and the cell nucleus is not clear. Meanwhile, with a concentration of 0.7 g/mL without the addition of 1% HCl with a soaking time of 15 minutes, the cells can be stained well and the cell nucleus and cytoplasm are clearly visible [6]. Other studies on the use of hibiscus extract also state that hibiscus extract is less able to stain the cell nucleus well [11]. A number of studies have been conducted to find alternative dyes to replace eosin, such as rosella flower (*Hibiscus sabdarifa*), henna leaves [12], black plum [13], curcumin [14] and beet root [15]. Research to find alternative dyes to replace eosin must continue to be carried out sustainably. Natural ingredient extracts as mentioned can be used as candidates.

CONCLUSION

It can be concluded that hibiscus flower extract has the potential as an alternative dye for tissue staining. However, the quality of the staining results has an intensity and color gradation that is still lacking when compared to staining using Eosin. Nevertheless, hibiscus extract can still be developed into an alternative dye with a more precise extraction method to obtain intact anthocyanins.

ACKNOWLEDGMENT

We would like to thank STIKES Guna Bangsa Yogyakarta for providing the opportunity and assistance in writing this manuscript.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in the writing of this academic work.

REFERENCES

[1] S. Sarode, G. Pradeep, N. Prakash, A. Mahajan, and N. Mangle, "Exploring a safer alternative to eosin in soft tissue staining," *Journal of Oral and Maxillofacial Pathology*, vol. 26, no. 4, p. 603, Feb. 2022, doi: 10.4103/jomfp.jomfp_27_22.

[2] K. Pradeepthi, K. Rajani, G. Rao, T. Sravya, S. Wahed, and J. Sailaja, "Evaluation of biosafe alternative to eosin in hematoxylin and eosin staining procedure: A comparative study," *Journal of Oral and Maxillofacial Pathology*, vol. 27, no. 2, pp. 424–425, Apr. 2023, doi: 10.4103/jomfp.jomfp_146_22.

[3] W. Weerasingha, M. Chandrasiri, K. Hewawitharana, and J. Sampath, "Evaluation of anthocyanin extracted from Hibiscus rosa sinensis as a natural food colorant," in *10th Annual Science Research Session*, 2021, pp. 17–20. [Online]. Available: https://www.researchgate.net/publication/356749627

[4] B. Enaru, G. Dreţcanu, T. D. Pop, A. Stănilă, and Z. Diaconeasa, "Anthocyanins: Factors affecting their stability and degradation," *Antioxidants*, vol. 10, no. 12. MDPI, Dec. 01, 2021. doi: 10.3390/antiox10121967.

[5] R. Ningsih, S. Salnus, and A. H. Novriani, "Pemeriksaan Telur Cacing Soil Transmitted Helminths," *Jurnal Farmasi, Kesehatan dan Sains (FASKES)*, vol. 1, no. 3, 2023, doi: 10.32665/faskes.v1i3.2394.

[6] L. Mutoharoh, S. D. Santoso, and A. A. Mandasari, "Pemanfaatan Ekstrak Bunga Sepatu (Hibiscus rosa-sinensis L.) sebaga Alternatif Pewarna Alami Sediaan Stologi Pengganti Eosin pada Pengecatn Diff-Quick," *Jurnal SainHealth*, vol. 4, no. 2, 2020.

[7] T. Ariyadi and H. Suryono, "Kualitas Sediaan Jaringan Kulit Metode Microwave Dan Convetional Histoprocessing Pewarnaan Hematoxylin Eosin," *Jurnal Labora Medika*, vol. 1, no. 1, pp. 7–11, 2017.

[8] M. Warnis, L. Adelia Aprilina, L. Maryanti, and J. Farmasi Poltekkes Palembang, "Pengaruh Suhu Pengeringan Simplisia terhadap Kadar Flavonoid Total Ekstrak Daun Kelor (Moringa oleifera L.)," in *Seminar Nasional Kahuripan I*, 2020, pp. 264–268.

[9] R. Sri Raghavi, M. Visalakshi, S. Karthikeyan, G. Amutha Selvi, S. Thamaraiselvi, and K. Gurusamy, "Standardisation of anthocyanin extraction techniques from hibiscus (Hibiscus rosa-sinensis) petals for biocolour utilisation," *Pharma Innov*, vol. 11, no. 8, pp. 303–309, Aug. 2022, doi: 10.22271/tpi.2022.v11.i8d.14647.

[10] B. Alappat and J. Alappat, "Anthocyanin pigments: Beyond aesthetics," *Molecules*, vol. 25, no. 23. MDPI AG, Dec. 01, 2020. doi: 10.3390/molecules25235500.

[11] M. N. Mizan, M. Damayanti, and F. Nuroini, "Gambaran Sitologi Epitel Mukosa Rongga Mulut Pewarnaan Ekstrak Bunga Sepatu (Hibiscus Rosa-sinensis L.)," *Prosiding Seminar Nasional UNIMUS*, vol. 4, pp. 1790–1796, 2021.

[12] B. Joshua, K. Ndam, G. Danjuma, and D. Gunya, "Assessing the use of Lawsonia Inermis and Hibiscus Sabdariffa Aqueous Extracts as a Possible

Substitute for Eosin Stain in Paraffin-embedded Tissues," *Article in Upsala Journal of Medical Sciences*, 2021, [Online]. Available: http://scientificpublications.in/index.php/ijmsar

[13] R. Ashi, "Black Plum Fruit Extract as a Substitute for Haematoxylin in Haematoxylin and Eosin Staining Technique," *Cytology & Histology International Journal*, vol. 7, no. 1, 2023, doi: 10.23880/chij-16000143.

[14] A. M. Krishnan, "Assessment of Staining Quality of Curcumin as a Substitute for Eosin in Hematoxylin and Eosin Staining in Histopathology," *Journal of Research in Medical and Dental Science* |, vol. 8, no. 5, 2020, [Online]. Available: www.jrmds.in

[15] M. Udonkang, C. Ene, A. Archibong, A. Egbe, and I. Inyang, "Aqueous beetroot dye as an alternative to haematoxylin and eosin in the diagnosis of breast tumours," *Global Journal of Pure and Applied Sciences*, vol. 27, no. 4, pp. 417–423, Nov. 2021, doi: 10.4314/gjpas.v27i4.9.