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Antibacterial Activity of *Staphylococcus capitis, Bacillus cereus, Pantoea dispersa* From Telang Flower (*Clitoria ternatea* L.) Kombucha Bath Soap as a Pharmaceutical Biotechnology Product

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Abstract

Telang lower kombucha is a probiotic drink that has the potential to enhance the immune system, active cosmetic ingredients, and its waste has been proven to be used as an organic liquid fertilizer preparation. The purpose of this study was to make a formulation and preparation of liquid bath soap with an active ingredient in a solution of seagrass kombucha fermentation at a sugar concentration of 20%, 30%, and 40% in inhibiting the growth of S. capitis, B. cereus, and P. dispersa bacteria. via the disc diffusion method. The results of the study have proven that based on the post hoc test analysis, kombucha bath soap at a concentration of 40% is significantly different from the concentration of 20% and 30% but not significantly different from the positive control and the concentration of 40% is the best concentration in inhibiting the growth of the three test bacteria. compared with the treatment and the two comparisons.

Abstrak

Kombucha bunga telang merupakan minuman probiotik yang memiliki potensi sebagai peningkat sistem kekebalan tubuh, bahan aktif kosmetik, dan limbahnya telah terbukti dapat dimanfaatkan sebagai sediaan pupuk cair organik. Tujuan dari penelitian ini adalah untuk membuat formulasi dan sediaan sabun mandi cair yang berbahan aktif larutan fermentasi kombucha bunga telang pada konsentrasi gula sebesar 20%, 30%, dan 40% dalam menghambat pertumbuhan bakteri S. capitis, B. cereus, dan P. dispersa melalui metode difusi cakram. Hasil penelitian telah membuktikan berdasarkan analis uji pos hoc adalah sediaan sabun mandi kombucha bunga telang pada konsentrasi 40% berbeda nyata dengan konsentrasi 20%, dan 30% namun tidak berbeda nyata dengan kontrol positif serta konsentrasi 40% merupakan konsentrasi terbaik dalam menghambat ketiga pertumbuhan bakteri uji dibandingkan dengan perlakuan dan kedua pembandingnya.

Keywords: *Body Wash, Kombucha, Telang Flower*

Kata kunci: Sabun Mandi, Kombucha, Bunga Telang.

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INTRODUCTION

We have entered an era where the era is growing from various sides of life in producing practical efforts for health, food, agriculture, pharmacy, and the development of life technology or biotechnology[1]. The use of living things to produce goods and services on a conventional and modern scale is one of the definitions of biotechnology[2] in responding to environmental challenges.

One of the conventional biotechnology products is kombucha fermentation. It is known that kombucha is a tea-based functional drink and acts as a probiotic to boost the immune system[3], has antioxidant properties[4], anti-cholesterol[5], and anticancer[6]. Another ingredient in making kombucha is Telang flower (*Clitoria ternatea* L) which has a secondary metabolite source as an antibacterial[7].

Qualitatively, butterfly pea flower kombucha contains secondary metabolites from alkaloids, flavonoids, and saponins[8] which have the potential as antibacterial sources[9-11], antimicrobial sources[12], and antifungal sources[13].

Telang flower kombucha (*Clitoria ternatea* L), besides having the ability as a functional drink to enhance the immune system[14], has potential as an active cosmetic ingredient such as a liquid bath soap preparation that has been tested as an antibacterial for *Staphylococcus aureus*[15], *Escherichia coli*[16], *Vibrio parahaemolyticus* and *Klebsiella pneumonia*[17], antifungal *Trichophyton rubrum* and *Trichophyton mentagrophytes*[18], antifungal *Candida albicans*[19], dish soap preparations as gram-positive and negative antibacterials[20], antifungal shampoo[21].

Even the Telang flower kombucha can be used as an organic liquid fertilizer[22-23]. The many benefits of Telang flower kombucha in health, pharmacy, and agriculture made the authors want to be interested in conducting this latest research to make Telang flower kombucha bath soap preparations in inhibiting the growth of *Staphylococcus capitis*, *Bacillus cereus*, and *Pantoea dispersa*.

Previous research conducted by Saddam et al. (2022)[22] has stated that telang flower kombucha (*Clitoria ternatea* L) at a concentration of 40% is the best concentration in inhibiting the growth of *S. capitis* with an average diameter of the resulting inhibition zone of 17.25 mm (strong category), *B. cerreus* with an average inhibition zone diameter of 14.86 mm (strong category), and *P. dispersa* with an average inhibition zone diameter of 12.24 mm (strong category).

This study aims to make formulations and preparations for bath soap with an active ingredient of telang flower kombucha fermented solution as an antibacterial for *Staphylococcus capitis* (a Gram-positive bacterium that lives on the scalp, neck, face, scrotum, and human ears), *Bacillus cereus* (a Gram-positive bacterium that inhabits the soil and causes food poisoning), and *Pantoea dispersa* (a gram-negative bacterium that causes sepsis)[24].

METHODOLOGY

Types of Research

Making as many as four bodies wash preparations consisting of a body wash base as a negative control that tends to be designed without active substances. The soap base is added with a solution of fermented Telang flower kombucha with a concentration of 20%, 30%, and 40% as the active substance. This research is an experimental laboratory providing liquid body wash circulating in the market as a comparison (positive control).

Materials

The materials used in this study included *Staphylococcus capitis*, *Bacillus cereus*, and *Pantoea dispersa*. *Muller Hinton Agar* (MHA) media, a solution of fermented Telang flower kombucha at a sugar concentration of 20%, 30%, and 40% as an active substance (antibacterial).

The main ingredients of liquid body soap consist of 40% KOH (Potassium Hydroxide) of 8 g as a foaming agent. Na-CMC of 1 g as a thickener. Sodium Lauryl Sulfate (SLS) of 1 g as a surfactant. 0.5 mL of Olive Oil Infused as a fatty oil. Phenoxyethanol of 0.5 g as a preservative. BHT of 1 g as an antioxidant. 1 mL of essential oil as a perfume[17].

Other ingredients as additional ingredients for liquid bath soap include 1 mL of castor oil as a fluid. Sodium lactate of 1 g as a moisturizer. White sugar of 1 g as a foam enhancer. Yoghurt of 1 g as a softness enhancer. Kaolin Klay of 1 g as an addition to the slip and silky effect when bathing. 100 mL of distilled water as a solvent[20].

Stages of Making Bath Soap

Weigh the ingredients as a whole. Put 15 mL of olive oil into a beaker glass, then add 8 mL of 40% KOH little by little while heating it at 60-70^{o}C until getting a paste of soap and add 15 mL of distilled water (first mixture).

Add hot water and Na-CMC, then let stand until it swells. Add SLS, then stir until homogeneous. Add the ingredients for soap and stir until homogeneous. Add the active substance as a fermented butterfly pea flower kombucha solution with a sugar concentration of 20%, 30% and 40%. Enter the distilled water up to

100 mL in each soap preparation, and put it in the soap bottle (Pertiwi *et al*., 2022).

Bath Soap Preparation Formulation

Bath soap formulations and preparations consist of 5 formulas. The bath soap preparations referred to include F0, namely as a soap base / comparison / negative control / without active substance. F1, namely bath soap, has been stable and is circulating in the market as a positive control. F2 is a bath soap base that is added to the active substance as a fermented butterfly pea flower kombucha solution with a concentration of 20%. F3 is a bath soap base added to a solution of fermented butterfly pea flower kombucha with a concentration of 30%. F4 is a bath soap base added to a solution of fermented Telang flower kombucha with a concentration of 40%. Bath soap formulations and preparations are listed in table 1 below.

Table 1. Bath Soap Formulation and Preparation[18]

Ingredients	Function	F0 (-)	F1 (+)	F2 (20%)	F3 (30%)	F4 (40%)
Fermentation Solution Telang Kombucha	Active substance	0	X	20	30	40
Olive oil	Soap base	15	15	15	15	15
KOH 40%	Foam maker	8	8	8	8	8
Na-CMC	Thickner	1	1	1	1	1
SLS	Surfactant	1	1	1	1	1
Infused in olive oil	Oil	0.5	0.5	0.5	0.5	0.5
Phenoxyethanol	Preservative	0.5	0.5	0.5	0.5	0.5
BHT	Antioxidants	1	1	1	1	1
Essence oil	Parfume	1	1	1	1	1
Castor oil	Additional liquid	1	1	1	1	1
Sodium lactat	Moisturizer	1	1	1	1	1
Sugar	Foam booster	1	1	1	1	1
Yoghurt	Soft enhancer	1	1	1	1	1
Kaolin clay	Slik and silky effect enhancer	1	1	1	1	1
Aquadest	Solvent	100	100	100	100	100

Bacterial Inhibitory Test of Bath Soap Preparation

The inhibition test on the growth of *Staphylococcus capitis*, *Bacillus cereus*, and *Pantoea dispersa* from liquid soap preparations with an active ingredient of kombucha-fermented Telang Flower is generally carried out to determine the clear zone of an antibacterial agent in the form of an active substance. This

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study refers to the results of previous research conducted by Rezaldi et al. (2022)[25], namely in the form of a liquid bath soap with an active ingredient of telang flower kombucha fermented solution.

The tool used to measure the growth of the diameter of the inhibition zone is the analytic caliper. The formula for measuring the average diameter of the inhibition zone is as follows:

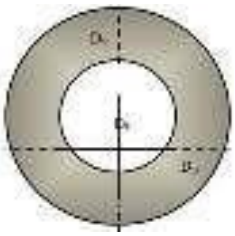

Figure 1. Calculation of the Average Diameter of the Inhibition Zone.

Description:

DV: Vertical Diameters

DH: Horizontal Diameters

DC: Cakram Diameters[13].

Data Analysis

Data obtained from the average diameter of the inhibition zone of all soap preparations in inhibiting the growth of *S. capitis*, *B. cerreus* and *P. dispersa* were processed through one-way ANALYSIS OF VARIANCE (ANOVA) with a 95% confidence level. If there is a significant difference in all bath soap preparations in inhibiting the growth of the three test bacteria, it will be followed by a post hoc analysis[19].

RESULT AND DISCUSSION

Preparation of liquid bath soap made from telang flower kombucha fermented solution, which includes a sugar concentration of 20%, 30%, and 40%, has been shown to correlate positively in inhibiting the growth of the three test bacteria, namely *S. capitis*, *B. cerreus*, and *P. dispersa*.

The average diameter of the inhibition zone produced from telang flower kombucha bath soap at a concentration of 20% in inhibiting the growth of *S. capitis* bacteria was 7.56 mm (moderate), 30% 8.17 mm (moderate), and 18.78 mm (strong). 0 mm body soap base (no response) and 17.67 mm commercial soap (strong)

The average diameter of the inhibition zone produced from telang flower kombucha bath soap at a concentration of 20% in inhibiting the growth of *B. cereus* bacteria was 7.40 mm (moderate), 30%, namely 8.05 mm (moderate), and 40%, namely 15.20 (strong). Bath soap base 0 mm (no inhibition), and market soap as a positive control, namely 14.90 mm (strong)

The average diameter of the inhibition zone produced from telang flower kombucha bath soap in inhibiting the growth of *P. dispersa* bacteria at a concentration of 20% was 7.09 mm (medium), 30% was 7.15 mm (moderate), and 40% 13.52 mm (strong). The body wash base is 0 mm, and the commercial body wash is 12.70 mm (strong).

The results of the one-way ANOVA analysis have proven that each bath soap preparation inhibiting the growth of the three test bacteria has a lower F table value than the calculated F, namely with a p value> 0.05 mm, so that it can be continued through post hoc analysis.

Post hoc analysis has proven that the concentration of 20% in preparing butterfly pea kombucha bath soap significantly differs from the concentration of 30%, 40%, soap base, and market soap. The concentration of 30% in the preparation of telang flower kombucha bath soap was significantly different from the 40% concentration, bath soap base, and commercial body wash as a positive control. However, it was not significantly different from the butterfly pea kombucha bath soap at a concentration of 20%. The Telang flower kombucha bath soap at 40% was significantly different from the eggplant kombucha bath soap at a concentration of 20% and a 30% soap base. However, it was not significantly different from market soap as a positive control in inhibiting the growth of *S. capitis*, *B. cereus*, and *P. dispersa* bacteria.

The results of this study have proven that the higher the concentration of bath soap, the higher its potential to inhibit the growth of the three test bacteria. The concentration of 40% is the best in inhibiting the growth of the three test bacteria and exceeds that of the market bath soap as a positive control.

The aim of using positive controls in the form of bath soap preparations that are spread on the market is to compare the average diameter of the inhibition of the preparations that have been circulating with the bath soap preparations used in this study[26].

The results of research conducted by Pertiwi et al. (2022) regarding the strength of antibiotics (antibacterial), which refers to the David-Stout method, states that if the clear zone is formed < 5 mm, the inhibition zone criteria are as weak antibacterial if the average value of the diameter of the inhibition zone is produced by 6 to 10 mm has inhibition zone criteria as moderate antibacterial, if the average diameter of the resulting inhibition zone is 10 to 20 mm, has inhibition zone criteria as strong antibacterial, and if the average diameter of the resulting inhibition zone exceeds 20 mm, then the criteria as an antibacterial inhibition zone are very strong.

Referring to this standard, it has been proven that formulations II and III in inhibiting the growth of the three tested bacteria have a moderate category, and Formulation IV has a strong category. The higher the concentration of the telang flower kombucha bath soap, the higher it is potential to inhibit the growth of the three test bacteria.

Antibacterial compounds that enter will ideally result in a higher

osmotic pressure inside the cell, thus causing cell lysis or rupture in pathogenic bacteria[2]. This is due to the influence of the secondary metabolites in the telang flower kombucha's fermentation[27].

The secondary metabolites contained in the telang flower kombucha fermented solution have been qualitatively studied by Abdilah et al. (2022)[28], namely from the alkaloids, flavonoids, and saponins, each of which has a different potential to inhibit the growth of pathogenic bacteria.

The alkaloid group contained in the telang flower kombucha fermented solution works cellularly in inhibiting the growth of pathogenic bacteria, including inhibiting the synthesis of enzymes and proteins, resulting in disturbances in the metabolism of pathogenic bacteria[29].

The cellular mechanism of the secondary metabolites contained in the telang flower kombucha fermentation solution acts as an antibacterial from the flavonoid group, namely by inactivating proteins and enzymes in the cell membrane of pathogenic bacteria[8].

The cellular mechanism of the secondary metabolites contained in the telang flower kombucha fermentation solution as an antibacterial from the saponin group is to synthesize complex compounds in pathogenic bacterial cells through hydrogen bonds until damage to the protein structure occurs and has the potential to influence imbalances in the pathogenic bacterial cell membrane[23].

CONCLUSION

This study concluded that the telang flower kombucha bath soap positively inhibited the growth of the three test bacteria. The concentration of 40% is the best in inhibiting the growth of the three test bacteria compared to the concentration of the two concentrations, namely 20% and 30%, and the two comparisons are positive and negative controls.

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REFERENCES

1. Rezaldi, F., Rachmat, O., Fadillah, M. F., Setyaji, D. Y., & Saddam, A. (2022). Bioteknologi Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Antibakteri Salmonella thypi dan Vibrio parahaemolyticus Berdasarkan Konsentrasi Gula Aren. Jurnal Gizi Kerja dan Produktivitas, 3(1), 13-22. http://dx.doi.org/10.52742/jgkp.v3i1.14724

2. Rezaldi, F., Ningtyas, R. Y., Anggraeni, S. D., Ma'ruf, A., Fatonah, N. S., Pertiwi, F. D., Fitriyani, F., A, L. D., US, S., Fadillah, M. F., & Subekhi, A. I. (2021). PENGARUH METODE BIOTEKNOLOGI FERMENTASI KOMBUCHA BUNGA TELANG (Clitoria ternatea L) SEBAGAI ANTIBAKTERI GRAM POSITIF DAN

NEGATIF. Jurnal Biotek, 9(2), 169-185. https://doi.org/10.24252/jb.v9i2.25467

3. Rezaldi, F., Fadillah, M. F., Mu'jijah, M., Abdilah, N. A., & Meliyawati, M. (2022). POTENSI KOMBUCHA BUNGA TELANG SEBAGAI HIMBAUAN KEPADA WISATAWAN PANTAI CARITA DALAM MENINGKATKAN IMUNITAS. SELAPARANG Jurnal Pengabdian Masyarakat Berkemajuan, 6(2), 867-871. https://doi.org/10.31764/jpmb.v6i2.8472

4. Situmeang, B., Shidqi, M. M. A., & Rezaldi, F. (2022). The Effect Of Fermentation Time On Antioxidant And Organoleptic Activities Of Bidara (Zizipus Spina Cristi L.) Kombucha Drink. Biotik: Jurnal Ilmiah Biologi Teknologi dan Kependidikan, 10(1), 73-93. http://dx.doi.org/10.22373/biotik.v10i1.11370

5. Rezaldi, F., Setiawan, U., Kusumiyati, K., Trisnawati, D., Fadillah, M. F., & Setyaji, D. Y. (2022). Bioteknologi Kombucha Bunga Telang (Clitoria ternatea L) dengan Variasi Gula Stevia sebagai Antikolesterol pada Bebek Pedaging. Jurnal Dunia Farmasi, 6(3), 156-169.

6. Taupiqurrohman, O., Rezaldi, F., Fadillah, M.F., Amalia, D., & Suryani, Y. (2022). Anticancer Potency of Dimethyl 2-(2-Hydroxy-2-Methoxypropilidine) Malonate in Kombucha. Jurnal Biodjati, 7(1), 86-94. https://doi.org/10.15575/biodjati.v7i1.14634

7. Pertiwi, F. D., Rezaldi, F., & Puspitasari, R. (2022). Uji Aktivitas Antibakteri Ekstrak Etanol Bunga Telang (Clitoria ternatea L.) Terhadap Bakteri Staphylococcus epidermidis. BIOSAINTROPIS (BIOSCIENCE-TROPIC), 7(2), 57-68. https://doi.org/10.33474/e-jbst.v7i2.471

8. Abdilah, N. A., Rezaldi, F., Pertiwi, F. D., & Fadillah, M. F. (2022). Fitokimia Dan Skrining Awal Metode Bioteknologi Fermentasi Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Bahan Aktif Sabun Cuci Tangan Probiotik. MEDFARM: Jurnal Farmasi dan Kesehatan, 11(1), 44-61. https://doi.org/10.48191/medfarm.v11i1.72

9. Rezaldi, F., Fadillah, M. F., Agustiansyah, L. D., Trisnawati, D., & Pertiwi, F. D. (2022). Pengaruh Metode Bioteknologi Fermentasi Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Penurun Kadar Kolesterol Bebek Pedaging Berdasarkan Konsentrasi Gula Aren Yang Berbeda-Beda. Jurnal Biogenerasi, 7(2), 57-67. https://doi.org/10.30605/biogenerasi.v7i2.1772

10. Fadillah, M.F., Hariadi, H., Kusmiyati, & Setyaji, D. Y. (2022). Karakteristik Biokimia dan Mikrobiologi pada Larutan Fermentasi Kedua Kombucha Bunga Telang (*Clitoria ternatea* L.) Sebagai Inovasi Produk Bioteknologi Terkini. Jurnal Pendidikan Biologi: Biogenerasi. 7 (2), 19-34.

11. Rochmat, A., Aditya, G., Kusmayanti, N., Kustiningsih, I., Hariri, A., & Rezaldi, F. (2022). Invitro Activity and Docking Approach In Silico Leaf Extract Syzygium polyanthum (Wight) Walp. as a Salmonella typhi Inhibitor. Trends in Sciences, 19(16), 5654-5654. https://doi.org/10.48048/tis.2022.5654

12. Puspitasari, M., Rezaldi, F., Handayani, E. E., & Jubaedah, D. (2022). KEMAMPUAN BUNGA TELANG (Clitoria ternatea L) SEBAGAI ANTIMIKROBA (Listeria monocytogenes, Staphylococcus hominis, Trycophyton mentagrophytes, dan Trycophyton rubrum) MELALUI METODE BIOTEKNOLOGI

FERMENTASI KOMBUCHA. Jurnal Medical Laboratory, 1(2), 1-10. https://ejournal.stikeskesosi.ac.id/index.php/Medlab/article/view/36

13. Rezaldi, F., Agustiansyah, L. D., Safitri, E., Oktavia, S., & Novi, C. (2022). Antifungi Candida albicans, Aspergilus fumigatus, dan Pitosporum ovale Dari Sediaan Sampo Probiotik Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Produk Bioteknologi Farmasi. Pharmaqueous: Jurnal Ilmiah Kefarmasian, 4(1), 45-52. https://doi.org/10.36760/jp.v4i1.385

14. Oktavia, S., Novi, C., Handayani, E. E., Abdilah, N. A., Setiawan, U., & Rezaldi, F. (2021). Pelatihan Pembuatan Immunomodulatory Drink Kombucha untuk Meningkatkan Perekonomian Masa New Normal pada Masyarakat Desa Majau dan Kadudampit Kecamatan Saketi Kabupaten Pandeglang, Banten. Jurnal Pengabdian Pada Masyarakat, 6(3), 716-724. https://doi.org/10.30653/002.202163.811

15. Rezaldi, F., Sasmita, H., Somantri, U. W., Kolo, Y., & Meliyawati, M. (2022). Pengaruh Metode Bioteknologi Fermentasi Kombucha Bunga Telang (Clitoria Ternateaa L) Sebagai Antibakteri Gram Positif-Negatif Berdasarkan Konsentrasi Gula Tropicanaslim Yang Berbeda-Beda. Pharmaqueous: Jurnal Ilmiah Kefarmasian, 4(1), 80-91. https://doi.org/10.36760/jp.v4i1.373

16. FATONAH, Nisa Siti et al. UJI AKTIVITAS ANTIBAKTERI ESCHERICHIA COLI PADA FORMULASI SEDIAAN SABUN CAIR MANDI PROBIOTIK DENGAN METODE BIOTEKNOLOGI FERMENTASI KOMBUCHA BUNGA TELANG (Clitoria ternatea L). AGRIBIOS, [S.l.], v. 20, n. 1, p. 27-37, june 2022. ISSN 2723-7044. https://doi.org/10.36841/agribios.v20i1.1510

17. Subagiyo, A., Rezaldi, F., Ma'ruf, A., Pertiwi, F.D., Yunita, Y., Safitri, A., Rustini, R. (2022). Antibakteri Vibrio parahaemolyticus dan Klebsiella pneumonia pada Sediaan Sabun Mandi Probiotik Fermentasi Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Produk Bioteknologi Farmasi. Journal oF Biotechnology and Conservation in WALLACEA, 2(2), 89-98. http://doi.org/10.35799/jbcw.v2i2.43886

18. Pamungkas, B. T., Safitri, A., Rezaldi, F., Andry, M., Agustiansyah, L. D., Fadillah, M. F., Hidayanto, F., & Hariadi, H. (2022). ANTIFUNGAL Trycophyton rubrum AND Trycophyton mentagrophytes IN LIQUID BATH SOAP FERMENTED PROBIOTIC KOMBUCHA FLOWER TELANG (Clitoria ternatea L) AS A PHARMACEUTICAL BIOTECHNOLOGY PRODUCT. BIOTIK: Jurnal Ilmiah Biologi Teknologi dan Kependidikan, 10(2), 179-196. http://dx.doi.org/10.22373/biotik.v10i2.15160

19. Ma'ruf, A., Safitri, E., Ningtias, R. Y., Pertiwi, F. D., & Rezaldi, F. (2022). Antibakteri Gram Positif Dan Negatif Dari Sediaan Sabun Cuci Piring Fermentasi Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Produk Bioteknologi Farmasi. Jurnal Kesehatan dan Kedokteran, 1(2), 16-25. https://doi.org/10.56127/jukeke.v1i2.115

20. Ma'ruf, A., Safitri, E., Pertiwi, F. D., Ningtias, R. Y., Trisnawati, D., Rezaldi, F., Kusumiyati, K., & Andayaningsih, P. (2022). PRODUK BIOTEKNOLOGI FARMASI BERUPA SABUN MANDI KOMBUCHA BUNGA TELANG (Clitoria ternatea L) SEBAGAI ANTIFUNGI Candida albicans. Jurnal Pertanian, 13(2), 78-84. https://doi.org/10.30997/jp.v13i2.6920

21. Rezaldi, F., Eman, E., Pertiwi, F. D., Suyamto, S., & Sumarlin, U. S. (2022). POTENSI BUNGA TELANG (Clitoria ternatea L) SEBAGAI Antifungi Candida albicans, Malasezia furfur, Pitosprorum ovale, dan Aspergilus fumigatus DENGAN METODE BIOTEKNOLOGI FERMENTASI KOMBUCHA. Jurnal Ilmiah Kedokteran dan Kesehatan, 1(2), 1-9. https://doi.org/10.55606/klinik.v1i2.381

22. Saddam, A., Fathurrohim, M. F., Rezaldi, F., Kolo, Y., & Hidayanto, F. (2022). PENGARUH LIMBAH FERMENTASI METODE BIOTEKNOLOGI KOMBUCHA BUNGA TELANG (Clitoria ternatea L) SEBAGAI PUPUK CAIR TERHADAP PERTUMBUHAN TOMAT (Lycopersicum esculantum L). AGRIBIOS, 20(2), 179-186. https://doi.org/10.36841/agribios.v20i2.2291

23. Rezaldi, F., & Hidayanto, F. (2022). POTENSI LIMBAH FERMENTASI METODE BIOTEKNOLOGI KOMBUCHA BUNGA TELANG (ClitoriaternateaL) SEBAGAI PUPUK CAIR TERHADAP PERTUMBUHAN CABAI RAWIT (CapsiumfrutencesL. Var Cengek). JURNAL PERTANIAN CEMARA, 19(2), 79-88. https://doi.org/10.24929/fp.v19i2.2239

24. Saddam, A., Rezaldi, F., Ma'ruf, A., Pertiwi, F.D., Suyamto, S., Hidayanto, H., & Kusumiyati, K. (2022). Uji Daya Hambat Bakteri Staphylococcus capitis Bacillus cereus dan Pantoea dispersa Melalui Metode Bioteknologi Fermentasi Kombucha Bunga Telang (Clitoria ternatea L), Jurnal Gizi Kerja dan Produktivitas, 3(2), 65-71 http://dx.doi.org/10.52742/jgkp.v3i2.17481

25. Rezaldi, F., Junaedi, C., Ningtias, R. Y., Pertiwi, F. D., Sasmita, H., Somantri, U. W., & Fathurrohim, M. F. (2022). Antibakteri Staphylococcus Aureus dari Sediaan Sabun Mandi Probiotik Kombucha Bunga Telang (Clitoria Ternatea L) Sebagai Produk Bioteknologi. Jurnal Biotek, 10(1), 36-51. https://doi.org/10.24252/jb.v10i1.27027

26. Pertiwi, F. D., Rezaldi, F., & Puspitasari, R. (2022). UJI AKTIVITAS DAN FORMULASI SEDIAAN LIQUID BODY WASH DARI EKSTRAK ETANOL BUNGA TELANG (Clitoria ternatea L) SEBAGAI ANTIBAKTERI Staphylococcus epidermidis. Jurnal Ilmiah Kedokteran dan Kesehatan, 1(1), 53-66. https://doi.org/10.55606/klinik.v1i1.257

27. Rezaldi, F., Hidayanto, F., Setyaji, D. Y., Fathurrohim, M. F., & Kusumiyati, K. (2022). Bioteknologi Kombucha Bunga Telang (Clitoria ternatea L) Sebagai Antibakteri Streptococcus mutan dan Klebsiella pneumoniae Berdasarkan Konsentrasi Gula Yang Berbeda Beda. Jurnal Farmagazine, 9(2), 21-27. http://dx.doi.org/10.47653/farm.v9i2.608

28. Abdilah, N. A., Mu'jijah, M., Rezaldi, F., Ma'ruf, A., Safitri, E., & Fadillah, M. F. (2022). ANALISIS KEBUTUHAN BIOKIMIA GIZI BALITA DAN PENGENALAN KOMBUCHA BUNGA TELANG (Clitoria ternatea L) TERHADAP ORANG TUA BALITA DALAM MENINGKATKAN IMUNITAS: ANALYSIS OF NUTRITIONAL BIOCHEMICAL REQUIREMENTS OF TOddlers and the Introduction of KOMBUCHA FLOWER (Clitoria ternatea L) ON PARENTS OF TOTAL CHILDHOOD IN INCREASING IMMUNITY. Medimuh: Jurnal Kesehatan Muhammadiyah, 3(2), 59-66. https://doi.org/10.37874/mh.v3i2.446

29. Rezaldi, F., Pertiwi, F. D., Yunita, Y., Rustini, R., & Hidayanto, F. (2022). Potensi Buah Nanas Madu Subang (Ananas comasus) sebagai Antibakteri Gram Positif Negatif Melalui Metode Bioteknologi Fermentasi Kombucha Berdasarkan

Konsentrasi Gula Aren Berbeda. Biofarmasetikal Tropis, 5(2), 119-126. https://doi.org/10.55724/jbt.v5i2.400

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TESTING THE EFFECTIVENESS OF BIDARA LEAF ETHANOL EXTRACT (*Ziziphus mauritiana* L.) IN GEL PREPARATIONS FOR BURN WOUNDS IN MALE RABBIT (*Oryctolapus cuniculus*)

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Abstract

Bidara plants have many benefits in the treatment of sore skin, ulcers, and burns. Burns are a form of tissue damage or loss caused by contact with a heat source such as fire, hot water, electricity, radiation, or corrosive substances. This study aims to determine the effectiveness of the ethanol extract of bidara leaf in healing burns in male rabbits compared to bioplacenta. This study used an experimental method of maceration extraction using 96% ethanol. To test the effect of burns, 15 test animals were grouped into five groups, each of which used three test animals. The treatment group was a gel-based negative control group, a positive bioplacenta control group, and a treatment group with bidara leaf ethanol extract at concentrations of 5%, 10%, and 15%. The results of the research on the effectiveness of healing burns in the three formulations, namely a concentration of 15%, showed faster healing of wound diameter in male rabbits compared to concentrations of 5% and 10% but were not more effective when compared to bioplacenta ointment.

Abstrak

Tanaman bidara memiliki banyak manfaat dalam pengobatan sakit kulit, bisul dan luka bakar. Luka bakar adalah suatu bentuk kerusakan atau kehilangan jaringan yang disebabkan oleh kontak dengan sumber panas seperti api, air panas, listrik, radiasi atau zat korosif. Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak etanol daun bidara dalam penyembuhan luka bakar pada kelinci jantan dibandingkan dengan bioplacenton. Penelitian ini menggunakan metode eksperimen dengan ekstraksi maserasi menggunakan etanol 96%. Untuk menguji pengaruh luka bakar menggunakan 15 hewan uji yang dikelompokkan menjadi lima kelompok, masing-masing kelompok menggunakan tiga hewan uji. Kelompok perlakuan adalah kelompok kontrol negatif berbasis gel, kelompok kontrol positif bioplacenton, dan kelompok perlakuan dengan ekstrak etanol daun bidara konsentrasi 5%, 10%, 15%. Hasil penelitian efektivitas penyembuhan luka bakar pada ketiga formulasi yaitu konsentrasi 15% lebih cepat penyembuhan diameter luka pada kelinci jantan dibandingkan konsentrasi 5% dan 10% tetapi tidak lebih efektif jika dibandingkan dengan salep bioplacenton.

Keywords: *Bidara leaf ethanol extract, burns, ointment and bioplacenton*

Kata kunci: *Ekstrak etanol daun bidara, luka bakar, salep dan bioplasenton.*

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INTRODUCTION

The bidara plant is a tiny, evergreen, fruit-bearing tree that is native to northern and tropical Africa as well as western Asia. It can be found in Israel in the valleys up to 500 meters above sea level. especially

in Indonesia, where the Sumbawa region is where this plant thrives in profusion (West Nusa Tenggara). The bidara plant's entire body can be used for medicinal purposes. Bidara plants are saponin-rich plants, as evidenced by the foam that is created when the leaves of a bidara plant are crushed. This foam has a strong scent.[1]. The content of secondary metabolites in Binara leaves are flavonoids, saponins, tannins[2]. The content of saponin secondary metabolites contained in plants really has an effect that can trigger the formation of collagen, which plays a very important role in the process of wound healing[3]. Flavonoids in plants act as antibacterial[4] and anti-inflammatory agents in wounds, as do tannins, which have an astringent function by shrinking skin pores[5].

Bidara leaf extract (*Ziziphus mauritiana* L.) has not yet been the subject of any scientific studies on how well it heals burns in rabbits used as test subjects. On the back of a rabbit that had been injured with a hot metal plate that had a 20 mm diameter, bidara leaf extract was made using the maceration method and tested for its impact on the healing of superficial second-degree burns. Gel preparations are used because they have a cooling impact on the skin due to their high water content[3].

METHODOLOGY

This research was conducted experimentally.

Apparatus

Laboratory glassware, a blender, a caliper, a razor blade, filter paper, a dropper pipette, a PH meter, an analytical balance, a mortar and stamper, an oven, a rotary evaporator, a 1 ml injection syringe (a Terumo), a metal plate 2.2 cm in diameter, a shaver, and a rabbit hutch were all used in this study.

Ingredient

The materials used in the study were bidara leaves (*Ziziphus Mauritiana* L.), 96% ethanol, carbopol 940, TEA, glycerin, propyleneglycol, metal paraben, paraben propellant, distilled water, 70% alcohol, bioplacenton, Mayer reagent, Molish reagent, Bochart reagent, Dragendroff reagent, chloroform, concentrated hydrochloric acid, acetic anhydrous acid, lead acetate, and rabbits as test animals.

Preparation of Bidara Leaf Ethanol Extract

As much as 1000 grams of bidara leaf simplicia were soaked in 96% ethanol with a ratio of 1 part simplicia dissolved in 10 parts of solvent for 5 days using 75 parts of 96% ethanol solvent. As much as 1000 grams of bidara leaf simplicia

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were soaked in 96% ethanol with a ratio of 1 part simplicia dissolved in 10 parts of solvent for 5 days using 75 parts of 96% ethanol solvent. Then it was filtered, re-macerated with 25 parts of 96% ethanol solvent for 2 days, then filtered, and concentrated with a rotary evaporator at 40°C [6,7]

Phytochemical Screening

Phytochemical screening tests can be seen in Table 1 below.

Bidara Leaf Extract Gel Formulation

Bidara leaf extract gel formula can be seen in Table 2 below.

Table 1. Phytochemical screening test

Secondary Metabolites	Procedure	Result
Alkaloids	The addition of 2N HCL and distilled water, heated, filtered, filtered 3 times each added reagent mayer, dragendroff and bouchardat.	Mayer : White precipitate Dragendroff : Brown precipitate Bouchardat : A brick red precipitate [8]
Saponins	Hot water was added, shaken and 2N HCL added	Stable foam is formed and 1 – 10 cm high[9]
Tannins	The addition of 10% $FeCl_3$ to the simplicia that had been macerated beforehand for 15 minutes	A blue color is formed in the filtrate [10]
Flavonoids	Add concentrated HCl and amyl alcohol	A red or yellow-orange color is formed on the amyl alcohol layer [11]
Steroid/Triterpenoid	The simplicia was macerated with N-Hexane	green color is formed

Table 2. Bidara leaf ethanol extract gel formula

Ingredients	F1	F2	F3	F4
Extract	5	10	15	-
Carbopol	4	4	4	4
TEA	4	4	4	4
Glycerin	20	20	20	20
Propylene glycol	10	10	10	10
Methyl paraben	0.4	0.4	0.4	0.4
Propyl paraben	0.4	0.4	0.4	0.4

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Evaluation of Bidara Extract Gel
Homogenity

Homogeneity testing is carried out using a glass object where the nanogel preparation is smeared on a piece of glass or other suitable transparent material and strives for transparency. [12].

pH

A pH meter was used to determine the preparation's pH. by calibrating the device using a buffer solution that has a neutral pH standard (pH 7.01) and an acidic pH standard (pH 4.01) until the tool displays the pH value. The electrodes were afterwards cleaned with distilled water and dried with paper towels. The gadget then displays a consistent pH value after the electrode has been submerged in the sample. The pH value of the preparation is represented by the number the pH meter displays[12].

Viscosity

A Brookfield DV-E viscometer was used to measure viscosity by placing the preparation within a 100 ml beaker glass and choosing the proper spindle number [13].

Making burns

Each rabbit was shaved on its back, then anesthetized with lidocaine HCL subcutaneously at a dose of 1 ml, then the metal was heated over a fire for 5 minutes then affixed to the rabbit's back for 5 seconds [14].

Burn tests were performed on five groups of rabbits, with one group consisting of three rabbits.

Group 1: Administration of gel base as a negative control
Group 2: Administration of burn ointment as a positive control
Group 3: 5% bidara leaf extract gel
Group 4: 10% bidara leaf extract gel
Group 5: Bidara leaf extract gel 15%

Observation of burn wound healing was carried out for 14 days, with the parameter being seen as a decrease in wound diameter from the diameter of the wound covered with drug therapy.

RESULTS AND DISCUSSION

Phytochemical screening results

The results of the phytochemical screening of bidara leaves can be seen in Table 3 below. Skrinning fitokimia adalah pengujian awal secara kualitatif untuk mengetahui kandungan metabolit sekunder yang terdapat pada tumbuhan. Metabolit sekunder yang terdapat pada tumbuhan adalah penentu aktivitas farmakologi dari suatu tanamana. Kandungan metabolit sekunder yang terdapat pada daun bidara diantaranya adalah alkaloid, flavonoid, tannin, saponin, glikosida, steroid/triterpenoid [15].

The presence of secondary metabolites in plants affects their ability to exert pharmacological effects. Alkaloids, saponins, tannins, flavonoids, steroids, and triterpenoids are all present in binara positive plants.

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In addition, saponins are crucial in increasing the production of fibronectin by fibroblasts and altering the gene expression of TGF- receptors. The presence of flavonoids can activate macrophages, which leads to macrophage proliferation and an increase in TGF- secretion. Tannin has astringent properties that induce the skin pores to constrict, stop mild bleeding, and produce fibroblast precursors [16].

Table 3. Phytochemical screening results

Secondary Metabolite	Result
Alkaloids	Positive
Saponins	Positive
Tannins	Positive
Flavonoids	Positive
Steroids/Triterpenoids	Positive

Table 4. Homogeneity of bidara extract gel preparations

Formula	Result
Gel Base	Homogeneous
Bidara extract gel 5%	Homogeneous
Bidara extract gel 10%	Homogeneous
Bidara extract gel 15%	Homogeneous

Evaluation of Bidara Extract Gel Preparations

Homogenity

Homogeneity test can be seen in Table 4 below. Homogeneity is done to ensure that there are no more coarse particles in the preparation at the time of its application [17].

pH

Secondary metabolite content in plants is a determinant of plant activity as a pharmacological effect. Binara-positive plants contain alkaloids, saponins, tannins, flavonoids, steroids, and triterpenoids. Flavonoids can activate macrophages, which causes macrophage proliferation, which increases TGF- secretion, and saponins play an important role in stimulating fibronectin by fibroblasts and changing TGF- receptor gene expression. Tannin acts as an astringent, shrinking skin pores and stopping light bleeding, while alkaloids form fibroblast precursors [18]. From the tests carried out it showed that all preparations met the requirements. The results of pH testing can be seen in table 5 below.

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Table 5. pH of bidara extract gel preparations

Formula	pH mean ± SD
Gel Base	6,14 ± 0,01
Bidara extract gel 5%	5,84 ± 0,00
Bidara extract gel 10%	5,77 ± 0,01
Bidara extract gel 15%	5,66 ± 0,01

Viscosity

The results of testing the bidara extract gel preparations can be seen in Table 6 below.

Table 6. Viscosity of bidara extract gel preparations

Formula	Viskositas mean ± SD
Gel Base	6,14 ± 0,01
Bidara extract gel 5%	5,84 ± 0,00
Bidara extract gel 10%	706 ± 8,6
Bidara extract gel 15%	804,3 ± 4,04

The good viscosity of semisolid preparations can be seen in their ability to be easily removed from their containers for easy application when used. The viscosity results are influenced by the formula used to create nanogel preparations as well as the materials used to increase the viscosity of the preparation[19].

Burn Effectiveness

The results of burn healing can be seen visually with the parameter of decreasing wound diameter.

The results of the average decrease in wound diameter can be seen in Table 7 below.

Table 7. wound reduction rate

Formula	Wound reduction ± SD
Gel Base	0.8 ± 0,21
Bioplasenton gel	2.4 ± 0.14
Bidara extract gel 5%	1.4 ± 0,00
Bidara extract gel 10%	1.6 ± 1.21
Bidara extract gel 15%	1.8 ± 4,04

The average percentage of wound recovery is the three formulations of bidara leaf ethanol extract gel with concentrations of 5%, 10%, and 15%, which is the effect of fast wound closure after formula administration, based on observations and data obtained. A gel preparation with a concentration of 15%, which obtained an average of 1.2 cm; a concentration of 10%, which obtained an average of 1.1 cm; a concentration of 5%, which obtained an average of 1 cm; while the negative control obtained an average of 0.88 cm, burn recovery can occur. If you give the gel, there is no extract because the wound that occurs can heal by itself, but it takes a very long time to give a gel containing extract [20].

In the bioplacenta gel, which is a positive control, recovery of burns with healing occurs at an average depth of 1.4 cm. This shows that in the recovery process using the three preparations, it can speed up normal wound recovery. The recovery results obtained showed that the positive control (bioplacenta gel) had a better effect than the ethanol extract of kedondong leaves with concentrations of 5%, 10%, and 15%, and the ethanol extract of bidara leaves with concentrations of 5%, 10%, and 15% gave a very good recovery effect. significantly different in gel preparations without extract (control basis); this is burn wounds in rabbits, and bidara leaf ethanol extract gel preparation with a concentration of 15% gives the best wound healing effect.The healing activity of burns treated with 5%, 10%, and 15% bidara leaf ethanol extract gel preparations is due to the presence of secondary metabolites, namely alkaloids, which have antibacterial properties[21]. Flavonoids can inhibit bacterial growth by damaging the bacterial walls, microsomes, and lysosomes. [22]. Tannins, which are found in plants, act as astringents. and can shrink pores on the skin, tighten skin, stop exudate, and lighten bleeding. Saponins contained in a plant can stimulate the formation of collagen and can help in the wound healing process [23].

Based on the results of the study, burn recovery had the best activity, namely bioplacenton as a comparison, as a test extract, namely bidara leaf extract gel with a concentration of 15%, 10% and 5%.

CONCLUSION

The ethanol extract of bidara leaves can be formulated into a gel preparation that can provide a healing effect on burns in rabbits and the most effective concentration is 15%.

REFERENCES

1. Bintoro, A., Ibrahim, A. M., Situmeang, B., Kimia, J. & Cilegon, B. Analisis dan identifikasi senyawa saponin dari daun

bidara (Zhizipus mauritania L.). *Jurnal Itekima* **2**, 84–94 (2017).

2. Andreas, H. UJI AKTIVITAS ANTIOKSIDAN DAN ANTIBAKTERI TERHADAP ESCHERICHIA COLI DARI EKSTRAK ETANOL DAUN TUMBUHAN BINARA (ARTEMISIA VULGARIS L.) SETELAH BLANCHING. (UNIMED, 2017).

3. Mappa, T., Edy, H. J. & Kojong, N. Formulasi gel ekstrak daun sasaladahan (Peperomia pellucida (L.) HBK) dan uji efektivitasnya terhadap luka bakar pada kelinci (Oryctolagus cuniculus). *Pharmacon* **2**, (2013).

4. Nasri, N., Kaban, V. E., Gurning, K., Syahputra, H. D. & Satria, D. Aktivitas Antibakteri Ekstrak Etanol Daun Pepaya (Carica papaya Linn.) Terhadap Bakteri Pseudomonas aeruginosa. *INSOLOGI: Jurnal Sains dan Teknologi* **1**, 252–259 (2022).

5. Wijonarko, B., Anies, A. & Mardiono, M. Efektvitas Topikal Salep Ekstrak Binahong (AnrederaCordifolia (Tenore) Steenis) terhadap Proses Penyembuhan Luka Ulkus Diabetik pada Tikus Wistar (Rattus Novergicus). *Jurnal Ilmiah Kesehatan* **9**, 96955 (2016).

6. Nasri, N., Harahap, U., Silalahi, J. & Satria, D. Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of Carp (Cyprinus carpio) against diarrhea-causing pathogenic bacteria. *Biodiversitas Journal of Biological Diversity* **22**, (2021).

7. Situmorang, S. M. P. Formulasi dan Uji Aktivitas Antibakteri Nanogel Asam Azelat Terhadap Bakteri Propionibacterium acnes dan Staphylococcus epidermidis. (2021).

8. Martiningsih, N. W. & Suryanti, I. A. P. Skrining Fitokimia dan Aktivitas Antijamur Minyak Atsiri Daun Kemangi (Ocimum sp.). in *Seminar Nasional Riset Inovatif* vol. 2 631–636 (2017).

9. Yulianto, S. Identifikasi Alkaloid Daun Kelor (Moringa oleifera L). *Jurnal Kebidanan dan Kesehatan Tradisional* **5**, 55–57 (2020).

10. Sulasmi, E. S., Saptasari, M., Mawaddah, K. & Zulfia, F. A. Tannin identification of 4 species pterydophyta from Baluran National Park. in *Journal of Physics: Conference Series* vol. 1241 012002 (IOP Publishing, 2019).

11. Fagbohun, E. D., Egbebi, A. O. & Lawal, O. U. Phytochemical screening, proximate analysis and in-vitro antimicrobial activities of methanolic extract of Cnidoscolus aconitifolius leaves. *Int. J. Pharm. Sci. Rev. Res* **13**, 28–33 (2012).

12. Vera, V. E. K. & Silalahi, J. Testing Of The Cream Formula Turmina Right Extract Against The Inhibition Of The Development Of Melanoma Cells. *International Journal of Science, Technology & Management* **3**, 525–529 (2022).

13. Ariani, L. W. Formulasi Sediaan Nanogel Minyak Zaitun Sebagai Antiacne. *REPOSITORY STIFAR* (2020).

14. Suriawanto, N. & Setyawati, E. PENGARUH PEMBERIAN EKSTRAK PROPOLIS LEBAH TANPA SENGAT PADA PENYEMBUHAN LUKA BAKAR TIKUS PUTIH (Rattus norvegicus). *Jurnal Bioteknologi & Biosains Indonesia (JBBI)* **8**, 68–76 (2021).

15. Gurning, K. Characterization and Screening of Phytochemical Secondary Metabolite of Seri (Muntingia calabura, L) Leaves which is Potential as an Anti-Diabetic based on Indonesian Herbal

Medicine Standard. *Journal of Drug Delivery and Therapeutics* **10**, 92–94 (2020).

16. Giri, I. M. D. S., Wardani, I. G. A. A. K. & Suena, N. M. D. S. Peran Metabolit Sekunder Tumbuhan dalam Pembentukan Kolagen pada Kulit Tikus yang Mengalami Luka Bakar. *Usadha: Jurnal Integrasi Obat Tradisional* **1**, (2021).

17. Ahmed, S., Alhareth, K. & Mignet, N. Advancement in nanogel formulations provides controlled drug release. *International Journal of Pharmaceutics* **584**, 119435 (2020).

18. Wulandari, W., Wildan, A. & Ariani, L. W. SIFAT FISIK DAN INDEKS IRITASI MASKER SHEET NANOGEL MINYAK BIJI MATAHRI. *CENDEKIA EKSAKTA* **4**, (2019).

19. Jannah, R. R. & Resti Erwiyani, A. KAJIAN VARIASI KONSENTRASI CMC-NA (Sodium Carboxymethyle Cellulose) TERHADAP pH, DAYA SEBAR, DAYA LEKAT DAN VISKOSITAS PADA SEDIAAN GEL. (Universitas Ngudi Waluyo, 2021).

20. Ananta, G. P. Potensi Batang Pisang (Musa Pardisiaca L.) Dalam Penyembuhan Luka Bakar. *Jurnal Ilmiah Kesehatan Sandi Husada* **9**, 334–340 (2020).

21. Muhammad, M., Nasri, N., Kaban, V. E., Satria, D. & Cintya, H. Antibacterial Potential Ethanol Extract of Papaya Leaves (Carica papaya Linn.) Towards Salmonella typhi. *BEST Journal (Biology Education, Sains and Technology)* **5**, 265–270 (2022).

22. Kaban, V. E., Aritonang, J. O., Hasibuan, Y. C. & Meliala, D. I. P. EFEKTIVITAS PENYEMBUHAN LUKA SAYAT MENGGUNAKAN SALEP EKSTRAK ETANOL DAUN SENGGANI (Melastoma malabathricum L.) PADA KELINCI. *Jurnal Penelitian Farmasi & Herbal* **2**, 8–14 (2020).

23. Istiana, S. & Tanti Azizah, S. Formulasi sediaan gel basis Na-CMC ekstrak etanol daun cocor bebek (Kalanchoe pinnata (Lmk.) Pers.) sebagai penyembuh luka bakar pada kelinci. (Universitas Muhammadiyah Surakarta, 2016).

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Analysis of Embryo Development and Early Performance of Larvae of *Barbonymus schwanenfeldii*: A Systematic Review

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Abstract

Tengadak fish is a freshwater fish that can be found in rivers, lakes and canals and ditches. One of the important phases in fish development is the embryo. Embryo development (embryogenesis) is more in the process of formation and development of the embryo, not only the increase in the number and mass of embryonic blastomer cells but also the activity of blastomeric cells.The purpose of this study was to analyze the stages of embryonic development and early larval performance in Tengadak fish (*Barbonymus schwanenfeldii*). This study used systematic literature review using data from PubMed, NCBI, Google Scholar databases using keywords, namely, "embryo, performance, Tengadak fish (*Barbonymus schwanenfeldii*)". Approximately, 350 articles were obtained for the keywords "embryo, performance, Tengadak fish (*Barbonymus schwanenfeldii*)". All articles were selected based on inclusion criteria and exclusion and obtained as many as 27 articles that meet the inclusion criteria From the results of research conducted, it can be concluded that this research has sequentially described the embryonic development and early performance of the larvae of the *Barbonymus schwanenfeldii*. Need research on the other animal for future research.

Keywords: *Analysis, Embryo development, form of larvae, Tegadak fish*

Abstrak

Ikan Tengadak merupakan ikan air tawar yang dapat ditemukan di sungai, danau dan kanal serta parit. Salah satu fase penting dalam perkembangan ikan adalah embrio. Perkembangan embrio (embriogenesis) lebih pada proses pembentukan dan perkembangan embrio, tidak hanya peningkatan jumlah dan massa sel blastomer embrio tetapi juga aktivitas sel blastomer. perkembangan embrionik dan penampilan larva awal pada ikan Tengadak (Barbonymus schwanenfeldii). Penelitian ini menggunakan sistematik literature review dengan menggunakan data dari database PubMed, NCBI, Google Scholar dengan menggunakan kata kunci yaitu, "embrio, performance, ikan Tengadak (Barbonymus schwanenfeldii)". Didapatkan sekitar 350 artikel untuk kata kunci "embrio, performance, ikan Tengadak (Barbonymus schwanenfeldii)". Semua artikel diseleksi berdasarkan kriteria inklusi dan eksklusi dan diperoleh sebanyak 27 artikel yang memenuhi kriteria inklusi Dari hasil penelitian yang dilakukan, dapat disimpulkan bahwa penelitian ini secara berurutan menggambarkan perkembangan embrio dan performa awal larva larva Barbonymus schwanenfeldii. Perlu penelitian pada hewan lain untuk penelitian selanjutnya.

Kata kunci: Analisis, bentuk larva, ikan tengadak, perkembangan embrio

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INTRODUCTION

Fish is one of the sources of animal protein that is very popular with the public in general. Currently, the demand for public consumption of fish is quite high, given the increasing public awareness of the importance of consuming fish [1]. This goes in reverse with fish stocks, especially cultivated products, so there is a need for development in terms of fish cultivation by utilizing the potential of local fish as aquaculture commodities [2].

As consumption fish, the existence of local fish in nature is threatened due to overfishing. Indonesian-specific local fish that are marketed today are generally caught from fresh waters, be it lakes or rivers [3]. Tegadak fish is one of the local fish that must be prioritized in the development of fish farming commodities in Indonesia. Bearing in mind that the stilt fish is a fish native to Indonesian waters which has economic value, its growth weight can reach 1 kg/head. Tegadak fish is a freshwater fish that can be found in rivers, lakes and canals and ditches. This fish is an omnivore whose main food is phytoplankton, zooplankton, aquatic invertebrates and detritus [4]

One of the important phases in fish development is the embryo. This information on embryonic development is a key step in improving the quality and survival of larvae [5]. Embryo development (embryogenesis) is part of the study of progressive changes in the structure and function of the body in living things, this is more in the process of formation and development of the embryo, not only the increase in the number and mass of embryonic blastomer cells but also the activity of blastomeric cells [6]. The purpose of this study was to analyze the stages of embryonic development and early larval performance in Tengadak fish (*Barbonymus schwanenfeldii*).

METHODOLOGY

A literature search was carried out systematically through the PubMed, NCBI, Google Scholar databases using keywords, namely "embryo, performance, Tengadak fish (*Barbonymus schwanenfeldii*)". Based on these keywords, the articles obtained were first selected by setting several inclusion criteria including journals are not paid / free articles, research results focus on "embryo, performance, Tengadak fish (*Barbonymus schwanenfeldii*)" [7]. Articles that do not meet the inclusion criteria are eliminated and articles that meet the criteria will be analyzed to obtain data.

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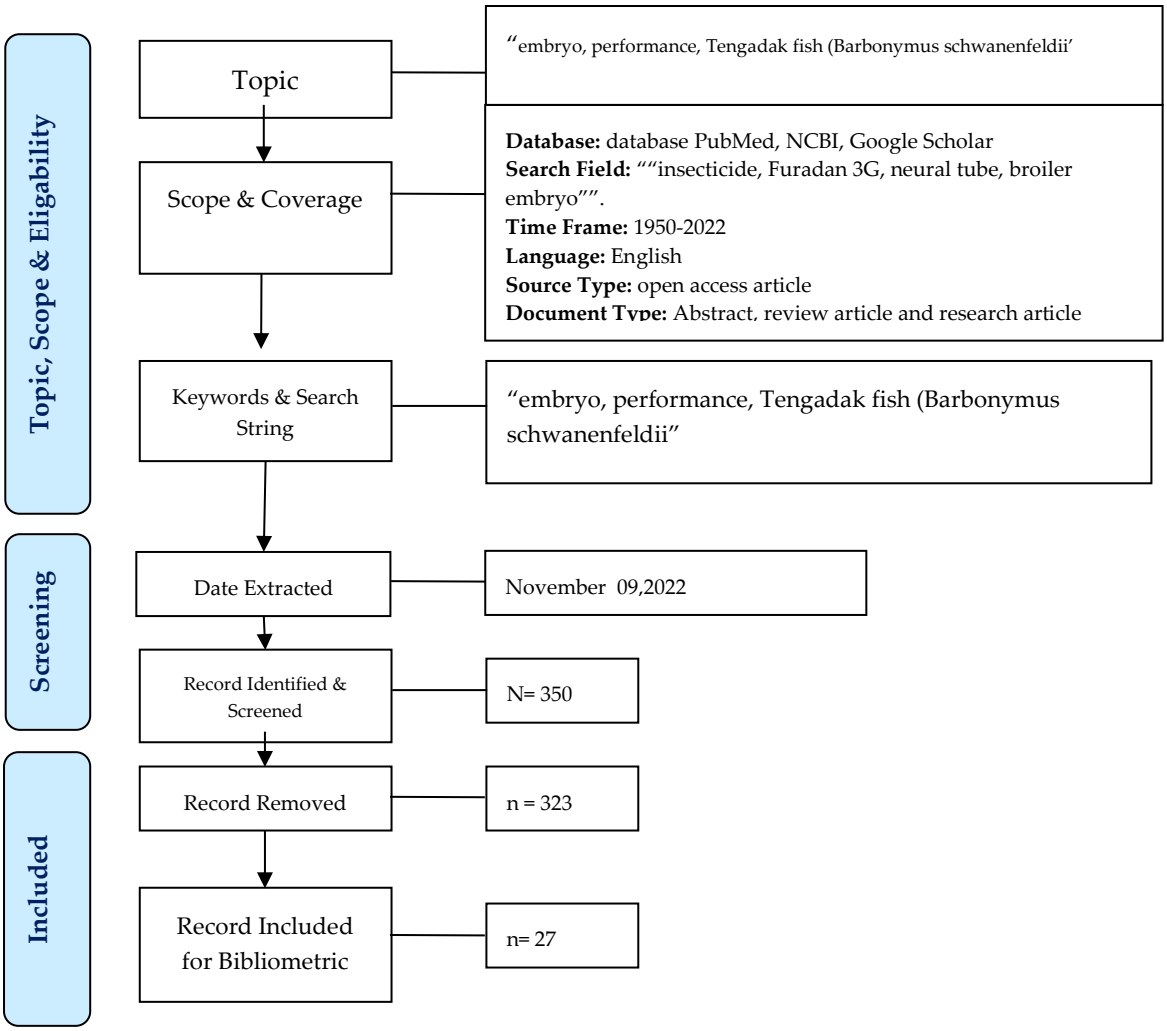

Figure 1. Flow diagram of the search strategy

RESULT AND DISCUSSION

Based on the search results in the PubMed, NCBI, Google Scholar databases using predetermined keywords, 350 articles were obtained for the keywords embryo, performance, Tengadak fish (*Barbonymus schwanenfeldii*). All articles were re-selected based on inclusion criteria and exclusion and obtained as many as 27 articles that meet the inclusion criteria.

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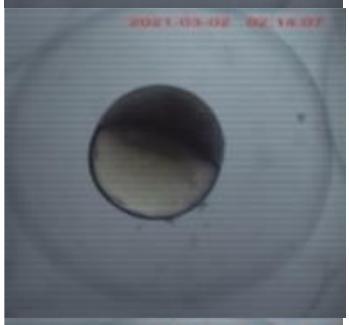
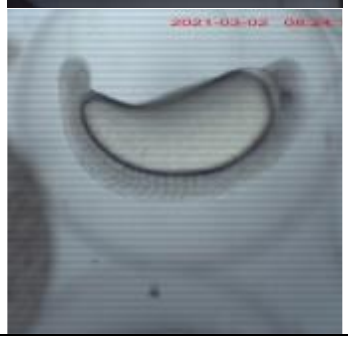
Table 1. Embryo development of the scorpion fish (*Barbonymus schwanenfeldii*)

Development Phase	Minute-	Embryo Development	Description
Division	10		Within the cell, a small space is formed called the segmentation cavity.
	30		Oil droplets have accumulated at the vegetal pole
Morula	50		There is division to produce cells with double the number until many cells are formed. Phase 2 cell division-34 cells
Blastula	80		The formation of a group arrangement that looks denser than the yolk. Final division phase 64 cells
	100		An empty cavity or layer of the area will form the main organ

Gastrula	120		The final blastula phase occurs when the yolk is invaded and enters the gastrula phase producing a germinal ring.
	150		The blastoderm covers almost the entire yolk sac and the germination ring is thicker. Move 50% Epiboly
	240		The cell begins to close the yolk completely. movement 90% Epiboly
Segmentasi	300		The final phase of the gastrula begins to form a tail bud
	360		The head and tail can be distinguished and 6 somites are formed

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	420		The eyes are getting rounder, and the heart and blood vessels have not yet formed. Formed 10 Somites
	480		The eyes are getting rounder, the body size is getting bigger. Formed 20 Somites
Hatching	640		The embryo hatches and a transparent segmented larva appears.

Table 2. Initial performance of the scorpion fish larvae (*Barbonymus schwanenfeldii*)

Parameter	Measurement Data
Egg yolk diameter	39.42 ± 4.41 µm
Initial length of larva	18.46 ± 1.32 µm
Abnormality	5.71 ± 2.5 %

Embryogenesis is the whole process that includes the process of developing fish eggs after fertilization (fertilization) to organogenesis before the fish eggs hatch (Raharjo et al. 2011). The development and embryogenesis of squirrel fish in general can go through several phases, including: single cell, blastomere, blastula, gastrula, neurula, and organogenesis. The development of the fish embryos begins with the rapid division of the zygote into small cell units that divide into 2 cells, 4 cells, 8 cells, 16 cells, until the final morula phase [8].

The cleavage phase occurs every 10 minutes. The results of observations in the cleavage phase of the catfish embryo occurred during 30 minutes after egg fertilization, this result was

faster when compared to other freshwater fish. In the cleavage phase, the embryo of Torfish species lasts for 10 hours. Furthermore, the process of embryo development in Oryzias woworae species occurs for 1 hour 20 minutes [9]. While the results of observations on the cleavage phase of the embryo in Tawes fish lasted for 2 hours 24 minutes after fertilization (Naskuroh et al. 2018). Each type of fish in the embryonic development phase is very dependent on environmental conditions, especially water temperature [10].

According to Wahyuningtias et al. (2015) at a good environmental temperature embryonic development, egg hatching, and early survival of fish larvae can take place normally. At high temperatures it will accelerate metabolism, so that embryonic development will be faster, and can inhibit the hatching process or cause death in larvae [11]. A good temperature for hatchery of freshwater fish ranges from 25-31 C

Furthermore, the fish eggs enter the morula phase. The morula phase is the end of the cleavage phase which will then be followed by the stage of embryo organ formation (Budianita et al. 2019). In this phase, a group arrangement that is denser than the yolk sac begins to form (Gusrina 2014). The morula phase ends when it has produced a blastomere. The blastomeres then condense into small blastodic cells forming two layers of cells and at the end of the division two groups will be produced [12].

After going through the morula phase, the egg develops into the blastula phase. The blastula phase begins with more and more blastomeric cells in the fluid-filled cavity as blastocoels. The blastula phase occurs between 80 and 100 minutes after fertilization [13]. During the blastula stage, the blastomere divides several times to form blastomeres of smaller size. At the end of the blastula phase, the blastoderm cells consist of neural, epidermal, notochordal, mesodermal, and endodermal [14].

The gastrula stage in fish begins with the movement of the epiboly which reaches 50% of the viteline vesicle. The blastoderm covers most of the yolk sac and the thicker posterior germ ring is called the embryonic shield [15]. In this phase, the curves of the body become more noticeable and visible, occurring in the 120-150 minute period after conception . Epiboly movement reaching 90% occurs at 240 minutes after fertilization and the last phase of the gastrula is marked by the appearance of a tail bud (Ath-Thar 2014). In this phase, the head will protrude at the animal pole and the tail will appear at the vegetal pole [16].

After the gastrula phase, the segmentation phase begins with the formation of the head, tail, and the appearance of 6 somites (body segments) (Budianita et al. 2019). This phase occurs at 300 minutes after fertilization. This segmentation process

lasts longer when compared to other phases. According to Nurjanah (2014) in the segmentation process successively will form organs including nerves, notochord, eyes, bone segments (somites), Kuffer cavity, olfactory sac, kidney cavity, intestines, subnotchord bone, lateral line, heart, aorta, gills, infundibulum, and fin folds.

In the next phase, it is marked by the eyes getting rounder, the body size getting bigger, and the formation of 10-20 somites occurring in 360-420 minutes (Ath-Thar 2014). At 480 minutes, the embryo resembles a larva with the eyes getting thicker and black pigment dots appear on the edges of the eyes (Budianita et al. 2019). In the segmentation phase, there is a differentiation process in the embryo, body organs such as eyes, head, tail, segments, heart, body, egg yolk, crystalline, and melanophores will be more clearly visible. In addition, this phase shows the movement of the embryo. The movement of the embryo is caused by the increasing length of the tail and starting to separate from the yolk sac and the heart has begun to be active [17].

The hatching time of fish eggs in each fish species will vary (Budianita et al. 2019). The results of the research by Cahyanti et al. (2020) stated that the Tor douronensis species hatched at 100 hours after fertilization. Meanwhile, the Tor soro species hatched 120 hours after fertilization and the Tor tambroides species hatched 140 hours after fertilization. According to Poto (2019), hatching can occur due to mechanical work, namely the embryo often changes its position due to lack of space in its shell or because the embryo is longer than its shell environment and enzymatic work, namely enzymes and other chemical elements released by the endodermal glands in the pharyngeal area of the embryo. In addition, hatching can also be caused by movements due to an increase in temperature, light intensity or absorption of oxygen pressure (Huwoyon et al. 2010).

Agatha et al. (2021) stated that fertilized eggs will develop and hatch normally if supported by good environmental conditions, including sufficient oxygen, appropriate temperature and clean water free of microorganisms. Embryos have a temperature tolerance limit in the process of development (Yuliani et al. 2020). In general, mackerel fish can live well at a temperature of 28-30 oC (Kusmini et al. 2018). This is in line with the research results of Prakoso et al. (2010) stated that the water temperature is quite low, namely 20-24°C, the growth of albino and black-tailed mackerel fish becomes slower.

After the complete embryonic development phase, the eggs will hatch into larvae. The development of fish larval organs has a main and specific character of larval development. The larvae develop to resemble adults (juveniles). In the early stages of larval development the eyespots are visible but not functioning, the mouth has not

opened, the digestive tract is in the form of a short alimentary canal, and the fins are new in the form of a caudal fin and a newly fused anal fin and a preanal fin on the abdomen in front of the anus [18]. Larvae rely on egg yolks as an energy source (endogenous energy). Larvae that carry food reserves for the development of body organs are called endogenous feeding [19].

The critical phase in the development of the squid embryo occurs in the pre-hatching phase and the hatching phase into larvae. According to Herjayanto et al. (2017) fertilized eggs can fail to hatch due to abnormal sperm cells and environmental factors that are not suitable for embryo development such as unsuitable temperature. Abnormalities that occur in each species of fish larvae cause the organs of the fish body to not develop properly [20].

During the larval observation period, it was found that there were abnormalities in the larvae of the cockroach fish. The results of the observation of the abnormality of the mackerel larvae with an incubation temperature of 28 oC showed an abnormality value of 16%. The results of Lestari's research (2016) on hormonal induction to improve the reproductive performance of squirrel fishshowed an abnormality value of 7.79-9.49%. Abnormalities in fish larvae occur due to a deviation process during embryogenesis or defective larvae after hatching . This statement is reinforced by Effendi (2004) which states that weak embryos that hatch successfully have the opportunity to become abnormal larvae. Defective fish larvae can be caused by the outermost layer of the egg (chorion) which has hardened so that the embryo will be difficult to come out. After the chorion can be broken, the embryo will come out in a deformed body state.

CONCLUSION

Based on the search results in the PubMed, NCBI, Google Scholar databases using predetermined keywords, 350 articles were obtained and 27 articles met the inclusion criteria. From the results of research conducted, it can be concluded that this research has sequentially described the embryonic development and early performance of the larvae of the *Barbonymus schwanenfeldii*. Need research on the other animal for future research.

REFERENCES

1. Barkah, T., Rusgianto, S. & Wardhana, A. Impact of Agricultural Land and the Output of Agricultural Products Moderated with Internet Users toward the Total export of Agricultural Product in Three Islamic South East Asian Countries. *Media Agribisnis* **6**, (2022).

2. Ali, M. A. Bin, Manuputty, A., Ashri, M. & Hendrapati, M. State Authorization to Fishery Resources in Indonesia: Management and Conservation Effort. *JL Pol Glob.* **73**, 148 (2018).

3. Febriyanti, A. R., Ratnasari, R. T. & Wardhana, A. K. The Effect of Economic Growth, Agricultural Land, and Trade Openness Moderated By Population Density on Deforestation in OIC Countries. *Quant. Econ. Manag. Stud.* **3**, (2022).

4. Natasya, A. THE EFFECT OF STORAGE TIME AT COLD TEMPERATURES ON THE QUALITY OF PRESTO SHORT FISH (*Barbonymus schwanenfeldii*). *J. Kesehat. Tambusai* **2**, 398–403 (2021).

5. Mafruchati, M., Wardhana, A. K. & Ismail, W. I. W. Disease and viruses as negative factor prohibiting the growth of broiler chicken embryo as research topic trend: a bibliometric review. *F1000Research* **11**, 1124 (2022).

6. Santiparadon, M., Rongpan, S., Payanglee, K., Hounkong, K. & Koedpuech, K. Morphologic Changes of the 3-day-old Chick Embryos after Curcumin Exposure. *Princess Naradhiwas Univ. J.* **13**, 309–318 (2021).

7. Mafruchati, M. Duck meat, delicious yet low in unsaturated fat; comparative study between duck meat consumption in asean toward the number of covid cases. *Syst. Rev. Pharm.* **11**, (2020).

8. Mellisa, S., Hasri, I., Nurfadillah, N. & Arisa, I. I. The effectiveness of feeding artemia enriched with vitamin c on the growth performance and survival of Lemeduk fish larvae (*Barbonymus schwanenfeldii*). in *IOP Conference Series: Earth and Environmental Science* vol. 674 12061 (IOP Publishing, 2021).

9. Kusmini, I. I., Gustiano, R., Radona, D. & Kurniawan, K. Domestication Strategies of Tinfoil Barb *Barbonymus schwanenfeldii* (Bleeker, 1854): Potential Candidate for Freshwater Aquaculture Development. in *IOP Conference Series: Earth and Environmental Science* vol. 934 12003 (IOP Publishing, 2021).

10. Mellisa, S., Ramadhan, R. D., Hasri, I. & Nurfadillah, N. The incubation of Tinfoil barb (*Barbonymus schwanenfeldii*) eggs using funnel system at different temperatures. *Depik* **11**, (2022).

11. Vutukuri, M., Ghode, G., Pawase, A. S., Kouser, S. & Tibile, R. M. Low level incorporation of Lactobacillus Sporogenes in feed of Tinfoil Barb (*Barbonymus schwanenfeldii* (Bleeker, 1854)) Fingerlings Positively Improves Growth, Gut Microflora and Intestinal Morphology. (2022).

12. Mafruchati, M. Global big data confirm Remdesivir to be a recommended antiviral drug to fight COVID-19. *J. Southwest Jiaotong Univ.* **55**, (2020).

13. Luqman, E. M., Mafruchati, M., Rahardjo, B. P. S. & Hestianah, E. P. Crosstalk between Necrosis and Apoptosis of Embryonal Cerebral Cortex Neuron Mice (Mus musculus) Caused by Carbofuran Exposure. *Res. J. Pharm. Technol.* **12**, 5492–5498 (2019).

14. Faria, M. *et al.* Screening anti-predator behaviour in fish larvae exposed to environmental pollutants. *Sci. Total Environ.* **714**, 136759 (2020).

15. Wahyudi, N. T., Suwanti, L. T., Sosiawati, S. M., Yudaniayanti, I. S. & Mafruchati, M. Prevalence of helminth eggs in cat feces contaminating public areas in Surabaya. *Indones. J. Trop. Infect. Dis.* **6**, 154–159 (2017).

16. Mahaputra, L., Mafruchati, M., Triakoso, N. & Aries, R. D. Pemisahan Spermatozoa Sapi Limousin yang Memiliki Kromosom X dan Y dengan Percoll dan Putih Telur Ayam. *J. Bina Praja* **14**, 172–175 (2012).

17. Mafruchati, M. Korelasi antara membran spermatozoa dan motilitas spermatozoa pada semen beku domba setelah dicairkan (post thawing). *MIPA* **4**, 161–166 (1999).

18. Nafees, M. S. M., Kamarudin, M. S., Karim, M., Hassan, M. Z. & de Cruz, C. R. Effects of dietary starch sources on growth, nutrient utilization and liver histology of juvenile tinfoil barb (*Barbonymus schwanenfeldii*, Bleeker 1853). *Aquac. Rep.* **23**, 101069 (2022).

19. Mawarid, M. M., Mafruchati, M., Suprayogi, T. W. & Widjiati, H. V. F. Potency of Kebar Grass (Biophytum petersianum) Extract to Histopathological of Mice (Mus musculus) Duodenum at Lactation Period Exposed to Carbofuran. *J Vet Mar. Res* **1**, 1–9 (2021).

20. Mafruchati, M. & Makuwira, J. Number of research papers about Agricultural production, Meat, and Egg During COVID-19 Pandemic: Does it Changed than Before? *Pharmacogn. J.* **13**, 995–998 (2021).

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Analysis of Medicinal Plants on Embryo Development of Mice (*Mus musculus* L.) And White Rats (*Rattus novergicus*): A Systematic Review

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Abstract

White rats are often used to assess protein quality, toxicity, carcinogenic, and pesticide content of an agricultural food product. M. Musculus mice are rodents that are very easy to breed, and easy to maintain. Embryogenesis is the process of growth and development of the embryo. The purpose of this study was to analyze medicinal plants on the embryonic development of Mice (Mus musculus L) and White Rats (Rattus Novergicus). This method uses a Systematic Review approach which is based on the Preferred Guide to Reporting Items for Systematic Review and Meta-analysis (PRISMA). Total of 700 articles were obtained which then entered the elimination stage resulting in 50 articles on medicinal plants (Mus musculus L) and White Rats (Rattus Novergicus). Based on the results of the study, it can be concluded that the identification of medicinal plants that are toxic to the embryonic development of Mice White Rats (Rattus Novergicus).

Keywords: *Ecosystem Health, Embryo of white rats, Extracts of Ajwa Dates, Resource Recycling.*

Abstrak

Tikus putih sering digunakan untuk menilai kualitas protein, toksisitas, karsinogenik, dan kandungan pestisida dari suatu produk pangan pertanian. Mencit M. musculus merupakan hewan pengerat yang sangat mudah berkembang biak, serta mudah dipelihara. Embriogenesis adalah proses pertumbuhan dan perkembangan embrio. Tujuan dari penelitian ini adalah menganalisis tumbuhan berkhasiat obat terhadap perkembangan embrio Mencit (*Mus musculus* L) dan Tikus Putih (*Rattus Novergicus*). Metode ini menggunakan pendekatan Systematic Review yang didasarkan pada Preferred Guide to Reporting Items for Systematic Review and Meta-analysis (PRISMA). Didapatkan sebanyak 700 artikel yang kemudian memasuki tahap eliminasi sehingga diperoleh 50 artikel tentang tanaman obat (*Mus musculus* L) dan Tikus Putih (*Rattus Novergicus*). Berdasarkan hasil penelitian dapat disimpulkan bahwa identifikasi tumbuhan obat yang bersifat toksik terhadap perkembangan embrio Mencit Tikus Putih (*Rattus Novergicus*).

Kata kunci: Ekstrak kurma Ajwa, embrio tikus putih, kesehatan ekosistem, daur ulang sampah

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INTRODUCTION

In Indonesia, there are no less than 2039 species of medicinal plants originating from tropical forests [1]. This situation makes Indonesia superior as one of the world's important biodiversity warehouses for pharmaceutical ingredients or drugs for human health [2]. Testing of medicinal substances intended for use in humans needs to be investigated by including human subjects as the final test tube. Human volunteers are ethically allowed to be included in the material to be tested has passed thorough laboratory testing, followed by using experimental animals for feasibility and safety.

Various small animals have certain characteristics that are relatively similar to humans, while other animals have similarities with the physiological aspects of human metabolism. White rats are often used to assess protein quality, toxicity, carcinogenic, and pesticide content of an agricultural food product[3]. M. Musculus mice are rodents that are very easy to breed, and easy to maintain. This is very advantageous in experimentation/research because mice can breed throughout the year. The mouse is one of the few mammalian species whose genome sequence can be determined and technically does not undergo genetic changes from each generation of development. So that mice can be categorized conditionally as experimental animals [4].

Embryogenesis is the process of growth and development of the embryo. The developmental stage of mammals (including humans) begins with the event of fertilization, namely the meeting/fusion of sperm cells with ovum cells. This fertilization will then produce a new individual cell called the zygote and will carry out growth and development towards the embryo. Organogenesis is the process of forming organs in living things (humans and animals) [5]. The stages of embryogenesis and organogenesis in their development are always in line, one of which is the development of the organs of the body. Teratogenesis is the formation of congenital defects or abnormal embryonic development, and these abnormalities are a major cause of morbidity and mortality in newborns. This happens because of chemicals, ionizing radiation, viruses, and nutritional deficiencies [6]. The purpose of this study was to analyze medicinal plants on the embryonic development of Mice (*Mus musculus* L.) and White Rats (*Rattus Novergicus*).

METHODOLOGY

This review uses a Systematic Review that was carried out in August-September 2022, in several databases, namely Pubmed, Sciencedirect, NCBI, and Elsevier with an article publication time span of 1973-2022 [7]. The inclusion

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criteria consisted of articles reporting "medicinal plants, embryos, mice (*Mus musculus L.)* and white mice (*Rattus Novergicus*)". Based on the results of the search carried out which then entered the elimination stage, there were 50 articles that were then included in the review results of this manuscript, according to the study by [8].

RESULT AND DISCUSSION

Table 1. The number of embryos and the percentage of morula and blastocyst in mice given red fruit extract (EBM) (Eriani).

Treatment	Number of embryos	Morula and blastocyst percentage	
		Morula	Blastosis
red fruit extract 0 ml	105	6,63±1,28a	93,36±1,28a
red fruit extract 0.05 ml	105	15,88±3,37b	84,12±3,37b
red fruit extract 0.1 ml	105	28,47±2,46c	71,53±2,46c

Note: different superscripts in the same column show significantly different (P<0.05).

Table 2. Number of embryos in mice given Pandanus conoideus var. yellow fruit (Muna L, Astirin OP, Sugiyarto. 2011)

Parameter	Dosage (mL)				
	Control	0,02	0,04	0,08	0,16
Number of pregnant women	5	5	5	5	5
Number of implants	58	52	44	46	44

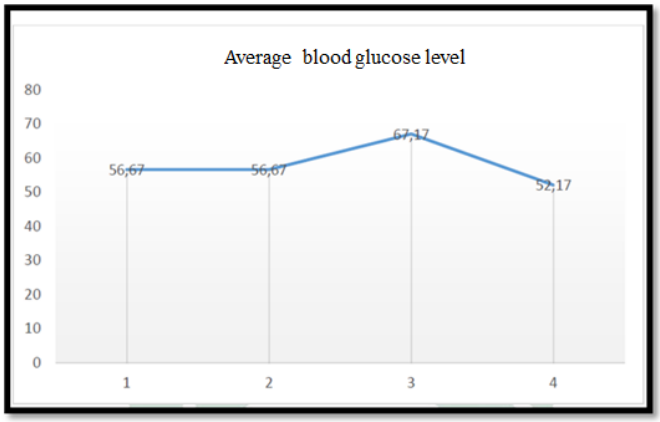

Figure 1. Average blood glucose levels in Mice Embryo after administration of ajwa date palm pulp extract (Phoenix dactylifera L.) (Setyaningsih N, 2018)

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Development of Mice Embryos Given Red Fruit Extract

Giving red fruit extract resulted in delays in embryo development until the blastocyst stage. The decline in the developmental ability of mammalian embryos is due to mitochondrial dysfunction and low available ATP. According to Silva (2006), during embryonic development, metabolism in cells forms ROS in their extracellular surroundings[9]. The effects of ROS on embryonic development can block or retard normal development in embryos. The more cells the embryo develops, the more ROS are formed. After fertilization at the beginning of embryonic development, the embryo is still dependent on gene expression from the mother, so it can still counteract ROS with the help of GSH from the mother. The embryonic genome in cows is activated at the 8-16 cell stage, without the synthesis of GSH from the mother's body [10]. The embryonic genome in mice is activated at the 2-cell stage. In the absence of gene expression from the mother in the embryo to counteract ROS, the embryo requires an increase in ATP for development. Oxidative stress has implications for various cell damage including lipid peroxidation layer, amino acid oxidation, apoptosis and necrosis which will reduce the success of in vitro embryo production [1112].

The decrease in the ability of embryo development to the blastocyst stage in this study, may also be caused by the administration of red fruit extract for a long time (7 days before fertilization and continued 3 days after fertilization) which resulted in damage to uterine organs. Red Fruit extract can cause degeneration and congestion of the uterine organs. Congestion is a condition caused by disruption of blood flow, so that blood circulation becomes slow and oxygenation to tissues decreases because blood is still in the blood vessels. This event can reduce the supply of oxygen in the uterine fluid, while the preimplantation embryo is very dependent on the secretions of the oviduct and uterine glands. Embryo development is an activity under aerobic conditions, namely activities that require oxygen [13]. Embryo development will be hampered by reduced oxygen supply due to uterine congestion.

Another thing that can reduce the ability of egg cells to develop to the blastocyst stage in vivo in mice is because Read Fruit (*Buah Merah*) extract also functions as an anticancer or inhibits cell development. Furthermore, anticancer substances can interfere with microtubule activity. Disruption of the spindle microtubules can interfere with the activity of dividing cells. Red fruit extract which

has been shown to have anticancer and antitumor activity, can also interfere with the formation of microtubule spindles which may contribute to the decrease in the ability of egg cells to develop to the blastocyst stage [14].

Pandanus conoideus var. yellow fruit against white mouse embryos (Rattus Novergicus)

Pandanus conoideus var. Yellow fruit is an alternative medicine to treat cancer [15]. This anti-cancer drug is used by all cancer patients, including pregnant women, while pregnant women are very susceptible to drugs, especially during organogenesis [16]. Anti-cancer drugs are teratogenic; not only affects cancer cells, but can affect normal cells in the vicinity[1718]. Fetal tissue grows at high speed, its cells divide rapidly so it is very susceptible to anti-cancer drugs. In addition, the drugs consumed by the mother will pass to the fetus through the placenta, namely through the same route that is passed by the nutrients needed for fetal growth and development. Drugs that reach the fetus can cause miscarriage, malformations or even death of the fetus [19].

PKH states that fat consumption can affect the production of progesterone. This is because the number of embryos in the uterus also affects the availability of space for embryo development and blood supply[20]. It is known that the fewer the number of implantations in the uterus, the availability of nutrients for the fetus will be fulfilled, so that the weight of the fetus with its nutrition will increase.

Provision of Ajwa Dates Flesh Extract (Phoenix dactylifera L.) Against Blood Glucose Levels of Mice Embryo (Mus musculus)

Annisafitria explained that dates are also believed to be good antioxidants. Not only as antioxidants, dates can also be anti-microbial, anti-mutagenic, anti-inflammatory, and anti-diabetic[21]. Rahmani et al. (2014) stated that ajwa dates can cure various types of diseases that play a protective role in liver toxicity so that the body's metabolism remains balanced[22]. This protective benefit is due to the fact that Ajwa dates contain several active compounds that are beneficial to the body such as tannins, saponins, flavonoids, polyphenol alkaloids and so on. Polyphenols in ajwa dates have the highest concentration compared to other types of dates[232425].Saryono stated that dates can prevent anemia, help the involution process and improve milk quality. Ajwa dates are also useful for fetal development and growth[26]. Suroso's researchstates that there is an effect between regular consumption of date palm juice at the end of pregnancy and the amount of labor bleeding. Ajwa dates are also beneficial for the

development of the embryo[27]. The content in it can increase embryo immunity, prevent anemia, improve digestion and help growth [28]. The administration of ajwa date fruit extract on the blood glucose levels of mice did not show a significant effect. This is due to the presence of fructose in ajwa dates which can significantly suppress the increase in blood glucose levels.

Fructose enters the cell with the help of GLUT-5. GLUT-5 functions to transfer fructose into cells. The action of GLUT-5 is independent of the presence of insulin. Fructose has a simpler structure than glucose so it is easier to enter cells. After that, glucose, galactose and fructose that have been absorbed in the intestinal cells diffuse into the blood through GLUT-2. Fructose can be consumed by diabetics because the transport of fructose to the body's cells does not require insulin, so it does not affect insulin secretion. In addition, the excess of fructose is 2.5 times as sweet as glucose [29]. Although there is no increase in blood glucose levels in the embryo, it does not mean there is no difference at all. There was a difference between the administration groups but it was not significant.

There was an increase in blood glucose levels at several doses when compared to blood glucose levels in the control group. The group that experienced an increase was the P5 group, which was 67.17 mg/dL. Meanwhile, the P3 group had the same mean value as the mean blood glucose level in the PK group, which was 56.67 mg/dL. However, in the P7 group, glucose levels decreased from all treatment groups, namely 52.17 mg/dL. This is because the content of ajwa date fruit extract contains various kinds of active compounds that are useful for the body. Dates contain carbohydrates, glucose, fructose, sucrose, magnesium, potassium, phosphorus, phosphate, protein, calcium, salicylic acid and several vitamins and a high content of polyphenols which are useful as antidiabetic, anti-inflammatory and good for fetal growth and development[21,26].

The increase in blood glucose levels in the P5 group was thought to be due to fructose. Fructose has a low glycemic index. Carbohydrates that have a low glycemic index trigger a slight increase in blood glucose levels, while those with a high glycemic index trigger high blood glucose levels as well. The increase in blood glucose levels in the P5 group did not exceed the blood glucose levels of normal mice. Normal mice blood glucose levels are 55-175 mg/dL.

This is also due to the influence of estrogen and progesterone. In normal pregnancy, pregnancy is said to be a diabetogenic condition, where the need for glucose will increase. Maternal metabolism undergoes changes for an

adequate and constant supply of glucose for the developing fetus. Maternal glucose is transferred to the fetus by a process of facilitated diffusion. Maternal insulin cannot cross the fetal placenta. At ten weeks' gestation, the fetus can secrete high amounts of its own insulin, allowing the use of glucose obtained from the mother. In the first trimester of pregnancy, maternal glucose levels drop rapidly below normal glucose levels of between 55 and 65 mg/dl. This happens because of the influence of the hormones estrogen and progesterone, so that the pancreas increases insulin production and increases the use of glucose. At the same time, the use of glucose by the fetus increases, thereby lowering maternal glucose levels. In addition, the first trimester is also marked by nausea, vomiting, and decreased food intake so that maternal glucose levels decrease and during the second and third trimesters there is an increase in levels of human placental lactogen, estrogen, progesterone, cortisol, prolactin, and insulin which causes increased insulin resistance.Insulin resistance is a glucose saving mechanism and ensures an adequate glucose supply for the fetus [30].

Another mechanism of increased blood glucose levels in the fetus is caused by the influence of the hormone progesterone. Progesterone can increase insulin secretion, increase fasting blood glucose levels, but decrease insulin effectiveness in peripheral tissues. Cortisol can inhibit the absorption and oxidation of glucose, increase the formation of glucose by the liver and can increase the secretion of glucagon. Thus, in the last trimester, fasting can lead to mobilization of maternal triglyceride stores causing maternal fatty acid levels to increase dramatically. This fatty acid is an alternative substrate for maternal metabolism so that glucose can be used for the brain and meet the needs of the fetus. As glucose uptake by tissues is suppressed, glucose levels increase which leads to stimulation of insulin secretion from the pancreas because hyperinsulinemia.

Hyperinsulinemia is a normal development in late pregnancy because insulin levels double in the third trimester [31]. In the P7 group, there was a decrease in blood glucose levels. This is because the consumption of fructose in the mother mice is too much. Fructose can have a hypoglycemic effect and fructose also has a low glycemic index so that its consumption in large quantities can reduce blood glucose levels [32].

Ajwa dates contain much higher levels of fructose compared to other dates, ie per fruit has a fructose content of 48.7 grams [33]and is safe for consumption if the levels are 25-40

grams per day [34]. When symptoms arising from mild hypoglycemia are manifested by blood glucose levels 55 mg/dL[35]. The mechanism of hypoglycemic activity is thought to be due to the presence of flavonoid glycoside compounds. The hypoglycemic mechanism is thought to be caused by flavonoid glycoside compounds that are absorbed in the blood and increase the solubility of blood glucose so that it is easy to be excreted through urine [36]. Another hypoglycemic mechanism is the ability of quercetin which is a type of flavonoid in inhibiting GLUT 2 of the intestinal mucosa so that it can reduce glucose absorption. This causes a reduction in the absorption of glucose and fructose from the intestine so that blood glucose levels fall. GLUT 2 is suspected**.** It is the major glucose transporter in the intestine under normal conditions. In a study conducted by Songit was found that flavonoids can inhibit glucose absorption [37]. When quercetin is ingested with glucose, hyperglycemia is significantly decreased. This shows that quercetin can inhibit glucose absorption through GLUT 2 [37].

The decrease in blood glucose in mice was also caused by the presence of xanthone compounds which are flavonoid compounds. Xanthones are antioxidant compounds [38]. This compound studied in mangosteen rind extract can neutralize free radicals and can help lower blood sugar levels and overcome fatigue caused by unbalanced blood sugar levels [39].

Polyphenols are antioxidant compounds. Ajwa dates have a very high polyphenol content. Polyphenols are compounds that have activity as antioxidants. It is known that antioxidants can lower blood glucose levels. Polyphenolic compounds in ajwa dates that are thought to be able to lower blood glucose levels are polyphenol acid (gallic, protocatechuic, hydroxybenzoic, vanillis, siovanilic, syirigic, caffeic, ferulic, sinapic, pcoumaric, isoferulic), flavonoid glycosides (quercetin, and kaempferol, apigenin) and anthocyanidins [40]. Giving dates with a dose of 5 grains is considered the right dose to be given to pregnant mice, because at that dose glucose levels are highblood increased slightly. While at a dose of 7 grains, there was a decrease because the polyphenol content in Ajwa dates was too high so that the antioxidant activity was greater and resulted in indications of mild symptoms of hypoglycemia in mouse embryos [41].

In addition to fructose, another possibility that can lower blood glucose levels is flavonoids. Flavonoids are able to reduce oxidative stress and reduce ROS (Reactive Oxygen Species) which have a protective effect on pancreatic beta cells and increaseinsulin

sensitivity. Flavonoid compounds, especially quercetin, are inhibitors of GLUT 2 in the intestinal mucosa, a pathway for glucose and fructose absorption in the intestinal membrane [42]. This mechanism is non-competitive, causing a reduction in the absorption of glucose and fructose from the intestine so that blood glucose levels fall [43].

Flavonoids have a mechanism in inhibiting phosphodiesterase so that cAMP levels in pancreatic beta cells increase. This stimulates insulin secretion via the Ca pathway. Increased levels of cAMP will cause the closure of K+ ATP channels in the plasma membrane of beta cells. This situation results in membrane depolarization and the opening of voltage-dependent Ca channels thereby accelerating the entry of Ca ions into the cell. This increase in Ca ions in the cytoplasm of beta cells will cause insulin secretion by pancreatic beta cells [44].

CONCLUSION

Based on the results of the search, 50 articles were obtained which were used as analysis material, where it can be concluded that more in-depth research is needed on medicinal plants that are toxic to the embryonic development of Mice (*Mus musculus* L.) and White Rats (*Rattus Novergicus*).

REFERENCES

1. Perdaniar, O. B. Uji patch ekstrak etanol bawang merah (allium ascalonicum l.) Terhadap penurunan temperatur dan jumlah limfosit pada tikus putih galur wistar. Preprint at (2018).

2. Ridwan, E. Etika pemanfaatan hewan percobaan dalam penelitian kesehatan. *J Indon Med Assoc* **63**, 112–116 (2013).

3. Mafruchati, M., Wardhana, A. K. & Ismail, W. I. W. Disease and viruses as negative factor prohibiting the growth of broiler chicken embryo as research topic trend: a bibliometric review. *F1000Research* **11**, 1124 (2022).

4. Luqman, E. M., Mafruchati, M., Rahardjo, B. P. S. & Hestianah, E. P. Crosstalk between Necrosis and Apoptosis of Embryonal Cerebral Cortex Neuron Mice (*Mus musculus*) Caused by Carbofuran Exposure. *Res. J. Pharm. Technol.* **12**, 5492–5498 (2019).

5. Mawarid, M. M., Mafruchati, M., Suprayogi, T. W. & Widjiati, H. V. F. Potency of Kebar Grass (Biophytum petersianum) Extract to Histopathological of Mice (*Mus musculus*) Duodenum at Lactation Period Exposed to Carbofuran. *J Vet Mar. Res* **1**, 1–9 (2021).

6. Pratama, Y. B., Wardhana, A. K. & Nugroho, P. A. HUBUNGAN ANTARA ARTIKEL MENGENAI GAME DAN TEKNOLOGI INFORMASI PADA SCOPUS: STUDI BIBLIOGRAFI. *VISI PUSTAKA Bul. Jar. Inf. Antar Perpust.* **22**, (2020).

7. Dolhnikoff, M. *et al.* Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19. *J. Thromb. Haemost.* (2020).

8. Da Silva, P. D. N. Physiology of peroxidation processes in mammalian sperm (Cholesterol oxidation in sperm cell membranes). Preprint at (2006).

9. Ali, A. A., Bilodeau, J. F. & Sirard, M. A. Antioxidant requirements for bovine oocytes varies during in vitro maturation, fertilization and development. *Theriogenology* **59**, 939–949 (2003).

10. Kitagawa, Y., Suzuki, K., Yoneda, A. & Watanabe, T. Effects of oxygen concentration and antioxidants on the in vitro developmental ability, production of reactive oxygen species (ROS), and DNA fragmentation in porcine embryos. *Theriogenology* **62**, 1186–1197 (2004).

11. Surya, S. K. H. K. S., Abdi, M. H. K. S. H., Unipdu, S. B. M. P. F. I. K., STIKES, J. S. P. H. B. & Dharma, S. B. N. A. K. Proceeding-UMY Repository.

12. Eriani, K., Tifani, P. L. & Said, S. Fertilization and development of mice (*Mus musculus*) embryo in vitro after supplementing the extract of Pandanus conoideus. *Nusant. Biosci.* **9**, 202–208 (2017).

13. Wasito, R. Cytotoxicity study of red fruit extract (Pandanus conoideus lam) as anti-goitrogenic herbal medicine in sprague dawley rats. *J. Sain Vet.* **32**, (2014).

14. Briggs, G. G., Freeman, R. K. & Yaffe, S. J. *Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk*. (Lippincott Williams & Wilkins, 2012).

15. Baldessarini, R. J. Current status of antidepressants: clinical pharmacology and therapy. *J. Clin. Psychiatry* (1989).

16. Foye, W. O. *Foye's principles of medicinal chemistry*. (Lippincott Williams & Wilkins, 2008).

17. Zairina, E. *et al. Unity in Diversity and the Standardisation of Clinical Pharmacy Services: Proceedings of the 17th Asian Conference on Clinical Pharmacy (ACCP 2017), July 28-30, 2017, Yogyakarta, Indonesia*. (CRC Press, 2017).

18. MUNA, L., ASTIRIN, O. P. & SUGIYARTO, S. Teratogenic test of Pandanus conoideus var. yellow fruit extract to development of rat embryo (Rattus norvegicus). *Nusant. Biosci.* **2**, (2010).

19. El-Sohaimy, S. A. & Hafez, E. E. Biochemical and nutritional characterizations of date palm fruits (Phoenix dactylifera L.). *J Appl Sci Res* **6**, 1060–1067 (2010).

20. Rahmani, A. H., Aly, S. M., Ali, H., Babiker, A. Y. & Srikar, S. Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. *Int. J. Clin. Exp. Med.* **7**, 483 (2014).

21. Shafaghat, A. Phytochemical investigation of quranic fruits and plants. *J. Med. Plants* **9**, 61–66 (2010).

22. Abdelrahman, A. H., Ibrahim, F. S., Abdelrahman, A., Jun, H. K. & Kim, D. H. Protective effect of dates (Phoenix dactylifera L.) and licorice (Glycyrrhiza glabra) on carbon tetrachloride–induced hepatotoxicity in dogs. 한국식품영양과학회학술대회발표집 402 (2015).

23. Ismail, W. I. W. & Radzi, M. N. F. M. Evaluation on the Benefits of Date Palm (Phoenix dactylifera) to the Brain. *Altern. Integr. Med.* 1–3 (2013).

24. Saryono, M. & Rahmawati, E. Effects of dates fruit (Phoenix dactylifera L.) in the

female reproductive process. *Int. J Recent Adv. Multidiscip. Res* **3**, 1630–1633 (2016).

25. Ulya, N. U. Peer to peer lending on financial technology under perspective positive law and Islamic law. *J. Islam. Bus. Law* **2**, (2018).

26. Putri, K. *et al.* LITERATURE REVIEW PEMBERIAN KURMA TERHADAP PERCEPATAN KALA I FASE AKTIF PERSALINAN. (2020).

27. BUDIAMAN, B. SISTEM AGROFORESTRI BERBASIS LEBAH Trigona incisa (APISILVIKULTUR) MENUJU SISTEM PERTANIAN BERKELANJUTAN= AGROFORESTRY SYSTEM BASED ON Trigona incisa HONEY BEES (APISILVICULTURE) TOWARDS A SUSTAINABLE AGRICULTURE SYSTEM. Preprint at (2021).

28. Gabbe, S. G. *et al. Obstetrics: normal and problem pregnancies e-book*. (Elsevier Health Sciences, 2016).

29. Djelmi, J. & Desoye, G. *Diabetology of pregnancy*. vol. 17 (Karger Medical and Scientific Publishers, 2005).

30. Stanhope, K. L. & Havel, P. J. Fructose consumption: considerations for future research on its effects on adipose distribution, lipid metabolism, and insulin sensitivity in humans. *J. Nutr.* **139**, 1236S-1241S (2009).

31. GASIM, A. A. Changes in sugar quality and mineral elements during fruit development in five date palm cultivars in AI-Madinah AI-Munawwarah. *Science* **6**, (1994).

32. Sánchez-Valle, V., C Chavez-Tapia, N., Uribe, M. & Méndez-Sánchez, N. Role of oxidative stress and molecular changes in liver fibrosis: a review. *Curr. Med. Chem.* **19**, 4850–4860 (2012).

33. Boyle, P. J. & Zrebiec, J. Physiological and behavioral aspects of glycemic control and hypoglycemia in diabetes. *South. Med. J.* **100**, 175–183 (2007).

34. Khairullah, A. R. *et al.* A Review of Methicillin-Resistant Staphylococcus aureus (MRSA) on Milk and Milk Products: Public Health Importance. *Syst. Rev. Pharm.* **11**, 59–69 (2020).

35. Song, J. *et al.* Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. *J. Biol. Chem.* **277**, 15252–15260 (2002).

36. Mafruchati, M. Curcumin vs Chloroquine in Coronavirus Global Pandemic: Trend analysis Study in Google. *Indian J. Forensic Med. Toxicol.* **14**, 3138–3143 (2020).

37. Rohman, A., Arifah, F. H., Alam, G. & Rafi, M. A review on phytochemical constituents, role on metabolic diseases, and toxicological assessments of underutilized part of Garcinia mangostana L. fruit. *J. Appl. Pharm. Sci.* **10**, 127–146 (2020).

38. Eid, N. *et al.* The impact of date palm fruits and their component polyphenols, on gut microbial ecology, bacterial metabolites and colon cancer cell proliferation. *J. Nutr. Sci.* **3**, (2014).

39. Himawan, R. G., Primarizky, H., Mafruchati, M., Triakoso, N. & Maslachah, L. Green Tea Leaves Extract Effect on Histopathology of Mercury Chloride Induced Rat's Liver. *Pollut. Res.* **40**, 326–329 (2021).

40. Mafruchati, M. Duck meat, delicious yet low in unsaturated fat; comparative study between duck meat consumption in asean toward the number of covid cases. *Syst. Rev. Pharm.* **11**, (2020).

41. Dewi, S. K. *et al.* PENGARUH PEMBERIAN EKSTRAK KULIT PISANG KEPOK (Musa acuminata) TERHADAP PENURUNAN KADAR MALONDIALDEHID (MDA) PARU MENCIT JANTAN (*Mus musculus*) YANG DIPAPAR ASAP ROKOK. *J. Basic Med. Vet.* **8**, 123–130 (2019).

42. Kusumaningsih, T., Luthfi, M. & Moffan, M. D. B. Macrophages Analysis on Gingival Tissue of Diabetic Rats after Insulin Leaf Extract Administration. *J. Int. Dent. Med. Res.* **11**, 308–311 (2018).

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Apis Mellifera Propolis Extract as A Stimulator of Brain Cell Development (Study on Experimental Animals)

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Abstract

Apis mellifera could have potential as cell growth stimulator ability of the propolis. It also expected to increase the growth and proliferation of brain cells. This study aimed to analyze Apis mellifera Propolis Extract as a Stimulator of Brain Cell Development in Experimental Animals. This research is an experimental study using a completely randomized design (CRD) with 6 treatments and 4 replications. The research consisted of making Propolis Extract, Making Media (DMEM Stock Media, Washing Media, Culture and Treatment Media), Brain Cell Isolation and Culture, Observation of Culture Results (Cell Viability, Population Doubling Time, The results found that all treatments with propolis extract showed higher average viability than the control, which had average viability of 86.3%. Based on PDT (Population Doubling Time value), propolis extract has a good effect on brain cell growth, hence increasing cell proliferation. In addition, the confluent brain cells of rats given propolis extract had higher average confluency than those not given propolis extract. Propolis extract can protect neurons from damage by increasing the brain-derivated neurotrophic factor (BDNF) gene expression in cell line neuron cultures.

Keywords: *Apis mellifera, Brain Cell, Propolis Extract, Stimulator.*

Abstrak

Apis mellifera berpotensi sebagai stimulator pertumbuhan sel propolis. Selain itu juga diharapkan dapat meningkatkan pertumbuhan dan proliferasi sel-sel otak. Penelitian ini bertujuan untuk menganalisis Ekstrak Propolis Apis mellifera sebagai Stimulator Perkembangan Sel Otak pada Hewan Percobaan. Penelitian ini merupakan penelitian eksperimen dengan menggunakan Rancangan Acak Lengkap (RAL) dengan 6 perlakuan dan 4 ulangan. Penelitian terdiri dari pembuatan Ekstrak Propolis, Pembuatan Media (Media Stok DMEM, Media Pencucian, Kultur dan Media Perawatan), Isolasi dan Kultur Sel Otak, Pengamatan Hasil Kultur (Viabilitas Sel, Waktu Penggandaan Populasi, Hasil penelitian didapatkan bahwa semua perlakuan dengan propolis viabilitas rata-rata ekstrak propolis lebih tinggi dibandingkan dengan kontrol yang memiliki viabilitas rata-rata 86,3%. Berdasarkan nilai PDT (Population Doubling Time), ekstrak propolis berpengaruh baik terhadap pertumbuhan sel otak sehingga dapat meningkatkan proliferasi sel. Selain itu, sel otak konfluen tikus yang diberi ekstrak propolis memiliki rata-rata pertemuan yang lebih tinggi daripada yang tidak diberi ekstrak propolis.Ekstrak propolis dapat melindungi neuron dari kerusakan dengan meningkatkan ekspresi gen Brain Derivated Neurotrophic Factor (BDNF) pada kultur cell line neuron.

Kata kunci: Apis Mellifera, ekstrak propolis, sel otak, stimulator

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INTRODUCTION

In the 1970s, initiated by the Apiary Scout Center, modern honey bee cultivation began to be developed using European bee species (Apis mellifera) imported from Australia. Starting from 20 stup (bee boxes), Apis mellifera as a gift from President Suharto's visit to Australia in 1974, given to the Scout Movement [1]. It has grown to tens of thousands of colonies in a few years and involves hundreds of breeders. In 2006, the Directorate General of Land Rehabilitation and Social Forestry (RLPS) of the Ministry of Forestry documented at least 33,000 Apismellifera colonies (Ditjen RLPS, 2006). Apis mellifera cultivation is essential to Indonesia's beekeeping and honey production. Kuntadi (2008), citing data from the Directorate General of RLPS, said that Apis mellifera accounts for about 25% of Indonesia's total honey production, which averages 4,000 tons per year.

The priority area for developing European bee cultivation is Java. Until now, the production and grazing bases of Apis mellifera bee are mainly around the north coast of Central Java, East Java, and West Java [2]. It is related to the availability of good bee-feeding plants in the area and road infrastructure that reaches remote areas following the presence of the food source plant itself [3].

Propolis is a substance created by bees in hive defense. In addition, it can also strengthen structural stability, prevent parasites and diseases from entering, prevent decay, and reduce vibrations from outside the nest, as well as close the cracks of damaged nests (Siregar, 2011). The function of propolis is not only for the benefit of bees but is also widely used by humans as an alternative medicine to cure various diseases. According to [4], propolis is called bee glue because the material is sticky like glue. Bees produce propolis by collecting resin or sap from various plants mixed with saliva and various enzymes present in bees.

Propolis contains active compounds including vitamins, minerals, enzymes, flavonoids, terpenoids, steroids, amino acids, caffeic acid and its derivatives, caffeic acid phenyl ester-caffeic acid phenylethyl ester (CAPE) (Lawal et al., 2015). These active compounds give propolis a variety of biological and pharmacological properties, including anti-cancer, anti-bacterial, anti-inflammatory, anti-microbial, anti-fungal, tumoricidal, immunomodulator, antioxidant, anti-bacterial, parasitic, anti-diabetic, anti-histamine, antacid, anti-protozoal, anti-Helicobacter pylori (ulceration treatment), as a therapeutic agent, cell stimulator, and the ability to neuroprotective [5].

Propolis extract, which has potential as a neuroprotective, is expected to protect brain cells from damage. The brain is an organ composed of many neurons and is very sensitive to free radical induction. It

causes the brain to have high polyunsaturated fatty acids and low antioxidant molecules compared to other organs [6]. This condition causes a low rate of cell proliferation in it. Moreover, to increase the proliferation of brain cells, it is necessary to induce compounds that can increase the number of cells due to cell growth and division so that new cells are available to replace damaged cells [7].

Propolis extract could stimulate growth and increase the number of cells in rat spleen cells, human lymphocytes, rat kidney cells, and rat liver cells in vitro. Based on the results of the MTT test in 1 mg/mL of propolis, the maximum yield of cell growth was shown by spleen cells as much as 48%, and the minimum results found in liver cells by 18%. The number of rat spleen cells, human lymphocytes, rat kidney cells, and rat liver cells increased by 65%, 55%, 35%, and 25%, respectively, at a dosage of 2 mg/mL. Direct observations indicated that all normal cells induced by propolis had a longer life span than cells that were not induced by propolis extract (control). It shows that propolis extract can increase cell viability and work as a stimulator of cell growth.

The cell growth stimulator ability of the propolis extract is also expected to increase the growth and proliferation of brain cells. Proliferation is the initial part of forming neurons. With the proliferation process, brain cells will increase and replace damaged cells to maintain the integrity between neurons. In vitro, the rate of cell proliferation can be determined from the value of Population Doubling Time (PDT). The PDT value is when it takes for the cell population to double its number [8]. If the PDT value is low, cell proliferation is fast, and vice versa [9].

Propolis extract has compounds that can help the growth of neurons in vitro, such as polyphenols and terpenoids that can protect neurons from damage and tannins that can maintain the integrity of cell membranes so that they can increase cell survival, growth, and proliferation. With this ability, propolis extract can protect cells from damage, increase cell viability, and speed up the time for proliferation [10]. This study aimed to analyze Apis Mellifera Propolis Extract as a Stimulator of Brain Cell Development in Experimental Animals.

METHODOLOGY

This research is an experimental study using a completely randomized design (CRD) with 6 treatments and 4 replications. The treatments used were control (without treatment) and rat brains given propolis with 5 different concentrations. The distribution of the treatment is as follows:

- P1 (treatment 1): brain cell culture without propolis extract (control)
- P2 (treatment 2): brain cell culture with propolis extract 20 g/mL
- P3 (treatment 3): brain cell culture with propolis extract 40 g/mL

- P4 (treatment 4): brain cell culture with propolis extract 60 g/mL
- P5 (treatment 5): brain cell culture with propolis extract 80 g/mL
- P6 (treatment 6): cultured brain cells with propolis extract 100 g/mL.

Propolis Extract Manufacturing

300 grams of raw propolis was obtained from beekeeping in East Java which was then extracted using the maceration method with 1200 ml of 96% ethanol solvent. The maceration results were then evaporated using a rotary evaporator for 1 hour.

Media Creation

DMEM Stock Media

DMEM stock media was made in 100 ml with a composition of 1.35 g DMEM, 0.37 g NaHCO3, 0.238 g HEPES, 0.006 g penicillin, 0.01 g streptomycin, and 100 ml sterile DI. These materials were dissolved until homogeneous, then filtered using a 0.22 m Millipore membrane. The media stock was added with 10% FBS as a culture medium.

Washing Media

The washing medium was made of 0.9% NaCl and antibiotics (penicillin and streptomycin), 4 ml of DMEM 0% media, and 1 ml of 10% DMEM media. The washing medium was made directly during cell implantation.

Culture Media and Treatment

Culture media were prepared from DMEM and 10% FBS added with propolis with different concentrations (20 g/mL, 40 g/mL, 60 g/mL, 80 g/mL, and 100 g/mL). The media was then put into a TC dish of 3 ml and incubated in an incubator for 60 minutes at 37oC and 5% CO2. The treatment used propolis which was diluted using DMSO first. A 10% DMSO stock solution was made by dissolving 1 ml of DMSO into 10 ml of sterile DI. Then a stock extract solution was made by dissolving 10 mg of propolis extract into 10 ml of 10% DMSO to obtain a stock solution of propolis extract with a concentration of 1 mg/ml or 1000 ug/ml. Furthermore, propolis extract was made with specific concentrations from the solution, namely 20 g/mL, 40 g/mL, 60 g/mL, 80 g/mL, and 100 g/mL.

Brain Cell Isolation and Culture

Neonatal rats aged 3-4 days were dislocated, the head was dissected, and the whole brain was taken. Then washed with 0.9% NaCl + antibiotics 3 times. After that, it was chopped into pieces and homogenized using a 3 ml syringe. Then put it into a 10 ml centrifugation tube. Centrifugation was carried out at a speed of 3500 rpm for 10 minutes and 3 times. The first centrifugation results from homogenization using a syringe, and the pellet is taken.

The second centrifugation consisted of pellets from the results of the first centrifugation, which added 2 ml of 0% DMEM media, and the pellets were taken. The third centrifugation was pelleting from the results of the

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second centrifugation, which was added with 1 ml of 10% DMEM. The result of the last centrifugation has discarded the supernatant and left a little with the pellet, which was then homogenized. 50 l of suspension was taken and put into a TC dish containing each treatment. Incubated, and the media was changed every 3 days [11].

Observation of Culture Results
Cell Viability

The first step before observing cell viability is that the cell culture media is removed first. Then the cells in the media were washed twice using PBS, then 500 l of trypsin-EDTA 0.25% was added and homogenized. After being homogeneous, the cells were incubated for 3 minutes at 37°C and 5% CO2. The result of trypsinization was taken as much as 10 l to be stained with 10 l 0.4% trypan blue. Cells added with trypan blue were observed under a microscope for viability.

Cell viability can be seen from the number of dead and living cells. Live cells will not be stained, while dead cells will be stained with trypan blue. Observation of cell viability using cell countess by taking pellets as much as 5 l and trypan blue as much as 5 l then homogenized. Take 5 l of pellet that has been mixed with trypan blue and put it into the chamber. The cell count will appear on the cell countess screen with green color for the percentage of live cells and red for the percentage of dead cells.

Population Doubling Time

Population Doubling Time is the time it takes for the cell population to double its original number. Population Doubling Time (days) is calculated using the formula: 1 per ((log of final cell count minus log of initial cell count) x3.32) per culture time.

Cell Confluence

Cell confluence was observed under an inverted microscope after incubation. Determination of cell confluence was carried out using ImageJ Fiji software. The software can be used for the calculation of confluence in cell growth. The analysis result is the area fraction, representing the total surface area covered by cells in the photographed area. These results will show the value of cell confluence on the TC dish as a whole.

RESULT AND DISCUSSION

Cell Viability

Cell viability can be measured using a stain containing 0.4% trypan blue. The general protocol used to observe cell viability is to clarify the plasma membrane by staining. Damaged or dead cell membranes will bind the dye so that the cells are stained, while regular or living cell membranes will not be stained because the cell membrane is impermeable to dyes, as shown in Figure 1.

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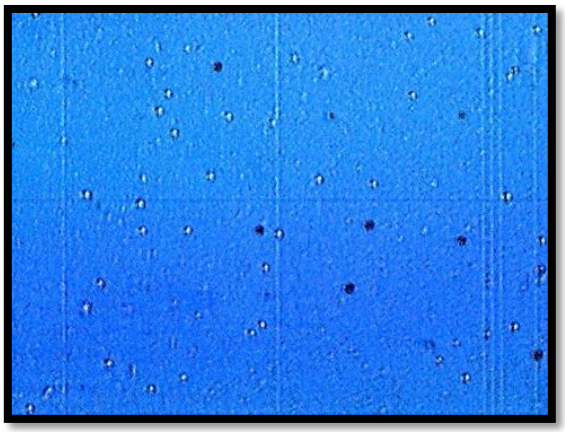

Figure 1. Cell viability with trypan blue 0.4%
staining under an inverted microscope with 200x magnification; black arrow: colored cells (dead); white arrows: cells that are not stained or clear (live).

After comparing the number of living cells and the number of dead cells, the data was obtained from each treatment's percentage of live cells. Based on table 1, it can be seen that the culture of rat brain cells (Rattusnorvegicus) in 10% DMEM media supplemented with propolis extract at doses of 20 g/mL, 40 g/mL, 60 g/ml, and 80 g/mL showed average results. The average viability increased successively, namely 86.3%, 88.0%, 89.5%, 89.8%, and 90.3%. Meanwhile, at a 100 g/mL concentration, the average viability decreased to 87.5%. All treatments with propolis extract showed higher average viability than the control, which had average viability of 86.3%. The difference in the viability results of brain cells cultured with propolis extract showed that in propolis extract, there were compounds that could help cells increase their survival ability; therefore, the viability of brain cells cultured with propolis extract was higher than brain cells cultured without extract propolis.

Table 1. Average viability (%) of rat brain cells (Rattusnorvegicus) in vitro on day 10 with and without propolis extract

Treatment	Average Viability (%)
P1 (0 µg/mL)	86.3
P2 (20 µg/mL)	88.0
P3 (40 µg/mL)	89.5
P4 (60 µg/mL)	89.8
P5 (80 µg/mL)	90.3
P6 (100 µg/mL)	87.5

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***Population Doubling Time* (PDT)**

Another parameter in this study is the value of Population Doubling Time (PDT) to determine the level of proliferation of brain cells. The faster the cell proliferation process, the faster the PDT value is achieved. Determine the value of PDT and it can be done by counting the number of cells before and after being cultured. The higher the PDT value, the lower the rate of cell proliferation, and vice versa.

Based on table 2, it can be seen that the administration of propolis extract in 10% DMEM media to brain cell culture can accelerate the PDT value or time for cell proliferation as the concentration of the extract increases with the average PDT value of 5.03 days (P2), 3.69 days (P2) P3), 3.41 days (P4), and 2.88 days (P5). The fastest PDT value was achieved by P5, which was 2.88 days, which means that in the P5 treatment, brain cells could double their original number in 2.88 days or about three days. While at P6, the PDT value was higher than in the previous treatment, which was 4.65 days, which means the proliferation rate decreased compared to the previous treatment but was still higher than P1 or control. It indicates that the treatment of propolis extract has a beneficial effect on the growth of brain cells to increase cell proliferation.

Table 2. Average PDT values (days) of rat brain cells (Rattusnorvegicus) on day 10 in vitro with and without propolis extract

Treatment	Average PDT Value (days)
P1 (0 µg/mL)	5.03
P2 (20 µg/mL)	4.55
P3 (40 µg/mL)	3.69
P4 (60 µg/mL)	3.41
P5 (80 µg/mL)	2.88
P6 (100 µg/mL)	4.65

Confluency

Confluency is the constant growth of cells to cover the culture dish. The cells have reached confluent, then the cells in the culture medium have used the substrate, and the cells are interconnected with one another. Table 3 shows the average confluency with and without in vitro administration of propolis extract in rat brain cells (Rattusnovergicus).

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Table 3. The results of the average confluency (%) of rat brain cells

Treatment	Average confluency(%)
P1 (0 µg/mL)	7.68
P2 (20 µg/mL)	10.11
P3 (40 µg/mL)	9.80
P4 (60 µg/mL)	12.23
P5 (80 µg/mL)	11.48
P6 (100 µg/mL)	13.58

Table 3 shows that the brain cell confluency of mice not given propolis extract (P1) had an average of 7.68 %. The concentration of propolis extract, which had the highest confluent value of rat brain cells, was P4 12.23 %, P5 and P6 13.58 %, P2 10.11%, and P3 9.80 %. Based on these results, it can be seen that the confluent of rat brain cells that were given propolis extract had a higher average confluent than those that were not given propolis extract. It can be seen that propolis extract can accelerate the confluent process of rat brain cells in vitro. Propolis can accelerate brain cell confluent because it contains compounds that help cell growth.

Cell culture is the culture of cells derived from organs or tissues that have been decomposed into cell suspensions. This cell suspension is then cultured in vitro in a container such as a vial, tube, or cup or into a cell suspension in a growth medium [12]. In vitro technique is used to maintain or reproduce parts of living tissue grown on a medium adapted to the physiological conditions of these living things [13]. Primary cell cultures can be obtained by growing cells from disaggregating tissue fragments using enzymes or obtained mechanically. In primary explants, there will be a selection based on the ability of cells to migrate from explants and grow into primary cell cultures [7].

Primary culture denotes the culturing stage in which tissue-isolated cells are grown until they occupy all accessible substrates under proper and controlled circumstances (reach confluent). At this point, the cells must be subcultured by transferring them to a fresh substrate and growth medium to allow for continued expansion [14]. Primary neuronal cultures from mice have been widely used to study neuronal properties such as axon elongation, synaptic transmission, and excitotoxicity (neuronal death due to excess glutamate) [15].

Brain cells develop from undifferentiated progenitor cells.

Neuron progenitor cells in in vitro culture have a spherical morphology, spherical with short extensions and elongated spindle-shaped (Riyacumala, 2010). In contrast, neuroglia cells will be shaped like multipolar fibroblasts before confluent and in the form of polygonal single-layered epithelial cells with regular sizes when confluent [16]. The primary brain cell cultures in this study were obtained from the brains of postnatal rats. According to Puspitasari (2013), the network is a source of several neurons with multipotent characters and many neuronal progenitor cells. To determine with certainty, the type of cells that develop in the primary culture of neurons can be confirmed by immunohistochemical assays.

The brain is the organ most susceptible to free radical attack because of its high-fat content (about 80%); thus, the risk of oxidative damage is very high (Aksenova, 2005). In addition, damaged brain nerve tissue cannot regenerate, and it can cause disease (Horner, 2005). In vitro, the problem of brain nerve cells is also not far from the in vivo state. Primary brain nerve cell culture is notoriously difficult. It is because the brain nerve cell culture has a short survival. Therefore, additional materials are needed in culture media that can increase survival and increase the proliferation of brain nerve cells. It can also be helpful for efforts to repair brain nerve cells so that diseases originating from oxidative damage can be avoided [17].

In this study, the additive used was propolis extract. According to Ni (2017), propolis extract can protect neurons from damage by increasing the brain-derivatedneurotrophic factor (BDNF) gene expression in cell line neuron cultures. The mechanism of propolis extract in increasing BDNF gene expression in cells occurs because propolis extract contains Caffeic acid phenylethyl ester (CAPE) compounds which can increase extracellular signal-regulated kinase (ERK) activation. ERK activation then alters the localization and phosphorylation of different target molecules, including transcriptional regulators (Moosavi, 2015). ERK activation is essential in proliferation because ERK translocation can trigger cells to enter the cell cycle through growth factors, namely BDNF [18].

BDNF will eventually result in phosphorylation of the CREB protein. Target gene transcription is enhanced by phosphorylation of CREB binding to CREB-binding protein. These genes are involved in cell survival, differentiation, growth, synaptic plasticity, and long-term memory activities (Moosavi, 2015). Brain-derived neurotrophic factor is one of CREB's target genes. BDNF belongs to the family of neurotrophins. With a structure similar to growth factors and plays a role in the proliferation, differentiation, and survival of nerve cells (Juananda, 2015). With the increased expression of this BDNF

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gene, cells will enter the cell cycle and carry out mitosis; therefore, proliferation increases.

Another compound in propolis extract that has a role in brain cell culture is polyphenols. According to Farooqui (2012), propolis is one of the most abundant sources of polyphenols (50-55%) and mainly contains flavonoids, phenolic acids, and their esters. Moosavi (2015) added that polyphenol compounds have potent antioxidant and neuroprotective activities. Polyphenols can protect neurons from damage by involving neurotrophic effects so that they can increase the survival, growth, proliferation, and differentiation of neurons.

Polyphenols are not only known as antioxidants but also as regulators of cell metabolism. It can interact with the cell surface and then penetrate through the plasma membrane into the cytoplasm. Moreover, polyphenols can affect lipid membranes' physical properties, including diffusion, solubility, osmotic stability, permeability to water-soluble compounds, and can interact with cell membranes and fusion. One group of polyphenols, such as flavonoids, also have a role in brain cell culture. Its antioxidant ability can protect cells from oxidative damage caused by reactive oxygen species (ROS) [19].

Flavonoids can protect cell line neurons from damage caused by hydrogen peroxide (H2O2) induction. They can increase cell viability at concentrations of 5 M, 10 M, 25 M, 50 M, 100 M, and 250 M (Dajas et al., 2003). According to Widayati (2019), flavonoids can stabilize ROS by reacting with free molecules, forming relatively stable radicals and lasting a long time until they react with non-radical products. In addition to polyphenols, propolis extract also contains terpenoids and tannins. Terpenoids are dehydrogenating and oxygenating derivatives of terpene compounds that can protect neurons against -amyloid peptides, glutamate, NO, oxygen, glucose loss, and other toxic stimuli by preventing apoptosis by targeting multiple kinases, increasing ROS clearance, and protecting mitochondrial integrity [20].

Meanwhile, tannins are a group of complex phenolic compounds, polyphenols, and flavonoids that can maintain cell membrane integrity because they have sufficient gallic acid residue to interact with all lipid head groups and cover the surface of the bilayer. If the integrity of the cell membrane is maintained, the permeability of the membrane can function correctly [21]. It causes material transportation in and out of the cell to run smoothly so that cell biological activities such as cell growth and development can run optimally. The compounds contained in the propolis extract can ultimately protect and support the survival of brain cells, thereby increasing cell viability. If the cell viability increases, the time used to proliferate will be faster [22].

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CONCLUSION

Propolis extract can protect neurons from injury by enhancing the brain-derived neurotrophic factor (BDNF) gene expression in neuron cultures obtained from cell lines. The mechanism of propolis extract in increasing BDNF gene expression in cells occurs because propolis extract contains Caffeic acid phenylethyl ester (CAPE) compounds which can increase extracellular signal-regulated kinase (ERK) activation. Another compound in propolis extract that has a role in brain cell culture is polyphenols. Polyphenols are not only known as antioxidants but also as regulators of cell metabolism. In addition to polyphenols, propolis extract also contains terpenoids and tannins. Terpenoids are dehydrogenated and oxygenated derivatives of terpene compounds that can protect neurons against -amyloid peptide, glutamate, NO, oxygen, glucose loss, and other toxic stimuli by preventing apoptosis by targeting multiple kinases, increasing ROS clearance, and protecting mitochondrial integrity. Propolis from Apismellifera can be used as a stimulator of brain cell development, and further studies are needed using other experimental animals, including non-human primates.

REFERENCES

1. Douglas, M. R., Sponsler, D. B., Lonsdorf, E. V & Grozinger, C. M. County-level analysis reveals a rapidly shifting landscape of insecticide hazard to honey bees (Apis mellifera) on US farmland. *Sci. Rep.* **10**, 1–11 (2020).
2. Febriyanti, A. R., Ratnasari, R. T. & Wardhana, A. K. The Effect of Economic Growth, Agricultural Land, and Trade Openness Moderated By Population Density on Deforestation in OIC Countries. *Quant. Econ. Manag. Stud.* **3**, (2022).
3. Barkah, T., Rusgianto, S. & Wardhana, A. Impact of Agricultural Land and the Output of Agricultural Products Moderated with Internet Users toward the Total export of Agricultural Product in Three Islamic South East Asian Countries. *Media Agribisnis* **6**, (2022).
4. Himawan, R. G., Primarizky, H., Mafruchati, M., Triakoso, N. & Maslachah, L. Green Tea Leaves Extract Effect on Histopathology of Mercury Chloride Induced Rat's Liver. *Pollut. Res.* **40**, 326–329 (2021).
5. El-Seedi, H. R. *et al.* A Spotlight on the Egyptian Honeybee (Apis mellifera lamarckii). *Animals* **12**, 2749 (2022).
6. Yudaniayanti, I. S., Primarizky, H. & Nangoi, L. The effects of honey (Apis dorsata) supplements on increased bone strength in ovariectomized rat as animal model of osteoporosis. in *AIP Conference Proceedings* vol. 1945 20004 (AIP Publishing, 2018).
7. Mafruchati, M. Identification of Moniezia, Sp in goat intestines in Indonesia which can impede goat productivity. *Syst. Rev. Pharm.* **11**, 1341–1345 (2020).
8. Ghabanchi, J., Bazargani, A., Afkar, M. D., Foroshan, S. B. & Ayeen, S. D. In vitro assessment of anti-Streptococcus mutans potential of honey. *Iran. Red Crescent Med. J.* **12**, 61 (2010).

9. Mafruchati, M., Wardhana, A. K. & Ismail, W. I. W. Disease and viruses as negative factor prohibiting the growth of broiler chicken embryo as research topic trend: a bibliometric review. *F1000Research* **11**, 1124 (2022).

10. Bicudo de Almeida-Muradian, L. *et al.* Standard methods for Apis mellifera honey research. *J. Apic. Res.* **59**, 1–62 (2020).

11. Nur, M. O. *et al.* Profil bakteri non spesifik dalam lendir serviks sapi perah pada fase folikuler dan fase luteal. *Ovozoa J. Anim. Reprod.* **9**, 17–22 (2020).

12. Luqman, E. M., Mafruchati, M., Rahardjo, B. P. S. & Hestianah, E. P. Crosstalk between Necrosis and Apoptosis of Embryonal Cerebral Cortex Neuron Mice (Mus musculus) Caused by Carbofuran Exposure. *Res. J. Pharm. Technol.* **12**, 5492–5498 (2019).

13. Yahya Al-ma'ruf, A. *et al.* Morphology and histology of paryphasmata and hemibaculum of Varanus salvator based on sexual maturity. *Open Vet. J.* **11**, 330–336 (2021).

14. Yip, C. W., Hon, C. C., Zeng, F. & Leung, F. C. C. Cell culture-adapted IBDV uses endocytosis for entry in DF-1 chicken embryonic fibroblasts. *Virus Res.* **165**, 9–16 (2012).

15. Mahaputra, L., Mafruchati, M., Triakoso, N. & Aries, R. D. Pemisahan Spermatozoa Sapi Limousin yang Memiliki Kromosom X dan Y dengan Percoll dan Putih Telur Ayam. *J. Bina Praja* **14**, 172–175 (2012).

16. Sunariani, J., Khoswanto, C. & Irmalia, W. R. Difference of brain-derived neurotrophic factor expression and pyramid cell count during mastication of food with varying hardness. *J. Appl. Oral Sci.* **27**, (2019).

17. Mafruchati, M. Duck meat, delicious yet low in unsaturated fat; comparative study between duck meat consumption in asean toward the number of covid cases. *Syst. Rev. Pharm.* **11**, (2020).

18. Murtaza, G. *et al.* Caffeic acid phenethyl ester and therapeutic potentials. *BioMed Res. Int.* **2014**, (2014).

19. Aengwanich, W. & Suttajit, M. Effect of polyphenols extracted from Tamarind (Tamarindus indica L.) seed coat on physiological changes, heterophil/lymphocyte ratio, oxidative stress and body weight of broilers (Gallus domesticus) under chronic heat stress. *Anim. Sci. J.* **81**, 264–270 (2010).

20. Dewi, S. K. *et al.* PENGARUH PEMBERIAN EKSTRAK KULIT PISANG KEPOK (Musa acuminata) TERHADAP PENURUNAN KADAR MALONDIALDEHID (MDA) PARU MENCIT JANTAN (Mus musculus) YANG DIPAPAR ASAP ROKOK. *J. Basic Med. Vet.* **8**, 123–130 (2019).

21. Mafruchati, M. Curcumin vs Chloroquine in Coronavirus Global Pandemic: Trend analysis Study in Google. *Indian J. Forensic Med. Toxicol.* **14**, 3138–3143 (2020).

22. Yunus, M. & Mafruchati, M. Eimeria tenella Pathogenicity Attenuation Through The Serial Passage of Precocious Lines Toward Number of Schizont and Cecal Histopathology In Broiler. *J. Poult. Sci.* **4**, 41–43 (2011).

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Testing Honey on Chicken Embryos as Candidates for Herbal Medicine in Indonesia

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Abstract

The nutrients in honey had function as antioxidants. The aim of this study was to testing honey on chicken embryos as candidates for herbal medicine in Indonesia. This study used treatment method, consisting of 6 (six) doses of honey products. The solution volume of each treatment dose was fixed at 10 l/egg via the route to the air bag. At the age of 13 days, embryo morphology and possible abnormalities were observed in all treatments. Each two eggs are cracked. The remainder was continued by incubation in the incubator until day 21. During this time the eggs were observed and the number was recorded to find out which eggs would die and which eggs would hatch at the end of the incubation period. Newborn chicks (DOC) were weighed and observed for possible morphological abnormalities. From the results of the research conducted, it was found that the percentage of viable chicken eggs sprouted after being injected with honey (days 13-21 after inoculation) and the morphology of chicken embryos after being injected with honey. Chicken Embryo Weight and Body Length Measurements were carried out in chicken embryos. The conclusion of the study is that propolis from honey products can be used as herbal medicine.

Keywords: Chicken, Embryo, honey, herbal medicine

Abstrak

Nutrisi dalam madu berfungsi sebagai antioksidan. Penelitian ini bertujuan untuk menguji madu pada embrio ayam. Penelitian ini menggunakan metode pengobatan yang terdiri dari 6 (enam) dosis produk madu. Volume larutan dari masing-masing dosis pada observasi dengan perlakuan ditetapkan pada 10 l/telur melalui rute ke kantong udara. Pada umur 13 hari diamati morfologi embrio dan kemungkinan kelainan pada semua perlakuan. Setiap dua telur dipecahkan. Selebihnya dilanjutkan dengan inkubasi di dalam inkubator hingga hari ke-21. Selama itu telur diamati dan dicatat jumlahnya untuk mengetahui telur mana yang mati dan telur mana yang menetas pada akhir masa inkubasi. Anak ayam yang baru lahir (DOC) ditimbang dan diamati kemungkinan kelainan morfologinya. Dari hasil penelitian yang dilakukan didapatkan persentase telur ayam yang hidup berkecambah setelah diinjeksi madu (hari ke 13-21 setelah inokulasi) dan morfologi embrio ayam setelah diinjeksi madu. Pengukuran Berat Embrio Ayam dan Panjang Badan dilakukan pada embrio ayam. Kesimpulan dari penelitian ini adalah propolis dari produk madu dapat digunakan sebagai obat herbal.

Keywords: ayam, embrio, madu, obat herbal

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INTRODUCTION

Honey is a thick liquid produced by bees from various nectars that still contain the active diastase enzyme. Various advantages of honey as a high nutrient food have been known since ancient times [1]. Total cholesterol concentrations could be reduced by administering 75 grams of honey every day. Research by [2] showed that honey can reduce lipid peroxide and malonaldehyde or often abbreviated as MDA. The mechanism for reducing cholesterol and MDA in honey administration is thought to be due to the antioxidant activity contained in honey.

Pure honey contains several nutrients such as carbohydrates, proteins, amino acids, vitamins and minerals [3]. The vitamins contained in honey include Vitamins, flavonoids, as well as antioxidant minerals [4]. The nutrients in honey that function as antioxidants are vitamins C, B3, organic acids, enzymes, phenolic acids, flavonoids, vitamin A and vitamin E, thus honey contains many nutrients that function as antioxidants [5].

Embryo development requires increased nutrition, oxygen, and removal of cellular metabolic wastes [6]. This increased need cannot be met by diffusion, so a new system is needed to ensure the survival and development of the embryo. The first thing that happens is the differentiation of mesenchymal cells (hemangioblasts) into endothelial cells so that new blood vessels are formed de novo. This event is called vasculogenesis [7].

Vasculogenesis is regulated by various factors with a complex sequence of events. Hemangioblast precursor cells depend on a variety of growth factors [8], including basic fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF). VEGF is very important in the events of embryonic angiogenesis [9]. VE-cadherin (Vascular-endothelial cadherin), a protein that plays a role in adhesion to the endothelium also has molecular interactions with the VEGF receptor [10].

VE-Cadherin is exclusively expressed in endothelial cells and expressed only in angioblasts. VE-Cadherin is known to play a role in regulating adherent junctions in endothelial cells that regulate intercellular adhesion, permeability, and endothelial cell migration [11]. This event is followed by angiogenesis, which is the formation of new blood vessel branches through the elongation of pre-existing small blood vessels [12]. Angiogenesis is required in the process of embryonic growth, as well as the process of postnatal physiological repair[13]. The process of vasculo-angiogenesis in embryos is often studied using

chicken embryos (and other aves families) because it is easier to study, and to reduce the use of mammals as experimental animals [14].

Chicken eggs are one of the media for the growth of various types of viruses such as the New Castle Disease (ND) virus, Avian Influenza, and Measles [15]. In recent years, embryonic chicken eggs have been widely used as a model for studying the process of tumor development and tumor treatment in humans and the impact of addictive substances such as alcohol on human fetuses [16]. This in vivo testing method has several advantages over in vitro testing using cell culture because it does not require media and complicated laboratory conditions so that the costs required are relatively low [10].

Embryo egg as a dynamic biological system is expected to describe in vivo conditions [17]. The in vivo condition in question is the ongoing metabolism and development of embryonic cells in the egg. Chemicals, including antiviral agents, can also be inoculated into eggs [18]. The effect of these substances on viruses and embryos is influenced by the age of the embryo, the application of the route of administration to the egg (embryo, allantois, yolk sac, air sac, amnion), the ability to absorb substances by the embryo, and the pharmacological structure of the substance itself [19]. The aim of this study was to examine the toxicity of honey products as a potential protective and therapeutic agent in chicken embryos. The aim of this study was to testing honey on chicken embryos as candidates for herbal medicine in Indonesia.

METHODOLOGY

This study used experimental method to determine the effect of Honey products on the development of chicken embryos, according to the study conducted by [20]. An experiment was designed by giving a series of doses of Honey to a number of chicken eggs that sprout [21]. In this case the treatment consists of 6 (six) levels of honey product dosages, namely:

(1) control (0 ng of honey /eggs);
(2) 15.6 ng of honey /egg products;
(3) 31.2 ng of honey /egg products;
(4) 62.5 ng of honey /egg product;
(5) 125 ng honey /egg product;
(6) 250 ng honey /egg.

The volume of solution from each treatment dose was set at 1 ml/egg, injected via the allantois space route. Each treatment (treatments 1 to 6) was injected into 100 brood chicken eggs aged 12 days in the air bag line with a volume of 1 ml/egg. The control (treatment 1) was only injected with a solution without honey products with the same volume of 1 ml in 12-day-old sprouted chicken eggs. The injection

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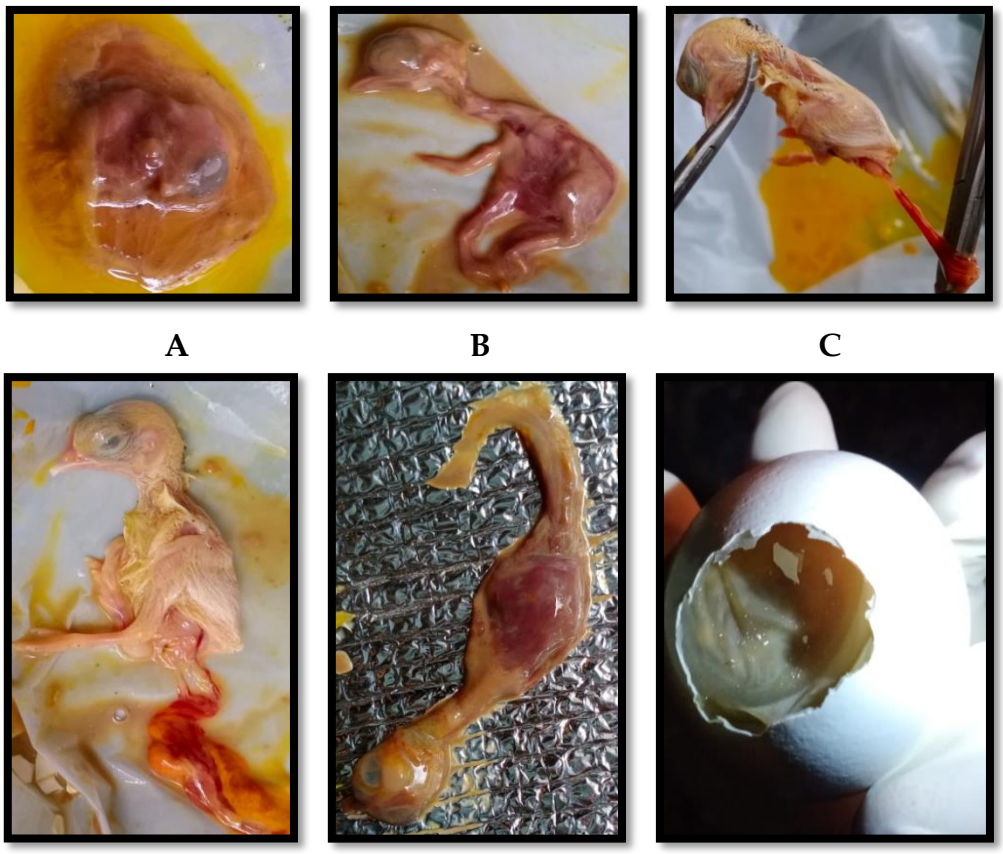
hole was closed again with liquid paraffin and incubated in an incubator and observed (candling) every day. At the age of 21 days, the embryo morphology and possible abnormalities were observed in all treatments, two eggs were broken each.

Newborn chicks (DOC) were weighed and observed for possible morphological abnormalities. The design used in this study was a simple completely randomized design (CRD) with 6 (six) treatments. To see the effect of treatment on chick body weight (DOC), the data obtained were analyzed by means of variance [22]. While other data are presented based on the percentage of embryonic mortality by comparing between treatments. This was according to the study conducted by [23].

RESULT AND DISCUSSION

From the results of the research conducted, it was found that the chicken embryos that had been injected with honey with various concentrations were as follows:



A **B** **C**

D **E** **F**

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Figure 1 (A-F). Observation of embryo morphology in each treatment. A. control (0 ng of honey /egg); B.15.6 ng of honey /eggt; C. 31.2 ng of honey /egg; D. 62.5 ng honey /egg; E.125 ng honey /egg products; F. 250 ng honey /egg products

From the results of the research conducted, it was found that on day 21 obtained embryo morphology of each treatment, in the control treatment group (0 ng honey/eggs); 15.6 ng honey/eggs; 31.2 ng of honey/egg products; 62.5 ng of honey/egg product; 125 ng of honey/egg product an abnormal embryological picture was obtained. At a concentration of 250 ng of honey/egg product, an abnormal embryological picture was obtained.[24]

Study by Dewi et alreported that hatchability was not affected when eggs were injected with amino acids, vitamin C, hormones, carbohydrates, and pollen extracts, respectively. Moreover, it also reported that in-ovo injection with vitamin C, ascorbic acid, carbohydrates, and glucose, respectively, had no significant effect on embryonic mortality.In this study, it was found that the weight of the chicken embryo after being injected with honey on the 21st day in each treatment and the length of the body part of the chicken embryo at the age of 21 days after being injected with honey in each treatment. In-ovo injection of some nutrients can cause nutritional imbalances in the eggs, and consequently can limit the maximal growth and development of the embryo during incubation [25].

CONCLUSION

The conclusion of this study was that the morphological description of the embryo on the 21st day after injection, at a concentration of 250 ng of honey/egg product, an abnormal embryological picture was obtained. Chicken Embryo Weight and Body Length Measurements were carried out in chicken embryos. The conclusion of the study is that honey can be used asherbal medicine. Suggestions for this research are: further testing was carried out using various experimental animals, further testing was carried out using different parameters and doses.

REFERENCES

1. Febriyanti, A. R., Ratnasari, R. T. & Wardhana, A. K. The Effect of Economic Growth, Agricultural Land, and Trade Openness Moderated By Population Density on Deforestation in OIC Countries. *Quant. Econ. Manag. Stud.* **3**, (2022).

2. Mundo, M. A., Padilla-Zakour, O. I. & Worobo, R. W. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *Int. J. Food Microbiol.* **97**, 1–8 (2004).

3. Barkah, T., Rusgianto, S. & Wardhana, A. Impact of Agricultural Land and the Output of Agricultural Products Moderated with Internet Users toward the Total export of Agricultural Product in Three Islamic South East Asian Countries. *Media Agribisnis* **6**, (2022).

4. Bicudo de Almeida-Muradian, L. *et al.* Standard methods for Apis mellifera honey research. *J. Apic. Res.* **59**, 1–62 (2020).

5. Prasetyo, R. & Safitri, E. TOPICAL HONEY TO TREAT AN ABSCESS CAUSED BY STAPHYLOCOCCUS AUREUS: A CASE REPORT. *SOUTHEAST ASIAN J. Trop. Med. PUBLIC Health* **49**, 835–838 (2018).

6. Santiparadon, M., Rongpan, S., Payanglee, K., Hounkong, K. & Koedpuech, K. Morphologic Changes of the 3-day-old Chick Embryos after Curcumin Exposure. *Princess Naradhiwas Univ. J.* **13**, 309–318 (2021).

7. Blatchley, M. R., Hall, F., Wang, S., Pruitt, H. C. & Gerecht, S. Hypoxia and matrix viscoelasticity sequentially regulate endothelial progenitor cluster-based vasculogenesis. *Sci. Adv.* **5**, eaau7518 (2019).

8. Mahaputra, L., Mafruchati, M., Triakoso, N. & Aries, R. D. Pemisahan Spermatozoa Sapi Limousin yang Memiliki Kromosom X dan Y dengan Percoll dan Putih Telur Ayam. *J. Bina Praja* **14**, 172–175 (2012).

9. Zarychta, E. & Ruszkowska-Ciastek, B. Cooperation between angiogenesis, vasculogenesis, chemotaxis, and coagulation in breast cancer metastases development: pathophysiological point of view. *Biomedicines* **10**, 300 (2022).

10. Mafruchati, M. Identification of Moniezia, Sp in goat intestines in Indonesia which can impede goat productivity. *Syst. Rev. Pharm.* **11**, 1341–1345 (2020).

11. Duong, C. N. *et al.* Interference with ESAM (endothelial cell-selective adhesion molecule) plus vascular endothelial-cadherin causes immediate lethality and lung-specific blood coagulation. *Arterioscler. Thromb. Vasc. Biol.* **40**, 378–393 (2020).

12. Wardhana, A. K. The Application of Waqf and Endowment Fund Based on the Principles in the Sharia Maqashid Pillar Society. *Prosper. J. Soc. Empower.* **1**, 107–119 (2021).

13. Yu, W.-K. *et al.* Vascular endothelial cadherin shedding is more severe in sepsis patients with severe acute kidney injury. *Crit. Care* **23**, 1–9 (2019).

14. Mafruchati, M. Duck meat, delicious yet low in unsaturated fat; comparative study between duck meat consumption in asean toward the number of covid cases. *Syst. Rev. Pharm.* **11**, (2020).

15. Sato, M., Tachibana, T., … M. F. P. P. A. M. & & 2006, undefined. Heat production and lipid metabolism in broiler and layer chickens during embryonic development. *Elsevier*.

16. Mafruchati, M., Epy, L. M., Neni, H. & Widjiati, P. B. Pemeriksaan Membran Spermatozoa Pada Semen Beku Domba Setelah Diencerkan. *Penelit. Fak. Kedokt. Hewan Univ. Airlangga Hal* 1–6 (1997).

17. Wardhana, A. K. & Ratnasari, R. T. Analisis sitasi publikasi tentang repositori bidang studi perpustakaan pada Web of Science selama pandemi. *Daluang J. Libr. Inf. Sci.* **2**, 53–61 (2022).

18. Yahya Al-ma'ruf, A. *et al.* Morphology and histology of paryphasmata and hemibaculum of Varanus salvator based on sexual maturity. *Open Vet. J.* **11**, 330–336 (2021).

19. Nur, M. O. *et al.* Profil bakteri non spesifik dalam lendir serviks sapi perah pada fase folikuler dan fase luteal. *Ovozoa J. Anim. Reprod.* **9**, 17–22 (2020).

20. Mawarid, M. M., Mafruchati, M., Suprayogi, T. W. & Widjiati, H. V. F. Potency of Kebar Grass (Biophytum

petersianum) Extract to Histopathological of Mice (Mus musculus) Duodenum at Lactation Period Exposed to Carbofuran. *J Vet Mar. Res* **1**, 1–9 (2021).

21. Mafruchati, M. & Makuwira, J. Identification of CF Mutations and Clinical Symptoms in CBAVD Patients. *Pharmacogn. J.* **14**, (2022).

22. Juliansyah, A. F., Putri, A. E., Suryadana, M. L., Endyana, C. & Wardhana, A. K. Global Muslim Response to Bandung Halal Tourism Branding. *Int. J. Appl. Sci. Tour. Events* **5**, 197–206 (2021).

23. Himawan, R. G., Primarizky, H., Mafruchati, M., Triakoso, N. & Maslachah, L. Green Tea Leaves Extract Effect on Histopathology of Mercury Chloride Induced Rat's Liver. *Pollut. Res.* **40**, 326–329 (2021).

24. Dewi, S. K. *et al.* PENGARUH PEMBERIAN EKSTRAK KULIT PISANG KEPOK (Musa acuminata) TERHADAP PENURUNAN KADAR MALONDIALDEHID (MDA) PARU MENCIT JANTAN (Mus musculus) YANG DIPAPAR ASAP ROKOK. *J. Basic Med. Vet.* **8**, 123–130 (2019).

25. Pratama, Y. B., Wardhana, A. K. & Nugroho, P. A. HUBUNGAN ANTARA ARTIKEL MENGENAI GAME DAN TEKNOLOGI INFORMASI PADA SCOPUS: STUDI BIBLIOGRAFI. *VISI PUSTAKA Bul. Jar. Inf. Antar Perpust.* **22**, (2020).

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Familiar Edible Flowers in Indonesia

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Abstract

Flowers besides being used as ornamental plants, they can also be consumed. Flowers that can be consumed are called Edible Flowers. Edible flowers in general can be consumed directly, usually in tea or can be served in the form of processed food. Edible flowers contain phytochemical compounds such as anthocyanins, flavonoids, phenolics, carotenoids which are useful as antioxidants. Indonesia is rich in biodiversity with a variety of plant species that can grow, including edible flowers. There is diversity, but only a few edibles that can grow and are familiar to Indonesian people will be reviewed in this article.

Abstrak

Bunga selain berguna sebagai tanaman hias, ternyata juga bias dikonsumsi. Bunga yang dapat dikonsumsi dinamakan Edible Flowers atau bunga edible. Bunga edible secara umum dapat dikonsumsi secara langsung, biasanya dalam seduhan teh maupun dapat disajikan dalam bentuk olahan pangan. Bunga edible mengandung senyawa-senyawa fitokimia seperti antosianin, flavonoid, fenolik, karotenoid yang bermanfaat sebagai antioksidan. Indonesia kaya akan keanekaragaman hayati dengan berbagai macam spesies tanaman yang dapat tumbuh, termasuk diantaranya bunga edible. Keanekaragaman yang yang ada, namun hanya beberapa edible yang dapat tumbuh dan familiar di masyarakat Indonesia yang akan diulas dalam artikel ini.

Keywords: *Antioxidants, Biodiversity, Edible Flowers, Phytochemicals,*

Kata kunci: Antioksidan, Bunga edible, Fitokimia, Keanekaragaman Hayati.

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INTRODUCTION

Flowers can not be separated from the part of human life. Since ancient times, flowers have been used as ornamental plants, ceremonial events, flower gardens, food coloring, flavoring, and dish decoration. Interest based on consumption is divided into two, edible and non-edible flowers. Edible flowers are flowers that can be consumed, both in tea and processed food and drinks[1]. Edible flowers are a cultural heritage that has been known for centuries, such as natural medicine, coloring, dish decoration, flavoring, and also used as a religion[2]. Several edible flowers such as roses (*Rosa sp*) have been used since ancient Rowawi times as sweeteners, jams and drinks and desserts[1]. Gemitir flowers (*Tagetes Erecta*) in Mexico are used as traditional medicines, analgesics, antiseptics and in India as religious ceremonies [3]. Edible flowers can thrive in tropical countries including in Indonesia.

Edible flowers are found in Indonesian residential areas even though the name of the edible flower itself is not familiar to Indonesians. There are various species of edible

flowers, but some of them can grow in Indonesia. This literature study aims to provide information on edible flowers that grow in Indonesia.

METHODOLOGY

This review article uses the scientific literature study method. Data collection was collected online through ScienceDirect, Google Scholar and Google. The search for library sources was carried out by collecting literature studies on flowers that grow and are familiar in Indonesia. Flowers in Indonesia are grouped into edible and non-edible flowers.

Flowers in the edible flowers group and found in Indonesia are searched for the keyword "Antioxidant activity of Tagetes erecta Linn extract." or "Antioxidant activity of *Ordorato Canaga* Linn. extracts". The literature used in compiling this review article comes from national and international journals. Appropriate journals are then reviewed in their entirety, and presented in the form of a review of literature studies. The number of journals used is 28 journals which are then ranked into 1 literature study.

RESULT AND DISCUSSION

Buteterfly pea Flowers (Clitoria ternatea L.)

Butterfly pea (*Clitoria ternatea* L.) is a medicinal plant that thrives in Asia. Based on the identification of the seeds, this flower belongs to the Fabaceae family (*legumes*). Butterfly pea has potential health benefits, such as antioxidant, anticancer, antipyretic, anti-inflammatory, hypolipidemic, cardiovascular and analgesic. The anthocyanin content causes this flower to have a bright blue color which is the main characteristic of this flower[4].

Previous studies reported that butterfly pea has phenolic activity as hepaprotective and nephronprotective which is characterized by a decrease in liver function parameters such as *serum glutamate pyruvate transaminase* (SGPT), *serum glutamate oxaloacetate transaminase* (SGOT), alkaline phosphate (ALP) & total serum bilirubin (SB) and decreased kidney function parameters such as urea and creatinine levels in experimental animal models of hepatotoxicity and nephrontoxicity[5].

Butterfly pea functions as an antiproliferative. Previous research reported that Butterfly pea can significantly increase apoptosis and necrosis in colorectal cancer cells (HCT116), TT (thyroid cancer cells), and MCF-7 (breast cancer cells)[4]. This is because butterfly pea extract contains many polyphenolic compounds including anthocyanins, which have antioxidant activity with implications for cancer therapy[6]

Marigold Flower (Tagetes erecta L.)

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Marigold flowers (*Tagetes erecta* L.) are ornamental flowers used in religious ceremonies in Bali and India. Marigold flowers are characterized by yellow or orange color with flowers that are fat, slightly rounded, and have overlapping petals[7]. This flower has activity as an antioxidant, antibacterial, and hepatoprotective activity due to the content of bioactive compounds in this flower, such as alkaloids, essential oils and flavonoids, terpenoids, alkaloids, and saponins[8,9].

Marigold Flowers has antioxidant activity. Previous studies reported that essential oil of marigold flowers is effective as a free radical scavenger, and the flavonoid content present in marigold flowers has antioxidant activity such as radical scavengers and the ability to chelate metal ions[10]. Previous studies reported that the activity of lutein compounds contained in marigold flower supplements acts effectively as an antioxidant in the eyes, this is due to lutein's ability to neutralize free radicals formed by ultraviolet radiation on the retina[11]

Marigold flowers have activity as a hepatoprotective. Previous research reported that marigold flower extract can significantly reduce serum levels of markers of liver enzymes damaged by CCl4 (SGOT, SGPT, ALP, and total bilirubin). Liver histopathology showed that marigold flower extract reduced liver lesions such as liver cell enlargement, lymphocyte infiltration, liver necrosis, and proliferation of fibrous connective tissue caused by CCl4. Therefore, the results of this study indicate that marigold flower extract can protect the liver against oxidative damage due to CCl4[12].

Marigold flowers function as antidiabetic and antihyperlipidemic. Previous research reported that the activity of quercetagetin extracted from marigold flowers showed that gemitir flowers could prevent the activity of α-glucosidase and pancreatic lipase, as well as α-amylase, so that quercetagetin acts as a treatment for diabetes and obesity[13].

Rosella Flower (Hibiscus sabdariffa L.)

Rosella flowers (*Hibiscus sabdariffa* L.), known as rosella or red tea, belongs to the *Malvaceae* family, which is rich in anthocyanins and other bioactive compounds, which are associated with a number of health benefits such as decreased blood pressure and plasma cholesterol[14].

Rosella flowers have long been known as herbal medicine in the world. Rosella flowers contain phenolic compounds such as alkaloids, saponins, anthocyanins, sterols and tannins. Rosella flowers

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have long been consumed in the form of decoctions or teas which are useful in preventing diseases such as nephrontoxicity, hypertension, diabetes, coronary heart disease and cancer[15].

Rosella flowers have antidiabetic activity. Previous research reported that Rosella Flower Extract can reduce blood glucose levels in animal models of diabetic models[16]. Rosella flower has antihyperlipidemic activity. Previous research reported the effect of infusion of sabdariffa hibiscus in animal models of hyperlipidemia. The results of the study showed that infusion of rosella flowers can reduce lipid function parameters such as total cholesterol levels and triacylglycerides but not as high as the reduction with standard drugs (statins)[17].

Ylang-ylang Flower (Cananga ordorata L.)

Ylang-ylang flower (*Cananga ordorata* L.) is a plant that thrives in tropical and subtropical regions with a plant height of approximately 10 meters. Ylang ylang plants have stems that break easily at a young age. Ylang-ylang flower is one of the essential oil producers. Ylang ylang flowers are found in Southeast Asia, especially the Philippines, Thailand and Indonesia. Yellow-green and yellow ylang flowers can produce good quality oil[18].

Ylang ylang flower has hepatoprotector activity based on its ability as an antioxidant and anti-inflammatory. One of the hepatoprotector mechanisms in overcoming liver damage is by stabilizing free radicals that cause damage to liver tissue. Previous research showed that there was a decrease in SGOT/SGPT levels after being given ethanol extract of ylang flowers (*Cananga ordorata* L.) in experimental animals induced by CCL4[19].

Ylang-ylang flower has antihyperlipidemic activity. Previous research reported that there was a decrease in serum cholesterol levels after being given ylang flower extract. The content of flavonoids in ylang flowers functions as an antioxidant which has an effect on repairing serum lipids, modifying oxidized LDL, and increasing the basal metabolic rate. As antioxidants, flavonoids act as reducers of LDL levels[18].

Rose Flower (Rosa damascene L.)

Rose flower (*Rosa damascena* L.) is an ornamental flower plant that is much loved because of the beauty and fragrance of flowers. Roses originate from China which has spread to various countries, including Indonesia. In Indonesia, roses are enjoyed as cut flowers or used for traditional

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ceremonies. Since ancient times, rose essential oil has been used as a raw material for cosmetics, fragrances, aromatherapy, and various medical purposes. Red rose extract (*Rosa damascena* Mill) contains tannins, geraniol, nerol, citronellol, flavonoids which have antibacterial effects[20].

Rose flowers have phenolic compounds that act as antioxidants in hepatotoxicity. Hepatoprotective rosehip was carried out in experimental animals induced by CCl4 as a model of hepatotoxicity. In particular, hepatotoxicity may prolong sleep duration due to pentobarbital after carbon tetrachloride poisoning. The results showed that there was a 63% reduction in sleep duration. The observed hepatoprotective effect of rose flower extract was due to the flavonoids present in the extract, because these phytochemicals were claimed to be involved as hepatoprotectors in CCl4-induced toxicity[21].

Soka Flower (Ixora coccinea L)

Soka flower (*Ixora coccinea* L) is a flower in the Rubiaceae family. This flower is in the form of this shrub tree, spread in tropical countries of Asia and Africa. Soka flowers are used as ornamental plants and also traditionally to treat diarrhea, fever, headaches, wound medicine, and stomach ulcers[22].

Phytochemical tests from previous studies revealed that asoka flowers contain various bioactive compounds including flavonoids, alkaloids, glycosides, terpenoids and carbohydrates[23]. The compound content can inhibit bacterial growth by inhibiting bacterial cell wall synthesis, protein synthesis, and the formation of complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane [24].

Asoka flower has potential as an antimicrobial. Based on the results of research that has been done, extracts of sunflower seeds can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. This result proved the formation of a clear zone around the disc. Based on the results of this study, soka flower extract has the potential to have greater inhibition against Gram-positive bacteria (*Staphylococcus aureus*) compared to Gram-negative bacteria (*Escherichia coli*)[25].

Ashoka flower has the potential as an anti-infection. Previous studies reported that gel formulation with a concentration of 10% extract of softwoods (*Ixora coccinea* L) has the potential as a therapy for infections caused by Staphylococcus aureus bacteria. 10% dose of soft-shelled flower extract is proven to be able to heal infected wounds in experimental animals caused by the Staphylococcus aureus bacteria seen in a reduction in wound diameter until the 11th day[23].

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Chrysanthemum flower (Chrysanthemum Indicum L.)

Chrysanthemum flower (*Chrysanthemum Indicum* L.) is used as an ornamental flower plant which is classified as a herbaceous plant, with slitted leaf edges andserrated, and arranged alternately onbranch or trunk. Chrysanthemum flowers belong to the plants of the kenikir-kenikir tribe or Asteraceae which includes various types of Chrysanthemums Plant stems growerect, soft in structure and green in color[26].

Chrysanrhemum flower has the potential to cure various diseases such as atherosclerosis, diabetes and others. Chrysanthemum flowers have been used in traditional medicine to relieve inflammatory diseases, gastroenteric problems, hypertension, bladder-related disorders, and uterine diseases such as menstrual irregularities and infertility. Chrysanthemum flowers contain flavonoids such as linarin, apigenin, acetin, and luteolin. Linarin is known as the best representative compound, which has been reported to have neuroprotective, hepatoprotective, and osteogenic differentiation effects. Previous studies reported that chrysanthenum flowers can significantly reduce fasting glucose levels, hemoglobin A1C (HBA1C) levels and increase insulin levels in animal models of diabetes[27].

Chrysanthenum flowers have the potential as an antihyperglycemic. Previous studies reported that chrysanthemum flowers can reduce the function of lipid parameters such as triacylglycerides (TG), *total cholesterol*, and *low-density lipoprotein* (LDL-C), *high-density lipoprotein cholesterol* (HDL-C) and can reduce glucose levels and liver function parameters such as total protein, *serum glutamic pyruvic transaminase* (SGPT), *serum glutamic oxaloacetic transaminase* (SGOT) in animal models of atherosclerosis [28].

CONCLUSION

This literature study aims to provide information about flowers found in Indonesia that are safe and can be consumed and as a reference for further researchers about the potential for edible flowers that are familiar in Indonesia.

REFERENCES

1. Santos, I. C. dos & Reis, S. N. Edible Flowers: Traditional And Current Use. *Ornam. Hortic.* **27**, 438–445 (2021).
2. Suryanti, I., Mulyadiharja, S. & Widiyanti, N. Pertumbuhan Tanaman Gemitir (Tagetes erecta) Dengan Penggunaan Pupuk Organik dan Anorganik. *J. Mat.* **13**, 40–48 (2019).
3. Singh, P. *et al.* Chemistry and biology of industrial crop Tagetes Species: A review. *J. Essent. Oil Res.* **28**, 1–14 (2015).
4. ALshamrani, S. M. *et al.* Antiproliferative Effect of Clitoria ternatea Ethanolic Extract against Colorectal, Breast, and Medullary Thyroid Cancer Cell Lines. *Separations* **9**, 331 (2022).
5. Chandra, S. *et al.* Evaluation of Methanolic Extract of Clitoria ternatea

Hepatoprotective & Nephroprotective Activity in Rats. *J. Drug Deliv. Ther.* **9**, 313–319 (2019).

6. Fu, X. *et al.* Spectral characteristic, storage stability and antioxidant properties of anthocyanin extracts from flowers of butterfly pea (Clitoria ternatea l.). *Molecules* **26**, (2021).
7. Pramitha, D. A. I., Suaniti, N. M. & Sibarani, J. Aktivitas Antioksidan Bunga Pacar Air Merah (Impatiens balsamina L.) dan Bunga Gemitir (Tagates erecta L.) dari Limbah Canang. *Chim. Nat. Acta* **6**, 8 (2018).
8. XU, L. wei, CHEN, J., QI, H. yang & SHI, Y. ping. Phytochemicals and Their Biological Activities of Plants in Tagetes L. *Chinese Herb. Med.* **4**, 103–117 (2012).
9. Karwani, G. & Sisodia, S. S. Hepatoprotective activity of Chenopodium album Linn. in ethanol induced hepatotoxicity in rats. *Sch. Acad. J. Pharm.* **8**, 669–673 (2015).
10. Dubey, P. K., Ganeshpurkar, A., Dubey, S. & Kakkar, A. Isolation and studies on chemotherapeutic potential of aloin. *Int. J. Green Pharm.* **9**, 45–49 (2015).
11. Šivel, M., Kráčmar, S., Fišera, M., Klejdus, B. & Kubáň, V. Lutein content in marigold flower (Tagetes erecta L.) concentrates used for production of food supplements. *Czech J. Food Sci.* **32**, 521–525 (2014).
12. Vidyadhar, K. M. & Sharma, H. K. Hepatoprotective Effect of Tagetes Erecta L. Extract on Carbon Tetrachloride Induced Hepatotoxicity in Rats. *J. Drug Deliv. Ther.* **120**, 30–34 (2019).
13. Wang, W. *et al.* In vitro antioxidant, anti-diabetic and antilipemic potentials of quercetagetin extracted from marigold (Tagetes erecta L.) inflorescence residues. *J. Food Sci. Technol.* **53**, 2614–2624 (2016).
14. Zulfiqar, S., Benton, K., Hassan, T., Marshall, L. & Boesch, C. In vitro and in vivo anti-diabetic properties of Hibiscus sabdariffa. *Proc. Nutr. Soc.* **78**, 2019 (2019).
15. Pratiwi, A. T. Pengaruh Ekstrak Rosella (Hibiscus Kadar Gula Darah untuk Diabetes Melitus. *J. Ilm. Mhs. Kedokt. Indones.* **6**, 138–142 (2018).
16. Herdiani, N. & Wikurendra, E. A. Efek Antioksidan Ekstrak Kelopak Rosella Terhadap Glukosa Darah Tikus Diabetes Mellitus Tipe 2. *An-Nadaa J. Kesehat. Masy.* **7**, 89 (2020).
17. Tarmooz, A., Al-Yasiri, T., Archives, Y. A.-P. & 2020, U. the Use of Hibiscus Sabdariffa Infusion for the Prevention and Treatment of High Blood Lipids Compared With Statin Treatment. *Plant Arch.* **20**, 812–817 (2020).
18. Hamdan & Musniati, N. Ekstrak Bunga Kenanga terhadap Dislipidemia. *J. Holist. Tradit. Med.* **03**, 321–326 (2019).
19. Udayani, N. N. W., Meriyani, H. & Adrianta, K. A. Efektivas Bunga Kenanga (Cananga odorata Hook.F & TH) Sebagai Hepatoprotektor pada Tikus Putih (Rattus norvegicus) yang Diinduksi Carbon Tetra Chloride. *J. Ilm. Medicam.* **3**, 79–84 (2017).
20. Pasril, Y. & Okasari, D. Pengaruh Daya Anti Bakteri Ekstrak Bunga Mawar Merah (Rosa damascena Mill) Terhadap Pertumbuhan Enterococcus faecalis. *Insisiva Dent. J. Maj. Kedokt. Gigi* **9**, 26–30 (2020).
21. Karen Mulkijanyan *et al.* Pharmacological assessment of the aqueous extract of rose oil waste from Rosa x damascena Herrm cultivated in Georgia. *World J. Biol. Pharm. Heal. Sci.* **7**, 001–008 (2021).

22. Pratiwi *et al.* Evaluation of antibacterial and antioxidant activity of extracts of endophytic fungi isolated from Indonesian Zingiberaceous plants. *Nusant. Biosci.* **8**, 306–311 (2016).

23. Shafriyani, R. & Lestari, W. Formulasi Sediaan Gel Ekstrak Etanol Bunga Soka (Ixora coccinea L) Sebagai Terapi Infeksi Pada Kulit Yang Disebabkan Oleh Bakteri Staphyloccocus aureus. *J. Heal. Technol. Med.* **6**, 2615–109 (2020).

24. Fatimah, S., Nadifah, F. & Burhanudin, I. Uji Daya Hambat Ekstrak Etanol Kubis (Brassica oleracea var. capitata f. alba) Terhadap Bakteri Staphylococcus aureus Secara In Vitro. *Biog. J. Ilm. Biol.* **4**, 102–106 (2016).

25. Munira, Maisarah, R. & Nasir, M. Potensi Antibakateri Ekstrak Bunga Soka (Ixora coccinea L) terhadap Staphylococcus aureus dan Escherichia coli. *J. AcTion Aceh Nutr. J.* **1**, 130–134 (2016).

26. Dalaila, I., Kusrinah & Syaifudin, A. MORFOLOGI DAN ANATOMI Chrysanthemum morifolium Ramat. var. puspita nusantara dan var. tirta ayunisertaChrysanthemum indicum L.var. mustika kaniya. *Al-Hayat J. Biol. Appl. Biol.* **2**, 53 (2019).

27. Kim, Y. J., Kim, H. K. & Lee, H. S. Hypoglycemic effect of standardized Chrysanthemum zawadskii ethanol extract in high-fat diet/streptozotocin-induced diabetic mice and rats. *Food Sci. Biotechnol.* **27**, 1771–1779 (2018).

28. Sravanthi, P. & Shakir Basha, S. Anti-atherosclerotic activity of ethanolic extract of Chrysanthemum Indicum L. Flowers against high-fat diet-induced atherosclerosis in male Wistar rats. *Asian J. Pharm. Clin. Res.* **10**, 52–56 (2017).

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