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Phytochemical Profiling of *Xylocarpus granatum* Extract Using Thin-Layer Chromatography and Selective Derivatization Reagents

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Abstract
This study aims to conduct phytochemical screening and evaluate the pharmacological activities of Bulistem bark extract, particularly its antioxidant and antidiabetic properties. The research began with the preparation of an optimized standardized extract as the test material, followed by phytochemical screening to identify groups of secondary metabolite compounds, and continued with biological activity screening as preclinical testing. The phytochemical analysis revealed that the extract contains flavonoid, phenolic,saponin, coumarin, alkaloid.

Keywords: Buli stem bark, phytochemical screening, *Xylocarpus granatum*

Introduction

Buli or Nyirih (*Xylocarpus granatum* J. Koenig) is a mangrove tree belonging to the family Meliaceae. This species grows widely along various coastal areas of Sulawesi Island. It has long been used in traditional medicine to treat a range of ailments, including diarrhea, cholera, dysentery, fever, malaria, and viral infections. In addition, several parts of this plant have been reported to exhibit anticancer, antioxidant, antidiabetic, antimicrobial, antimalarial, and neuroprotective activities (1,2,3). The stem bark of Buli is one of the parts frequently utilized empirically, either as an ingredient in a traditional drink known as "tambu" or as a component of traditional herbal remedies. This plant part holds substantial potential for further development; however, research on its processing methods and biological activities remains very limited. Buli stem bark contains various classes of bioactive compounds, including alkaloids, flavonoids, lactones, and terpenoids. These compounds are presumed to be responsible for its biological activities (1). Research on *Xylocarpus granatum* remains relatively limited. Although this plant has considerable potential, the scientific data required for its optimal utilization are still insufficient. Other studies have also reported its anti-aging activity (4). Several previous investigations have documented its ethnopharmacological uses and some preliminary bioactivity findings. *X. granatum* is traditionally used by various ethnic groups to treat diarrhea, cholera, dysentery, fever, malaria, viral infections (5), and as a neuroprotective agent (6,7). Therefore, comprehensive and in-depth scientific studies are urgently needed.

At present, the government is actively promoting the development of standardized traditional medicines and encouraging their advancement toward standardized herbal medicines (OHT) and phytopharmaceuticals (FF). This study serves as an initial screening and exploratory investigation to scientifically substantiate empirical knowledge and provide foundational data for increasing the value and evidence-based utilization of Indonesian natural products as OHT and FF. This mandate is stipulated in the Regulation of the Head of the National Agency for Drug and Food Control (BPOM) Number 25 of 2023 concerning the criteria and procedures for the registration of traditional medicines, standardized herbal medicines, and phytopharmaceuticals. The stem bark of Buli represents a highly promising candidate as a raw material source for extract development.

A. *Research Method*

***Tools*:** Extraction apparatus set, a rotary vacuum evaporator, TLC kit set.

Materials: Ethanol 96%, TLC plate, chemical reagent

Procedure:

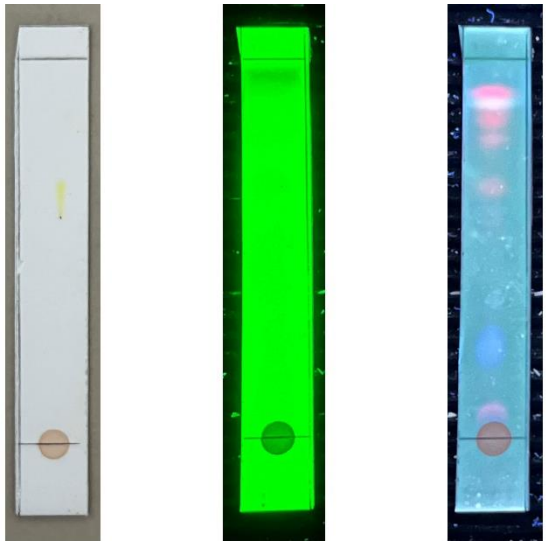
1. Extraction
   Extraction was carried out using the Ultrasonic-Assisted Extraction (UAE) method for 5, 15, and 30 minutes at temperatures of 10°C, 15°C, and 20°C. After the extraction process was completed, the extract was removed from the apparatus and stored in appropriate containers (8,14).
2. Phytochemical Screening with TLC Plate
   Phytochemical screening included tests for flavonoid, steroid, phenolic, saponin, coumarin, and alkaloid using the TLC method (9).

B. *Results and Discussion*

1. Results
   a. Phytochemical screening

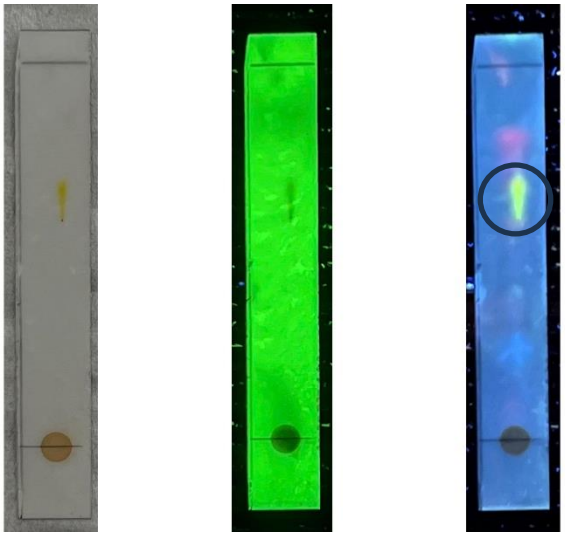
Table 1. Results of phytochemical screening using TLC method

Compound	Reagent	Result
Flavonoid	AlCl3	+
Steroid	Liebermann-Burchard	-
Phenolic	FeCl3	+
Saponin	Liebermann-Burchard	+
Coumarin	KOH	+
Alkaloid	Dragendorff	+



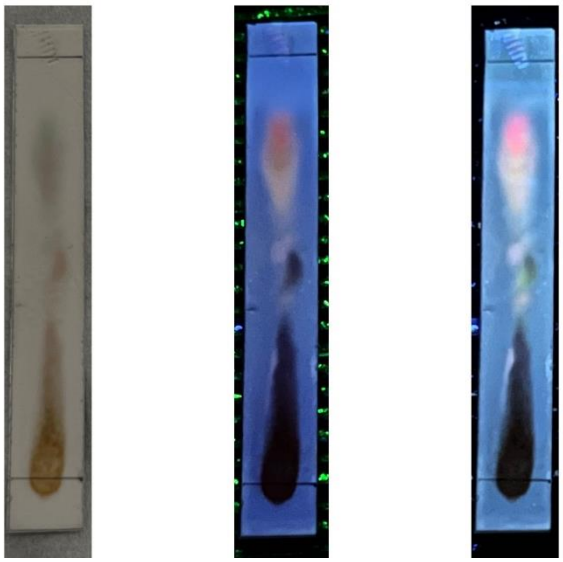
(a) (b) (c)

Figure 1. TLC Plate before sprayed



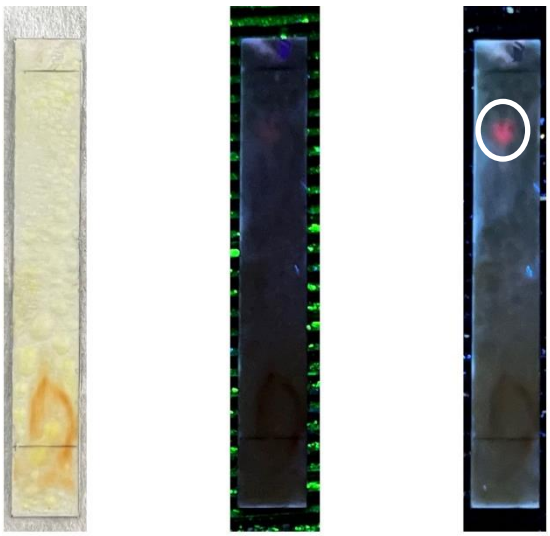
(a) (b) (c)

Figure 2. TLC Plate after sprayed with AlCl3 reagent for Flavonoid



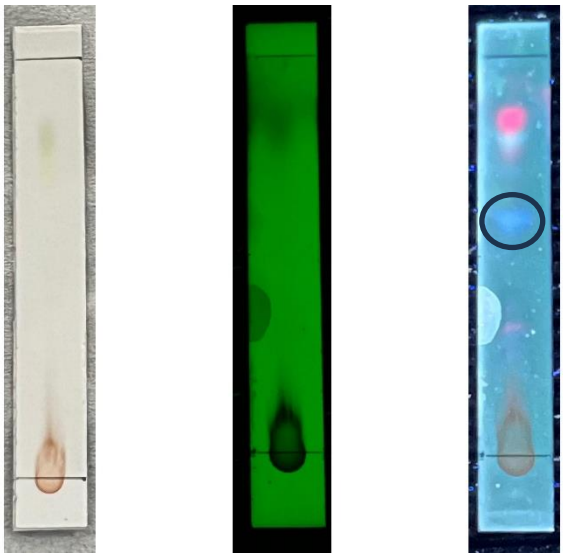
(a) (b) (c)

Figure 3. TLC Plate after sprayed with Liebermann-Burchard reagent for Steroid



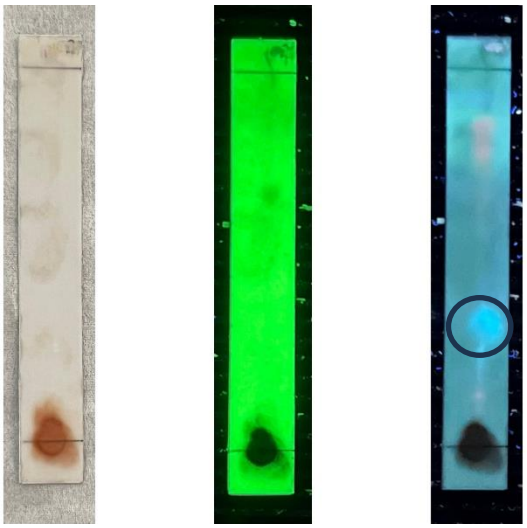
(a) (b) (c)

Figure 4. TLC Plate after sprayed with $FeCl_3$ reagent for Phenolic



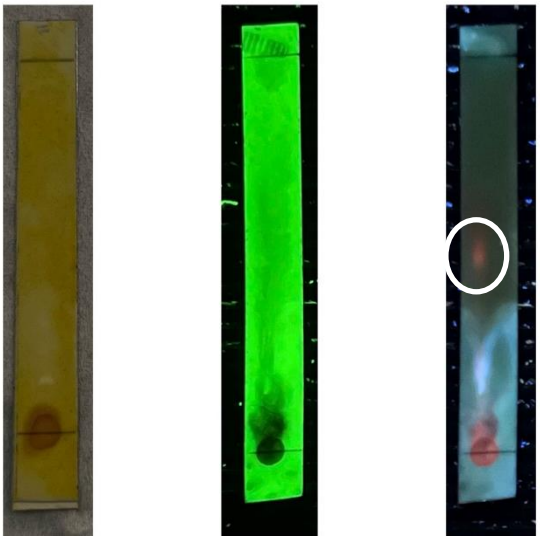
(a) (b) (c)

Figure 5. TLC Plate after sprayed with Liebermann-Burchard reagent for Saponin



(a) (b) (c)

Figure 6. TLC Plate after sprayed with KOH reagent for Coumarin



(a) (b) (c)

Figure 7. TLC Plate after sprayed with Dragendorff reagent for Alkaloid

2. Discussion

The findings demonstrate that *Xylocarpus granatum* extract exhibits marked antioxidant and α-glucosidase inhibitory activities. The confirmed presence of flavonoids strongly supports the extract's bioactivity, as these compounds commonly contribute to both radical scavenging and antidiabetic mechanisms. The extract nonetheless shows considerable pharmacological promise.

Thin-layer chromatography (TLC) analysis was conducted to identify the phytochemical constituents of *Xylocarpus granatum*. Prior to treatment with $AlCl_3$ (Figure 2), several chromatographic spots were visible under visible and UV light; however, the fluorescence was relatively faint. After spraying with $AlCl_3$ reagent (Figure 2), the spots exhibited much stronger fluorescence under UV 366 nm. This enhancement is typical of flavonoid–$AlCl_3$ complexes and confirms the presence of flavonoid compounds in the extract. These findings support earlier studies reporting that *X. granatum* contains flavonoids and phenolic metabolites associated with antioxidant activity.

Phenolics were confirmed using Ferric Chloride ($FeCl_3$) after TLC plate development. Upon spraying with $FeCl_3$, phenolic-containing spots produced a dark blue, green, or black coloration, indicating complex formation between phenolic –OH groups and Fe^{3+}. This response verifies the presence of phenolic compounds such as tannins and simple phenols, which often exhibit strong radical-scavenging properties.

Saponins were detected using TLC followed by derivatization with the Liebermann–Burchard (LB) reagent, a classical reagent for identifying steroidal and triterpenoid-based compounds. After developing the TLC plate and applying the LB reagent, saponin-containing spots typically underwent a color transformation to blue, green, or violet, depending on their triterpenoid or steroidal backbone. This color change results from the reaction between unsaturated sterol or triterpenoid nuclei within saponins and the acetic anhydride–sulfuric acid components of the LB reagent. The presence of saponins in X. granatum is notable, as these amphiphilic glycosides are associated with antioxidant and antidiabetic activity.

Coumarins were detected through TLC observation under UV 366 nm and subsequent treatment with potassium hydroxide (KOH). Coumarin-containing spots displayed blue or blue-green fluorescence, which intensified upon application of alcoholic KOH. This enhancement occurs due to the formation of ionized coumarin derivatives, confirming the presence of coumarin compounds known for antioxidant and enzyme-inhibitory activity.

Alkaloids were identified using Dragendorff's reagent following TLC separation. Spraying the plate with Dragendorff's reagent produced orange to reddish-brown precipitate-like spots, characteristic of alkaloid–bismuth iodide complex formation. This reaction confirms the presence of alkaloidal constituents, which may contribute to the extract's antidiabetic mechanism through interaction with metabolic enzymes.

C. Conclusion

Xylocarpus granatum extract was confirmed to contain flavonoids, phenolic, saponin, coumarin, and alkaloid that was tested using TLC method.

D. Acknowledgement

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References

1. Dey, D., Quispe, C., Hossain, R., Jain, D., Ahmed Khan, R., Janmeda, P., Islam, M. T., Ansar Rasul Suleria, H., Martorell, M., Daştan, S. D., Kumar, M., Taheri, Y., Petkoska, A. T., & Sharifi-Rad, J. (2021). Ethnomedicinal Use, Phytochemistry, and Pharmacology of Xylocarpus granatum J. Koenig. Evidence-based complementary and alternative medicine : eCAM, 2021, 8922196. https://doi.org/10.1155/2021/8922196
2. Lakshmi, V., Srivastava, S., Mishra, S. K., Srivastava, M. N., Srivastava, K., & Puri, S. K. (2012). Antimalarial activity in Xylocarpus granatum (Koen). Natural product research, 26(11), 1012–1015. https://doi.org/10.1080/14786419.2010.535000
3. Darmadi, J., Batubara, R. R., Himawan, S., Azizah, N. N., Audah, H. K., Arsianti, A., Kurniawaty, E., Ismail, I. S., Batubara, I., & Audah, K. A. (2021). Evaluation of Indonesian mangrove Xylocarpus granatum leaves ethyl acetate extract as potential anticancer drug. Scientific reports, 11(1), 6080. https://doi.org/10.1038/s41598-021-85383-3
4. Heryanto, R., Putra, C. A., Khalil, M., Rafi, M., Putri, S. P., Karomah, A. H., & Batubara, I. (2023). Antioxidant Activity and Metabolite Profiling of *Xylocarpus granatum* Extracts Using Gas Chromatography-Mass Spectrometry. *Metabolites*, *13*(2), 156. https://doi.org/10.3390/metabo13020156
5. Das, S. K., Samantaray, D., Sahoo, S. K., Pradhan, S. K., Samanta, L., & Thatoi, H. (2019). Bioactivity guided isolation of antidiabetic and antioxidant compound from *Xylocarpus granatum* J. Koenig bark. *3 Biotech*, *9*(5), 198. https://doi.org/10.1007/s13205-019-1711-y
6. Zhou, Z.-F. *et al*. Novel and Neuroprotective Tetranortriterpenoids from Chinese Mangrove *Xylocarpus granatum* Koenig. *Sci. Rep.* 6, 33908; doi: 10.1038/srep33908 (2016)
7. Noviarni I, Batubara I, Putri SP. Antiglycation and Antioxidant Activity from Methanol Extract and Fraction of Xylocarpus granatum Stem. *Jurnal Kimia Sains dan Aplikasi* [Online]. 2020 Jan;23(1):21-27. https://doi.org/10.14710/jksa.23.1.21-27.
8. Khasanah NDN, Rahmat M, Al-Kindi H. ANALISIS DISTRIBUSI UKURAN PARTIKEL TEH HIJAU HASIL EKSTRAKSI DENGAN METODE ULTRASONIC ASSISTED EXTRACTION (UAE) COLD BREW. ALMIKANIKA [Internet]. 2022 Feb. 19.
9. Syarif RA, Handayani V, Angraeni A. Standarisasi Ekstrak Etanol Buah Bintaro (Cerbera odollam Gaertn.) sebagai Obat Tradisional. J Fitofarmaka Indones. 2022;9(2):7–13. doi:10.33096/jffi.v9i2.592.
10. Chaves N, Santiago A, Alías JC. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. Antioxidants. 2020;9(1):76. doi:10.3390/antiox9010076
11. Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. 2nd ed. Springer; 2009.
12. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. Springer; 1998.
13. Mabry TJ, Markham KR, Thomas MB. *The Systematic Identification of Flavonoids*. Springer; 1970.
14. Markham KR. *Techniques of Flavonoid Identification*. Academic Press; 1982.
15. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates by means of Folin–Ciocalteu reagent. *Methods Enzymol*. 1999;299:152-178.

16. Harborne JB. *The Flavonoids: Advances in Research Since 1986*. Chapman & Hall; 1994.
17. Houghton P, Raman A. *Laboratory Handbook for the Fractionation of Natural Extracts*. Springer; 1998.
18. Hostettmann K, Marston A. *Saponins*. Cambridge University Press; 1995.
19. Sherma J, Fried B. *Handbook of Thin-Layer Chromatography*. 3rd ed. Marcel Dekker; 2003.
20. Dewick PM. *Medicinal Natural Products: A Biosynthetic Approach*. 2nd ed. Wiley; 2002.
21. Geissman TA. *The Chemistry of Flavonoid Compounds*. Macmillan; 1963.
22. Stahl E. *Thin-Layer Chromatography: A Laboratory Handbook*. Springer; 1969.
23. Vijayavel K, Martinez J. Phytochemical screening and antioxidant activity of *Xylocarpus granatum*. *J Med Plants Res*. 2012;6(7):1108-1112.
24. Abeysinghe PD. Bioactive constituents and antidiabetic properties of *Xylocarpus granatum*. *Asian J Tradit Med*. 2010;5(4):121-130.