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PHYTOCHEMICAL SCREENING OF *Phaleria Macrocarpa* (Scheff.) Boerl AND TOTAL PHENOLIC CONTENT OF WATER EXTRACT AS PRELIMINARY STUDY ON ANTIBACTERIAL ACTIVITY

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ABSTRACT

Phaleria macrocarpa (Scheff.) Boerl is a native flora grown in the tropical areas of Papua Island with notable medicinal properties. Biologically active compounds from the plant can be isolated using alcohol to isolate large amounts of phenolic contents. However, alcohol remains a main and controversial issue in the production of halal pharmaceuticals. This study explores the phytochemical constituents and total phenolic content of water extracts from the exocarp, mesocarp, and endosperm of *Phaleria macrocarpa* (Scheff.) Boerl as a halal-compliant alternative. The extraction was carried out with a maceration technique using water as a solvent. A preliminary phytochemical screening was conducted to identify the constituents and Thin-Layer Chromatography (TLC) was used to verify the results. Total phenolic content (TPC) was quantitatively assessed with UV-Vis Spectrophotometry using Folin-Ciocalteu`s colorimetric method. The results revealed the presence of flavonoids, polyphenols, tannins, alkaloids, and saponins. The TPC values measured in the exocarp, mesocarp, and endosperm extracts were 0.016, 0.004, and 0.003 mg GAE/g, respectively. These findings hold significant promise for the potential development of water extracts from *Phaleria macrocarpa* (Scheff.) Boerl is a halal-compliant antibacterial agent.

Keywords: *P. macrocarpa*, Phytochemistry, Phenolic Content, Halal Medicine.

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INTRODUCTION

Phaleria macrocarpa (Scheff.) Boerl popularly referred to as `Mahkota Dewa' is indigenous to the tropical areas of Papua Island in Indonesia.[1] It can grow between 1 and 18 meters tall and thrives at elevations of up to 1200 meters above sea level.[2] *Phaleria macrocarpa* (Scheff.) Boerl is a fully developed tree, showcasing all its vital components: roots, stems, leaves, flowers, and fruits.[1,2] Almost all parts of the plant have beneficial properties with medicinal activities.[1] Its fruit and seed are rich in secondary metabolites including phenols, tannins, flavonoids, and saponin glycosides, which contribute to their antioxidant and cytotoxic activities.[3] The leaves exhibited substantial antioxidant potential and showed a beneficial impact on heart health by reducing cholesterol levels and increasing high-density lipoprotein (HDL) levels.[4,5] Furthermore, the bark of the plant has been found to possess inhibitory properties against mouse leukemia L1210, attributed to the presence of benzophenone glucoside.[6] The diverse beneficial properties of this plant highlight its significance in traditional medicine as well as its potential uses in modern pharmacology. The extraction of biologically active compounds from plants has been a focus of extensive research. Various conventional methods have been employed to achieve this goal, including maceration, steam distillation, and Soxhlet extraction, each offering unique advantages and challenges.[7] Researchers have studied the impact of different solvents on the extraction process, specifically targeting bioactive compounds found in various parts of plants. Their findings revealed that highly polar solvents, particularly methanol, are effective in isolating substantial amounts of phenolic contents compared to other solvents.[8]

However, alcohol remains a main and controversial issue in the production of halal pharmaceuticals. Although solvents used in extractions might be removed at the end of the process, some residuals might unintentionally remain. It is imperative to ensure that pharmaceutical products comply with halal

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standards, as those containing non-halal ingredients are not considered halal until meeting these requirements. To address this concern, the authors conducted research focusing on *Phaleria macrocarpa* (Scheff.) Boerl by examining the phytochemical and phenolic content of water extract from the exocarp, mesocarp, and endosperm of the plant since no previous work on water extract of this plant has been reported. This research was a preliminary study of our research focusing on analyzing the antimicrobial activity of this plant as a halal-compliant source of bioactive compounds.

EXPERIMENTAL

Extraction Method

The plant materials were collected from Bandung, Indonesia, and determined at Institut Teknologi Bandung`s School of Life Sciences and Technology (SITH-ITB). Freshly collected plant materials were washed carefully with distilled water and allowed to air dry at room temperature until they became suitable for grinding. Extraction was carried out using the maceration technique. As much as 500 mg of the samples were put into a 1 L extraction flask. Into the samples were added 1 L of water and then heated at 90ºC for about 15 minutes.[9] The mixtures were filtered, and the filtrates were concentrated to obtain the water extract of *Phaleria macrocarpa* (Scheff.) Boerl.

Phytochemical Analysis

Phytochemical analysis was performed using qualitative methods as described by Wahab *et al.* (2020) and Shaikh & Patil (2020).[10,11] A detailed list of phytochemical constituents investigated, and identification methods of the samples can be found in Table-1.

Thin-Layer Chromatography

Silica gel 60 GF_{254} TLC plates were utilized for the separation and analysis of secondary metabolites. To initiate the process, a TLC chamber was pre-saturated with the mobile phase to ensure optimal conditions for development. Each TLC plate was developed to a height of 5.0 cm, allowing sufficient distance for the separation of the components within the sample. Once the development was complete, the plates were carefully removed and set aside to air dry. The spots were then visualized under visible light, as well as UV light at 254 and 366 nm. Subsequently, the plates were sprayed with various reagents: ammonium hydroxide for flavonoids, ferric chloride for tannins as polyphenols' derivatives, Dragendorff's reagent for alkaloids, and Liebermann-Burchard's reagent for saponins. Different mobile phases were utilized to characterize the secondary metabolites present in the samples. Each mobile phase was prepared using the solvent ratios specified in Table-2.

Total Phenolic Content

Initially, 100 mg of dried extracts were diluted in 100 mL of distilled water. Each 75 µL extract was piped into 4 mL of a 7% sodium carbonate solution and 0.4 mL of a 10% Folin-Ciocalteu reagent. This mixture was incubated for 2 hours to facilitate the reaction. Following the incubation, the absorbance was measured three times at its maximum wavelength. Using a calibration curve of standard gallic acid ranging from 2 to 6 µg/mL, the results were expressed as milligrams of gallic acid equivalents (mg GAE/g) per gram of extract.[12]

RESULTS AND DISCUSSION

Extraction

The plant materials were cut into small pieces to facilitate the drying process. Following drying, the samples were thoroughly ground to maximize the surface area, thereby enhancing the extraction efficiency of secondary metabolites through a maceration technique. As much as 1 L of water was added to 500 mg of the samples and then macerated at 90°C for 15 minutes, allowing for optimal extraction. The crude extracts were then concentrated to obtain viscous extracts of *Phaleria macrocarpa* (Scheff.) Boerl. The extraction yields obtained from the exocarp, mesocarp, and endosperm were 33.67%, 46.83%, and 9.64%, respectively. These percentages provide insight into the quantities of bioactive constituents extracted during the process.[13] The variability in yield can be attributed to several factors, such as the physical condition and the size of plant materials, the duration of extraction, temperature, and distribution of different compounds among different parts of the plants.[14]

Phytochemical Analysis

Phytochemical analysis serves as a crucial preliminary examination, providing valuable insights into the compounds present in extracts that could potentially exhibit antibacterial properties. The findings from this analysis are summarized in Table-1. The presence of flavonoids was confirmed through a color reaction with concentrated HCl and magnesium powder. This reaction results in the formation of an orange-to-red color, which serves as a visual indicator of the presence of flavonoids.[15] In addition to flavonoids, the extracts were also tested for polyphenols and tannins by employing iron (III) chloride as a reagent.[10] The phenolic hydroxyl groups present in tannins can interact with Fe^{3+} ions found in iron (III) chloride, leading to distinctive color changes. Specifically, hydrolyzed tannins produce a blue color upon reaction, while condensed tannins yield a green color.[16] The analysis demonstrated that both flavonoids and condensed tannins were present in all tested extracts.

Table-1: Phytochemical Analysis of *Phaleria macrocarpa* (Scheff.) Boerl

Phytochemical constituents	Identification method	Exocarp extract	Mesocarp extract	Endosperm extract
Flavonoids	HCl + Mg	+	+	+
Polyphenols and tannins	$FeCl_3$	+	+	+
Alkaloids	Dragendorff	+	+	+
	Wagner	+	+	+
	Mayer	+	+	+
Saponins	Foam formation	+	-	-
Triterpenoids	Liebermann-Burchard	+	+	+
Anthraquinone	Ammonia	-	-	-
Cyanohydrin	Picric acid	-	-	-

The identification of alkaloid compounds was performed through a systematic approach, involving the addition of Wagner, Mayer, and Dragendorff reagents to the water extracts that had been previously dissolved in ethyl acetate. A positive result for alkaloids was indicated by distinct precipitate formation: a red precipitate formed in the presence of Dragendorff's reagent, a brown precipitate in the presence of Wagner's reagent, and a white precipitate in the presence of Mayer's reagent, resulting from change of the ligand.[13] The water extracts from the exocarp, mesocarp, and endosperm of *Phaleria macrocarpa* (Scheff.) Boerl displayed positive results across all Dragendorff, Wagner, and Mayer tests. Moreover, saponin is identified by the formation of stable foam measuring between 1 and 3 centimeters in height that remained stable for 15 minutes.[10] The foam indicates the presence of glycoside that is hydrolyzed into glucose and its aglycone compounds in water.[18] The analysis result showed that among the different extracts tested, only the exocarp extract demonstrated the presence of saponins. In contrast, the mesocarp and endosperm extracts yielded negative results, as they did not produce any observable stable foam when subjected to the same conditions. Triterpenoids were identified by the Liebermann-Burchard test method. The development of a reddish-violet color complex indicated a favorable test result, with the hue varying based on the specific terpenoid present.[11] The phytochemical analysis conducted confirmed that all extracts tested contained triterpenoid, as evidenced by the appearance of red complexes following the addition of the reagents. Nevertheless, all the extracts showed negative results on anthraquinone and cyanohydrin tests. Overall, this study highlights the specific phytochemicals present in water extracts from different parts of *Phaleria macrocarpa* (Scheff.) Boerl provides insight into their potential applications and benefits.

Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a reliable separation technique that is essential for identifying and characterizing chemical compounds in various samples. This method relies on the distribution of two phases (a stationary phase and a mobile phase) with different polarity. The varying polarities of the two phases allow for the effective separation of compounds based on their affinities for each phase. This procedure focused on a range of phytochemicals, including flavonoids, tannins, alkaloids, and saponins that showed positive results during the initial screening. The comprehensive TLC results are shown in

Fig.-1 and the R_f values are summarized in Table-2, validating the effectiveness and reliability of TLC as a technique for the analysis and characterization of bioactive chemicals in plant materials.

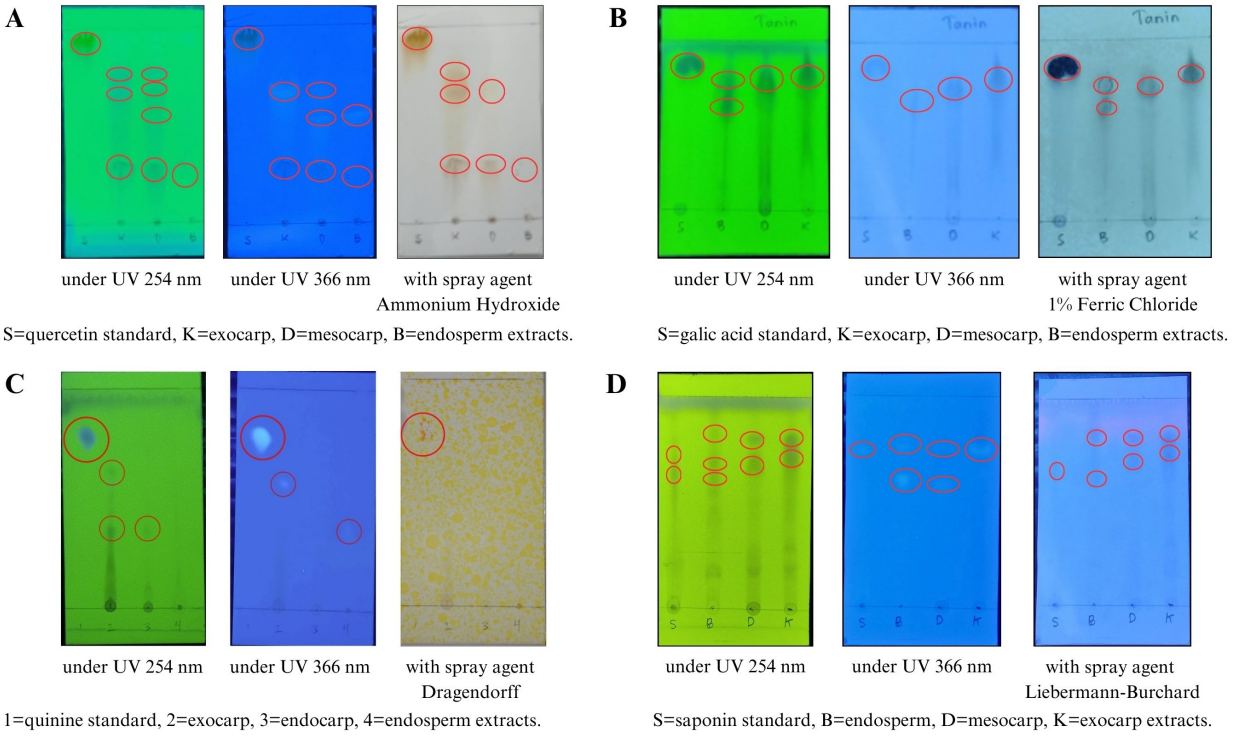

Fig.-1: TLC Separation of Flavonoid (A), Tannins (B), Alkaloids (C), and Saponin (D)

The water extract of *Phaleria macrocarpa* (Scheff.) Boerl has been identified as containing a diverse combination of bioactive compounds as evidenced by their respective R_f values shown in Table-2. The diverse R_f values of these compounds provide valuable information about their polarity and chemical properties. Moreover, the conducted TLC has successfully confirmed the presence of flavonoids, tannins, alkaloids, and saponins. Despite the negative results for saponins in the mesocarp and endosperm extracts during the phytochemical analysis, the detection of spots in the TLC test suggests the possibility of saponin in different parts of the plant.[19] In contrast, the alkaloids demonstrated positive outcomes during the phytochemical analysis, indicating their presence and potential benefits. However, upon conducting a TLC analysis, no distinct spots were observed after applying the Dragendorff reagent. This discrepancy might be attributed to the specific TLC system utilized in the analysis, which could have influenced the visibility of the alkaloids.

Table-2: R_f Values of Phytochemical Constituents Detected in the Extracts

Phytochemical constituents	Solvent system	Detection	R_f values*			
			Std	Exo	Meso	Endo
Flavonoids	glacial acetic acid-butanol-water (1:4:5)	UV 254 nm	0.82 0.66 0.78	0.30 0.55 0.69 0.77	0.28	0.25
		UV 366 nm	0.82	0.30 0.66	0.28 0.55 0.69	0.25 0.55
		NH_4OH	0.82	0.30 0.66 0.78	0.28 0.69	0.25
Tannin	methanol-ethyl acetate (8:2)	UV 254 nm	0.72	0.57 0.68	0.64	0.70
		UV 366 nm	0.72	0.70	0.64	0.57

		1% $FeCl_3$	0.72	0.57 0.68	0.64	0.70
Alkaloids	chloroform-methanol (4.7:0.3)	UV 254 nm	0.57	0.35 0.50	0.35	-
		UV 366 nm	0.57	0.50	-	0.35
		Dragendorff	0.57	-	-	-
Saponins	butanol-glacial acetic acid-water (84:14:17)	UV 254 nm	0.64 0.73	0.73 0.83	0.69 0.81	0.60 0.66 0.78
		UV 366 nm	0.73	0.73	0.59 0.75	0.60 0.78
		Liebermann-Burchard	0.64	0.73 0.83	0.69 0.81	0.60 0.78

* Std = Standard, Exo = Exocarp Extract, Meso = Mesocarp Extract, Endo = Endocarp Extract

Total Phenolic Content

The calibration curve presented in Fig.-2 provides a clear visual representation of the relationship between absorbance (y-axis) and concentration (x-axis) of standard gallic acid. The equation y = 0.0582x + 0.097 (R^2= 0.993) describes this relationship. The strong linear regression equation, indicated by an R^2 value close to 1, demonstrates the reliability of the calculations performed.[21] The extracts` TPC was determined as milligrams of gallic acid equivalents (mg GAE/g) per gram of extract. The TPC values determined for the water extracts from the exocarp, mesocarp, and endosperm of *Phaleria macrocarpa* (Scheff.) Boerl were 0.016, 0.004, and 0.003 mg GAE/g, respectively.

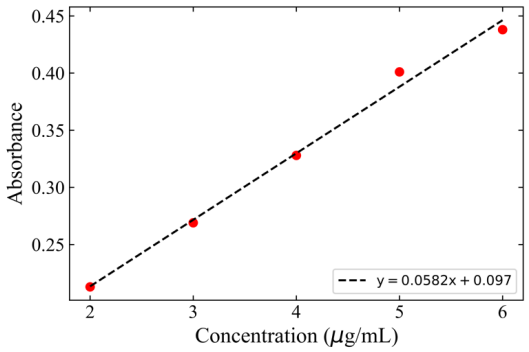

Fig.-2: A standard calibration curve of gallic acid for TPCs

Phenolic compounds are crucial secondary metabolites known for their effectiveness against a broad spectrum of bacteria, which encompass both Gram-positive bacteria and Gram-negative bacteria[20]. The TPC values in this study reveal that the exocarp has a significantly higher phenolic content than the mesocarp and endosperm, indicating its potential as a valuable source of phenolic compounds. However, to fully utilize its antibacterial properties, further investigations are needed to optimize the extraction efficiency. These findings could provide valuable insights into its potential as a halal antibacterial agent and promote its use in various health-related fields.

CONCLUSION

This study aimed to identify the secondary metabolites present in the water extracts of *Phaleria macrocarpa* (Scheff.) Boerl. The results revealed the presence of flavonoids, polyphenols, tannins, alkaloids, and saponins. Total phenolic content (TPC) was evaluated as a preliminary assessment of its antibacterial activity. The findings indicated significant variations in TPC values between different parts of the plants: the exocarp exhibited the highest value at 0.016 mg GAE/g, followed by the mesocarp at 0.004 mg GAE/g, and the endosperm at 0.003 mg GAE/g. These results hold promise for future research aiming to develop the water extract of *Phaleria macrocarpa* (Scheff.) Boerl is a halal alternative to antibacterial agents.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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