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Quantification of Phenolic Content in Bridelia Micrantha Bark Extract Using Folin-Ciocalteu Method

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Abstrak

The scientific validation of medicinal plants is essential to ensure the safety, efficacy, and reproducibility of traditional remedies in modern healthcare. *Bridelia micrantha*, although widely used in Southeast Asian and African ethnomedicine, has not been fully standardized despite claims of antioxidant and anti-inflammatory properties. This study determined the total phenolic content (TPC) of *B. micrantha* stem bark extract to support its pharmacological relevance. An experimental design was implemented using 80% methanol maceration for extraction, qualitative screening with $FeCl_3$, and quantitative analysis via the Folin-Ciocalteu method. Absorbance was measured at 760 nm using UV-Vis spectrophotometry, with gallic acid as a calibration standard ($r^2 = 0.9978$). The extract showed a phenolic content of 22.14% gallic acid equivalent (GAE), indicating a substantial presence of antioxidant-related compounds. These findings confirm the traditional use of *B. micrantha* and highlight its potential for standardized herbal formulations. The study also bridges a gap between ethnobotanical knowledge and phytochemical evidence, contributing to the integration of traditional plant-based remedies into evidence-based healthcare systems.

Keywords: *Bridelia micrantha*, phenolic content, Folin–Ciocalteu, antioxidant activity, medicinal plant, gallic acid equivalent, phytochemical screening.

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INTRODUCTION

Medicinal plants have long been integral to global healthcare systems, especially in tropical and subtropical regions where modern pharmaceuticals may be inaccessible. The World Health Organization (WHO) reports that approximately 80% of the global population depends on herbal remedies for primary health care (Efendi, 2020). These traditional practices are increasingly recognized for their scientific value, as many plants contain bioactive compounds with therapeutic potential. Among these, phenolic compounds are notable for their antioxidant properties that neutralize reactive oxygen species, thereby mitigating chronic diseases such as cancer, cardiovascular disorders, and diabetes (Ranneh et al., 2021; Mahomoodally et al., 2020). Additionally, phenolics support dermatological health, immune function, and

metabolic processes (Das et al., 2024; Sumantri et al., 2020). Given their extensive biological activity, phenolic-rich plants merit rigorous phytochemical investigation.

Despite their widespread use, many medicinal plants in Indonesia lack empirical validation and quality standardization. One such species is *Bridelia micrantha*, known locally in East Lombok as *melandean*, which is traditionally prepared by boiling its bark to treat inflammation, gastrointestinal disturbances, and respiratory ailments (Maroyi, 2017; Kevin et al., 2023; Tamokou et al., 2023). Although anecdotal and ethnomedicinal evidence supports its use, scientific studies analyzing its phytochemical composition remain limited. Preliminary studies have identified secondary metabolites such as flavonoids, alkaloids, tannins, and phenolics (Anywar et al., 2021; Asumang et al., 2022), but comprehensive and quantitative validation—especially of its antioxidant constituents—remains insufficient. This gap between traditional use and laboratory validation constitutes a major research problem.

The current literature reveals that very few studies have applied the Folin-Ciocalteu method specifically to quantify total phenolic content (TPC) in *Bridelia micrantha* bark. Among those that do exist, methodological inconsistencies, such as variations in solvent polarity, calibration standards, and spectrophotometric conditions, limit their comparability and reproducibility. Furthermore, many prior studies employed semi-quantitative or qualitative approaches without robust standard curves, replicates, or modern instrumentation, which compromises data accuracy (Das et al., 2024; Assogba et al., 2021; Loko et al., 2025). This study aims to address those limitations by introducing a validated spectrophotometric method using gallic acid as the calibration standard under controlled laboratory conditions.

Given these issues, the present research explicitly asks: *Does the methanol extract of* Bridelia micrantha *stem bark contain significant phenolic content when quantified using the Folin-Ciocalteu method with gallic acid calibration?* The working hypothesis is that *Bridelia micrantha* stem bark contains a high concentration of phenolic compounds, supporting its traditional use as an antioxidant agent.

This study focuses on extract preparation, qualitative identification of phenolics using $FeCl_3$, and quantitative assessment using UV-Vis spectrophotometry at 760 nm. By limiting the analysis to phenolic compounds, the research provides foundational phytochemical data to support future pharmacological applications, particularly in antioxidant and anti-inflammatory therapies. This approach contributes to bridging traditional ethnomedicine with standardized herbal drug development based on empirical evidence.

METHOD

Research Design and Overview

This study employed a quantitative experimental design focusing on the phytochemical analysis of *Bridelia micrantha* stem bark. The primary objective was to determine the total phenolic content (TPC) using the Folin-Ciocalteu method. This design is particularly suitable for validating traditional medicinal knowledge through standardized laboratory protocols. A diagrammatic overview of the methodological stages—plant collection, extraction, qualitative testing, and quantitative

spectrophotometric analysis—is presented in Figure 1 and referred to within this section.

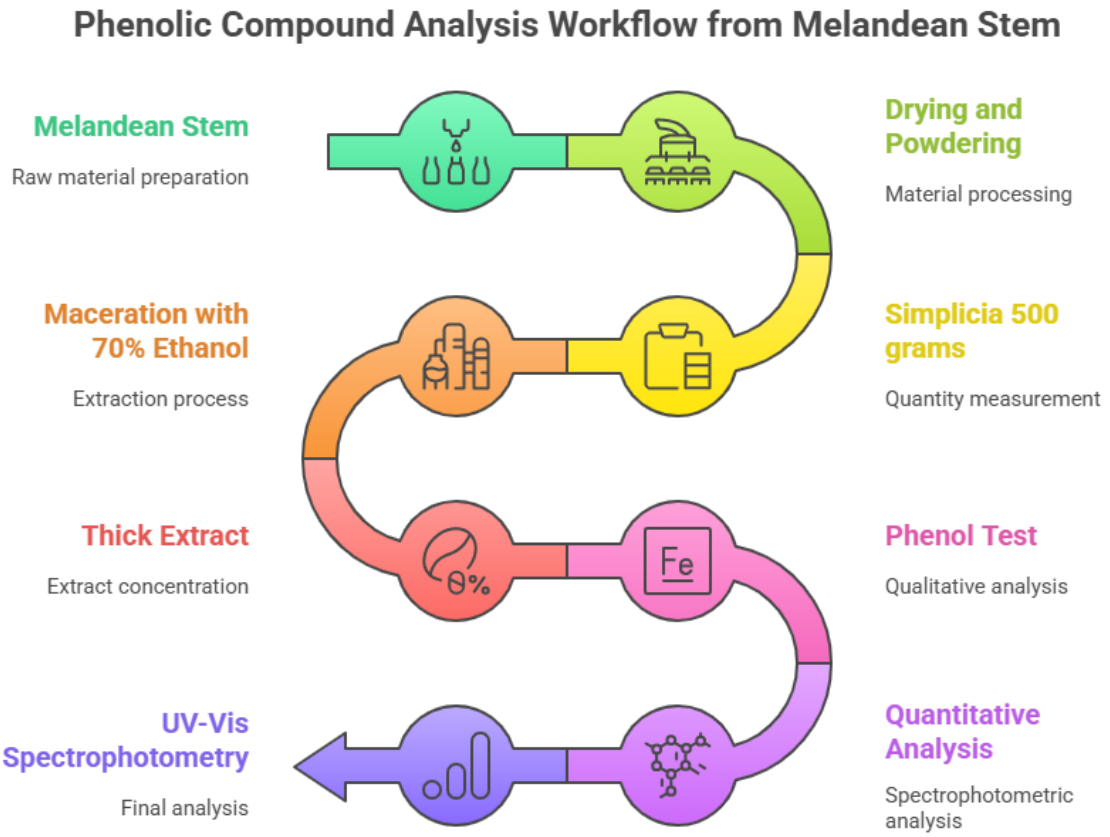

Figure 1. Workflow diagram illustrating the sequential stages of phenolic analysis in Bridelia micrantha bark extract: sampling, extraction, phytochemical testing, and quantification.

The experimental workflow follows a structured sequence in which the biological material is authenticated, extracted, and analyzed under controlled conditions. This approach aligns with pharmacognostic research practices emphasizing reproducibility, accuracy, and transparency (Anywar et al., 2021; Mahomoodally et al., 2020). Methanol maceration was selected due to its effectiveness in preserving thermolabile phenolic compounds. The study contributes to the broader aim of bioactive standardization in traditional herbal remedies.

Research Samples and Characteristics

The sample analyzed was the stem bark of *Bridelia micrantha*, collected from Selong, East Lombok, Indonesia. Botanical authentication was carried out by taxonomists at the Laboratory of Advanced Biology, Faculty of Mathematics and Natural Sciences, University of Mataram. The specimen was registered with voucher number BM-2024-07. A total of 500 grams of dried bark (simplisia) was prepared for extraction.

Sample preparation involved multiple stages: washing, air-drying in shaded conditions for 20 days, dry sorting, and mechanical grinding to obtain uniform particle size. This precision ensures the sample's purity and maximizes extraction efficiency. The species was selected based on its ethnomedicinal prominence and accessibility in the local environment, supporting its potential for scientific validation.

Instruments and Research Procedures

The instruments used included UV-Visible spectrophotometer, rotary evaporator, standard laboratory glassware, and Whatman filter paper. Analytical-grade reagents were utilized throughout, including Folin-Ciocalteu reagent, sodium carbonate, gallic acid (as the standard), and 1% ferric chloride ($FeCl_3$).

Extraction was conducted by macerating 500 g of powdered simplisia in 2.5 liters of 80% methanol, establishing a 1:5 solvent-to-solid ratio. The mixture was allowed to stand for 72 hours with manual agitation every 24 hours. The resulting extract was filtered and concentrated using rotary evaporation at 60–65°C, then stored in amber containers at room temperature.

Qualitative detection of phenolics was performed using $FeCl_3$, which produced a characteristic dark greenish color. Quantitative analysis followed the standard Folin-Ciocalteu protocol by reacting the extract with the reagent and sodium carbonate, with absorbance measured at 760 nm after 60 minutes of incubation. Each assay was replicated three times for consistency.

Data Analysis and Interpretation

Microsoft Excel was used for regression modeling and calculation of phenolic content. A calibration curve was generated using gallic acid standards ranging from 10–50 ppm, resulting in the regression equation $y = 0.0168x + 0.0425$ with a high correlation coefficient ($r^2 = 0.9978$). The sample's absorbance value was interpolated to determine phenolic concentration in mg GAE/g, which was subsequently expressed as a percentage.

While the Folin-Ciocalteu method is known to be susceptible to interference by non-phenolic reducers (Fagbohun & Bamikole, 2019; Bayani et al., 2023), it remains practical due to its reproducibility and cost-effectiveness. Interpretations of TPC were supported by existing literature correlating phenolic levels with antioxidant potential (Adesina et al., 2019; Singh et al., 2024). The use of triplicate sampling and the linearity of the standard curve enhance the reliability and validity of the findings, providing a robust basis for further bioactivity assays and pharmacokinetic investigations.

RESULTS

Botanical Identification

The plant specimen used in this study was positively identified as *Bridelia micrantha* through morphological assessment at the Laboratory of Advanced Biology, University of Mataram. The authentication was based on key diagnostic features such as alternate phyllotaxy, elliptic leaves with entire margins, axillary flower clusters, and the presence of fleshy drupe fruits. Additional taxonomic confirmation was

derived from the identification of multicellular uniseriate trichomes and pronounced leaf venation patterns (Etono et al., 2023; Xu et al., 2022; Saha et al., 2015; Maroyi, 2017).

These morphological traits are consistent with documented botanical descriptions of *B. micrantha*, supporting accurate species-level identification critical for phytochemical studies. Proper identification ensures the validity and reproducibility of pharmacognostic analysis.

Extraction Yield

A total of 500 grams of dried *B. micrantha* bark powder yielded 60 grams of thick extract following 80% methanol maceration, translating to a 12% extraction yield. This efficiency was influenced by multiple factors, including solvent polarity, maceration duration, ambient temperature, and the matrix's structural characteristics (Zali et al., 2023; Gómez-Urios et al., 2022).

Methanol at 80% concentration was chosen for its ability to dissolve both polar and semi-polar phenolic compounds efficiently. The yield reflects not only solvent performance but also the bark's secondary metabolite content and cellular architecture, which favor compound solubilization (Thomas et al., 2020; Alchera et al., 2022).

Qualitative and Spectral Analysis

The presence of phenolics was confirmed qualitatively using 1% $FeCl_3$, which produced a dark greenish color, indicative of phenol-Fe complex formation. This classical test supports the presence of hydroxylated aromatic structures (Olalekan, 2023; Zhao et al., 2024). For quantitative analysis, gallic acid standards scanned at 400–800 nm showed maximum absorbance at 760 nm, which became the basis for further measurements (Oniszczuk et al., 2019; Luaces et al., 2021).

The constructed calibration curve exhibited high linearity with the regression equation $y = 0.0168x + 0.0425$ and $R^2 = 0.9978$, confirming excellent reproducibility (Abumelha et al., 2024; Park et al., 2021). Figure 2 presents both the qualitative test result and the calibration curve.

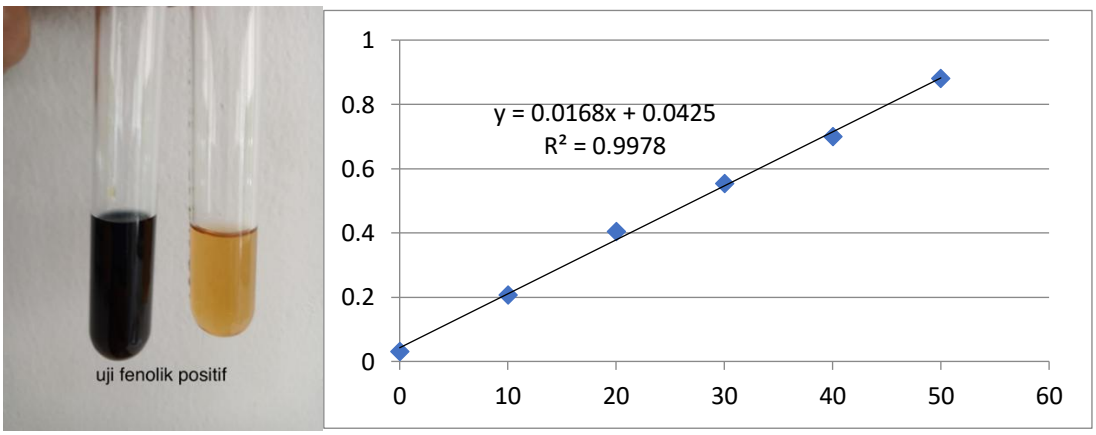

Figure 2. Qualitative color change of phenol-$FeCl_3$ complex (left) and linear gallic acid calibration curve (right).

Total Phenolic Content Estimation

Using the mean absorbance value of 1.536 and interpolation via the gallic acid calibration curve, the calculated concentration of phenolics was 88.90 ppm. When expressed as gallic acid equivalents (GAE), the total phenolic content was 22.14%.

This TPC value is considered high and comparable to or exceeding values reported in related species such as *Bridelia ferruginea* (approx. 19.6% GAE) and *Bridelia montana* (approx. 17.3% GAE), which have also been studied for their antioxidant capacities. Thus, *B. micrantha* demonstrates significant phenolic richness, positioning it as a strong candidate for herbal standardization (Souiy et al., 2023; Gontar et al., 2024).

DISCUSSION

The research findings confirm the high total phenolic content (22.14% GAE) in *Bridelia micrantha* stem bark extract, supporting its traditional use in treating oxidative and inflammatory conditions. The use of 80% methanol optimized compound recovery, while UV-Vis spectrophotometry and validated regression analysis ensured precision. This substantial phenolic yield implies strong potential antioxidant activity and therapeutic application in traditional and modern medicine (Sompong & Adisakwattana, 2015; Deocaris et al., 2023).

Theoretically, phenolics are linked to free radical scavenging and metal chelation. Their electron-donating ability enables mitigation of oxidative stress—a common pathway in chronic diseases such as cardiovascular and metabolic disorders (Oguntimehin et al., 2021; Firew et al., 2020). The results align with prior research on *Bridelia ferruginea*, whose TPC correlates with antioxidative bioactivity, indicating that *B. micrantha* could exhibit similar pharmacodynamics.

In comparison with earlier studies on congeners such as *Bridelia ferruginea* and *Bridelia montana*, the current TPC value is notably higher. These differences may arise from species variation, geographic origin, and extraction protocols. Such comparative analysis strengthens the claim that *B. micrantha* offers superior phenolic yield under optimal maceration conditions (Adesina et al., 2019; Singh et al., 2024).

However, the study acknowledges limitations. The Folin-Ciocalteu method, although validated, is susceptible to interference from non-phenolic reducing agents such as vitamin C, reducing sugars, and some amino acids, which may inflate TPC values (Fagbohun & Bamikole, 2019; Bayani et al., 2023). While this does not negate the findings, it underscores the need for method triangulation in future studies.

Additionally, this study did not conduct specific antioxidant bioassays such as DPPH or ABTS. Though TPC provides a strong indicator of antioxidative potential, future integration of in vitro radical scavenging tests and in vivo pharmacological assessments would yield more comprehensive conclusions. Advanced profiling techniques like HPLC could also help isolate specific phenolic constituents and validate their bioactivities.

Standardization remains essential for herbal product development. The consistent TPC results from triplicate trials, combined with validated spectrophotometric methods, can serve as a model for assessing batch-to-batch phytochemical quality. This contributes to the broader goal of integrating ethnomedicinal knowledge with evidence-based practices.

To advance this field, future research should focus on complete phytochemical profiling, toxicological safety evaluations, and exploration of synergistic effects among plant constituents. The high phenolic concentration reported here substantiates *B. micrantha*'s medicinal relevance and supports its further exploration as a therapeutic agent rooted in traditional practice.

CONCLUSION

This study addressed a critical gap in the scientific validation of *Bridelia micrantha* by quantifying its total phenolic content using the Folin-Ciocalteu method. The findings confirmed that the stem bark extract contains a high concentration of phenolic compounds, reinforcing its ethnomedicinal use as a natural antioxidant source. These results fulfilled the research objective by demonstrating the plant's potential as a standardized phytochemical ingredient suitable for herbal formulations.

The study underscores the value of integrating traditional medicinal knowledge with modern analytical tools to ensure reproducibility and scientific credibility. Its implications are relevant for advancing herbal pharmacology, as *B. micrantha* emerges as a promising candidate for antioxidant-based therapeutic applications.

Although limited to phenolic quantification, the study provides a foundational reference for future investigations into the plant's broader bioactivities, pharmacological mechanisms, and safety profile. Subsequent research should incorporate functional bioassays, compound isolation, and clinical validation to fully harness its therapeutic potential. Therefore, government-supported standardization efforts should prioritize indigenous species like *Bridelia micrantha* to preserve local medicinal heritage while advancing evidence-based phytotherapy.

RECOMMENDATION

Future research should focus on expanding the pharmacological profile of *Bridelia micrantha* by conducting in vitro and in vivo antioxidant assays to complement the current phenolic quantification findings. Isolation and characterization of individual phenolic compounds using chromatographic and spectrometric techniques such as HPLC and LC-MS are recommended to better understand the specific bioactive agents responsible for its therapeutic potential.

In addition, toxicity assessments and dose-response studies are necessary to ensure safety and establish standardized dosage parameters. Long-term pharmacological safety evaluations, including sub-chronic and chronic toxicity testing, should be prioritized to support the plant's integration into formal therapeutic systems.

Moreover, integrating ethnomedicinal knowledge with molecular studies could help identify mechanisms of action, enhancing the plant's scientific validation.

Research should also explore the impact of environmental variables, plant age, and seasonal variation on phytochemical composition to improve the reproducibility and scalability of herbal formulations.

Challenges encountered in this study, such as potential interference in the Folin-Ciocalteu assay by non-phenolic reducing agents, emphasize the need for multi-assay validation in future experiments. Limited access to advanced analytical instruments and standardized reference materials remains a barrier, suggesting that collaborative research with well-equipped laboratories or institutions may improve methodological robustness. Additionally, the development of a regional herbal pharmacopoeia could support broader utilization and recognition of *Bridelia micrantha* as a scientifically endorsed medicinal plant.

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