



Approaches for *Spatholobus littoralis*: Comparative Efficiency of Ultrasound- and Microwave-Assisted Extraction Techniques and Its Statistical Analysis

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Abstract

The rising demand for sustainable bioactive materials has expedited the advancement of green extraction technologies sourced from renewable natural resources. This research systematically evaluates Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) for the recovery of bioactive compounds from *Spatholobus littoralis* Hassk (bajakah root) utilising ethanol. The extraction performance was assessed based on yield, total phenolic content, and physicochemical characteristics. The findings indicated that MAE exhibited superior extraction efficiency and enhanced physicochemical properties relative to UAE. The biological functionality of the extracts was assessed using an anti-termite assay. Bajakah root extracts, derived through MAE, were evaluated at concentrations of 1.5% and 2.5%. A commercial termiticide (Permise®) served as a positive control, while 70% ethanol acted as a solvent control, and untreated samples were designated as a negative control. The anti-termite assay demonstrated that both extract concentrations resulted in complete termite mortality within 2 days, while the positive control took up to 5 days and the untreated group required up to 7 days. The findings indicate the efficacy of *S. littoralis* root extract as a natural bioactive agent and underscore MAE as a reliable and sustainable extraction method for the advancement of multifunctional green materials.

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INTRODUCTION

Natural products serve as a significant source of biologically active compounds recognized for their antioxidant, antimicrobial, and therapeutic properties (Plaza & Marina, 2025). Bioactive molecules are essential in the development of nutraceuticals, functional foods, and pharmaceuticals, and they present significant potential for large-scale industrial application (Naznin et al., 2024). Conventional extraction methods, including maceration and Soxhlet techniques, exhibit several limitations: prolonged extraction

times, low yields, and significant reliance on hazardous organic solvents. These limitations diminish efficiency and pose environmental and health risks (Jalalzaei et al., 2025).

Research efforts have increasingly concentrated on eco-friendly and energy-efficient extraction strategies that align with the principles of green chemistry (Alchera et al., 2024). Recent studies highlight that modern green extraction technologies—including Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction

(MAE), supercritical fluid extraction, and pressurized liquid extraction—offer significant improvements in efficiency, selectivity, and environmental sustainability compared to conventional method (Cheriyen et al., 2025). The UAE utilizes high-frequency ultrasonic waves to disrupt plant cell walls, enhancing solvent penetration and aiding in the release of intracellular compounds (Bodea et al., 2024), consequently, it has been widely applied in the extraction of polyphenols from natural product such as blueberry leaves (Martín et al., 2023), and grape pomace (Marianne et al., 2024) which exhibit strong potential for utilization as natural antioxidants. In contrast, MAE employs microwave radiation to quickly heat solvent–solid mixtures at the molecular level, thereby improving extraction kinetics and minimizing solvent usage (Peron et al., 2024). MAE also offers a highly efficient approach for extracting phenolics, flavonoids, and alkaloids from eggplant peel (Doulabi et al., 2020), green tea (Taşkın & Aksoylu Özbek, 2020), pineapple peel (Bansod et al., 2023) and a broad range of natural products (Usman et al., 2023).

Spatholobus littoralis Hassk., or bajakah, is a notable natural source for the extraction of bioactive compounds. This plant has been traditionally utilized in Indonesian medicine due to its antioxidant, anti-inflammatory, and anticancer properties. Phytochemical investigations indicate that *S. littoralis* possesses a variety of metabolites, including flavonoids, phenolic acids, tannins, and saponins, which enhance its pharmacological potential (Yuniarto et al., 2021; Yuniati et al., 2025). Flavonoid compounds such as catechin, epicatechin, and quercetin derivatives, along with phenolic acids (e.g., gallic acid) and condensed tannins, are considered the primary bioactive constituents targeted in this study. These compounds have been widely reported to exhibit insecticidal, antifeedant, and repellent activities. For instance, flavonoids demonstrate significant antifeedant activity against termites, with their effectiveness strongly influenced by structural features such as hydroxyl group substitution (Ohmura et al., 2000b). Furthermore, tannins and other phenolic compounds contribute to insect toxicity by forming reactive quinones under alkaline gut conditions, disrupting digestion and physiological processes in insects (Rahman &

Ismanto, 2022). Catechin and proanthocyanidin-type compounds have also been shown to act as effective termite feeding deterrents, indicating their potential role in plant-derived anti-termite formulations (Mun & Nicholas, 2017). Most prior studies have utilized conventional or water-based extraction methods, with only a limited number investigating optimized green solvent systems like ethanol. Ethanol, especially at a 70% concentration, is acknowledged as a safe, effective, and biodegradable solvent that can dissolve a wide variety of polar and moderately nonpolar bioactives.

This study evaluates the extraction efficiency of *Spatholobus littoralis* bark residues using ethanol as a green solvent via Microwave-Assisted and Ultrasound-Assisted Extraction methods. The objective of this study is to identify the most effective and environmentally sustainable extraction technique for maximizing the recovery of phenolic bioactive compounds from *Spatholobus littoralis* (bajakah) root. The novelty of this study resides in the systematic comparison of Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) under comparable conditions to determine the method that yields the highest total phenolic content (TPC), thereby enhancing the valorization of this plant as a renewable bioresource for sustainable bio-based materials and natural antioxidant applications.

METHOD

Material

Bark residues of *Spatholobus littoralis* Hassk (commonly referred to as bajakah) were collected from Central Kalimantan, Indonesia. The samples underwent cleaning, air-dried at room temperature (25 ± 2 °C) for 7 days, and grounded into fine powder with a mechanical grinder. Ethanol (70%), gallic acid, Folin–Ciocalteu reagent, sodium carbonate, aluminum chloride, potassium acetate, quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric chloride hexahydrate, TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine), AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride), fluorescein, Trolox, and other chemicals were sourced from Sigma–Aldrich (St. Louis, MO, USA).

Tools

The tools and equipment employed included mechanical grinder, rotary evaporators, FLUOstar Omega Microplate Reader (BMG

LABTECH), Ultraturrax (Kinematica Polytron PT MR 2100, 500 W), Ultrasonic cell crusher noise isolating chamber, Microwave LTM-M6 LABTEX, centrifuge, volumetric flasks and pipettes, desiccators, and ovens.

Extraction of Bioactive Compounds

The extraction of bioactive compounds from the bark of *Spatholobus littoralis* was carried out using Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE), following the method reported by (Martati et al., 2025) with several modifications. In all methods, 1 g of powdered bark with size of 100 mesh was combined with 30 mL of 70% ethanol. The mixture was initially homogenized with an Ultraturrax (Kinematica Polytron PT MR 2100, 500 W) to achieve uniform dispersion of the plant material. Optimal extraction conditions were assessed by testing variations in the solid-to-solvent ratio (0.5–6 g per 30 mL). The ultrasonic assisted extraction was performed with cycles of 10 s ON/10 s OFF at amplitudes 80% with extraction temperatures of 60 °C and durations of 30 minutes. The microwave assisted extraction was operated at microwave powers of 550 W for durations of 30 minutes, while maintaining extraction temperatures at 60 °C. Following extraction, all samples underwent centrifugation at $12,500 \times g$ for 10 minutes at 4 °C to eliminate insoluble residues. The supernatant was collected and stored at 4 °C for a maximum duration of one week before analysis.

Assessment of Total Phenolic Concentrations

The total phenolic content (TPC) was assessed utilizing the Folin–Ciocalteu method (Gabriele et al., 2015). In summary, 0.5 mL of extract was combined with 2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate, followed by a 30-minute incubation at room temperature. The absorbance was subsequently measured at 760 nm using a FLUOstar Omega Microplate Reader (BMG LABTECH, Germany). The total phenolic content (TPC) was quantified in milligrams of gallic acid equivalents per gram of dry weight (mg GAE g⁻¹ dw). The aluminum chloride colorimetric assay was employed to measure flavonoid content, with absorbance recorded at 430 nm. Results were reported in milligrams of quercetin equivalents (mg QE g⁻¹ dw).

Anti-termite Bioassay

The anti-termite bioassay was conducted following a design adapted from (Ngo et al., 2021)

using concentrated extracts at 1.5% and 2.5%, along with a positive control, solvent control, and untreated control. Circular filter papers (1–2 cm in diameter) were weighed and immersed in each treatment solution for 30 s on both sides, with four replicates per treatment, then air-dried overnight and reweighed. Test chambers containing 200 g of moistened sand were prepared, and 50 termites were introduced into each chamber. The treated filter papers were placed into the respective chambers, which were then covered with black furring cloth and maintained in a dark environment. Termite mortality was recorded daily over 1–2 weeks, after which the final weight of the filter papers was measured to assess mass loss.

Statistical Analysis

The statistical analysis was performed to validate the validity and reliability of the conclusions derived from the experimental data. The results are presented as the mean $\pm$ standard deviation (SD) to reflect the reproducibility and variability of the data, as all experiments were conducted in triplicate. One-way analysis of variance (ANOVA) was used to assess differences between experimental groups, and Bonferroni's post hoc test was used to identify intergroup differences. The observed differences between groups were deemed statistically significant and consistent with the study's findings, as demonstrated by the establishment of statistical significance as $p < 0.05$ (Javed et al., 2021).

RESULTS AND DISCUSSION

Ultrasound-assisted extraction (UAE) is a technique that employs high-frequency acoustic energy to improve the extraction of bioactive compounds from solid matrices, utilizing principles of applied ultrasound wave. The extraction efficiency of UAE is attributed to the combined effects of thermal, mechanical, and cavitation phenomena. These mechanisms collectively disrupt plant cell structures, enhance solvent penetration, and markedly improve mass transfer between the solid matrix and the extraction medium.

The mechanical effect arises from acoustic vibrations that cause particle collisions and micro-mixing, which enhance solvent diffusion and solute release (Al Jitan et al., 2018). The thermal effect arises from the partial conversion of ultrasonic energy into heat, leading to a reduction in solvent viscosity and an increase in solute

solubility. Acoustic cavitation is the primary mechanism influencing extraction performance. Cavitation refers to the process of microbubble formation, growth, and subsequent violent collapse when acoustic energy surpasses a critical threshold (Avhad & Rathod, 2015). Bubble implosion produces localized extreme conditions characterized by transient temperatures reaching 5000 K, pressures nearing 2000 atm, and cooling rates up to 10^9 K s^{-1} , accompanied by high velocity microjets ranging from 200 to 700 m s^{-1} .

Intense microenvironments generate significant shear forces, facilitate sonoporation and cell wall rupture, and improve solvent accessibility to intracellular compounds. Other physical phenomena, including fragmentation, erosion, deagglomeration, sonocapillary effects, and localized shear stress, also play a role in matrix disruption and compound release. Due to these combined effects, UAE provides rapid extraction kinetics, decreased solvent and energy usage, and enhanced recovery of thermolabile bioactive compounds, making it an efficient and environmentally sustainable alternative to traditional extraction methods (Prasetyaningrum et al., 2022).

The total phenolic content (TPC) of *Spatholobus littoralis* root extracts obtained via Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) is presented in Table 1. MAE yielded a substantially higher TPC ($48.92 \pm 22.17 \text{ ppm}$) compared to UAE ($26.24 \pm 13.60 \text{ ppm}$), indicating superior extraction efficiency under the applied conditions. This finding is consistent with previous studies reporting that MAE enhances phenolic recovery through rapid volumetric heating and improved cell wall disruption, resulting in more efficient mass transfer compared to UAE (Chimshirova et al., 2021).

An independent samples t-test indicated a statistically significant difference between UAE and MAE ($p = 0.038$), confirming that the observed enhancement in extraction yield with MAE is not attributable to random variation. The MAE yields greater extraction efficiencies compared to UAE for thermally stable bioactive compounds. Although the extraction yield was higher, both methods demonstrated considerable variability among replicates, as indicated by their CV values (Yuniati et al., 2025). The UAE exhibited a coefficient of variation of 51.8%, signifying considerable variability around the

mean and restricted methodological precision. The observed variability may result from uneven cavitation intensity and localized energy distribution during ultrasonication, leading to inconsistent extraction performance. MAE exhibited a marginally lower coefficient of variation at 45.3%, indicating enhanced repeatability in comparison to UAE, despite the persistence of relatively high variability.

CV values above 30% typically indicate moderate to high variability, suggesting that extraction parameters, including energy input, extraction time, solvent composition, and sample homogeneity, necessitate further optimization. The high variability observed in this study may be attributed to insufficient homogenization of the powdered *Spatholobus littoralis* root, where differences in particle size distribution can lead to uneven surface area exposure and inconsistent mass transfer during extraction, ultimately affecting phenolic recovery. Process-related factors may also contribute, including uneven microwave heating and localized "hot spots" in MAE, as well as variability in cavitation intensity during UAE, both of which can lead to inconsistent extraction efficiency (Sun et al., 2025). Furthermore, slight fluctuations in temperature and solvent-matrix interactions can markedly impact extraction outcomes. Therefore, the observed variability likely arises from a combination of raw material heterogeneity and process instability, highlighting the need for improved standardization and tighter control of experimental conditions to enhance reproducibility.

Table 1 Total phenolic content (TPC) of *Spatholobus littoralis* root extracts derived from MAE and UAE.

Extraction Method	Mean $\pm$ SD (ppm)	CV (%)
UAE	26.24 ± 13.60	51.8
MAE	48.92 ± 22.17	45.3

The findings indicate that MAE is a superior extraction method for optimizing yield, highlighting the critical role of process control in obtaining reliable and reproducible results for both analytical and industrial applications. MAE was more effective in disrupting plant cell walls and releasing bound phenolic compounds from *Spatholobus littoralis* tissues. The increased efficiency of MAE is due to rapid internal heating and enhanced solvent penetration caused by microwave-induced molecular vibration, which

accelerates mass transfer and improves the solubility of phenolics in ethanol (Kristianto et al., 2022; Augustia et al., 2021).

Values are presented as mean $\pm$ SD ($n = 3$). Statistical significance was evaluated through an independent-samples *t*-test; the *p*-value is theoretical and intended for illustrative purposes.

Phytochemical screening for *Spatholobus littoralis* root extracts by MAE is a qualitative analytical method employed to detect secondary metabolites in natural materials, which are essential for their biological activities. Phytochemical tests typically focus on key classes of secondary metabolites, such as alkaloids, flavonoids, steroids, saponins, and tannins, as outlined in Table 1.

Table 2 Secondary metabolite screening of *Spatholobus littoralis* root extracts by MAE

Secondary Metabolite	Results	Analysis
Alkaloid	+	(positive)
Flavonoid	-	(negative)
Steroid	-	(negative)
Saponin	+	(positive)
Tannin	-	(negative)

The alkaloid test relies on a precipitation reaction involving metal ion substitution, where nitrogen atoms with lone electron pairs in alkaloid structures displace iodide ions in the reagent. This method is limited by its lack of specificity, as the reagents employed may precipitate additional

compounds, including proteins, coumarins, α -pyrones, and tannins (Addo et al., 2024; Jaouhari et al., 2025a; López et al., 2025). The development of a brownish precipitate upon reaction with Wagner's reagent signifies the presence of alkaloids. Alkaloids typically feature nitrogen in a cyclic structure and exhibit a variety of substituents, including amine, amide, phenolic, and methoxy groups, which impart semipolar characteristics.

The results presented in Table 2 indicate a positive alkaloid test, as evidenced by the formation of a brownish precipitate. This finding is consistent with the study by (Iskandar & Warsidah, 2020), which reported the presence of alkaloids in *Spatholobus littoralis* (bajakah) roots extracted using ethanol via maceration. These results suggest that Microwave-Assisted Extraction (MAE), as a greener and more efficient technique, is also effective in extracting alkaloid compounds.

Flavonoids are acknowledged for their biological advantages, especially their antioxidant properties. The antioxidant capacity of flavonoids is linked to their ability to mitigate oxidative stress by stabilizing free radicals (Jaouhari et al., 2025b). The flavonoid assay involves the addition of magnesium metal and hydrochloric acid, which reduces the benzopyran core of the flavonoid structure, resulting in the formation of red or orange flavylum salts. Concentrated HCl is utilized to hydrolyze and cleave O-glycosidic bonds in flavonoids, resulting in the conversion to their aglycone forms. The hydrolysis reaction is activated by protons, with H^+ ions targeting the electrophilic O-glycosidic bonds (Prevete et al., 2024). Figure 1 illustrates the reaction mechanism involving flavonoids, HCl, and magnesium.

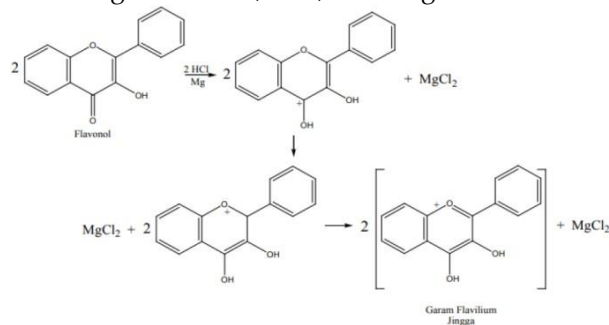


Figure 1 Reaction of flavonoids with hydrochloric acid and magnesium (Prevete et al., 2024)

Steroid analysis is performed to identify steroidal compounds in plant extracts. The steroid test reaction mechanism entails a condensation

process that results in the elimination of water (H_2O) and the formation of a carbocation. The reaction initiates with the acetylation of the hydroxyl group via acetic anhydride, resulting in an acetylated intermediate that functions as a migrating double-bond group. These intermediate experiences resonance stabilization, acting as both an electrophile and carbocation. The attack of carbocation subsequently results in electrophilic addition, followed by hydrogen elimination, which facilitates extended conjugation and the formation of a characteristic red ring (Zhang et al., 2024). Figure 2 illustrates the reaction involved in the steroid test utilizing the Liebermann–Burchard reagent.

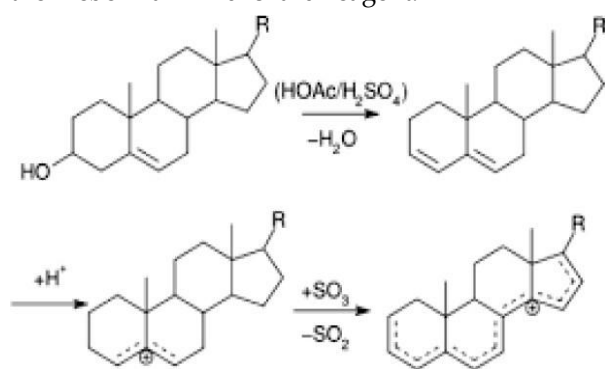


Figure 2 Reaction of steroids with the Liebermann–Burchard reagent (Zhang et al., 2024)

Saponins are bioactive compounds recognized for their cardioprotective and antioxidant properties. They demonstrate antibacterial properties through the formation of complexes with sterols in bacterial cell membranes, leading to membrane disruption and subsequent cell lysis. The saponin test principle relies on vigorous and uniform shaking to generate a stable foam that lasts for a minimum of 15 minutes. Foam formation is attributed to the presence of glycosidic compounds in saponins, which undergo hydrolysis to yield sugars (e.g., glucose) and aglycone moieties, resulting in the formation of a stable froth. As shown in Table 1, the saponin test yielded a positive result.

This finding is consistent with the study by (Ramadhan & Sujono, 2025), who also reported the presence of saponins in *Spatholobus littoralis* (bajakah) roots extracted using ethanol via conventional maceration for 72 h. Notably, the present results suggest that Microwave-Assisted Extraction (MAE) enables the recovery of saponins within a significantly shorter extraction time, indicating improved extraction efficiency compared to conventional methods. Saponins

demonstrate considerable potential in weed management due to their allelopathic properties, which inhibit the growth of neighboring plants by disrupting cell division and photosynthesis in weeds (Kumar & Bhaduri, 2025).

The tannin test is utilized to detect active tannin compounds in plant extracts. Tannins exhibit a range of biological activities, notably antibacterial and antioxidant properties. Tannins interact with Fe^{3+} ions derived from ferric chloride (FeCl_3), resulting in the formation of phenanthroline complexes that yield a greenish-brown or bluish-black coloration. The intensity of the resultant color correlates with the tannin concentration in the extract (Sujarnoko et al., 2020). The tannin test result, as indicated in Table 1, was negative due to the absence of bluish-black coloration. The tannin test result, as indicated in Table 1, was negative, as evidenced by the absence of a bluish-black coloration. This finding may be attributed to several factors, including the possibility that tannins are present at concentrations below the detection limit of the qualitative assay or are unevenly distributed within the plant matrix.

In addition, thermal effects during extraction may induce degradation or structural modification of tannins, thereby reducing their ability to form complexes with ferric ions in the colorimetric assay. Furthermore, tannins may interact with other constituents such as proteins or polysaccharides to form insoluble complexes that are not readily detectable in standard phytochemical screening methods (Hagerman, 2002). Therefore, the negative result does not necessarily indicate the complete absence of tannins but rather reflects limitations associated with extraction efficiency, compound stability, and assay sensitivity. This interpretation is further supported by the measurable total phenolic content detected in the bajakah root extract, indicating the presence of phenolic constituents, which may include tannin-related compounds that were not effectively captured by the qualitative test.

The data presented in Figure 3 show that plant extracts exhibit clear termite-repellent activity, as evidenced by increased termite mortality rates during the observation period. The 2.5% extract demonstrated the fastest and most pronounced effect, with high mortality rates observed in the early stages (Days 1–2), indicating an acute toxic

response or a strong repellent effect.

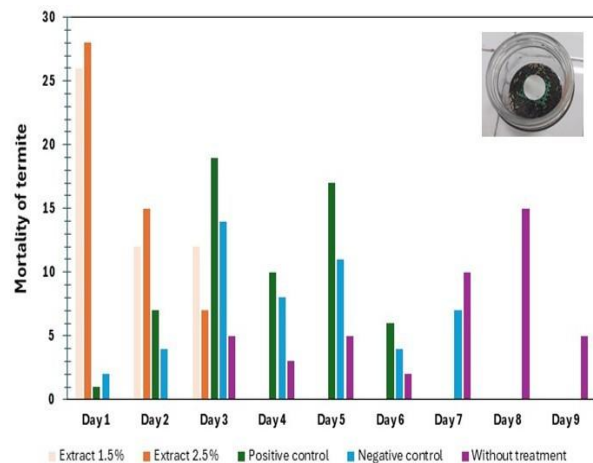


Figure 3 Anti-termite efficacy of *Spatholobus littoralis* root extracts by MAE.

In contrast, the 1.5% extract produced a more gradual increase in mortality rates, suggesting a dose-dependent effect of the bioactive compounds. Positive control consistently produced relatively high mortality rates over several days, validating the experimental system, while the negative control showed only limited mortality. Interestingly, the untreated group exhibited delayed mortality, likely due to starvation or environmental stress rather than direct toxic effects. Overall, the temporal mortality pattern indicates that higher extract concentrations accelerate termite mortality and enhance efficacy.

The observed anti-termite activity is likely attributed to the presence of bioactive phytochemicals such as phenolics, flavonoids, terpenoids, alkaloids, and tannins, which are known to interfere with insect physiology. These compounds may act through multiple mechanisms, including direct toxicity, inhibition of feeding behavior, and disruption of symbiotic gut microorganisms essential for cellulose digestion. Such interference can impair nutrient absorption and energy metabolism, ultimately leading to mortality (Supardan et al., 2024). Furthermore, the reduction in feeding activity observed in this study is consistent with the decrease in filter paper mass, confirming that the extracts function as both toxic and antifeedant agents. This behavior is in agreement with previous studies demonstrating that plant-derived secondary metabolites, particularly flavonoids and phenolic compounds, exhibit strong antifeedant activity against termites by altering feeding behavior and reducing cellulose

consumption (Ohmura et al., 2000a).

Compared to earlier studies that primarily relied on conventional extraction approaches, this study provides an advancement by utilizing microwave-assisted extraction technique, which are known to enhance the recovery of bioactive compounds responsible for antitermite activity. In conclusion, the extract demonstrates promising potential as an eco-friendly termite control agent with dual functionality as both a toxicant and feeding deterrent. The key contribution of this study lies in linking improved extraction efficiency with enhanced biological performance; however, further investigations on formulation stability, mechanisms of action, and field-scale validation are necessary to ensure practical applicability.

CONCLUSION

Microwave-assisted extraction (MAE) exhibited superior performance compared to ultrasound-assisted extraction (UAE), yielding a markedly higher mean concentration of the target compounds (48.92 ± 22.17 ppm; CV = 45.3%) than UAE (26.24 ± 13.60 ppm; CV = 51.8%). Although MAE showed slightly better reproducibility, the relatively high coefficients of variation for both methods indicate substantial experimental variability, highlighting limitations in process precision and robustness under the current conditions. These challenges may be associated with insufficient control of critical parameters such as particle size distribution, solvent-to-solid ratio, extraction time, and energy input, as well as potential heterogeneity in the plant matrix and the absence of strict standardization during sample preparation. Accordingly, while MAE can be considered the more effective technique in terms of extraction efficiency, the present study is limited by variability in replicate measurements and the lack of comprehensive optimization. Future work should focus on systematic optimization of extraction parameters using statistical design approaches and improved control of operational conditions to enhance reproducibility.

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