

## Green Extraction of *Kleinhovia hospita* Saponins: Superiority of Microwave Irradiation over Maceration and the Critical Role of Solvent Polarity

Baso Didik Hikmawan<sup>1\*</sup>, Khusnul Khotimah<sup>2</sup>, Mutia Ramadhani Rose Permana<sup>2</sup>,  
Islamudin Ahmad<sup>1</sup>, Lizma Febrina<sup>1</sup>, Muhammad Arifuddin<sup>1</sup>, Arsyik Ibrahim<sup>1</sup>, Supriatno  
Salam<sup>1</sup>

<sup>1</sup>Pharmaceutical Research and Development Laboratory of FARMAKA TROPIS, Faculty of Pharmacy, Universitas Mulawarman, Kampus Unmul Jalan Gunung Kelua, Samarinda, East Kalimantan 75119, Indonesia

<sup>2</sup>Undergraduate Pharmacy Program, Faculty of Pharmacy, Universitas Mulawarman, Kampus Unmul Jalan Gunung Kelua, Samarinda, East Kalimantan 75119, Indonesia

Submitted 02 December 2025; Revised 10 December 2025; Accepted 16 December 2025; Published 30 December 2025

\*Corresponding author: [didikhikmawan@unmul.ac.id](mailto:didikhikmawan@unmul.ac.id)

### Abstract

*Kleinhovia hospita* is a hepatoprotective plant rich in bioactive saponins, yet conventional extraction methods remain inefficient. This study aimed to optimize saponin recovery by comparing Microwave-Assisted Extraction (MAE) against conventional maceration and assessing the effect of solvent polarity. Saponins were extracted using MAE (15 minutes) and maceration (72 h) with various ethanol concentrations ranging from 0 to 96%. Total Saponin Content (TSC) was quantified colorimetrically using the vanillin-sulfuric acid method. MAE significantly outperformed maceration, attaining a two-fold increase in saponin purity (106.67 and 52.92 mg Diosgenin Equivalent (DE)/g extract, respectively) in a fraction of the time. Solvent analysis revealed a trade-off: whereas 96% ethanol yielded the highest specific purity (133.89 mg DE/g), it resulted in low mass recovery. In contrast, 50% ethanol extracted the highest Total Saponin Yield (TSY) (18.84 mg/g powder), effectively balancing matrix swelling with solubility. MAE utilizing 50% ethanol is identified as the optimal, rapid, and green strategy for the industrial production of standardized *K. hospita* extracts.

**Keywords:** cycloartane-triterpenoid saponins, microwave-assisted extraction, saponin, Tahongai

## Ekstraksi Hijau Saponin *Kleinhovia hospita*: Keunggulan Iradiasi Gelombang Mikro Dibandingkan Maserasi dan Peran Penting Polaritas Pelarut

### Abstrak

*Kleinhovia hospita* adalah tanaman hepatoprotektif yang kaya akan saponin bioaktif, namun metode ekstraksi konvensional masih kurang efisien. Studi ini bertujuan untuk mengoptimalkan perolehan saponin dengan membandingkan *Microwave-Assisted Extraction* (MAE) dengan maserasi konvensional dan menilai pengaruh polaritas pelarut. Saponin diekstraksi menggunakan MAE (15 menit) dan maserasi (72 jam) dengan berbagai konsentrasi etanol mulai dari 0 hingga 96%. Kandungan Saponin Total (TSC) diukur secara kolorimetri menggunakan metode vanillin-asam sulfat. MAE secara signifikan lebih unggul dari maserasi, dimana peningkatan kemurnian saponin mencapai dua kali lipat (106,67 dan 52,92 mg Diosgenin Ekuivalen (DE)/g ekstrak, masing-masing) dalam waktu yang lebih singkat. Analisis pelarut menunjukkan adanya kompromi: meskipun etanol 96% menghasilkan kemurnian spesifik tertinggi (133,89 mg DE/g), namun menghasilkan perolehan massa yang rendah. Sebaliknya, ekstraksi etanol 50% menghasilkan *Total Saponin Yield* (TSY) tertinggi (18,84 mg/g serbuk), secara efektif menyeimbangkan *swelling* matriks dengan kelarutan. MAE dengan etanol 50% diidentifikasi sebagai strategi optimal, cepat, dan ramah lingkungan untuk produksi industri ekstrak *K. hospita* yang terstandarisasi.

**Kata Kunci:** Saponin sikloartana-triterpenoid, *microwave-assisted extraction*, saponin, Tahongai

## 1. Introduction

The worldwide pharmaceutical sector is increasingly shifting towards the exploration of natural products as safer, sustainable alternatives for the treatment of chronic degenerative diseases.<sup>1,2</sup> Within this broad context, *Kleinhovia hospita* L. (locally known as Tahongai) has emerged as a promising candidate, having been traditionally utilized in Indonesia for the treatment of liver ailments. Recent studies have validated this empirical use, exhibiting potent hepatoprotective effects against drug-induced toxicity.<sup>3–5</sup> These therapeutic effects, along with reported anticancer and anti-inflammatory properties, are largely attributed to specific bioactive constituents concentrated in the leaves, primarily cycloartane-type triterpenoid saponins.<sup>6,7</sup> As the specific glycosylation pattern of these scaffolds significantly affects their solubility and bioavailability<sup>8</sup>, optimizing extraction to enrich these saponin constituents is a critical step toward producing standardized, biologically meaningful extracts.

Despite the therapeutic potential of *K. hospita*, current extraction processes predominantly use conventional methods such as maceration.<sup>9,10</sup> Although prevalent, solid–liquid maceration is frequently constrained by prolonged processing durations, excessive consumption of organic solvents, and low extraction yields.<sup>11</sup> Moreover, non-selective solvents often co-extract high levels of impurities, raising concerns regarding efficiency, scalability, and environmental impact.<sup>8,12</sup> These limitations are in contrast with the fundamental scientific shift toward "Green Extraction," which emphasizes minimum solvent consumption and greater energy efficiency while maintaining yield integrity.<sup>13,14</sup> Therefore, there is an urgent need to move from diffusion-limited conventional methods to advanced technologies.

Microwave-Assisted Extraction (MAE) is increasingly positioned as a superior alternative within this green extraction landscape. In contrast to maceration, MAE employs microwave-induced volumetric heating, which enhances mass transfer and disrupts plant cell walls, thus increasing extraction efficiency.<sup>12,15</sup>

For metabolite classes such as saponins, where plant matrix effects and solvent–solute interactions significantly influence recovery, the selection of method (MAE versus maceration) might determine not only the yield but also the feasibility of producing standardized extracts with minimized processing demands.<sup>1</sup>

The extraction process of *K. hospita* leaf saponins was optimized in this study to overcome these constraints. We evaluated the efficiency of MAE, a rapid technique that employs volumetric heating, in comparison to conventional maceration in terms of yield, time, and saponin purity. Despite the limitations of laboratory-scale MAE regarding batch sample capacity in comparison to traditional large-scale maceration vessels, this contemporary approach was intentionally chosen for its enhanced extraction kinetics. The improved extraction parameters at the laboratory scale are designed to function as a fundamental model for upscaling to high-throughput, continuous industrial microwave systems. Additionally, we investigated the effects of ethanol polarity on the trade-off between extract purity and total mass recovery by varying the ethanol concentrations from 0% to 96% during the extraction of the samples. This study aimed to provide a systematic, rapid, and energy-efficient protocol for the industrial production of standardized *K. hospita* saponin extracts, facilitating their prospective application potential in pharmaceutical and other fields.

## 2. Methods

### 2.1. Equipment

Modified Microwave (Modena MG 3116), Spectrophotometer UV-VIS (Thermo Scientific), Rotary Evaporator, Quartz Cuvette (Hellma), Water Bath, Micropipette, Beaker Glass, Glass Jar Volumetric Flask, Analytical balance (Ohaus PR224).

### 2.2. Materials

#### 2.2.1. Plant Material

The leaves of *Kleinhovia hospita* (voucher specimen number: 011/BRN/08/2024) were collected from Berau, East Kalimantan. This plant was taxonomically identified

and authenticated by the Laboratory of Ecology and Tropical Rainforest Biodiversity Conservation, Faculty of Forestry, Universitas Mulawarman, Indonesia.

### 2.2.2. Chemical materials

Diosgenin (standard reference) was purchased from Sigma-Aldrich. All reagents used in this study were analytical grade and purchased from Merck, including vanillin, sulfuric acid, ethanol p.a., potassium iodide, glacial acetic acid, hydrochloric acid, iron (III) chloride, mercuric chloride, acetic anhydride, and sulfuric acid. Distilled water was used for all aqueous preparations.

## 2.3. Procedures

### 2.3.1. Extraction

#### a. Conventional Maceration Extraction

Conventional maceration was performed based on our previous study to provide a baseline for extraction efficiency.<sup>17</sup> Briefly, 200 grams of dried leaf powder was extracted with 2 liters of 75% ethanol at room temperature for 3 days with occasional stirring. The mixture was filtered and separated from the residue, after which it was concentrated with a rotary evaporator at 60°C until viscous extract was obtained. The obtained extract was then subjected to a food dehydrator at 45°C until the extract was dried.

#### b. Microwave-assisted Extraction (MAE)

MAE was conducted using a modified microwave (Modena MG 3116, Modena Electromedistici, Italy) equipped with a reflux condenser to prevent solvent loss. To compare MAE performance against maceration, extraction was conducted using 75% ethanol under fixed conditions: microwave power of 450 watts, irradiation time of 15 min, and a solid-to-solvent ratio of 1:10 g/mL (100 grams of dried leaf powder was extracted with 1 liter of 75% ethanol). These operating parameters were selected based on previous optimization studies, which demonstrated that 450 W provides sufficient volumetric heating to rupture plant cell walls without causing thermal degradation of the bioactive constituents.<sup>18</sup>

#### c. Optimization of Solvent Polarity

To evaluate the effect of solvent polarity on saponin recovery, MAE was conducted using five different solvent compositions: distilled water (0% ethanol) and aqueous ethanol at concentrations of 25%, 50%, 75%, and 96% (v/v). All other operational parameters (power: 450 watts; time: 15 mins; solid-to-solvent ratio: 1:10 g/mL) were kept constant. After irradiation, extracts were cooled to room temperature, filtered, concentrated, and dried as described in the Conventional Maceration Extraction section.

### 2.3.2. Phytochemical screening

The crude extract was subjected to qualitative phytochemical screening to identify major types of secondary metabolites, including alkaloids, saponins, flavonoids, phenolics, tannins, terpenoids, and steroids.<sup>19</sup> Phytochemical screening was conducted to detect major secondary metabolites using standard methods as described below:

#### a. Test for Alkaloids

The presence of alkaloids was assessed using standard precipitation reagents. Approximately 500 mg of the extract was dissolved in 5 mL of 2 N hydrochloric acid (HCl) and heated on a water bath for 2 min. The mixture was filtered, and the filtrate was divided into three aliquots. These were treated individually with Dragendorff's, Mayer's, and Wagner's reagents. The formation of an orange-red precipitate (Dragendorff), a creamy-white precipitate (Mayer), or a reddish-brown precipitate (Wagner) indicated the presence of alkaloids.

#### b. Test for Saponins (Froth Test)

Saponins were detected using the foam test. The extract (500 mg) was suspended in a test tube containing 10 mL of distilled water and boiled for 5 min in a water bath. The mixture was allowed to cool and then shaken vigorously in a vertical direction for 15 s. The formation of a persistent honeycomb froth stable for more than 3 min was considered a positive indication of saponins.

c. Test for Flavonoids (Shinoda Test)

Flavonoid analysis was performed using the cyanidin reaction. Approximately 0.5 g of the extract was dissolved in 5 mL of hot distilled water. To this solution, 0.1 g of magnesium powder and 1 mL of concentrated HCl were added. The development of a red, yellow, or orange coloration indicated the presence of flavonoids.

d. Test for Phenolics and Tannins

**Phenolics:** The presence of phenolic compounds was assessed by adding 2–3 drops of 1% (w/v) ferric chloride ( $\text{FeCl}_3$ ) reagent to the aqueous extract solution. The change of color to intense green, purple, blue, or black indicated a positive result.

**Tannins:** To assess the presence of tannins, 500 mg of the extract was dissolved in 5 mL of hot water and subsequently filtered. A few drops of 1% gelatin solution were added to the filtrate. The formation of a white precipitate confirmed the presence of tannins.

e. Test for Terpenoids and Steroids (Liebermann-Burchard Test)

The extract (0.5 g) was dissolved in 2 mL of chloroform and filtered. The filtrate was treated with 2–3 drops of the Liebermann-Burchard reagent (acetic anhydride mixed with concentrated sulfuric acid). The solution was observed for color changes at the interface. The appearance of a brownish-red or violet ring at the interface indicated the presence of triterpenoids, while the formation of a blue-green ring indicated the presence of steroids.

### 2.3.3. Determination of Total Saponin

Total saponin content was quantified colorimetrically using the vanillin-sulfuric acid method as described by Le et al. (2018) with minor modifications.<sup>20</sup> Prior to the determination of total saponin content, the maximum wavelength, the operating time of diosgenin-sulfuric acid-vanillin reagent, and the diosgenin calibration curve were carried out.

a. Preparation of Standard Curve

A stock solution of diosgenin was

prepared in methanol. A series of working standard solutions were prepared to obtain concentrations ranging from 200 to 800  $\mu\text{g/mL}$ . A 0.1 mL aliquot of each standard solution was mixed with 0.5 mL of 4% (w/v) vanillin solution (in ethanol) and 2.5 mL of 72% (v/v) sulfuric acid. The mixture was incubated in a water bath at 60°C for 20 min to develop the color, then cooled in an ice-water bath. Absorbance was measured at 450 nm against a reagent blank using a UV-Vis spectrophotometer (Genesys 1XX, ThermoFisher Scientific).

b. Sample Analysis

Dried extracts were redissolved in methanol to a known concentration. The sample solutions were treated identically to the standards. TSC was calculated from the linear regression equation of the calibration curve ( $R^2 > 0.9937$ ) and expressed as milligrams of diosgenin equivalents per gram of dry extract (mg DE/g extract) or per gram of dry powder (mg DE/g powder). All measurements were performed in triplicate.

### 2.4. Statistical Analysis

All extraction experiments were performed in triplicate ( $n=3$ ), and data are expressed as Mean  $\pm$  Standard Deviation (SD). Statistical analysis of the extract yields and total saponin contents was conducted using the Python programming language utilizing the SciPy and NumPy libraries. To compare the extraction efficiency between the two methods (Maceration vs. Microwave-Assisted Extraction), an independent samples t-test was performed. The effects of different ethanol concentrations on extraction yield and saponin content were analyzed using One-Way Analysis of Variance (ANOVA) followed by post-hoc testing where applicable. A p-value of  $< 0.05$  was considered statistically significant. Data visualization and graphical representation were generated using the Matplotlib library.

## 3. Results

### 3.1. Calibration Curve

The quantitative analysis of Total

Saponin Content (TSC) was performed using diosgenin as the standard. The calibration curve showed good linearity ( $R^2 = 0.9937$ ) within the concentration range of 200–800  $\mu\text{g/mL}$ . The regression equation was determined as  $y = 0.0006x + 0.192$ .

### 3.2. Comparison of Extraction Methods

The extraction efficiency of MAE was compared to conventional maceration using 75% ethanol. As shown in Figure 1, MAE resulted in a significantly higher TSC ( $106.67 \pm 2.92$  mg DE/g extract) compared to maceration ( $52.92 \pm 2.92$  mg DE/g extract) ( $p < 0.05$ ). Moreover, MAE attained this yield in a significantly shorter duration (15 minutes) compared to maceration (3x24 hours).

### 3.3. Effect of Solvent Polarity on Extract Yield and Saponin Recovery

The polarity of the extraction solvent significantly affected both the crude extract yield and the total saponin (diosgenin equivalent) recovered from *K. hospita* leaves (Figure 2). The extract yield had a non-linear pattern, reaching a notable maximum at 50% ethanol ( $20.37 \pm 2.25\%$ ) before sharply decreasing as the ethanol concentration approached 96% ( $8.73 \pm 1.62\%$ ).

Conversely, the total saponin content (TSC) of the extract, measured in diosgenin equivalent (DE), showed a consistent increase with rising ethanol concentrations. The lowest

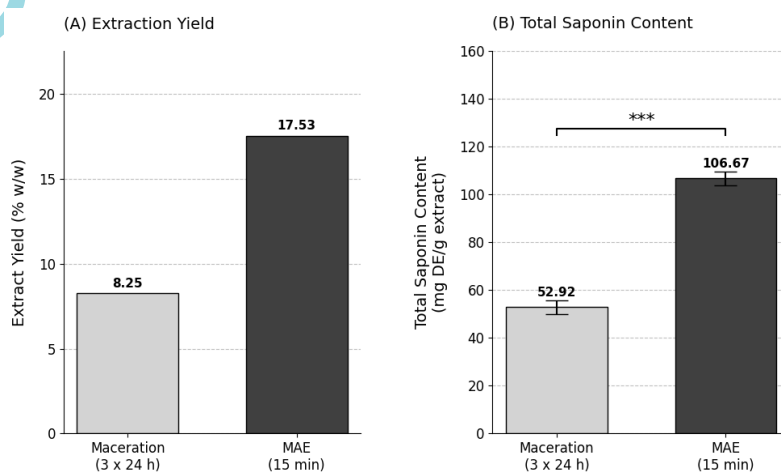
TSC was observed in the aqueous extract ( $39.72 \pm 1.88$  mg DE/g extract), whereas the highest TSC was achieved using 96% ethanol ( $133.89 \pm 1.96$  mg DE/g), representing a 3.3-fold increase in saponin concentration.

However, when analyzing the Total Saponin Yield (TSY), calculated as the total mass of saponins recovered per gram of dry leaf powder, a distinct optimum was revealed (shown in Table 1). Although 96% ethanol yielded the highest purity, its low extract yield resulted in a poor total recovery from the plant matrix (11.69 mg/g dry leaves powder). In contrast, 50% ethanol achieved the highest TSY (18.84 mg DE/g leaves powder), statistically comparable to 75% ethanol (18.70 mg DE/g) but significantly superior to both water (5.47 mg/g) and 96% ethanol. This indicates that while 96% ethanol is more selective for saponins, 50% ethanol is the most effective solvent for maximizing total saponin recovery from the raw biomass.

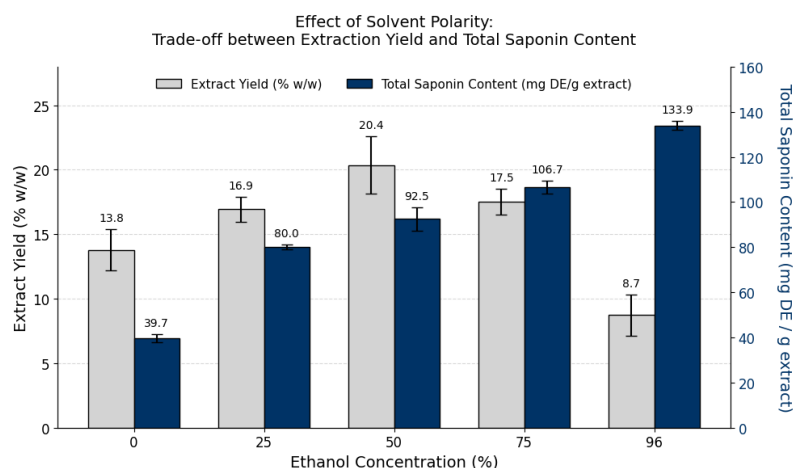
## 4. Discussion

### 4.1. Efficiency of Microwave-Assisted Extraction

The significant elevation in total saponin content observed with Microwave-Assisted Extraction (MAE) compared to maceration can be attributed to the unique mechanism of microwave heating. Unlike maceration, which relies on superficial diffusion and passive mass transfer, microwaves produce heat throughout



**Figure 1.** Comparison of extraction efficiency between conventional maceration (3 x 24 h) and Microwave-Assisted Extraction (MAE; 15 minutes) using 75% ethanol. (A) Extract yield (% w/w); (B) Total Saponin Content (mg DE/g extract). Data are expressed as mean  $\pm$  standard deviation ( $n=3$ ). The asterisk (\*\*\*) indicates a statistically significant difference ( $p < 0.001$ ).



**Figure 2.** Impact of ethanol concentration on the extract yield and saponin purity of *K. hospita* leaves. The grey bars indicate the extract yield (% w/w, left axis), whereas the blue bars represent the Total Saponin Content (mg Diosgenin Equivalent/g extract, right axis). Data are expressed as mean  $\pm$  standard deviation ( $n=3$ ).

the plant material by ionic conduction and dipole rotation.<sup>21</sup> This rapid internal heating causes moisture within the plant cells to vaporize, creating high internal pressure that ruptures the cell walls and facilitates the release of intracellular saponins into the solvent.<sup>22</sup> The substantial reduction in extraction time (from 72 hours to 15 minutes) further prevents the thermal degradation of sensitive saponin structures that might occur during prolonged exposure to ambient conditions. Consequently, MAE offers a dual advantage of higher purity and superior energy efficiency, making it a feasible eco-friendly option for the industrial extraction of *K. hospita* saponins. The findings are aligned with the study on *Sapindus mukorossi* pericarps, indicating that MAE attained a saponin yield of 280.55 mg/g under optimal conditions (40% ethanol, 425 W power), surpassing conventional methods. This contrasts with the slower, surface-limited extraction of maceration.<sup>23</sup> In addition, MAE typically extracts 1.3–7.8 times more

saponins than maceration or heat reflux, as seen in *Gymnemagenin* yields (4.3% w/w in 6 minutes) and *Jatropha curcas* leaves (35.04 mg/g). Variables such as microwave power, solvent ratio, and short exposure time minimize degradation while enhancing efficiency.<sup>24–26</sup>

#### 4.2. Effect of Solvent Polarity and Selection of Optimal Solvent

Contrary to studies on other saponin-rich plants, which typically report an optimum recovery at 50–70% ethanol<sup>27–30</sup>, our findings demonstrated that 96% ethanol yielded the highest specific Total Saponin Content (TSC) per gram of extract. This improved purity can be attributed to the selectivity against polar impurities: water and low-concentration ethanol exhaustively extract highly polar primary metabolites, such as polysaccharides and soluble proteins, which act as 'ballast' and dilute the final extract purity.<sup>31</sup> By using 96% ethanol, these hydrophilic impurities are minimized. Furthermore, the cycloartane-

**Table 1.** Calculated Total Saponin Yield of *Kleinhovia hospita* leaves

Solvent	Extract Yield (%)	Total Saponin Content (mg/g extract)	Total Saponin Yield (mg/g leaves powder)
0% (Water)	13.77 $\pm$ 1.60	39.72 $\pm$ 1.88	5.47
25% Ethanol	16.93 $\pm$ 0.97	80.00 $\pm$ 1.10	13.54
50% Ethanol	20.37 $\pm$ 2.25	92.50 $\pm$ 5.22	18.84
75% Ethanol	17.53 $\pm$ 1.01	106.67 $\pm$ 2.92	18.70
96% Ethanol	8.73 $\pm$ 1.62	133.89 $\pm$ 1.96	11.69

type triterpenoid saponins dominant in *K. hospita* likely possess significant lipophilic character, adhering to the 'like dissolves like' principle. This suggests that the saponins in this species may contain methylated aglycones or shorter sugar chains, making 96% ethanol the preferred solvent for obtaining highly refined saponin fractions.

However, a critical trade-off exists between extract purity and overall yield. While 96% ethanol yielded the highest purity, it resulted in poor Total Saponin Yield (TSY) due to the limited capacity of the solvent to penetrate the dry plant matrix. In industrial contexts, maximizing Total Saponin Yield (TSY) is prioritized over achieving the highest specific purity (TSC), since it is crucial for the process to remain economically viable by extracting the maximum bioactive compounds from the raw biomass. The optimal total recovery observed at 50% ethanol suggests that this binary mixture provides the ideal balance: the aqueous component hydrates the plant cellulose to facilitate mass transfer, while the ethanol component solubilizes the saponin aglycones.

This non-linear trend, where intermediate ethanol concentrations optimize total recovery, aligns with research on other saponin-rich species. For instance, saponin yield from steamed *Panax notoginseng* was found to peak at 60% ethanol (31.96% yield), declining significantly at extreme concentrations.<sup>28</sup> Similarly, steroidal saponins from *Polygonatum kingianum* achieved maximum TSY at 85% ethanol, with recovery dropping sharply at 95%.<sup>32</sup> These indicate that while high ethanol levels often yield purer extracts, the reduced crude extract yield leads to lower overall recovery. Our results mirror these patterns, where intermediate concentrations (50–75%) effectively promote cell swelling and saponin desorption without the excessive hydrolysis or low yields associated with water or absolute ethanol.

Consequently, 50% ethanol is identified as the optimal solvent for large-scale processing. It maximizes the harvest of bioactive saponins from the raw leaf biomass (high TSY) while offering significant economic

advantages and supporting Green Chemistry principles by minimizing organic solvent consumption. Although the higher aqueous content of 50% ethanol necessitates longer downstream concentration times and higher evaporation energy compared to 96% ethanol, potential long-term stability issues, such as saponin hydrolysis or microbial growth from residual moisture are effectively mitigated by completely drying the concentrated extracts at 45°C.

## 5. Conclusion

This study successfully demonstrates that Microwave-Assisted Extraction (MAE) is a vastly superior, rapid, and green alternative to conventional maceration for extracting bioactive saponins from *K. hospita* leaves. Furthermore, solvent polarity plays a critical role in the extraction dynamics, revealing a distinct trade-off between extract purity and total mass recovery. While near-absolute ethanol (96%) provides the highest saponin purity, a 50% aqueous ethanol mixture proved to be the optimal solvent for maximizing the Total Saponin Yield (TSY) from the raw biomass. Consequently, MAE coupled with 50% ethanol represents an efficient, scalable, and economically viable protocol for the industrial production of *K. hospita* saponin extracts. Future studies should focus on the isolation and structural characterization of the specific cycloartane glycosides responsible for the observed biological activities.

## References

1. Xie S, Zhan F, Zhu J, Xu S, Xu J. The latest advances with natural products in drug discovery and opportunities for the future: a 2025 update. *Expert Opin Drug Discov* 2025;20:827–43. <https://doi.org/10.1080/17460441.2025.2507382>.
2. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov* 2021;20:200–16. <https://doi.org/10.1038/s41573-020-00114-z>.
3. Djabir YY, Arsyad MA, Sartini S, Lallo S. Potential Roles of *Kleinhovia hospita* L.

- Leaf Extract in Reducing Doxorubicin Acute Hepatic, Cardiac and Renal Toxicities in Rats. *Pharmacogn Res* 2017;9:168–73. [https://doi.org/10.4103/pr.pr\\_129\\_16](https://doi.org/10.4103/pr.pr_129_16).
4. Djabir YY, Arsyad A, Murdifi M, Tayeb R, Amir MN, Kamaruddin FA-F, Najib NH. *Kleinhovia hospita* extract alleviates experimental hepatic and renal toxicities induced by a combination of antituberculosis drugs. *J Herbm Pharm* 2020;10:102–8. <https://doi.org/10.34172/jhp.2021.10>.
  5. Arung ET, Kusuma IW, Kim Y-U, Shimizu K, Kondo R. Antioxidative compounds from leaves of Tahongai (*Kleinhovia hospita*). *J Wood Sci* 2012;58:77–80. <https://doi.org/10.1007/s10086-011-1217-7>.
  6. Rahim A, Saito Y, Miyake K, Goto M, Chen C-H, Alam G, Morris-Natschke S, Lee K-H, Nakagawa-Goto K. Kleinhospitine E and Cycloartane Triterpenoids from *Kleinhovia hospita*. *J Nat Prod* 2018;81:1619–27. <https://doi.org/10.1021/acs.jnatprod.8b00211>.
  7. Tofrizal A, Zulfikri E, Arya B, Maliza R. Anticancer Activity of Ethanol Extract of *Kleinhovia hospita* L. Leaves Against HCT-116 Colorectal Cancer Cells. *Trop J Nat Prod Res* 2025;9:6046–55. <https://doi.org/10.26538/tjnpr/v9i12.119>.
  8. Fordos S, Amin S, Abid N, Pasha I, Khan MKI, Amin A, Gulzar M, Subtain M, Abdi G. Saponins: Advances in extraction techniques, functional properties, and industrial applications. *Appl Food Res* 2025;5:101146. <https://doi.org/10.1016/j.afres.2025.101146>.
  9. Suryani AI, Yusrini Y, Rahim A, Minarfa T. Protective Effects of *Kleinhovia hospita* Leaf Extract against Triton X-100-Induced Hypercholesterolemia in Rats. *Iran J Toxicol* 2024;18:1–5. <https://doi.org/10.61186/IJT.18.1.1>.
  10. Solihah I, Herlina H, Anggraini F, Fitria N, Kirana M. The potential healing effect of *Kleinhovia hospita* L. leaves extract on second-degree burns in rats. *J Adv Pharm Educ Res* 2023;13:109–18. <https://doi.org/10.51847/VusD92SyAU>.
  11. Osorio-Tobón JF. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *J Food Sci Technol* 2020;57:4299–315. <https://doi.org/10.1007/s13197-020-04433-2>.
  12. Usman M, Nakagawa M, Cheng S. Emerging Trends in Green Extraction Techniques for Bioactive Natural Products. *Processes* 2023;11. <https://doi.org/10.3390/pr11123444>.
  13. Ahmad I, Hikmawan BD, Mun'im A, Sulistiarini R. *Peperomia pellucida* (L.) Kunth herbs: A comprehensive review on phytochemical, pharmacological, extraction engineering development, and economic promising perspectives. *J Appl Pharm Sci* 2022. <https://doi.org/10.7324/JAPS.2023.130201>.
  14. Ahmad I, Kurnya, Iswahyudi I, Suhardi H, Hikwaman BD, Putra AR, Angelina M, Mun'im A. The Optimum Condition Design of Microwave-assisted Extraction Combined Lactic Acid-Sucrose Based Natural Deep Eutectic Solvent on Polyphenols Enrichment from *Eleutherine bulbosa* Mill. Urb. Bulbs. *J Res Pharm* 2023;27:1086–95. <https://doi.org/10.29228/jrp.401>.
  15. Melikoglu M. Microwave-assisted extraction: Recent advances in optimization, synergistic approaches, and applications for green chemistry. *Sustain Chem Clim Action* 2025;7:100122. <https://doi.org/10.1016/j.scca.2025.100122>.
  16. Ahmad I, Hikmawan BD, Febrina L, Junaidin J, Rusman A, Salam S, Suhartono E, Nugroho Y, Iskandar I, Zein M, Julianto V, Rahmanto O, Mun'im A. Application of microwave-assisted extraction using glucose-citric acid deep eutectic solvent for enhancement of polyphenols extraction from *Peperomia pellucida* (L) Kunth herb. *J Appl Pharm Sci* 2025;15:089–97. <https://doi.org/10.7324/JAPS.2025.231184>.
  17. Ahmad I, Hikmawan BD, Maharani DF, Nisrina N, Arifianti AE, Mun'im A. Natural Deep Eutectic Solvent based Ultrasound-assisted extraction: A green approach for extraction of sulfhydryl and mimosine from *Leucaena leucocephala* (Lam) de Wit seeds. *Heliyon* 2023;9.

18. Ahmad I, Shakti SOP, Prabowo WC, Hikmawan BD, Arifuddin M, Angelina M, Prastya ME, Okselni T, Alwi RS, Sadikin R, Iswahyudi I, Febrina L, Herman H, Junaidin J, Faisal M, Samsul E, Ibrahim A, Mun'im A. Citric acid-glycerol-based NADES for microwave-assisted extraction enhances the polyphenols level of *Eleutherine bulbosa* mill. Urb. Bulbs. J Appl Pharm Sci 2024;14;:189–97. <https://doi.org/10.7324/JAPS.2024.180291>.
19. Febrina L, Hikmawan BD, Adyama MD. Secondary Metabolite Profiles and Antibacterial Activity of *Mitragyna speciosa* Leaf Extracts According to Vein Color Variation. J Pharm Nat Sci 2025;2:109–18. <https://doi.org/10.70392/jpns.v2i3.39>.
20. Le AV, Parks SE, Nguyen MH, Roach PD. Improving the Vanillin-Sulphuric Acid Method for Quantifying Total Saponins. Technologies 2018;6. <https://doi.org/10.3390/technologies6030084>.
21. Mandal V, Mohan Y, Hemalatha S. Microwave Assisted Extraction – An Innovative and Promising Extraction Tool for Medicinal Plant Research. Pharmacogn Rev 2007;1.
22. Chan C-H, Yeoh HK, Yusoff R, Ngoh GC. A first-principles model for plant cell rupture in microwave-assisted extraction of bioactive compounds. J Food Eng 2016;188:98–107. <https://doi.org/10.1016/j.jfoodeng.2016.05.017>.
23. Deng B, Liu Z, Zou Z. Optimization of Microwave-Assisted Extraction Saponins from *Sapindus mukorossi* Pericarps and an Evaluation of Their Inhibitory Activity on Xanthine Oxidase. J Chem 2019;2019:5204534. <https://doi.org/10.1155/2019/5204534>.
24. Dahmoune B, Houma-Bachari F, Chibane M, Akroure-Aissou C, Guégan J-P, Vives T, Jehan P, Dahmoune F, Mouni L, Ferrières V, Hauchard D. Microwave assisted extraction of bioactive saponins from the starfish *Echinaster sepositus*: Optimization by response surface methodology and comparison with ultrasound and conventional solvent extraction. Chem Eng Process - Process Intensif 2021;163:108359. <https://doi.org/10.1016/j.cep.2021.108359>.
25. Aryanti N, Khoiriyah L, Nafiunisa A, Ratnawati, Widiasta IN, Zakki A, Adina AR. Microwave-assisted extraction of eco-friendly surfactant from *Jatropha curcas* for sustainable solubilization of reactive dyes. Commun Sci Technol 2025;10:52–8. <https://doi.org/10.21924/cst.10.1.2025.1636>.
26. Mandal V, Dewanjee S, Mandal SC. Microwave-assisted extraction of total bioactive saponin fraction from *Gymnema sylvestre* with reference to gymnemagenin: a potential biomarker. Phytochem Anal PCA 2009;20:491–7. <https://doi.org/10.1002/pca.1151>.
27. Kwon J-H, Lee G-D, Bélanger JMR, Jocelyn Paré JR. Effect of ethanol concentration on the efficiency of extraction of ginseng saponins when using a microwave-assisted process (MAPTM). Int J Food Sci Technol 2003;38:615–22. <https://doi.org/10.1046/j.1365-2621.2003.00688.x>.
28. Hu Y, Cui X, Zhang Z, Chen L, Zhang Y, Wang C, Yang X, Qu Y, Xiong Y. Optimisation of Ethanol-Reflux Extraction of Saponins from Steamed *Panax notoginseng* by Response Surface Methodology and Evaluation of Hematopoiesis Effect. Mol J Synth Chem Nat Prod Chem 2018;23:1206. <https://doi.org/10.3390/molecules23051206>.
29. Fang Y, Kan W, Yimamu Y, Cui X, Luo Y, Cao X. Extraction, purification, and identification of total saponins from hazel mushroom and its application evaluation. Front Nutr 2026;12. <https://doi.org/10.3389/fnut.2025.1737642>.
30. Jin Z, Zhou A, Wang S, Zhao Y, Shu J, Song Y, Liu M, Tian C. Study on the extraction process, chemical compositions, and anti-inflammatory activity of total saponins extract from *Anemone raddeana* Regel. Pharmacol Res - Mod Chin Med 2023;9:100332. <https://doi.org/10.1016/j.prmcm.2023.100332>.
31. Zhang Q-W, Lin L-G, Ye W-C. Techniques for extraction and isolation of natural

- products: a comprehensive review. *Chin Med* 2018;13:20. <https://doi.org/10.1186/s13020-018-0177-x>.
32. He S, Wang X, Chen J, Li X, Gu W, Zhang F, Cao G, Yu J. Optimization of the Ultrasonic-Assisted Extraction Technology of Steroidal Saponins from *Polygonatum kingianum* Collett & Hemsl and Evaluating Its Quality Planted in Different Areas. *Molecules* 2022;27:1463. <https://doi.org/10.3390/molecules27051463>.

GALLEY PROOF