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Catalytic Enhancement of Gmelinol Yield from *Gmelina arborea* Leaves: Process Optimization and Reproducibility

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Abstract. *Gmelinol, a bioactive lignan derived from Gmelina arborea, exhibits significant antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. Despite its pharmacological potential, existing extraction methods are largely qualitative, poorly reproducible, and lack quantitative yield metrics. This study addresses these gaps by developing a reproducible and statistically validated catalytic process for gmelinol production from Gmelina arborea leaves using barium chloride-catalyzed thermal hydrolysis. The effects of reaction time (10-50 min) on gmelinol yield were systematically investigated at 80 °C. Optimal conditions were identified at 40 min, yielding 17.90% (437.12 mg/g) of gmelinol with excellent reproducibility (coefficient of variation = 3.95%). Statistical analyses, including paired t-tests and one-way ANOVA, confirmed that reaction time significantly influenced yield ($p<0.001$), while Tukey HSD post-hoc tests validated 40 min as the optimal duration. Prolonged reaction times led to yield reduction, indicative of product degradation. This work provides the first quantitative, mass-based yield benchmark for gmelinol extraction, establishing a reliable and scalable catalytic route that enhances both efficiency and reproducibility. The findings support the sustainable valorization of G. arborea leaf biomass for pharmaceutical and industrial applications.*

Keywords: *Gmelinol; Gmelina Arborea; Barium Chloride; Thermal Hydrolysis; Catalytic Extraction*

1. Introduction

Many pharmaceutical drugs currently in use are synthesized using chemical agents that may produce adverse effects when administered over prolonged periods (Odiba *et al*., 2016; Kümmerer, 2009). In contrast, numerous ailments treated with conventional pharmaceutical formulations can also be managed using bioactive compounds derived directly from forest-based medicinal resources, including leaves, bark, stems, and roots (Tiwari *et al*., 2025; Hemmami *et al*., 2024; Rana *et al*., 2021; Arya *et al*., 2021; Murugesan & Senthilkumar, 2017). Traditional herbal therapies are generally associated with improved biocompatibility and a lower incidence of long-term toxicity, as they typically involve minimal chemical modification or synthetic additives (Jităreanu *et al*., 2022; Bilia *et al*., 2019). In addition to their therapeutic roles, medicinal plants often possess significant nutritional value, contributing to overall physiological well-being (Radha *et al*., 2021; Awuchi, 2019). These forest-derived resources are rich in diverse bioactive constituents, such as alkaloids, flavonoids, terpenoids, lignans, and phenolic compounds, which have been reported to exhibit anticancer, anti-inflammatory, antibacterial, antiviral, and antioxidant activities (Tiwari *et al*., 2025; Tchamgoue *et al*., 2024; Stevanovic *et al*., 2009). Consequently, plant-based

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remedies represent a valuable and sustainable alternative for disease management and drug discovery.

Gmelinol is a naturally occurring lignan belonging to the polyphenolic compounds biosynthesized via the phenylpropanoid pathway in plants (Ortiz & Sansinenea, 2023; Patyra et al., 2022; Martinengo et al., 2021). The compound was first isolated from species of the *Gmelina* genus, notably from the leaves of *Gmelina arborea* (Kadam et al., 2025; Goswami et al., 2024; Warrier et al., 2021), a fast-growing tree native to several regions of Asia and Africa (Waswa et al., 2022; Adesina et al., 2017). Structurally, gmelinol exhibits a dibenzylbutane skeleton typical of lignans (Figure 1) and is commonly obtained as a crystalline solid (Alfonzo, 2020; Linder, 2016). Owing to its diverse biological activities, gmelinol has attracted increasing attention in natural product and pharmaceutical research (Ben Ammar, 2023; Riyadi et al., 2023).

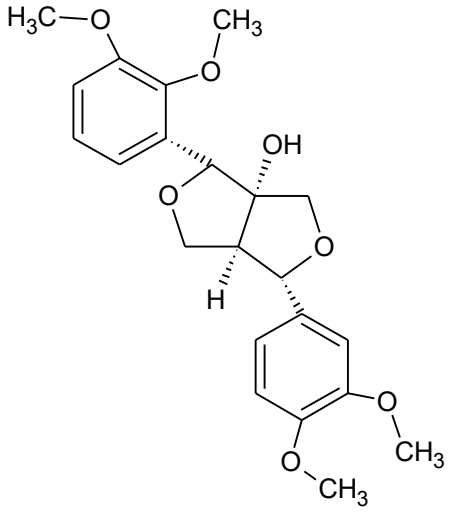

Figure 1. Structural formula of gmelinol

Gmelinol has demonstrated broad-spectrum antimicrobial activity, particularly against Gram-positive bacteria such as *Staphylococcus aureus* and fungal species including *Candida albicans* (Wiart et al., 2023; Waswa et al., 2022; Martinengo et al., 2021). These effects are attributed to its ability to compromise microbial cell membranes and disrupt essential enzymatic pathways (Górniak et al., 2019; Khameneh et al., 2019; Natvarlal et al., 2018). In addition, gmelinol exhibits notable antioxidant activity by scavenging reactive oxygen species (ROS), thereby reducing oxidative stress and cellular damage (Goswami et al., 2024; Waswa et al., 2022; Natvarlal & Bothara, 2018). Anti-inflammatory activity has been reported through the suppression of pro-inflammatory mediators, including prostaglandins and leukotrienes (Ríos, 2008; Saleem et al., 2005). Gmelinol has also been shown to possess antipyretic properties, which are believed to arise from inhibition of prostaglandin synthesis in the hypothalamus, resulting in reduced febrile responses (Na Nongkhai et al., 2024; Goswami et al., 2024; Adesina et al., 2017; Warrier et al., 2015; Chakraborty et al., 2024). These pharmacological attributes highlight its potential utility in managing inflammatory and fever-related conditions.

Beyond antimicrobial and anti-inflammatory effects, gmelinol has demonstrated gastroprotective activity by reducing gastric lesions and preserving mucosal integrity in chemically induced ulcer models, including ethanol- and NSAID-induced gastric injury (Beiranvand, 2022; Serafim et al., 2021; Fulga et al., 2020). Moreover, gmelinol has been

reported to exhibit antidiabetic potential through multiple mechanisms, including enhancement of insulin sensitivity (Kadam et al., 2025; Patel & Tirgar, 2023), suppression of hepatic glucose production (Sreelakshmi et al., 2025; Suneetha et al., 2023; Wadasinghe et al., 2022; Doungwichitrkul, 2018), and stimulation of glucose uptake in skeletal muscle cells (Kaushik et al., 2023; Jeeva et al., 2019).

In *Gmelina arborea*, gmelinol is predominantly localized in the heartwood, with additional occurrence in the leaves and bark (Warrier et al., 2021; Natvarlal & Bothara, 2018; Sankh, 2015). Its presence contributes to the plant's intrinsic defense mechanisms against pathogenic organisms (Sharma et al., 2022; Jamiołkowska, 2020) and supports the extensive ethnomedicinal use of *G. arborea* in the treatment of infections and inflammatory disorders (Kadam et al., 2025; Bhat et al., 2023). From an applied perspective, gmelinol and related lignans have been investigated as natural preservatives, bioactive surface coatings, and lead compounds for pharmaceutical development (Waswa et al., 2022; Martinengo et al., 2021; Belaid et al., 2024; Amarasiri et al., 2022).

Several methods have been reported for the isolation of gmelinol, including conventional solvent extraction (Falah et al., 2008; Yan et al., 2007), steam distillation (Hillis, 2012; Kadam et al., 2025), cold pressing (Ho, 2016; Górnaś et al., 2014), and supercritical fluid extraction (Tian et al., 2025). Supercritical techniques have also been successfully applied to the recovery of structurally related lignans from other botanical sources, such as *Acanthopanax senticosus* and *Podophyllum peltatum* (Patyra et al., 2025). From a synthetic perspective, gmelinol can be obtained via oxidative transformation of pinoresinol (Shettigar et al., 2020; Runeberg et al., 2019) or through acid-catalyzed hydrolysis routes (Patel & Tirgar, 2023; Dziadas & Jeleń, 2016).

Although *Gmelina arborea* has been widely investigated for its ethnomedicinal value and phytochemical composition, existing studies on gmelinol are largely qualitative, emphasizing isolation, identification, and biological activity rather than quantitative production metrics. Most reported extraction methods lack mass-based yield data, reaction optimization, and reproducibility analysis, making meaningful comparison across studies difficult. Moreover, research has predominantly focused on heartwood, while leaf biomass, an abundant and renewable resource, remains underexplored despite its phytochemical relevance. Reported extraction approaches are often solvent-intensive or energy-demanding, with limited attention to mild, aqueous, and catalytically assisted processes suitable for sustainable scale-up. In addition, the use of simple inorganic Lewis acid catalysts, such as barium chloride, for promoting gmelinol release via thermal hydrolysis has not been systematically examined, and statistical validation of process parameters is largely absent. Accordingly, a clear gap exists for a quantitative, reproducible, and statistically validated catalytic route for gmelinol production from *Gmelina arborea* leaves. This study addresses this gap by establishing an optimized barium chloride-catalyzed thermal hydrolytic process with explicit yield reporting and rigorous statistical evaluation. By valorizing an underutilized, renewable leaf biomass through a mild, catalytically assisted approach, the work advances SDG 9 (Industry, Innovation and Infrastructure) by promoting scalable and innovation-driven bioprocess development, while simultaneously supporting SDG 12 (Responsible Consumption and Production) through waste minimization, efficient resource utilization, and the development of environmentally responsible chemical production pathways.

This study aims to develop a reproducible and statistically validated catalytic process for the efficient extraction of gmelinol from *Gmelina arborea* leaves using barium chloride-catalyzed thermal hydrolysis. This was achieved by optimizing reaction time for maximum gmelinol yield under mild thermal conditions (80 °C), evaluating the reproducibility of the extraction process across duplicate experimental runs, statistically assessing the significance of reaction time on gmelinol yield using paired t-tests and one-way ANOVA, identify the optimal reaction duration that balances hydrolysis efficiency with product stability while minimizing variability, and provide quantitative yield data for gmelinol production, addressing the gap in existing qualitative studies.

2. Methods

Dried *Gmelina arborea* leaves served as the biomass feedstock for this study. The materials and equipment employed included a ceramic mortar and pestle, 250 µm and 300 µm sieves, 1000 mL conical flasks and beakers, a 500 mL separating funnel, thermometer, distilled water, a Gallenkamp hot plate equipped with a magnetic stirrer, filter cloth, and filter paper. Analytical-grade reagents consisted of barium chloride, used as the catalyst, and sodium sulphate as the drying agent. The *Gmelina arborea* leaves were obtained from Kaduna Polytechnic, manually sorted to remove extraneous materials, washed with distilled water, and air-dried at ambient conditions. The dried biomass was then pulverized using a ceramic mortar and pestle to obtain a fine, homogeneous powder. For catalyst preparation, 0.25 g of barium chloride (0.5 % w/w) was dissolved in 500 mL of distilled water in a 1000 mL conical flask, following the method reported by Ibrahim et al. (2023) and Ali and Ibrahim (2023a). Thereafter, 50 g of the pulverized leaf biomass was introduced into the catalyst solution. The reaction mixture was heated to 80 °C and agitated at 2000 rpm using a magnetic stirrer for 10 min. Upon completion of the reaction, the mixture was first filtered through filter cloth and subsequently through filter paper into a 1000 mL beaker, in accordance with procedures described by Ibrahim et al. (2022) and Ibrahim and Ali (2023).

The resulting filtrate was transferred into a separating funnel, and sodium sulphate (0.2 % w/w relative to the filtrate mass) was added as outlined by Ali and Ibrahim (2023b,c). The mixture was allowed to stand for several hours to facilitate phase separation. The organic phase was then carefully separated, dehydrated, collected, and weighed. The experiment was repeated at extended reaction times of 20, 30, 40, and 50 min, with each condition performed in duplicate to assess experimental reproducibility. Samples obtained from all experimental runs were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) to elucidate the chemical composition and identify individual reaction products.

For derivatization, 200 µL of the standard solution was combined with 100 µL of trimethyl sulfonium hydroxide (TMSH) and 20 µL of triethylamine (TEA). The mixture was sealed in vials and heated at 70 °C for 1 h before GC-MS analysis, following the procedure described by Ibrahim et al. (2025). GC-MS measurements were conducted using a Varian 3800/4000 gas chromatograph–mass spectrometer fitted with a DB-5 capillary column (30 m × 0.25 mm × 0.25 µm). Nitrogen served as the carrier gas, with the column head pressure regulated at 10 psi. The oven temperature was programmed to start at 100 °C with a 3 min hold, then increased at a rate of 8 °C min^{-1} to 300 °C. The transfer line temperature was maintained at 290 °C, and mass spectral detection was performed on a VG 7070E magnetic sector mass spectrometer operating under electron impact ionization conditions.

3. Results and Discussion

Table 1 summarizes the yield of gmelinol obtained from the barium chloride-catalysed thermal hydrolysis of Gmelina arborea leaf biomass conducted at 80 °C over reaction times ranging from 10 to 50 min, as determined by GC–MS analysis. The data comprise results from two independent experimental runs and their corresponding average values, enabling evaluation of the effect of reaction time on gmelinol formation as well as assessment of the reproducibility of the catalytic process under mild thermal conditions. In addition to gmelinol, several other industrially relevant compounds were identified in the product mixture, including 2,5-dimethylfuran, 2-methyltetrahydrofuran, ethyl acetate, furan-2-ylmethanamine, and stearic acid, highlighting the chemical diversity of the hydrolysate.

Table 1. Gmelinol yield (%) from Gmelina arborea leaf

Time (min)	1st Run (%)	2nd Run (%)	Ave. Run (%)
10	1.85	2.21	2.03
20	2.10	2.46	2.28
30	8.90	9.56	9.23
40	17.40	18.40	17.90
50	10.85	11.61	11.23

The results show a clear time-dependent evolution of gmelinol yield during the barium chloride-catalysed thermal hydrolysis of *Gmelina arborea* leaves at 80 °C. At 10 and 20 min, the average yields are low (2.03 % and 2.28 %, respectively), indicating that hydrolysis is still at an early stage. At these short reaction times, the interaction between $BaCl_2$ and chlorophyll-bound phytol is limited, resulting in incomplete cleavage and low gmelinol release. A pronounced increase in yield is observed at 30 min, where the average yield rises sharply to 9.23 %. This marks the onset of effective catalytic activity, suggesting that sufficient thermal energy and reaction time have been achieved to promote $BaCl_2$-assisted hydrolysis. The maximum gmelinol yield (17.90 %) occurs at 40 min, identifying this duration as the optimal reaction time under the studied conditions. The close agreement between the first and second runs at this point further indicates good reproducibility and process stability.

Extending the reaction time to 50 min results in a decline in average yield to 11.23 %, despite being higher than at shorter times. This decrease suggests the onset of secondary reactions, such as thermal degradation or further transformation of gmelinol into other derivatives, under prolonged heating. Overall, the results demonstrate that reaction time strongly influences gmelinol production, with 40 min at 80 °C providing the most favourable balance between hydrolysis efficiency and product stability in the $BaCl_2$-catalysed system.

The result of the reproducibility analysis presented in Table 2 shows that the coefficient of variation (CV) decreases markedly with time, from >11 % at 10–20 min to <5 % at 30–50 min. CV values below 5-10 % are generally considered good experimental reproducibility for biomass conversion systems (Jokhi *et al*., 2009; Svoboda *et al*., 2008). The lowest CV (3.95 %) occurs at 40 min, indicating excellent run-to-run consistency at the optimum yield condition. Higher variability at shorter times likely reflects incomplete hydrolysis and greater sensitivity to mass-transfer and catalytic initiation effects. The yields were converted from % to mg/g in Table 2 in accordance with the expression in Equation 1.

$$Yield\ \left(\frac{mg}{g}\right) = \%yield * M_p * \frac{1000}{M_f} \quad (1)$$

where Mp is the weight of the dry product. Mf is the weight of feed (pulverised leaves).

Table 2. Reproducibility analysis of gmelinol yield

Time (min)	Run 1 (mg/g)	Run 2 (mg/g)	Mean (mg/g)	SD (mg/g)	CV (%)
10	118.03	141.00	129.52	16.24	12.54
20	135.20	158.38	146.79	16.39	11.17
30	223.75	240.34	232.05	11.73	5.06
40	424.91	449.33	437.12	17.27	3.95
50	182.28	195.05	188.67	9.03	4.79

A paired t-test was used to assess whether Run 1 and Run 2 differ significantly. The p-value < 0.01 as presented in Table 3 indicates a statistically significant difference between Run 1 and Run 2. Run 2 consistently produced higher gmelinol yields across all reaction times. This systematic offset suggests a bias rather than random error, potentially due to: Slight differences in effective catalyst dispersion, Minor variation in biomass moisture or particle size, Improved thermal equilibration or handling in the second run. Importantly, despite this bias, the low CV values confirm that the process remains highly reproducible.

Table 3. Paired t-test for Run-to-Run consistency

Parameter	**Value**
t-statistic	−8.83
p-value	0.00091

Gmelinol yield increases steadily from 10 to 40 min, reaching a maximum of 437.12 mg/g. A sharp decline at 50 min suggests secondary reactions, such as: Thermal degradation of gmelinol, further transformation into other phytochemical or hydrocarbon derivatives. An optimal condition is 40 min at 80 °C represents the optimal hydrolysis time, combining: Highest yield, Lowest variability, and Strong catalytic effectiveness of $BaCl_2$. Although a paired t-test indicated a statistically significant difference between Run 1 and Run 2 ($p = 0.00091$), the low coefficient of variation (3.95%) suggests a minor systematic offset rather than random variability, likely arising from factors such as experiment order, catalyst conditioning, or heating profile stabilization. Importantly, both runs displayed identical time–yield trends and the same optimal reaction time (40 min), confirming reproducible process behavior despite small differences in absolute yield.

The calculated F-value (146.65) is very large, as shown in Table 3, indicating that variation in gmelinol yield is dominated by reaction time rather than experimental error. The p-value (2.28×10^{-5}) is far below 0.05, confirming that reaction time has a statistically significant effect on gmelinol yield at 80 °C. The relatively small within-group mean square (209.96) further supports good experimental precision.

Table 4. One-way ANOVA of gmelinol yield versus reaction time

Source of variation	Sum of squares (SS)	Df	Mean square (MS)	F-value	p-value
Between times	123,164.52	4	30,791.13	146.65	2.28×10^{-5}
Within times (error)	1,049.79	5	209.96		
Total	124,214.31	9			

The statistically significant ANOVA outcome validates the strong time dependence of $BaCl_2$-catalysed thermal hydrolysis. The observed yield enhancement from 10 to 40 min reflects the progressive catalytic cleavage of lignan-associated linkages and matrix-bound precursors within the leaf biomass, facilitated by the Lewis acidic character of $BaCl_2$ under mild thermal conditions. The decline in yield at 50 min, despite statistically significant differences across groups, indicates over-processing, likely arising from secondary degradation or further transformation of gmelinol into downstream products under prolonged heating. When considered alongside the reproducibility analysis, the ANOVA confirms that 40 min represents the optimal reaction time, delivering the highest gmelinol yield with minimal variance. One-way ANOVA further demonstrated that reaction time significantly influenced gmelinol yield during $BaCl_2$-catalysed thermal hydrolysis of Gmelina arborea leaves ($F = 146.65$, $p = 2.28 \times 10^{-5}$). The pronounced between-group variance relative to experimental error confirms reaction time as a dominant process variable, with maximum yield consistently observed at 40 min.

Tukey HSD analysis in Table 4 shows that early reaction times (10 vs. 20 min) are statistically indistinguishable, indicating limited progress in hydrolysis. Significant yield enhancement begins at 30 min, confirming the onset of effective $BaCl_2$-catalysed gmelinol release. 40 min differs significantly from all other times, statistically validating it as the optimum reaction duration. The drop at 50 min is statistically significant relative to 40 min, confirming yield loss due to secondary degradation rather than random variability.

Table 5. Tukey HSD comparison of gmelinol yield at different reaction times ($\alpha = 0.05$)

Comparison (min)	Mean difference (mg/g)	p-value	Significance
10 vs 20	+17.28	>0.05	Not significant
10 vs 30	+102.53	<0.01	Significant
10 vs 40	+307.61	<0.001	Highly significant
10 vs 50	+59.15	<0.05	Significant
20 vs 30	+85.26	<0.01	Significant
20 vs 40	+290.33	<0.001	Highly significant
20 vs 50	+41.88	>0.05	Not significant
30 vs 40	+205.07	<0.001	Highly significant
30 vs 50	−43.38	<0.05	Significant
40 vs 50	−248.46	<0.001	Highly significant

(*Positive values indicate higher yield at the later reaction time*)

Tukey HSD post-hoc analysis following one-way ANOVA revealed that gmelinol yields at 30 and 40 min were significantly higher than those obtained at 10 and 20 min ($p < 0.01$). The 40-minute reaction time differed significantly from all other durations ($p < 0.001$), confirming it as the optimal condition. The subsequent yield decline at 50 min was also statistically significant, indicating the onset of secondary degradation reactions.

The plot in Figure 1 shows a clear time-dependent evolution of gmelinol yield during barium chloride-catalyzed thermal hydrolysis of *Gmelina arborea* leaves at 80 °C. From 10 to 30 min, the yield increases steadily, indicating progressive hydrolytic cleavage of chlorophyll-derived precursors and enhanced release of gmelinol under Lewis acidic catalysis. A pronounced maximum is observed at 40 min for both runs (≈420-450 mg g^{-1}), identifying this duration as the optimal reaction time. Beyond 40 min, the yield declines sharply at 50 min, suggesting secondary reactions such as degradation, recondensation, or product instability under prolonged heating. The close agreement between Run 1 and Run 2 across all times demonstrates good reproducibility of the process. Overall, the results confirm that controlled reaction time is critical, with 40 min providing the best balance between gmelinol formation and degradation under the applied conditions.

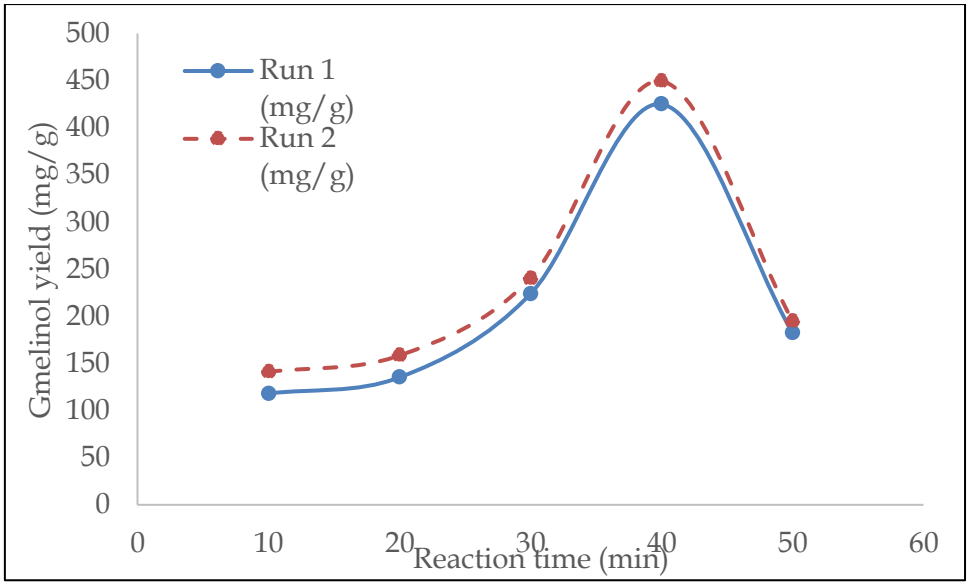

Figure 2. Gmelinol yield from Gmelina arborea leaf

Although several studies have reported the isolation of gmelinol from *Gmelina* species, these reports are largely qualitative or semi-quantitative, providing limited or no explicit yield data that would enable direct comparison with the present work. Notable examples include the studies of Bhattacharyya et al. (2025), Sreelakshmi *et al*. (2025), Chowdhary (2021), MangindaanI *et al*. (2017), and the early work of Anjaneyulu *et al*. (1977), all of which documented the extraction of gmelinol alongside other lignans from *Gmelina* trees without reporting production yields on a mass basis. In contrast, the current study establishes a clear quantitative benchmark, recording a notably high gmelinol yield of 449.33 mg g^{-1} in 40 minutes at 80 ^{o}C via a barium chloride-catalyzed thermal hydrolytic process applied to *Gmelina arborea* leaves. This represents a significant advancement in both the quantification and efficiency of gmelinol production, enabling meaningful comparison and highlighting the effectiveness of the proposed catalytic route.

Table 6. Comparison of gmelinol from different methods

Study	Gmelina Species / Part Used	Method / Notes	Reported Yield	Comments / Comparison to Current Work
Bhattacharyya *et al.*, 2025	*Gmelina* sp.	Conventional extraction	Not reported	Qualitative/semi-quantitative; no mass-based yield
Sreelakshmi *et al.*, 2025	*Gmelina* sp.	Solvent extraction	Not reported	Focused on identification and biological activity
Chowdhary, 2021	*Gmelina* sp.	Extraction of lignans	Not reported	Yield data absent; mostly qualitative analysis
MangindaanI *et al.*, 2017	*Gmelina* sp.	Phytochemical isolation	Not reported	No explicit mass-based quantification
Anjaneyulu *et al.*, 1977	*Gmelina* sp.	Early extraction studies	Not reported	Historical qualitative reference
This study	*Gmelina arborea* leaves	$BaCl_2$-catalyzed thermal hydrolysis	449.33 mg g^{-1} (40 min, 80 °C)	Provides explicit, quantitative yield; demonstrates efficiency and reproducibility; establishes benchmark for comparison

Conclusions

This study establishes a reproducible and statistically validated catalytic thermal hydrolysis process for gmelinol production from *Gmelina arborea* leaves using barium chloride as a Lewis acid catalyst. Reaction time optimization identified a clear time-yield relationship, with a maximum average yield of 17.90% (437.12 mg g^{-1}) achieved at 40 min and 80 °C, while prolonged heating led to yield deterioration due to secondary degradation. Statistical analyses (paired t-tests, one-way ANOVA, and Tukey HSD; $p < 0.001$) confirmed reaction time as a critical process variable, and the low coefficient of variation (3.95%) demonstrated excellent reproducibility. By valorizing an abundant, renewable leaf biomass through a mild, catalytically assisted route with explicit mass-based yield reporting, this work advances SDG 9 by supporting innovation-driven, scalable bioprocess development and SDG 12 by promoting responsible resource utilization and sustainable production practices. The framework provides a viable foundation for environmentally responsible gmelinol manufacture, with further gains anticipated through catalyst recycling and integration of greener process strategies.

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Conflicts of Interest

The authors declare no conflict of interest. No one outside the authors has any role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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