International Journal of Applied Science and Engineering

# Simultaneous green extraction of tea saponins from *Camellia oleifera* leaf waste using deep eutectic solvents for potential animal feed and bioethanol applications

**Apinya Kaoloun [1], Malinee Sriariyanun [1,2], Atthasit Tawai [1,2*], Saranya Sedtananun [3]**

[1] *Department of Chemical Engineering and Management, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand*
[2] *Biorefinery and Process Automation Engineering Center (BPAEC), King Mongkut's University of Technology North Bangkok, Bangkok, Thailand*
[3] *Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand*

## ABSTRACT

Saponins exhibit significant potential in animal feed applications due to their roles in enhancing nutrient absorption and providing antimicrobial properties. However, conventional extraction methods using organic solvents pose potential health risks. This study proposes a sustainable extraction approach using deep eutectic solvents (DESs), a green solvent, to recover saponins from *Camellia oleifera* leaf waste, a food industry byproduct. Choline chloride (ChCl) and lactic acid (Lac) are selected as DES components, with response surface methodology (RSM) and genetic algorithm (GA) employed for process optimization. RSM achieves a saponin yield of 249.87 mg/g (raw material) at a ChCl-Lac ratio of 1:4, solid content of 17.5%, and pretreatment temperature of 57°C, while GA yields 232.17 mg/g at a ChCl-Lac ratio of 1:1, solid content of 21.5%, and pretreatment temperature of 54°C. Both DES extraction outperforms ethanol-based extraction (157.76 mg/g). Fermentation of hydrolysates produces ethanol concentrations of 6.14 mg/mL (RSM) and 7.07 mg/mL (GA), with yields of 42.35% and 41.58%, respectively, based on initial reducing sugar content. Minimal inhibition from residual saponins confirms the compatibility of DES-based pretreatment with fermentation. These findings establish DES-based extraction as a sustainable and efficient method for saponin recovery, with strong potential for integration into biorefinery applications, including bioethanol production.

***Keywords***: Bioethanol, Biorefinery, Deep eutectic solvents, Genetic algorithm, Response surface methodology, Saponin extraction.

**OPEN ACCESS**

**Received:** February 21, 2025
**Revised:** April 17, 2025
**Accepted:** April 29, 2025

**Corresponding Author:**
Atthasit Tawai
atthasit.t@tggs.kmutnb.ac.th



**Publisher:**
Chaoyang University of Technology
**ISSN:** 1727-2394 (Print)
**ISSN:** 1727-7841 (Online)

## 1. INTRODUCTION

Tea saponins, natural triterpenoid glycosides derived from *Camellia oleifera*, are highly regarded for their bioactive properties, including antimicrobial and emulsifying effects. These attributes have positioned saponins as a key focus of research for improving nutrient absorption and promoting overall health (Gua et al., 2018; Chen et al., 2024). The processing of *C. oleifera* for both oil and beverage production generate substantial solid byproducts, such as shells (COS), cake (COC), and leaf residues. Unlike conventional lignocellulosic biomass, these byproducts are rich in lignocellulosic materials and bioactive compounds, including saponins. Their unique composition necessitates advanced extraction strategies to efficiently recover these valuable compounds for potential use in animal feed supplement formulations (Liu et al., 2024; Zhang et al., 2024). Saponins are commonly extracted from natural sources utilizing

conventional solvents such as ethanol, methanol, and ethyl acetate. However, these solvents present significant limitations, including environmental concerns, excessive consumption, and inherent toxicity (Lei et al., 2022). As a result, there is growing academic and industrial interest in developing environmentally sustainable and efficient alternatives to replace traditional organic solvents (Wu et al., 2021).

To address the limitations of traditional extraction methods, DESs have emerged as a promising green alternative. Composed of eutectic mixtures of natural components, DESs offer notable advantages, including environmental compatibility, cost-effectiveness, biodegradability, and non-toxicity (Tang et al., 2021). Their demonstrated ability to efficiently extract various bioactive compounds from plant materials underscores their potential as a sustainable and effective medium for saponin extraction (Mavai et al., 2024). Recent studies have highlighted the versatility of DES-based extraction techniques for saponins. Zhang et al. (2023) developed a natural deep eutectic solvent (NADES), butyric acid-urea, coupled with ultrasound-assisted extraction for food and pharmaceutical applications, achieving 95% solvent recovery, stable yields over five cycles, and extracts with superior saponin concentration and antioxidant activity compared to ethanol-based methods. Similarly, a NADES system (proline and glycerol, 2:5) was optimized for extracting saponins from COC, yielding 702.22 ± 1.28 mg/g, outperforming water and methanol while enriching bioactive compounds such as flavonoids, alkaloids, and phenolic acids (Wei et al., 2024). Deng et al. (2024) optimized the extraction of saponins from *Gleditsia sinensis Lam.* pods using DES (choline chloride and lactic acid, 1:2) and ultrasound-assisted extraction to enhance antioxidant activity and surface properties. The method achieved a yield of 183.61 mg/g within 12 mins, showcasing the utility of DES-extracted saponins as natural surfactants and antioxidants in the food industry. Another study utilized NADES (chloride/acrylic acid, 1:2) with ultrasound to extract saponins from purple yam root, achieving an extraction rate of 0.935% (96.5 mg/g, based on raw material) under optimized conditions: 24% water content, a 20 mL/g liquid-solid ratio, and 85 mins at 81°C and 600 W. This method proved more efficient and cost-effective ($1.53/g) than conventional techniques, with molecular dynamics and FT-IR analyses identifying hydrogen bonding as the extraction mechanism (Hou et al., 2024). Guo et al. (2024) demonstrated the potential of DES (betaine and ethylene glycol, 1:3) combined with ultrasonic extraction for extracting tea saponins from tea meal. This method achieved an extraction efficiency of 20.93 ± 0.48% within 20 mins, and purification resulted in 95.94% saponin purity, surpassing commercially available standards.

Leveraging these developments, this study investigates the use of *Camellia oleifera* leaf waste, an abundant byproduct of the food industry, as a sustainable source of saponins. By adopting a DES-based approach, the research aims to develop an environmentally sustainable extraction method that aligns with the dual objectives of generating value-added products for animal feed and facilitating bioethanol production. Choline chloride and lactic acid are selected as DES components due to their favorable environmental and functional properties, including low toxicity, biodegradability, and cost-effectiveness. Notably, both choline chloride and lactic acid are widely utilized as feed supplements in animal nutrition. Choline chloride supports lipid metabolism, enhances fat absorption, and mitigates fatty liver syndrome in livestock, while lactic acid serves as a feed preservative and acidifier, promoting gut health and improving nutrient digestibility. The established safety and compatibility of these compounds in animal feed applications further validate the suitability of DES-based extractions for producing saponin-enriched extracts intended for feed use (Ma et al., 2020; Bermúdez-Oria et al., 2023). To optimize the extraction process, RSM and GA are employed to refine critical parameters, including DES composition, solid loading, and extraction temperature, ensuring efficient recovery of saponins. These complementary optimization techniques contribute to the development of a green, scalable process that remains compatible with downstream bioprocessing steps. By integrating DES-based technology with advanced optimization methods, this study makes a significant contribution to the sustainable valorization of *C. oleifera* leaf waste, paving the way for its application in high-value animal feed formulations and bioethanol production.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Reagents

Vanillin (99%) is obtained from Thermo Fisher Scientific Inc., while the saponin standard (Technical Grade, ≥ 98%) is sourced from Toronto Research Chemicals Inc. Choline chloride (98% Extra Pure) is supplied by Loba Chemie PVT. LTD., and lactic acid (85%) as well as acetic acid (glacial, ≥ 99%) are purchased from Daejung Chemicals and Metals Co., LTD. Acetonitrile (HPLC grade) is procured from RCI Labscan Group, whereas sulfuric acid (98%) is supplied by Quality Reagent Chemical (QReC)™. Ethyl alcohol anhydrous (99.9%) is also obtained from Daejung Chemicals and Metals Co., LTD.

### 2.2 Sample Preparation

*C. oleifera* leaf waste was obtained in 2024 from a tea beverage processing factory in Pathum Thani Province, Thailand. During the processing of tea leaves at the factory, the leaves were ground and extracted with hot water, after which the residual material was separated as waste. The process is categorized under food processing operations. For this study, the leaf waste was used as a sample. To control its moisture content, the tea leaf waste was dried at 60°C for 24 hrs in a hot-air drying oven. The dried sample was then passed through a 10-mesh sieve to ensure uniform

particle size. Finally, the dried *C. oleifera* leaf waste was stored in a sealed desiccator to maintain its stability.

## 2.3 Extraction of Tea Saponins

### 2.3.1 Preparation and Selection of DESs

Deep eutectic solvents are prepared following a modified literature procedure (Duan et al., 2016). This method investigates the use of DESs for extracting bioactive compounds from herbal medicines, with the extraction efficiency optimized using response surface methodology. For this study, DESs were selected for their animal-friendly properties, making them potentially suitable for animal feed applications. ChCl was used as the hydrogen bond acceptor (HBA), and Lac was used as the hydrogen bond donor (HBD). The ChCl-Lac system was prepared with molar ratios varying from 1 to 5, and 20% water was added to reduce the viscosity of the system. The mixture was magnetically stirred at 80°C for 1 hr. For extraction, 1 g of sample was mixed with 20 mL of the prepared DESs and stirred in a water bath at 60°C for 1 hr. After extraction, the mixture was steeped for 12 hrs. The extracts were then centrifuged at 4,100 rpm for 15 mins, and the supernatant was collected for the determination of tea saponin content.

### 2.3.2 RSM-based Optimization of Tea Saponin Extraction

Response surface methodology is applied to optimize the saponin extraction process by evaluating the effects of three key parameters: ChCl-Lac ratio (1:1 to 1:5), solid loading (10%–20%), and the pretreatment temperature (45–60°C), with an extraction time of 60 mins. These parameters are selected for their critical role in disrupting the lignocellulosic matrix, improving extraction efficiency, and enabling cost-effective production under mild conditions. To achieve this, a Box-Behnken design (BBD) within the RSM framework is utilized, offering an efficient approach to examine quadratic response surfaces while minimizing experimental runs. This design systematically explores the parameter ranges to evaluate their impact on the physicochemical properties of tea leaves, with the goal of maximizing saponin yield. The total saponin yield, quantified using a specific analytical method (Li and Wu, 2014; Yu et al., 2023), serves as the primary response variable. The relationship between the independent variables and the response is described using a second-order polynomial equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \qquad (1)$$

where $Y$ represents the reducing sugar yield, $X_i$ and $X_j$ are the independent variables, and $\beta_0, \beta_i, \beta_{ii}$ and $\beta_{ij}$ are the regression coefficients. Table 1 presents the independent variables along with their corresponding levels used to optimize the extraction conditions of tea leaf waste. Table 2 outlines the experimental design for saponin extraction from tea leaf waste within the RSM framework. Response surface plots illustrate the interactive effects of the ChCl-Lac ratio, solid loading, and temperature on the total saponins yield.

**Table 1.** Variables and their levels for optimizing tea leaf waste extraction

| Independent variable | Coded symbols | Levels | | |
|---|---|---|---|---|
| | | -1 | 0 | 1 |
| ChCl-Lac ratio | $X_1$ | 1:1 | 1:3 | 1:5 |
| Solid loading (%) | $X_2$ | 10 | 15 | 20 |
| Temperature (°C) | $X_3$ | 45 | 52.5 | 60 |

**Table 2.** RSM-based experimental design for saponins extraction from tea leaf waste

| Run number | Pretreatment condition | | |
|---|---|---|---|
| | $X_1$: ChCl-Lac ratio (%) | $X_2$: Solid loading (%) | $X_3$: Temperature (°C) |
| 1 | 1:3 | 15 | 52.5 |
| 2 | 1:1 | 20 | 60 |
| 3 | 1:1 | 20 | 45 |
| 4 | 1:5 | 15 | 45 |
| 5 | 1:1 | 10 | 52.5 |
| 6 | 1:1 | 20 | 52.5 |
| 7 | 1:3 | 15 | 52.5 |
| 8 | 1:3 | 15 | 52.5 |
| 9 | 1:3 | 20 | 45 |
| 10 | 1:3 | 10 | 60 |
| 11 | 1:5 | 10 | 52.5 |
| 12 | 1:3 | 10 | 45 |
| 13 | 1:5 | 15 | 60 |
| 14 | 1:5 | 20 | 52.5 |
| 15 | 1:3 | 20 | 60 |
| 16 | 1:3 | 15 | 52.5 |
| 17 | 1:3 | 15 | 52.5 |

### 2.3.3 Genetic Algorithm Based Optimization

The optimization process for saponins extraction from tea leaf waste combines the predictive capabilities of the RSM with the global search efficiency of the GA. First, a second-order polynomial model, derived through RSM, represents the relationship between the total saponins yield (*TS*) and the key parameters–ChCl-Lac ratio ($X_1$), solid loading ($X_2$), and temperature ($X_3$). The RSM model is expressed as:

$$TS = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (2)$$

where $\beta_0$ is the intercept, $\beta_1, \beta_2, \beta_3$ are the linear coefficients, $\beta_{11}, \beta_{22}, \beta_{33}$ are the quadratic coefficients, and $\beta_{12}, \beta_{13}, \beta_{23}$ are the interaction coefficients. The GA then uses this RSM derived model to define the optimization objective. The total saponins yield is expressed as an objective function, $f(X_1, X_2, X_3)$ , to maximize the yield. This objective function is formulated as:

$$f(X_1, X_2, X_3) = -(b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_1^2 + b_5 X_2^2 + b_6 X_3^2 + b_7 X_1 X_2 + b_8 X_1 X_3 + b_9 X_2 X_3) \quad (3)$$

where $b_0$, $b_1$, …, $b_9$ represent the respective coefficients obtained from the RSM model. GA uses this objective function to iteratively search for the optimal combination of parameters through selection, crossover, and mutation processes, ensuring a global exploration of the parameter space. The integration of the RSM model with the GA objective function ($f(X_1, X_2, X_3)$) ensures both accuracy in modeling and robustness in optimization, effectively maximizing the total saponins yield.

## 2.4 Determination of Total Tea Saponins Content

The total saponin content of tea leaf waste is analyzed using a modified method adapted from Yu et al. (2023) and Li and Wu (2014). A 0.078 mL aliquot of the extract is combined with 0.065 mL of 8% vanillin-anhydrous ethanol solution and 5 mL of 77% sulfuric acid solution, sequentially, in an ice-water bath. The mixture is incubated at 60°C for 15 mins and then cooled for 10 mins. Absorbance is recorded at 550 nm, and the saponin content is determined using standard curves ($r^2 = 0.9992$) prepared from tea saponin standards at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL.

## 2.5 Conventional Extraction

A conventional extraction method is employed for comparison. One gram of sample was mixed with 20 mL of 50% ethanol and extracted in a water bath at 60°C for 1 hr. After extraction, the mixture was centrifuged at 4,100 rpm for 15 mins, and the supernatant was collected for tea saponin analysis.

## 2.6 Enzymatic Hydrolysis of Substrates

The extracted tea leaf waste, regarded as pretreated biomass, is subjected to enzymatic hydrolysis to convert cellulose into reducing sugars, facilitating the production of bioproducts, particularly bioethanol. For the hydrolysis process, 0.3 g of residual solids is precisely weighed and transferred into a 15 mL centrifuge tube. An acetic acid-sodium acetate buffer solution (pH 4.8) is added to achieve a solid consistency of 5.0 wt%. Cellulase is applied at a dosage of 50 FPU/g of substrate to catalyze the reaction. The enzymatic hydrolysis is conducted in a constant-temperature shaking reactor at 50°C for 72 hrs to ensure adequate enzymatic activity. This duration is selected based on findings (Wu et al., 2023), which indicate that extending the reaction time beyond 48 hrs does not significantly increase the yield. After hydrolysis, the mixture is centrifuged, and the glucose concentration in the supernatant is quantified using the 3,5-dinitrosalicylic acid (DNS) method. The glucose conversion yield is calculated following the formula outlined in the reference (Mujtaba et al., 2023), which accounts for the theoretical glucose content derived from the initial cellulose composition of the substrate.

## 2.7 Bioethanol Fermentation

This study evaluates batch fermentation for bioethanol production using hydrolysate derived from tea leaf lignocellulosic biomass, with *S. cerevisiae* as the fermenting microorganism. The process involves 19 mL of hydrolysate and 1 mL of yeast inoculum, with cell concentration standardized to an optical density (OD) of 1 at 660 nm. To support yeast acclimatization, glucose (1% w/v) and yeast extract (1% w/v) are added as carbon and nitrogen sources, respectively (Chuetor et al., 2022). Fermentation is conducted at pH 5, 30°C, and 150 rpm for 48 hrs in a shaking incubator. After fermentation, yeast cells are separated via centrifugation at 6,000 rpm for 5 mins, and ethanol concentration is analyzed using a spectrophotometric method (Sriariyanun et al., 2019). Ethanol is extracted with tri-n-butyl phosphate (TBP) and centrifuged to create two phases. The TBP phase is reacted with a dichromate reagent to oxidize ethanol, producing a blue-green solution. The product is diluted, and its absorbance is measured at 595 nm using a UV/Vis spectrophotometer. Ethanol concentration is calculated using a calibration curve prepared with absolute ethanol (99.8%), with triplicate measurements ensuring accuracy. Ethanol yield, sugar yield, and ethanol conversion are determined using standard methods (Hatzis et al., 1996). Ethanol conversion, calculated as the ratio of ethanol produced to the initial reducing sugar content, serves as an indicator of fermentation efficiency.

## 2.8 Combined Process for Saponin Extraction and Ethanol Fermentation

The simultaneous saponin extraction and bioethanol production process is conducted as illustrated in Fig. 1. This integrated approach begins with the preparation of *Camellia*

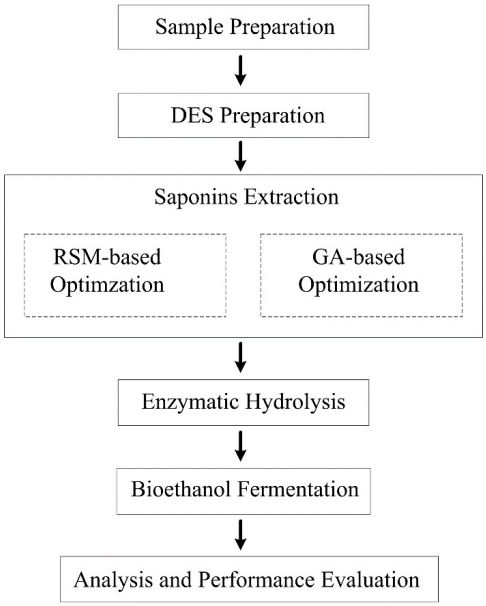


**Fig. 1.** Process flow diagram for simultaneous saponin extraction and bioethanol production from *C. oleifera* leaf waste

*oleifera* leaf waste, which undergoes drying, sieving, and storage to ensure consistent quality. Deep eutectic solvents are then prepared and utilized for the efficient extraction of saponins, optimized using RSM and further refined through GA based optimization. The optimal conditions obtained from both RSM and GA based approaches are further applied, and their performance is compared in terms of saponin extraction efficiency, sugar yield, and ethanol production. The pretreated biomass is subsequently subjected to enzymatic hydrolysis to convert cellulose into reducing sugars, which serve as the primary substrate for bioethanol fermentation. The final step, analysis and performance evaluation, involves assessing ethanol yield, sugar conversion, and overall process efficiency to validate the feasibility and effectiveness of this dual-purpose system.

## 3. RESULTS AND DISCUSSION

### 3.1 Application of RSM and GA for Optimizing Saponins Extraction Yield

The effects of experimental variables on saponin extraction yield are analyzed using three-dimensional and two-dimensional response surface plots as illustrated in the Fig. 2. Analysis of variance (ANOVA) indicates that the interaction between the ChCl-Lac ratio and solid loading has the most significant impact on saponin extraction yield, while variations in extraction temperature also exhibit a notable effect. The experimental data are further applied to optimize extraction conditions using a GA. The GA optimized conditions are experimentally validated and compared with those obtained through RSM to evaluate their effectiveness in maximizing saponin extraction yield.

### 3.2 Saponin Extraction Results and Comparison

The optimal conditions for saponin extraction, determined using RSM and a GA, are summarized in Table 3. RSM identified optimal conditions at a ChCl-Lac ratio of 1:4, solid content of 17.5%, and pretreatment temperature of 57°C, achieving a saponin yield of 249.87 mg/g (raw material). In contrast, GA optimization resulted in slightly different conditions, a ChCl-Lac ratio of 1:1, solid content of 21.5%, and the pretreatment temperature of 54°C–yielding 232.17 mg/g. Both sets of optimized conditions were experimentally validated to confirm their effectiveness. Fig. 3 illustrates the total saponin content extracted under various conditions. Compared to ethanol-based (157.76 mg/g) and water-based extractions (15.98 mg/g), both RSM and GA optimizations demonstrate superior efficiency, with RSM yielding 7.64% more than GA and 58.37% more than ethanol. The minimal yield from water-based extraction suggests that prior processing from the tea beverage industry removes only small amounts of saponins, leaving most bioactive compounds available for subsequent extraction. Although the yield obtained through GA optimization is 47.09 % higher than that achieved with ethanol extraction, its slightly lower yield compared to RSM can be attributed to the broader parameter search inherent in the GA approach. While this method is effective for global optimization, it may overlook subtle interactions that RSM is able to identify. The integration of GA with RSM, or the application of machine learning techniques, has the potential to enhance parameter refinement and improve predictive accuracy, thereby facilitating a more comprehensive understanding of complex nonlinear interactions. In comparison with previous studies, Yu and He (2018a) developed a rapid method for extracting tea-leaf saponins through ultrasonic-assisted water extraction and acetone precipitation, achieving a concentration of 3.832 mg/mL without reporting an extraction yield per g of raw material. In a subsequent study, the same authors extracted saponins from aged tea leaves (Longjing 43) using optimized water extraction, obtaining a 12.19% yield under optimal conditions (75 mL/g, 1 hr, 80°C) (Yu and He, 2018b). These findings suggest that tea leaves may offer higher extraction potential than tea seeds. Meanwhile, Ye et al. (2023) extracted saponins from *C. oleifera* seed meal using DESs, achieving the highest yield of 81.51 mg/g, whereas Zhao et al. (2020) reported a 7.28% yield from COS using 75% ethanol. However, the present study focuses on tea leaf waste, which is rarely mentioned in the literature, making direct comparisons with raw tea leaf extractions difficult. Additionally, variations in extraction methods and solvents further complicate yield comparisons.

### 3.3 Comparison of Ethanol Yield from RSM and GA Optimal Conditions

The optimal saponin extraction conditions, considered a pretreatment step, are determined using pretreated tea leaves from prior extractions with 5% biomass loading. The

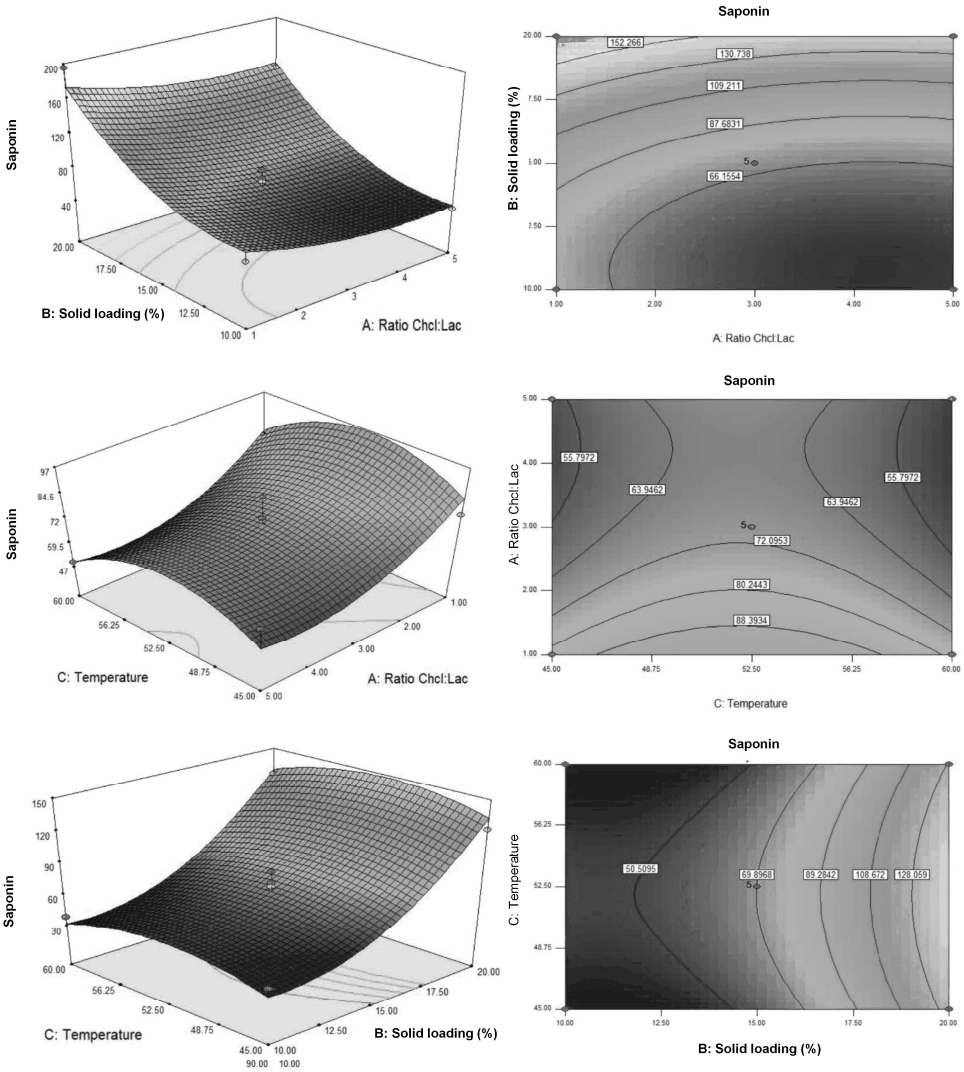


**Fig. 2.** Response surface analysis of factors influencing saponin yield

effectiveness of both RSM and GA optimized conditions is experimentally validated. Following extraction, the pretreated biomass undergoes enzymatic hydrolysis and ethanol fermentation using *Saccharomyces cerevisiae* with hydrolysates derived from the optimized pretreatment conditions. Fig. 4 shows saponin yield and ethanol concentration under RSM and GA optimized conditions, while Fig. 5 presents ethanol yield and initial sugar concentration under the same conditions. As shown in these figures, ethanol yield, based on initial reducing sugar content, is 42.35% for RSM and 41.58% for GA, indicating that pretreatment conditions influence fermentable sugar availability and conversion efficiency. Ethanol concentration, presented in Fig. 4, is 6.14 mg/mL for RSM and 7.07 mg/mL for GA, suggesting that GA optimization, despite lower saponin extraction yield, produces a slightly higher ethanol concentration after hydrolysis and fermentation. This difference may result from variations in the ChCl:Lac ratio and pretreatment conditions. The ethanol yields, though high compared to the theoretical maximum of 51.10%, highlight the need for improved enzymatic hydrolysis to enhance sugar recovery (Im et al., 2016; Tsegaye et al., 2024). Minimal inhibitory effects from residual compounds are evident, as the yields remain comparable. Ethanol yield calculations are based solely on reducing sugars, excluding other fermentable sugars in the hydrolysate that may contribute to yeast metabolism.

Mild pretreatment conditions, with temperatures maintained below 60°C, reduce energy consumption but result in lower reducing sugar concentrations, with GA optimization improving only 29.84% compared to untreated biomass. Consequently, ethanol yields are lower than those reported in studies using deep eutectic solvents at temperatures above 150°C. For example, Jose et al. (2023) reported ethanol yields of 0.200 g/g biomass using separate hydrolysis and fermentation (SHF), and 0.483 g/g biomass

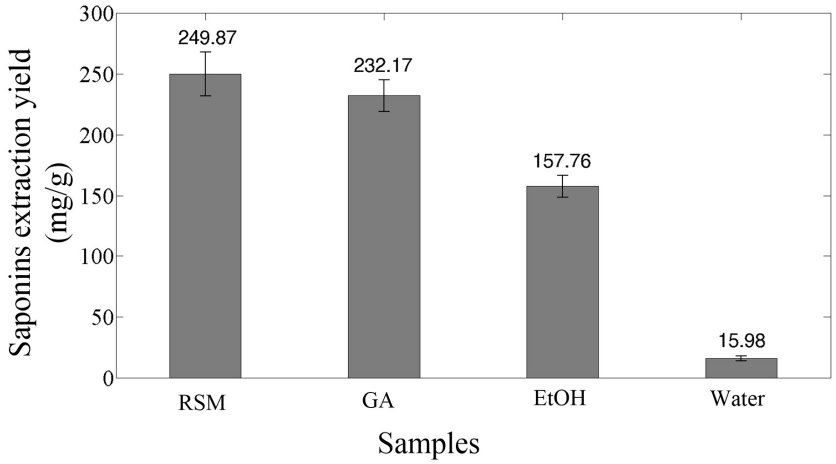


**Fig. 3.** Comparison of saponin extraction yields under optimal conditions identified by RSM, GA, ethanol, and water samples

**Table 3.** Optimal conditions and saponin yields from RSM and GA

| Method | Optimal extraction parameter | | | Saponins extraction yield (mg/g) |
|---|---|---|---|---|
| | $X_1$ | $X_2$ (%) | $X_3$ (°C) | |
| RSM | 1:4 | 17.5 | 57 | 249.87 ± 18 |
| GA | 1:1 | 21.5 | 54 | 232.17 ± 13 |

using a one-pot process, both applied to Napier grass. Although these values are higher, the referenced study focused solely on ethanol production using a different lignocellulosic feedstock and process objective, while our study integrates saponin extraction with ethanol production under milder, energy-efficient conditions. However, this approach produces crude saponin extracts with potential applications, such as in animal feed, while lowering overall solvent related costs by using the same solvent for both saponin extraction and biomass pretreatment. The lower ethanol yields emphasize the need for additional processing steps before hydrolysis to improve sugar recovery. Optimizing hydrolysis can enhance fermentable sugar availability, thereby increasing ethanol yields and improving both technical performance and economic feasibility of the bioconversion system.

## 4. CONCLUSION

This study establishes the efficacy of DESs as a sustainable and efficient medium for the extraction of saponins from *Camellia oleifera* leaf waste, a byproduct of the food industry. The optimization of extraction parameters using RSM and GA demonstrates that the ChCl-Lac ratio, solid content, and pretreatment temperature exert significant influences on saponin extraction yield. Under optimal conditions, RSM achieves a saponin yield of 249.87 mg/g, while GA yields 232.17 mg/g, both of which are markedly higher than those obtained through conventional ethanol-based extraction methods. The pretreated biomass, subjected to enzymatic hydrolysis and subsequent fermentation, yields ethanol concentrations of 6.14 mg/mL (RSM) and 7.07 mg/mL (GA). The presence of residual saponins in the hydrolysate does not exhibit a significant inhibitory effect on yeast activity, thereby confirming the compatibility of DES based pretreatment with downstream bioconversion processes. Further studies should focus on enhancing the efficiency of enzymatic hydrolysis to maximize sugar conversion, as well as investigating the long-term effects of residual saponins on microbial fermentation performance.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## ACKNOWLEDGMENT

This research was funded by King Mongkut's University of Technology North Bangkok, Contract no. KMUTNB-68-BASIC-56.

## REFERENCES

Bermúdez-Oria, A., Fernández-Prior, A., Castejón, M.L., Rodríguez-Gutiérrez, G., Fernández-Bolaños, J. 2023. Extraction of polyphenols associated with pectin from olive waste (alperujo) with choline chloride. Food Chemistry, 419, 136073.

Chen, S., Kang, J., Zhu, H., Han, Z., Wang, L., Wang, K., Liu, J., Wu, Y., He, P., Tu, Y, Li, B. 2024. Tea seed saponins ameliorate cyclophosphamide-induced intestinal

injury, immune disorder and gut microbial dysbiosis in mice. Food Bioscience, 57, 103504.

Chuetor S., Panakkal E.J., Ruensodsai T., Cheenkachorn K., Kirdponpattara S., Cheng Y.S., Sriariyanun M. 2022. Improvement of enzymatic saccharification and ethanol production from rice straw using recycled ionic liquid: The effect of anti-solvent mixture, Bioengineering, 9, 115.

Deng, Y., Wang, X., Zhang, C., Xie, P., Huang, L. 2024. Enhanced and green extraction of saponins from Gleditsia sinensis Lam. Pods by ultrasound-assisted deep eutectic solvents: optimization and comprehensive characterization. Food and Bioprocess Technology, 1–20.

Duan, L., Dou, L.L., Guo, L., Li, P., Liu, E.H. 2016. Comprehensive evaluation of deep eutectic solvents in extraction of bioactive natural products. ACS Sustainable Chemistry Engineering, 4, 2405–2411.

Guo, J., Zhao, N., Zhao, Y., Jin, H., Sun, G., Yu, J., Zhang, H., Shao, J., Yu, M., Yang, D., Liang, Z. 2024. The extraction using deep eutectic solvents and evaluation of tea saponin. Biology, 13, 438.

Guo, N., Tong, T., Ren, N., Tu, Y., Li, B. 2018. Saponins from seeds of Genus Camellia: Phytochemistry and bioactivity. Phytochemistry, 149, 42–55.

Hatzis C., Riley C., Philippidis G. 1996. Detailed material balance and ethanol yield calculations for the biomass-to-ethanol conversion process. Applied Biochemistry and Biotechnology, 57, 443–459.

Hou, Y.J., Wang, P.W., Zhang, H., Fan, Y.Y., Cao, X., Luo, Y. Q., Li, Q., Njolibimi, M., Li, W., Hong, B, Zhao, C.J. 2024. A high-permeability method for extracting purple yam saponins based on ultrasonic-assisted natural deep eutectic solvent. Food Chemistry, 457, 140046.

Im, K.H., Nguyen, T.K., Choi, J., Lee, T.S. 2016. Ethanol production from various sugars and cellulosic biomass by white rot fungus *Lenzites betulinus*. Mycobiology, 44, 48–53.

Jose, D., Tawai, A., Divakaran, D., Bhattacharyya, D., Venkatachalam, P., Tantayotai, P., Sriariyanun, M. 2023. Integration of deep eutectic solvent in biorefining process of lignocellulosic biomass valorization. Bioresource Technology Reports, 21, 101365.

Lei, J., Wang, Y., Li, W., Fu, S., Zhou, J., Lu, D., Wang, C., Sheng, X., Zhang, M., Xiao, S., Sun, C., Wang, G. 2022. Natural green deep eutectic solvents based eco-friendly and efficient extraction of flavonoids from *Selaginella moellendorffii*: Process optimization, composition identification and biological activity. Separation and Purification Technology, 283, 120203.

Li, T., Zhang, H., Wu, C. E. 2014. Screening of antioxidant and antitumor activities of major ingredients from defatted *Camellia oleifera* seeds. Food science and biotechnology, 23, 873–880.

Liu, X., Wu, Y., Gao, Y., Jiang, Z., Zhao, Z., Zeng, W., Mingyu, X., Liu, S., Liu, R., Chao, Y., Nie, S., Zhang, A., Li, C., Xiao, Z. 2024. Valorization of *Camellia oleifera* oil processing byproducts to value-added chemicals and biobased materials: A critical review. Green Energy and Environment, 9, 28–53.

Ma, X., Gao, M., Yin, Z., Zhu, W., Liu, S., Wang, Q. 2020. Lactic acid and animal feeds production from Sophora flavescens residues by Rhizopus oryzae fermentation. Process biochemistry, 92, 401–408.

Mavai, S., Bains, A., Sridhar, K., Chawla, P., Sharma, M. 2024. Emerging deep eutectic solvents for food waste valorization to achieve sustainable development goals: Bioactive extractions and food applications. Food Chemistry, 141000.

Mujtaba M., Fraceto L.F., Fazeli M., Mukherjee S., Savassa S.M., Medeiros G.A., Pereira A.E.S., Mancini S.D., Lipponen J., Vilaplana F. 2023. Lignocellulosic biomass from agricultural waste to the circular economy: A review with focus on biofuels, biocomposites and bioplastics, Journal of Cleaner Production, 402, 136815.

Sriariyanun M., Mutrakulcharoen P., Tepaamorndech S., Cheenkachorn K., Rattanaporn K. 2019. A rapid spectrophotometric method for quantitative determination of ethanol in fermentation products. Oriental Journal of Chemistry, 35, 744–750.

Tang, Y., He, X., Sun, J., Liu, G., Li, C., Li, L., Sheng, J., Zhou, Z., Xin, M., Ling, D., Yi, P., Zheng, F., Li, J., Li, Z., Yang, Y., Tang, J., Chen, X. 2021. Comprehensive evaluation on tailor-made deep eutectic solvents (DESs) in extracting tea saponins from seed pomace of *Camellia oleifera* Abel. Food Chemistry, 342, 128243.

Tsegaye, K.N., Alemnew, M., Berhane, N. 2024. Saccharomyces cerevisiae for lignocellulosic ethanol production: a look at key attributes and genome shuffling. Frontiers in Bioengineering and Biotechnology, 12, 1466644.

Wei, Z., Zhang, W., Du, M., Zhong, H., Fang, X. 2024. Widely targeted metabolomic and KEGG analyses of natural deep eutectic solvent-based saponins extraction from *Camellia oleifera* Abel.: Effects on composition. Food Chemistry, 450, 139333.

Wu, L., Chen, Z., Li, S., Wang, L., Zhang, J. 2021. Eco-friendly and high-efficient extraction of natural antioxidants from *Polygonum aviculare* leaves using tailor-made deep eutectic solvents as extractants. Separation and Purification Technology, 262, 118339.

Wu R., Li Y., Wang X., Fu Y., Qin M., Zhang Y. 2023. In-situ lignin sulfonation for enhancing enzymatic hydrolysis of poplar using mild organic solvent pretreatment. Bioresource Technology, 369, 128410.

Yu, X., Zhao, Z., Yan, X., Xie, J., Yu, Q., Chen, Y. 2023. Extraction optimization of tea saponins from *Camellia oleifera* seed meal with deep eutectic solvents: Composition identification and properties evaluation. Food Chemistry, 427, 136681.

Yu, X.L., He, Y. 2018a. Development of a rapid and simple method for preparing tea-leaf saponins and investigation on their surface tension differences compared with tea-seed saponins. Molecules, 23, 1796.

Yu, X.L., He, Y. 2018b. Optimization of tea-leaf saponins water extraction and relationships between their contents and tea (*Camellia sinensis*) tree varieties. Food Science and Nutrition, 6, 1734–1740.

Zhang, H., Li, X., Kang, M., Li, Z., Wang, X., Jing, X., Han, J. 2023. Sustainable ultrasound-assisted extraction of *Polygonatum sibiricum* saponins using ionic strength-responsive natural deep eutectic solvents. Ultrasonics Sonochemistry, 100, 106640.

Zhang, P., Xiong, Y., Bi, L., Zhong, H., Ren, J., Zhou, B. 2024. Non-antibiotic feed additives production by *Acremonium terricola* solid-fermented *Camellia oleifera* meal. Bioresources and Bioprocessing, 11, 90.

Zhao, Y., Su, R., Zhang, W., Yao, G. L., Chen, J. 2020. Antibacterial activity of tea saponin from *Camellia oleifera* shell by novel extraction method. Industrial crops and products, 153, 112604.