RASĀYAN *J. Chem.*
Vol. 19 | No. 3 | 1588-1596 | July-September | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

OPTIMIZED HIGH-YIELD POLYPHENOL EXTRACTION FROM *Curcuma aeruginosa* AND *Curcuma xanthorrhiza* USING SUSTAINABLE CHOLINE CHLORIDE-BASED NATURAL DEEP EUTECTIC SOLVENTS

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ABSTRACT

The increasing demand for bioactive compounds from rhizomes necessitates sustainable and high-efficiency extraction approaches. This study reports the optimization of polyphenol extraction from *Curcuma aeruginosa* and *Curcuma xanthorrhiza* using choline chloride-based Natural Deep Eutectic Solvents (NADES). Extraction performance was systematically evaluated by varying hydrogen bond donors (glucose, fructose, maltose, and xylose), molar ratios (1:2 and 1:1), and water content (20–30%). Optimal conditions were achieved using Choline Chloride–Fructose (1:2) with 20% water, yielding enhanced polyphenol recovery and antioxidant activity. The extracts exhibited strong radical scavenging capacity at polyphenol concentrations of 1599.18 mg/L and 832.36 mg/L, with total phenolic contents of 31.98 mg GAE/g and 16.65 mg GAE/g for *Curcuma xanthorrhiza* and *Curcuma aeruginosa*, respectively. These results demonstrate that NADES significantly improves extraction efficiency while providing an environmentally benign alternative to conventional organic solvents. This work advances green extraction methodologies and supports the utilization of *Curcuma*-derived polyphenols for pharmaceutical and functional food applications.

Keywords: *Curcuma aeruginosa, Curcuma xanthorrhiza*, NADES, Polyphenols, Green Extraction.

RASĀYAN *J. Chem.*, Vol. 19, No.3, 2026

INTRODUCTION

The *Zingiberaceae* family, which includes the *Curcuma* genus, is widely utilized in food, herbal medicine, and traditional practices.[1] *Curcuma* is a perennial, rhizomatous plant belonging to one of the most extensive genera within the *Zingiberaceae* family, traditionally used to treat various illnesses and diseases in Indonesia, Malaysia, and Thailand.[2] Most *Curcuma* species possess aromatic properties in at least one part of the plant and serve as rich sources of diverse bioactive compounds, including monoterpenoids, diarylheptanoids—particularly curcuminoids in certain species—and phenolics found in extracts.[3] Species of the *Curcuma* genus possess many medicinal effects such as anti-inflammatory[4], antioxidant[5], anticancer[6], antimicrobial[7], antihyperglycemic[8], antidiabetic[9], nitric oxide inhibition[10], antinociceptive[11], analgesic[12], antiandrogenic[13], anti-aging[14], and hepatoprotective effects.[15] Antipyretic, skin penetration, and uterine muscle relaxation[1] effects have also been reported. In Indonesia, various species of Curcuma are well known as cooking condiments; they also possess medicinal values, especially *Curcuma aeruginosa*[1,13,16] and *Curcuma xanthorrhiza*.[2,3] Wang *et al.* (2024) reported that the remarkable antioxidant activity and associated health benefits of these plants are largely attributed to their polyphenol content. Polyphenols are widely acknowledged as bioactive compounds with antioxidative functions, playing a pivotal role in mitigating oxidative stress and lowering the likelihood of chronic disease development.[17] Despite their recognized bioactivity, the efficient and sustainable extraction of polyphenols from these specific Indonesian *Curcuma* species remains insufficiently explored, particularly using environmentally benign solvent systems. In recent years, researchers have focused on extracting polyphenols to choose sustainable and eco-friendly extraction methods as compared to conventional

methods. Conventional methods include maceration[18], digestion[19], decoction[20], Soxhlet extraction[21], hydrodistillation[22], infusion[23] and percolation.[24] The utilization of conventional organic solvents in these studies has raised concerns due to their high energy requirements for recovery, environmental toxicity, volatility, non-degradability, flammability, and adverse effects on both human health and the ecosystem.[25] These limitations highlight a critical need for greener extraction strategies that minimize environmental impact while maintaining or enhancing extraction efficiency. In this context, the present study aligns with the United Nations Sustainable Development Goal (SDG) 12: Responsible Consumption and Production, by promoting safer solvent systems and sustainable resource utilization. To address these challenges, several studies have investigated environmentally friendly solvent alternatives. An ideal solvent should demonstrate stability for efficient recycling and reuse, non-volatility to reduce air pollution, non-flammability for improved safety, and derivation from renewable sources to ensure long-term sustainability. All these criteria can be fulfilled by NADES.[26] NADES are customizable solvents composed of two or more natural, cost-effective, and biodegradable components. They are formed through the combination of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) in a specific molar ratio to create a eutectic mixture.[27] Furthermore, NADES-based extracts are increasingly gaining attention due to their ability to be directly integrated into final products, eliminating the need for solvent removal and thereby reducing energy consumption and water usage. Another significant advantage of NADES lies in their contribution to sustainability, including applications in biodegradable materials such as food films, which have demonstrated complete degradation within seven days.[28] In addition, NADES have shown superior extraction efficiency compared to conventional organic solvents due to their tunable solubility properties. For example, NADES exhibited significantly higher efficiency in extracting polyphenols from sunflower meal compared to traditional methods.[29] Similarly, higher polyphenol yields were obtained from Juniperus chinensis Roxb. using NADES, supporting their application in food, pharmaceutical, and cosmetic industries.[30] Moreover, NADES extraction of baicalin from *Oroxylum indicum* fruit resulted in yields over 2.5 times greater than those obtained using 70% ethanol. This NADES approach markedly enhances the extraction efficiency and converts baicalin to baicalein, boosting the pharmacological potential of plant extracts.[31] NADES have also demonstrated enhanced antimicrobial and antioxidant activities compared to conventional solvent extracts.[28]

However, despite these promising advantages, the application of NADES for extracting polyphenols from Indonesian rhizomes, particularly *Curcuma aeruginosa* and *Curcuma xanthorrhiza*, remains limited. In addition, systematic optimization of NADES composition and operational parameters for maximising polyphenol yield from these species has not been comprehensively investigated. Therefore, this study aims to develop and optimize a sustainable and eco-friendly extraction method using choline chloride-based NADES combined with glucose, fructose, maltose, and xylose for the efficient recovery of polyphenols from *Curcuma aeruginosa* and *Curcuma xanthorrhiza*. Furthermore, the physicochemical properties of the NADES systems (viscosity and density) were evaluated to better understand their extraction performance. This work not only addresses the existing research gap but also contributes to the advancement of green extraction technologies in line with SDG 12, with potential applications in biomedical and functional food industries.

EXPERIMENTAL

Material and Methods

Plant materials, namely *Curcuma aeruginosa* and *Curcuma xanthorrhiza*, were collected from Bogor, West Java, Indonesia. The reagents used in the experimental procedures comprised choline chloride, glucose, fructose, xylose, maltose, Folin–Ciocalteu reagent, sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and methanol. All chemicals were bought from Merck. In this study, a Shimadzu UV-1800 UV-Vis spectrophotometer was used for the analytical measurements.

Sample Preparation

The specimens analyzed in this study were *Curcuma aeruginosa* and *Curcuma xanthorrhiza*. The samples were cut into smaller pieces and air-dried at room temperature for 24 hours, ensuring no direct exposure to sunlight. The dried samples were then stored at room temperature until further use.

Preparation of NADES

NADES was produced by reacting the HBA component obtained from choline chloride with different HBD compounds in a molar ratio of 1:2. The HBD compounds used in this study were glucose, fructose, maltose, and xylose. The HBA and HBD compounds were mixed and heated at 150 °C.[32] The solution was stirred at a speed of 500-800 rpm until a homogenous solution was achieved. The solution was subsequently allowed to reach room temperature and was prepared for use as a solvent in the extraction process. The density and viscosity of the prepared NADES were measured.

Viscosity

The viscosity of the obtained NADES was then measured at room temperature using the flow method.

Density

The density of NADES and the extracted products of *Curcuma aeruginosa* and *Curcuma xanthorrhiza* was measured gravimetrically at room temperature using a 25 mL pycnometer.

Extraction of Active Substance in *Curcuma aeruginosa* and *Curcuma xanthorrhiza* using NADES

The active compound separation process was performed by dispersing 1 g of the dried plant material into 10 mL of NADES, followed by stirring at 120 rpm at room temperature for 60 minutes in an Erlenmeyer flask. After extraction, the mixture is filtered using filter paper to obtain the supernatant. The extracted solution is then ready for active compound analysis.[32] The density of the extracted products of *Curcuma aeruginosa* and *Curcuma xanthorrhiza* was measured.

Determination of Total Phenolic Content

The overall phenolic concentration was quantified by employing gallic acid as the calibration standard, with concentrations of 10, 20, 30, 40, and 50 ppm. An aliquot of 1 mL from the standard solution was combined with 5 mL of a 10% Folin–Ciocalteu reagent solution and incubated at room temperature for 10 minutes. Subsequently, 4 mL of 7.5% (w/v) Na_2CO_3 solution was incorporated, and the mixture was stirred until homogeneous. The solution was subsequently maintained in the dark at room temperature for 60 minutes. Deionised water was employed as the reference solution. The absorbance was recorded with a UV-Vis spectrophotometer at a detection wavelength of 765 nm. The sample was prepared by dissolving the extract in a 1:10 ratio. An aliquot of 1 mL extract was combined with 5 mL of 10% Folin–Ciocalteu reagent and 4 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3). The mixture was then incubated in the dark at room temperature for 60 minutes. Subsequently, 200 µL of the solution was placed into a cuvette, and absorbance was recorded at 760 nm using a UV-Vis spectrophotometer. The total phenolic concentration was quantified by reference to the gallic acid calibration curve, and results were expressed as gallic acid equivalents (GAE) per gram of dry weight.[32]

RESULTS AND DISCUSSION

Physicochemical Properties of NADES

The physicochemical properties of the synthesized NADES, including viscosity and density, are summarized in Table-1.

Table-1: Physicochemical Properties of NADES

NADES (HBA-HBD)	Abbreviation	Mole ratio	Water content	Visual appearance	Viscosity (mPa·s)	Density (g/mL)
Choline chloride–Glucose	ChCl–Glu	1:2	20 %	colorless	60.98	1.272
Choline chloride–Fructose	ChCl–Fr	1:2	20 %	colorless	88.73	1.298
Choline chloride–Maltose	ChCl–Mal	1:1	20 %	light yellow	250.09	1.305
Choline chloride–Xylose	ChCl–Xyl	1:1	30 %	dark yellow	5.99	1.194

Among the synthesized NADES, significant differences in viscosity and stability were observed depending on the hydrogen bond donor. ChCl–Glu and ChCl–Fr formed colorless liquids; however, ChCl–Glu exhibited recrystallisation during storage, likely due to glucose supersaturation, indicating lower physical stability. ChCl–Fr remained stable without observable changes. ChCl–Mal exhibited the highest viscosity, which may hinder solvent transfer and reduce extraction efficiency, whereas ChCl–Xyl showed the lowest viscosity due to its higher water content (30%), resulting in improved fluidity.

Numerous studies have demonstrated that, compared to low-viscosity solvents, NADES generally require higher energy input to enhance solute–solvent interactions and mass transfer efficiency.[33] The influence of temperature and water content on NADES viscosity has also been widely reported. Pan *et al.* (2025) showed that viscosity decreases with increasing temperature, while systems without added water can exceed 400 mPa·s.[28] Consistently, Jovanović *et al.* (2024) reported that water addition reduces viscosity by weakening intermolecular interactions.[34] In addition, water content up to 30% has been shown to improve stability and extraction performance of bioactive compounds.[35] These findings support the selection of 20–30% water content in this study. In addition to viscosity, density ranged from 1.194 to 1.305 g/mL, which is consistent with reported values for sugar-based NADES systems.[36] This observation is further supported by Koh et al. (2024), who reported comparable density ranges for similar systems. Variations in density are governed by molecular interactions and water content, where increased water reduces density by weakening intermolecular forces. Together, viscosity and density govern mass transfer efficiency and phase behavior, thereby influencing extraction performance. The visual characteristics of the synthesized NADES, as shown in Fig.-1, provide additional qualitative confirmation of these physicochemical differences.

Fig.-1: Visual Characteristics of Synthesized Choline Chloride–Based Natural Deep Eutectic Solvents (NADES) with different Hydrogen Bond Donors and Molar Ratios: ChCl–Glu (1:2, 20% water), (b) ChCl–Fr (1:2, 20% water), (c) ChCl–Mal (1:1, 20% water), and (d) ChCl–Xyl (1:1, 30% water)

The visual appearance further confirms differences in composition and intermolecular interactions among NADES systems. Homogeneous and transparent systems indicate effective hydrogen bonding, whereas variations in color and stability reflect differences in hydrogen bond donor type and water content. These observations are consistent with the physicochemical trends reported in Table-1. Building on these observations, improper molar ratios between hydrogen bond acceptor and donor may lead to phase transitions from homogeneous liquids to semi-solid systems, as previously reported.[37] This highlights the importance of maintaining appropriate component ratios to preserve eutectic behavior. The selection of the hydrogen bond donor also significantly influences solvent performance. NADES systems such as ChCl–lactic acid (1:2) and ChCl–glycerol (1:2) have been reported to exhibit high extraction efficiency under optimized conditions, particularly at approximately 20% water content.[38] Furthermore, Scandar *et al.* (2024) demonstrated that ChCl–based NADES systems can significantly modify the chemical profile of extracted compounds in coriander essential oil systems, highlighting the versatility of NADES in tuning extraction selectivity.[39]

Extraction of Polyphenols in *Curcuma aeruginosa* and *Curcuma xanthorrhiza* using NADES

The rhizomes used in this study were obtained from a conventional market in Bogor, West Java, and identified as *Curcuma aeruginosa* and *Curcuma xanthorrhiza*. Prior to extraction, the samples were washed, chopped, and air-dried at room temperature. The prepared dried rhizomes are shown in Fig.-2.

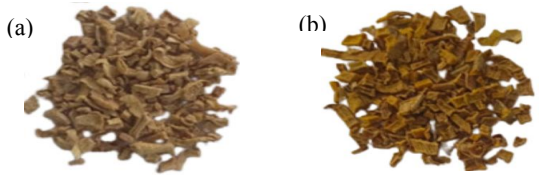


Fig.-2: Dried Rhizomes of (a) *Curcuma aeruginosa*, (b) *Curcuma xanthorrhiza*

To evaluate the extraction efficiency of the synthesized NADES, a solid-to-liquid ratio of 1:20 (g/mL) was applied under stirring at 120 rpm for 60 minutes. The resulting extracts and corresponding density values are presented in Fig.-3 and 4.

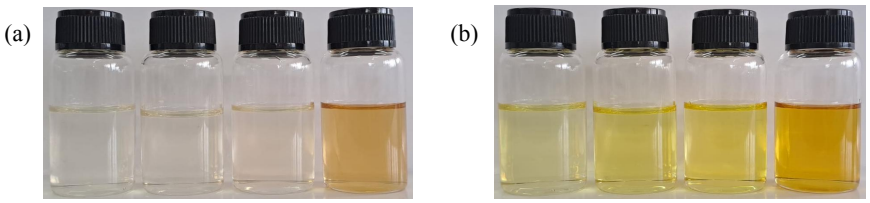
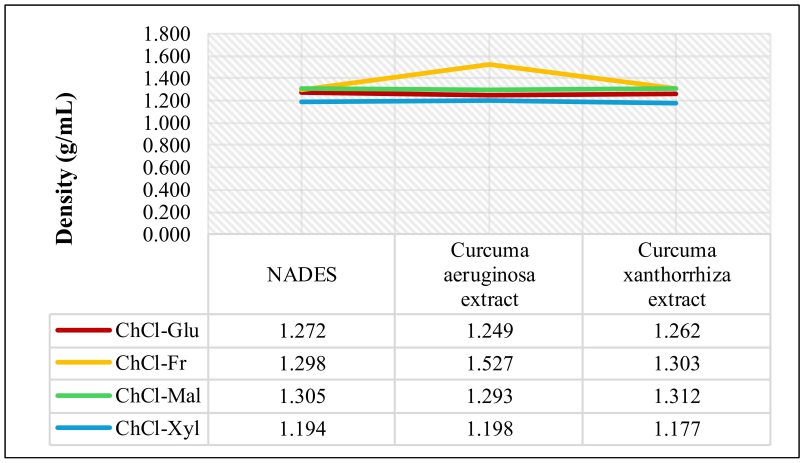


Fig.-3: Polyphenols Extracts from (a) *Curcuma aeruginosa* and (b) *Curcuma xanthorrhiza*, using ChCl–Fr 1:2 (20%), ChCl–Glu 1:2 (20%), ChCl–Mal 1:1 (20%), ChCl–Xyl 1:1 (30%), Respectively



	NADES	Curcuma aeruginosa extract	Curcuma xanthorrhiza extract
ChCl-Glu	1.272	1.249	1.262
ChCl-Fr	1.298	1.527	1.303
ChCl-Mal	1.305	1.293	1.312
ChCl-Xyl	1.194	1.198	1.177

Fig.-4: Density of NADES and Extracts from *Curcuma aeruginosa* and *Curcuma xanthorrhiza*

Density is a key physicochemical parameter influencing phase behavior and mass transfer during extraction. All NADES systems exhibited higher densities than water, consistent with their hydrophilic nature.[28,40] The density of sugar-based NADES typically falls within a comparable range, as previously reported.[36] Variations in density arise from molecular interactions and water content, where increased hydration reduces density by weakening intermolecular forces. Although higher density can facilitate phase separation due to stronger density gradients, it does not directly determine extraction efficiency, as observed in this study. Notably, ChCl–Mal exhibited the highest density but did not produce the highest polyphenol yield, likely due to its high viscosity, which limits mass transfer. In contrast, ChCl–Fr, with relatively high density and moderate viscosity, achieved the highest extraction performance for both plant species, indicating that viscosity plays a more dominant role than density in this system. To quantitatively assess extraction performance, total polyphenol content of the rhizome extracts was determined using the Folin–Ciocalteu method, with gallic acid as the standard. The results are summarized in Table-2.

Table-2: Polyphenol Content (mg/L) of *Curcuma aeruginosa* and *Curcuma xanthorrhiza* Extracts

NADES	Polyphenol Content of extracts (mg/L)	
	Curcuma aeruginosa	*Curcuma xanthorrhiza*
ChCl–Glu 1:2 (20 %)	296.49	301.97
ChCl–Fr 1:2 (20 %)	832.36	1599.18
ChCl–Mal 1:1 (20 %)	361.46	498.96
ChCl–Xyl 1:1(30 %)	694.52	700.61

ChCl–Fr–based NADES exhibited the highest extraction efficiency, yielding 832.36 mg/L for *Curcuma aeruginosa* and 1599.18 mg/L for *Curcuma xanthorrhiza*, while ChCl–Glu showed the lowest

performance. The extraction efficiency followed the order: ChCl–Fr > ChCl–Xyl > ChCl–Mal > ChCl–Glu, indicating that the hydrogen bond donor structure strongly influences solute affinity and extraction efficiency. The superior performance of ChCl–Fr can be attributed to its optimal balance of polarity and viscosity, which enhances solute diffusion and solvation of phenolic compounds. This behavior is consistent with the principle of "like dissolves like," where polyphenols, as polar compounds containing multiple hydroxyl groups, exhibit higher affinity toward more polar solvent systems. The effectiveness of NADES in polyphenol extraction has been widely reported. For instance, Soukaina *et al.* (2024) demonstrated that NADES-based extraction significantly outperformed methanol, achieving up to 1.73-fold higher yield compared to conventional solvents. Similarly, NADES systems such as ChCl–lactic acid and ChCl–glycerol have shown enhanced performance in essential oil extraction under optimized conditions.[38] In addition, ChCl–urea-based NADES has been reported to increase extraction yield in hydrodistillation processes by up to 31.5%.[39] Furthermore, NADES have been shown to outperform hydroalcoholic solvents in extracting phenolic compounds from plant matrices due to strong hydrogen bonding interactions.[17] These findings collectively confirm the superior capability of NADES as green solvents for polyphenol extraction.

Overall, this study demonstrates that NADES based on ChCl–Fr provide the most efficient medium for polyphenol extraction from both *Curcuma* species. The extraction performance is governed primarily by viscosity and hydrogen bonding interactions rather than density alone. These results highlight the critical role of solvent composition in tuning extraction efficiency and confirm the potential of NADES as sustainable green solvents for bioactive compound recovery. To further visualize and compare the extraction performance of the different NADES systems, the polyphenol yields are presented in Fig.-5, highlighting the relative efficiency of each solvent system across both *Curcuma* species.

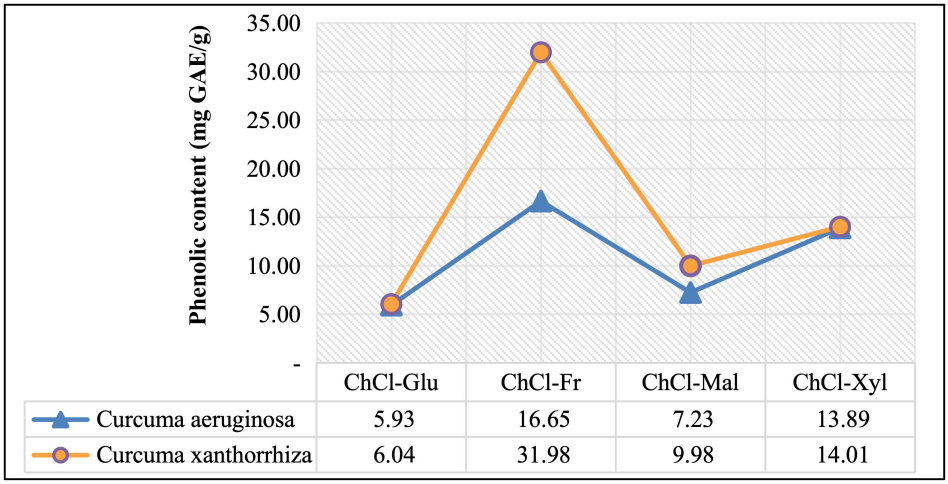


Fig.-5: Polyphenol Extraction Results of *Curcuma aeruginosa* and *Curcuma xanthorrhiza* using NADES

Figure-5 illustrates the same extraction trends observed in Table-2, with ChCl–Fr–based NADES consistently exhibiting the highest polyphenol yield for both *Curcuma aeruginosa* and *Curcuma xanthorrhiza*, while ChCl–Glu shows the lowest performance. The overall extraction efficiency follows the order: ChCl–Fr > ChCl–Xyl > ChCl–Mal > ChCl–Glu.

This trend indicates that extraction efficiency is strongly influenced by physicochemical compatibility between solute and solvent, particularly viscosity and polarity. In this system, ChCl–Fr provides an optimal balance of these properties, facilitating enhanced solvation and diffusion of polyphenolic compounds within the plant matrix. These findings further confirm that solvent composition exerts a stronger influence on extraction performance than density, supporting the earlier conclusion that viscosity is the key controlling parameter in NADES-assisted extraction. From a sustainability perspective, the present work aligns with Sustainable Development Goal (SDG) 12: Responsible Consumption and Production, by promoting the use of NADES as green and biodegradable alternatives to conventional

organic solvents. The use of NADES reduces reliance on volatile and toxic organic solvents while improving extraction efficiency, thereby supporting cleaner and more sustainable extraction processes. In addition, the utilisation of plant-based raw materials and low-energy extraction conditions contributes to reduced environmental impact, further reinforcing the potential of this approach in sustainable bioactive compound recovery.

CONCLUSION

This work confirms the effectiveness of choline chloride-based NADES as sustainable extraction media for polyphenols from *Curcuma aeruginosa* and *Curcuma xanthorrhiza*. Extraction performance is strongly governed by the hydrogen bond donor composition. Choline chloride–fructose (1:2, 20% water) was identified as the optimal system, achieving 1599.18 mg/L and 832.36 mg/L total polyphenols, with total phenolic contents of 31.98 and 16.65 mg GAE/g extract for *Curcuma xanthorrhiza* and *Curcuma aeruginosa*, respectively. The system further exhibited recyclability and enabled direct extract utilisation without solvent removal, thereby reducing energy and water inputs. Overall, NADES offer a green and efficient alternative to conventional solvents, supporting sustainable processing strategies aligned with SDG 12 (Responsible Consumption and Production).

ACKNOWLEDGMENTS

The research presented in this article is supported by the AKA Bogor Polytechnic.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: B. Mellisani *et al.*, *Rasayan J. Chem.*, 19(3), 1588-1596(2026), https://doi.org/10.31788/RJC.2026.1939729

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[RJC-9729/2026]