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Extraction of bamboo cellulose via sequential hydrated alkaline-acidic deep eutectic solvent pretreatment

Melody Kimi[1*] and Nurfitri Syafawani Kadai[2]

*Correspondence:
Melody Kimi
kmelody@unimas.my
[1]Centre for Pre-University Studies, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak 94300, Malaysia
[2]Faculty of Resource Science and Technology, Universiti Malaysia, Sarawak, Kota Samarahan, Sarawak 94300, Malaysia

Abstract

The extraction of cellulose from bamboo residues remains challenging because lignin and hemicellulose are strongly integrated within the fibre matrix. Green pretreatment using a two-step DES method allows efficient fractionation and selective valorisation of lignocellulosic components. In this study, we aimed to extract cellulose from bamboo residues by selectively removing lignin and hemicellulose using a sequential hydrated alkaline-acidic deep eutectic solvent (DES) method. The process involved an initial treatment with an alkaline DES prepared from choline chloride and monoethanolamine, followed by an acidic DES made from choline chloride and oxalic acid. Hydrated dual treatment improved cellulose recovery, yielding 64.8% cellulose compared to 39.1% in untreated bamboo. Fourier Transform Infrared (FTIR) spectroscopy confirmed effective chemical fractionation through the disappearance of hemicellulose and lignin-associated peaks. Scanning electron microscopy (SEM) images showed that cellulose fibres extracted via dual-DES treatments exhibited network-like structures with widths of 20–30 µm. Thermogravimetric analysis (TGA) results also confirmed the efficient removal of non-cellulosic components, with 10.4% residue left compared to 20.8% for the untreated bamboo. Overall, these findings show that sequential hydrated DES pretreatment improved cellulose extraction and offers a sustainable route for processing bamboo biomass.

Article Highlights

- Sequential alkaline–acidic green solvent treatment enhanced cellulose recovery of bamboo residues from ~39% to ~65%.
- Incorporating water into the solvent enhanced fibre penetration, leading to markedly improved cellulose extraction from bamboo residues.
- Effective removal of lignin and hemicellulose produced cellulose with higher purity.

Keywords Bamboo residues, Cellulose extraction, Deep eutectic solvents, Dual-step pretreatment, Lignocellulosic fractionation

1 Introduction

In Malaysia, bamboo is widely available due to its rapid growth and renewability and is used in handicrafts, furniture and even in construction. Bamboo waste is often disposed of by open burning or landfilling, thereby contributing to air and soil pollution and increasing greenhouse gas emissions. According to the National Biomass Action Plan 2023–2030 [1], approximately 182.6 million tonnes of biomass including bamboo waste are generated annually in Malaysia. This highlights the pressing need for effective valorisation methods to be developed to convert bamboo waste into value-added materials.

Bamboo, being a common lignocellulosic biomass, is mainly composed of lignin, hemicellulose, and cellulose. Among the components, cellulose accounts for approximately 30–50% of the dry weight, making it the most abundant natural polymer. However, cellulose is strongly embedded in the lignin-hemicellulose matrix; therefore, extracting it is rather challenging and is achieved through effective delignification [2, 3]. At present, conventional cellulose extraction from bamboo is based on Kraft or alkaline pulping, which requires high energy and harsh chemicals. These methods raise serious ecological concerns since they generate large amounts of harmful waste materials [4, 5].

To overcome these drawbacks, greener fractionation methods have received growing attention, including extraction using deep eutectic solvents. Deep eutectic solvents (DESs) have been identified as a novel alternative due to their low toxicity, tunable physicochemical properties, and ability to fractionate lignin and hemicellulose selectively while leaving cellulose intact [6, 7]. DESs typically consist of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), which enables strong hydrogen-bonding interactions that improve solubility and efficiency of fractionation [8].

The dense and complex structure of bamboo suggests that single-step DES pretreatment may not be sufficient for effective fractionation. Sequential or dual-step DES approaches may be required, combining alkaline and acidic formulations designed to target different biomass components. This approach may offer improved delignification and cellulose purification. However, limited literature exists on the fractionation of bamboo biomass using sequential alkaline-acidic DESs. In addition, the effect of DES hydration, where controlled water addition adjusts the viscosity of the solvent, hydrogen bonding and penetration have not been sufficiently explored for bamboo materials [7, 8].

In this study, a novel dual-step sequential treatment using two types of DES, alkaline DES (choline chloride-monoethanolamine) and acidic DES (choline chloride-oxalic acid), is developed to extract cellulose with high purity from bamboo residues (*Bambusa vulgaris*). Unlike conventional single-step DES treatments, this sequential method applies an alkaline DES first to disrupt lignin and hemicellulose linkages, followed by an acidic DES to further purify the cellulose fraction. Both alkaline and acidic-based DES systems were evaluated at a fixed hydration level of 50%, which was selected as a practical compromise to reduce solvent viscosity while maintaining sufficient molecular interactions to enhance mass transport during extraction. To promote faster solvent penetration and enhance the bamboo matrix disruption, ultrasonication-assisted treatment is applied. The thermal behaviour associated with the removal of non-cellulosic components following sequential DES pretreatment was also evaluated.

2 Materials and methods

2.1 Materials

Bamboo residues (*Bambusa vulgaris*) in this study were obtained locally from Kota Samarahan, Sarawak, Malaysia. The chemicals used in this study are all analytical grade, including choline chloride ($C_5H_{14}ClNO$, 98%), oxalic acid ($C_2H_2O_4$, 99.5–102.5%), and sodium hydroxide (NaOH, 98.6%), supplied by Sigma-Aldrich. Monoethanolamine (C_2H_7NO, 99%), glacial acetic acid (CH_3COOH, 100%), sulphuric acid (H_2SO_4, 95–97%), and sodium chlorite ($NaClO_2$, 80.3%), obtained from Merck. Also, ethanol absolute (C_2H_5OH, 99.5%) was supplied by Systerm. All chemicals were used as received without any further purification.

2.2 Preparation of the bamboo sample

The bamboo residues used as the raw material were obtained locally. Initially, the bamboo residues were divided into small pieces, rinsed with distilled water to remove surface impurities, and air-dried at room temperature. Subsequently, the dried residues were ground into 0.5 mm powder using a CT 293 Cyclotec™ laboratory mill (Foss Analytical). To remove its moisture content, the powder was then dried in an oven at 105 °C until constant weight. About 10 g of bamboo powder was placed in a cellulose thimble and subjected to a Soxhlet extraction for 6 h with ethanol as a solvent to remove extractives. Before further treatment, the Soxhlet-treated powder was oven-dried at 105 °C until constant weight.

2.3 Preparation of deep eutectic solvents (DESs)

In this study, pure alkaline DES (Alk-DES100) was formed by mixing choline chloride (ChCl) as HBA and monoethanolamine (MEA) as HBD at a molar ratio of 1:2, followed by mechanical agitation at 80 °C for 1.5 h until a clear and uniform medium was obtained. ChCl was selected as the HBA in this study because the chloride anion (Cl^-) provides high electron density for strong hydrogen-bond interactions with HBDs, enabling the disruption of intermolecular interactions in lignocellulosic matrices. MEA was chosen as the HBD due to the presence of hydroxyl (–OH) and primary amine ($–NH_2$) groups that readily donate hydrogen for strong interactions with the chloride anion, while its alkaline nature promotes disruption of lignin–carbohydrate linkages during biomass pretreatment. This molar ratio was selected based on reported DES systems in which an excess HBD increases hydrogen-bond interactions with the chloride anion, thereby disrupting the crystal lattice of the HBA and promoting eutectic formation [9]. The formation of the DES was indicated by the conversion of the solid components into a stable, clear, and homogeneous liquid upon heating.

By diluting the pure DES with deionised water according to a 1:1 mass ratio, hydrated alkaline DES (Alk-DES50) was prepared. Equivalently, pure acidic DES (Acd-DES100) was synthesised by mixing ChCl and oxalic acid (OA) at a molar ratio of 1:2, stirred at 80 °C for 1 h to obtain a homogenised acidic DES. Subsequently, deionised water was added to the acidic DES at a 1:1 mass ratio to produce hydrated acidic DES (Acd-DES50). All DES preparations were cooled at 25 °C and kept in a vacuum desiccator containing silica gel to prevent moisture uptake for later use.

2.4 Dual-step DES pretreatment

For dual-step pretreatment, bamboo powder was treated sequentially with alkaline DES first, followed by acidic DES. The experimental procedure for dual-step DES treatment of bamboo residues is shown in Fig. 1. For the first step, 2 g of Soxhlet-treated bamboo powder was immersed in 20 mL of Alk-DES (either Alk-DES100 or Alk-DES50) in a Teflon-lined stainless-steel reactor. The mixture was heated and maintained at 150 °C for about 16 h to encourage delignification and promote the elimination of hemicellulose. After the pretreatment, the solid fraction was separated by centrifugation at 3,000 rpm for 10 min, repeatedly washed with distilled water until neutral pH, then rinsed with ethanol, and oven-dried at 60 °C until constant weight. The pretreatment conditions for the alkaline DES were optimised in the preliminary stage, where several factors, including the extractive removal, reaction time, temperature, and hydration level, were taken into account. Hydrothermal treatment with a reaction time of 16 h at 150 °C was found to be an optimal condition for effectively breaking down lignin-carbohydrate bonds, thereby increasing cellulose accessibility.

In the second step, the dried solids were subjected to treatment with acidic DES using Acd-DES (either Acd-DES100 or Acd-DES50). The mixture was preheated for 30 min at 80 °C due to the higher viscosity of the acidic DES, then the treatment was immediately subjected to ultrasonic sonication cycles of 10 min sonication followed by 5 min cooling, repeated four times. The sonication was conducted using an ultrasonic bath (WUC-A02H, DAIHAN Scientific, Korea) with a cooling system to regulate the temperature. After the treatment, the solids were filtered, washed with distilled water to neutral pH, ethanol-rinsed, and oven-dried. Ultrasonication was used in the acidic DES pretreatment step to enhance mass transfer and solvent penetration due to the high viscosity of the acidic DES system. Ultrasonic energy was used to further disrupt the biomass structure and increase the contact between the solvent and the biomass components [10]. The sonication parameters, including the sonication time and cooling time during the sonication process, were varied in the preliminary stage to avoid over-degradation of the sample.

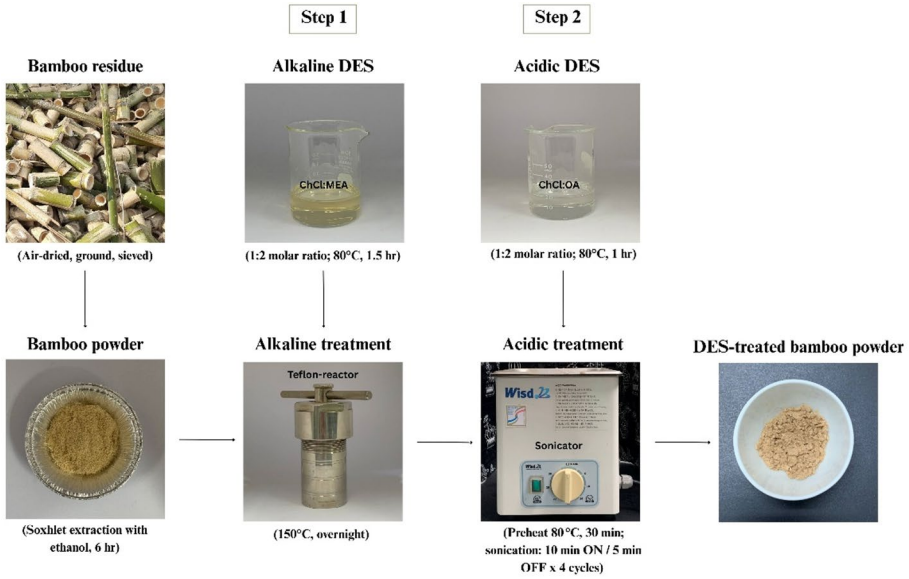

Fig. 1 Flowchart of the experimental procedure for dual-step DES treatment of bamboo residues

For comparison, control samples treated with a single-step alkaline DES (Alk-DES), as well as samples treated with a single-step acidic DES (Acd-DES), were also prepared. To evaluate the effects of hydration on the extraction efficiency, each DES system was applied in both its pure form (100% DES) and hydrated form (50% DES).

2.5 Lignocellulosic composition analysis

The lignocellulosic composition of the bamboo samples before and after pretreatment was analysed to determine the changes that occurred. The determination of the holocellulose, α-cellulose, and lignin contents in the samples was done using wet chemical methods [11]. In this study, the percentage of hemicellulose was calculated as the difference between holocellulose and α-cellulose. The ash content was estimated by burning α-cellulose residue at 550 ℃ for 2 h in a furnace.

2.5.1 Holocellulose determination

1 g of the sample (m_0) was accurately measured and then transferred into a flask. Then, 32.5 mL of deionised water, followed by 1.25 g of sodium chlorite and 0.5 mL of acetic acid, were added to the flask. The sample was then heated for reaction at 70 °C for 4 h. After cooling, the sample was filtered, washed with distilled water until neutral pH, and oven-dried until a constant weight was achieved. The dried residue (m_1) was weighed and recorded. The holocellulose percentage was determined using the following equation:

$$\%\ \text{Holocellulose} = \frac{\text{Holocellulose mass } (m_1)}{\text{Initial mass } (m_0)} \times 100 \tag{1}$$

2.5.2 Alpha cellulose determination

The holocellulose sample obtained was measured and transferred into a flask. The sample was then soaked with 12.5 mL of 17.5% sodium hydroxide solution for 30 min. The mixture then was thoroughly agitated with 30 mL of deionised water for another 30 min of reaction, followed by filtration using a glass filter, washed until neutral, and dried until constant weight. The final weight was recorded as the alpha-cellulose content (m_2) of the sample. The percentage of alpha cellulose was calculated using the equation below:

$$\%\ \text{Alpha cellulose} = \frac{\text{Alpha cellulose mass } (m_2)}{\text{Initial mass } (m_{0)}} \times 100 \tag{2}$$

2.5.3 Lignin determination

A 0.3 g sample (n_0) was accurately measured and transferred into a flask. Subsequently, 3 mL of 72% concentrated sulphuric acid was added to the flask and then placed in a water bath maintained at a temperature of 30℃ for 1 h. Then, 84 mL of deionised water was added to the flask and placed for 1 h at 121℃ in the autoclave. The sample was then filtered, washed until neutral, and dried in the oven until reaching a constant weight. The dried residue (n_1) was weighed and recorded. The following equation was used to determine the lignin percentage.

$$\%\ \text{Lignin} = \frac{\text{Lignin mass } (n_1)}{\text{Initial mass } (n_{0)}} \times 100 \tag{3}$$

2.6 Characterisation of samples

In this experiment, the viscosity of the DES systems was measured at 20 °C using a rotary viscometer (NDJ-5 S, China) and operated for 1 min at 60 rpm. The Fourier-transform infrared (FTIR) spectra of both DES systems and treated bamboo samples were analysed using an Agilent Cary 630 FTIR spectrometer with an attenuated total reflectance (ATR) accessory. The spectra were recorded in the range of 500 to 4000 cm^{-1} to monitor the chemical changes and identify the functional groups of the DES systems and the bamboo samples. The morphology and structure of the raw and DES-treated bamboo powders were observed using a scanning electron microscope (SEM, JSM-6390, JEOL, USA) to examine their fibrous structures and surface textures. SEM images were captured at a magnification of x500 to expose surface texture and structural changes. Lastly, thermogravimetric analysis (TGA) was performed by using a thermogravimetric analyser (DTG-60 H, Shimadzu, Japan) under a nitrogen atmosphere flowing at 25 mL/min. Samples (roughly 5–40 mg) were heated at 10 °C/min from 30 °C to 600 °C to analyse compositional changes after DES treatment and also the thermal stability.

3 Results and discussion

3.1 Viscosity of DES systems

According to Feher (2017) [12], viscosity is the resistance of a flowing fluid to deformation at a specific shear rate, or the internal friction the fluid experiences. Table 1 summarises both the formulation parameters (hydrogen bond acceptor, hydrogen bond donor, molar ratio, and water content) and the corresponding viscosities of the DES systems at 20 °C, highlighting the strong dependence of viscosity on DES composition and hydration level. Under the same conditions, pure alkaline DES (Alk-DES100) has a very high viscosity of 976.0 mPa·s, significantly higher than pure acidic DES (Acd-DES100), measured at 95.0 mPa·s. Even though acidic DESs are often assumed to show higher viscosities than alkaline DESs due to stronger hydrogen bonding from acidic HBDs, it has been revealed by earlier studies that alkaline HBDs also formed dense hydrogen-bonding interactions. This result is supported by recent experimental data, showing that viscosities for alkaline DESs surpass 700 mPa·s, which are higher than acidic systems (70–110 mPa·s) [13].

ChCl/MEA was selected as the alkaline DES due to its strong alkalinity [13], which is effective in breaking down the lignin-carbohydrate complexes and thereby promoting delignification to the extent of 94% among the alkaline DESs [14]. Although ChCl/MEA has relatively high intrinsic viscosity, the use of hydrothermal pretreatment conditions (150 °C, 16 h) helped overcome this limitation, enhancing molecular mobility and mass transfer within the biomass matrix. Additionally, the partial hydration of the DES system also helped reduce the viscosity and improve solvent penetration.

Table 1 Viscosity of DES systems at 20 °C

Sample	HBA	HBD	Molar ratio (HBA: HBD)	Water content (%)	Viscosity (mPa·s)
Alk-DES100	ChCl	MEA	1:2	0	976.0
Alk-DES50	ChCl	MEA	1:2	50	< 1.0
Acd-DES100	ChCl	OA	1:2	0	95.0
Acd-DES50	ChCl	OA	1:2	50	59.0

*Viscosity of Alk-DES50 was below the lower detection limit of the viscometer at 20 °C and is therefore reported as < 1.0 mPa·s

ChCl/OA (Acd-DES100) viscosity (94.7 mPa·s) is slightly lower but comparable to the reported value for ChCl/acetic acid (108.22 mPa·s) [15]. This indicates that the viscosities of acidic DES formed from small carboxylic acids with ChCl are in a similar moderate range around 70–110 mPa·s at 20 °C. Meanwhile, alkaline DES (ChCl/MEA) shows much higher viscosity (976.0 mPa·s) compared to the reported value for ChCl/Urea (725 mPa·s) [15]. Both are significantly more viscous than acidic DES, which supports the idea that DES formed with neutral or alkaline HBDs like urea or MEA tend to have denser hydrogen-bonding networks with high viscosity.

In the case of Alk-DES50, the dense gel-like network in the alkaline DES is effectively broken down by the high-water content, leading to a fluid with viscosity approaching that of pure water (≈ 1 mPa·s at 20 °C) [16, 17]. Water acts as a plasticiser, weakening chloride–HBD interactions and increasing molecular mobility, which explains the drastic viscosity reduction [18]. The viscosity of Alk-DES50 was recorded as < 1.0 mPa.s in Table 1 because the measured value fell below the lower detection limit of the viscometer at 20 °C. This indicates that Alk-DES50 behaves as a low-viscosity liquid with viscosity approaching that of pure water. For Acd-DES50 (59.0 mPa·s), the DES sample resulted in a significant decrease in viscosity with the addition of 50% water. This reduction in viscosity is in agreement with previous observations, where the viscosity of ChCl: OA (1:1 molar ratio) was reduced to 44 mPa·s upon the absorption of 19.40% of moisture [13]. This reduction happened when the extensive hydrogen bonding network formed was being disrupted between ChCl and the HBDs, due to the plasticising effect of water, similar to the observation for Alk-DES50. In contrast, Acd-DES50 retains some viscosity due to the relatively stronger and less disrupted hydrogen bonding network in the acidic system, even after dilution. A study by Gygli et al. (2020) [19] shows that while water decreases viscosity broadly, acidic DESs maintain stronger intermolecular structures, resulting in less viscosity reduction compared to neutral and alkaline DESs.

In addition to viscosity and acidity, the pretreatment performance of DESs is strongly influenced by their Kamlet–Taft (KT) solvent parameters, which describe hydrogen-bond donating ability (α), hydrogen-bond accepting ability (β), and dipolarity/polarizability (π*). Previous multivariate analyses of DES-based lignocellulosic pretreatment have demonstrated that KT parameters, particularly hydrogen-bond basicity (β) and acidity (α), play a crucial role in governing lignin solubilisation, hemicellulose removal, and overall fractionation efficiency [20, 21]. Further modifications of KT behaviours are possible due to the nature of the HBDs. Alkaline DESs containing amine and hydroxyl functionalised HBDs usually exhibit balanced α and β values, promoting biomass swelling and lignin extraction without compromising cellulose integrity. In contrast, acidic DESs based on organic acids possess higher α values, enhancing the cleavage of ether linkages and preferential hemicellulose hydrolysis. However, it may also increase the risk of cellulose degradation under severe conditions. The addition of water to the DES systems moderates these KT interactions by reducing excessive hydrogen-bond strength and lowering effective solvent polarity, thereby improving selectivity and overcoming cellulose loss [19].

3.2 FTIR analysis of DES systems

The FTIR analysis was carried out to identify the functional groups present and to assess the structural changes upon dilution with 50% water, which is shown in Fig. 2. All spectra

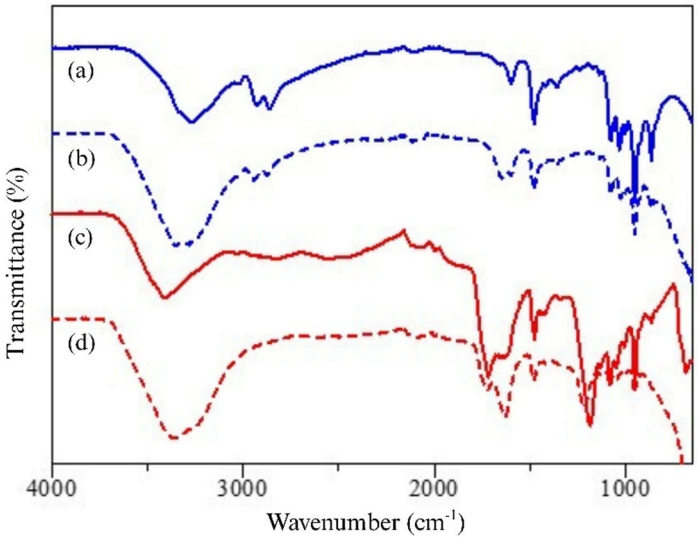

Fig. 2 FTIR spectra of different types of DESs

exhibit broad O–H stretching bands around 3200–3400 cm^{-1}, indicative of extensive hydrogen bonding in both DES systems. In alkaline DES systems, the observed C–H stretching bands near 2907 cm^{-1} represent the aliphatic ($–CH_2–$) groups of monoethanolamine, while the broad O–H stretching band in the range of 3400–3200 cm^{-1} corresponds to the hydroxyl group involved in hydrogen bonding. These hydrogen-bonding interactions play an important role in influencing DES viscosity, solvent mobility, and interactions with lignocellulosic components during bamboo pretreatment. Apart from this, the overlapping N–H stretching vibrations from the primary amine group may also contribute to the band observed near 3400 cm^{-1}, whereas the weak vibrations of the N–H bond appeared near 1600 cm^{-1} [22].

Furthermore, a slight broadening of the O–H band was observed along with a minor spectral shift upon dilution in Alk-DES50. This result confirmed the moderate enhancement of hydrogen bonding and also a reduction in the viscosity of the alkaline system. This structural modification enhances solvent penetration into the dense bamboo cell wall matrix, improving mass transfer and accessibility of the DES to lignin–carbohydrate complexes, which is essential for effective cellulose liberation. In acidic DESs, a sharp C=O stretching band near 1700 cm^{-1} and strong C–O vibrations near 1084 cm^{-1} [23] were observed, which correspond to the carboxylic groups of oxalic acid. Meanwhile, in Acd-DES50, the O–H band appeared clearer, and the carbonyl band shifted slightly upon dilution, indicating extensive hydrogen bonding reconfiguration. These changes suggest enhanced interaction between acidic DES components and lignin structures. Compared to pure DESs, hydrated DESs showed a stronger broad peak near 3300 cm^{-1}. This is a typical behaviour when water is present in the existing hydrogen-bond network, increasing the system's viscosity [17, 24]. This balance between hydrogen bonding strength and solvent fluidity is crucial for optimising bamboo pretreatment, as lower viscosity improves impregnation while maintaining sufficient chemical interaction for selective lignin and hemicellulose removal.

Moreover, although Alk-DES100 showed lower intensities near peaks 1040 and 1430 cm^{-1}, it still exhibited higher viscosity (976.0 mPa·s) than Acd-DES100 (95.0 mPa·s).

This finding suggests that the behaviour of the alkaline DES is influenced more by the hydrogen bonding rather than its simple ionic interactions. MEA might also contribute to a more flexible or entangled structure, which makes the liquid more resistant to flow [13]. Besides the formation of additional hydrogen bonds (resulting in increased absorbance in the O-H stretch region), the presence of water molecules disrupts the structured DES hydrogen bonding network that is responsible for the high degree of viscosity in the system [25].

3.3 Lignocellulosic composition analysis

In this study, bamboo residues were subjected to Soxhlet extraction with ethanol, producing an extract with a yellowish-green hue in contrast to the dark green hue exhibited by the extracts of leaves high in chlorophyll [26]. According to Pan et al. (2017) [27], removing these extractives is a critical step in the pretreatment process that facilitates delignification, improving cellulose yield and lignocellulosic biomass accessibility and enzymatic digestibility. The pretreatment sequence was intentionally designed to apply an alkaline-based DES prior to an acidic DES to achieve controlled and selective lignocellulosic fractionation. Alkaline DES disrupts lignin–carbohydrate complexes and partially solubilises lignin, thereby enhancing biomass accessibility [6]. Direct application of acidic DES to untreated biomass was avoided, as acidic conditions can induce polysaccharide hydrolysis and result in excessive cellulose degradation. Preconditioning the biomass with alkaline DES weakens the lignin network, allowing the subsequent acidic DES treatment to selectively remove hemicellulose [8] under milder conditions and thus preserve cellulose integrity. Ultrasonication was not applied during the alkaline step to minimise excessive structural disruption [10] of the lignocellulosic matrix during initial delignification.

Following pretreatment with DESs, the chemical composition of the bamboo powder underwent significant changes, as evident from the compositional analysis. The compositional changes in bamboo residues before and after DES pretreatments are summarised in Table 2.

In Table 2, the raw bamboo sample contained 66.96% holocellulose, comprising 39.08% α-cellulose and an estimated 27.88% hemicellulose, alongside 32.33% lignin. These values align well with prior findings by Li et al. (2024) [28], who reported holocellulose contents between 65.2% and 66.7% in Moso bamboo. Also, according to earlier studies, the chemical composition of raw *Bambusa vulgaris* is often more than 60% for holocellulose (39–55% for α-cellulose and 28–48% for hemicellulose) and around 25–33% for lignin, with variations based on age and culm part [29, 30].

Pretreatment using acidic DES led to a significant reduction in α-cellulose content. The lowest values were observed in Acd-DES100, with holocellulose at 53.66% and

Table 2 Lignocellulosic compositions of raw and DES-treated bamboo powder

Sample	Holocellulose (%)	α-Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Raw bamboo	66.96	39.08	27.88	32.33	9.25
Alk-DES100	76.22	56.13	20.09	0.73	5.87
Alk-DES50	82.92	61.46	21.46	4.17	5.98
Acd-DES100	53.66	36.96	16.70	27.57	15.21
Acd-DES50	68.72	43.82	24.90	25.13	11.32
Dual-DES100	60.66	47.41	13.25	6.07	10.63
Dual-DES50	81.37	64.81	16.56	0.37	7.78

α-cellulose at 36.96%, suggesting partial removal of hemicellulose and limited cellulose retention. This indicates that acidic DES may be less effective in preserving the cellulose-rich fraction, consistent with literature suggesting that acidic environments tend to hydrolyse polysaccharides more aggressively [31], especially for pure acidic DES. According to Chen et al. (2022) [31], hemicellulose is more readily broken down than cellulose and lignin when exposed to acidic DES pretreatment, resulting in less holocellulose and cellulose retention, especially at high temperatures. Moreover, an increase in apparent lignin content was observed following acidic DES pretreatment, particularly in the Acd-DES100-treated sample. This result agrees with earlier studies, showing that acidic DES can lead to pseudo-lignin formation by breaking down hemicellulose sugars like xylose. Chen et al. (2018) [32] reported that acidic DES breaks the glycosidic bonds in xylan, producing xylose, which then dehydrates into furfural. The formed reactive furfural could then react with itself and its derivatives via aldol condensation, ring opening, and other reactions to form pseudo-lignin. This suggests that the elevated lignin percentage in Acd-DES100-treated bamboo may not solely reflect residual native lignin but also the deposition of newly formed pseudo-lignin.

The higher α-cellulose content observed in Acd-DES50 compared to Acd-DES100 may be attributed to the dilution effect of water in the DES system. The presence of water in Acd-DES50 likely reduced the overall acidity of the system, thereby better preserving cellulose and reducing pseudo-lignin formation. This view aligns with a study by Zhang et al. (2024) [33] that observes the function of water as an auxiliary solvent in DES pretreatment. The study revealed that the addition of water has an impact on the preservation of cellulose and the fractionation process of lignocellulosic biomass, based on the adjustment of the viscosity and acidity level of the DES system. On the contrary, it was found that alkaline DES treatment has efficiently improved the purification level of cellulose. Alk-DES50 and Alk-DES100 resulted in α-cellulose contents of 61.46% and 56.13%, respectively, accompanied by a substantial reduction in lignin below 5%, reflecting effective delignification and hemicellulose removal, promoting cellulose recovery.

A dual-step DES approach (alkaline followed by acidic) further improved cellulose purity. Dual-DES50 achieved the highest α-cellulose content (64.81%) and most efficient lignin removal (0.37%), highlighting its superior fractionation performance. Although Dual-DES100 showed a lower α-cellulose yield (47.41%) compared to Alk-DES50, it still demonstrated significant lignin reduction (6.07%). This shows that sequential application of alkaline and acidic DES effectively targets distinct lignocellulosic components. Alkaline DES disrupts lignin–carbohydrate complexes, enhancing biomass porosity and enabling better penetration of subsequent acidic DES, which further solubilises residual non-cellulosic fractions. This sequence also reduces pseudo-lignin formation, an issue common when acidic DES is used first due to rapid hemicellulose hydrolysis, furfural formation, and its condensation into pseudo-lignin, which can redeposit on cellulose and reduce purity. This trend aligns with conventional pretreatment methods, where dilute acid and steam explosion primarily solubilise hemicellulose, while alkaline treatments are more efficient at lignin removal and biomass disruption [34]. Thus, the alkaline–acidic DES sequence offers a more selective and synergistic route for efficient lignocellulose fractionation.

The lower α-cellulose yield from Dual-DES100 compared to Alk-DES100 alone is attributed to the harsher conditions of Dual-DES100 using pure acidic DES. High

viscosity and strong hydrogen bonding in pure DES can promote biomass disruption but also risk partial cellulose hydrolysis and degradation. Without water to moderate these effects, cellulose may become soluble or degrade, lowering recovery. This is consistent with previous findings showing that strong hydrogen bond acceptors and high DES viscosity significantly influence cellulose solubilisation [35, 36].

Conversely, Dual-DES50, which has 50% water content, exhibits the highest yield of α-cellulose due to increased solvent penetration as well as the inhibition of cellulose degradation caused by the decrease in acidity and viscosity of the DES system. According to Xu et al. (2023) [37], the incorporation of water decreases hydrogen bonding within the DES, modifying the acidity strength to an extent where lignin and hemicellulose removal is not at the cost of cellulose degradation. This leads to greater cellulose purity as well as fractionation efficiency, which is in agreement with earlier findings showing that the addition of water to DES systems improves cellulose extraction and saccharification more than in pure DES [37, 38]. Thus, the cellulose content in Alk-DES50 is also higher than that in Alk-DES100 due to the hydrated system having better cellulose preservation during pretreatment.

Figure 3 presents the relative changes in α-cellulose, hemicellulose, and lignin contents of the DES-treated bamboo samples, calculated with respect to the raw bamboo. Alkaline DES treatments exhibit the greatest lignin removal, in which the lignin content decreased more than 85% relative to the raw bamboo. This result indicates that the alkaline DES system has a high efficiency of delignification. There is also a substantial increase in the level of α-cellulose relative to raw bamboo, up to 57% for Alk-DES50 and 43% for Alk-DES100. This shows that a larger reduction in lignin has a strong correlation with cellulose enrichment, demonstrating that the lignin-carbohydrate complexes are effectively disrupted by alkaline DES. While achieving a great removal of lignin (81 to 98%), it is apparent that Dual-DES50 treatment shows the highest relative increase in α-cellulose content by 65% among all the DES treatments. This suggests a synergistic effect of the sequential DES approach, in which the initial disruption of the matrix

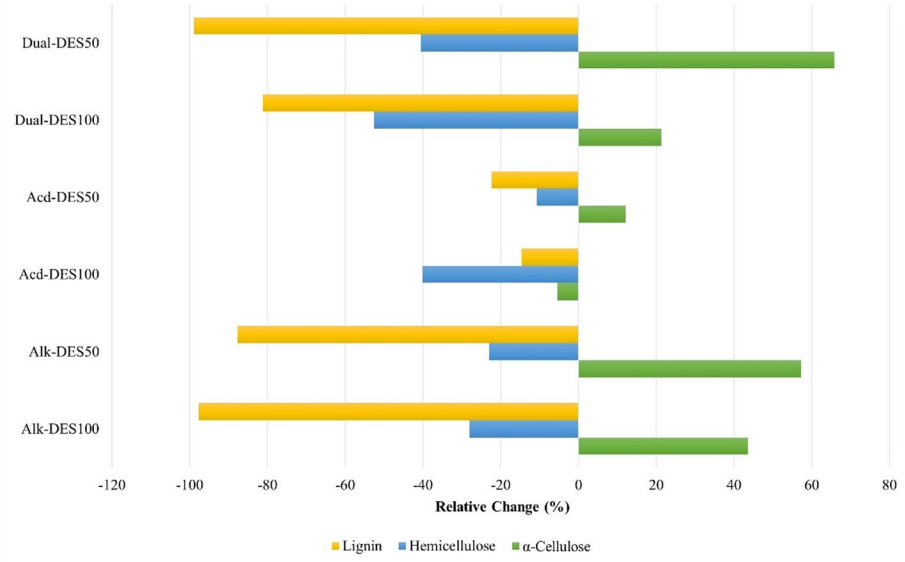

Fig. 3 The relative changes in α-cellulose, hemicellulose, and lignin contents of DES-treated samples, with respect to raw bamboo

would promote much more effective removal of the amorphous parts during the following treatment steps.

To the contrary, acidic DES treatments result in rather moderate lignin removal (14 to 22%) and slight increases in α-cellulose yield. From Fig. 3, it is apparent that the composition of hemicellulose was reduced in all DES-treated samples, with the hemicellulose decrease obtained in alkaline (23 to 28%) and dual-step DES (40.6 to 52.5%) systems. Selective hemicellulose and lignin removal contribute to the relative cellulose enrichment, highlighting the effectiveness of the DES systems in the fractionation of targeted biomass. Overall, the relative change suggests that the visualisation of DES performance is clear and that the hydrated dual-step DES system is a better method for bamboo biomass pretreatment to increase cellulose yield.

To contextualise the effectiveness of the proposed sequential DES pretreatment, its performance (relative changes to raw bamboo) was compared with reported DES-based systems and conventional acid and alkaline pretreatments. The single alkaline DES pretreatments in this study removed 87.1 to 97.7% of the lignin content, which is consistent with the lignin removal values reported for the alkaline DES methods (~ 84.8 to 97.6%) [39, 40]. Meanwhile, the single acidic DES pretreatments exhibited lower lignin removal with only 14.7 to 22.3%, which agrees with the value obtained by Ling et al. (2020) [41] (~ 22.76% lignin removal). The dual-step hydrated alkaline-acidic DES system showed a high delignification of 98.9% with an enrichment of α-cellulose content up to 65.8%, which is in line with or better than the hydrated acid/alkaline DES pretreatments that report lignin removal of ~ 86.9 to 93.2% [42, 43].

The current dual-step DES approach, as well as the single alkaline DES system, showed significantly higher lignin removal in milder DES conditions. In comparison, conventional alkaline pretreatments using hydrogen peroxide, aqueous ammonia, or lime have achieved comparable lignin removal (~ 30 to 70%). Meanwhile, in kraft pulping, 35 to 45% lignin was removed, and 45 to 50% cellulose was yielded. In addition, they also perform better compared to the sequential acid-alkaline pretreatment, which removed 52.48% of the lignin. However, these treatments often rely on longer reaction times and give low cellulose yields [34, 44]. Therefore, the present sequential hydrated DES system can be seen to have a high level of delignification, comparable to alkaline pretreatments, and it exceeds most single-step DES and acid-based methods, highlighting the improved selectivity of the dual-step approach.

3.4 Characterisations of bamboo samples

FTIR spectra of cellulose contained in untreated bamboo and solid residues obtained after various DES treatments are shown in Fig. 4. The spectrum of raw bamboo (Fig. 4(a)) exhibited characteristic peaks corresponding to cellulose, hemicellulose, and lignin. A broad absorption band around 3330 cm^{-1} assigned to –OH stretching vibrations and a peak at 2884 cm^{-1} from C–H stretching in polysaccharides are consistent with earlier reports [35]. The distinct band at 1718 cm^{-1}, corresponding to C=O stretching of acetyl ester groups, indicates the presence of hemicellulose. Additionally, peaks around 1509 cm^{-1} and 1595 cm^{-1}, associated with aromatic C=C vibrations, confirmed the presence of lignin [32, 43]. The region near 1237 cm^{-1} was ascribed to C–O stretching in lignin and hemicellulose, while peaks at 1028 cm^{-1} and 894 cm^{-1} reflected C–O and β-glycosidic linkages in cellulose, respectively [36].

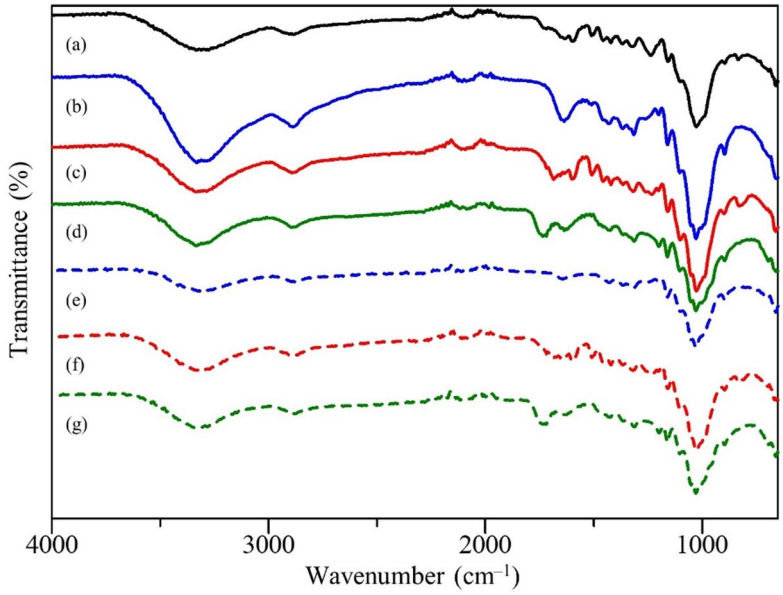

Fig. 4 FTIR spectra of (**a**) raw bamboo, (**b**) Alk-DES100, (**c**) Acd-DES100, (**d**) Dual-DES100 (**e**) Alk-DES50, (**f**) Acd-DES50 and (**g**) Dual-DES50

Treatment with Alk-DES100 led to a clear reduction in the 1718 cm^{-1} and 1509 cm^{-1} bands, indicating effective removal of hemicellulose and partial delignification, while enhanced intensities at 1028 cm^{-1} and 894 cm^{-1} suggested cellulose enrichment (Fig. 4(b)). Acidic DES treatment in Fig. 4(c) showed a reduction in hemicellulose-related peaks but was less effective in removing lignin, as shown by the persistent aromatic peaks (C=C stretch) at 1509 cm^{-1} and 1595 cm^{-1}.

Among all treatments, the Dual-DES50 system presented the most balanced performance with significant suppression of lignin and hemicellulose peaks, alongside sharper and stronger cellulose-associated bands at 1028 cm^{-1} and 894 cm^{-1}. This performance indicates a better enrichment and preservation of cellulose under milder conditions. Compared to its pure DES counterpart (Dual-DES100), which led to partial cellulose degradation, Dual-DES50 retained cellulose quality better due to reduced acidity and viscosity resulting from the addition of water. Samples treated with other hydrated DESs (Alk-DES50 and Acd-DES50) showed incomplete removal of non-cellulosic components, which is likely due to limited disruption of the biomass material. Dual-DES50 overcame this issue through the synergistic action of alkaline and acidic components, enhanced by water-assisted penetration [33, 35]. Based on the composition analysis, this spectral evidence indicates the preservation of the molecular structure of cellulose with the selective removal of biomass components.

SEM analysis presented in Fig. 5 reveals significant morphological changes in bamboo powder treated with different DES systems compared to the raw material. Raw bamboo fibre bundles appear compact with smooth surfaces, indicating a well-preserved state of lignocellulosic components, which agrees with observations of untreated bamboo fibres that show compact cell walls with smooth surfaces [45].

Treatment with pure DES systems, as shown in Figs. 5(b) and 5(c), results in noticeable delamination and fibrillation. Specifically, Alk-DES100 causes fibre structures that are more individualised, which is consistent with alkaline DES treatments reported to

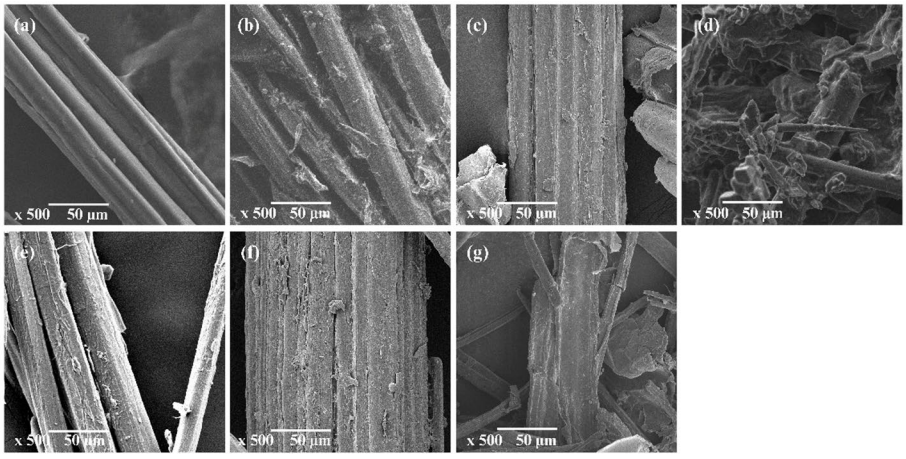

Fig. 5 SEM images of (**a**) raw bamboo, (**b**) Alk-DES100, (**c**) Acd-DES100, (**d**) Dual-DES100, (**e**) Alk-DES50, (**f**) Acd-DES50, and (**g**) Dual-DES50. All images were taken at 500x magnification, with a scale bar of 50 µm

remove hemicellulose and lignin, exposing finer fibrils and roughening fibre surfaces [46]. Acd-DES100, on the other hand, shows smoother yet more fragmented fibres, which matches the literature showing that acidic DES primarily depolymerises hemicellulose and partially removes lignin. This causes less physical fibre separation but more fibre fragmentation [11, 47].

The sequential Dual-DES100 treatment induces the greatest changes, characterised by extensive fibre separation, rough surfaces, and fine fragmentation, indicating an effective removal of lignin and hemicellulose. In contrast, hydrated DES systems (Alk-DES50 and Acd-DES50) exhibit generally less severe changes, with slightly loosened fibre networks and limited structural disintegration, as shown in Figs. 5(e) and 5(f). Notably, it can be observed that the Dual-DES50 treatment demonstrates clearer fibre separation and surface roughness than the individual diluted DESs and a better retention of fibre structure than Dual-DES100. This confirms that Dual-DES50 effectively disrupts lignin–carbohydrate complexes without over-degrading cellulose due to the moderating effects of water on DES viscosity and reactivity, as reported in studies on hydrated DES systems [37, 48]. These morphological alterations correlate with chemical compositional changes where alkaline and sequential DES treatments effectively remove hemicellulose and lignin, as confirmed by complementary FTIR and chemical assays, thus weakening the lignin-carbohydrate matrix and exposing cellulose fibrils.

TGA curves in Fig. 6 illustrate the thermal stability and degradation patterns of raw bamboo and DES-treated bamboo residues to assess their processing suitability. The changes in the onset of degradation temperatures and the residual mass of the samples can be taken as an indirect indicator to confirm that lignin and hemicellulose present in the bamboo biomass have been removed by the DES treatments. This is due to both lignin and hemicellulose degrading at higher temperatures than cellulose.

In this study, raw bamboo exhibits a gradual loss of weight starting around 200 °C, with major degradation taking place between 250 °C and 350 °C. This is due to the degradation of hemicellulose and cellulose, with lignin increasing the thermal stability at higher temperatures. This degradation profile aligns closely with reports on thermal analyses of bamboo species, with the initial gradual loss of weight attributed to the evaporation of

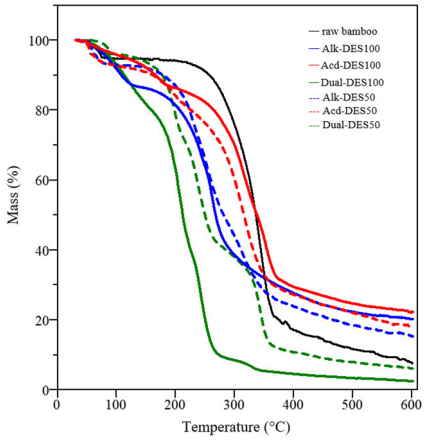

Fig. 6 Thermogravimetric analysis (TGA) curves of samples

moisture and the removal of extractives, followed by a significant weight loss due to the breakdown of polysaccharides [45, 49].

The DES-treated samples exhibit reformed degradation profiles, reflecting the removal of non-cellulosic components such as lignin and hemicellulose. This is consistent with literature reports that alkali and chemical treatment remove lignin and hemicellulose, thus exposing cellulose and altering thermal degradation [45, 50]. Pure DES-treated samples (Alk-DES100, Acd-DES100, Dual-DES100) retain a slightly higher level of thermal stability, initiating degradation at higher temperatures compared to their hydrated counterparts due to residual lignin and less disruption of cellulose crystallinity. Among these, Dual-DES50 demonstrates the greatest thermal degradation shift, with a sharp weight loss starting around 200 °C and nearly complete degradation before 400 °C. This suggests effective lignin removal, though the degree of degradation may also reflect partial cellulose breakdown under harsh treatment [51].

The thermal properties of hydrated DES-treated bamboo residues (Alk-DES50, Acd-DES50, and Dual-DES50) show a greater extent of degradation and weight loss compared to the untreated bamboo sample. This implies a higher lignin and hemicellulose degradation, resulting in lower thermal stability but suggesting improved cellulose exposure and purification [45, 51]. Thus, hydrated DES systems produced thermally unstable samples due to the more effective removal of lignin and hemicellulose, while pure DES-treated samples retained relatively higher thermal resistance. The degradation behaviour of the sequentially hydrated DES-treated samples supports their use as cellulose-rich materials for downstream processing applications.

4 Conclusion

In this study, cellulose was effectively extracted from bamboo residues using a hydrated dual-step deep eutectic solvent (Dual-DES50) treatment. This method improves cellulose purity and preserves its structural strength, compared to pure and single-step DES treatments. Pure cellulose was extracted by first using alkaline DES to disrupt lignin-carbohydrate complexes and then using acidic DES to remove hemicellulose selectively. Although pure DES systems like Dual-DES100 removed more lignin and hemicellulose, they also caused some cellulose loss due to their harsh conditions, achieving the lowest α-cellulose content (36.96%). In contrast, Dual-DES50 gave the highest α-cellulose

content (64.81%), which represents a great increase compared to the untreated bamboo (39.08%). Dual-DES50 also yielded the lowest lignin residue (0.37%) compared to the raw bamboo (32.33%), as determined by the compositional analysis. The addition of water reduced the viscosity and acidity of the DES system, which improved lignocellulosic fractionation with little cellulose damage. These findings show that the dual-step DES approach can be used as a milder and greener option for treating bamboo residues. The method yields cellulose with preserved structure and high purity, making it suitable for further processing and potential development of materials.

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Author contributions
M.K: Supervision, Visualization, Methodology, Data curation. Validation, Writing – review & editing, Writing – original draft. N.F.K: Validation, Investigation, Formal analysis, Writing – original draft.

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Data availability
The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethical approval
This article does not contain any studies with human participants or animals performed by any of the authors.

Clinical trial
Not applicable.

Human or animal rights
This article does not contain any studies involving animals or human participants performed by any of the authors.

Informed consent
None.

Consent to Publish
Not applicable.

Competing interests
The authors declare no competing interests.

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