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Original Article

Changes in phenolic content and biological activities of *Plectranthus amboinicus* (Lamiaceae) leaf extracts resulting from pectinase and cellulase treatments

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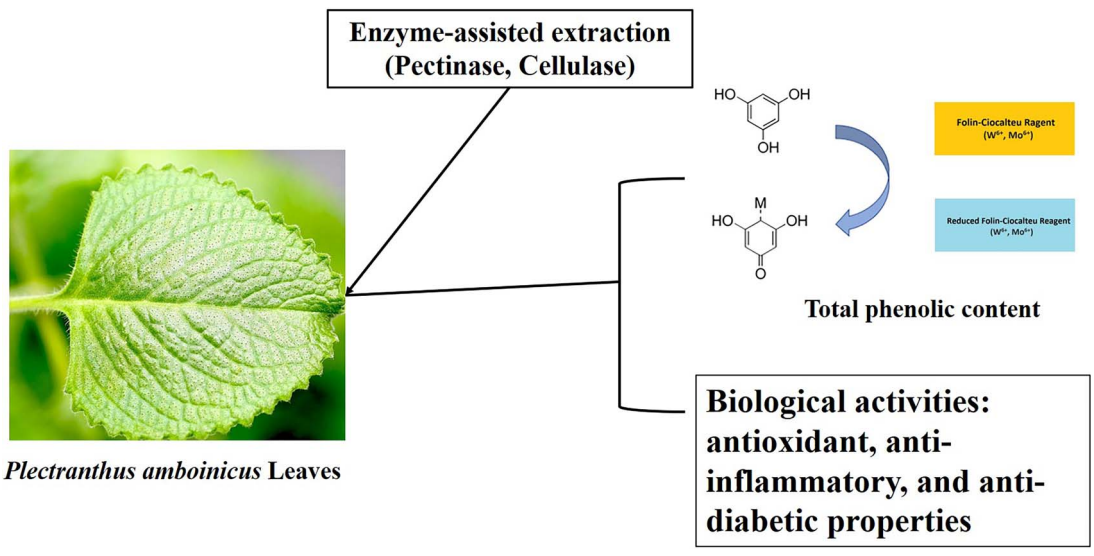
Abstract

Enzyme-assisted extraction has emerged as an eco-friendly and effective method for enhancing the yield of phenolic compounds from plant materials. This study examined the effectiveness of pectinase and cellulase in extracting phenolic compounds from *Plectranthus amboinicus* leaves (PAL) and evaluated the biological activities of the resulting extracts. The effect of key extraction factors, including enzyme concentration, extraction time, and solid-to-liquid ratio, on the total phenolic content (TPC) was examined. Under optimal conditions, cellulase treatment increased the TPC by 24%, 39%, and 56% compared to pectinase, 30% ethanol, and water extractions, respectively. Phenolic-rich extracts obtained through enzymatic methods exhibited significantly superior bioactivities, including antidiabetic, anti-inflammatory, and antioxidant properties, compared to conventional methods. Among the tested extracts, the cellulase-treated extract demonstrated the highest 2-diphenyl-1-picrylhydrazyl radical scavenging activity (IC_{50} value of 57.81 μg/ml), albumin denaturation inhibition (IC_{50} value of 136.36 μg/ml), and α-amylase inhibition (IC_{50} value of 135.25 μg/ml). Hierarchical clustering and principal component analyses further revealed strong correlations between the biological activities of PAL extracts and the extraction method employed. These findings highlight cellulase-assisted extraction as a promising eco-friendly and efficient alternative to solvent-based techniques for recovering phenolic compounds with enhanced bioactivities, offering potential applications in functional foods, nutraceuticals, and herbal medicine.

Keywords: *Plectranthus amboinicus*, enzyme, extraction, antioxidant, antidiabetic

Graphical abstract



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Introduction

Phenolic-rich plant extracts have gained significant scientific attention for their diverse biological activities, particularly their potential in preventing and treating diseases associated with oxidative stress, such as diabetes, cancer, cardiovascular diseases, and inflammation (Paun et al., 2020; Rahman et al., 2021). Numerous studies have highlighted the roles of polyphenols, flavonoids, and terpenoid compounds derived from *Plectranthus* species in mitigating oxidative damage, inflammation, and diabetes mellitus (Barbosa et al., 2023; Bhatt & Negi, 2012; Monzote et al., 2020; Nguyen et al., 2020; Sindhu et al., 2022). Among these, *P. amboinicus* stands out due to its remarkable physiological effects, including anti-inflammatory, antioxidant, wound-healing, antidiabetic, and anticancer properties. These effects are largely attributed to its high phenolic content, making it a valuable resource for herbal medicine and the pharmaceutical industry (Arumugam et al., 2016; Bhatt & Negi, 2012; Kumar et al., 2020; Rodrigues et al., 2021).

Despite its promising applications, the efficient recovery of phenolics from *P. amboinicus* remains a significant challenge. Conventional extraction methods, often relying on toxic organic solvents such as methanol, ethanol, or ethyl acetate, are widely used for extracting phenolic compounds due to their efficiency in solubilising target compounds (Ashaari et al., 2021; Chakka & Babu, 2022; Machado et al., 2024). However, these methods not only raise environmental and health concerns but also struggle to release tightly bound phenolic compounds from cellular components such as proteins, hemicellulose, cellulose, and acidic pectin (Boulet et al., 2023; Ilyas et al., 2021; Paun et al., 2020). To address these limitations, advanced techniques such as ultrasound-assisted extraction have been developed for extracting phenolic compounds from *P. amboinicus* (Malpica-Acosta Sheila et al., 2024). Ultrasound-assisted extraction stands out as a more sustainable alternative and enhances phenolic recovery through cavitation effects that disrupt cell walls, increase solvent penetration, and improve mass transfer efficiency (González-Silva et al., 2022). However, its industrial application still faces certain technical challenges.

Enzyme-assisted extraction (EAE) has recently emerged as a novel and highly effective approach for recovering phenolic compounds from various materials (Domínguez-Rodríguez et al., 2021; Xavier Machado et al., 2021; Nguyen et al., 2024a). This method employs enzymes such as pectinases, cellulases, and tannases to hydrolyse structural plant components, enabling the release of bioactive molecules into the extraction solvent (Xavier Machado et al., 2021). In this method, the type of enzyme used along with the extraction conditions play a crucial role in the phenolic extraction efficiency and the biological activity of the phenolic extract (Ghandahari Yazdi et al., 2019). Recent studies have highlighted the potential of EAE to outperform the traditional methods in terms of phenolic yield and sustainability (Çinar, 2005; Ghandahari Yazdi et al., 2019; Jiang et al., 2024; Ngadze et al., 2018). However, the application of EAE to *P. amboinicus*, particularly using specific enzyme cellulase and pectinase, remains underexplored. Furthermore, although the biological properties of conventionally extracted *P. amboinicus* are well documented, the bioactivities of enzymatically treated extracts, including antioxidant, anti-inflammatory, and antidiabetic effects, have not been reported yet.

Therefore, this work aimed to examine the influence of enzymatic treatments, specifically pectinase and cellulase, on the recovery of phenolic compounds from *P. amboinicus* and evaluate their biological activities. To maximise phenolic recovery, the effects of key extraction factors (enzyme concentration, extraction time, and solid-to-liquid ratio) on total phenolic content (TPC) were systematically investigated. Additionally, the enzymatic methods were compared with conventional extraction methods using 30% ethanol (EtOH) and water to provide a comprehensive assessment of their relative efficiency. Finally, the extracts were evaluated for their antioxidant, anti-inflammatory, and antidiabetic activities. By combining enzymatic optimisation with a comparative analysis of extraction methods, this study not only advances our understanding of phenolic recovery from *P. amboinicus* but also highlights the potential of enzymatic extraction as a sustainable and efficient alternative to produce biologically active extracts.

Materials and methods

Plant materials

P. amboinicus (Lour.) Spreng. leaves (PAL) were collected from a farm in the Phu Loi Residential Area (Ho Chi Minh City, Vietnam) from November to December 2023. The plant was authenticated by Dr Van-Son Dang and deposited as a herbarium specimen (voucher number VNM00070337) at the Institute of Tropical Biology, Vietnam. After drying in the shade at 32–35 °C, *P. amboinicus* leaves were ground into powder (particle size $\leq$0.3 mm) using a grinder (ZXMOTO, China). The powdered samples were then stored in a freezer at −30 °C until further use.

Chemicals and reagents

Albumin from human serum (HSA) (≥ 96%), 2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), formic acid, gallic acid (GA; ≥ 95%, HPLC), α-amylase (≥ 5 units/mg solid), and Folin–Ciocalteau were purchased from Sigma (Singapore). Aspirin, agarose, and ascorbic acid (vitamin C) were obtained from Merck Pte Ltd. (Singapore). Pectinase and cellulase were also supplied by Sigma (Singapore), with their characteristics detailed in Table 1.

Extraction of phenolic compounds from *P. amboinicus* leaves

Enzymatic extraction

The enzymatic extraction was carried out following the method of Ghandahari Yazdi et al. (2019) with minor modifications. The extractions were conducted under the optimal pH and temperature conditions for pectinase and cellulase as provided by the suppliers (Table 1). To optimise the extraction conditions, this study investigated the effects of three variables: enzyme concentrations (0, 1.0, 1.5, and 2.5 U/ml), extraction times (1, 3, 5, and 7 hr), and solid-to-liquid ratios (1:20, 1:60, 1:80, and 1:100 g/ml) on the TPC. Briefly, 20.0 g of PAL powder was mixed with 150 ml of enzyme solution (in 50 mM sodium phosphate buffer) in a 250 ml beaker. The extraction was conducted in an orbital shaker (IKA homogeniser control, Germany) with gentle agitation (120 rpm). After the extraction was completed, the sample was centrifuged at 4 °C for 10 min at 6,000 × g to collect the supernatant. The resulting pellet was re-extracted twice. The extracts were combined, evaporated using a rotary evaporator (Pollabm, India) at 35 °C, and stored at −20 °C for further use.

Aqueous ethanol and water extraction

Ethanol and water extractions were conducted as comparative methods against enzymatic extraction. Briefly, 20.0 g of powdered PAL material was mixed with 150 ml of aqueous ethanol (30%) or distilled water in Pyrex beakers. The extractions were carried out

Table 1. Enzyme characteristics.

Enzyme	Source	Optimum conditions		
		Activity	pH	T (°C)
Pectinase	*Aspergillus niger*	≥ 5,000 U/ml	4.0	40
Cellulase	*Trichoderma reesei* ATCC 26921	≥ 2,500 U/ml	5.0	37

at the optimised temperature and extraction time. The mixtures were filtered three times using filter paper (Whatman No. 1) to collect all the extracts. The aqueous ethanol and water extracts were evaporated using a rotary evaporator (Pollabm, India) at 35 °C and used for further analysis.

Determination of TPC

The TPC of PAL extracts was measured by the Folin–Ciocalteau method (Aryal et al., 2019) with minor modifications. Dried extracts were dissolved in distilled water (1.0 mg/ml). Subsequently, 10% Folin–Ciocalteau reagent (5 ml) and 7.5% Na_2CO_3 (4 ml) were added to 1.0 ml of the extract solution, then vortexed thoroughly. The solution was subsequently incubated at 40 °C for 30 min. Absorbance of the blue-coloured solution was measured at 765 nm using a UV-visible spectrophotometer (V-730, Jasco, USA). For quantification of TPC in the extract, a standard calibration curve was prepared using GA. The experiments were performed in triplicate, and the results were shown as milligrams of GA equivalents per gram of dry weight (DW) of extract (mg GAE/g DW).

Identification and quantification of GA using high-performance liquid chromatography

Gallic acid content, a prevalent phenolic compound in PAL extracts, was quantified using the HPLC-2160 system (Agilent, USA) equipped with a C18 column (4.6 × 250 mm) and a UV detector (Agilent Series 1,100), following the method of Rodrigues et al. (2021). The mobile phase comprised water containing 0.1% acetic acid (60%) and methanol (40%), with a flow rate of 1.0 ml/min. A stock solution of GA (0.02–0.20 mg/ml) was prepared to establish a standard curve at 272 nm ($y = 0.007x - 0.008$; $R^2 = 0.992$) for quantification of GA in the sample. The chromatographic peaks were confirmed by comparing UV detector spectra (200–600 nm) and retention time with reference standard. Operations were performed at a constant temperature of 30 °C in triplicate.

DPPH radical scavenging activity assay

The DPPH radical scavenging activity (DRSA) of PAL extracts was estimated using the method described by Rodrigues et al. (2021) with minor modifications. Extract solutions (25, 50, 75, and 100 μg/ml) in 50% EtOH (1 ml) were added to 1 ml of 0.004% DPPH solution. The mixtures were incubated in the dark at 37 °C for 30 min, followed by the measurement of absorbance at 517 nm using a V-730 UV-visible spectrophotometer (Jasco, USA). The percentage of DRSA was calculated as follows:

$$\%\text{DRSA} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance of the control (using 50% EtOH instead of the extract) and A_1 is the absorbance of the test extracts. The IC_{50} value, representing the extract concentration required to achieve 50% inhibition, was determined from RSA percentage *versus* extract concentration plots. Ascorbic acid (25–100 μg/ml) served as the standard.

Albumin denaturation inhibition assay

The anti-inflammatory activity of PAL extracts was evaluated using the human albumin (HSA) denaturation inhibition assay, modified from the method of Nguyen et al. (2024b). Reaction mixtures of extract samples (50–200 μg/ml in DMSO) and 1% HSA were incubated for 20 min at 37 °C, followed by heating at 70 °C for 5 min. The solutions were then cooled to room temperature for 20 min. Absorbance of the reaction mixtures before and after denaturation was measured at 660 nm using a UV-V is spectrophotometer. Aspirin (Merck, Singapore) served as a positive control. The protein denaturation inhibition was calculated as follows:

$$\text{Protein denaturation inhibition } (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

where A_c and A_s denote the absorbance of the control and the sample, respectively. The IC_{50} value was then determined.

α-Amylase inhibition assay

α-Amylase inhibition was performed using the method described by Pham et al. (2023), with minor modifications to evaluate the *in vitro* antidiabetic activity of the extract. The PAL extracts (50–200 μg/ml in DMSO) were mixed with 250 μl of α-amylase (2 U/ml in phosphate buffer solution, pH 6.9) and incubated at 37 °C for 20 min. Starch solution (1% in phosphate buffer solution, pH 6.9) was added to the mixture, followed by an additional incubation at 37 °C for 20 min. Subsequently, 500 μl of dinitrosalicylic acid (DNS) reagent (96 mM DNS and 5.31 M sodium potassium tartrate in 2 M NaOH) was added, and the mixture was incubated at 90 °C for 10 min. After cooling, the mixture was diluted with 5 ml of distilled water and measured the absorbance at 540 nm using a spectrophotometer. The α-amylase inhibition was estimated as follows:

$$\alpha - \text{amylase inhibition } (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (3)$$

where A_c and A_s are the absorbance of the control (DMSO) and the samples, respectively. The IC_{50} values were calculated, and acarbose was used as a positive control.

Statistical analysis

All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical significance ($p < .05$) was determined using Tukey's multiple-comparison test and *t*-test in Minitab 20 software (State College, PA, USA).

Multivariate analysis

Multivariate analyses, principal component analysis (PCA), and hierarchical cluster analysis (HCA) were conducted using Minitab 20 software to identify patterns of variation and clustering among treatments (water, 30% ethanol, cellulase, and pectinase). Square Euclidean distances were employed to calculate the distances between the extracts of *P. amboinicus* leaves. Principal components (PCs) were validated through full cross-validation with a 95% confidence level. Transformed values of variables, expressed as Z scores (mean of 0 and SD of 1), were used. For HCA, the complete linkage method was employed. The dendrogram similarity scales ranged from 0 (greater similarity) to 0.21 (lower similarity).

Results

Effect of extraction factors on TPC from *P. amboinicus* leaf extracts

Effect of enzyme concentration

The work examined the effect of pectinase and cellulase concentrations on the TPC of PAL extracts. Extractions were performed under the optimal pH and temperature conditions for each enzyme (Table 1), while the enzyme concentrations range from 1.0 to 2.5 U/ml. The extraction time (2 hr) and solid-to-liquid ratio (1:40 g/ml) were kept constant throughout the experiments. As can be seen from Figure 1A, varying concentrations of pectinase and cellulase significantly influenced the extraction of phenolic compounds from PAL. At a concentration of 1.0 U/ml, pectinase and cellulase increased the TPC by 32.17% and 39.43%, respectively, compared to the control sample (enzyme-free extraction). Increasing the enzyme concentration to 1.5 U/ml further enhanced the TPC, with increases of approximately 17.25% for pectinase and 23.15% for cellulase relative to the 1.0 U/ml treatments. However, a further increase in enzyme concentration (from 1.5 and 2.5 U/ml) led to a significant reduction in the TPC ($p < .05$). Therefore, the optimal concentration for both pectinase and cellulase was determined to be 1.5 U/ml.

Extraction time

The current study examined the effect of different extraction durations (1, 3, 5, and 7 hr) on the TPC from PAL under constant conditions (enzyme concentration of 1.5 U/ml and solid-to-solvent ratio of 1:40 g/ml). The results showed that the TPC of PAL extracts increased significantly, reaching 1.52-fold and 1.48-fold higher TPC for pectinase and cellulase, respectively, after 5 hr of incubation. Beyond 5 hr, the TPC slightly decreased, though the decrease was not statistically significant. Based on these findings, a 5-hr incubation time was determined to be the optimal duration for extracting phenolic compounds from PAL.

Solid-to-liquid ratio

To investigate the influence of solid-to-liquid ratio on the TPC of PAL extracts, the extraction was performed at different solid-to-liquid ratios (1:20, 1:60, 1:80, and 1:100 g/ml), while the enzyme concentration (1.5 U/ml) and extraction time (5 hr) were kept constant. The solid-to-liquid ratio significantly impacted the TPC of PAL extracts. Increasing the solid-to-liquid ratio from 1:40 to 1:60 enhanced the phenolic content by approximately 49.74% with pectinase and 48.29% with cellulase. However, a further increase in the solid-to-solvent ratio reduced the extraction efficiency. Specifically, increasing the ratio from 1:60 to 1:80 resulted in a decrease in TPC by approximately 17.24% and 21.37% for pectinase and cellulase treatments, respectively. Increasing the solid-to-liquid ratio to 1:100 further reduced the TPC by about 19.70% and 18.33% for pectinase and cellulase, respectively (Figure 1C). These findings indicated that a solid-to-liquid ratio of 1:60 g/ml was optimal for extracting phenolic compounds from PAL.

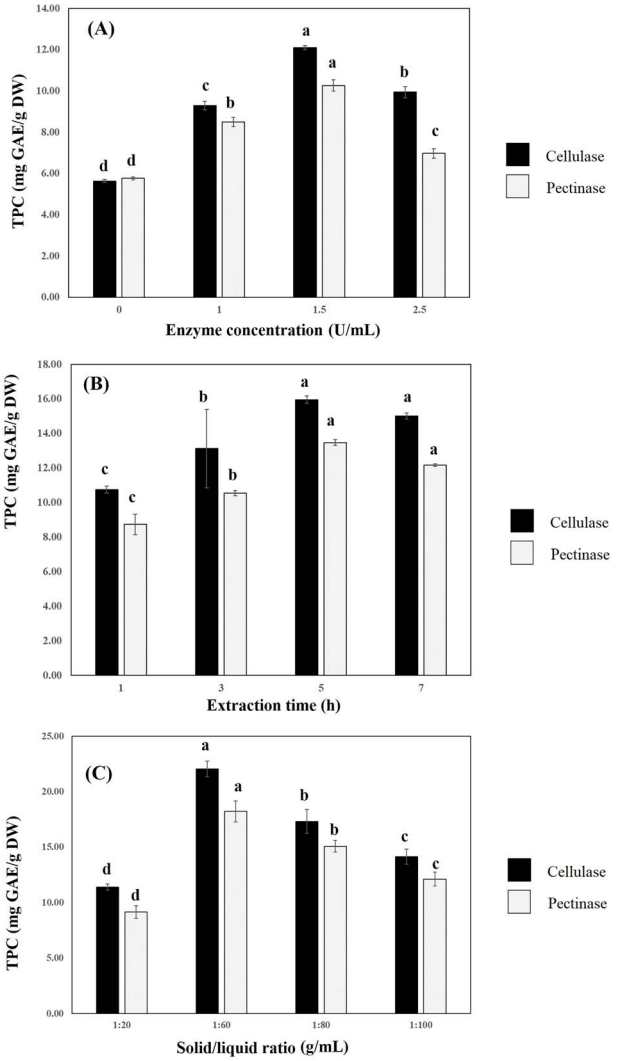

Figure 1. Total phenolic content of *Plectranthus amboinicus* leaf extracts obtained from different enzyme concentrations (A), extraction times (B), and solid-to-solvent ratios (C). Means within the same bar colour with different letters indicate significant differences by Tukey's test at $p < .05$.

Through the single-factor experiments evaluating enzyme concentration, extraction time, and solid-to-solvent ratio, the optimal extraction conditions were identified as: an enzyme concentration of 1.5 U/ml, an extraction time of 5 hr, and a solid-to-solvent of 1:60 mg/ml. These conditions were determined to provide the highest extraction efficiency for phenolic compounds in this study.

The t-test was also performed to analyse the statistically significant differences between cellulase and pectinase in the phenolic extraction under different conditions. As shown in Table 2 and Figure 1, the use of cellulase resulted in a significantly higher TPC than pectinase at any enzyme concentration, extraction time, and solid-to-liquid ratio ($p < .05$). These findings indicated that cellulase is more effective than pectinase in extracting phenolic compounds from PAL.

Table 2. *t*-Test analysis comparing cellulase and pectinase in phenolic extraction from *Plectranthus amboinicus* leaves under different conditions.

Enzyme concentration (U/ml)	*t*-value*	*p*-value	Extraction time (hr)	*t*-value*	*p*-value	Solid-to-liquid ratio (g/ml)	*t*-value*	*p*-value
0	−2.329	.080	1	5.557	.019	1:20	6.017	.011
1	4.594	.010	3	10.491	.006	1:60	5.582	.006
1.5	10.952	.003	5	15.879	.000	1:80	3.212	.053
2.5	14.102	.000	7	11.615	.002	1:100	3.815	.019

*t-values were obtained from a *t*-test analysis comparing the effect of different enzymes (cellulase and pectinase) at each concentration, extraction time, and solid-to-liquid ratio.

Table 3. Total phenolic content and gallic acid content of *Plectranthus amboinicus* leaf extracts using different extraction methods.

Treatment	TPC (mg GAE/g DW)	Gallic acid (μg/g DW)
Pectinase	19.32 ± 0.70^{b}	32.44 ± 0.57^{b}
Cellulase	22.79 ± 0.92^{a}	42.54 ± 0.45^{a}
30% Ethanol	16.50 ± 0.92^{c}	25.76 ± 0.80^{c}
Water	11.16 ± 0.95^{d}	18.72 ± 0.92^{d}

Note. Values are shown as mean ± SD ($n = 3$). Values with different superscript lowercase letters within a column indicate statistically significant differences ($p < .05$). TPC = Total phenolic content; GAE = Gallic acid equivalents; DW = Dry weight.

Effect of enzymatic treatment on the TPC and GA content of PAL extracts

The effects of different extraction methods—pectinase, cellulase, 30% EtOH, and water—on the TPC and GA content of PAL extracts were investigated (Table 3 and Figure 2). The TPC of PAL extracts from cellulase (CEE), pectinase (PEE), 30% EtOH (EOE), and water (WAE) treatments were 22.79, 19.32, 16.50, and 11.16 mg GAE/g DW, respectively. A similar trend was recorded for the GA content, with the highest value in CEE (42.54 μg/g DW), followed by PEE (32.44 μg/g DW), EOE (25.76 μg/g DW), and WAE (18.72 μg/g DW). Notably, the enzymatic treatments significantly enhanced both the TPC and GA content of PAL extracts compared to the enzyme-free methods (30% EtOH or water). This enhancement is attributed to the enzymatic degradation of plant cell wall polysaccharides, which facilitated the release of phenolic compounds into the solvent. Among the extraction methods, cellulase-assisted extraction resulted in the highest TPC and GA content ($p < .05$). Besides the presence of GA peak in the HPLC chromatograms, other peaks were also detected in the extract, which can be further isolated for identification as well as investigation of their properties.

Effect of enzymatic treatment on *in vitro* biological activities of PAL extracts

DPPH radical scavenging activity

The antioxidant potential of PAL extracts from different extraction methods was assessed using the DRSA assay (Tables 4 and 5). Enzymatic treatments with pectinase and cellulase significantly increased the DRSA of the extracts, with IC_{50} values of 65.52 and 57.81 μg/ml, respectively, compared to the enzyme-free extractions using 30% EtOH (IC_{50} value of 83.12 μg/ml) or water (> 100 μg/ml). Among the samples, cellulase-treated extracts exhibited the highest DPPH radical scavenging capacity.

In vitro inhibition of protein denaturation

The *in vitro* anti-inflammatory potential of PAL extracts was evaluated using the human albumin denaturation inhibition method. The inhibition of albumin denaturation increased gradually with the concentration of the extracts (Table 4). Enzymatic extracts showed significantly greater inhibition compared to non-enzymatic extracts across all tested concentrations (50–200 μg/ml). The IC_{50} values for pectinase, cellulase, 30% EtOH, and water-treated extracts were 144.53, 136.36, 155.36, and 172.31 μg/ml, respectively. However, the inhibition observed in pectinase- and cellulase-treated extracts was 43.51% and 40.13% lower, respectively, than the reference standard, aspirin (Table 5).

In vitro α-amylase inhibitory effect

The inhibition of digestive enzymes involved in polysaccharide breakdown (e.g., α-glucosidase and α-amylase) can help regulate postprandial blood glucose levels (Mechchate & Es-Safi, 2021). In the present study, the α-amylase inhibitory activity of PAL extracts was assessed to evaluate their antidiabetic potential (Tables 4 and 5). Significant differences in the α-amylase inhibition were observed among the treatment methods ($p < .05$). The cellulase-treated extract demonstrated the strongest inhibitory effect, with an IC_{50} value of 135.25 μg/ml, followed by pectinase (167.73 μg/ml), 30% EtOH (185.32 μg/ml), and water (198.67 μg/ml).

Hierarchical clustering and principal component analysis

Hierarchical clustering analysis and PCA were employed to explore the relationships between different treatments (pectinase, cellulase, 30% EtOH, and water) and their effects on biological activities (DPPH radical scavenging, albumin denaturation inhibition, and α-amylase inhibition) of the PAL extracts. The HCA dendrogram (Figure 3A) revealed three distinct clusters. Cluster 1 (pectinase and cellulase treatments) was clearly differentiated from 30% EtOH and water treatment. This clustering highlights the significant differences in biological activity based on the extraction method.

The PCA further confirmed the differences in the biological activities of PAL extracts (Figure 3B). The score plots of PC1 and PC2 revealed distinct patterns reflecting the effect of treatments on DRSA (at extract concentrations ranging from 25 to 100 μg/ml), as well as albumin denaturation and α-amylase inhibition (at extract concentrations ranging from 50 to 200 μg/ml). The first two principal components accounted for 96.2% of the total variation, with PC1 and PC2 explaining 94.1% and 2.1%, respectively. These components clearly highlighted the correlations between variables. PC1 was positively correlated with enzymatic treatments, including pectinase (4.504; 4.621,

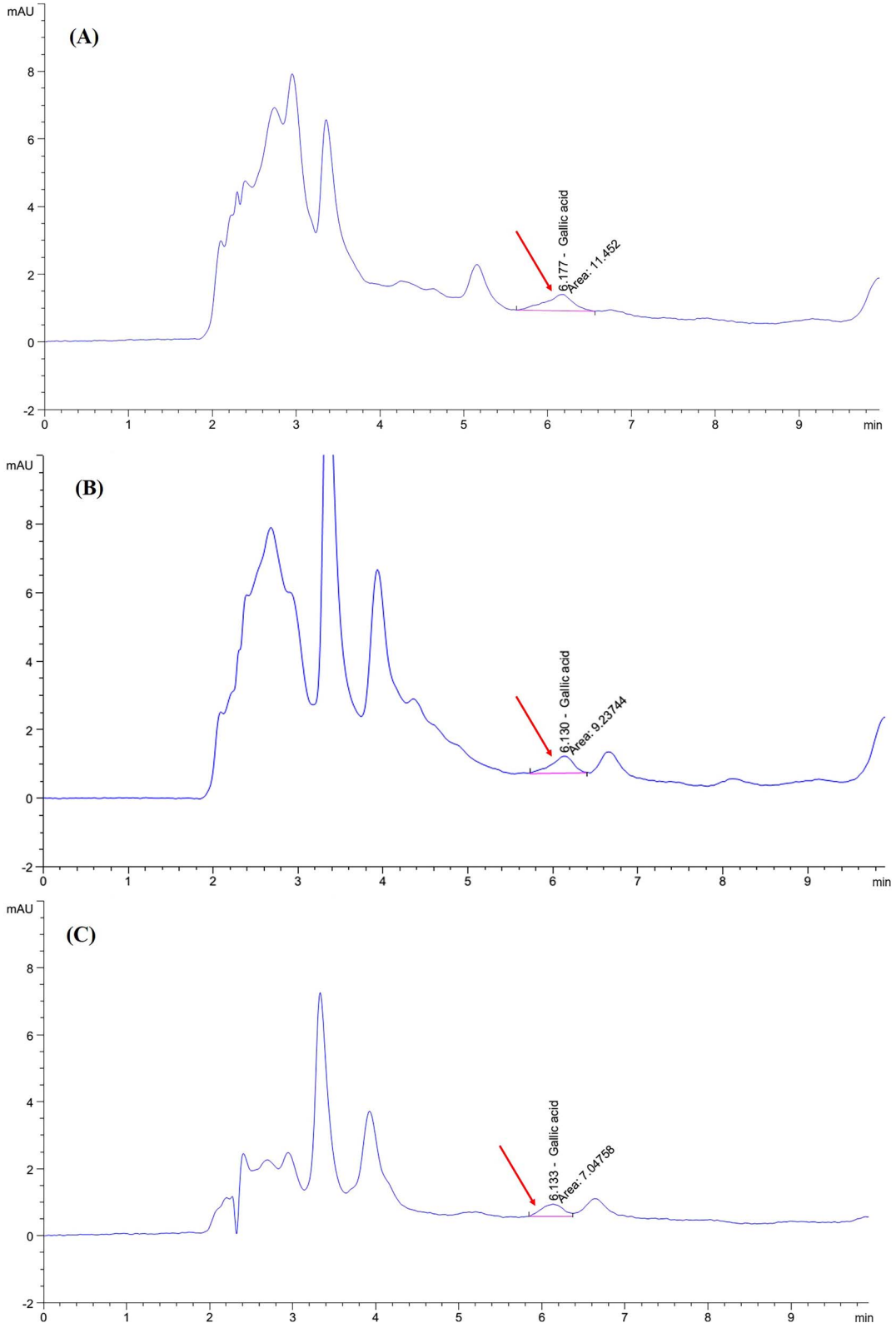

Figure 2. HPLC chromatogram of *Plectranthus amboinicus* leaf extracts obtained using cellulase (A), 30% ethanol (B), and water (C). Arrows indicate the gallic acid peak.

4.650) and cellulase (0.810; 1.026; 1.251), and negatively correlated with nonenzymatic treatments: water (−4.279; −4.183; −3.882) and 30% EtOH (−1.641; −1.436; −1.440). PC2 showed a positive correlation with water (0.384; 0.494; 0.497) and cellulase treatments (0.116; 0.633; 0.580) but a negative correlation with 30% EtOH (−0.420; −0.323; −0.146) and pectinase treatments (−0.649; −0.547; −0.619). Overall, the combined HCA and PCA analyses demonstrated that the biological activities of PAL extracts were primarily influenced by the extraction method and extract concentration.

Table 4. Percentage inhibition of DPPH radical, albumin denaturation, and α-amylase by PAL extracts obtained using different treatments.

Activities	Concentration (μg/ml)	Inhibition (%)						
		PEE	CEE	EOE	WAE	Vitamin C	Aspirin	Acarbose
DPPH radical scavenging	25	12.31 ± 0.37^{c}	17.68 ± 0.88^{b}	9.15 ± 0.93^{d}	5.12 ± 0.49^{e}	47.73 ± 1.17^{a}		
	50	33.15 ± 0.26^{c}	39.03 ± 0.59^{b}	26.48 ± 0.51^{d}	20.36 ± 1.69^{e}	60.80 ± 1.65^{a}		
	75	52.18 ± 0.79^{c}	55.32 ± 1.08^{b}	44.90 ± 0.67^{d}	33.38 ± 0.15^{e}	74.06 ± 1.05^{a}		
	100	65.33 ± 0.22^{c}	72.77 ± 0.93^{b}	52.28 ± 1.10^{d}	47.80 ± 1.47^{e}	88.57 ± 0.97^{a}		
Albumin inhibition	50	17.09 ± 0.73^{c}	21.15 ± 1.76^{b}	15.11 ± 0.84^{c}	11.15 ± 1.76^{d}		34.17 ± 1.14^{a}	
	100	29.92 ± 1.71^{c}	34.41 ± 0.32^{b}	28.65 ± 1.61^{c}	24.41 ± 0.32^{d}		48.92 ± 1.33^{a}	
	150	48.74 ± 1.26^{c}	56.27 ± 4.43^{b}	41.47 ± 1.17^{d}	39.60 ± 1.97^{d}		67.87 ± 1.56^{a}	
	200	63.25 ± 0.73^{c}	77.65 ± 1.21^{b}	60.83 ± 1.19^{d}	57.65 ± 1.21^{d}		83.73 ± 1.70^{a}	
α-Amylase inhibition	50	20.64 ± 0.30^{c}	24.37 ± 0.74^{b}	14.91 ± 0.28^{d}	13.11 ± 0.29^{e}			35.26 ± 0.28^{a}
	100	32.07 ± 0.13^{c}	40.33 ± 0.91^{b}	28.05 ± 1.08^{d}	22.67 ± 0.80^{e}			50.79 ± 1.08^{a}
	150	46.19 ± 0.38^{c}	55.27 ± 0.83^{b}	42.28 ± 0.73^{d}	36.89 ± 0.49^{e}			64.05 ± 0.73^{a}
	200	62.33 ± 0.33^{c}	69.43 ± 1.00^{b}	58.02 ± 1.60^{d}	51.65 ± 0.31^{e}			78.82 ± 1.60^{a}

Note. Data are shown as mean ± SD ($n = 3$). Means within a row with different letters indicate statistically significant differences at $p < .05$ by Tukey's test. PEE = pectinase-treated extract; CEE = cellulase-treated extract; EOE = ethanolic extract; WAE = water extract.

Table 5. IC_{50} values for *in vitro* antioxidant, anti-inflammatory, and anti-diabetic activities of *Plectranthus amboinicus* leaf extracts obtained from different treatments.

Treatment	IC_{50} values (μg/ml)		
	DPPH inhibition	Albumin inhibition	α-Amylase inhibition
Pectinase	65.52 ± 0.20^{b}	144.53 ± 3.20^{c}	167.73 ± 0.77^{c}
Cellulase	57.81 ± 0.37^{c}	136.36 ± 4.74^{d}	135.25 ± 1.34^{d}
30% Ethanol	83.12 ± 1.53^{a}	156.36 ± 4.40^{b}	185.32 ± 2.55^{b}
Water	>100	172.31 ± 4.31^{a}	198.67 ± 0.56^{a}
Vitamin C	4.20 ± 0.04^{d}		
Aspirin		91.64 ± 0.64^{e}	
Acarbose			80.60 ± 0.65^{e}

Note. Values are shown as mean ± SD ($n = 3$). Values with different superscript lowercase letters within a column indicate statistically significant differences ($p < .05$). IC_{50} = half-maximal inhibitory concentration.

Discussions

In recent years, cell wall–digesting enzymes such as cellulase, pectinase, protease, and amylase have been increasingly employed for extracting phytochemical compounds from herbal plants, providing an eco-friendly alternative to organic solvents (Ghandahari Yazdi et al., 2019; Granato et al., 2022; Nadar & Rathod, 2019). Studies have reported that enzymatic treatments significantly enhance extraction yield and polyphenol content (Ghandahari Yazdi et al., 2019; Namdar et al., 2019; Streimikyte et al., 2022). Additionally, variations in extraction techniques and conditions (e.g., extraction time, temperature, and solid-to-liquid ratio) are known to affect the composition and concentration of phenolics (Kapasakalidis et al., 2009; Nizar Ahamed et al., 2023; Streimikyte et al., 2022). This study aimed to recover phenolic compounds from *P. amboinicus* leaves using enzymatic treatments.

To investigate the effect of extraction factors on TPC, a series of experiments were performed under varying conditions, including enzyme concentrations (0, 1.0, 1.5, and 2.5 U/ml), extraction times (1, 3, 5, and 7 hr), and solid-to-liquid ratios (1:20, 1:60, 1:80, and 1:100 mg/ml). The results showed that these factors significantly affected extraction efficiency. The use of cellulase and pectinase significantly increased the TPC. However, high enzyme concentrations (2.5 U/ml) led to a reduction in the TPC. This finding aligns with previous studies (Fernández et al., 2015; Ghandahari Yazdi et al., 2019; Wang et al., 2021). Fernández et al. (2015) reported a 2.5-fold increase in the extraction yield of phenolic compounds from grape skin and seeds using pectinase under optimal extraction conditions compared to the control. However, excessive enzyme concentrations may have a negative effect due to potential enzyme degradation or interference with other components (Ghandahari Yazdi et al., 2019; Wang et al., 2021). Extraction time is another crucial factor in enzymatic extraction. Studies have shown that increasing extraction time when using cellulase and pectinase generally enhances phenolic content in plant leaf extracts, as the enzymes require sufficient time to break down plant cell walls and release phenolic compounds into the extraction solvent. However, excessively long extraction times can lead to the degradation of phenolic compounds (Ghandahari Yazdi et al., 2019; Zheng et al., 2009). The solid-to-liquid ratio is also a key parameter influencing the yield of phenolic compounds. Increasing the liquid-to-solid ratio in enzymatic treatment generally enhances phenolic content, as a higher ratio provides an optimal environment, including sufficient water and enzyme availability, and improves the mass transfer, facilitating more efficient cell wall breakdown and greater phenolic compound release (Ghandahari Yazdi et al., 2019; Guo et al., 2023). However, excessively high liquid-to-solid ratios can reduce extraction efficiency, which was similar to previous studies (Ghandahari Yazdi et al., 2019; Guo et al., 2023). Ghandahari Yazdi et al. (2019) reported that increasing the solid-to-solvent ratio from 1:20 to 1:80 significantly enhanced phenolic yield from pistachio green hulls by approximately 25% with pectinase, 63% with cellulase, and 97% with tannase. However, further increasing the ratio beyond 1:80 led to a decrease in extraction yield (Ghandahari Yazdi et al., 2019). In this study, the highest TPC was achieved with an enzyme concentration of 1.5 U/ml, an

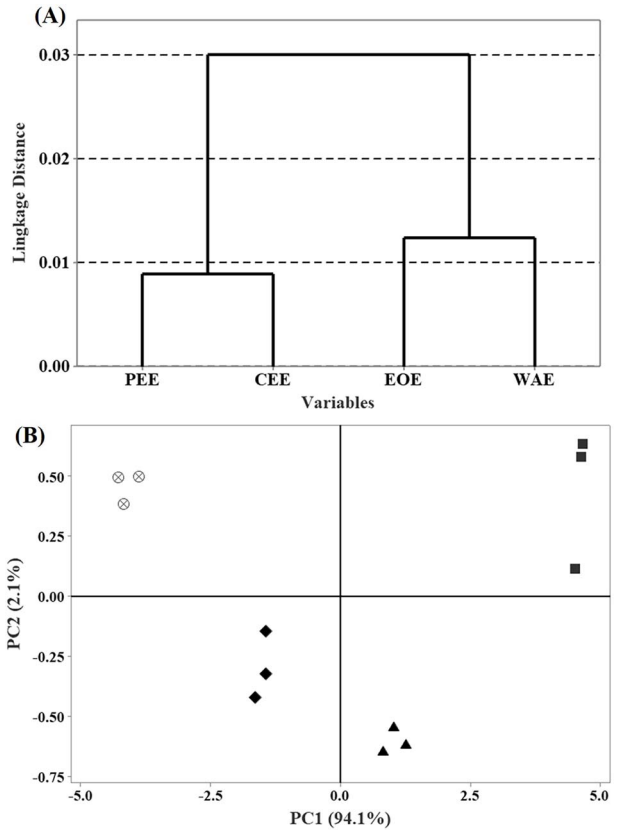

Figure 3. Dendrogram from (A) hierarchical cluster analysis and (B) principal component analysis (PCA) score plot for *Plectranthus amboinicus* leaf extracts obtained using different treatments. The analysis was based on the total phenolic content and percentage inhibitions of DPPH radical, albumin denaturation, and α-amylase.

extraction time of 5 hr, and a solid-to-liquid ratio of 1:60 mg/ml (Figure 1).

The efficiency of different extraction techniques—including enzymatic treatments (pectinase and cellulase), solvent extraction (30% ethanol), and water extraction—was compared based on TPC, GA content, and biological activities (DPPH radical scavenging, protein denaturation inhibition, and α-amylase inhibition). Among these methods, cellulase-assisted extraction yielded the highest TPC and GA content (Table 3), followed by pectinase treatment. Enzymatic extractions significantly outperformed the solvent- and water-based methods, demonstrating that the enzyme-assisted techniques effectively enhance the recovery of phenolic compounds from PAL. These findings align with studies by Belkheiri et al. (2021) and Wang et al. (2021), which reported increased phenolic yields with cellulase and pectinase treatments. Similarly, Zheng et al. (2009) observed a threefold increase in TPC in apple pomace treated with cellulase and pectinase. Ghandahari Yazdi et al. (2019) found that enzymatic hydrolysis altered the phenolic profile in plants, while Granato et al. (2022) and Machado et al. (2025) demonstrated cellulase's ability to enhance GA extraction from grape pomace. These effects are attributed to the hydrolysis of plant cell walls by enzymes, which release both free and bound phenolic compounds (Çinar, 2005; Ghandahari Yazdi et al., 2019; Granato et al., 2022).

Table 6 compares the TPC of PAL extracts obtained using different extraction methods. The enzymatic extraction employed in this study resulted in a significantly higher TPC compared to all other methods. These results suggest that cellulase-assisted extraction is a highly effective method for recovering phenolics from *P. amboinicus* leaves and represents an eco-friendly alternative to conventional solvent-based extraction techniques for herbal plants.

The enzymatic extracts showed significantly higher antioxidant, anti-inflammatory, and antidiabetic activities than enzyme-free extracts (30% EtOH and water). Notably, the cellulase-treated extract exhibited the strongest DRSA, followed by pectinase, 30% EtOH, and water extracts (Tables 4 and 5). These results are similar to the findings of Yin et al. (2023) study, which reported the highest DRSA in cellulase-assisted extracts of *Equisetum arvense* L. The biological activities of PAL extracts correlated with their TPC and GA content (Table 3), consistent with previous studies showing that phenolic compounds contribute to antioxidant properties (Leangnim et al., 2023; Zheng et al., 2009). Phenolic compounds, including GA, neutralise free radicals by donating electrons and activating antioxidant enzymes (El-shiekh et al., 2019; Mba & Zouheira, 2022).

Tissue protein denaturation is a marker of inflammation (Banerjee et al., 2014; Das et al., 2022; Derbel et al., 2023). Therefore, compounds that can inhibit protein denaturation can be considered promising candidates for anti-inflammatory drug development. Enzyme-treated extracts, particularly cellulase-treated ones, demonstrated higher albumin denaturation inhibition than enzyme-free extracts and were comparable to the standard aspirin (Tables 4 and 5). The inhibition of albumin denaturation is attributed to phenolic compounds, particularly GA. These findings correspond with previous studies reporting the anti-inflammatory properties of *Plectranthus* species (Chang et al., 2010; Chiu et al., 2012; Dutra da Silva et al., 2022). Similarly, Cortes-Ferre et al. (2022) and Liu et al. (2022) demonstrated that cellulase treatment enhanced the release of bioactive components, including GA, which contributes to the increased anti-inflammatory activity observed in herbal extracts.

Plant-based medicines are increasingly recognised as a cost-effective and efficient approach to managing type 2 diabetes by targeting key enzymes associated with the disorder (Paun et al., 2020; Tran et al., 2024). In this work, the α-amylase inhibitory activity of PAL extracts was highest in the cellulase-treated sample, followed by pectinase, 30% EtOH, and water extracts (Tables 4 and 5). This inhibitory effect on α-amylase, a key enzyme in starch digestion, suggests the potential of PAL extracts as natural antidiabetic agents. The results align with studies highlighting the antidiabetic properties of *Plectranthus* species (Etsassala et al., 2022; Kidane et al., 2018). Phenolic compounds play a critical role in diabetes management by regulating carbohydrate metabolism, stimulating insulin secretion, and influencing fat metabolism (Anhê et al., 2013; Bahadoran et al., 2013).

Conclusion

This study reported the enzymatic methods for extracting phenolic compounds from *P. amboinicus* leaves. A series of experiments were performed to optimise the extraction conditions for maximising the TPC of the extract. Under the optimal extraction conditions, cellulase treatment outperformed pectinase and conventional methods (30% EtOH and water) in terms of TPC, GA content, and the biological activities of the extract. The cellulase-treated extract showed significantly enhanced antioxidant (DPPH radical scavenging), anti-inflammatory (albumin denaturation inhibition), and antidiabetic (α-amylase inhibition) activities. These findings suggest that cellulase-assisted extraction is a promising, environmentally friendly, and efficient alternative

Table 6. TPC of *Plectranthus amboinicus* leaves from different extraction methods.

Extraction method	TPC (mg GAE/g)	Reference
Pectinase	19.32	Current study
Cellulase	22.79	Current study
Organic solvent	8.4	El-hawary et al. (2012)
Water bath ultrasound	3.70	Malpica-Acosta Sheila et al. (2024)
Probe ultrasound	4.40	Malpica-Acosta Sheila et al. (2024)
Heat-assisted extraction	7.20	Malpica-Acosta Sheila et al. (2024)

to conventional solvent-based methods for recovering phenolic compounds with enhanced biological activities for potential applications.

Supplementary material

Supplementary material is available at *International Journal of Food Science and Technology* online.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Hoai K. Tran (Data curation, Formal analysis, Investigation, Methodology, Writing—original draft [equal]), Thi T.H. Nguyen (Data curation, Investigation, Validation [equal]), Thanh T. Huynh (Formal analysis, Investigation [equal]), Ngoc M.T. Vo (Data curation, Investigation [equal]), Hoai O. Le (Data curation, Validation, Visualization [equal]), Dieu-Hien Truong (Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing—original draft [equal]), Hoang C. Nguyen (Conceptualization, Methodology, Project administration, Resources, Supervision, Visualization, Writing—review & editing [equal]), and Colin J. Barrow (Conceptualization, Project administration, Resources, Supervision, Writing—review & editing [equal]).

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Conflicts of interest

The authors have declared no conflicts of interest for this article.

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