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Enhancement of dieckol extraction yield from *Ecklonia cava* through optimization of major variables in generally recognized as safe solvent-based process

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Ecklonia cava (EC), an edible brown macroalga abundant in intertidal areas of East Asia (Korea, Japan, and China), contains high-value bioactive compounds such as dieckol, which has antifungal, anti-inflammatory, antitumor, and antihyperlipidemic activities. However, no studies have been reported on the utilization of EC as a biorefinery feedstock, and the design of a more economical and high-yield process is required for the utilization of dieckol for the human healthcare industry. In this study, we designed a bioprocess for the high-yield recovery of dieckol from EC in a generally recognized as safe (GRAS) solvent to facilitate its application in the food and healthcare industries. Preliminary studies identified ethanol as an efficient solvent with the highest dieckol extraction yield (2.9 mg/g biomass). In order to maximize the recovery of dieckol from EC, the major extraction variables (solvent concentration, reaction temperature, and reaction time) were optimized based on statistical methods. Based on the predictive model, the numerical optimization determined that the solution with the highest dieckol content per weight of extract (62.6 vol% ethanol concentration, 54.2°C temperature, 13.2 min) was the optimal extraction condition. Under the determined conditions, the dieckol yield from EC achieved 6.4 mg dieckol/g EC (95.5% agreement with the predicted value). The designed process offers several advantages, including improving the utilization feasibility of EC, utilizing GRAS solvents with potential human applications, short extraction time (13.2 min), maximized process yield, and the highest dieckol recovery compared to previous reports.

KEYWORDS

***Ecklonia cava*, dieckol, macroalgal biorefinery, green process, mass balance, optimization**

1 Introduction

Biorefinery, defined as the production of biomaterials from biomass, is a vital concept for a circular bioeconomy (Yoo & Kim, 2021; Paliwal et al., 2022). Recently, the strategy of including marine resources as feedstock for biorefineries has gained attention to overcome the limited availability and low economic viability of lignocellulosic biomass due to limited agricultural land (Paliwal and Jutur, 2021; Vo et al., 2022; Yarkent and Oncel, 2022). In particular, marine biomass has a relatively high potential as a bioresource because it contains bioactive compounds that can be applied to related industries such as food, feed, cosmetics, and pharmaceuticals. For instance, polysaccharides from *Sargassum dentifolium*, a brown algal strain, can promote growth performance of fish (Abdelrhman et al., 2022), and *Gammarus pulex*, a freshwater amphipod, can be used as a partial replacement for fish meal due to its high protein content (Abo-Taleb et al., 2020). The extracts from *Sinularia maxim*, a soft coral, have antibacterial, antifungal, and anticancer activities, revealing potential pharmaceutical uses (Metwally et al., 2020).

In general, the economic feasibility of biorefineries can be improved by recovery of high-activity functional substances from biomass and application of high content carbohydrates. According to integrated or multi-step biorefineries, an extraction process is generally chosen as a first step because valuable components (e.g., carbohydrates) still remain in the residual biomass after the extraction, which can be further valorized through additional processes such as hydrothermal liquefaction and pyrolysis (Guo et al., 2019; Kim et al., 2021; López-Linares et al., 2021; de Almeida-Couto et al., 2022). Among various marine resources, macroalgae sequester carbon dioxide as primary producers, have high value as a sustainable resource due to their fast growth, and contain high content of carbohydrates and bioactive substances, thus are suitable for the described biorefinery feedstock (Choi, 2019; Lim et al., 2019; Woo et al., 2022).

Ecklonia cava (EC), an edible brown macroalga, is abundant in the subtidal areas of South Korea, Japan, and China (Lin et al., 2021). Globally, the economic values of *Ecklonia* (Laminariales, Phaeophyta) was reported to be about 920 kg ($28,000) per hectare per year (Eger et al., 2023). EC is used as feed for aquaculture operations (e.g., abalone) and as a raw material in the food, additive, and cosmetic industries (Choi et al., 2020). However, rejected materials generated during the harvesting and processing stages significantly impact environmental problems. In particular, most of the unmarketable seaweeds generated in the aquaculture stage are directly dumped into the sea (Kang and Kim, 2019). For more sustainable use of EC biomass, the identification and evaluation of functional materials in EC extractives have been actively studied in the research field of bioprocessing for EC (Kang et al., 2013; Hwang et al., 2021).

As one of the substances isolated from EC, dieckol is gaining attention as a high-value material with pharmacological effects (Abraham et al., 2021). Besides EC, dieckol has also been isolated from brown algae such as *Eisenia bicyclis* and *Ecklonia stolonifera*, but EC is the major feedstock for dieckol (Rajan et al., 2021). The nature of dieckol is as follows: chemical formula, $C_{36}H_{22}O_{18}$; molecular weight, 742.52 g/mol; boiling point, 999.6°C; appearance, white to faint yellow; and stability, 2 years (Rajan et al., 2021; Jo et al., 2022).

Dieckol exhibits several bioactivities, including anti-fungal, anti-inflammatory, anti-tumorigenic, anti-hyperlipidemic, anti-platelet, anti-aging, and radioprotective activities, as reported by various studies (Lee et al., 2010a; Yoon et al., 2008; Jang et al., 2015; Lee et al., 2019; Abraham et al., 2021). Evaluating bioactivities of dieckol recovered from EC has been actively conducted, but research on process design and optimization for efficient recovery of dieckol from biomass is limited. Therefore, we hypothesized that designing and optimizing the dieckol extraction process would be a viable option as a first step for the valorization of EC. As mentioned earlier, the activities of dieckol enable its application in the food, cosmetic, and pharmaceutical industries, so the use of generally recognized as safe (GRAS)-grade solvents is recommended. Numerous studies have prepared microalgae (*Spirulina platensis*) and macroalgae (*Chondrus crispus*, *Laminaria ochroleuca*, and *Ulva* sp.) extracts using GRAS solvents such as ethanol, acetone, and ethyl acetate, considering potential human body applications (Herrero et al., 2005; Amaro et al., 2022).

The main objective of this study was to develop an eco-friendly process for efficient recovery of dieckol from EC and optimize the process variables using statistical methodology. The effects of various GRAS solvents, including distilled water (DW), ethanol, acetone, and ethyl acetate, on the extraction yield of dieckol from EC were investigated to determine the best solvent with the highest extraction efficiency. In addition, the process variables for solid-liquid extraction were optimized to maximize the dieckol yield from EC. The optimization process involved experimental design, modeling, and optimization. Finally, the potential of EC as a feedstock for dieckol production was evaluated. This study was the first attempt to maximize the yield of a GRAS solvent-based dieckol extraction to achieve economic feasibility and human safety.

2 Materials and methods

2.1 Materials

EC (Figure 1) was used as biomass in this study. EC powders were purchased from Myoungmunjungyakcho (Seoul, Republic of Korea). The obtained EC powders were freeze-dried using a freeze dryer (TFD8501, ilShinBioBase Co. Ltd., Dongducheon, Republic of Korea). Acetone (99.8% purity), ethanol (99.9% purity), and trifluoroacetic acid (TFA) were purchased from Daejung Chemicals & Metals Co., LTD. (Siheung, Republic of Korea). Ethyl acetate (99.8% purity) and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (99.9% purity) was purchased from J.T. Baker (New Jersey, USA). Dieckol standard was purchased from Aktin Chemical, Inc. (Chengdu, P.R. China). All chemicals and reagents used in this study were of analytical grade.

2.2 Procedure for EC extraction

A 1 g portion of freeze-dried EC was soaked in 10 mL of each of the different solvents (acetone, DW, ethanol, and ethyl acetate or

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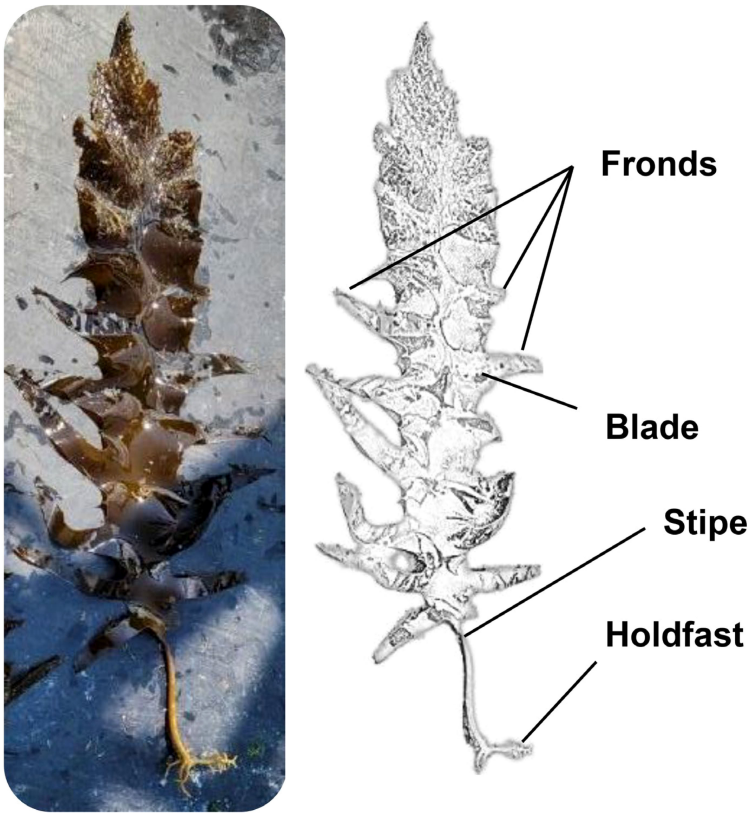

FIGURE 1
The photograph and the structure of Korean *Ecklonia cava*.

ethanol-DW mixture) in a 50 mL plastic tube. To evaluate the extraction capacity of the selected pure GRAS solvents (acetone, DW, ethanol, and ethyl acetate), the reactions were performed at 30°C for 3 hr in a water bath (C-WBD3, Changshin Science, Seoul, Republic of Korea). In a preliminary study investigating the effects of ethanol concentration (mixed with DW) and extraction time on dieckol yield, reactions were carried out at 30°C for either 1 or 3 hours in the water bath. In the optimization study, the extraction reaction followed the designed conditions. Subsequently, each EC extract was centrifuged for 10 min at 12,000 × g, and the supernatant was used for dieckol detection via high-performance liquid chromatography (HPLC) analysis. All experiments were performed in triplicate, and data were expressed as mean ± standard deviation.

2.3 Experimental design and statistical optimization

Design-Expert software (version 7, Stat-Ease, Inc., USA) was used for experimental design, empirical model development, and numerical optimization in order to optimize extraction conditions for efficient dieckol extraction from EC (Rizqullah et al., 2022). Here, the experimental design was based on the central composite rotatable design (CCRD) of response surface methodology (RSM). A 5-level-3-factor CCRD was chosen to optimize extraction conditions for dieckol recovery from EC (Table 1). The levels of several variables (i.e., major extraction parameters) were set based on the results of the preliminary study mentioned above. Then, twenty extraction conditions were created by the CCRD, and the EC

TABLE 1 A central composite rotatable design (CCRD) with three variables affecting dieckol recovery from *Ecklonia cava*.

Variable	Unit	Symbol	Coded level				
			−1.682	−1	0	1	1.682
Ethanol concentration	vol%	X_1	7.95518	25	50	75	92.0448
Extraction temperature	°C	X_2	23.1821	30	40	50	56.8179
Extraction time	min	X_3	9.54622	30	60	90	110.454

was extracted according to each of them. The dieckol yield (mg/g biomass) was set as the response value (i.e., dependent variable), and finally, an empirical model for predicting the dieckol yield was developed. A model equation was generated according to the following Eq. (1):

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

where Y is the response value (i.e., dieckol yield [mg/g biomass]), β_0 is the constant coefficient, β_i, β_{ii}, and β_{ij} are the linear coefficients, quadratic coefficients, and the interaction coefficients, respectively, and X_i and X_j are the coded values of the variables (Kim et al., 2022a).

Analysis of variance (ANOVA) was carried out on the developed model to investigate statistical significance (Esfe et al., 2022). A reduced model was then created by eliminating the model terms that were not statistically significant (Mamelkina et al., 2019; Jin et al., 2022). Finally, numerical optimization was performed using the reduced model to derive the optimal extraction conditions for maximizing Y.

2.4 HPLC analysis

The dieckol concentration in the supernatant prepared according to the extraction procedure was determined using HPLC analysis (Agilent, 1260 Infinity II, Santa Clara, CA, USA). The samples were diluted 5 times and then filtered through a syringe filter (DISMIC-13HP, Advantec, Tokyo, Japan). The analysis was performed using an INNO column C18 (5 µm, 4.6 mm × 250 mm); a variable wavelength detector at 230 nm; and a mobile phase of 0.1vol% TFA in water (A) and 0.1vol% TFA in acetonitrile (B) with a flow rate of 1.0 mL/min. The gradient elution for the analysis was as follows: start, 90% A and 10% B; 40 min, 60% A and 40% B; 55 min, 90% A and 10% B. The sample injection volume was 5 µL. A standard curve (R^2 = 0.9999) was prepared by analyzing various concentrations of dieckol solution and used for dieckol quantification. Based on the results of HPLC analysis, dieckol yield from biomass was calculated according to the following Eq. (2):

$$\text{Dieckol yield (mg/g biomass)} = \text{dieckol concentration (mg/mL)} \times \text{solvent volume (mL)} / \text{biomass weight (g)}, \quad (2)$$

where solvent volume (mL) and biomass weight (g) were 10 mL and 1 g dry wt (of EC), respectively.

2.5 FTIR analysis

In order to investigate the chemical properties of the EC extracts and dieckol (standard material), Fourier-transform infrared (FTIR) analysis was performed using FTIR spectroscopy (JASCO FTIR-4600). The spectra were monitored in the range of 4000–650 cm^{-1}.

3 Results and discussion

3.1 Extraction capacity of the selected GRAS solvents for dieckol recovery from EC

The effects of selected GRAS solvents on dieckol yield from EC were investigated in order to identify the most efficient solvent for dieckol recovery. Acetone, DW, ethanol, and ethyl acetate were tested in the experiment, and the dieckol yields were analyzed, as shown in Figure 2A. The dieckol yield was highest in the following order of solvents: ethanol (2.9 mg/g biomass), acetone (1.1 mg/g biomass), DW (0.7 mg/g biomass), and ethyl acetate (0.3 mg/g biomass). Among the selected pure GRAS solvents, ethanol demonstrated the highest extraction capacity for dieckol, and it is the most favored solvent in bioindustries due to its low toxicity (Fathordoobady et al., 2016; Ma et al., 2019). Although methanol exhibited a dieckol yield of 3.6 mg/g biomass (data not shown), it is considered a toxic solvent, leading us to choose ethanol for further process design and optimization. The dieckol yield in the ethanol extraction process can be improved by (1) preparing ethanol-based solvents with enhanced extraction capacity for dieckol, and (2) optimizing process variables. Aqueous organic solvents are generally used as efficient solvents for recovering polar phenolic compounds (Kim et al., 2022b). According to previous reports, binary solvents are more efficient in recovering phenolic compounds than mono-solvent, and water-solvent mixtures create a more polar medium, which can improve the extraction of phenolic compounds (Chaves et al., 2020; Sonar and Rathod, 2020). Therefore, we further investigated the effect of ethanol concentration on dieckol yield from EC and subsequently optimized extraction variables.

3.2 A preliminary study for experimental design

Figure 2B depicts the effect of ethanol concentration on dieckol recovery from EC. The dieckol yield (mg/g biomass) in extraction processes using various concentrations of ethanol were as follows (for 1 hr and 3 hr extraction, respectively): 0 vol% (0.6 and 0.7), 25 vol% (4.1 and 4.6), 50 vol% (6.0 and 6.1), 75 vol% (5.7 and 6.0), and 100 vol% (2.2 and 2.7). From 100 vol% to 50 vol% ethanol concentration, the dieckol yield gradually increased as more water was added to ethanol. This finding supports the notion that the extraction capacity of the ethanol solvent was enhanced by the addition of water, in line with earlier reports (Kim et al., 2022b; Brahmi et al., 2022). According to this preliminary study, ethanol concentration significantly affects the dieckol yield from EC, prompting us to designate "ethanol concentration" as one of the variables in the optimization study (Table 1). Preliminary investigations of dieckol stability under severe conditions (70°C, 99% ethanol) showed that about 99% of the initial dieckol was detected at 2 hr, indicating that it was stable. In contrast, about 50% of the initial

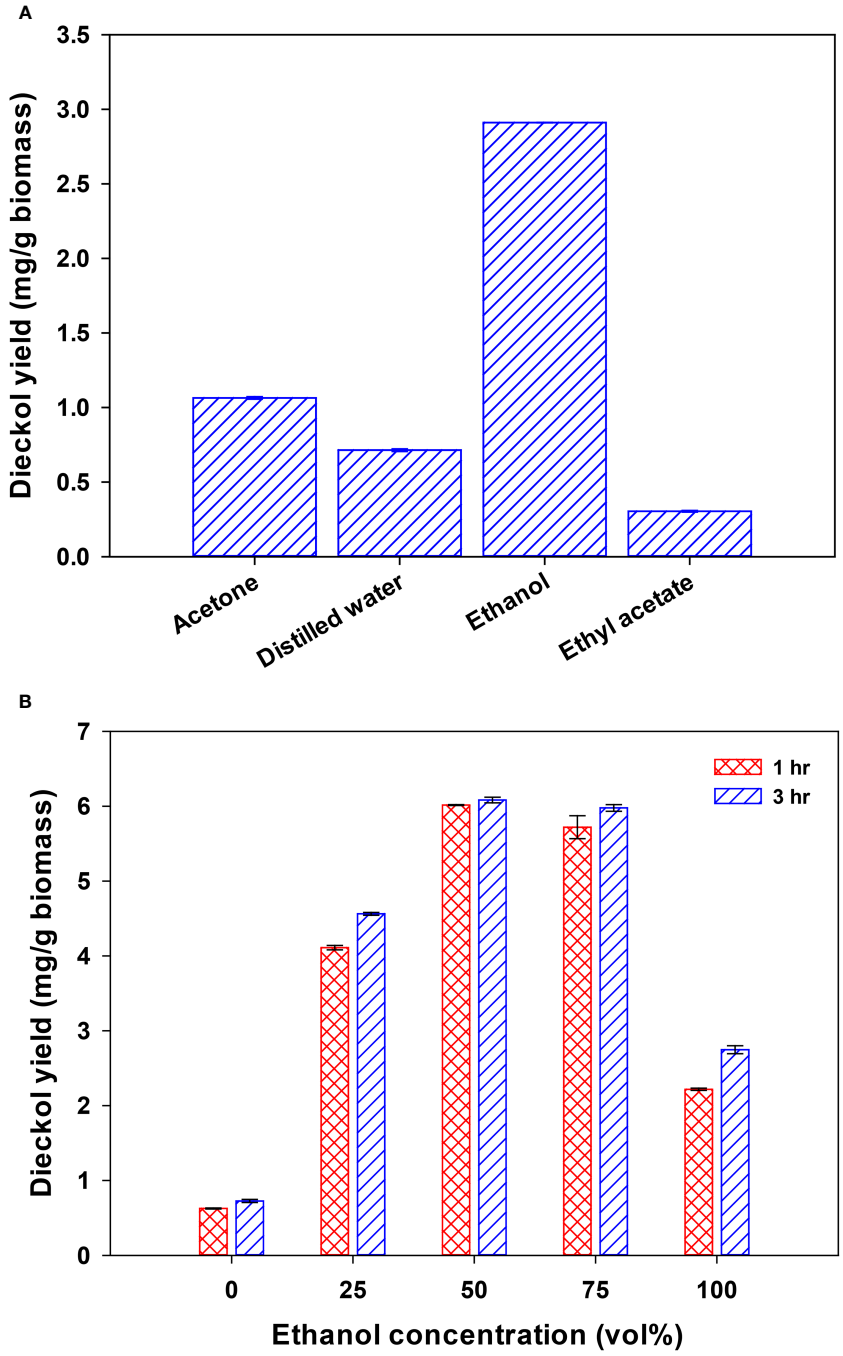

FIGURE 2
Dieckol extraction capacity in pure GRAS solvents (acetone, distilled water, ethanol, and ethyl acetate) **(A)** and ethanol-distilled water mixtures **(B)** from *Ecklonia cava*.

dieckol was detected at 24 hr, suggesting that close to half of the component was degraded (data not shown). Therefore, extraction time is considered an important parameter in the dieckol extraction process. The parameter of extraction time was designed within a stable range based on this finding. On the other hand, extraction times exceeding 1 hr did not result in a substantial increase in dieckol yield (Figure 2B). In the extraction using 50 vol% ethanol, which demonstrated the highest dieckol extraction capacity, the increase was a mere 0.1 mg/g biomass. Consequently, we planned to investigate the effect of extraction time by setting level 0 of the CCRD as 1 hr.

3.3 Development of empirical model for predicting dieckol yield

In order to develop the empirical model for predicting dieckol yield from EC, experiments were performed according to the

designed extraction conditions. The designed conditions and corresponding experimental results are listed in Table 2. Regression analysis based on the experimental data resulted in model Eq. (3) in terms of actual factors:

$$Y = 8.3830 + 0.1094\,X_1 - 0.2163\,X_2 - 0.0455\,X_3 + 0.0003\,X_1X_2 + 0.0003\,X_1X_3 + 0.0001\,X_2X_3 - 0.0013\,X_{1^2} + 0.0026\,X_{2^2} + 0.0002\,X_{3^2}, \quad (3)$$

where Y is the predicted dieckol yield (mg/g biomass), X_1, X_2, and X_3 are ethanol concentration (vol%), extraction temperature (°C), and extraction time (min), respectively. The Y values predicted by Eq. (3) are listed in Table 2.

The terms with a positive sign imply the synergistic effect that increases dieckol yield, while the terms with a negative sign imply a hostile effect (Nayak & Vyas, 2019). Table 3 presents the ANOVA result for regression validation of Eq. (3). The model F-value of 33.12 implies that the model is statistically significant, with only a 0.01% chance that a model F-value this large could occur due to noise. In general, a low p-value (< 0.05) of a model term means that the corresponding term has a statistically significant effect (Lee et al., 2022a). Therefore, it was confirmed that the following model terms all have significant effects on dieckol yield from EC: the linear term of ethanol concentration (X_1) and extraction time (X_3), the quadratic term of ethanol concentration (X_1^2), extraction temperature (X_2^2), and extraction time (X_3^2), and the interaction term of ethanol concentration-extraction time (X_1X_3). On the other hand, the interaction term of ethanol concentration-extraction temperature (X_1X_2) and extraction temperature-extraction time (X_2X_3) had no significant effect on the dieckol yield (Table 3).

The empirical model can be improved by excluding insignificant model terms from the initial quadratic model Eq. (3) (Mamelkina et al., 2019; Jin et al., 2022). Thus, the insignificant model terms (X_1X_2 and X_2X_3) were removed from the model, while the linear term X_2 was not removed because its significant quadratic term (X_2^2) should be included in the final model (Yu and Kim, 2018). The final empirical model was finally developed by model reduction using backward elimination, as shown in Eq. (4) in terms of actual factors:

$$Y = 7.5098 + 0.1200X_1 - 0.1944X_2 - 0.0398X_3 + 0.0003X_1X_3 - 0.0013\,X_{1^2} + 0.0026\,X_{2^2} + 0.0002\,X_{3^2}, \quad (4)$$

TABLE 2 Extraction conditions according to the CCRD and corresponding response values (dieckol yield).

No.	Point type	Coded level			*Y*: dieckol yield (mg/g dry wt. biomass)		
		X_1	X_2	X_3	Experimental [a]	Predicted by model Eq. (3)	Predicted by model Eq. (4)
1	Factorial	−1	−1	−1	5.63 ± 0.12	5.47	5.36
2	Factorial	1	−1	−1	5.34 ± 0.14	5.31	5.33
3	Factorial	−1	1	−1	5.61 ± 0.23	5.49	5.60
4	Factorial	1	1	−1	5.70 ± 0.07	5.59	5.57
5	Factorial	−1	−1	1	4.82 ± 0.23	4.68	4.66
6	Factorial	1	−1	1	5.43 ± 0.11	5.32	5.43
7	Factorial	−1	1	1	5.08 ± 0.13	4.88	4.90
8	Factorial	1	1	1	5.85 ± 0.40	5.77	5.66
9	Axial	−1.682	0	0	2.86 ± 0.19	3.12	3.12
10	Axial	1.682	0	0	3.64 ± 0.18	3.73	3.73
11	Axial	0	−1.682	0	6.08 ± 0.24	6.23	6.23
12	Axial	0	1.682	0	6.43 ± 0.19	6.63	6.63
13	Axial	0	0	−1.682	6.27 ± 0.24	6.41	6.41
14	Axial	0	0	1.682	5.70 ± 0.27	5.90	5.90
15	Center	0	0	0	5.66 ± 0.26	5.70	5.70
16	Center	0	0	0	5.93 ± 0.46	5.70	5.70
17	Center	0	0	0	5.80 ± 0.14	5.70	5.70
18	Center	0	0	0	5.50 ± 0.06	5.70	5.70
19	Center	0	0	0	5.65 ± 0.03	5.70	5.70
20	Center	0	0	0	5.69 ± 0.58	5.70	5.70

[a]The experiments were carried out in randomized order. The data were represented as mean ± standard deviation (n = 3).

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TABLE 3 Analysis of variance (ANOVA) results for the initial quadratic model.

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value Prob > F	Remarks
Model	12.94	9	1.44	33.12	< 0.0001	Significant
X_1	0.46	1	0.46	10.53	0.0088	
X_2	0.19	1	0.19	4.45	0.0611	
X_3	0.31	1	0.31	7.14	0.0234	
X_1X_2	0.04	1	0.04	0.81	0.3902	
X_1X_3	0.32	1	0.32	7.29	0.0223	
X_2X_3	0.01	1	0.01	0.34	0.5723	
X_1^2	9.30	1	9.30	214.32	< 0.0001	
X_2^2	0.96	1	0.96	22.09	0.0008	
X_3^2	0.37	1	0.37	8.61	0.0149	
Residual	0.43	10	0.04			
Lack of fit	0.33	5	0.07	3.02	0.1252	Not significant
Pure error	0.11	5	0.02			
Cor total	13.37	19				

Coefficient of determination (R^2): 0.97; Adjusted R^2: 0.94; Predicted R^2: 0.80; Coefficient of variation (CV): 3.83%; Adequate precision (AP): 23.82.

where Y is the predicted dieckol yield (mg/g biomass), X_1, X_2, and X_3 are ethanol concentration (vol%), extraction temperature (°C), and extraction time (min), respectively. The Y values predicted by Eq. (4) are listed in Table 2.

Table 4 shows the ANOVA result for regression validation of Eq. (4). The ANOVA result proved that the R^2, adjusted R^2, and predicted R^2 of the model are 0.96, 0.94, and 0.85, respectively. In general, an R^2 close to 1 indicates a good degree of correlation between predicted and experimental values (Lee et al., 2023a). Because the R^2 always increases by adding variables, in the statistical areas, the adjusted R^2 is generally used to judge the accuracy of a model (Lee et al., 2022b). Additionally, the difference between the adjusted R^2 and the predicted R^2, which implies the accuracy of the model in predicting the response value (Y) for the new trials, should be less than 0.2 (Haji and Rahimi, 2020; Li et al., 2022). The predicted R^2 of the final model Eq. (4) is in reasonable agreement with the adjusted R^2 (with a difference value of 0.09). Compared to the difference value (0.14) between adjusted R^2 and predicted R^2 in the initial model Eq. (3), the value of the final model was decreased, resulting in improved model accuracy, which

TABLE 4 Analysis of variance (ANOVA) results for the reduced quadratic model.

Source	Sum of square	Degree of freedom	Mean square	F-value	p-value Prob > F	Remarks
Model	12.89	7	1.84	45.66	< 0.0001	Significant
X_1	0.46	1	0.46	11.33	0.0056	
X_2	0.19	1	0.19	4.79	0.0491	
X_3	0.31	1	0.31	7.68	0.0169	
X_1X_3	0.32	1	0.32	7.84	0.0160	
X_1^2	9.30	1	9.30	230.71	< 0.0001	
X_2^2	0.96	1	0.96	23.78	0.0004	
X_3^2	0.37	1	0.37	9.27	0.0102	
Residual	0.48	12	0.04			
Lack of fit	0.38	7	0.05	2.49	0.1666	Not significant
Pure error	0.11	5	0.02			
Cor total	13.37	19				

Coefficient of determination (R^2): 0.96; Adjusted R^2: 0.94; Predicted R^2: 0.85; Coefficient of variation (CV): 3.69%; Adequate precision (AP): 27.64.

may be a result of model reduction. The model F-value was also improved from 33.12 to 45.66, with a p-value of< 0.0001 (Table 2). In the reduced model, all linear terms and quadratic terms were found to be statistically significant. A lack of fit F-value should be insignificant to make the model fit well (Rajewski and Dobrzyńska-Inger, 2021), and in the final model Eq. (4), the lack of fit F-value of 2.49 (p-value = 0.17) indicates that the lack of fit is insignificant relative to the pure error. As shown in Figure 3A, the predicted dieckol yields match the experimental values well. Further, the adequate precision (AP) and coefficient of variation (CV) of the final model were 27.64 (> 4) and 3.69% (< 10%), respectively. Relatively high AP implies that the model is suitable for exploring the designed space, and relatively low CV indicates the accuracy and reliability of the predicted response value (Kim et al., 2022b). Overall, the ANOVA results demonstrated that the final model is more improved than the initial model, and thus, the final model is suitable for predicting dieckol yield from EC.

Figures 3B, C display the effect of extraction parameters on dieckol yield. As discussed previously, the extraction temperature (X_2) was not included in interaction terms, while ethanol concentration (X_1) and extraction time (X_3) were included in interaction terms as well as linear terms. One factor plot for extraction temperature was plotted at a fixed solvent concentration (50 vol%) and extraction time (60 min). Extraction temperatures of 30°C, 40°C, and 50°C resulted in dieckol yields of 5.8, 5.7, and 6.1 mg/g biomass, respectively, which were minor differences (Figure 3B). This finding is presumably due to the low F-value. A higher model term F-value indicates a greater effect on the response value (Balasundram et al., 2023). In this context, although extraction temperature (X_2) was significant in the final model Eq. (4), its F-value of 4.79 (p-value = 0.0491) was relatively lower than other variables (Table 4). In the case of the interaction effect of ethanol concentration and extraction time (X_1X_3), it was plotted at a fixed extraction temperature of 40°C. Figure 3C clearly shows that there is an interaction effect of those two variables. Decreasing the extraction time from 90 min (red line in Figure 3C) to 30 min (black line in Figure 3C) did not always increase the yield of dieckol. If there is no interaction effect of ethanol concentration and extraction

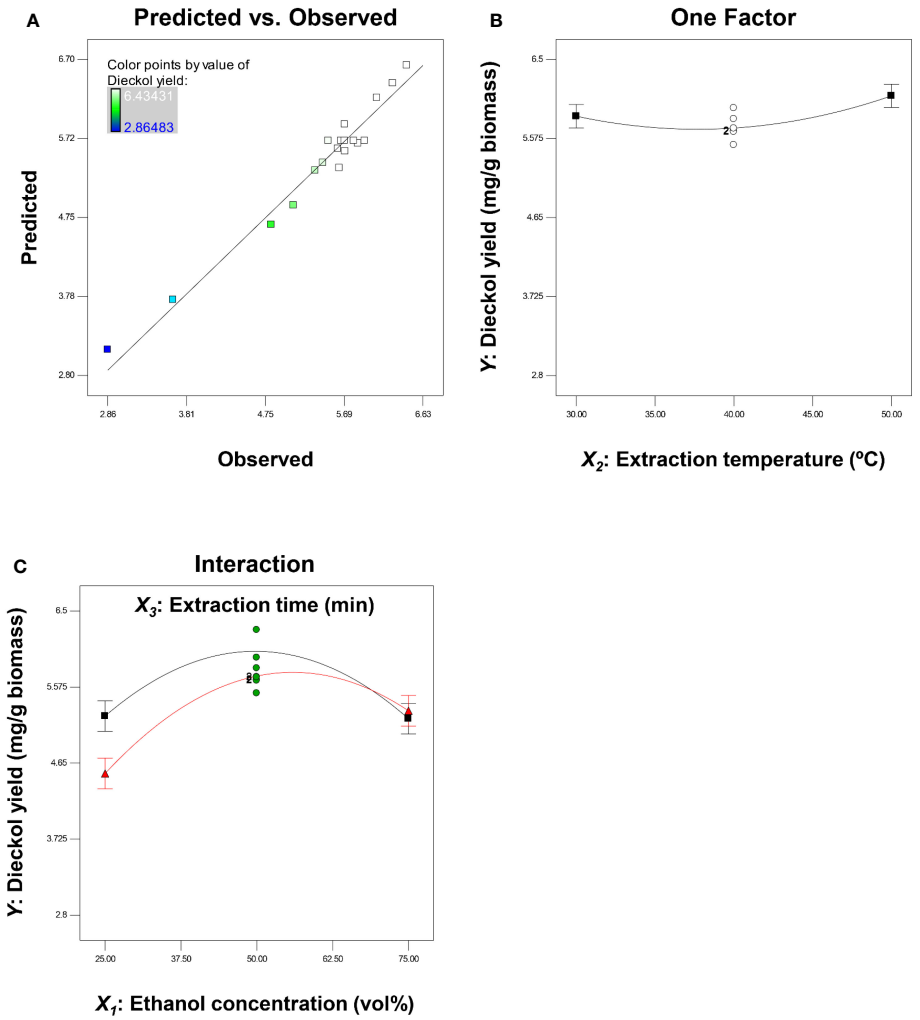

FIGURE 3
Plots representing the predicted dieckol yield versus observed values **(A)**, one factor effect of extraction temperature on dieckol yield **(B)**, and interaction effect of ethanol concentration and extraction time on dieckol yield **(C)**.

time, the graph must plot the tendency irrespective of any ethanol concentration. Therefore, Eq. (4), which includes this interaction as a significant term, is promising as a tool for optimizing dieckol extraction conditions.

3.4 Optimization of dieckol extraction parameters using the final model

Numerical optimization was carried out using the final model Eq. (4) to determine the optimal extraction conditions for maximizing dieckol yield from EC. The goals were set as ethanol concentration, "in range"; extraction temperature, "in range"; extraction time, "in range"; and dieckol yield, "maximize". As a result, RSM solutions for obtaining the maximized dieckol yield (mg/g biomass) were derived, and several solutions with the desirability of 1.0 and the highest dieckol yield were sorted in Table 5. Under the optimal suggested conditions, the dieckol yield was predicted as 6.7–7.0 mg/g biomass. For the model validation, extraction processes were performed according to each solution, and then the dieckol yield in each process was calculated. As a result, the maximum error was 15.25% and the minimum error was only 3.40%. The average error was about 7.0%, which can successfully predict the dieckol yield in the EC extraction process.

The experimental results show that RSM solutions 1, 2, 4, and 5 give similar dieckol yields (Table 5). In order to finally select the best one, we further investigated the dieckol content in the EC extract obtained under each condition. The high dieckol content at the extract level is expected to provide ease of separation and purification. Figure 4 shows the dieckol content in the EC extract obtained following each solution. The contents (mg dieckol/g freeze-dried extract from EC) were as follows: 28.0, 24.8, 24.2, 27.4, and 29.4 mg/g under the solution 1, 2, 3, 4, and 5, respectively. Solution 5 is considered to be a condition that can effectively recover dieckol from EC in a shorter time (13.2 min) than other solutions. Taken together, solution 5 was finally determined as the best extraction condition for efficient dieckol recovery from EC.

TABLE 5 RSM solutions to obtain EC extracts with maximized dieckol yield.

Solution[a]	X_1 (vol%)	X_2 (°C)	X_3 (min)	Predicted Y (mg/g biomass)	Experimental Y (mg/g biomass)	Actual error (%)
1	41.7	56.2	20.9	7.0	6.77 ± 0.01	3.40
2	46.3	55.2	23.2	6.9	6.37 ± 0.07	8.32
3	41.9	23.3	13.6	6.8	5.90 ± 0.04	15.25
4	57.8	56.8	100.7	6.8	6.57 ± 1.20	3.50
5	62.6	54.2	13.2	6.7	6.41 ± 0.09	4.52

[a]Solutions with desirability of 1.0 were sorted in order of highest dieckol yield.

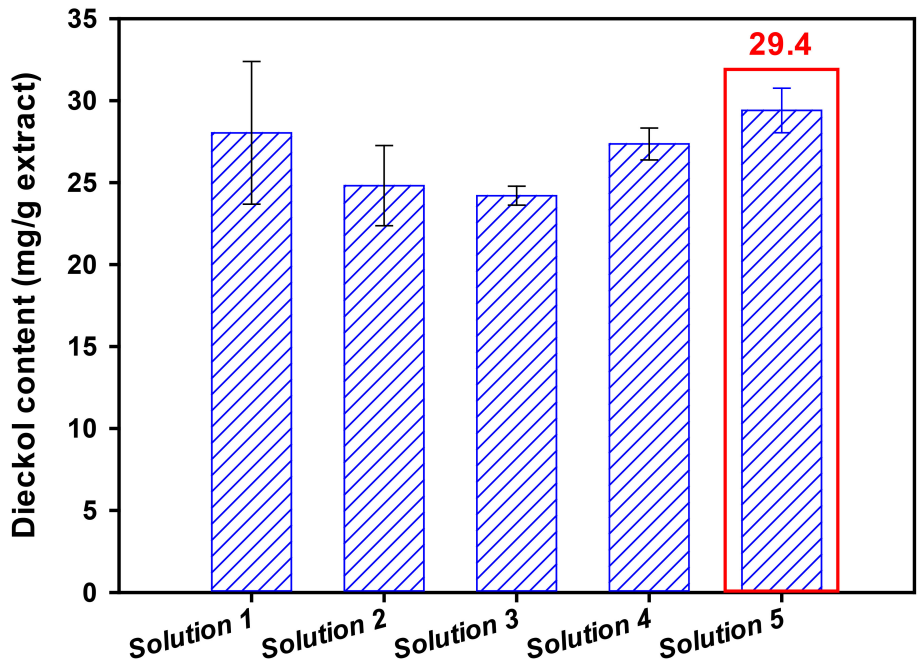

FIGURE 4
Dieckol content in EC extract prepared under the suggested RSM solutions.

3.5 Characterization of EC extract

Figure 5 shows that the HPLC chromatogram of the EC extract from solution 5. The peak of dieckol was detected at a retention time of 32.4 min, which is the same as that of standard material, implying dieckol-rich EC extract.

Figure 6 shows the FTIR spectra of the EC extract and dieckol standard. The broad peak at 3200 cm^{-1} was observed in both samples, especially strong in the standard compound. This peak is assigned to the phenolic hydroxyl band (Xing et al., 2020). Previous reports on FTIR studies of phlorotannins also observed this peak (Xiaojun et al., 1996; Yeo et al., 2012). Other significant peaks at 1607, 1482, and 1270 cm^{-1} were observed, which are assigned to the aromatic ring structure of phlorotannin including dieckol (Yeo et al., 2012). Overall, the FTIR pattern of the EC extract is similar to that of the dieckol standard and shows signature peaks, implying that the produced extract is dieckol-rich.

3.6 Mass balance

The potential of feedstocks can be estimated based on the mass balance. Figure 7 shows the mass balance in the extraction processes for recovering dieckol from 1 g of dried EC. The GRAS solvents recovered 0.3–2.9 mg dieckol, in which ethanol showed the best extraction capacity. It was found that dieckol extraction capacity can be enhanced by using an ethanol-DW mixture, and drastically improved dieckol yield (6.0 mg/g EC) was observed in the extraction using 50 vol% ethanol. Dieckol yield from EC could be further improved by optimizing process variables such as ethanol concentration, extraction temperature, and extraction time. The statistical model for dieckol yield suggested several solutions to maximize process yield, and finally, 6.4 mg of dieckol was recovered from 1 g EC. Previous studies have focused on the bioactivities of dieckol, and the results have scientifically proven the potential value of dieckol (Zhang et al., 2011; Lee et al., 2010a; Lee et al., 2008; Yoon et al., 2013; Yayeh et al., 2014; Lee et al., 2010b; Yoon et al., 2008; Kim et al., 2012; Jung et al., 2014). In previous studies, dieckol extracts were generally prepared for function evaluation but not extracted under the optimized processes. In earlier reports, dieckol extraction processes have been performed using methanol under room temperature for 3 hr–10 days, resulting the dieckol yields of up to 0.6 mg/g biomass. Therefore, the present study is significant in that it improved the extraction yield for the economic feasibility of the dieckol recovery process. EC is expected to be a sustainable feedstock for the production of dieckol for human use.

4 Conclusion

The current study designed and optimized a green process for dieckol recovery from EC as part of sustainable biorefinery strategies. Existing research has focused on the evaluation of bioactivities of EC extracts, but not maximizing dieckol extraction yield. Ethanol was efficient for dieckol extraction, with ethanol concentration demonstrating a significant impact on dieckol yield. Under severe conditions, dieckol was degraded by about 50% after 24 hr, although it was found to be stable up to 2 hr. Therefore, the RSM design to optimize the dieckol extraction parameters was set to less than 2 hr. RSM was successfully employed in the process optimization, leading to the determination of optimal conditions: ethanol concentration at 62.6%, extraction temperature at 54.2°C, and extraction time at 13.2 min. Under the optimal conditions, the dieckol yield was found to be 6.4 mg/g EC, which was the highest

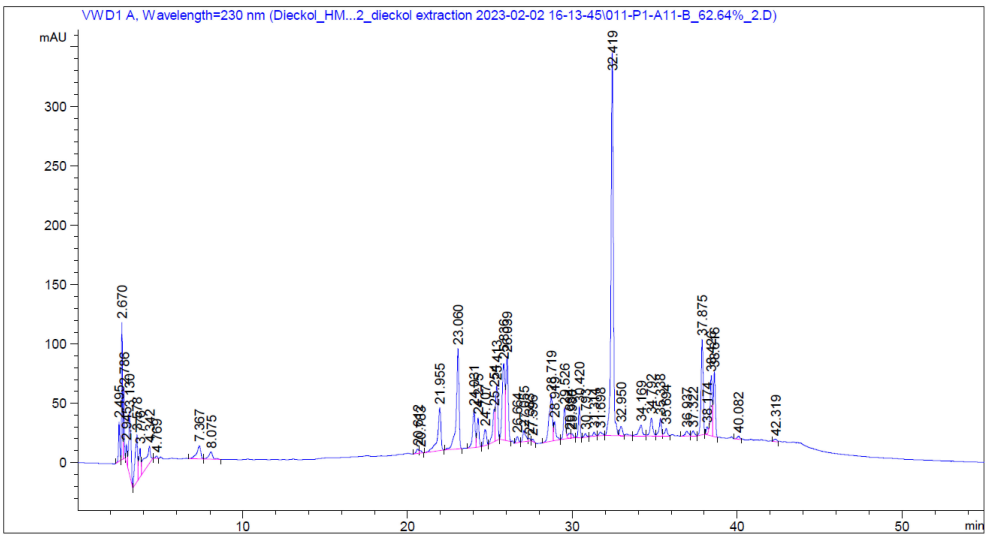

FIGURE 5
HPLC chromatogram of *Ecklonia cava* extract obtained in optimized conditions (62.6% ethanol solvent, 54.2°C temperature, and 13.2 min extraction time). Dieckol was detected at 32.4 min.

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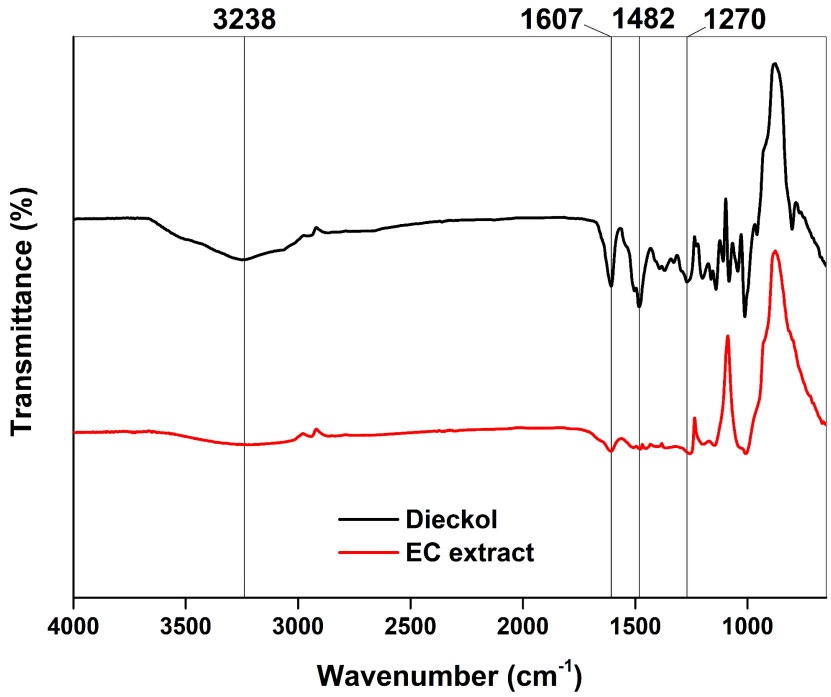

FIGURE 6
FTIR spectra of dieckol standard material and *Ecklonia cava* (EC) extract.

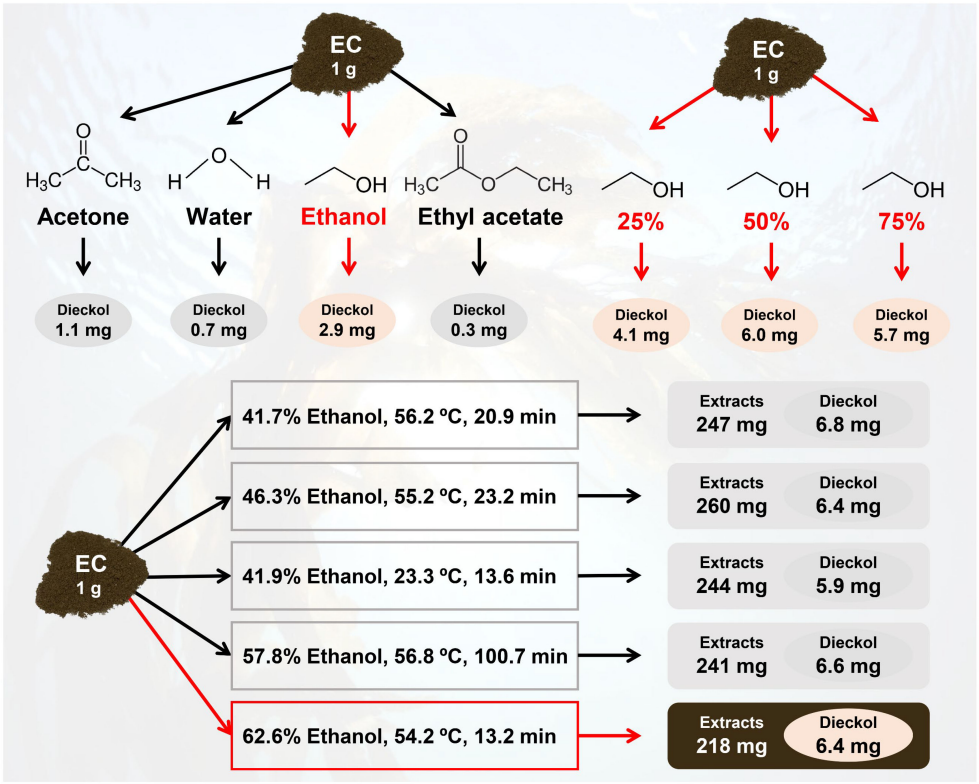

FIGURE 7
Mass balance for dieckol recovery from 1 g of dried *Ecklonia cava* (EC).

yield compared to previous reports. This study offers an economical dieckol production process from EC. In the near future, we aim to further valorize the residual biomass after EC extraction to contribute to a circular bioeconomy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

HS: Conceptualization, Formal Analysis, Methodology, Writing – original draft. JL: Conceptualization, Formal Analysis, Methodology, Writing – original draft. JB: Investigation, Methodology, Visualization, Writing – original draft. KL: Investigation, Methodology, Validation, Writing – original draft. HY: Conceptualization, Project administration, Validation, Writing – review & editing. CP: Conceptualization, Funding acquisition, Project administration, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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