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HPLC/DAD Analysis and Antioxidant Activity of Adlay Sprouts and Seeds

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Abstract: Adlay is an annual plant known for its abundant bioactive compounds and diverse pharmacological activities. Coixol, a key component found in various parts of adlay, significantly contributes to its biological activity. This study was conducted to extract adlay sprouts and seeds using different solvents (methanol and ethanol) and extraction methods (reflux and ultrasonic extraction). The extracts were then evaluated for their total polyphenol and flavonoid contents, as well as antioxidant ability (DPPH and $ABTS^{+}$). Additionally, the coixol content of these extracts was analyzed using HPLC/DAD analysis. The results showed that the extraction methods and solvents used impacted the bioactive compounds and their activities in the samples. Adlay sprouts exhibited a higher compound content and stronger antioxidant capacity than adlay seeds. Moreover, a substantial amount of coixol was found in the sprouts, while it was not detected in the seeds. This study emphasizes the importance of selecting appropriate extraction methods to optimize the biological activities of adlay sprouts and seeds. Adlay sprouts, with their enriched phytochemical compounds and enhanced antioxidant ability, could serve as a valuable material for health product applications.

Keywords: adlay; coixol; seeds; sprouts; polyphenols; flavonoid; antioxidant; HPLC analysis

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1. Introduction

Oxidation is a crucial process in various domains such as food, chemicals, and living systems. However, a notable consequence of this process is the generation of free radicals, specifically reactive oxygen species (ROS) [1]. Additionally, significant amount of ROS are produced within the human body as a result of natural physiological processes, interactions with the external environment, and dietary practices, posing potential risks such as damage to proteins, lipids, and nucleic acids [2]. These contributions from ROS extend to phenomena like food spoilage, the deterioration of chemical materials, and the development of over a hundred human disorders [3,4]. Nevertheless, the introduction of antioxidant substances can mitigate the oxidation process. Even at low concentrations, these compounds can substantially delay or completely prevent the oxidation of easily susceptible substrates [5]. Recently, the isolation, characterization, and widespread application of natural compounds possessing antioxidant properties have been demonstrated in various medical contexts [6]. Numerous methods are employed to assess the effectiveness of natural antioxidants, including assays such as the ferric reducing antioxidant power assay [7], the β-carotene/linoleic acid assay [8,9], the Rancimat method [10], the inhibition of low-density lipoprotein oxidation [11], and the 2,2-diphenyl-1-picrylhydrazyl (DPPH)

assay [12]. This array of methods is necessary due to the complex nature of the analyzed substrates, often presenting as intricate mixtures with numerous compounds exhibiting diverse functional groups, polarities, and chemical behaviors [13].

Phenolic compounds, prevalent in plants, constitute a diverse group of chemical compounds distinguished by the presence of one or more phenol rings in their molecular structure. These compounds, known for their aromatic nature, exhibit varied chemical properties. The term "phenolic compounds" functions as a comprehensive label for a diverse array of molecules, each possessing unique structures and functional groups [14–16]. Renowned for their antioxidative attributes, phenolic compounds showcase a range of biological activities that offer potential advantages for human health [17]. Their ability to safeguard cells and tissues from oxidative stress, neutralizing harmful free radicals that can harm DNA, proteins, and lipids, underscores their significance. Additionally, the consumption of dietary sources rich in phenolic compounds has been linked to numerous health benefits, including reduced susceptibility to chronic ailments such as cardiovascular diseases, malignancies, and neurodegenerative conditions. Furthermore, these compounds may exhibit anti-inflammatory and antimicrobial effects [18,19].

Within the complex biochemical milieu of adlay, coixol, a phenolic compound, assumes significance as a by-product of 2,4-dihydroxy-7-methoxy-2–11-24-benzoxin-3-(4H)-one. This key glucoside, inherent in growing monocotyledonous plants like adlay, plays a crucial role in providing protection against pathogens and herbivores [20–22]. It has been reported to demonstrate antibacterial and antifungal activities, and the ability to prevent convulsions [23–25]. Additionally, there is suggestive evidence indicating that coixol may regulate gene expression and the production and secretion of mucin by directly acting on airway epithelial cells [26].

Adlay or Job's tears, otherwise commonly known as *Coix lacryma-jobi* L. var. *ma-yuen* Stapf., stands as a prominent annual plant within the grass family (Poaceae) and is widely cultivated in East and Southeast Asia [27,28]. Beyond its agricultural significance, adlay has made notable inroads into various consumable forms, including beverages, snacks, and traditional medicines [29]. The seeds derived from the adlay plant are commonly used as a cereal grain and have a mild, nutty flavor, making them highly versatile for various culinary applications, similar to other grains like rice or barley. The cultural importance of adlay in Asian gastronomy is particularly evident in regions such as China, Japan, and Korea, where it is embraced for its medicinal properties [30,31].

The seeds of adlay contain a wide range of health-beneficial bioactive components, such as protein, polysaccharides, polyphenols, coixenolide, and oil. Traditionally, these elements have been harnessed in oriental medicine for centuries, with adlay being employed to treat conditions such as edema, rheumatism, and neuralgia [32,33]. Scientific investigations have expanded our understanding of the therapeutic potential inherent in adlay seeds, showcasing their ability to prevent tumor formation, reduce inflammation, ameliorate metabolic syndrome, and regulate the gastrointestinal tract [34–36]. The accumulating body of evidence also underscores adlay's antioxidant and anti-inflammatory properties, its anticancer potential, antimicrobial activity, immunomodulatory effects, and cardiometabolic benefits [37–40]. In response to its perceived nutritional and health advantages, adlay seeds are progressively gaining recognition within the food industry.

Adlay sprouts, the young and tender shoots emerging from germinated adlay seeds, result from the sprouting process, wherein seeds are soaked and allowed to germinate [41]. This process enhances the digestibility of seeds and grains by activating enzymes that break down complex compounds into simpler forms. As a result, adlay sprouts emerge as nutrient-rich entities, boasting a composition replete with vitamins, minerals, and antioxidants [42]. The inclusion of flavonoids and phenolic compounds further augments their potential for promoting health and well-being [43,44].

The present study embarked on a comprehensive exploration, extracting methanol (MeOH) and ethanol (EtOH) extracts from both adlay sprouts and seeds, employing diverse extraction methods such as reflux extraction and ultrasonic extraction. Subsequent analyses

included assessments of their total polyphenol and total flavonoid contents, coupled with evaluations of their antioxidant capabilities using the $ABTS^+$ and DPPH methods. Notably, the amount of coixol in these extracts was meticulously quantified using high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD). This meticulous approach aspires to unravel the intricate nutritional and bioactive profile of adlay, shedding light on its multifaceted potential applications in promoting human health.

2. Materials and Methods

2.1. Plant Materials

The adlay sprouts utilized in this experiment were cultivated under conditions of 27 °C temperature and 98% humidity, with irrigation applied three times and illumination provided by a metal halide lamp of 100 μmol/m^2/s. Sprouts were harvested on the 9th day after germination following pre-hydration, as detailed in a previous experiment [15]. Adlay sprouts exhibited the initial signs of germination as light green shoots on the second day after sowing. By the third day, these sprouts had developed into a shape with an aboveground part measuring approximately 2.75 cm in length. The length of the aboveground part expanded from 15 cm to 20 cm over the course of 7 to 9 days after sowing (Figure 1).

Figure 1. Adlay sprouts.

2.2. Chemicals and Apparatus

The extraction solvents were MeOH and EtOH (Pyeongtaek, Republic of Korea). HPLC was performed using an Agilent 1260 Infinity II Quat Pump (Santa Clara, CA, USA), and DAD WR detector (Santa Clara, CA, USA) with INNO C18 column (25 cm × 4.6 mm, 5 μm). HPLC-grade solvents were MeOH, water, trifluoroacetic acid (TFA), and acetonitrile (ACN) of J. T. Baker (Radnor, PA, USA). Coixol (Figure 2) was provided by the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 1 December 2023), Anseong, Republic of Korea.

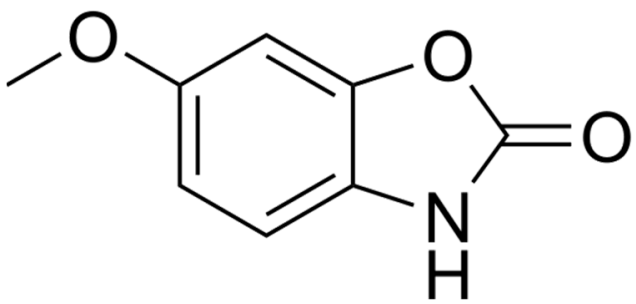


Figure 2. Chemical structure of coixol.

2.3. Sample Extraction

The adlay sprouts (SP) and seeds (S) underwent a meticulous process, in which they were finely crushed and extracted with MeOH and EtOH using two extraction methods: reflux extraction and ultrasonic extraction. Samples were extracted for 3 h at 80 °C and 3 h at 50 °C using a reflux extractor and ultrasonic extractor, respectively. The names of the samples were assigned based on the extraction method and solvent used, and these are presented in Table 1. The extraction yields were computed and are provided in Table 2.

Table 1. Sample information and extraction details.

Sample	Plant Part	Solvent	Extraction Method
SPM1	Sprouts	MeOH	Ultrasonic extraction
SPE1		EtOH	
SPM2		MeOH	Reflux extraction
SPE2		EtOH	
SM1	Seeds	MeOH	Ultrasonic extraction
SE1		EtOH	
SM2		MeOH	Reflux extraction
SE2		EtOH	

Note: The abbreviated names are derived from the plant part, solvent, and extraction method used; SP: sprout; S: seed; M: methanol; E: ethanol; 1: ultrasonic; 2: reflux. Hence, SPM1 stands for sprout extracted with methanol using ultrasonic extraction. The rest of the samples follow the same naming pattern.

Table 2. Extraction yield.

Sample	Dry Sample (g)	Extract (g)	Yield (%)
SPM1	2.0	0.5	25.0
SPE1	2.0	0.4	20.0
SPM2	4.0	1.1	27.5
SPE2	4.0	1.0	25.0
SM1	2.5	0.2	8.0
SE1	2.5	0.3	12.0
SM2	5.0	0.3	6.0
SE2	5.0	0.3	6.0

The meanings of the abbreviated sample names are the same ones as shown in Table 1.

2.4. Total Polyphenol Content

The total polyphenol content in the samples was determined by adding 40 µL of 2 N Folin–Ciocalteu reagent (Sigma-Aldrich, St. Lewis, MO, USA) to 60 µL of each sample. Subsequently, 100 µL of 7.5% Na_2CO_3 was added, and the mixture was allowed to react at room temperature in the dark for 30 min [45]. The absorbance was measured at 760 nm using a microplate reader (Epoch; BioTek, Winooski, VT, USA). The total polyphenol content was computed based on a standard curve constructed using different concentrations of the standard compound—tannic acid (TA).

2.5. Total Flavonoid Content

The total flavonoid content in the samples was determined by adding 100 µL of 2% $AlCl_36H_2O$ to 100 µL of the extract; followed by incubation for 10 min [45]. The absorbance was read at 430 nm using a microplate reader. The total flavonoid content was calculated based on a standard curve constructed using different concentrations of the standard compound—quercetin (QE).

2.6. $ABTS^+$ Radical Scavenging Assays

$ABTS^+$ and potassium persulfate were dissolved in distilled water (pH 7.4) to the concentration of 7.4 and 2.6 mM, respectively. The solutions were then stored in a dark place at 4 °C for 24 h. Then, 10 µL of each sample was combined with 200 µL of an $ABTS^+$

stock solution, prepared by diluting it with distilled water to achieve an absorbance of 1.00 ± 0.2 at 734 nm. Following a 30 min incubation in darkness, the concentration of the residual radicals was measured at 734 nm using a microplate reader [45,46]. To assess the $ABTS^+$ radical scavenging activity, the following formula was used:

$$[ABTS^+ \text{ radical scavenging activity } (\%) = (\text{Blank O.D} - \text{Sample O.D})/\text{Blank O.D} \times 100]$$

As a positive control, ascorbic acid at a concentration ranging from 0.0–0.2 mg/mL was employed. The results were expressed as the sample concentration required for 50% inhibition (IC_{50}) of the $ABTS^+$ radicals.

2.7. DPPH Radical Scavenging Assays

DPPH (200 µL, 0.2 mM; Sigma-Aldrich, St. Lewis, MO, USA) was dissolved in MeOH, and 10 µL of each extract was added to this solution. Following a 30 min incubation in darkness, the absorbance of the remaining radicals was measured at 514 nm using a microplate reader [45,46]. To assess the DPPH radical scavenging activity, the following formula was employed:

$$[\text{DPPH radical scavenging activity } (\%) = (\text{Blank O.D} - \text{Sample O.D})/\text{Blank O.D} \times 100]$$

As a positive control, ascorbic acid was employed. The results were reported as the IC_{50} values.

2.8. Preparation of Samples and Standard Solutions for HPLC Analysis

Adlay sprout and seed extracts (6 mg) and coixol (0.5 mg) were dissolved in MeOH (1 mL). Subsequently, the solutions underwent 15 min of sonication, and were filtered using 0.45 µm PVDF membrane filter (Cat No. 6779, Piscataway, NJ, USA).

2.9. HPLC Conditions

Adlay samples were quantitatively analyzed using a reversed-phase HPLC system equipped with an INNO C18 column (25 cm × 4.6 mm, 5 µm). The column's temperature was set to 30 °C. The mobile phase consisted of 0.1% TFA in water (A) and ACN (B). Elution was performed using a gradient system. The gradient elution conditions were 95% A from 0 to 5 min, 80% A at 15 min, 70% A at 25 min, 55% A at 30 min, and 0% A from 40 to 45 min. The sample injection volume was 10 µL, the mobile phase flow rate was 1.0 mL/min, and the detector wavelength was set to 290 nm.

2.10. Calibration Curve

The coixol standard solutions were serially diluted to six concentrations (15.625–500 µg/mL) for the construction of the calibration curve. The linearity of the calibration curve was assessed by determining the correlation coefficient (R-value). The content of coixol was then computed using the equation of the calibration curve. In the calibration correction function, the concentration (µg/mL) was plotted on the *X*-axis, the peak area on the *Y*-axis, and the value to be substituted as the mean value (n = 3) ± standard deviation.

2.11. Statistical Analysis

All statistical analyses were performed using the software Minitab 16.0. Significant differences between the results were calculated by using anova analysis (ANOVA) and multiple comparisons of the Tukey test, with a significance level of $p < 0.05$.

3. Results

3.1. Total Polyphenol and Total Flavonoid Contents

The detailed examination of the results presented in Table 3 revealed a wide-ranging spectrum for both total polyphenol and total flavonoid contents within adlay sprouts and seeds. Our analysis revealed a range in total polyphenol content of 1.77 to 62.05 mg TAE/g

of extract and a range of 1.75 to 17.89 mg QE/g of extract for total flavonoid content. Notably, the majority of sprout samples exhibited higher amounts of both total polyphenol and total flavonoid contents compared to the seed samples. Despite observed differences in total polyphenol content among various sprout and seed samples, a meticulous statistical analysis revealed that these disparities were statistically insignificant. Thus, the variations in the solvent used and extraction methods did not exert a discernible impact on the total polyphenol content in either the sprout or seed samples.

Table 3. The total polyphenol and flavonoid contents in samples.

Sample	Total Polyphenol Content (mg TAE/g Extract)	Total Flavonoid Content (mg QE/g Extract)
SPM1	50.91 ± 8.10^{a}	6.42 ± 0.73^{c}
SPE1	60.05 ± 4.57^{a}	17.89 ± 2.16^{a}
SPM2	62.05 ± 5.38^{a}	11.72 ± 0.75^{b}
SPE2	54.14 ± 3.80^{a}	14.64 ± 2.42^{ab}
SM1	3.02 ± 1.68^{a}	5.32 ± 1.53^{ab}
SE1	2.95 ± 2.18^{a}	6.58 ± 2.40^{a}
SM2	1.77 ± 2.02^{a}	4.53 ± 0.98^{ab}
SE2	2.49 ± 1.28^{a}	1.75 ± 0.22^{b}

The data represent the mean value ± SD of triplicates. The meanings of the abbreviated sample names are the same ones as shown in Table 1. Note: TAE, tannic acid equivalent; QE, quercetin equivalent. $^{a–c}$ Different letters in the same column of the same sample indicate significant statistical differences ($p < 0.05$).

For total flavonoid content in the sprout samples, SPE1 (17.89 mg QE/g extract) had the highest content, followed by SPE2 (14.64 mg QE/g extract) and SPM2 (11.72 mg QE/g extract), and SPM1 had the lowest (6.42 mg QE/g extract). Notably, the differences between SPE1 and SPE2, as well as between SPE2 and SPM2, were deemed statistically insignificant. However, variations among SPE1, SPM2, and SPM1 were identified as being statistically significant. Therefore, EtOH extraction emerged as the preferred strategy for achieving an elevated total flavonoid content in sprout samples.

Turning to the seed samples, SM1 (5.32 mg QE/g extract) yielded lower results than SE1 (6.58 mg QE/g extract). However, SM2 (4.53 mg QE/g extract) had a higher total flavonoid content than SE2 (1.75 mg QE/g extract) but was still lower than SE1. Additionally, the differences between SM1, SE1, and SM2 as well as between SM1, SM2, and SE2, were statistically insignificant. In contrast, differences between SE1 and SE2 were statistically significant. Hence, for high levels of total flavonoids, seed samples should not undergo EtOH extraction under reflux conditions.

3.2. Antioxidant Activity

DPPH is commonly used in laboratory experiments to assess the antioxidant activity of different substances [47]. As a stable free radical with a distinctive deep purple color and an unpaired electron, DPPH is highly reactive. Antioxidants contribute to the reduction in DPPH by donating an electron, resulting in a color change to yellow, quantifiable through spectrophotometry [48]. The degree of this color change is directly linked to the antioxidant activity of the tested compound.

Similar to DPPH, $ABTS^{+}$ is frequently employed in laboratory experiments to evaluate antioxidant activity [49]. In its oxidized state, $ABTS^{+}$ transforms into a stable radical cation, displaying a distinctive blue-green color attributed to unpaired electrons. When antioxidants are introduced into a solution containing $ABTS^{+}$, they contribute electrons to the radical, leading to its reduction and a subsequent color change from blue-green to colorless [50].

In general, adlay sprouts exhibited significantly stronger antioxidant abilities than adlay seeds; however, both were weaker than ascorbic acid (Table 4). Regarding the sprout samples, results from both the $ABTS^{+}$ and DPPH methods indicated that those extracted with MeOH under reflux extraction had the lowest antioxidant capacity. Meanwhile, the

antioxidant abilities of sprout samples extracted by other methods showed statistically insignificant differences. Consequently, extracting sprout samples with MeOH under reflux conditions results in diminished antioxidant ability.

Table 4. Antioxidant activity ($ABTS^+$ and DPPH) of adlay extracts.

Sample	$ABTS^+$ (IC_{50}, mg/mL)	DPPH (IC_{50}, mg/mL)
SPM1	3.16 ± 0.43^{b}	6.94 ± 0.29^{b}
SPE1	2.73 ± 0.26^{b}	7.20 ± 0.55^{b}
SPM2	5.47 ± 0.44^{a}	12.28 ± 0.81^{a}
SPE2	2.41 ± 0.30^{b}	6.00 ± 0.22^{b}
SM1	8.84 ± 0.11^{c}	27.30 ± 1.50^{b}
SE1	22.55 ± 1.38^{a}	49.38 ± 1.92^{a}
SM2	7.77 ± 0.57^{c}	22.85 ± 3.19^{b}
SE2	11.32 ± 0.42^{b}	44.78 ± 2.66^{a}
Ascorbic acid	0.11 ± 0.00	0.15 ± 0.01

The data represent the mean value ± SD of triplicates. The meanings of the abbreviated sample names are the same ones as shown in Table 1. $^{a–c}$ Different letters in the same column of the same sample indicate significant statistical differences ($p < 0.05$).

Concerning the seed samples, both the $ABTS^+$ and DPPH assays revealed that SM1 and SM2 exhibited stronger antioxidant activity than SE1 and SE2. Furthermore, the difference between SM1 and SM2 was statistically insignificant. Thus, extraction with MeOH using either ultrasonic or reflux is proved to be the suitable approach for obtaining adlay seed extracts with a strong antioxidant capacity.

3.3. HPLC Analysis

The coixol content in all adlay samples was determined using HPLC/DAD analysis. As a result, coixol was identified with a retention time of 25.45 min, and the method demonstrated excellent linearity, indicated by an R-value of 1.00. The chromatogram and calibration equation for coixol are presented in Figure 3 and Table 5.

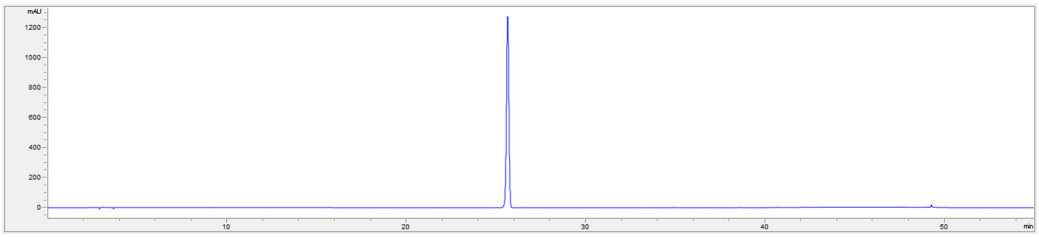


Figure 3. HPLC chromatogram of coixol.

Table 5. The calibration curve for coixol.

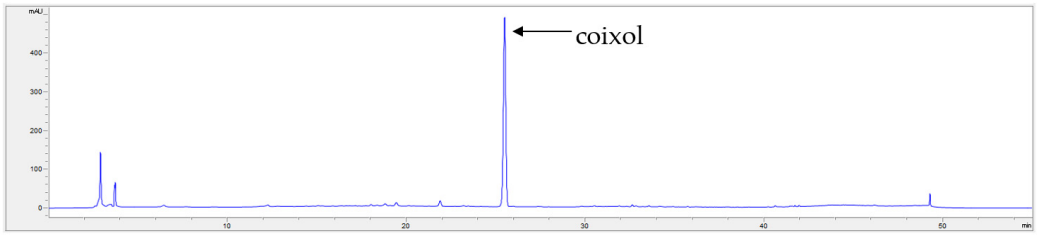
Compound	t_R	Calibration Equation	Correlation Factor, R-Value
coixol	25.45	Y = 24.092X + 169.16	1.0000

Y = peak area, X = concentration of standards (μg/mL). R-value = correlation coefficient of five calibration data points ($n = 3$).

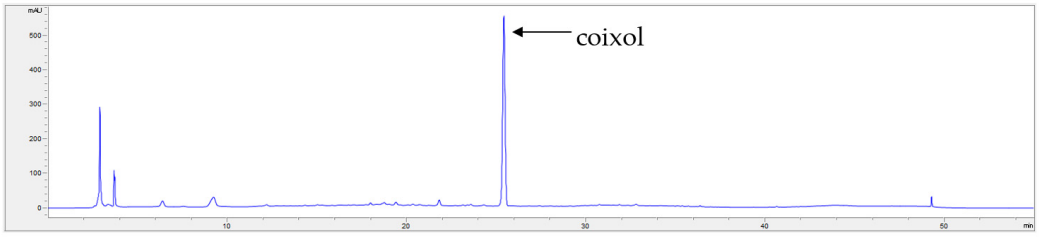
Based on the retention time, in the experiment which used the matrix spike samples, UV spectrum, and calibration equation, the presence of coixol in the chromatograms of all samples was determined (Figures 4 and 5), and its contents were calculated (Table 6).

In general, the analysis revealed that the coixol content in the sprout samples ranged from 29.97 to 34.81 mg/g extract, while coixol was not detected in the seed samples. Among the two extraction methods employed, SPM2 (32.36 mg/g extract) and SPE2 (34.81 mg/g extract) exhibited a higher coixol content compared to SPM1 (29.97 mg/g extract) and

SPE1 (33.17 mg/g extract). Particularly noteworthy was the observation that SPE2 showed a higher coixol content than SPM2. It can be observed that extraction with EtOH under reflux conditions is a suitable method for optimizing the extraction of sprouts to enhance antioxidant ability.



(**a**)



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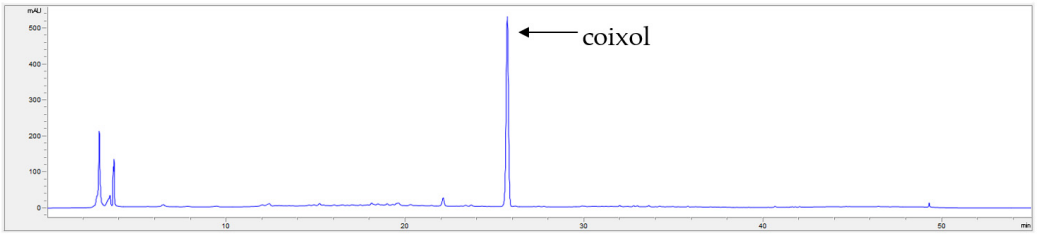

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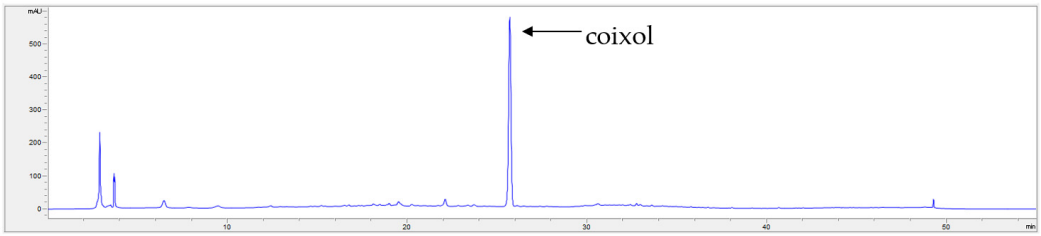
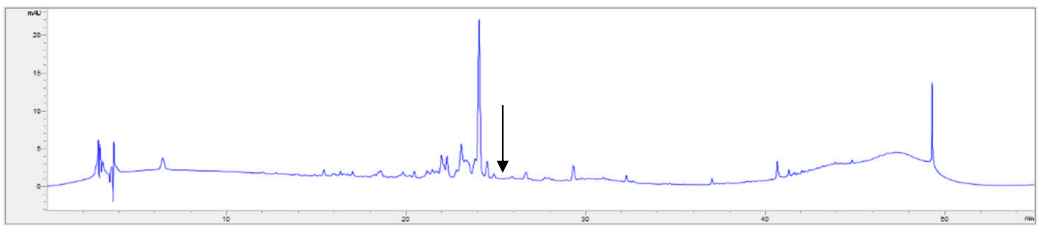


(**d**)

Figure 4. HPLC chromatograms of sprout samples SPM1 (**a**), SPE1 (**b**), SPM2 (**c**), and SPE2 (**d**). The meanings of the abbreviated sample names are the same ones as shown in Table 1.



(**a**)

Figure 5. *Cont*.

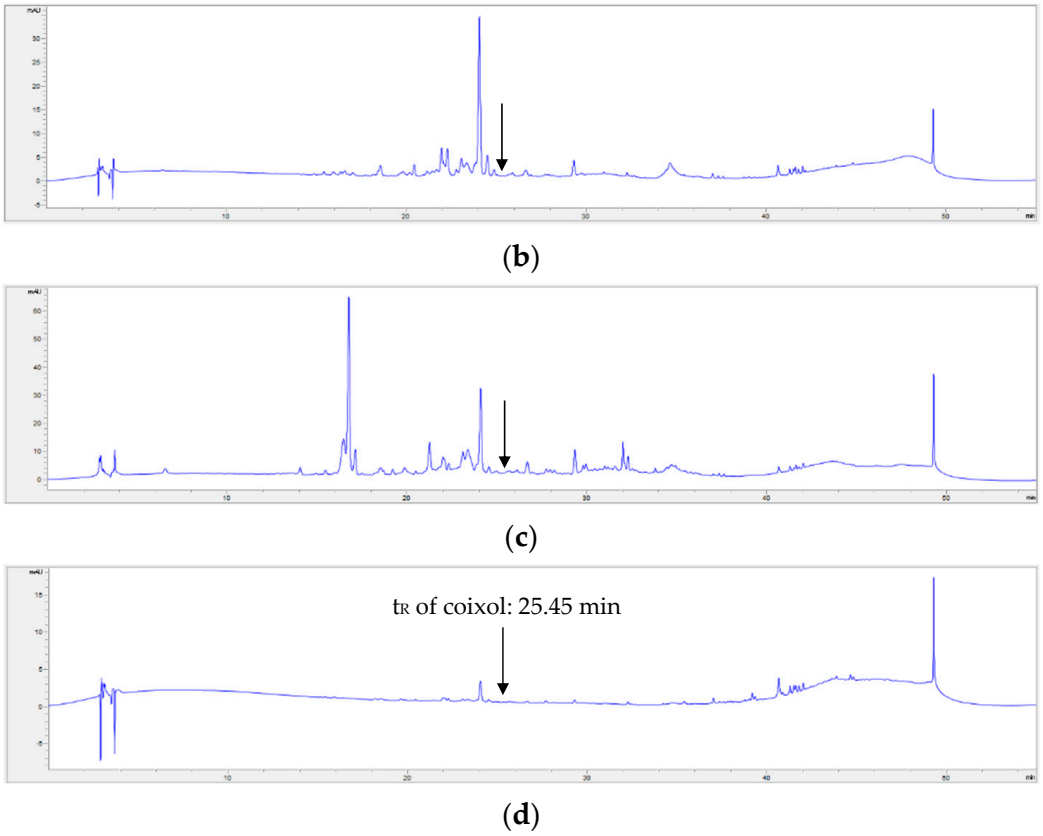


Figure 5. HPLC chromatograms of seed samples SM1 (**a**), SE1 (**b**), SM2 (**c**), and SE2 (**d**). The meanings of the abbreviated sample names are the same ones as shown in Table 1.

Table 6. Coixol content in adlay extracts.

Samples	Coixol (mg/g Extract)
SPM1	29.97 ± 0.20 ^{d}
SPE1	33.17 ± 0.22 ^{b}
SPM2	32.36 ± 0.22 ^{c}
SPE2	34.81 ± 0.06 ^{a}
SM1	ND
SE1	ND
SM2	ND
SE2	ND

The data represent the mean value ± SD of triplicates. The meanings of the abbreviated sample names are the same ones as shown in Table 1. ND: not detected. $^{a–d}$ Different letters in the same column of the same sample indicate significant statistical differences ($p < 0.05$).

4. Discussion

Ultrasonic extraction and reflux extraction are both methods which are used in the process of extracting compounds from various substances. Ultrasonic extraction involves the use of ultrasonic waves to create cavitation in the solvent, leading to the formation and collapse of bubbles [51]. This process generates intense local heating and pressure changes, facilitating the extraction of compounds from the material [52]. It typically operates at lower temperatures, thereby reducing the risk of degradation in heat-sensitive compounds [53]. In contrast, reflux extraction involves the boiling of a solvent to generate vapor, which rises through a condenser and then returns to the sample as a liquid. This continuous cycle enhances extraction efficiency but requires higher temperatures due to the boiling of the solvent, potentially affecting the stability of certain compounds [54,55].

Due to the differences between the two extraction methods, the total polyphenol, flavonoid, and coixol contents, as well as the antioxidant capacity of both the sprout and seed samples, varied. It can be observed that the optimized solvent and extraction method which was appropriate for adlay sprouts was EtOH and reflux extraction. Meanwhile, for seed samples, extraction with MeOH, employing either ultrasonic or reflux methods, exhibited higher total polyphenol and flavonoid contents and demonstrated a stronger antioxidant ability. This underscores the importance of tailoring solvent and extraction methods based on specific preferences, intended applications, and available equipment, ensuring the development of effective extraction strategies aligned with the desired outcomes.

In a prior study, the total polyphenol (2.71 mg/g DW) and flavonoid contents (0.60 mg/g DW), coixol (59.70 mg/g DW), and antioxidant capacity (453.93 μg/mL) of adlay sprouts were found to be the highest when investigating these components at various growth stages [56]. Another study reported that the coixol content in adlay sprouts, extracted at 80 °C under reflux, was 2.55 mg/g DW [33]. Additionally, adlay seeds were reported to contain 8.58 mg GAE/g of extract of total polyphenol, 6.09 mg QE/g of extract of flavonoid content, and 3.12 mg/mL for antioxidant ability with the DPPH assay [57]. The variations in total polyphenol, flavonoid, and antioxidant ability in adlay sprout and seed extracts in the present study compared to previous research may be attributed to differences in extraction conditions, such as solvent, temperature, and extraction time, as well as variations in plant agronomic conditions, including weather, soil condition, and sun exposure [58].

On the other hand, coixol has been proven to possess a range of pharmacological effects, including anti-inflammation, the inhibition of multiple synaptic reactions, antipyretic properties, blood sugar concentration reduction, muscle relaxation, anticonvulsant, and antithrombotic effects [59–61]. It has been identified in various parts of adlay, including the root, testa, stem, seeds, and sprouts [62]. Furthermore, the coixol content in adlaysprouts was observed to be higher than that in the seeds [41]. Nonetheless, the content of coixol in the same plant part of adlay from different habitats varies [63]. In this study, coixol was not detected in the seeds but was found exclusively in the sprouts. These findings were not documented in previous studies; however, they can be explained by differences in cultivation conditions, drying methods, preservation techniques, and adlay varieties, which may contribute to the absence of coixol in adlay seeds.

5. Conclusions

The extraction of adlay sprouts and seeds was carried out using two solvents, MeOH and EtOH, employing ultrasonic and reflux extraction methods. Subsequent analysis involved the determination of total polyphenol and flavonoid content, as well as antioxidant capacity using the $ABTS^+$ and DPPH methods. Additionally, the content of coixol was quantified using HPLC/DAD analysis. The outcomes underscore the significant impact of both extraction solvent and method on compound content and antioxidant capacity. For adlay sprouts, EtOH and reflux extraction emerged as the optimal method, demonstrating superior efficacy. Conversely, in the case of adlay seeds, MeOH proved to be the preferred solvent, yielding heightened antioxidant ability and elevated compound contents. Notably, coixol was exclusively detected in adlay sprouts, with a peak content recorded at 34.81 mg/g extract. Comparing the two components of adlay, sprouts exhibited a more robust profile, showcasing higher bioactive compound content and enhanced antioxidant ability in contrast to seeds. These findings carry practical implications for selecting extraction methods tailored to specific applications. Moreover, the revelation of substantial coixol content in adlay sprouts augurs well for their potential utilization as a valuable natural resource. This suggests promising applications in medical, functional, and cosmetic domains, further emphasizing the multifaceted benefits inherent in adlay sprouts. The study not only provides essential insights for optimizing extraction processes

but also underscores the potential of adlay sprouts as a versatile and valuable resource in various industries.

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