Evaluation of Proximate Composition, Multielement, and Bioactive Phenolics Contents of Different Coix Seed Varieties using Multivariate Analysis Techniques

Izzah Hayati Yahya¹, Hazrulrizawati Abd Hamid^{1*}, Ade Chandra Iwansyah²

ABSTRACT

Coix lacryma-jobi L. is a plant that serves as a source of food, medicine, cosmetics, and forage in Asian countries. Due to the distinct geographic environments, Coix seed germplasm resources are extremely diverse. In this study, we evaluated the proximate composition, multi-elemental content by ICP-OES, and phenolic bioactives by UPLC-QTOF/MS of five varieties of Coix seeds from different Asian countries, including China, Thailand, Indonesia, and Malaysia. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) were used to classify the different varieties of Coix seeds. The C. lacryma-jobi var. ma-yuen seeds from Origin 1 (China) and Origin 2 (Thailand) contained high levels of energy, total fat, and calcium, while C. lacryma-jobi var. agrotis (Indonesia) and C. lacryma-jobi var. lacryma-jobi (Malaysia) had high levels of crude fiber and carbohydrates. Twenty-three phenolic compounds were identified. Protein, carbohydrate, crude fiber, magnesium, zinc, meliadanoside, and 3,4-dihydroxyphenethyl-3-O-β-D-glucopyranoside were the dominant variables and contributed the most to data variability in PCA. The HCA results were consistent with the PCA, classifying the samples into three groups: those rich in nutrients, those rich in phenolics, and those with a mixture of nutrients and phenolics. This comprehensive analysis provides valuable insights into the nutritional and bioactive composition of Coix seed varieties, with potential applications in nutrition, food science, and pharmaceuticals.

Keywords: Coix lacryma-jobi; Coix seed, mineral content, nutritional composition; phenolic compounds, multivariate analysis.

1. INTRODUCTION

Coix lacryma-jobi L., a perennial plant native to Southeast Asia, belongs to the Poaceae (or Gramineae) family and the Coix genus (1). Coix seeds, in particular, are highly nutritious and can be used in medicine (2). They contain polysaccharides, lipids, flavonoids, phenols, proteins, vitamins, and other beneficial compounds (3). Modern pharmacological research has

revealed that these bioactive components possess potential in tumor inhibition, antibacterial and antiviral activities, blood lipid regulation, pancreatic protein suppression, anti-inflammatory, analgesic, and antioxidant properties (4-7).

Also known as Job's Tears, Coix lacryma-jobi originated in India and is now indigenous to Southeast Asia, including China, Japan, the Philippines, Burma, and Thailand. Numerous products are made from these seeds. After cleaning and dehulling, the mature seeds are boiled and served with rice. When used as a cooling beverage similar to barley or flour water, the pounded flour is occasionally mixed with water (8). Among the

*Corresponding author: Hazrulrizawati Abd Hamid hazrulrizawati@umpsa.edu.my

Received: 29/01/2024

Accepted: 04/04/2024. DOI: https://doi.org/10.35516/jjps.v17i3.2302

¹ Faculty of Industrial Science and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuh Persiaran Tun Khalil Yaakob, Kuantan, Pahang, Malaysia.

² Research Center for Food Technology and Processing, National Research and Innovation Agency, Jl. Jogja-Wonosari, Km 31,5, Gading-Playen, Gunungkidul, Yogyakarta, Indonesia.

Garo, Karbi, and Naga tribes, Coix seeds are used to make beer from pounded grains and for ornamental purposes such as rosaries and necklaces. In China, Coix is used as a medicinal food supplement and traditional medication. In Thailand, the seeds are consumed after removing the seed covering and are also used to create a beverage sold in stores. The benefits of Job's Tears include reducing liver fat buildup, inhibiting tumor growth, preventing viral infections, decreasing the risk of allergic reactions, coronary artery disease, atherosclerosis, and osteoporosis (3, 9, 10).

Coix lacryma-jobi exhibits wide distribution across tropical and temperate regions, showcasing its remarkable ability to thrive in various environments, including wetlands and forest fringes (11). There are four varieties of Coix: C. lacryma-jobi var. ma-yuen (Rom. Caill.) Stapf., C. lacryma-jobi var. lacryma-jobi, C. lacryma-jobi var. puellarum (Balansa) A. Camus, and C. lacryma-jobi var. stenocarpa Oliv (12). C. lacryma-jobi var. lacryma-jobi is commonly found as a wild type, recognized for its durable shells and traditional use in crafting beads for necklaces and rosaries. The cultivated form, C. lacryma-jobi var. ma-yuen, has a softer shell. The genetic diversity of Coix seeds is evident in their varied shapes and colors, reflecting extensive genetic variation. This diversity may contribute to variations in Coix's chemical composition, a topic that warrants further investigation. Limited research has been conducted on the phytochemical profiles of different Coix seed varieties. To address this gap, we conducted a study on the proximate composition, multi-elemental, and chemical contents of Coix seed varieties. To the best of our knowledge, this is the first comparative study of the chemical composition of Coix seed varieties.

2. MATERIALS AND METHODS

2.1 Plant characterization, sampling, and preparation

The botanical classification of the five samples was

conducted by comparing them with descriptions provided in taxonomic reviews and scientific botanical resources, such as botanical atlases and species photographs. Coix seeds originating from Indonesia, specifically Coix lacryma-jobi var. ma-yuen (CMY3) and C. lacryma-jobi var. agrotis (CA), were sourced from KWT Pantastik in Sumedang, West Java, Indonesia. C. lacryma-jobi var. mayuen (CMY1) from Origin 1 (China) and CMY2 from Origin 2 (Thailand) were purchased from different companies that claimed their seeds originated from these countries. C. lacryma-jobi var. lacryma-jobi (CLJ) was sourced from Murni Herbs in Perak, Malaysia (Figure 1). All samples were carefully stored in a cool, dry environment until analysis. The experimental research, including the collection of plant material, adhered to institutional, national, international and guidelines and legislation.

2.2 Proximate composition

The moisture, ash, protein, lipid, and carbohydrate levels were evaluated using procedures specified in AOAC methods (13). All chemical analyses were carried out in triplicate to ensure reliability. The total carbohydrate content was determined by subtracting the combined percentages of moisture, ash, lipid, and protein from 100%.

2.3 Multielement analysis

microwave-enclosed jar was filled with approximately 300 mg of dried powder, and 7.0 mL of 65% (v/v) HNO3 and 1 mL of 30% (v/v) H2O2 solutions were added. The heating program involved four successive steps at a constant pressure of 35 bar: Step 1 for 6 minutes at 750 watts, Step 2 for 4 minutes at 750 watts, Step 3 for 8 minutes, and Step 4 for 15 minutes at 750 watts and 180°C. After digestion, the samples were cooled via ventilation for 20 minutes. The digested samples were then diluted with deionized water to a final volume of 50.0 mL. Elemental analysis was performed using ICP-OES (Perkin Elmer, Optima 8300) to determine the concentrations of lead (Pb), sodium (Na), zinc (Zn), indium (In), silver (Ag), bismuth (Bi), thallium (Tl), potassium (K), magnesium

(Mg), gallium (Ga), aluminum (Al), nickel (Ni), cobalt (Co), chromium (Cr), calcium (Ca), strontium (Sr), iron

(Fe), barium (Ba), manganese (Mn), copper (Cu), and cadmium (Cd).



Figure 1: Different varieties of Coix seeds

2.4 Chromatographic analysis

The preparation of extracts for phenolic compound analysis was conducted in triplicate. Approximately 1 g of dried Coix seed powder was extracted with water and sonicated for 30 minutes. The water extracts were then dried using a freeze dryer. Each sample extract, consisting of 1 mg of dried powder, was dissolved in 1 mL of a 1:1 methanol solution and subjected to UPLC-QTOF/MS (Waters, VION Ion Mobility QTOF MS) analysis. Sample separation was performed on a Zorbax Eclipse Plus Acquity UPLC BEH C18 column (1.7 μ m particle size, 2.1 mm \times 50 mm). The column temperature

was maintained at 40° C. Aliquots of 2 μ L were injected into the UPLC-QTOF/MS at a flow rate of 0.50 mL/min. The following gradient was used for elution with A (water + 0.1% formic acid) and B (acetonitrile + 0.1% formic acid): the gradient started with 1% B for 5 minutes, increased linearly to 35% B from minutes 5 to 16, then to 100% B from minutes 16 to 18, and finally returned to 99% A from minutes 18 to 20. UPLC-QTOF/MS analysis was conducted in positive ion mode using an electrospray ionization (ESI) source. MS spectra were collected for each test sample within the mass range of 50 Da to 1500 Da. Ultra-high purity helium (He)

served as the nebulizing gas, and highly pure nitrogen (N_2) was used as the collision gas. For positive electrospray mode, the capillary voltage was set at 1500 V. The Traditional Chinese Medicine (TCM) library was used for the identification of phenolic groups.

2.5 Multivariate analysis

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed for initial exploration of the samples. PCA involved evaluating principal components based on a data matrix comprising 23 variables and five observations. The data were preprocessed by autoscaling. For HCA, a dendrogram was generated using Euclidean distance and Ward's method to represent the results. All data were collected in triplicate, and analyses were conducted using the XLSTAT 2019 add-on for data analysis within Excel.

2.6 Statistical Analysis

All data collected in triplicate were presented as means and standard deviations (Std). Statistical analysis was performed using Microsoft Excel 2019 data processing software with one-way analysis of variance (ANOVA). The significance of the data was determined by Duncan's test ($\alpha = 5\%$).

3. RESULTS AND DISCUSSION

3.1 Proximate composition

Table 1 shows the nutritional composition (total ash, moisture, protein, total fat, carbohydrate, energy, and crude fiber) of Coix seeds. The proximate composition of CMY seeds exhibited higher carbohydrate and crude fiber levels compared to the other samples in this study. CMY1 and CMY2 had higher protein, total fat, and energy content. The protein content of Coix varieties ranged from 8.7% to 14.6%. This finding aligns with previous research (14), which also noted that Coix seeds contain approximately 20% protein, with the major constituent being prolamin called coixin. Another study involving NIR analysis of 41 Coix seed samples (15) revealed that the major protein component, coixin, accounted for 79%

of the total protein. Coix prolamins, or coixins, are classified into two groups: α - and γ -coixins. γ -coixins consist of a single molecular weight class, while α -coixins are divided into four size classes. When prolamins were isolated from the endosperm during seed development, SDS-PAGE and Western blot analyses showed that αcoixins are synthesized earlier than y-coixins. Polysomal RNA coupled to protein bodies extracted from midmaturation endosperm was translated in vitro, revealing significant coixin message enrichment in these polyribosomes. cDNA clones representing α- and γcoixins were used for electrophoresis and probing of polysomal RNAs extracted from all developmental stages. These findings confirmed that α -coixins were expressed earlier and indicated that coixin RNA accumulation in the endosperm predominantly occurs during mid-seed maturation (16).

Additionally, 17 proteins were detected, supporting the high protein content of CMY seeds. Consequently, the substantial protein content contributes to the elevated nutritional value of Coix seeds. The study demonstrated that various Coix seed types can be predicted to have differing amounts of protein, asparagine, serine, glutamine, alanine, valine, isoleucine, leucine, phenylalanine, and proline, using NIRS and chemometrics techniques (17).

CLJ exhibited the highest total ash content at 11.1%, while CMY1 had the lowest at 1.6% (P < 0.05). The five samples showed a range between 1.6% and 11.1%. This study's findings slightly differ from those of reference 18, where the total ash content in Coix lacryma-jobi from different parts of North-Eastern India ranged from 2.67% to 4.27%. The moisture content of the five Coix seed samples varied between 10.1% and 13.9%. CMY3 exhibited the highest moisture content at 13.9%, while CLJ had the lowest at 10.1%. These results indicate slightly higher moisture content compared to the study conducted by reference 18, which reported moisture content ranging from 9.65% to 12.9%.

CMY2 exhibited the highest total fat content at 5.2%, while the fat content across samples ranged from 0.6% to 5.2% (P < 0.05). CMY2 can be classified as having a high total fat content, as Coix seeds naturally contain more lipids compared to most cereals and millets. This finding aligns with research (18) that reported a range of crude fat content from 2.22% to 6.26%, with the highest fat content recorded. The moisture content of all Coix seed samples ranged from 10% to 13% by mass. CLJ had the highest total ash content. Variations in plant composition can be attributed to factors such as temperature, rainfall, sun exposure, soil composition, growth stage, and interactions with other organisms in the ecosystem (19, 20).

3.2 Multielement analysis

Investigating the composition of inorganic elements is crucial for public health, as these elements play essential roles in human and animal health while also considering the potential presence of harmful components. The study conducted a multielement analysis of Coix seeds using microwave-assisted acid digestion and ICP-OES. The concentration values for elements in Coix seeds are presented in Table 2. Notably, macroelements such as Ca, K, Na, P, and Mg are significant for both plants and animals (19). CMY1 had the highest calcium content at 11,028.00 mg/kg compared to other samples (P < 0.05). The calcium content in Coix seeds was higher than that found in other grains (21).

Table 1: Proximate composition of Coix seeds

Tuble 1. I To Almate composition of Cola Secus									
Sample	Total ash	Moisture g/100 g	Protein g/100 g	Total fat g/100 g	Carbohydrate g/100 g	Energy kcal/100 g	Crude fiber g/100 g		
CMY1	1.6 ± 0.2	12.3 ± 0.1	14.6 ± 0.2	4.4 ± 1.5	67.1 ± 2.0	366.0 ± 1.5	13.1 ± 0.5		
CMY2	1.9 ± 0.3	12.4 ± 0.4	13.2 ± 0.5	5.2 ± 0.7	67.3 ± 0.9	369.0 ± 0.2	1.2 ± 0.5		
CMY3	8.2 ± 0.2	13.9 ± 0.1	10.1 ±0.9	0.3 ± 0.5	67.5 ± 0.5	313.0 ± 0.2	12.8 ± 0.3		
CA	9.3 ± 0.1	13.4 ± 0.2	8.7 ± 0.5	0.6 ± 1.0	68.0 ± 0.9	312.0 ± 1.0	26.5 ± 0.2		
CLJ	11.1 ± 0.2	10.1 ± 0.1	5.9 ± 0.2	1.3 ± 0.5	71.6 ± 0.1	322.0 ± 0.1	45.6 ± 0.2		

Total samples (n=3). Data presented as mean \pm standard errors. Values are significantly different by Duncan's test (p< 0.05).

Table 2: The concentration values obtained for Coix seeds elements

Sample	Zn (mg/kg)	P (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	
CMY1	47.62 ± 2.50	1271.00 ± 0.50	1414.00 ± 0.30	11028.00 ± 0.50	
CMY2)	45.89 ± 1.50	1483.00 ± 2.50	1418.00 ± 0.50	ND	
CMY3	52.48 ± 1.55	1593.00 ± 0.50	1423.00 ± 0.30	270.49 ± 0.50	
CA	ND	ND	ND	ND	
CLJ	ND	420.45 ± 0.30	622.73 ± 1.50	397.73 ± 1.60	

Total samples (n=3). Data presented as mean \pm standard errors. Values are significantly different by Duncan's test (p< 0.05).

ND: not detected

CMY3 showed the highest level of phosphorus at

1,593.00 mg/kg (P < 0.05). Extensive research has

investigated how Chloris gayana Kunth and Coix lacryma-jobi L. plants respond physiologically to water stress and phosphorus nourishment. Prior studies have suggested a potential link between elevated phosphorus levels and enhanced drought resistance in plants (22). Coix seeds are also rich in minerals essential for human health, such as calcium, magnesium, phosphorus, iron, zinc, and manganese (23). The mineral content varies significantly, with low concentrations in the seed shell and skin, but higher concentrations in the roots and leaves. The highest levels of phosphorus and zinc in *Coix* lacryma-jobi L. were found in the seed kernels, reaching 1.93 mg/g and 0.15 mg/g, respectively (P < 0.05). The highest concentrations of potassium, calcium, sodium, and magnesium were found in the leaves, with levels of 4.18 mg/g, 15.65 mg/g, 1.90 mg/g, and 5.85 mg/g, respectively (P < 0.05). The highest concentrations of copper, iron, and chromium were found in the roots, with levels of 0.01 mg/g, 1.46 mg/g, and 0.04 mg/g, respectively (23).

A study (24) that evaluated mercury, lead, cadmium, arsenic, chromium, and 116 pesticides in Coix seeds found that among the 123 Coix seed samples, the average levels of lead, cadmium, arsenic, and chromium were 0.0069 mg/kg, 0.0021 mg/kg, 0.0138 mg/kg, and 0.1107 mg/kg, respectively. Notably, no traces of mercury were detected in any of the Coix seed samples. These findings suggest that Coix seeds are considered safe for consumption based on the assessment of heavy metal and pesticide residue pollution indices and associated risks.

3.3 Chromatographic analysis

Phenolic compounds are currently being investigated in various fields, particularly in chemical and biological research aimed at discovering novel compounds with biological activities. These compounds are significant in industries such as food, chemicals, and pharmaceuticals. Figure 2 shows the components identified by UPLC/QTOF-MS in all Coix samples. Phenolic compounds identified in CMY1 and CMY2 include 3,4-dihydroxyphenothyl-3-O-β-D-glucopyranoside, koaburaside, meliadanoside A, and shogaol, each with varying ion intensities. CMY3 was found to contain abundant amounts of salidroside, meliadanoside A, mulberrofuran A, and shogaol. CLJ showed the presence of meliadanoside A and shogaol. The high abundance of

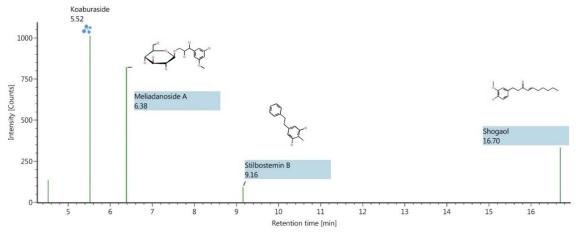
glucoside and shogaol was detected in CA (P < 0.05)).

3,7-dihydroxy-2,4-dimethoxyphenanthrene-3-O-

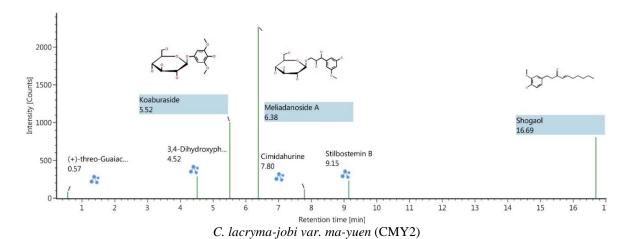
Based on Table 3, the results indicate the ion response of phenolic compounds in Coix seeds. CMY3 was found to contain the most abundant phenolic compounds, while CA had the least. All Coix samples showed a significant difference in the concentration of shogaol. Previous studies on polyphenols in Coix seeds have mainly focused on the total polyphenol content and their antioxidant activity. Recent research has led to the isolation of phenolic compounds from Coix husk, including pcoumaric acid, vanillic acid, chlorogenic acid, tannic acid, catechol, and ferulic acid. Additionally, polyphenols such as p-hydroxybenzoic acid, vanillic acid, syringic acid, ferulic acid, p-coumaric acid, caffeic acid, erucic acid, vanillin, 2-hydroxyphenylacetic acid, coixol, 4-ketopinol ester, syringaresinol, and catechin have been identified in Coix lacryma-jobi (23).

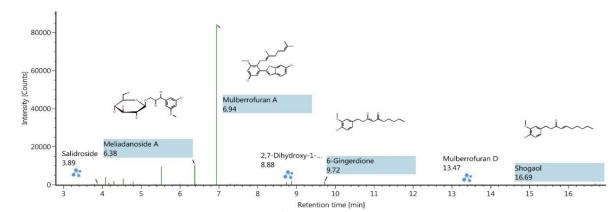
The observed differences in quality can be attributed to variations in geographical environments, soil composition, and climate conditions. Even within the same species, distinct DNA barcodes, elemental compositions, and chemical fingerprints can exhibit unique characteristics, aiding in the identification of their origins (25). Variation in geographical locations significantly influences the chemical compounds within the same species. To the best of our knowledge, there is no documented research on the chemical composition of CA.

Jordan Journal of Pharmaceutical Sciences, Volume 17, No. 3, 2024

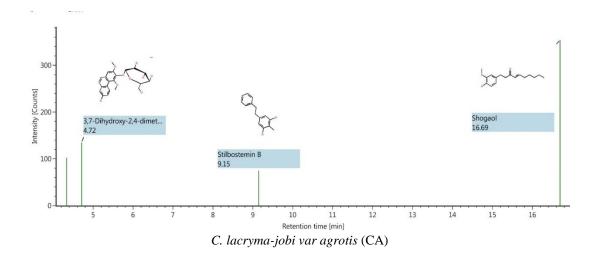


C. lacryma-jobi var. ma-yuen (CMY1)





C. lacryma-jobi var. ma-yuen (CMY3)



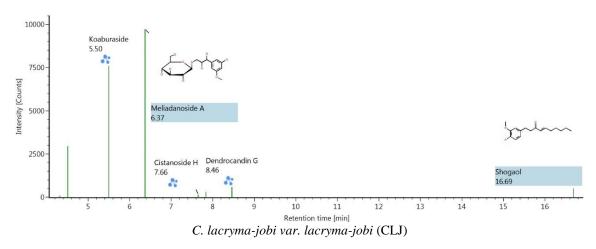


Figure 2: Chemical component identified by UPLC/QTOF-MS in Coix seeds water extract

3.4 Multivariate analysis

Exploratory analyses, such as Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA), were employed to assess nutritional compositions, multielement content, and phenolic compounds. PCA, functioning as an exploratory technique, offers a comprehensive overview of the analyses. It enables the derivation of significant conclusions for potential decision-making based on observed outcomes, effectively reducing data set dimensionality. This results in a statistical analysis involving fewer variables while

retaining critical information. Given the extensive data points, PCA was utilized as a multivariate statistical tool to evaluate outcomes across diverse variables.

The biplot obtained from PCA is shown in Figure 3. It is shown that the extracts are mainly distributed into three groups: group I–III. Two components, PC-1 and PC-2 showed, accounted for 63.73% of the total variance. From the scores plot, group I was in the positive PC-1 axis while groups II and III were in the negative PC-1 axis. Coix *C. lacryma-jobi var. ma-yuen* (Origin 1) and *C. lacryma-jobi var. ma-yuen* (Origin 2) in a group I, *C. lacryma-jobi var.*

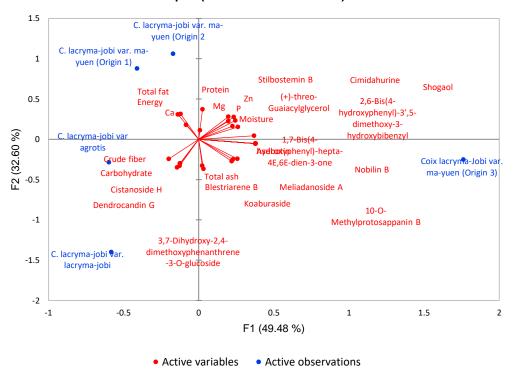
agrotis and C. lacryma-jobi var. lacryma-jobi in a group II while C. lacryma-jobi var. ma-yuen (Origin 3) in Group III. Based on the result, those samples with similar chemical profiles were grouped near to each other, while a larger distance was observed in samples with different chemical profiles. To identify the variables responsible for significant differences between various extracts, correlation loading plots for PC-1 and PC-2 were generated. These plots reveal that Coix Thailand and C. lacryma-jobi var. ma-yuen (Origin 1) exhibit moderate

levels of total fat, energy, and calcium, whereas *C. lacryma-jobi var. lacryma-jobi* has a lower content of these nutritional components. *C. lacryma-jobi var. mayuen* (Origin 3) contains the majority of the phenolic compounds. Additionally, *C. lacryma-jobi var. agrotis* and *C. lacryma-jobi var. lacryma-jobi* display moderate levels of carbohydrate, crude fiber, 3,7-dihydroxy-2,4-dimethoxyphenanthrene-3-O-glucoside, cistanoside H, and dendrocandin G.

Table 3: Phenolic compounds identified in different varieties of Coix seeds water extract

Table 3: Phenolic compounds identified in different varieties of Coix seeds water extract									
Component name	CMY(1)	CMY(2)	CMY(3)	CA	CLJ				
(+)-threo-Guaiacylglycerol	-	84.0 ± 1.3	76.5 ± 0.3	-	-				
1,7-Bis(4-hydroxyphenyl)-hepta-4E,6E-	-	-	114.0 ± 1.5	-	-				
dien-3-one									
10-O-Methylprotosappanin B	-	-	190.5 ± 0.5	-	-				
2,6-Bis(4-hydroxyphenyl)-3',5-dimethoxy-	-	-	523.5 ± 0.3	-	-				
3-hydroxybibenzyl									
2,7-Dihydroxy-1-(p-hydroxybenzyl)-4-	-	-	1924.0 ± 0.2	-	-				
methoxy-9,10-dihydrophenanthrene									
3,4-Dihydroxyphenothyl-3- <i>O</i> -β-D-	135.0 ± 2.5	288.0 ± 0.3	3078.5 ± 0.1	-	2946.5 ± 4.5				
glucopyranoside									
3,7-Dihydroxy-2,4-	-	-	-	135.0 ± 0.5	87.0 ± 0.6				
dimethoxyphenanthrene-3-O-glucoside									
Albaspidin AA	-	-	418.5 ± 0.1	-	-				
Asebotin	-	-	77.5 ± 1.0	-	-				
Blestriarene B	-	-	117.0 ± 0.5	-	300.5 ± 0.7				
Cimidahurine	-	117.5 ± 0.3	273.5 ± 0.3	-	-				
Cistanoside H	-	ı	-	-	133.0 ± 0.2				
Dendrocandin G	-	-	-	-	566.0 ± 0.5				
Isomucronustyrene	-	-	1307.0 ± 0.1	-	-				
	1013.0 ±	1005.0 ± 0.5	9552.0 ± 0.1	-	7599.5 ± 1.5				
Koaburaside	0.5								
Kuwanon P		-	249.5 ± 2.5	-	-				
Meliadanoside A	822.5 ± 0.3	2269.0 ± 0.1	10376.0 ± 4.5	-	9725.5 ± 0.5				
Mulberrofuran A	-	-	84344.5 ± 6.5	-	-				
Nobilin B	-	-	278.0 ± 0.5	-	-				
Salidroside	-	-	3893.0 ± 0.5						
Shogaol	332.0 ± 0.4	806.5 ± 1.5	453.0 ± 0.3	354.5 ± 0.3	508.5 ± 0.4				
Stilbostemin B	94.5 ± 1.3	229.0 ± 4.5	173.5 ± 3.0	75.0 ± 0.2	-				
Tachioside	_	-	485.5 ± 0.5	-	-				

Total samples (n=3). Data presented as mean \pm standard errors. Values are significantly different by Duncan's test (p<0.05).



Biplot (axes F1 and F2: 82.07 %)

Figure 3. PC-1 and PC-2 biplot chart of Coix seeds

This variation illustrates that plants possess inherent chemical traits, leading to differences in concentrations even among samples of the same species due to factors such as climate, soil composition, and harvest timing (26). To assess the contributions of these factors to the overall variability in phenolic compounds, we employed two analytical techniques: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). HCA evaluates data based on their similarities and differences, effectively grouping them according to their degrees of resemblance (27). The trends in correlations identified through PCA were further validated by constructing a dendrogram using HCA. For this purpose, Ward's method was used to minimize variance within each cluster, while Euclidean distances quantified the similarities between

different samples.

In today's technology-driven and fast-paced environment, trade globalization and the rapid evolution of e-commerce have led to increased product returns. This has heightened customer expectations for well-organized and efficient logistics, particularly in reverse logistics, making its role even more critical.

The HCA dendrogram shows that the samples were separated into three groups: in group I (in red) formed by *C. lacryma-jobi var. ma-yuen* from Origin 1 and Origin 2, while in group II (in blue) were *C. lacryma-jobi var. lacryma-jobi* and *C. lacryma-jobi var. agrotis* and group III (in blue) was *C. lacryma-jobi var. ma-yuen* from Origin 3. Analysis of the dendrogram (Figure 4) shows the same classification as in the PCA result.

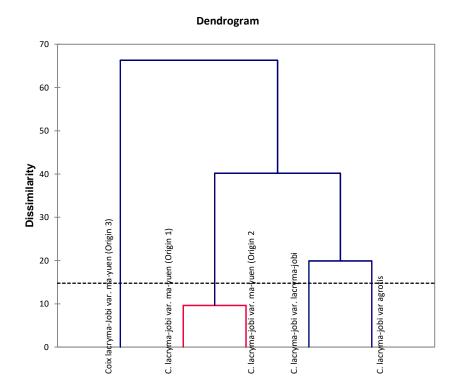


Figure 4. Dendrogram obtained from the analyzed Coix samples

4 CONCLUSIONS

In summary, the proposed methods for assessing nutritional content and microelements using ICP-OES, along with bioactive compound analysis through UPLC/QTOF MS and multivariate analysis, offer a robust framework for comprehensive component analysis of diverse Coix samples from different countries. This approach is highly relevant for studying Coix and has the potential to be applied to other edible plants as well. By addressing a gap in the existing literature on Coix seeds, this study provides valuable insights to the food composition database.

The findings reveal significant variation in the types and relative quantities of chemical constituents within Coix seeds. This establishes a foundation for broader applications, including setting quality standards and effectively utilizing these seeds. Notably, the presence of essential nutrients, potentially hazardous elements, and phenolic compounds in Coix seed samples suggests their potential for medicinal and edible purposes across various plant parts. This encourages further exploration into plant metabolic processes.

Data availability

All data generated or analyzed during this study are included in this published article.

Author Contribution statement

Izzah Hayati Yahya: Data collection and analysis. Hazrulrizawati Abd Hamid: Concept idea and review the manuscript. Ade Chandra Iwansyah: Preparation of sample and review of the manuscript

Conflict of Interest Declaration

The authors declared that there are no competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or

discussion reported in this paper

Acknowledgements

The financial support of Universiti Malaysia Pahang, RDU223009, is gratefully acknowledged. The authors would

REFERENCES

- Kang SH, Kim B, Choi BS, et al. Genome assembly and annotation of soft-shelled adlay (Coix lacryma-jobi Variety ma-yuen), a cereal and medicinal crop in the Poaceae Family. Frontiers in Plant Science. 2020;11:630.
- Zhou Q, Yu R, Liu T, et al. Coix seed diet ameliorates immune function disorders in experimental colitis mice. *Nutrients*. 2021;14(1):123.
- Devaraj RD, Jeepipalli SP, Xu B. Phytochemistry and health promoting effects of Job's tears (Coix lacrymajobi)-A critical review. *Food Bioscience*. 2020;34:100537.
- Kuo C-C, Chen H-H, Chiang W. Adlay seeds of Coix lachryma-jobi L. var. ma-yuen Stapf) is a Potential Cancer Chemopreventive Agent toward Multistage Carcinogenesis Processes. *J Tradit Chin Med*. 2012;2(4):267-275.
- Chen H-J, Chung C-P, Chiang W, Lin Y-L. Antiinflammatory effects and chemical study of a flavonoidenriched fraction from adlay bran. *Food Chem.* 2011;126(4):1741-1748.
- Wang L, Chen J, Xie H, Ju X, Liu RH. Phytochemical profiles and antioxidant activity of adlay varieties. *Journal of agricultural and food chemistry*. 2013;61(21):5103-5113.
- Puspitasari E, Triatmoko B, Dianasari D, Muslichah S, Nugraha AS. Assessment of Extraction Methods Effects on the Biological Activities (Antioxidant and Antiamylase) and Chemistry (Total Phenolics and Flavonoids) of Guazuma ulmifolia Leaves. *Jordan Journal of Pharmaceutical Sciences*. 2024;17(1).

like to thank the Ministry of Higher Education for providing financial support under Fundamental Research Grant Scheme FRGS/1/2018/TK05/UTM/02/18 (University reference RDU190125).

- 8. Kutschera M, Krasaekoopt W. The use of job's tear (Coix lacryma-jobi L.) flour to substitute cake flour in butter cake. *AU Journal of Technology*. 2012;15(4).
- Chang H-C, Huang Y-C, Hung W-C. Antiproliferative and chemopreventive effects of adlay seed on lung cancer in vitro and in vivo. *Journal of agricultural food chemistry*. 2003;51(12):3656-3660.
- 10. Acharya S, Gond H, Aslam S, KS S, Mishra S, Kumar S. Job's tears (Coix lacryma-jobi): a medicinal plant of India.
- 11. Zhu F. Coix: Chemical composition and health effects. *Trends in Food Science & Technology*. 2017;61:160-175.
- 12. Govaerts R, Nic Lughadha E, Black N, Turner R, Paton A. The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity. *Scientific Data*. 2021;8(1):215.
- Chemists A. Association of Official Analytical Chemists (AOAC) Official methods of analysis. AOAC Washington, DC, USA. 2004.
- 14. Ottoboni LM, Leite A, Targon MLN, Crozier A, Arruda P, chemistry f. Characterization of the storage protein in seed of Coix lacryma-jobi var. Adlay. *Journal of agricultural*. 1990;38(3):631-635.
- 15. Liu X, Rong Y-Z, Zhang X, Mao D-Z, Yang Y-J, Wang Z-W. Rapid determination of total dietary fiber and minerals in Coix seed by near-infrared spectroscopy technology based on variable selection methods. *Food analytical methods*. 2015;8:1607-1617.
- 16. Targon MLN, Ottoboni LM, Leite A, Ludevid D, Puigdomenech P, Arruda P. Synthesis and deposition of coixin in seeds of Coix lacryma-jobi. *Plant Sci*. 1992;83(2):169-180.

- 17. Liu X, Zhang X, Rong Y-Z, Wu J-H, Yang Y-J, Wang Z-W. Rapid determination of fat, protein and amino acid content in coix seed using near-infrared spectroscopy technique. *Food analytical methods*. 2015;8:334-342.
- Laxmisha K, Semwal DP, Gupta V, et al. Nutritional profiling and GIS-based grid mapping of Job's tears (Coix lacryma-jobi L.) germplasm. *Applied Food Research*. 2022;2(2):100169.
- 19. Moura HFS, de Souza Dias F, e Souza LBS, et al. Evaluation of multielement/proximate composition and bioactive phenolics contents of unconventional edible plants from Brazil using multivariate analysis techniques. Food Chem. 2021;363:129995.
- Alsarhan A, Salman KB, Olimat S. Chemical Composition of the Essential Oils of the Flowers Asphodelus aestivus Brot. Grown Wild in Jordan. *Jordan Journal of Pharmaceutical Sciences*. 2023;16(4):734-739.
- 21. Xie J, Liu L, Lu M, Wang Y. Research Progress on Nutritional Value and Edible Efficacy of Coix Seed. *Grain Processing*. 2016;41:50-52.

- Saneoka H, Fujita K, Ogata S. Effect of phosphorus on drought tolerance in Chloris gayana Kunth and Coix lacryma-jobi L. Soil Science Plant Nutrition. 1990;36(2):267-274.
- Weng WF, Peng Y, Pan X, et al. Adlay, an ancient functional plant with nutritional quality, improves human health. Frontiers in Nutrition. 2022;9:1019375.
- 24. Yu J, Wang X, Yao X, Wu X. Safety evaluation of heavy metal contamination and pesticide residues in Coix seeds in Guizhou province, China. *Foods*. 2022;11(15):2286.
- 25. Wang Y, Xiong F, Zhang Y, et al. Application of hyperspectral imaging assisted with integrated deep learning approaches in identifying geographical origins and predicting nutrient contents of Coix seeds. *Food Chemistry*. 2023;404:134503.
- 26. Teggar N, Bakchiche B, Abdel-Aziz ME-S, Bardaweel SK, Ghareeb MA. Chemical composition and biological evaluation of Algerian propolis from six different regions. *Jordan Journal of Pharmaceutical Sciences*. 2023:184-197.
- Ferreira MMC, Morgano MA, de Queiroz SCdN, Mantovani DMB. Relationships of the minerals and fatty acid contents in processed turkey meat products. *Food Chem.* 2000;69(3):259-265.

تقييم التركيب التقريبي والمحتوى الفينولي متعدد العناصر والفعال بيولوجيًا لأصناف مختلفة من بذور Coix تقييم التركيب التعليل متعدد المتغيرات

آدى شاندرا إيوانسياه 1، هاز رولريزاواتي عبد الحميد 1*، عزة حياتي يحيي2

1 كلية العلوم الصناعية والتكنولوجيا، جامعة ماليزبا باهانج السلطان عبد الله، ليبوه بيرسياران تون خليل يعقوب، كوانتان، باهانج، ماليزبا.

ملخص

نظرًا للبيئة الجغرافية المتميزة، فإن موارد الأصول الوراثية لبذور Coix واسعة للغاية ومتنوعة. في هذه الدراسة، تم تقييم التركيب التقريبي والعناصر المتعددة بواسطة ICP-OES والفينول الحيوي النشط بواسطة UPLC-QTOF/MS لخمسة التركيب التقريبي والعناصر المتعددة بواسطة ICP-OES والفينول الحيوي النشط بواسطة UPLC-QTOF/MS لخمسة أنواع من بذور Coix من دول آسيوية مختلفة. تم استخدام تحليل المكون الرئيسي (PCA) وتحليل المجموعات الهرمية (HCA) لتصنيف أنواع مختلفة من بذور Coix ج. لاكريما جوبي فار. تحتوي بذور ma-yuen من الأصل ا والأصل 2 على نسبة عالية من الطاقة والدهون الكلية والكالسيوم، في حين تحتوي بذور Iacryma-jobi var وعشرون 2 على نسبة عالية من الألياف الخام والكربوهيدرات. تم تحديد ثلاثة وعشرون مركباً فينولياً. كانت البروتينات والكربوهيدرات والألياف الخام والمغنيسيوم والزنك والميليادانوسيد و 4-3-ثنائي هيدروكسي فينوثيل O-β-D-glucopyranoside هي المتغيرات السائدة وساهمت في أكبر تباين في البيانات في PCA. تحليل فينوثيل -PCA وخليط العناصر الغذائية مع الفينولات. تساهم هذه الدراسة في التطوير العلمي واستخدام أصناف مختلفة المغنية، الفينولات وخليط العناصر الغذائية مع الفينولات. تساهم هذه الدراسة في التطوير العلمي واستخدام أصناف مختلفة من بذور الكوبكس.

الكلمات الدالة: كويكس لاكريما-جوبي، بذور جوز الهند، المحتوى المعدني، التركيب الغذائي، مركبات فينوليه.

hazrulrizawati@umpsa.edu.my

تاريخ استلام البحث 2024/01/29 وتاريخ قبوله للنشر 2024/04/04.

² مركز أبحاث تكنولوجيا الأغذية وتجهيزها، الوكالة الوطنية للبحث والابتكار، جي. جوجيا-ونوساري، كم 31،5، غادينغ-بلاين، b جونونجكيدول، يوجياكارتا، إندونيسيا.

^{*} المؤلف المراسل هازر ولريزاواتي عبد الحميد