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Job's Tears (*Coix Lacryma-Jobi*) Adaptability in Sa Kaeo Province, East of Thailand, and Their Chemical Properties in an Edible Group for Alternative Ruminant Animal Forage and Supplementary Feed

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Abstract

In the past decade, the cultivation of Job's tears in Thailand has decreased. By contrast, consumption of Job's tears in Thailand is still high as a functional food. Thus, it needs to be imported in large quantities from other nations. The objectives of this study were to determine the suitability of the different Job's varieties of Job's tears in Sa Kaeo province, East of Thailand and then to select a possible variety to evaluate its chemical properties. Then, the data will be used for the conservation and cultivation of Job's tears in Thailand. Seven varieties of Job's tears were collected from markets in Thailand from different sources such as Luang Phra Bang province in the Lao People's Democratic Republic, Nong Khai, Loei and Chiang Rai provinces in Thailand. Firstly, 7 varieties of Job's tears were grown and the plant growth characteristics were collected to evaluate their adaptability in Sa Kaeo province, Thailand. Secondly, the plant growth characteristics of 4 varieties of edible Job's tears were recorded chemical properties such as their chemical composition, total phenolic content (TPC), total flavonoid compounds (TFC), DPPH radical scavenging assay, ABTS decolorization scavenging effect and potassium ferricyanide reducing power assay. The statistical data were analyzed by using the one-way analysis of variance (ANOVA) to determine the difference between varieties. The results showed that there are differences between the varieties in plant morphology and chemical properties. Seven Job's tears plants were classified into 2 groups, an edible and an ornamental group. The Job's tears seeds of the ornamental group could not be dehulled into kernels. AL1 to AL4 showed a good adaptability in their growth characteristics. AL4 in kernels had the proper CP at 17.78 % ($p < 0.001$) for use in a ruminant diet. AL2 had high plant height (157 cm), leaf areas (1,714 cm^2) and a tiller number (4 - 6 tillers) which were good characteristics for forage utilization. While AL1 and AL4 had high TPC (46.15 and 24.55 mg GAE/g, respectively, $p < 0.001$) which showed a positive correlation with EE ($p < 0.05$). The kernels of AL1 and AL4 can be used as functional food.

Keywords: Job's tears, Adaptability, Chemical properties, Antioxidant activity, Ruminant animal feed

Introduction

In 2023, the Food and Agriculture Organization of the United Nations (FAO) announced the International Year of Millets (IYM2023) [1]. FAO would increase awareness and attention to the benefits of millets. Moreover, millets can be cultivated under climate change conditions. Millets include pearl millet, foxtail millet, sorghum and Job's tears. Job's tears or adlay (*Coix lacryma-jobi* L.) is a cereal crop belonging to the Gramineae family. Job's tears are cultivated in Asia countries, such as China, India, Thailand, Lao, Philippines, Sri Lanka and Myanmar. It is grown for human food, medicine, forage and ornaments. The plants and grain of Job's tears are used as herbal medicine in China and India. They are a source of nutrients for healthy diets. The kernels are used for the treatment of the lungs, liver, stomach and breast cancer with the official approval of the Chinese government. The compounds in the kernels that have this anti-tumor property are: coixenolide, palmitic acid, stearic acid, oleic acid and linoleic acid [2]. The grains from Job's tears have a higher chemical composition compared to other cereals such as rice, corn and wheat. Starch in the kernels of Job's tears also contains high amylopectin and is gluten-free [3]. The starch from the seeds of Job's tears has a high amount of crude protein (CP) (12.6 - 19.5 %), crude fat (EE) (2.2 - 9.1 %), dietary fibre (2.51 - 10.3 %), total soluble sugar (1.03 - 2.06 %), total phenols (20.5 mg GAE/g), copper (0.31 - 1.90 mg/100 g), iron (2.14 - 25.6 mg/100 g), potassium (216.5 mg/100 g), phosphorous (193.3 mg/100 g), magnesium (121.2 mg/100 g) and calcium (107 - 175 mg/100 g) [2,4,5]. These ingredients depend on genetics and the environment. In India and the Philippines, the stem and leaves of Job's tears are used for forage by cutting ratoons several times. The ratoon of Job's tears has a high CP and low dry matter (DM) [6]. The chemical compositions of the stems include 12.1 % CP, 43.4 % crude fiber (CF) and 2.4 % EE with a high content of vitamin E and 70 % of unsaturated fatty acids (oleic and linoleic acid). The leaves also contain a high CP content, vitamin E, Ca, Mg, K, Na and 79 % of unsaturated fatty acid [2]. The hulls are a by-product of the waste after the dehulling process is used to separate the kernels for sale. It has been reported that there are many antioxidants and phytochemical constituents [7].

In Thailand, Job's tears are a minor crop which hill tribes still cultivate in the highlands of such places as Chiang Rai, Phayao, Phrae, Nong Khai and in Loei province which are in northern Thailand. They are found on the slopes of hills at altitudes of up to 1,700 m above sea level [8]. Also, it has been reported that about 4 to 10 million tons of Job's tears were imported into Thailand in 2018 - 2022 [9]. This means that there is a high demand for Job's tears in Thailand but its cultivation has greatly decreased recently. Climate is an important factor in agriculture, particularly temperature and rainfall. Sa Kaeo province is in eastern Thailand where climate and topography differ from northern Thailand, for example, with higher temperatures (20 - 36 °C), higher rainfall (1,305.1 mm per year), elevation above sea level of 41 m and mostly flat areas [10]. In the last decade, rainfall has decreased both in the amount per day and the number of times a day per year as a result of climate change. Therefore, farmers have to select crops which will grow with little water. These plants must be able to adapt widely to different environmental conditions. They should be able to yield in areas with 3 or 4 consecutive months in the dry season (143 - 374 mm of rainfall) [11]. They must also be able to respond to chemical fertilizer and organic fertilizer (goat manure) and be cultivated in acidic soil [12]. The yield is an important factor for farmers in selecting suitable varieties to plant in each area. However, other factors such as chemical composition, TPC and TFC and antioxidant activity have to be considered as Job's tears is a functional crop.

This study evaluated the adaptability of the varieties of Job's tears in Sa Kaeo province with regard to plant height, area of leaves, plant morphology and seed weight. The chemical composition and antioxidant activity of different varieties (edible group) of Job's tears must also be considered. Such data will be a useful guideline for further cultivation of suitable varieties in Sa Kaeo province, Thailand. The kernels and plants of Job's tears might also be an alternative functional food for humans or be used as supplementary feed and/or forage for livestock in the east of Thailand.

Materials and methods

Job's tears samples

Seven varieties of Job's tears seeds were collected from local markets in Luang Phra Bang province in the Lao People's Democratic Republic (coded as AL1), in Nong Khai province, in the North-East Thailand (coded as AL2), in Loei province, in the North-East of Thailand (coded as AL3 and AL4), in Doi Mae Salong, in Mae Fah Luang District, Chiang Rai province, in the North of Thailand (coded as AL5) and in Mueang District, Chiang Rai province, in the North of Thailand (coded as AL6 and AL7) (**Figure 1**). The seed morphology of Job's tears such as shape and color were observed by the eye. The seeds of AL1, AL2, AL3 and AL4 varieties were edible but the other varieties were ornamental.

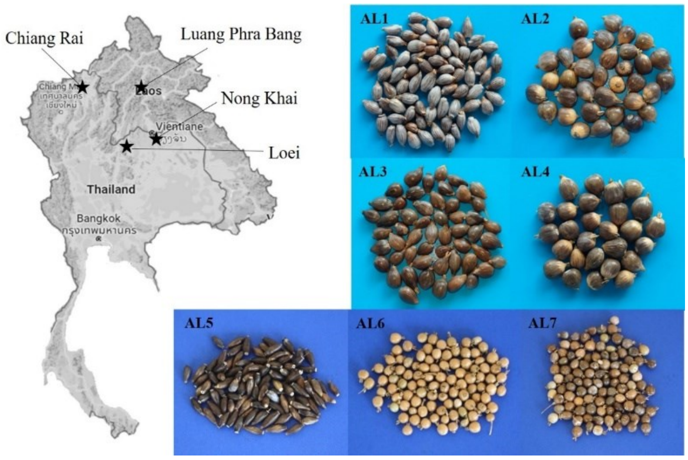

Figure 1 Job's tears seed morphology and sources. AL1: Luang Phra Bang province; AL2: Nong Khai province; AL3- AL4: Loei province; AL5 - AL7: Chiang Rai province, AL1 - AL4: edible group; AL5 - AL7: ornamental group (adapted from [13]).

Plant growth

A preliminary yield trial was conducted in an experimental field, at the Faculty of Agricultural Technology, Burapha University, Sa Kaeo Campus. Five varieties of Job's tears were evaluated in the field including AL1, AL2, AL3, AL4, AL5, AL6 and AL7. Each variety was planted in a plot (15×0.75 m^2), with 21 hills per plot and 3 seeds per hill. Seedlings were thinned to one plant per hill at 20 days after germination. Weeds were eliminated at 45 days after planting and 2 g. of N - P - K fertilizer ratio of 15 - 15 - 15 was applied to each plant. A steel net was installed around the base of the plant to prevent encroachments by rats. The characteristics of the growth of the plants were recorded randomly from 7 plants for each variety. Data included plant height, leaf area per plant (calculated from leaf length x leaf width×0.7 [14] of all leaves), 50 and 100 % flowering date, weight of 100 seeds, seed color before and after maturation, flower color, number of tillers and branches.

Analysis of colors

The seeds of AL1, AL2, AL3 and AL4 varieties in the edible group, were separated into the kernel and hull (**Figure 2**). Because the seeds of AL5, AL6 and AL7 did not shed their kernels and hulls separately, we decided to eliminate them from the seed evaluation. The color of the seed and kernels (Job's tears bran) were measured by a Hunter Lab-UltraScan VIS spectrophotometer (USVIS2329, USA), with the colors displayed according to the Commission on Illumination (CIE) as L*, a* and b*. The color evaluation by

L* or brightness (0 = black, 100 = white), a* (+ a = red, –a = green) and b* (+ b = yellow, –b = blue). C* (the apparent color intensity) and hues (H^0) were applied: H^0 approaches 0° (degree) = the red group, H^0 approaches 90° = the yellow group, H^0 approaches 180° = the green group and H^0 approaches 270° = the blue group.

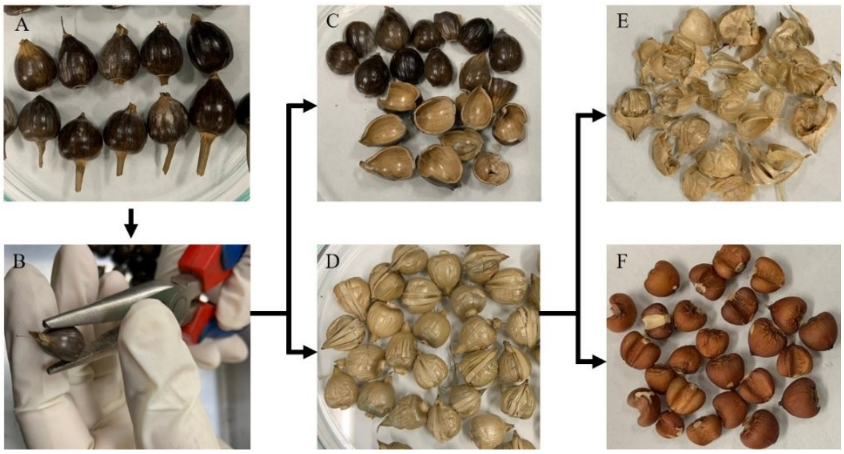

Figure 2 De-hulling process of Job's tears: whole seed (A), cracked by pliers (B), hard hull (C), kernel with soft hull (D), soft hull (E), and kernel (F), C and E were called hulls in our study.

Figure 3 Seed characteristics of 7 varieties of Job's tears.

Determination of chemical composition and antioxidative activity

Sample preparation

The seeds of Job's tears were separated into kernels and hulls. The kernels and hulls were dried at 60 °C for 24 h by using a hot air oven (UFE 550, Memmert GmbH+Co.KG, Germany) and then they were ground into powder (< 1 mm).

Chemical composition

The chemical composition of the Job's tears sample was determined: dry matter (DM), organic matter (OM), crude protein (CP) and ash using the standard methods of AOAC [15], neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Soest *et al.* [16].

Total phenolic content analysis

Total phenolic content (TPC) of the Job's tears sample was determined using the Folin-Ciocalteu reagent as adapted from Singleton *et al.* [17]. The Job's tears sample extract was mixed with Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3) solution. It was incubated at 20 °C for 2 h. The absorbance was measured at 765 nm using a spectrophotometer (SP-V1100, ONILAB, USA). Based on the gallic acid standard curve using concentrations of 0, 20, 40, 80, 160, 320 and 640 ug/mL, with the quantitative data represented in grams of the dry weight sample (mg GAE/gDW).

Analysis of total flavonoid compounds

The analysis of total flavonoid compounds (TFC) of the Job's tears sample was described by Chandra *et al* [18] The Job's tears sample extract was mixed with aluminum chloride ($AlCl_3$) and then the solution was incubated for 60 min at room temperature. The absorbance was measured at 420 nm with a spectrophotometer (SP-V1100, ONILAB, USA). The concentration of TFC was showed in mg quercetin equivalent (QE)/g of dried plant material. Concentrations of quercetin at 0, 25, 50, 100, 200 and 400 ug/mL were used to make a standard curve.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the Job's tears sample was estimated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay adapted from Aqil *et al.* [19]. A solution of Job's tears sample extract and DPPH was incubated for 30 min at room temperature. At 517 nm, the absorbance was determined using spectrophotometry (SP-V1100, ONILAB, USA). The free radical scavenging activity was calculated as: % inhibition = [(Absorbance of control-Absorbance of sample)/(Absorbance of control)]×100.

ABTS radical cation decolorization scavenging assay

The ABTS decolorization scavenging activity (ABTS) of the Job's tears sample was estimated by using a method adapted from Re *et al.* [20]. The solution of the Job's tears sample and ABTS was incubated in the dark for 30 min. The free radical scavenging activity was calculated as: % inhibition = [((Absorbance of ABTS radical + methanol) – (Absorbance of ABTS radical + sample extract/standard))/(Absorbance of ABTS radical + methanol)]×100. Vitamin C at concentrations of 0, 4, 8, 12, 16, 20 and 24 ug/mL was used as a standard substance.

Ferricyanide reducing antioxidant power assay

The Job's tears sample was analyzed using ferricyanide in a reducing antioxidant power assay (FRAP) method adapted from El Jemli *et al.* [21] A solution of the Job's tears sample and potassium ferricyanide [$K_3Fe(CN)_6$] was incubated at 50 °C for 20 min. Afterwards, trichloroacetic acid was added to the solution and centrifuged at 1,000 rpm for 10 min. The supernatant was taken and mixed with distilled water together with ferric chloride ($FeCl_3$). Using ascorbic acid at concentrations of 0, 50, 100, 200, 300, 400 and 500 ug/mL as the standard, the absorbance was measured at 700 nm.

Data analysis

All data were analyzed by using R statistics [22]. Duncan's new multiple range test at a confidence level of 95 % was used to compare the mean differences between the varieties.

Results and discussion

Plant growth

Table 1 shows the plant growth characteristics of 7 varieties of Job's tears. The germination date of all the varieties was in the range of 10 to 42 days after planting. AL3 and AL4 were able to germinate faster than the other varieties. The average plant height of was in the range of 85.7 to 157.1 cm. The plant heights of AL1 to AL2 were higher than for the AL3 - AL7 varieties. According to the age after germination of each variety, Job's tears had different growth stages and plant heights depending on the variety. The average leaf areas of the edible group (AL1 to AL4) were higher than AL5, AL6 and AL7 (the ornamental group). This corresponds to Ruminta *et al.* [11] which showed that Job's tears with high plant height also had high biomass. The average 100 seed weight of each cultivar was obtained after harvesting. AL2 and 4 were the highest followed by AL3, AL1, AL5, AL7 and AL6, respectively. The seed weights of AL5, AL6 and AL7 were similar because their seeds were small as they were in the ornamental group. All the varieties of Job's tears had tillering and branching while AL2, AL5, AL6 and AL7 had the highest number of tillers. The tiller number depended on the row spacing [11]. The number of branches for AL1, AL2 and AL5 were 2 - 3 branches while the others were 3 - 4 branches. Thus, AL1 and AL2 varieties might be suitable for forage utilization.

Table 1 Plant growth characteristics of 7 varieties of Job's tears.

Variety	Germination (days)	Plant height* (cm)	Leaf area* (cm^2)	100 Seed weight* (g)	Tiller number	Branch number
AL1	22	154.0 ± 3.3	1,528.0 ± 210.2	11.3 ± 1.0	3 - 4	2 - 3
AL2	31	157.1 ± 9.7	1,714.0 ± 256.5	31.4 ± 1.2	4 - 6	2 - 3
AL3	11	108.2 ± 3.8	1,635.5 ± 136.0	17.0 ± 0.8	2 - 4	3 - 4
AL4	10	125.7 ± 8.0	1,425.9 ± 204.4	32.6 ± 1.8	1 - 3	3 - 4
AL5	39	108.6 ± 25.7	530.7 ± 206.4	9.1 ± 0.5	4 - 6	2 - 3
AL6	42	106.0 ±16.1	481.6 ± 120.8	8.9 ± 0.7	4 - 6	3 - 4
AL7	35	85.7 ± 6.4	681.6 ± 116.8	9.1 ± 0.6	4 - 6	3 - 4

*mean ± SD

Table 2 Flower characteristics of 7 varieties of Job's tears.

Variety	Seed shape	Stigma color	Male inflorescences pedicel	50 % Flowering (days)	100 % Flowering (days)
AL1	Oval	Purple	Long	95	117
AL2	Globular with 1 flat side	White	Short	113	123
AL3	Oval	Purple	Long	123	139
AL4	Globular with 1 flat side	White	Short	120	129
AL5	Bottle	Purple	Short	93	95
AL6	Globular	Purple	Long	101	109
AL7	Globular	Purple	Long	93	119

The female flower characteristics of each variety (**Table 2**) show that the stigma of almost all varieties are purple, except for AL2 and AL4 which are white. The shape of the involucre or seed, which is a modified leaf sheath containing a kernel, is different. AL1 and AL3 were oval in shape. AL2 and AL4 were globular with 1 flat side while AL6 and AL7 were globular, but small in size. AL5 was a bottle shape. The male inflorescence of AL2, AL4 and AL5 had a short pedicel while the others had a long pedicel. The flowering duration of AL5 was shorter than the other varieties (3 days, from 50 to 100 % flowering), followed by AL6. The flowering duration of AL7 was longest. However, the 50 % flowering phases of AL3 and AL4 were more delayed than the other varieties (**Table 2**). However, Job's tears are a quantitative short-day plant that generates fewer flowers when the daylight time is less than 12 h [23]. The 50 % flowering duration of Job's tears in India was 92 - 115 days after planting [8]. While in the Philippines it was 72 - 86 days after planting with maturity at 135 - 148 days after planting [12]. Job's tears are normally harvested at 4 - 6 months after sowing, depending on the cultivar and the season [24]. This study found that the plant height, leaf areas and tiller numbers of the AL2 variety were high with large seeds. Therefore, the AL2 variety showed good adaptability in the Sa Kaeo province. Although the germination period is longer for the AL1 variety and has a later flowering period than any other variety. Therefore, AL2 variety are suitable for planting in the Sa Kaeo province followed by AL1. The seeds of all the varieties were green at beginning. Afterwards, the color of AL1 changed to gray with black streaks on maturity. AL2 and AL4 changed to dark yellow then dark brown with cream. The AL3 seeds were green which turned red-brown and black when ripe, with a smooth and shiny. The AL5 seed was pale yellow which turned light brown and black when ripe, with a smooth and shiny skin. AL6 and AL7 were cream and light brown, respectively, with a smooth and shiny skin (**Figures 1** and **3**). Numerous pigments are produced by the plants [25] such as green chlorophyll, yellow, orange, red, blue or brown. Compared to AL2 and AL4, AL1 and AL3's kernel colors were brighter and more yellow-hued (**Table 1**), while AL2 and AL4 displayed red-brown kernels, AL1 and AL3 displayed brown kernels. The yellow of the leaves is caused by carotenoids and flavones and brown is caused by fucoxanthol, while anthocyanins and phycobilins are responsible for the red and blue hues [25]. The percentage of the kernels of AL1 to AL4 varieties were 63.1, 40.9, 65.1 and 49.9 %, respectively (data not shown). These are the amounts of the kernels after harvesting.

Seed and kernel color

The colors of kernels of AL1 to AL4 varieties are shown in **Figure 4** and **Table 3**. The colors of AL1 to AL4 seed varieties are shown in **Figure 1** and **Table 3**. The colors of the kernels and seeds were consistent. All the characteristics of the kernels and seed colors of 4 varieties of Job's tears were significantly different. The brightness (L*), redness (a*), yellowness (b*) and hue (H^0) of the kernels of 4 varieties of Job's tears were significantly different that of the other varieties. The color of kernels AL1 and AL3 were yellower (b*) and brighter (L*) than AL2 and AL4. AL1 and AL3 had brown kernels but AL2 and AL4 had a reddish-brown kernel. AL1 was the brightest (L*) of all the seed varieties. AL2 was the reddest (a*), the most yellow (b*) and its apparent color intensity (C*) was the highest. Compared to the study of Xi *et al.* [26], we found that the seed color of AL2 and AL4 was similar to the seed color of wild accessions from China whereas the seed color of AL3 was similar to the seed color of Job's tears accessions from northern China. The kernel color of AL1 and AL3 was similar to Job's tears accessions from central China and southern China while the kernel color of AL2 and AL4 was similar to Job's tears accessions from northern China.

Figure 4 Kernel color of 4 varieties of Job's tears.

Table 3 Kernel and seed color characteristics (mean ± SD) of 4 varieties of Job's tears.

Variety	L*	a*	b*	C*	H^0
Kernel					
AL1	42.76 ± 0.36^a	9.54 ± 0.04^b	24.50 ± 0.42^a	26.29 ± 0.41	68.73 ± 0.25^a
AL2	33.47 ± 1.38^b	14.52 ± 0.23^a	18.80 ± 0.14^b	23.76 ± 0.02	52.32 ± 0.64^b
AL3	43.77 ± 1.72^a	10.01 ± 0.09^b	26.27 ± 0.69^a	28.11 ± 0.61	69.15 ± 0.66^a
AL4	35.33 ± 0.95^b	14.09 ± 1.48^a	20.40 ± 1.45^b	24.79 ± 2.04	55.40 ± 0.92^b
p-value	**	**	**	ns	***
Seed					
AL1	53.01 ± 1.84^a	0.49 ± 0.22b	1.39 ± 0.32^b	1.48 ± 0.36^b	71.14 ± 4.79^a
AL2	45.70 ± 1.82^b	3.31 ± 0.70a	6.64 ± 1.88^a	7.42 ± 2.00^a	63.16 ± 1.82^{ab}
AL3	41.79 ± 0.34^{bc}	1.66 ± 0.29b	2.74 ± 0.40^b	3.21 ± 0.49^b	58.96 ± 1.26^{ab}
AL4	38.78 ± 1.26^c	1.23 ± 0.27b	1.70 ± 0.75^b	2.11 ± 0.75^b	52.31 ± 6.65^b
p-value	***	***	***	***	***

* significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$; Different letters in the same column represent significant differences

Chemical composition

The chemical composition of the kernels and hulls of 4 varieties of Job's tears (edible group) are shown in **Table 4**. The DM, OM, CP, ash and EE of the kernels of 4 varieties of Job's tears contained between 92.09 to 93.89 %DM, 97.64 to 98.04 %OM, 13.46 to 17.78 %CP, 1.96 to 2.36 %ash and 8.61 to 10.47 %EE. The results show that the kernels of AL1 were the highest in OM and EE and also the lowest in ash and AL1 was also higher in DM than AL3 and AL4 ($p < 0.05$). The hulls of 4 varieties of Job's tears ranged from 94.08 to 95.77 %DM, 75.47 to 88.68 %OM, 1.5 to 2.0 %CP, 11.32 to 24.53 %ash and 0.32 to 0.65 %EE, respectively. AL4 had the lowest moisture content considering the DM, together with the lowest ash content resulting in the highest OM content ($p < 0.05$). In previous studies, it was found that the nutritional profile of the kernels of 32 accessions included moisture at 11.3%, of CP at 15.9 %, 3.34 %ash, 4.66 %crude fat and 5.53 % dietary fiber [4]. White whole grain flour had 13.54, 4.86 and 1.74 %CP, lipid and ash, respectively, while white degermed flour from Job's tears had 13.81, 0.91 and 0.59 % [3]. Department of Livestock Development [27] reported that the nutrients in Job's tears in terms of seed and husk. Job's tears' seeds had 89.80 %DM, 15.86 %CP, 1.79 %ash, 4.68 %EE, 4.31 %ADF, 23.40 %NDF, 0.05 %Ca and 0.37 %P, whereas Job's tears husks had 90.73 %DM, 15.71 %CP, 27.09 %EE and 4.68 %CF. The protein of raw Job's tears seeds was 13.6 g/100 g per edible portion [28]. Ding *et al.* [29] reported that the seeds of Job's tears which had been soaked at different temperatures contained an average minimum and maximum of CP, lipid and moisture at 13.60 - 13.70 g/100 g, wet base, 6.32 - 6.39 g/100 g, wet base and 28.08 - 35.83 g/100 g, wet base, respectively. The amount of nutrients in each form of feed varies because each type of feed contains a different amount of water [30]. Depending on the temperature and maturity, the water content of forage changes throughout the day. To compare the nutritional value of animal feed, it is necessary to adjust the animal feed to the same condition first and express it in terms of DM [30]. The average CP of the kernels of 4 varieties were all higher than the hulls (**Table 4**). Significant differences in the CP of 4 varieties of the both kernels and hulls of Job's tears varieties were observed ($p < 0.05$), which had a mean value of 14.92 and 2.69 %CP, respectively. The kernel of AL4 was the highest CP. The hull of AL3 showed the highest CP (3.99 %CP), followed by AL1, AL4 and AL2 respectively with significantly different ($p < 0.05$). Since nitrogen is a crucial component of proteins, the availability of nitrogen in the soil is necessary for plants to produce proteins. However, the age, type and variety of the crop are the main determinants of the protein content in forage. The Bureau of Animal Nutrition Development [30] stated that mold and aflatoxins are produced by the high moisture content of the hull of 4 varieties of Job's tears, which is detrimental to both animals and consumers. The hulls of 4 varieties were low in protein, but the hulls were high in ash and could have been contaminated by clay or sand. The ash content in the hulls of 4 varieties of Job's tears was exceptionally high, especially in AL1 followed by AL2, AL3 and AL4, respectively. According to Kochhar [25], the ash content of plants varies depending on the plant species and tissue divisions. Ash is beneficial to plants that grow in dry or saline soils where ash can account for 4 % or more of the plant's fresh weight. Because Job's tears are a field plant that grows in upland areas or areas with slopes, good drainage and no flooding, farmers tend to plant on hillside slopes outside the irrigation zone, relying only on seasonal rainwater. Job's tears require little water. With regard to climatic data for the hulls of AL1, AL2, AL3 and AL4 varieties, AL1 is cultivated in Luang Prabang province, Lao People's Democratic Republic with an average rainfall of 739.8 mm per year with temperatures between 14 - 34 °C [31]. AL2 is cultivated in Nong Khai province, Northeast Thailand with an average rainfall of 1,105.0 mm per year and temperatures between 17 - 35 °C [32]. AL3 is cultivated in Loei province which is in upper Northeast Thailand with an average rainfall of 1,022.8 mm per year, temperatures between 15 - 35 °C and AL4 is also cultivated in Loei province [33]. Based on the findings of this investigation, the average amount of ash in the hulls of AL1, AL2, AL3 and AL4 was approximately 10.65, 23.46, 16.71 and 12.87 % of plant fresh weight, respectively. In this study, the ash contents of the kernels of AL1, AL2, AL3 and AL4 Job's tears were 2.07, 1.84, 2.20 and 2.11 % of plant fresh weight, respectively, which had a higher moisture content than the hulls (See **Table 1**), which is consistent with data indicating that succulent tissue and fresh fruit have low mineral content [25].

In the kernel, A1 had the highest EE. The EE of the hulls in AL2 was lower than those in groups AL1, AL3 and AL4. Both of kernels and hulls of AL2 had significantly lower EE than AL1, AL3 and AL4 ($p < 0.05$). Job's tears have a higher EE content in the kernel than in the hull (**Table 4**), according to Chaisiricharoenkul *et al*. [3] who reported that the lipids of whole grain Job's tears have both white and black varieties which were higher than degermed Job's tears flowers. The fat of raw Job's tears seeds was 3.7 g/100 g edible portion with an energy at 365 Kcal [28]. The Department of Livestock Development [27] reported that the seeds of Job's tears had 4.68 %EE, while wheat bran had 3.48 %EE and sorghum (*Sorghum bicolor*) had 5.71 %EE. Compared to rice and wheat, Job's tears are richer in nutrient grains in terms of both fat and protein [1]. Fat and oil are almost exclusively found in seeds, while fat and oil can be a significant dietary reserve in some particular seeds [25]. They have a higher EE content in the kernels than in the hulls. The kernels are more likely to become rancid especially AL1 when used in animal feed due to its high EE content and the likelihood that animals do not like consuming also it is not possible to maintain it for extended periods of time [30]. The properties of this high fat content are similar to rice bran (fine bran), which had a high fat content of more than 15 %, exceeding the standard criteria [30]. Paengkoum [34] stated that roughage of ruminants generally contains approximately 1 - 2 % fat, less than the concentrate diet of about 3 - 4 %. Supplementation with high levels of fat in ruminants' diets will have an effect on the microorganisms in the rumen, directly reducing the digestibility of the fiber and the palatability of the feed. The fat coats the plant cell walls, which makes it difficult for microorganisms in the rumen to digest it. Raising ruminants in the tropics requires a supplementary diet of protein and energy sources. Due to the problem of low quality of roughage (grass), digestibility in the rumen and feed intake are low, affecting production performance and yield due to insufficient protein or nitrogen and energy to meet the animals' requirements. Microbes in the rumen absorb nutrients and utilize them in a metabolic process which enables the ruminants to generate further products, microorganisms produce end products, specifically volatile fatty acids (VFAs) including acetic acid (C_2), propionic acid (C_3) and butyric acid (C_4).

Table 4 The chemical composition in the kernels and hulls of 4 varieties of Job's tears.

Characteristics	Part use	Variety				*p*-value
		AL1	AL2	AL3	AL4	
Dry matter	Kernel	93.89 ± 0.59[a]	92.90 ± 0.50[ab]	92.38 ± 0.07[b]	92.09 ± 0.20[b]	**
	Hull	95.67 ± 0.13[a]	95.75 ± 0.26[a]	95.77 ± 0.47[a]	94.08 ± 0.09[b]	***
Organic matter[1]	Kernel	98.04 ± 0.07[a]	97.64 ± 0.10[b]	97.72 ± 0.04[b]	97.75 ± 0.04[b]	***
	Hull	75.47 ± 0.13[d]	82.55 ± 0.24[c]	86.56 ± 0.61[b]	88.68 ± 0.08[a]	***
Crude protein	Kernel	13.92 ± 0.03[c]	13.46 ± 0.14[d]	14.50 ± 0.08[b]	17.78 ± 0.18[a]	***
	Hull	3.21 ± 0.08[b]	1.46 ± 0.04[d]	3.99 ± 0.09[a]	2.11 ± 0.05[c]	***
Ash	Kernel	1.96 ± 0.07[b]	2.36 ± 0.10[a]	2.28 ± 0.04[a]	2.25 ± 0.04[a]	***
	Hull	19.78 ± 0.18[a]	7.42 ± 0.05[d]	13.78 ± 0.21[b]	11.34 ± 0.11[c]	***
Ether extract	Kernel	10.47 ± 0.62[a]	8.83 ± 0.06[b]	8.61 ± 0.09[b]	9.22 ± 0.12[b]	*
	Hull	0.53 ± 0.04[a]	0.32 ± 0.10[b]	0.58 ± 0.04[a]	0.65 ± 0.02[a]	*
Total carbohydrate[2]	Kernel	67.47 ± 1.38[a]	68.03 ± 0.30[a]	66.93 ± 0.16[a]	62.70 ± 0.05[b]	**
	Hull	76.48 ± 0.22[d]	90.80 ± 0.01[a]	81.65 ± 0.33[c]	85.90 ± 0.07[b]	***
Neutral detergent fiber	Kernel	26.98 ± 0.14[a]	22.19 ± 0.27[c]	25.95 ± 0.09[b]	22.58 ± 0.35[c]	***
	Hull	78.83 ± 1.19[b]	82.32 ± 0.57[a]	78.18 ± 0.52[b]	83.72 ± 0.66[a]	***
Acid detergent fiber	Kernel	7.25 ± 0.18[a]	5.24 ± 0.03[c]	6.13 ± 0.33[b]	5.15 ± 0.11[c]	**
	Hull	57.99 ± 0.10[b]	61.50 ± 0.01[a]	53.33 ± 0.19[c]	61.31 ± 0.08[a]	***
Acid detergent lignin	Kernel	1.65 ± 0.21	1.34 ± 0.23	1.74 ± 0.03	1.33 ± 0.16	ns
	Hull	14.74 ± 0.13[d]	17.15 ± 0.06[b]	15.68 ± 0.08[c]	18.80 ± 0.21[a]	***
Non-fiber carbohydrate[3]	Kernel	46.73±0.46[c]	53.08±0.34[a]	48.68±0.08[b]	48.33±0.21[b]	***
	Hull	(tr)[c]	8.54 ± 0.80[a]	3.53 ± 0.39[b]	2.00 ± 0.90[b]	**
Cellulose[4]	Kernel	19.65 ± 0.09[a]	17.08 ± 0.17[b]	19.78 ± 0.27[a]	17.25 ± 0.12[b]	***
	Hull	21.20 ± 1.32[b]	20.77 ± 0.79[b]	24.80 ± 0.92[a]	22.59 ± 0.75[ab]	*
Hemicellulose[5]	Kernel	5.61 ± 0.39[a]	3.91 ± 0.21[b]	4.39 ± 0.36[b]	3.83 ± 0.28[b]	*
	Hull	43.26 ± 0.04[b]	44.35 ± 0.07[a]	37.65 ± 0.28[d]	42.52 ± 0.29[c]	***

[1]Organic matter: %dry matter-%ash; [2]Total carbohydrate: 100-(%crude protein+%ether extract+%ash+%moisture); [3]Non-fiber carbohydrate: 100-(%crude protein+%ether extract+%neutral-detergent fiber+%ash); [4]Cellulose: %acid-detergent fiber-%acid-detergent lignin; [5]Hemicellulose: %neutral-detergent fiber-%acid-detergent fiber; tr: trace; ns: non-significance; * significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$; Different letters in the same row represent significant differences

The kernel of AL1, AL2 and AL3 had the highest total carbohydrate (TC), while the hull of AL1 had the lowest. The kernel of AL2 had the highest non-fiber carbohydrate (NFC), the hulls of AL2 were the highest of NFC. The NDF of the kernels AL1, AL2, AL3 and AL4 ranged from 22.19 to 26.98 % (**Table 4**). The AL1 kernel had the highest NDF and ADF, followed by AL3, whereas AL2 and AL4 were the lowest. The hemicellulose content of the kernels of AL1 was the highest and AL1 and AL3 had more cellulose than AL2 and AL4 (**Table 4**). All the hulls had high fiber in terms of NDF and ADF. AL2 and AL4 had the highest amounts of NDF and ADF. None of the kernels showed any significant differences in

ADL, while part of the hull in AL4 showed the highest ADL. AL1 in the kernel contains a high level of NDF, ADF, cellulose and hemicellulose. All the hulls contain a high level of NDF and ADF, especially AL2 and AL4. Forage plants contain fibre ranging from 23 - 90 % [35]. Cellulose of ten grass species grown in meadows has been found to range from 33.38 to 38.68 % [36]. In the rumen fermentation process of ruminants, microbes degrade structures of carbohydrate (plant cell wall) and generate VFA, which serve as an energy source [37]. Ruminants rely on these VFAs as a major energy source for both sustenance and meat and milk production. Ruminant animals receive large amounts of plant leaves, resulting in a low digestibility rate and the feed remains in the rumen for a long time, resulting in a decrease in the cattle's total energy intake. Cellulose may limit the energy level in the diets of free-grazing ruminants unless energy feed is supplemented. The non-structural carbohydrate (NSC) is a part of NFC which includes sugar, starch, various organic acids and pectin, while the value of NFC is similar to NSC in dairy cows [38]. NSC is an important source of energy that results in high production which can be easily degraded in the rumen and is proportional to the amount of NDF. Where the animal feed contains a high amount of NDF, the amount of NSC will be low as well. The fiber is inversely related to the protein content. The fiber content of plants increases with age. Both kernels and hulls had lower cellulose than the stem and leaves [39], which usually has about 70 % cellulose content in fresh leaves. All the kernels and hulls contained similar amounts of NDF and ADF when compared to the seeds [27]. South and East Asia are indigenous regions for Job's tears. The tropics and subtropics currently produce Job's tears as a minor cereal crop [1]. In comparison to temperate forage grass, tropical forage grass has higher silica and ADL in the ash and fiber, according to Wilaipon [40]. In general, feed digestibility decreases as the percentage of fiber in the feed increases. Feed components that contain a ratio of lignin fiber will result in decreased digestibility in ruminant animals. Lignin may act as a wrapper around the cellulose and hemicellulose surface structure, preventing microorganisms in the rumen from digesting them. Nonetheless, the digestibility of cellulose and hemicellulose can be increased when feed is ground very finely [41]. Furthermore, the forage production of Job's tears should be investigated as it could generate more tillers after several cuts [42].

Antioxidative activity

There were significant differences between the varieties in TPC, TFC and antioxidant activities (**Table 5**). The seed and kernel weights of AL1 were the lowest. This accords with the kernel size in **Figure 1** which is smaller than other varieties. Similarly, the seed and kernel weight of AL4 were highest according to the kernel size. The content of TPC and TFC in the kernels of AL1 and AL4 were higher than in the hulls while TPC and TFC of AL2 and AL3 showed the opposite content. The content of TPC and TFC in the kernels of AL1 and AL4 were higher than in AL2 and AL3. Previous studies reported that the TPC of the kernels was 7.3 - 20.5 mg GAE/g [3,5] and the TFC of the kernels was 11.5 mg RE/g, [5]. The secondary metabolites such as phenolic compounds and flavonoids are synthesised when plants are under stress [43]. Most varieties are cultivated under environmental stress such as drought, floods or dry conditions [23]. This might explain why some varieties produce high levels of TPC or TFC. The husk after shelling of some plants such as cacoa [44] and brazil nuts [45] have antioxidants which can be extracted and for utilized. Zeng *et al*. [2] showed that the flavonoid content in hulls was lower than in the kernels while phenol in the hulls was higher than in the kernels. The hulls in AL2 had the lowest in content of TPC and TFC compared to the other varieties ($p < 0.001$) (**Table 5**). It is still not possible to clearly conclude that AL1 to AL4 varieties have hulls with a lower TFC than in the seeds of the kernels. **Table 5** shows that only the content of TFC in the hulls of AL1 and AL4 were lower than in the kernels and similar to the TPC. The TPC in the ungerminated kernel seeds of AL1, AL2, AL3 and AL4 varied widely ranging from 46.15 ± 17.98, 0.16 ± 0.06, 0.22 ± 0.01 and 24.55 ± 0.16 mg GAE/g, respectively (**Table 5**). Khampang *et al*. [46] found that when Thai rice and corn germinated, more total phenolics were detected at 0.73 and 0.60 mg/100 g, respectively, compared to the ungerminated rice and corn at 0.29 and 0.58 mg/100 g, respectively. DPPH of kernels was not different between varieties. DPPH of hulls of AL3 and AL1 were the highest. AL4 showed the highest FRAP both in kernel and hull. The antioxidant activity of the kernels of 4 varieties was high which was similar to that reported by Chhabra and Gupta [5] who stated that the DPPH of kernel from India were 91.35 %. In contrast, the report of Chaisiricharoenkul *et al.* [3] that the DPPH of the kernels in Thailand was 5.4 - 6.5 %. Tseng *et al*. [47] found that the DPPH of kernels from Taiwan was 16 %. The DPPH of the hulls was lower than that of the hulls from Taiwan (65 %) [7]. Nevertheless, DPPH of the hull of AL3 and AL1 were higher than DPPH in kernels in the reports of Chaisiricharoenkul *et al.* [3] and Tseng *et al*. [47]. This shows that antioxidant activity depends on the different varieties and the environment. The TPC and TFC of the kernel showed a positive correlation with DM, OM, EE and HEM ($p < 0.05$) (**Table 6**). But TPC and TFC of the kernel showed a negative correlation with ash. Both kernel and hull showed a strong positive correlation between TPC and TFC with EE. There was not any significant correlation with antioxidants (DPPH, ABTS and FRAP) of the kernels and hulls (data not shown).

Table 5 Total phenolic content, total flavonoid compound, DPPH free radical scavenging activity (DPPH), ABTS radical cation decolorization scavenging activity (ABTS) and ferricyanide reducing antioxidant power (FRAP) (mean ± SD) in the kernels and hulls of the different varieties.

Characteristics	Part use	Variety				*p*-value
		AL1	AL2	AL3	AL4	
TPC (mg GAE/g)	Kernel	46.15 ± 17.98^{a}	0.16 ± 0.06^{b}	0.22 ± 0.01^{b}	24.55 ± 0.16^{ab}	***
	Hull	1.42 ± 0.03^{b}	0.79 ± 0.10^{c}	1.73 ± 0.02^{a}	1.72 ± 0.17^{a}	***
TFC (mg QE/g)	Kernel	13.64 ± 0.61^{a}	0.05 ± 0.03^{c}	0.54 ± 0.04^{c}	6.05 ± 0.61^{b}	***
	Hull	0.45 ± 0.01^{c}	0.18 ± 0.01^{d}	0.61 ± 0.02^{a}	0.54 ± 0.04^{b}	***
DPPH (%)	Kernel	58.35 ± 2.26	58.67 ± 1.42	58.83 ± 2.89	56.17±5.08	ns
	Hull	30.67 ± 8.79^{ab}	11.28 ± 4.58^{c}	38.21 ± 17.96^{a}	14.89 ± 1.01^{bc}	*
ABTS (%)	Kernel	43.84 ± 7.57	46.09 ± 3.45	47.00 ± 1.66	39.42 ± 4.50	ns
	Hull	6.32 ± 0.16^{b}	6.10 ± 0.03^{b}	6.18 ± 0.05^{b}	96.07 ± 2.53^{a}	***
FRAP (mgVitC/g)	Kernel	1.42 ± 0.27^{b}	0.26 ± 0.11^{c}	0.43 ± 0.00^{c}	2.48 ± 0.18^{a}	***
	Hull	0.75 ± 0.02^{b}	0.44 ± 0.03^{c}	0.97 ± 0.04^{b}	1.85 ± 0.19^{a}	***

TPC: total phenolic content; TFC: total flavonoid compounds; ns: non-significance; * significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$; Different letters in the same row represent significant differences.

Table 6 The correlation coefficients between total phenolic content, total flavonoid compounds, antioxidant activity and chemical composition of the kernels and hulls.

Items	DM	Ash	OM	CP	EE	NDF	ADF	ADL	CEL	HEM
					Kernel					
TPC	0.73*	–0.76*	0.76*	0.12	0.78*	0.44	0.65	0.01	0.28	0.74*
TFC	0.69	–0.90**	0.90**	0.08	0.92**	0.52	0.71*	0.18	0.36	0.76*
DPPH	0.21	–0.21	0.21	–0.60	0.07	0.45	0.42	0.67	0.43	0.29
ABTS	–0.14	–0.17	0.17	–0.37	0.18	0.35	0.20	0.52	0.42	0.07

Items	DM	Ash	OM	CP	EE	NDF	ADF	ADL	CEL	HEM
FRAP	–0.08	–0.23	0.23	0.85**	0.36	–0.18	–0.07	–0.29	–0.24	0.01
					Hull					
TPC	–0.51	–0.39	0.39	0.65	0.90**	–0.19	–0.50	0.01	0.70	–0.65
TFC	–0.38	–0.34	0.34	0.79*	0.86**	–0.37	–0.67	–0.13	0.76*	–0.78*
DPPH	0.24	0.37	–0.37	0.69	0.28	–0.73*	–0.66	–0.69	0.25	–0.44
ABTS	–0.94***	–0.63	0.63	–0.34	0.57	0.72*	0.48	0.83*	0.08	0.13
FRAP	–0.91***	–0.65	0.65	0.00	0.78*	0.45	0.16	0.64	0.34	–0.19

*, **, *** indicates a significant correlation at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

Conclusions

The characteristics of AL1 to AL4 varieties (in the edible group) showed better adaptability (e.g. plant height and leaf areas) than AL5 to AL7 in Sa Kaeo province, East Thailand. The vegetative part (stem and leaves) of AL1 to AL4 varieties is usually used as roughage for ruminant animal feed. AL4 in kernels had the proper chemical properties in terms of CP which were greater than those of AL3, AL1 and AL2 respectively. This study recommends that AL4 is suitable for cultivation for seed harvesting for use as a functional food. In a further study, AL1 to AL4 should be evaluated for ruminant animal forage.

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