

SPROUTS CONTAIN HIGHER TOTAL PHENOLICS, TOTAL FLAVONOIDS, AND ANTIOXIDANT ACTIVITY THAN SEED IN DIFFERENT THAI VARIETIES OF MUNGBEANS (*Vigna Radiata* L.)

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Abstract: It has been reported that mungbean seeds contain fat, protein, lecithin and amino acids, but the study of antioxidants and their activity in those seeds, particularly in comparison to the sprouts of Thai mungbean varieties, is rare. This research studied the antioxidants and antioxidant activity in seeds and sprouts of five Thai mungbean varieties (*Vigna radiata* L.), namely Chainat 36, Chainat 72, Chainat 84-1, M.T.S 1 and Kamphaengsaen 2. Total phenolics, total flavonoids, chlorophyll *a*, chlorophyll *b*, total chlorophyll and antioxidant activity (DPPH) in the seeds and sprouts were analyzed. The experiment protocols were arranged as a completely randomized design (CRD) (n=4). There was low variation of total phenolics, total flavonoids, chlorophyll *a*, chlorophyll *b*, total chlorophyll and DPPH radical scavenging in the seeds of all mungbean varieties. However, they increased sharply with germination. At Day 4, Chainat 36 sprouts were found to contain the highest total phenolics and total flavonoids (3,372.8 mg GAE/100g of DW and 6,808.6 mg QE/100g of DW, respectively), which were significantly higher than those in seeds and sprouts at other germination stages. The total phenolics in Chainat 36 also increased sharply by almost six-fold compared with the previous day, whereas it was three-fold in other varieties. The DPPH radical scavenging in the seeds of all varieties was low, but increased sharply during germination. The sprouts at Day 4 had significantly higher antioxidant activity than in seeds by almost 30-fold. At the same day, the highest DPPH radical scavenging was observed in the sprouts of M.T.S 1. In conclusion, antioxidants and their activity are low in mungbean seeds. After germinating, especially at Day 4, total phenolics and total flavonoids would rise significantly, especially in Chainat 36. This indicated that Thai mungbean sprouts are a good source of nutrition and health.

Keywords: Phenolics, flavonoids, antioxidant activity, mungbean, seed, sprout.

Introduction

Oxidants are unstable molecules that are sensitive to chemical reaction and harmful to human health (Pizzino *et al.*, 2017). They can continuously leave a deteriorative effect on cells and tissues (Wu *et al.*, 2013). The human body is constantly exposed to such elements and can neutralize its effects by using bioactive compounds (Pizzino *et al.*, 2017). Antioxidants can protect cells through three mechanisms, namely preventing, scavenging and chain-breaking of the oxidization process. Generally, bioactive compounds can be obtained by eating a good amount of fruits, vegetables, seeds and sprouts (Wu *et al.*, 2013).

Mungbeans (*Vigna radiata* L.; Fabaceae) are grown in countries like India, China and Thailand (Shi, 2016), which use it as an ingredient in many local dishes. It can be grown in all types of soil and under warm climate up to 35 °C. Mungbeans can be harvested after 75 to 90 days, which makes it a suitable rotation crop (Liu, 2006; Lim, 2012). They are commonly consumed as seeds and sprouts in Asia and Europe (Tang *et al.*, 2014). The mungbean seed contains 20-25 % protein and 55-65 % carbohydrate dry weight (Tang, 2014; Kudre *et al.*, 2013; Nair *et al.*, 2013). It also contains tannins, phytic acid, trypsin inhibitors and hemagglutinin (Lin, 1997). Most importantly, the seeds contain

bioactive compounds like flavonoids (flavones, isoflavones, and isoflavonoids), phenolic acids (gallic acid, vanillic acid, caffeic acid, cinnamic acid, protocatechuic acid, shikimic acid, p-hydroxybenzoic acid) and many other organic acids (Anwar *et al.*, 2007).

The Thai mungbean Chainat 84-1 is obtained by irradiating Chainat 36 with gamma-ray at 500 Gy. The seed is large and suitable for producing sprouts. Seed weight is 69 g / 1,000 seeds, which is higher than the original Chainat 36 and Kampangsan 1 varieties. When it is used to produce sprouts, its fresh weight is higher than the two latter varieties. The taste of the sprouts is also sweeter (Department of Agriculture, n.d.).

However, the study of antioxidants and antioxidant activity in Thai mungbean varieties is rare. Therefore, the objective of this research is to quantify the antioxidants and their activity in mungbean seeds and sprouts. It is hypothesised that there will be significant variation of antioxidants and antioxidant activity in seeds and sprouts of different mungbean varieties.

Materials and Methods

Experiment Setup

The experiment was designed according to a completely randomized design (CRD) protocol with four replications. Seeds of five mungbean varieties were used, namely Chainat 36, Chainat 72, Chainat 84-1, M.T.S 1 and Kamphaengsaen (KPS) 2. The seeds were placed in a moist environment and allowed to germinate for four days (Day 1 to Day 4).

Extraction of Crude Antioxidants

Antioxidants were extracted from seeds and sprouts daily throughout the germination period. A total of 5 g of mungbean seeds or sprouts were crushed and placed in Erlenmeyer flasks before adding 20 ml of 1 % hydrochloric acid in ethyl alcohol. The flasks were shaken before being allowed to rest at room temperature for 18 hrs. The solutions, which had a mungbean extract concentration of 0.25 g/ml, was then filtered and stored below 4°C.

Determination of antioxidants

Total phenolic content

The procedures to determine total phenolic content were optimized from Javanmardi *et al.* (2003). Standard solution of gallic acid was prepared at concentrations of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml. A stock solution was prepared by dissolving 0.051 g gallic acid powder in 25 ml water. The desired concentrations were achieved by taking the following volumes of the stock solution and topping them up with ethyl alcohol to 1 ml: 0.125 ml (for 0.025 mg/ml), 0.025 ml (0.05 mg/ml), 0.05 ml (0.1 mg/ml), 0.1 ml (0.2 mg/ml), 0.2 ml (0.4 mg/ml), 0.4 ml (0.8 mg/ml) and 0.8 ml (1.6 mg/ml).

A total of 50 µl of each mungbean extract was added to 2.5 ml of 10 % Folin-Ciocalteu reagent and shaken before leaving at room temperature for 15 min. Then 2 ml of 7.5 % sodium bicarbonate (Na₂CO₃) was added into the solutions before being shaken again and left at room temperature for another 15 minutes. The solutions' absorbance was then measured using a spectrophotometer at a wavelength of 765 nm.

The total phenolic content for each extract was then derived from the absorbance curve of the standard solution. The results of all extracts were described as mg of gallic acid equivalent per one hundred grammes of dry weight (mg GAE/100g of DW).

Total Flavonoid Content

Determination of flavonoid content was optimized from Yang *et al.* (2009). A standard solution of Quercetin at concentrations of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/ml were prepared from a stock solution by mixing 0.05 g of Quercetin powder in 25 ml of distilled water. The desired concentrations were achieved by pipetting the following volumes from the stock solution and topped up with ethyl alcohol to 3 ml: 0.01875 mL (for 0.025 mg/ml), 0.075 ml (0.05 mg/ml), 0.15 ml (0.1 mg/ml), 0.3 ml (0.2 mg/ml), 0.6 ml (0.4 mg/ml), 1.2 ml (0.8 mg/ml) and 2.4 ml (1.6 mg/ml).

The sample solution at concentration

of 0.25 g/ml was pipetted 25 μ L. 1.25 ml of distilled water was filled in before adding 5% NaNO_2 75 μ L of 5% NaNO_2 . The solution was shaken and left at room temperature for five minutes. 10% AlCl_3 at was added and the volume was adjusted to 150 μ L. It was shaken before leaving at room temperature for 6 min. 500 mL of 1 M NaOH was added before adding 775 μ L distilled water. Then the sample was measured by a Spectrophotometer at 510 nm.

The total flavonoid content for each extract was then extrapolated from the absorbance curve of the standard solution. The results were described as mg Quercetin equivalent per 100 g of dry weight (mg QE/100g of DW).

Chlorophyll a, chlorophyll b, total chlorophyll determination

The seed coat of mungbeans contains chlorophyll and carotenoid. The protocols to determine the content of chlorophyll *a*, chlorophyll *b* and total chlorophyll were optimized from Sumanta *et al.* (2014). A total of 0.3 ml mungbean extract was pipetted into 2.7 ml of ethyl alcohol. The solution was subjected to spectrophotometer measurements at 664, 649 and 470 nm wavelengths. Chlorophyll concentrations were calculated according to Equations 1, 2 and 3.

$$\text{Chlorophyll a} = (13.36)(A_{664}) - (5.19)(A_{649}) \quad (\text{Eq 1})$$

$$\text{Chlorophyll b} = (27.43)(A_{649}) - (8.12)(A_{664}) \quad (\text{Eq 2})$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (\text{Eq 3})$$

where (A664) is the absorbance at 664nm wavelength and (A649) is the absorbance at 649nm wavelength.

Antioxidant Activity (DPPH Scavenging)

DPPH radical scavenging assay was modified from the methods of Chan *et al.* (2007). A standard solution of BHA and L-ascorbic acid was prepared at concentrations of 1, 2, 10, 20, 30 and 40 μ g/ml. The desired concentrations were

achieved by taking the following volumes of the stock solution and topping them up with ethyl alcohol to 1ml: 0.025 ml (for 1 μ g/ml), 0.05 ml (2 μ g/ml), 0.25 ml (10 μ g/ml), 0.5 ml (20 μ g/ml), 0.75 ml (30 μ g/ml) and 0.4 ml (40 μ g/ml).

The mungbean extracts were diluted from 0.25 g/ml to the following concentrations by taking a desired volume and topping it up with ethyl alcohol to 1 ml: 0.1 ml (0.005 μ g/ml), 0.5 ml (0.025 μ g/ml), 1.0 ml (0.05 μ g/ml), 2.0 ml (0.1 μ g/ml), 5.0 ml (0.25 μ g/ml), 10.0 ml (0.5 μ g/ml) and 20.0 ml (0.8 μ g/ml).

Then 3 ml of 0.1 mM DPPH 2,2-diphenyl-1-picrylhydrazyl was added and the mixtures were shaken for 30 minutes. The absorbance of the solutions were then measured with a spectrophotometer (517 nm) and the IC_{50} was established based on the BHA and L-ascorbic acid curve.

Data Analysis

The data were subjected to one-way analysis of variance (ANOVA) using the using STATISTIX 8 software. The means were compared using the Least Significant Difference (LSD) test ($P < 0.05$).

Results

Total Phenolic Content

Different varieties of mungbean contained different concentrations of total phenolics. Chainat 36, Chainat 72, Chainat 84-1 had the highest concentration of total phenolics at between 50.57 and 57.47 mg GAE/100 g of DW. The concentration of total phenolics in the sprouts at Day 4 was significantly higher than in seeds and sprouts at Day 1, 2 and 3. At Day 4, the sprouts of Chainat 36 had a total phenolic concentration of 3,372.8 mg GAE/100 g of DW, which was significantly higher than other varieties. Total phenolic content of Chainat 36 Day 4 sprouts also increased almost six-fold compared to Day 3, and was about three times higher than other varieties on Day 3 (Table 1).

Table 1: Total phenolic content in seed and sprouts of mungbeans at Day 1, 2, 3 and 4 of germination (mg GAE/100 g of DW)

Mungbean	Total phenolic content				
	Seeds	Sprouts			
		Day 1	Day 2	Day 3	Day 4
Chainat 36	44.72 bC	200.74 bC	326.92 bBC	548.21 bB	3,372.8 aA
Chainat 72	50.57 abE	207.04 abD	423.15 aC	680.63 aB	2,068.3 cA
Chainat 84-1	57.47 aD	211.75 abCD	345.10 bC	642.11 aB	2,764.6 bA
M.T.S 1	47.20 bD	196.36 bCD	324.30 bC	701.09 aB	2,483.4 bcA
Kamphaengsaen 2	51.50 abE	235.74 aD	354.01 bC	634.86 aB	1,324.3 dA

abc Means within a column with different upper case letters are significantly different at $P < 0.05$.

ABC Means within a row with different upper case letters are significantly different at $P < 0.05$.

Total Flavonoid Content

Total flavonoids in seeds were very low, ranging from 3.93 to 4.53 mg QE/100g of DW. Little variation was observed among the mungbean varieties. However, the concentration of total flavonoids increased sharply when the seeds began germinating. KPS 2 was observed to have total flavonoid content of 2,789.0 mg QE/100g of DW, while Chainat 36 had 6,808 mg QE/100g of DW. At Day 4, total flavonoids in sprouts were significantly higher than that in seeds and sprouts at Day 1, 2 and 3 of germination. Chainat 36 and Chainat 72 showed no significant differences on the concentration of

total flavonoids in seeds and sprouts at every day of germination (Table 2).

Chlorophyll a

Chlorophyll *a* concentration was quite low in seeds and sprouts, ranging from 0.36-0.42 $\mu\text{g}/\text{ml}$. M.T.S 1 contained significantly higher chlorophyll concentration (0.38 $\mu\text{g}/\text{ml}$) than other varieties, while Chainat 72 contained chlorophyll *a* at 0.33 $\mu\text{g}/\text{ml}$. There was not much difference of chlorophyll *a* in different varieties of mungbeans both in seed and sprout form. During germination from Day 1 to Day 4, the chlorophyll *a* content in sprouts was quite stable and almost the same in seeds (Table 3).

Table 2: Total flavonoid content in seeds and sprouts at Day 1, 2, 3 and 4 of germination (mg QE/100g of DW)

Mungbean	Total flavonoids				
	Seed	Sprout			
		Day 1	Day 2	Day 3	Day 4
Chainat 36	3.93abD	2,008.6 aC	1,730.9 bC	2,716.0 cB	6,808.6 aA
Chainat 72	4.53 aD	2,012.9 aC	2,384.4 aC	4,231.3 aB	4,823.9 bA
Chainat 84-1	3.41bcD	1,922.5 aC	1,868.6 bC	3,263.8 bB	5,271.9 bA
M.T.S 1	3.65 bcD	1,664.6 bC	1,852.4 bC	3,079.8 bcB	4,757.5 bA
KPS 2	3.18 cD	1,860.1 aC	1,805.7 bC	3,523.8 bA	2,789.8 cB
F-test	*	*	*	*	**
LSD	0.30	91.44	188.21	238.19	463.26
CV (%)	11.42	6.83	13.44	10.02	13.40

abc Means within a column with different upper case letters are significantly different at $P < 0.05$.

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Table 3: Chlorophyll *a* in seeds and sprouts of mungbean at Day 1, 2, 3 and 4 of germination ($\mu\text{g/ml}$)

Mungbean	Chlorophyll <i>a</i>				
	Seed	Sprout			
		Day 1	Day 2	Day 3	Day 4
Chainat 36	0.36bBC	0.32 abC	0.42 aA	0.37 aB	0.38 abB
Chainat 72	0.33cBC	0.31 bC	0.37 bA	0.38 aA	0.36 abAB
Chainat 84-1	0.35 bB	0.32 abB	0.39 abA	0.40 aA	0.39 aA
M.T.S 1	0.38 aA	0.35 aC	0.38 abA	0.38 aAB	0.35 bBC
KPS 2	0.35bAB	0.32 abB	0.38 bA	0.37 aAB	0.36 bAB
F-test	*	*	*	*	*
LSD	7.845E-03	0.01	0.01	0.02	0.01
CV(%)	3.10	6.31	6.95	8.43	5.68

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Chlorophyll *b*

Chlorophyll *b* in seeds of every mungbean variety was about 0.83-0.92 $\mu\text{g/ml}$. There was not much difference in the concentration of chlorophyll *b* among varieties. The concentration

of chlorophyll *b* increased after germination. At Day 4, chlorophyll *b* in sprouts increased to around 1.06-1.30 $\mu\text{g/ml}$, which was significantly higher than in seeds (Table 4).

Table 4: Chlorophyll *b* in seeds and sprouts at Day 1, 2, 3 and 4 of germination ($\mu\text{g/ml}$)

Mungbean	Chlorophyll <i>b</i>				
	Seed	Sprout			
		Day 1	Day 2	Day 3	Day 4
Chainat 36	0.89 abC	0.89 abC	1.09 aB	1.04 bB	1.20 aA
Chainat 72	0.83 cC	0.85 bC	0.99 bB	1.02 bAB	1.08 bA
Chainat 84-1	0.87 bD	0.88 abD	1.08 abC	1.19 aB	1.30 aA
M.T.S 1	0.92 abB	0.94 aB	1.02 abA	1.04 bA	1.05 bA
KPS 2	0.89 aB	0.89 abB	1.03 abA	1.06 bA	1.06 bA

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Total chlorophyll

Total chlorophyll in seeds was quite low in all varieties (1.16-1.30 $\mu\text{g/ml}$). It increased with time after germination. At Day 4, total chlorophyll in every variety was significantly

higher than in seeds, but the increase was not much. The biggest increase in total chlorophyll concentration between seeds and sprouts was observed in Chainat 84-1, where it jumped from 1.22 $\mu\text{g/ml}$ to 1.70 $\mu\text{g/ml}$ (Table 5).

Table 5: Total chlorophyll in seeds and sprouts at Day 1, 2, 3 and 4 of germination ($\mu\text{g/ml}$)

Mungbean	Total chlorophyll				
	Seed	Sprout			
		Day 1	Day 2	Day 3	Day 4
Chainat 36	1.25 bC	1.22 abC	1.51 aAB	1.40 bB	1.58 abA
Chainat 72	1.16 cB	1.16 bB	1.36 bA	1.40 bA	1.45 bcA
Chainat 84-1	1.22 bC	1.20 abC	1.47 abB	1.60 aA	1.70 aA
M.T.S 1	1.30 aB	1.29 aB	1.40 abA	1.41 bA	1.40 cA
KPS 2	1.25abBC	1.22 abC	1.41 abAB	1.44 abA	1.42 cAB

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Antioxidant Activity

The DPPH radical scavenging in seeds was low at about 28-37 mg. It was 28.11 $\mu\text{g/ml}$ in Chainat 72, 30.66 $\mu\text{g/ml}$ in Chainat 84-1 and 37.63 $\mu\text{g/ml}$ in Chainat 36. Chainat 72 and Chinat 84-1 contained significantly higher antioxidant activity than other varieties. During germination, antioxidant activity increased sharply, the

sprouts at Day 4 contained antioxidant activity significantly higher than that in seeds by almost 30-fold. At Day 4, the highest DPPH radical scavenging was found in sprouts of M.T.S 1, but it was not much different with other varieties. However, antioxidant activity in all sprouts increased quite steadily from Day 1 to Day 4 (Table 6).

Table 6: Antioxidant activity in seeds and sprouts at Day 1, 2, 3 and 4 of germination (mg/ml).

Mungbean	IC ₅₀				
	Seed	Sprout			
		Day 1	day 2	day 3	day 4
Chainat 36	37.63 aA	3.75 aB	2.80 aB	1.95 aB	0.90 cB
Chainat 72	28.11 cA	3.02 bcB	2.18 bC	1.44 cD	1.10 bD
Chainat 84-1	30.66 bcA	2.74 cdB	2.23 bBC	1.55 bcBC	1.52 aC
M.T.S 1	33.53 abA	2.46 dB	2.30 bBC	1.61 bBC	0.83 dC
KPS 2	33.07 abA	3.23 bB	1.97 cB	1.42 cC	0.94 cC
F-test	*	**	**	**	**
LSD	2.21	0.17	0.08	0.07	0.02
CV(%)	9.57	7.95	4.67	6.38	2.92

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Discussion

There was low variation of antioxidants, total phenolics, total flavonoids, chlorophyll *a*, chlorophyll *b* and total chlorophyll in seeds of four mungbean varieties in this study. This confirmed earlier observations by Ebert *et al.* (2017) that there was less variation in antioxidant activity among mungbean genotypes at sprouting stage, with the exception of VI000197 having inferior content. There was not much difference of chlorophyll *a*, *b*, and total chlorophyll concentration in different varieties of mungbean both in seeds and sprouts. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll were low and did not increase with germination day. Earlier observations in mungbean sprouts from Afghanistan, Taiwan, Thailand by Ebert *et al.* (2017) indicated that their total carotenoids were lower compared with grain.

During germination, especially at Day 4, total phenolic and flavonoid contents were very high, particularly in Chainat 36, which were almost 10- to 15-fold more than the seeds. This was probably due to the accumulation of reactive oxygen species (ROS) (Job *et al.*, 2005). Plants, therefore, would attempt to maintain the balance of ROS in cells by synthesizing phenolics and flavonoids (Chen *et al.*, 2014; Chen *et al.*, 2019). The increase of phenolics differed among the types of seed (Cevallos-Casals & Cisneros-Zevallos, 2010). One study had found it increasing by 2,010 % in sprouts and 11 % in kale at Day 7. The study by Nair *et al.*, (2015) identified ferulic acid (1,540-3,400 mg/g) as the phenolic compound in mungbean seeds. Kim *et al.* (2013) showed that the IT104818 variety of the Korean mungbean contained higher phenolic compounds than other varieties. The concentration of total phenolics and total flavonoids in the sprouts in this study could be clearly compared with other studies if the experiment was extended beyond Day 4.

The total phenolic content in seeds of all mungbean varieties in this study was significantly higher than the Chinese toon bud, perilla leaf, looses-trife, soybean (green) and

pepper leaf (Deng *et al.*, 2013). When compared with vegetables like the ruby red lettuce (483 mg GAE/100 g of DW), the total phenolic content was far lower, but it was still higher than the garden cress (12.8 mg GAE/100 g of DW) and wild rocket leaves (8.1 mg GAE/100 g of DW) (Santos *et al.*, 2014).

Total flavonoids in mungbean seeds was very low compared with sprouts at Day 4. This figures were comparable with the findings of a study on 11 vegetables from west Java, Indonesia, where the flavonoid content was between 4.0 and 832 mg QE /100 g dry weight (Jiménez-Aguilar and Grusak, 2017).

The DPPH radical scavenging in seed of every variety of mungbean was low, but sharply increased with germination. They increased with the increase of total phenolic and total flavonoid content. At Day 4, total phenolics, total flavonoids and antioxidant activity were highest in every mungbean variety. The result of this research was in contrast with the review and report by Ebert *et al.* (2017) on another antioxidant like vitamin C, which found the content to be considerably lower at the fully mature stage of sprouting. In this study, DPPH radical scavenging might have increased during germination from the increase of total phenolics and total flavonoids. In this study, antioxidant activity in mungbean seeds was 28.11-37.63 mg/ml, and it was 0.83-1.52 mg/ml in sprouts at Day 4. Whereas in leafy vegetable of *Alternanthera sessilis* in India, percentages of DPPH radical scavenging activity was 57.6 and 64.73 % after extraction by acetone and ethyl acetate, respectively (Kota *et al.*, 2017). However, little variation in antioxidant activity was found both in seeds and sprouts. Our results are in agreement with Ebert *et al.* (2017), who reported little variation of mungbean genotypes at the sprouting stage.

Conclusion

Antioxidants studied in mungbean seeds were low and there was low variation among varieties. Chainat 72 and Chinat 84-1 had the highest

seed antioxidant activity than other varieties, but still in low variation. During germination, especially on Day 4, total phenolic and total flavonoid contents were very high, particularly in Chainat 36. They were almost 10- and 15-fold compared with the seeds, respectively. Chainat 36, Chainat 72, and Chainat 84-1, contained the highest concentration of total phenolic and total flavonoid contents than other varieties.

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