Investigation of Using Beijing Grass Extract as a Natural Antioxidant in Edible Oil

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Abstract
Beijing grass [*Murdannia loriformis* (Hassk) Rolla Rao et Kammathy] has been widely used as a Thai medicinal plant. In this study, the antioxidant activity of the ethanolic extract of Beijing grass was evaluated using the DPPH assay. Furthermore, the extract was added into soybean oil and pork lard, which were incubated at 75°C for 14 days. Peroxide value and color of the oil were determined during the storage in order to screen suitability of the extract as a natural antioxidant in edible oil. The antioxidant activity of the Beijing grass extract at 200, 400, 600 and 800 ppm were 60.38, 70.13, 85.38 and 87.29%, respectively according to the DPPH assay. Protection factor was calculated to show a relative increase of the induction period of lipid oxidation due to the addition of antioxidant. The protection factors of the Beijing grass extract in pork lard at 200, 400, 600 and 800 ppm were 1.4, 1.6, 1.6, and 1.6, respectively. This suggested that the extract helped increase oxidative stability of the oil compared to the control without antioxidant. However, antioxidant efficiency of the Beijing grass extract was relatively low compared to synthetic antioxidant BHT which possessed the protection factor of 3.3. The same trend was observed in soybean oil in the way that Beijing grass extract at 200, 400, 600 and 800 ppm showed protection factor of 1.2, 1.2, 1.2, and 1.3, respectively. TBHQ, a synthetic antioxidant, again exhibited significantly higher antioxidant activity compared to the Beijing extract in soybean oil. Measuring the Hunter *L*, *a*, *b* values of the oil suggested that the Beijing extract caused the oil lightness to decrease, while the greenness and blueness increased. In conclusion, the Beijing grass extract showed great potential as an effective natural antioxidant to protect edible oil from lipid oxidation. However, the impact of the extract on the color of the oil is still a great concern that needs to be further investigated.

Keywords: Beijing grass, Antioxidant, Edible oil

1 Introduction

Lipid oxidation is a great concern in the edible oil industry. It is a free radical chain reaction triggered by light, heat, or transition metals. Once lipid hydroperoxide forms as a primary lipid oxidation product, it can be further broken down by β-scission reactions into numerous secondary lipid oxidation products such as aldehydes, ketones, acids, esters, and alcohols that are responsible for unpleasant rancid flavor in edible oil [1]. Moreover, lipid oxidation involves deterioration of fatty acids, especially polyunsaturated fatty acids, thus leading to nutrition losses. In addition, lipid oxidation generates potentially toxic compounds that relate to diseases such as atherosclerosis, asthma and cancer [2]–[5]. Therefore, protection of edible oil from lipid oxidation is a major challenge for the edible oil industry.

A common strategy that is widely applied to prevent lipid oxidation is incorporation of antioxidants. Synthetic antioxidants such as butylated hydroxyanisole (BHA),

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butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ), work effectively in lipid foods, but consumers have concerns regarding their safety [6]. Thus, there is a trend to replace synthetic antioxidants with natural compounds. An advantage of using natural antioxidants in foods is that they are not subject to any legislative restrictions. Moreover, the use of natural antioxidants in edible oil could be multifunctional by acting as a nutraceutical component to promote consumers' health as well [7]. In recent years, special attention has been paid to a number of plant extracts that could be used as antioxidants in food lipids. There are several reports that revealed the efficacy of different natural antioxidants for retarding lipids oxidation in edible oil [8]–[10]. However, most of the plant extracts showed relatively low antioxidant activities. Thus, there is continuing research seeking for a potent natural antioxidant as an alternative.

Murdannia loriformis (Hassk) Rolla Roa et Kammathy, commonly called Beijing grass, is a medicinal plant that has been used as an alternative remedy for the treatment of colds, throat infections, pneumonia, diabetes mellitus, flu and inflammation. It is also claimed to have anti-inflammatory and anti-carcinogenic properties [11]–[12]. A phytochemical study showed that Beijing grass contains phytosterylglycoside and glycosphingolipid, which elicited immunomodulatory properties and a moderate cytotoxicity against human breast cell lines and colon cancer cell lines [11]. In addition, the antioxidant activity of Beijing grass has been investigated. Pinitsoontorn and co-workers (2012) reported that Beijing grass tea extract contained a phenolic content of 46.96 mg gallic acid equivalent (GAE)/ g dry wt with antioxidant activity of 4.14 mmol Fe(II)/g dry wt evaluated by ferric reducing antioxidant power (FRAP) assay [13]. Klomsakul and co-workers (2012) determined the antioxidant activity of Beijing grass extract by DPPH (2, 2′-diphenyl-1-picrylhydrazyl) assay and found that Beijing grass at 500 μg/mL exhibited a scavenging effect of 91.50% [7]. Nevertheless, to our knowledge, none of studies investigated antioxidant activity of Beijing grass in a food system. Therefore, this study aims at evaluating antioxidant activity of ethanol extract of Beijing grass in different types of edible lipids including pork lard and soybean oil at various concentrations. Moreover, the influence of the extract on color of the oil was also investigated.

2 Experimental

2.1 *Materials*

Dried Beijing grass was purchased from Ban Dong Bang medicinal plants cultivation group in Prachinburi province, Thailand. Pork lard and soybean oil were purchased from local market in Prachinburi province, Thailand. Ethanol, hexane, silicic acid, activated charcoal, propylene glycol, potassium iodide, chloroform, sodium thiosulphate, acetic acid, DPPH, BHT and TBHQ were acquired from Sigma Chemical Co. (St. Louis, MO., U.S.A).

2.2 *Extraction of Beijing grass*

Antioxidants, most of which were phenolic compounds in Beijing grass were extracted in ethanol according to a slightly modified method of Klomsakul and co-workers (2012) [7]. Briefly, 15 g of dried Beijing grass were soaked in 150 mL of 80% ethanol and shaken at 220 rpm for 24 h in the dark at room temperature. The extract solution was evaporated at 40°C under vacuum to dryness using a rotary evaporator (Buchi R-205, Germany), yielding the crude extract which was kept at –20°C in the dark for further experiment.

2.3 *Preparation of stripped oil*

Both pork lard and soybean oil were stripped of tocopherols and other minor components by column chromatography [14]. A chromatographic column was packed sequentially with 22.5 g of silicic acid, followed by 5.63 g of activated charcoal and another 22.5 g of silicic acid. Thirty grams of the oil dissolved in 30 ml of hexane were passed through the column by eluting with 270 ml of hexane. The solvent present in the stripped oils was removed with a vacuum rotary evaporator (Buchi R-205, Germany) at 37°C. The effectiveness of stripping was determined by reduction of tocopherol concentrations to below the level of detection using high performance liquid chromatography (HPLC) method as described by Chen and coworkers (2014) [15]. The stripped oils were used for the whole experiment.

2.4 *Antioxidant assay*

Radical scavenging activity of crude extract was determined using DPPH assay [16]. Briefly, the extract

was dissolved in 95% ethanol for various concentrations (200, 400, 600, and 800 ppm). Then, 5.0 ml aliquot of ethanolic extract of Beijing grass was added to 5.0 ml of 0.16 mM DPPH ethanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and its absorbance was read at 520 nm using a spectrometer (Optima SP-3000 plus, Japan). The ability to scavenge the DPPH radical was calculated as indicated in the Equation (1),

$$\%\ Radical\ scavenging = 100 \times (A_{control} - A_{sample})/\ A_{control} \quad (1)$$

where, $A_{control}$ is the absorbance of the control (DPPH solution without sample) and A_{sample} is the absorbance of the test sample (DPPH solution plus test sample).

2.5 *Sample preparation*

Crude extract of Beijing grass was dissolved in propylene glycol at ratio of 1:5 (v/v) which was added into stripped soybean oil and pork lard at 0, 200, 400, 600, and 800 ppm of the extract. In addition, synthetic commercial antioxidants including TBHQ and BHT at 200 ppm were added into stripped soybean oil and pork lard, respectively.

All the oil samples were shaken at 40°C for 20 minutes. Then, 100 g of oils were transferred into an amber glass with a closure. All the samples were stored at 75°C for 14 days for peroxide value, and color determination every 2 days.

2.6 *Determination of peroxide value*

Peroxide values of the oils were determined according to the AOCS method (1990) [17]. About 5 g samples were weighed into 250 ml glass erlenmeyer, then 30 ml of a mixture of acetic acid and chloroform (at ratio of 3:2 v/v) was added. After mixing, 0.5 ml of saturated KI solution was added. It was shaken for 1 min prior to adding 30 ml of water. The liberated iodine was titrated with 0.01 N sodium thiosulphate solution until the yellow color almost disappeared. Then, 1 ml of 1% starch solution was added before continuing the titration until the blue color disappeared. The peroxide value (*PV*) was calculated as indicated in the Equation (2), and expressed in milliequivalents per kg of oil (meq/kg oil).

$$PV = \frac{VT}{m} \quad (2)$$

where V is the volume, in ml, of the standardized sodium thiosulphate solution, T is the exact normality of the sodium thiosulphate solution used, and m is the mass, in g, of the test sample.

In addition, to show a relative increase of the induction period due to the addition of antioxidant, the protection factor was calculated as shown in the Equation (3).

$$Protection\ factor = \frac{Induction\ period\ of\ the\ sample\ containing\ antioxidant}{Induction\ period\ of\ the\ control\ sample} \quad (3)$$

where the induction period defined as the time (days) needed for the peroxide value to reach at 20 and 70 meq/kg oil in pork lard and soybean oil, respectively. At this point, the oils would become noticebly rancid. As a consequence, it is customary to use this subjective evaluation to define the induction period of lipid oxidation of oil [18].

2.7 *Color measurement*

The colors of the oils with and without Beijing grass extract were measured as Hunter L (lightness), a (±, redness/greenness), and b (±, yellowness/blueness) values using a colorimeter (Hunter Lab Colorflex 4510, USA).

2.8 *Statistical analysis*

All experiments were conducted in triplicate samples. Data were presented as means ± standard deviations. Data results were analyzed by analysis of variance (ANOVA) using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). The differences between mean values were compared using Duncan's multiple-range test with significance defined as $p \leq 0.05$.

3 Results and discussion

3.1 *DPPH radical scavenging activity of Beijing grass extract*

There are several methods commonly used to evaluate antioxidant activity of compounds such as 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP) and oxygen

radical absorbance capacity (ORAC) assay. Among others, DPPH assay is a rapid, simple and inexpensive method to measure antioxidant capacity of food. DPPH is a relatively stable radical that is widely used for investigating the free radical-scavenging activity of a compound. This assay is based on the ability of the antioxidant to reduce the DPPH• radical to the yellow-colored diphenylhydrazine.

Table 1: DPPH radical scavenging activity of Beijing grass extract

Beijing Grass Extract Concentration (ppm)	Free Radical Scavenging Activity (%)
200	60.38 ± 2.14^{c}
400	70.13 ± 2.88^{b}
600	85.38 ± 1.79^{a}
800	87.29 ± 2.46^{a}

a, b, c represent significant differences at $p \leq 0.05$

As shown in Table 1, the ethanolic Beijing grass extract exhibited a concentration-dependent DPPH radical scavenging activity. It showed the radical scavenging activity of 85.38 and 87.29% at the concentration of 600 and 800 ppm, respectively. These activities were significantly higher than those of the Beijing extract at 200 and 400 ppm ($p \leq 0.05$), which exhibited the radical scavenging activity of 60.38 and 70.13%, respectively. Klomsakun and coworkers (2012) reported that Beijing grass extract at 500 ppm showed radical scavenging activity of 91.50% which was comparable with that of the commercial antioxidant, BHT, which exhibited radical scavenging activity of 93.56% [7]. Phenolic compounds could be responsible for the observed antioxidant activity in the Beijing grass extract due to their well-known ability to scavenge free radical [7], [19]– [21]. Beijing grass extract has been reported to contain phenolic compounds of 4–5 mg GAE/g [7]. Antioxidant activity of Beijing grass extract in our study was lower than those observed by Klomsakun and coworkers (2012) [7]. This is possibly due to different conditions used in sample preparation including heat, light, and pH, all of which have been reported to cause loss of antioxidant activity of phenolic compounds [22]–[25]. Moreover, the phenolic content in Beijing grass could vary greatly based on growing conditions as well [26].

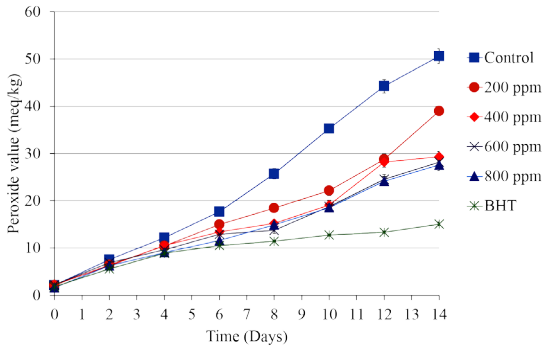

Figure 1: Peroxide value of pork lard containing Beijing grass extract at 200, 400, 600, 800 ppm and BHT at 200 ppm and storage at 75°C for 14 days.

3.2 *Antioxidant activity of Beijing grass extract in edible oils*

To evaluate the antioxidant activity of Beijing grass extract in edible oil, the extract was added into soybean oil and pork lard at 0, 200, 400, 600, and 800 ppm. Its antioxidant activity was compared with synthetic commercial antioxidants including TBHQ and BHT at 200 ppm in soybean oil and pork lard, respectively. Note that different synthetic antioxidants were used in different oils because TBHQ is most effective in stabilizing highly unsaturated vegetable oil including soybean oil, while BHT is commonly added to pork lard as antioxidants [27]. All the samples were stored under accelerated condition at 75°C for 14 days for the oxidation study. Peroxide value was determined during storage to define the content of lipid hydroperoxide which formed as lipid oxidation product.

As shown in Figure 1, peroxide value in pork lard significantly increased over the storage time. The increasing rate of peroxide value in the pork lard containing Beijing grass extract was relatively low compared to that in the control sample. This indicates that Beijing grass extract was able to decrease lipid oxidation rate in pork lard.

Moreover, the protection factor was calculated to show a relative increase of the induction period due to the addition of antioxidant. The protection factors of the Beijing grass extract in pork lard at 200, 400, 600 and 800 ppm were 1.4, 1.6, 1.6, and 1.6, respectively (Table 2). This suggested that the extract helped increase the oxidative stability of the oil compared to the control without antioxidant. However, antioxidant

efficacy of the Beijing grass extract was relatively low compared to synthetic antioxidant BHT which possessed the protection factor of 3.3.

Figure 2 shows the peroxide value in soybean oil containing the Beijing grass extract and TBHQ. Similar to what was observed in the pork lard system, the Beijing grass extract was able to protect soybean oil from lipid oxidation as seen from the lower peroxide value compared to the control sample. As observed in Table 2, the Beijing grass extract at 200, 400, 600 and 800 ppm in soybean oil showed protection factor of 1.2, 1.2, 1.2, and 1.3, respectively. TBHQ, a synthetic antioxidant, again exhibited significantly higher antioxidant activity (protection factor higher than 1.3) compared to the Beijing extract in soybean oil. This is in agreement with previous reports that TBHQ is a superior antioxidant in soybean oil [28]– [29]. Note that the induction time for soybean oil containing TBHQ was indicated as > 14.4 days as shown in Table 2 because this sample was oxidatively stable over the incubation time. Antioxidant activity of Beijing grass extract observed in both pork lard and soybean oil was likely due to phenolic compounds that act as free radical scavengers [7]. However, complementary studies on determination of active components are suggested.

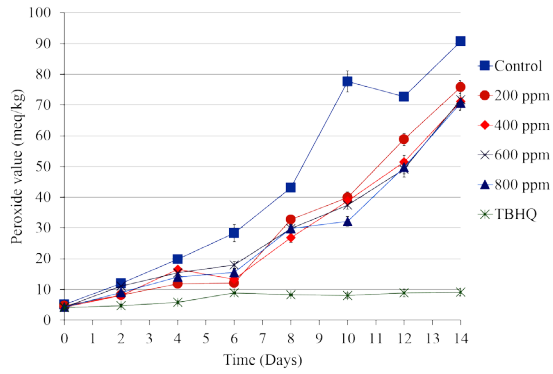

Figure 2: Peroxide value of soybean oil containing Beijing grass extract at 200, 400, 600, 800 ppm and TBHQ at 200 ppm and storage at 75°C for 14 days.

3.3 *Effect of addition of Beijing grass extract on color of the oil*

One of the limitations of natural antioxidant application in commercial food system could be the color interference of the antioxidant with food products. Moreover, the ethanolic extract of Beijing grass was brown green in color. Thus, the effect of addition of Beijing grass extract on color of the oil was evaluated as Hunter *L*, *a*, *b* values.

The Hunter *L*, *a*, *b* values of pork lard are shown in Table 3. The *L* value of the samples containing Beijing grass extract significantly decreased, while the greenness (*a* value) and yellowness (*b* value) increased in a concentration-dependent manner compared to the control.

However, the *L*, *a*, *b* value did not change over the storage time of 14 days. A similar trend was observed in soybean oil containing the Beijing grass extract as shown in Table 4. This effect could be linked to pigments, mostly chlorophylls, found in the Beijing grass. Therefore, further purification studies are needed so that the extract will be used without color interference with the color appearance of the food products.

Table 2: Induction time and protection factor of pork lard and soybean oil containing Beijing grass extract at 200, 400, 600, 800 ppm and BHT and TBHQ at 200 ppm after storage at 75°C for 14 days

Antioxidants	Concentration (ppm)	Pork Lard		Soybean Oil	
		Induction Time (days)	Protection Factor	Induction Time (days)	Protection Factor
Control	0	6.5 ± 0.0^{d}	1.0 ± 0.0^{d}	11.5 ± 0.0^{c}	1.0 ± 0.0^{c}
Beijing Grass Extract	200	8.8 ± 0.0^{c}	1.4 ± 0.0^{c}	13.6 ± 0.0^{bc}	1.2 ± 0.0^{bc}
	400	10.2 ± 0.1^{b}	1.6 ± 0.0^{b}	14.0 ± 0.0^{b}	1.2 ± 0.0^{bc}
	600	10.4 ± 0.0^{b}	1.6 ± 0.0^{b}	13.8 ± 0.1^{bc}	1.2 ± 0.0^{bc}
	800	10.4 ± 0.0^{b}	1.6 ± 0.0^{b}	14.4 ± 0.0^{b}	1.3 ± 0.0^{b}
BHT	200	21.2 ± 0.2^{a}	3.3 ± 0.1^{a}	-	-
TBHQ	200	-	-	$> 14.4^{a}$	$> 1.3^{a}$

a, b, c represent significant differences at $p \leq 0.05$

Table 3: Hunter *L*, *a*, *b* value of pork lard containing Beijing grass extract at 200, 400, 600, 800 ppm and BHT at 200 ppm on day 0 and after storage at 75°C for 14 days

Antioxidants	Concentration (ppm)	Color Values					
		Day 0			Day 14		
		L	*a*	*b*	*L*	*a*	*b*
Control	0	56.293 ± 2.139^{a}	-1.680 ± 0.013^{a}	5.050 ± 0.014^{e}	56.097 ± 0.019^{b}	-1.897 ± 0.001^{a}	7.590 ± 0.145^{f}
Beijing Grass Extract	200	56.013 ± 1.477^{a}	-2.167 ± 0.022^{c}	7.100 ± 0.127^{d}	55.283 ± 0.074^{c}	-2.777 ± 0.017^{c}	12.493 ± 0.577^{d}
	400	56.127 ± 0.988^{a}	-2.167 ± 0.019^{c}	7.077 ± 0.087^{d}	55.047 ± 1.113^{d}	-2.813 ± 0.014^{c}	12.937 ± 0.245^{c}
	600	54.577 ± 2.143^{b}	-3.150 ± 0.037^{d}	10.957 ± 0.145^{b}	54.560 ± 0.347^{e}	-3.077 ± 0.028^{d}	14.920 ± 0.136^{b}
	800	53.576 ± 1.137^{c}	-3.463 ± 0.039^{e}	13.120 ± 0.059^{a}	53.397 ± 1.278^{f}	-3.937 ± 0.023^{e}	15.617 ± 0.217^{a}
BHT	200	56.347 ± 1.349^{a}	-1.793 ± 0.014^{b}	7.863 ± 0.044^{c}	56.343 ± 1.159^{a}	-2.013 ± 0.017^{b}	9.310 ± 0.159^{e}

a, b, c represent significant differences at $p \leq 0.05$

Table 4: Hunter *L*, *a*, *b* value of soybean oil containing Beijing grass extract at 200, 400, 600, 800 ppm and TBHQ at 200 ppm on day 0 and after storage at 75°C for 14 days

Antioxidants	Concentration (ppm)	Color Values					
		Day 0			Day 14		
		L	*a*	*b*	*L*	*a*	*b*
Control	0	59.830 ± 1.119^{a}	-2.630 ± 0.019^{b}	7.600 ± 0.023^{e}	59.410 ± 0.024^{b}	-2.633 ± 0.081^{a}	7.030 ± 0.059^{f}
Beijing Grass Extract	200	57.880 ± 1.123^{b}	-3.600 ± 0.027^{c}	11.133 ± 0.102^{d}	57.443 ± 0.146^{c}	-3.790 ± 0.013^{c}	11.503 ± 0.412^{d}
	400	57.000 ± 0.728^{d}	-4.357 ± 0.029^{d}	14.730 ± 0.029^{c}	56.390 ± 1.271^{d}	-4.327 ± 0.025^{d}	14.693 ± 0.313^{c}
	600	56.500 ± 2.127^{e}	-4.407 ± 0.127^{e}	14.967 ± 0.139^{b}	56.183 ± 1.102^{e}	-4.637 ± 0.016^{e}	17.137 ± 0.214^{b}
	800	55.090 ± 2.017^{f}	-4.730 ± 0.129^{f}	17.207 ± 0.124^{a}	54.937 ± 1.124^{f}	-4.680 ± 0.017^{f}	18.530 ± 0.304^{a}
TBHQ	200	57.433 ± 1.129^{c}	-2.063 ± 0.015^{a}	5.953 ± 0.057^{f}	59.677 ± 1.023^{a}	-3.220 ± 0.043^{b}	10.260 ± 0.277^{e}

a, b, c represent significant differences at $p \leq 0.05$

4 Conclusions

This study reveals that the Beijing grass extract possessed significant free radical scavenging activity according to the DPPH assay. Moreover, the addition of Beijing grass extract results in lower lipid hydroperoxide formation in pork lard and soybean oil compared to the control oil without antioxidant. These results suggest that Beijing grass ethanolic extract has great potential as an effective natural antioxidant used to protect edible oil from lipid oxidation. However, the addition of Beijing grass extract caused the oil lightness to decrease, while the greenness and blueness increased. Thus, to utilize this natural antioxidant in food products, further studies on purification of the Beijing grass extract are needed.

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