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Research article

Bio-active compounds and antioxidant activity in flowers and seeds of Sunn hemp

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ABSTRACT

Sunn hemp (*Crotalaria juncea*) is generally used as green manure in agriculture because of its ability to fix nitrogen. It also has potential to be used as food and feed. The objectives of this study were to evaluate phytosterols, vitamin E, phenolic compounds and antioxidant activity in flower and seed and quantify fatty acid composition of Sunn hemp oil. Flower and seed of Sunn hemp had the phytosterols of 1,217.54 mg/100g and 1,283.82 mg/100g, respectively. β-sitosterol, campesterol and Δ5-stigmasterol constituted the major components of total phytosterols in both flower and seed. Vitamin E in seed (129.33 mg/100g) was higher than in flower (56.61 mg/100g), and the major components of total vitamin E were similar, consisting of α-tocopherol and γ-tocopherol. Total phenolic compound in flower (111.84 mgGAE/100g) was higher than in seed (80.44 mgGAE/100g), but antioxidant activity in seed (64.67 %) was higher than in flower (52.33 %). Sunn hemp oil had high unsaturated fatty acids of 68.90 %. Linolenic acid constituted 57.8 % of total fatty acids followed by palmitic acid (15.3 %) and stearic acid (11.5 %). The information is important for utilization of Sunn hemp as food and feed.

Introduction

Sunn hemp (*Crotalaria juncea*) is a plant used mainly for green manure. Sunn hemp known as Brown hemp, Indian hemp, Madras hemp or Sunn hemp is a tropical Asian plant of the legume family. However, it can be as animal feed. Its flowers and young shoots can be consumed as a cooked vegetable and an ingredient in many Thai recipes. Sunn hemp is also grown for aesthetic purpose in tourist attractions. According to Chaudhury et al. (1995), seed of Sunn hemp consisted mainly of carbohydrate (41.1 %), protein (30-35 %) and lipid (12.6 %).

High protein in Sunn hemp seed is interesting for use as food and feed. According to Mosjidis et al. (2012), some species of *Crotalaria* are toxic. Some species are less toxic, and they do not cause fatality in animals and birds (Savaris et al., 2019). Most studies in Sunn hemp oil have focused on transesterification of the oil into biodiesel (Sadhukhan & Sarkar, 2014). Sunn hemp oil might be suitable for human consumption. Unfortunately,

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the characteristics of the oil profile are not available in the literature.

Unsaturated lipid acids including linoleic acid, oleic acid and linolenic acid constituted the largest portion (79.7 %) of total lipid. These lipid acids are useful to health, and Sunn hemp oil can be used as a raw material for many value-added products such as cosmetics, soap and shampoo (Ahmad & Ahsan, 2020). Flower of Sunn hemp has medicinal properties such as prevention of cancer, appetizer, curation of throat inflammation and prevention of intestinal inflammation. For non-toxic Sunn hemp, Punchuklang et al. (2021), reported that leaves and flowers had high phenolic compounds, tannins and antioxidant activity. Therefore, Sunn hemp is interesting for use as human food, and the value-added products from Sunn hemp should be developed.

Currently, people are more concerned about their health. Health food products are more popular, and the demand for health food products are increasing. Sunn hemp is a promising plant for development of health food products. However, the information on phytosterols, antioxidant activity and bioactive chemicals such as phenolic compounds, and vitamin E in Sunn hemp is still limited. The objectives of this study were to quantify bioactive phytochemicals and antioxidant activity from flower and seed of Sunn hemp and develop value-added products from Sunn hemp.

Materials and Methods

Planting and crop management

Soil after rice harvest was ploughed twice. The second tillage was carried out at seven days after the first tillage. Harrowing was practiced after the second tillage. Mixed fertilizer (15-15-15) at the rate of 125 kg ha^{-1} was incorporated into the soil during harrowing, and Sunn hemp seed at the rate of 93.75 kg ha^{-1} was sowed after harrowing.

Sprinkler irrigation system was installed, and water was supplied to the field at field capacity level soon after sowing. After emergence, water was supplied to the crop at 3 to 5-day intervals or when wilting symptom was observed. Weeds were removed with uprooting, while insects and disease were not control.

Flower was harvested at 50-60 days after emergence for chemical analysis and product development. The seed was harvested at 150 days after emergence, sun-dried and threshed. The dry seed and dry flower were used for chemical analysis, and all analyses were done with three replicates.

Chemical analysis for phytosterols and antioxidant substances

Phytosterols and vitamin E

Phytosterols and vitamin E were analyzed according to the methods described by Thammapat et al. (2016) with minor modification. Briefly, the samples of 2 grams each were saponified to remove lipids. The finely-ground samples were loaded into the mixture consisting of KOH (2 ml), NaCl (2 ml), ethanol 95 % (2 ml) and ethanolic pyrogallol (5 ml). 5 α-Cholestane was used as an internal standard.

The mixed samples were warmed in a water bath at 70 °C for 10 minutes and set aside at room temperature. The extraction of phytosterols was carried out with the mixture of N-hexane and ethyl acetate at the ratio of 9:1 and the volume of 15 ml. The extractant was evaporated in a rotary evaporator at 40 °C. The samples were derivatized by using BSTFA: TMCS (99:1) at the volume of 10 µl and pyridine at the volume of 1 ml.

All volatile substances were evaporated in a hot-air oven at 60 °C for 30 minutes. The evaporated samples were further dissolved with hexane and filtered through filter membrane (2 µm). Phytosterols and vitamin E were quantified by GC-MS method.

Total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method (Noreen et al., 2017). Briefly, the finely-ground samples were extracted in 80 % ethanol at the ratio of 1:5 by weight for 24 hours. The samples were filtered with filter paper. The filtered samples were partially evaporated in a vacuum rotary evaporator at 40 °C. The concentration of the samples was adjusted to obtain the concentration of 5 % by adding 80 % ethanol. Folin-Ciocalteu reagent at the concentration of 10 % and volume of 3 ml was added into the samples, and the samples were shaken for 1 minute. Na_2CO_3 at the concentration of 10 % and the volume of 3 ml was further added into the samples. After adding Na_2CO_3, distilled water was added, and the samples were set aside at room temperature for 2 hours.

Optical density was measured in a spectrophotometer at the wave length of 760 nanometers. Gallic acid was used a standard reagent to measure polyphenol concentration. The absorbance was reported as equilibrium optical density of gallic acid per 100 mg of sample.

Antioxidant activity in flower and seed

Antioxidant activity

Antioxidant activity was determined by DPPH radical scavenging activity method (Loypimai et al. 2015) Briefly, the finely-ground samples were extracted in 80 % ethanol at the ratio of 1:5 by weight for 24 hours. The samples were filleted with filter paper. The filtered samples were partially evaporated in a vacuum rotary evaporator at 40 °C. The concentration of the samples was adjusted to obtain the concentration of 5 % by adding 80 % ethanol.

The extractant at the concentration of 100 µg/l, and the volume of 1 ml was added to the samples followed by DPPH agent at the concentration of 10 mmol and the volume of 3 ml. The samples were set aside at room temperature for 30 minutes, and the absorbance was measured at the wavelength of 517 nanometers. Antioxidant activity was reported as % scavenging as follows;

$$\% \text{ Scavenging} = A_{\text{control}} - A_{\text{sample}} \times 100,$$

where A sample is the absorbance of the sample, and A control is the absorbance of control.

Crude oil extraction and analysis of lipid composition

Crude oil extraction and lipid composition

Seed of Sunn hemp was finely ground and used for oil extraction. The extracted oil was analyzed for oil compositions using gas chromatography according to the methods described previously (Wanasundara & Shahidi, 1998). The oil was extracted according to the method described by Sunarya et al. (1996). Briefly, 10 g of finely-ground seed was loaded in an opaque bottle, and 100 ml of extractant containing chloroform and methanol at the ratio of 2:1 by volume with BHT 50 ppm was filled in the bottle. The sample was set aside at room temperature for 24 hours.

The sample was filtered through a filter paper, and the process was further repeated two times by using the extractant of 40 ml and 20 ml, respectively. The sample was transferred into a liquid separator. Twenty ml of sodium chloride (0.85 %) was added into the sample and the sample was shaken well.

The sample was set aside at room temperature until the water phase and oil phase were separated. Water phase was removed from the separator and evaporated in an evaporator at the temperature of 40 °C. The sample was stored at -25 °C until analysis of lipid components.

Methylation

The sample was further methylated to transform lipid into methyl ester. The sample of 100 mg was loaded into a test tube with screw cap. Internal standard (C15:0;10 ppm) of 1 ml was loaded into the test tube followed by sulfuric acid of 0.9M in methanol and toluene of 1 ml, and the test tube was closed tightly.

The sample was then put into water bath at 70 °C for 2 hours, and the water bath was shaken every 45 minutes. Hexane of 2 ml was filled into the test tube followed by sodium chloride 0.85 % of 1 ml. The upper part of the sample in the test tube was transferred into another test tube containing water of ½ of the test tube. The test tube was further centrifuged to mix the sample.

The upper part of the sample in the test tube was transferred into another test tube through filler paper containing sodium sulphate, and the contaminants in the sample were removed. The extractant was removed by nitrogen.

Elimination of non-lipid contaminants

A Sep-Pak™ C18 cartridge was set up, and the samples and solvents were applied to the system with a 10 ml syringe. The syringe was cleaned three times with chloroform and methanol at the ratio of 2:1v/v followed by petroleum ether.

The samples were loaded into the syringe. The test tubes of the samples were washed with the small amount of petroleum ether, and the washed samples were loaded into the syringe. The samples were passed through the Sep-Pak with high speed. 3.5 ml of 5 % petroleum ether was loaded into the syringe. The samples were passed through the Sep-Pak with low speed, and the drops were collected in clean test tubes. The syringe was washed with

chloroform and methanol and passed through the Sep-Pak with medium speed, and the washed samples were collected in a jar. The samples were evaporated with nitrogen and were diluted with hexane. The sample was analyzed for lipid compositions by gas chromatography method (Thammapat et al., 2015).

Statistical analyses

The results are presented as mean ± standard deviation of determinations for triplicate samples. Experimental design was a completely randomized design (CRD). Differences were considered statistically significant at $p < 0.05$. Data were subjected to Duncan's post hoc test where differences were detected for homogenous subsets. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences, SPSS Corporation, Chicago, IL) for Windows.

Results

Phytosterols

Phytosterols in flower and seed were rather similar for both their contents and their compositions. Total phytosterols in flower and seed of Sunn hemp were, 217.54 mg/100g and 1,283.82 mg/100g, respectively (Table 1). β-sitosterol was recorded as the highest composition in flower and seed, accounting for 595.88 mg/100g and 621.29 mg/100g, respectively. Campesterol was found in flower and seed of Sunn hemp at high contents of 316.24 mg/100g and 353.62 mg/100g, respectively. Δ5-stigmasterol was also found at high contents, accounting for 177.07 mg/100g in flower and 198.44 mg/100g in seed. α-amyrin, Δ5-avenasterol, campestanol and sitostanol were found in flower and seed at low contents (lower than 100 mg/100g), ranging from 7.15 mg/100g to 61.28 mg/100g in flower and 4.58 mg/100g to 61.63 mg/100g in seed, whereas cycloarternol and 24-methylenecycloartanol were not detected in both flower and seed.

Vitamin E

Vitamin E values in flower and seed were rather similar for the compositions in flower and seed, but they were different in their compositions (Table 2)

Table 1 Phytosterols in flower and seed of Sunn hemp

Phytosteral	Flower (mg/100g)	Seed (mg/100g)
Campesterol	316.24±7.88^{b}	353.62±5.11^{a}
Campestanol	19.77±1.30^{a}	14.49±1.97^{b}
Δ5-stigmasterol	177.07±7.40^{b}	198.44±3.83^{a}
β-sitosterol	595.88±6.05^{b}	621.29±9.00^{a}
Sitostanol	7.15±0.86^{a}	4.58±1.19^{b}
Δ5-avenasterol	40.15±1.42^{a}	29.76±4.58^{b}
α-amyrin	61.28±1.83	61.63±2.67
Cycloarternol	0.00±0.00	0.00±0.00
24-methylenecycloartanol	0.00±0.00	0.00±0.00
Total sterol	1,217.54±17.20^{b}	1,283.82±18.52^{a}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each row were significantly different ($p < 0.05$).

Table 2 Vitamin E in flower and seed of Sunn hemp

Vitamin E	Flower (mg/100g)	Seed (mg/100g)
α-tocopherol	32.99±3.03^{b}	89.80±2.48^{a}
β-tocopherol	0.13±0.03	0.14±0.04
γ-tocopherol	19.22±1.08^{b}	35.39±2.44^{a}
δ-tocopherol	0.33±0.15	0.27±0.12
α-tocotrienol	1.39±0.20	1.52±0.29
β-tocotrienol	0.77±0.06^{a}	0.43±0.15^{b}
γ-tocotrienol	1.78±0.09	1.79±0.15
δ-tocotrienol	0.00±0.00	0.00±0.00
Total vitamin E	56.61±3.81^{b}	129.33±0.88^{a}

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each row were significantly different ($p < 0.05$).

Vitamin E values in seed were about two folds higher than vitamin E values in flower for most compositions except

for δ-tocotrienol, which was not detected in both flower and seed. Total vitamin E in flower was 56.61 mg/100g, and total vitamin E in seed was 129.33 mg/100g. α-tocopherol contributed to the largest portions of total vitamin E in flower (32.99 mg/100g) and in seed (89.80 mg/100g) followed by γ-tocopherol, which contributed 19.22 mg/100g in flower and 35.39 mg/100g in seed. γ-tocotrienol, α-tocotrienol, β-tocotrienol, δ-tocopherol and β-tocopherol contributed to small portion of total vitamin E, ranging between 0.13 mg/100g and 1.78 mg/100g in flower and 0.14 mg/100g and 1.79 mg/100g in seed.

Total phenolic content and antioxidant activity

Total phenolic content in flower was 111.84 mgGAE/100g, whereas total phenolic compound in seed was 80.44 mgGAE/100g (Table 3). However, antioxidant activity in flower determined by DPPH method was 52.33 %, which was lower than antioxidant activity in seed (64.67 %).

Lipid compositions from Sunn hemp seed

Lipid acids are classified into two groups consisting of unsaturated fatty acids and saturated fatty acids (Table 4). Unsaturated fatty acids consisting of linoleic acid (57.8 %), oleic acid (8.7 %), palmitoleic acid (2.0 %) and linolenic acid (0.4 %) constituted the large portion of total lipid acids in Sunn hemp seed. Saturated fatty acids including palmitic acid (15.3 %), stearic acid (11.5 %), myristic acid (1.8 %), behenic acid (0.9 %), arachidic acid (0.7 %) and lauric acid (0.2 %) constituted the small portion of total lipid acids in Sunn hemp. Unknown fatty acids constituted the smallest portion (0.6 %)

Table 3 Free radical scavenging activity determined by DPPH method

Source	Total phenolics (mgGAE/100g)	DPPH (% inhibition)
Flower	111.84±7.74[a]	52.33±3.06[b]
Seed	80.44±5.94[a]	64.67±2.52[b]

Mean values ± standard deviation of determinations for triplicate samples. Values with different superscripts in each row were significantly different ($p < 0.05$).

Table 4 Lipid compositions in oil extract of Sunn hemp seed

Fatty acid	%
Lauric acid	0.2±0.04[1/]
Myristic acid	1.8±0.02
Palmitic acid	15.3±0.05
Stearic acid	11.5±0.03
Arachidic acid	0.7±0.03
Behenic acid	0.9±0.03
Palmitoleic acid	2.0±0.06
Oleic acid	8.7±0.03
Linoleic acid	57.8±0.10
Linolenic acid	0.4±0.03
Unknown	0.6±0.06
Total	100.0

1/ ± Standard deviation (SD)

Discussion

Phytosterols

Flower and seed of Sunn hemp had total phytosterols of 1,217.54 mg/100g and 1,283.82 mg/100g, respectively. Phytosterol contents in this study were lower than that reported in rice bran oil (1,830 mg/100g) and higher than those reported in maize oil (909 mg/100g), sunflower oil (411 mg/100g) and soybean oil (320 mg/100g) (Van Hoed et al., 2006). Phytosterols in cereals and pulses such as rice, sunflower seed, almond and hazelnut were in a range between 23 and 360 mg/100g (Van Hoed et al., 2006), which were lower than phytosterols in flower and seed of Sunn hemp. To the best of our knowledge so far, the authors have not found the information on phytosterols in flower and seed of Sunn hemp in the literature, and the authors are not able to compare the results directly with other studies. However, most studies have reported on industrial hemp oil. According to

Montserrat-de la Paz et al. (2014), the most interesting compounds were β-sitosterol (190.00 mg/100 g), campesterol (50.57 mg/100g), phytol (16.76 mg/100 g), cycloartenol (9.10 mg/100 g) and γ-tocopherol (7.34 mg/100 g). Blasi et al. (2022) found that β-sitosterol was the predominant phytosterol, followed by campesterol, as obtained by high-resolution gas chromatography (HRGC). Golimowski et al. (2022) reported that the predominant sterols were campesterol (32.00 mg/100 g), β-sitosterol (130.00 mg/100 g) and Δ5-avenasterol (15.00 mg/100 g). Most components of phytosterols in industrial hemp oil were lower than in this study.

According to Van Hoed et al. (2006), the highest phytosterols in natural food sources was recorded in rice bran oil. The use of cooking oil with high phytosterols such as rice bran oil with other food sources will help reduce the assimilation of cholesterols and inhibit cancer cells. Previous study in Sunn hemp oil was aimed to develop biodiesel (Sadhukhan & Sarkar, 2016). The results indicated that Sunn hemp oil had phytosterols slightly lower than rice bran oil and it is promising oil for use as cooking oil after processing to eliminate beany odor. Sunn hemp has potential as a dual-purpose crop for use as green manure and cooking oil as it is a hardy plant that can be planted with minimum cost.

Vitamin E, total phenolic content and antioxidant activity

Vitamin E and phenolic compounds receive more attention as natural antioxidants because they are safer than synthetic antioxidants (Pokorny et al., 2001; Yim et al., 2013). In this study, the components of vitamin E in flower and seed were similar. However, vitamin E contents in seed were higher than in flower, and the major components of vitamin E in both flower and seed were α-tocopherol, γ-rocopherol, γ-tocotrienol and α-tocotrienol. Vitamin E is higher in the seeds than in the flowers as the seeds store nutrients for germination and growth of young plants. Many previous studies have shown that the content of vitamin E of the sunflower seed (Zilic et al., 2010), soybean (Vasantharuba et al., 2007). However, a literature search revealed that there is limited information on the bioactive compounds and vitamin E of Sunn hemp.

Sunn hemp is used primarily as green manure, and it also used as animal feed. Flower is also consumed as a cooked vegetable (Yasar et al., 2022). However, the seeds of these related species are toxic to animals and birds. Inclusion of Sunn hemp seed at appropriate ratios did not affect motility of broiler chicken (Hess & Mosjidis, 2008). Scant information is available for nutrition benefits of Sunn hemp. In industrial hemp oil, Blasi et al. (2022) found high levels of vitamin E, with prevalence of γ-tocopherol.

Total phenolic content in flower of Sunn hemp was higher than in seed, and the ranges of total phenolic content were 111.84 mgGAE/100g in flower and 80.44 mgGAE/100g in seed. However, antioxidant activity determined by DPPH method in seed (64.67 %) was higher than in flower (52.33 %).

According to Punchuklang et al. (2021), total phenolic compounds in young shoot and flower of Sunn hemp were in the ranges between 125 mgGAE/100g and 230 mgGAE/100g and 80 mgGAE/100g and 160 mgGAE/100g, respectively. Phenolic compounds in previous study were slightly higher than the results in this study. Punchuklang et al. (2021) also found tannin contents in the ranges between 150 mgGAE/100g and 350 mgGAE/100g in shoot and 100 mgGAE/100g and 180 mgGAE/100g in flower.

The antioxidant activity determined by DPPH method of tea beverage from Sunn hemp flowers and young shoots were shown in the ranges between 49.40 % and 56.39 %, and 58.53 % and 72.41 %, respectively (Punchuklang et al., 2021). The results in this study were in agreement with those in previous report.

If flavor is acceptable, Sunn hemp flower can be used as tea because of its health benefits. The selection of flower or shoot for development of tea product should depend on consumer preference, and further investigations are still required.

Lipid compositions of Sunn hemp seed

Unsaturated fatty acids such as linoleic acid accounted for 57.8 % of total fatty acids. Linoleic acid helps reduce the assimilation of cholesterols. Linoleic acid is also
used as an indicator to evaluate the usefulness of cooking oil. In general, linoleic acid in cooking oil should be about 40 to 50 %. Therefore, Sunn hemp

oil is a promising source for use as cooking oil.

Most studies on Sunn hemp oil were for production of biodiesel (Sadhukhan & Sarkar, 2016), and the authors have not found any report on Sunn hemp oil compositions. According to Sadhukhan & Sarkar, (2014), oils from Sunn hemp seed ranged from 2.3 % to 11.4 % depending on extraction solvents. It was low compared to other sources such as peanut (40.2-42.3 %) (Bilal et al., 2020), soybean (16.5-25.5 %) (Huskey et al., 1990) and rice bran (15-25 %) (Okajima et al., 2022).

Conclusion

This study has shown that there were differences in phytosterols, phenolic compounds, vitamin E and fatty acid distribution amongst different parts of Sunn hemp. The contents of their bioactive compounds of seed were higher than that in flower. Unsaturated fatty acids constituted the largest portion of fatty acid in the seed, and the major components of unsaturated fatty acid was linoleic acid. These data will provide the nutritional basis for improving the product such as tea from flower, cosmetic from oil seed, and providing more information to the consumer.

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