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Nutritional Composition, Antioxidant Activity and Phytochemical Composition of *Tupistra albiflora* K. Larsen's Flowers

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Abstract

The purpose of this study was to investigate the nutrient composition, antioxidant activity, and chemical constituent of *Tupistra albiflora* K. Larsen's flowers. The methods used were AOAC, DPPH, and GC-MS, respectively. The results revealed that *T. albiflora* K. Larsen's flowers have nutritional value (protein, carbohydrate, fat, ash, fiber, energy, thiamin, riboflavin, and pyridoxine) and antioxidant properties. There was also a high ratio of protein content (26.00 - 29.60 %) and riboflavin (0.1 - 2.0 mg/100g dry weight). Phytochemical composition analysis presented high content of essential oils (cis,cis-linoleic acid, hexadecanoic acid and hexadeconic acid ethyl ester); it may be a potential source for the food and pharmaceutical industries.

Keywords: *Tupistra albiflora* K. Larsen's, nutrient composition, antioxidant, protein content, linoleic acid

Introduction

Many plant resources are commonly considered as agricultural food. Some plants, consumed by people in north of Tanzania, were reported to have a nutritional composition including protein, fat, starch, sugars, fiber, ash, and energy [1]. Moreover, wild food resources, gathered by native people, contain useful characteristics, such as vitamins and minerals [2]. In addition, natural antioxidants in plant resources were identified. Those such as polyphenols, flavonoids, or related compounds can prevent human disease, as they inhibit the development of free radicals which cause oxidative damage [3]. There are many parts of plant species that provide antioxidant properties, e.g., *Inula helenium* (root), *Silybum marianum* (seed), *Carum carvi* (fruit), *Echinacea purpurea* (leaf), *Curcuma longa* (rhizome) [4], and *Limnocharis flava* Buch (bud and flower) [5]. The antioxidant activity of 51 plant species of Jordanian origin was determined. It was found that the antioxidant capacity ranged from 10.1 to 720 µmol TE/g dry weight [6]. The crude extract of *Lens culinaris* also presented antioxidant activity of 0.75 µmol Trolox eq./mg extract [7]. The methanolic extract of *Mosla chinensis* Maxim was analyzed by DPPH method. It was found that the amount of phenol components was 47.3 ± 0.4 µg GAE/mg, with antioxidant activity of IC_{50} value 1482.5 ± 10.9 µg/ml [8].

Tupistra albiflora K. Larsen's flowers were harvested from Wiang Pa Pao District, Chiang Rai Province, Thailand. There are 3 types of *T. albiflora* K. Larsen's flowers, based on different colors (violet, green, and white flowers) (**Figure 1**). Flowers of *T. albiflora* K. Larsen are commonly utilized by native people; they are consumed and are an ingredient in local food. Native people widely plant and sell it (about 120 baht per kg). The nutritional and pharmaceutical information of this plant have not been studied before. Therefore, it is interesting to study its useful characteristics. The purpose of this research was to investigate the nutritional composition, antioxidant activity, and chemical constituent of 3 types of *T. albiflora* K. Larsen's flowers.

(a) (b) (c)

Figure 1 *T. albiflora* K. Larsen's flower: violet (a), green (b), and white (c) flowers.

Materials and methods

Nutritional and vitamins analysis of *T. albiflora* K. Larsen's flower

T. albiflora K. Larsen's flowers were dried at 60 °C in a hot air oven. Then, the proximate analysis of dried flower was performed, by AOAC method, to find the composition of fat, protein, carbohydrates, crude fiber, and ash [9]. Bomb calories method was used for energy quantification. Vitamins [Thiamin (B_1), Riboflavin (B_2), and Pyridoxine (B_6)] were quantified by HPLC (High Performance Liquid Chromatography). AOAC 942.23 was used for vitamin B_1 quantification [10]. Vitamin B_2 and B_6 were quantified by in-house method TE-CH-057 [11].

Antioxidant activity and phenolic content of *T. albiflora* K. Larsen's flowers

T. albiflora K. Larsen's flowers were dried, weighed, and ground. The samples were extracted by methanol (Merck, HPLC grade, Germany). The solution was filtered and evaporated to a crude extract by a rotary vacuum-evaporator. The crude extract was utilized for determination of DPPH (1,1-diphenyl-2-hydrazy) radical scavenging activity. The extract, in different concentrations, was added in an equal volume to a methanolic solution of DPPH. The absorbance (517 nm) was recorded after being placed in room temperature for 30 min. The standard control used was butylated hydroxytoluene (BHA). The test was examined in triplicate. The percentage inhibition of antioxidant activity was investigated and calculated by the following formula [12,13]:

$$\%\ \text{inhibition} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}] \times 100}{\text{Absorbance (control)}}$$

A plotted graph of percent inhibition against different concentrations was used to determine the IC_{50} value (total antioxidant presence necessary to decrease the initial DPPH radical concentration by 50 %).

The total phenolic content was determined by using Folin-Ciocalteu reagent with a gallic acid calibration curve. The extract solution of 0.2 ml and 0.2 ml of Folin-Ciocalteu reagent were mixed and, after 4 min, 15 % Na_2CO_3 (1 ml) was added. The mixture was stood at normal temperatures for 2 h. The absorbance was measured at 760 nm. The total phenolic concentration was performed in triplicate, and the average calculated as mg of gallic acid/ g extract [14].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *T. albiflora* K. Larsen's flowers

The extracts were analyzed by a GC-MS system with a GC 7890A Agilent Technology machine. A 30 m DB5-MS column (0.25 mm I.D., 0.25-µm film thickness) was used. The inlet temperature was 250 °C and the oven temperature was programmed to be 60 °C, then raised at a rate of 3 °C/min to 240 °C. Injection volume was 1 µl, and solvent delay was 4 min, with a total runtime of 60 min. A mass spectra scan covered a range from 50 to 550 amu.

Results and discussion

Nutritional and vitamins analysis of *T. albiflora* K. Larsen's flower

Nutrient compositions of *T. albiflora* K. Larsen's flowers are presented in **Table 1**. For the white flower, the level of fat, protein, carbohydrate, crude fiber, and ash were 3.24, 29.60, 35.83, 12.11, and 8.97 g per 100 g dry weight, respectively. Green flower compositions of fat, protein, carbohydrate, crude fiber, and ash were 4.30, 27.50, 35.08, 13.62, and 9.84 g per 100 g dry weight, respectively. Violet flower consisted of fat, protein, carbohydrate, crude fiber, and ash of 3.27, 26.00, 41.23, 11.71, and 9.40 g per 100 g dry weight, respectively. Green flower produced the highest energy (4.024 kcal/g), whereas white and violet flower provided 3.947 kcal/g. All flower types presented thiamine, riboflavin, and pyridoxine in different ratios (**Table 1**). The results revealed that *T. albiflora* K. Larsen's flowers had high amounts of protein. The protein content of *T. albiflora* K. Larsen's flowers was 26.00 - 29.60 %, and white flower had the highest content. It scored relatively highly compared with the WHO protein standard. Similarly, 3 plants, *Vigna* sp., *Hibiscus esculentus*, and *Parkiia biglobosa*, contained 20 to 37 % protein [15]. Moreover, the highest amount of protein (22.5 - 29.4 %) was found in *Parkia biglobosa*, *Leptadenia hastata*, and *Bombax costatum* [16]. The protein content of *T. albiflora* K. Larsen's flowers was also higher than *Moringa oleifera* Lam (6.7 %) [17]. Green flower contained higher crude fiber, fat, and ash than white and violet flower, whereas violet flower provided the highest amount of carbohydrate. However, the nutrient composition of all *T. albiflora* K. Larsen's flowers were different, because they may have had various pigments or other components, due to the varieties of this species. In addition, the amount of vitamin content of *T. albiflora* K. Larsen's flowers was more than other consumed plants. For example, average nutritional values of 829 wild vegetable foods consumed by Australian aboriginals was found to have a riboflavin content of 0.2 mg/100g [18], which was less than that found in *T. albiflora* K. Larsen's flowers (0.1 - 2.0 mg/100g).

Table 1 Nutrient compositions of *T. albiflora* K. Larsen's flowers extract.

Nutrient composition	Type of *T. albiflora* K. Larsen's flowers		
	White flower	Green flower	Violet flower
Unit: g/100 g dry weight			
Fat	3.24±0.049^{b}	4.30±0.066^{a}	3.27±0.026^{b}
Protein	29.60±0.917^{a}	27.50±0.361^{b}	26.00±0.400^{b}
Carbohydrate	35.83±0.603^{b}	35.08±0.144^{b}	41.23±0.070^{a}
Crude fiber	12.11±0.095^{b}	13.62±0.053^{a}	11.71±0.079^{c}
Ash	8.97±0.090^{b}	9.84±0.092^{a}	9.40±0.400^{ab}
Energy (kcal/g)	3.947±0.059^{a}	4.024±0.007^{a}	3.947±0.055^{a}
Unit: mg/100 g dry weight			
Thiamine	0.022±0.005^{a}	0.017±0.002^{a}	0.008±0.001^{b}
Riboflavin	0.426±0.010^{b}	0.109±0.004^{c}	2.020±0.053^{a}
Pyridoxine	0.147±0.003^{b}	0.425±0.004^{a}	0.145±0.006^{b}

Statistical significance was determined by analysis of variance (ANOVA) with adjustments for multiple comparisons with Tukey's test. Values are means ± standard deviation of triplicate determinations. Values on the same row with different superscripts are significantly different ($P \leq 0.05$).

Antioxidant activity and phenolic content of *T. albiflora* K. Larsen's flowers

The antioxidant activity of *T. albiflora* K. Larsen's flowers is shown in **Table 2**. It was found that antioxidant activity was 54.704 - 80.923 mg/ml of IC_{50} and phenolic content 231.33 - 246.98 mg gallic acid/g extract. The highest activity was found in white flower, with 54.704±0.853 mg/ml IC_{50}. Antioxidants are defined as compounds that can delay, repress, or obviate oxidative stress which causes the development of chronic degenerative diseases, including coronary heart disease, cancer, and aging. Phenolic compounds have been considered to be powerful antioxidants, as they reduce the risk of oxidative stress-associated diseases, such as cardiovascular diseases, cancer, or osteoporosis [19]. The relationship of antioxidant activity and phenolic content was described previously [20,21]. Many aspects were studied, such as the relationship between their antioxidant activities, hydrogen donation of free radical scavengers, and their chemical structures. The cumulative findings concerning structure-antioxidant activity relationships and reaction with relevant radicals defined the antioxidant activities. The total phenolic contents and total antioxidant capacity were examined in several plants, such as *Ballota nigra* L, *Cotinus coggygria* Scop, *Echium rubrum* L., *Echium vulgare* L., *Gentiana asclepiadea* L., and *Halacsya sendtneri* (Boiss.) Dorfl. The results revealed that total phenolic content complied with antioxidant activity [22]. Furthermore, the extract of *Thermopsis turcica* was found to have free radical scavenging activity and relate to total phenolic contents [23]. Nevertheless, the antioxidant activity of *T. albiflora* K. Larsen's flowers extract (IC_{50} value) was not correlated with total phenolic contents. The extraction of white flower had the highest antioxidant activity, but had the lowest total phenolic content. There were earlier studies that showed that antioxidant activity was not related to total phenolic content. It was found that some plant extract samples in the studies were not correlated between antioxidant activity (% inhibition or IC_{50} value) and total phenolic content [24-27].

All of *T. albiflora* K. Larsen's flowers provided antioxidant activity, but it was highly comparable with other effective antioxidant activity plants, such as *Cajanus cajan* (L.) Millsp. [28], *Coleus aromaticus* [29], and *Sesbania grandiflora* Desv. [5]. However, natural antioxidants gathered from all parts of the plant presented antioxidant activity in a range from extremely slight to very great [30].

Table 2 IC_{50} and total phenolic content of *T. albiflora* K. Larsen's flowers.

Type of *T. albiflora* K. Larsen's flowers	IC_{50} of the extract (mg/ml)±SD	Total phenolic content (mg gallic acid/g extract)±SD
White flower	54.704±0.853^{c}	231.33±5.619^{a}
Green flower	80.923±1.156^{a}	246.98±16.661^{a}
Violet flower	70.419±0.268^{b}	239.36±15.125^{a}

Statistical significance was determined by analysis of variance (ANOVA) with adjustments for multiple comparisons with Tukey's test. Values are means ± standard deviation of triplicate determinations. Values on the same column with different superscripts are significantly different ($P \leq 0.05$).

GC-MS analysis of *T. albiflora* K. Larsen's flowers

Phytochemical composition of *T. albiflora* K. Larsen's flowers was investigated (**Table 3**) and found to contain the essential compounds (i.e., cis,cis-linoleic acid of 16.10, 18.54, and 20.78 % of white, green, and violet flower, respectively). Linoleic acid is an essential polyunsaturated fatty acid in human low-density lipoprotein. An earlier study suggested that linoleic acid may initiate responses that lessen damage caused by oxidative stress [31]. There is evidence of the beneficial effects of polyunsaturated fatty acid on coronary heart diseases [32,33]. Linoleic acid could protect apoptotic renal cell death via inhibition of endoplasmic reticulum stress [34], and also prevent oxidative stress against DNA damage and apoptosis induced by palmitic acid [35]. Previous research indicated that linoleic acid and calcium intake of low daily doses during the third trimester of pregnancy reduced the incidence of preeclampsia significantly in women at high risk [36]. In addition, levels of cardiolipin in cultured skin fibroblasts of patients with Barth syndrome can be restored by the addition of linoleic acid [37]. *T. albiflora* K. Larsen's

flowers also consist of other essential fatty acids, such as hexadecanoic acid (21.95 - 26.99 %) and hexadeconic acid ethyl ester (3.38 - 5.07 %). The insulin plant *Costus pictus* is anti-hyperglycemic and provides insulin secretory activity. The chemical composition of this plant is similar to that of *T. albiflora* K. Larsen. It is composed of hexadecanoic acid (28.3 %) and cis,cis-linoleic acid (18.33 %) [38]. Moreover, *Phargmytes vallatoria*, which has medicinal properties, e.g., wound healing and benefits towards arthritis, antimetics, fabrifuges, rheumatism, and diabetes, also contains hexadeconic acid ethyl ester (30.88 %) and cis,cis-linoleic acid (19.44 %) [39]. The results revealed that essential fatty acid was found in all *T. albiflora* K. Larsen's flowers, and this may continue to be useful for studies on pharmaceutical activity.

Table 3 Phytochemical composition of *T. albiflora* K. Larsen's flowers.

Flower type	Retention time	Component	Percentage
White flower	16.578	5-Hydroxymethylfurfural	11.00
	32.714	Propylparaben	13.31
	44.667	Palmitic acid (Hexadecanoic acid)	21.95
	45.428	Hexadecanoic acid, ethyl ester	5.07
	49.863	cis-9,cis-12-Octadecadienoic acid (cis,cis-Linoleic acid)	16.10
	50.452	9,12-Octadecadienoic acid, ethyl ester	8.55
Green flower	16.429	2- Furancarboxaldehyde, 5-(hydroxymethyl)	4.95
	32.714	Propylparaben	15.21
	44.656	Palmitic acid (Hexadecanoic acid)	24.90
	45.422	Hexadecanoic acid, ethyl ester	4.54
	49.846	cis-9,cis-12-Octadecadienoic acid (cis,cis-Linoleic acid)	18.54
	50.446	9,12-Octadecadienoic acid, ethyl ester	7.88
Violet flower	16.578	2-Furancarboxaldehyde, 5-(hydroxymethyl)	8.94
	32.754	Propylparaben	14.56
	44.724	Palmitic acid (Hexadecanoic acid)	26.99
	45.428	Hexadecanoic acid, ethyl ester	3.38
	49.925	cis-9,cis-12-Octadecadienoic acid (cis,cis-Linoleic acid)	20.78
	50.452	9,12-Octadecadienoic acid, ethyl ester	6.06

Conclusions

The results showed that *T. albiflora K.* Larsen's flowers contained nutritional values and antioxidant properties (54.704 - 80.923 mg/ml of IC_{50}). High ratios of protein content (26.00 - 29.60 g/100 g dry weight) and vitamin B_2 (0.426 - 2.020 mg/100 g dry weight) were found. Phytochemical composition analysis presented a high content of essential oils of cis,cis-linoleic acid, hexadecanoic acid, and hexadeconic acid ethyl ester (16.10 - 20.78 %, 21.95 - 26.99 %, and 3.38 - 5.07 %, respectively). Therefore, *T. albiflora K.* Larsen's flowers can be used for nutrient consumption and may be used in pharmaceutical treatment.

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