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Nutritional and antinutritional values of leaves and stems of *Ocimum tenuiflorum* L.

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Abstract

Ocimum tenuiflorum L., commonly known as ruku in Malaysia, is usually cultivated as a garden ornamental plant because of its small purplish and some yellowish flower. Additionally, the young leaves of *O. tenuiflorum* L. are used to make Nasi Ulam. In this study, we investigated the nutritional values of *O. tenuiflorum* leaves and stems to find a rich source of essential nutrients needed in daily diet. High pressure liquid chromatography (HPLC) and gas chromatography/mass spectrophotometer (GC/MS) methods were used to measure the amino acid and fatty acid content of leaves and stems of *O. tenuiflorum* L. The result of antinutritional compositions of the leaves and stems displayed as a safe range of compounds that were effective to increase absorption of nutritional compound by body cells. The result of fatty acid profile showed that the butanoic acid as the highest amount of fatty acid content in stems and leave compared to other fatty acid compositions. The result of amino acids profile indicated that *α*-aminobutyric acid and cysteine (non-essential amino acid) showed the highest and the lowest amount of amino acid content in stem and leaves consequently. Vitamin E and C showed the high and lowest amount of vitamins content in the leave and stem, respectively. In conclusion, the result of antinutritional and nutritional contents of *O. tenuiflorum* L. leaves and stems clearly indicated that the antinutritional compounds were in the safe range. The amount and nutritional content of the leaves and stem proved that this plant a good source to improve our body health system.

1. Introduction

There has been a correlation conducted by epidemiological studies regarding certain diets, specific foods, and disease expression since the last three decades (Bidlack *et al*., 2000). Currently, the discovery of cheaper sources of protein and other nutrients is highly considered by researchers. This could be found from most plant materials, which are mostly under-utilized (Eknayake *et al*., 1999). Beside of that, each particular plant species or group having its own unique medicinal actions are consistent with this concept. There are many reports due to different unique compound from a different family of plants as a nutritional compound which is useful for the treatment of special disease (Bidlack *et al.*, 2000). However, between many plant varieties and family, the plant in Lamiaceae family showed valuable properties in medicinal preparations, flavoring, confectionery, cosmetics, and perfumery as an aromatic herbs plants (Magness, 2006). Due to the abundant content of essential oils within these plants family, they can perform antibacterial, antimicrobial, and suppressive activities against tumour formation (Farhat *et al.*, 2001).

Ocimum tenuiflorum L., commonly known as ruku in Malaysia, is usually cultivated as a garden ornamental plant because of its small purplish and some yellowish flower. Additionally, the young leaves of *O. tenuiflorum* L. are used to make Nasi Ulam. Due to its high medical potential, *O. tenuiflorum*, becomes one of the few wonder herbs (Kothari *et al.*, 2004). This can be seen from the content of essential oil within the leaves, where various compounds of medical value are available (Rai *et al.*, 2004). Due to the numerous curatives uses of this plant, it is thought to be highly sacred in India (Kothari *et al.*, 2004). Many studies regarding the effects of various drying methods performed on the antioxidant capacity of *O. tenuiflorum* have displayed several promising results (Rabeta and Lai, 2013). In addition, the

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reports have been recorded regarding the anti-microbial evaluation (Mousavi *et al.*, 2014), antidiabetic properties (Mousavi *et al.,* 2016) and promote wound healing (Rohini *et al.*, 2019) of *O. tenuiflorum*.

However, no report has been made regarding the nutritional and antinutritional values of *O. tenuiflorum* L. leaves and stems. Therefore, the potentials of the chemical composition (proximate analysis, amino acid profile, fatty acid, vitamins, and antinutritional composition) of frozen and dried leaves and stems of *O. tenuiflorum* L. were investigated in this research. Finally, the quantity of various nutrients present and the nutritional value of the leaves and stems will be determined separately by the parameters.

2. Materials and methods

2.1 Plant material and preparation of sample

The fresh sample *O. tenuiflorum* leaves and stem was collected from Perak (Malaysia). Then, the sample was identified and kept at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia (USM Herbarium number 11400). Flowers and leaves were separated from the stem and cleaned with tap water. Damaged leaves were removed and the remains were rinsed with distilled water. Then, the samples were freeze-dried for three days following Ratti (2001) protocol. After completely dried, the samples were ground into powder form using a blender. The powder was packaged in vacuum pack and kept at 4˚C (Toshiba, GR-M48MP, Minato-Ku, Japan) for further analysis (Eknayake *et al.*, 1999).

2.2 Proximate analysis

The test on the contents of moisture, ash, and fat were conducted following the methods of Association of the Official Analytical Chemists (AOAC, 2000). All measurements were performed in triplicate. The carbohydrate content was calculated with the equation below:

% Carbohydrate = 100 % - (% Crude protein + % Crude fat + % Crude fiber + % Ash)

2.3 Fatty acid analysis

Upon hydrolyzation, fatty acid analysis within the leaves and stems of *O. tenuiflorum* L. was performed by gas chromatography (GC) separately. Flame ionization detector was used for the injection process into a Gas Chromatography (GC-MS, 17-A-Shimadzu Scientific Inc., USA). The column used was the 30m × 0.25mm fused silica capillary, with 70% cyanopropylpolysilphenylene-siloxane of 0.25 µm film thickness coated on it. Hydrogen was used as a the carrier gas at a constant linear velocity, 28 cm/s. The temperature used for injection was 250˚C, while the temperature for the detector was 280˚C. The programming of the oven was as follows: 80°C set for 2 minutes,5˚C/min to 200˚C set for 10 mins, and 10 ˚C/ min to 230°C set for another 10 mins.

2.4 Amino acids analysis

Amino acids analysis was performed according to the method of Boogers *et al.* (2008). Samples were subjected to hydrolysis process using 6N HCl at 110˚C in screw-capped tubes. Reverse-phase HPLC with the AccQ-Tag 3.9 × 150 mm column at a temperature of 35˚C, a fluorescence detector Mod.474 (λ_{ecc}=285 nm; λ_{em}=345 nm), an auto sampler programmed with injector, and a water system with Alliance 2690 Separation Module pump and "AccQ-Fluor Reagent Kit Watres" (borate buffer, AQC, CH_3CN; Cat. No. 052880) was used in order to conduct the derivatization procedure. Eluents comprised of the gradient of phosphate buffer H_2O (C) (flow-rate 1.0 mL/min), CH_3CN (B), and pH 5.80 (A) (Strydom and Cohen, 1994).

2.5 Water soluble and fat-soluble vitamin analysis

All chemicals of analytical reagent grade were used for this analysis. Vitamins standards (purity>99.0%), such as α-tocopherol (E), folic acid (B12), niacin (B3), and ascorbic acid (C), butylatedhydroxytoluene (BHT), triethylamine (TEA), trans-β-Apo-8́-carotenal, hippuric acid, and internal standards were purchased from Sigma Aldrich (Madrid, Spain). Water soluble vitamin standard solutions and hippuric acid solution (1 mg/mL) was prepared in 10 mM of ammonium acetate (pH 4.5) and stored in the dark at 4˚C. The folic acid (0.01 mg/mL), pyridoxine (1 mg/mL), niacin, and ascorbic acid (5 mg/ mL) were prepared in different volume. Following that, trans-β-Apo-8́-carotenal, β-carotene, and α-tocopherol were dissolved in methanol (1 mg/mL) and kept at −20˚C in dark condition. Further analysis was performed by HPLC-MS/MS (Thermo Scientific, San Jose, CA) and the diode array detector (DAD) was used as an auto sampler, followed by a TSQ Quantum triple quadruple analyzer (Thermo Scientific).

In addition, an electro spray (ESI) interface was used to pair the chromatograph to MS analyzer. The column number ACE-100 C18 (100 × 2.1 mm i.d., 3 µm particle size) was used for the analysis (Advanced Chromatographic Technologies, Aberedeen, UK) (Tayade *et al.*, 2013). Meanwhile, the determination of FSV was conducted in an Agilent 1100 HPLC chromatograph (Agilent, Palo Alto, CA), the analytical column of YMC $C_{30,}$ a diode array detector (DAD,) and

auto sampler being included according to Santos *et al.* (2012).

2.6 Phytochemical determination

2.6.1 Total saponin

The spectrophotometric assay was referred to the method described by Baccou *et al*. (1977). Ground leave and stem of 0.5 g each was weighed and added with 10 mL of 80% aqueoue methanol. The mixture was stirred and then centrifuged at 3000 x *g* for 10 mins. The supernatant was collected and transferred into 25 mL measuring flasks. The residue was added with 5 mL of 80% aqueous methanol, centrifuged and the supernatant was collected into the measuring flasks. This step was repeated three times. The supernatant was added with 80% aqueous methanol until 25 mL. In order to determine the saponin, aliquot samples from the flasks were involved. The expression of the results was calculated by the diosgenin equivalents from a standard curve.

2.6.2 Total phytic acid

Analysis on the total phytic acid content was conducted according to the method described by Vaintraub and Lapteva (1988) with some modifications. In brief, 0.5 g of sample was weighed into a beaker and added with 10 mL of 3.5% HCl. The mixture was stirred at 1700 rpm for 1 hr followed by centrifugation at 3500 rpm for 10 mins. To remove the anthocyanin pigments which might disrupt the assay, centrifugation of the obtained supernatant was conducted once more, using activated carbon. The supernatant was decanted and filtered using Whatman No. 1 filter paper. Spectrophotometric assay was performed on the centrifuged mixture an aliquot (1 mL) of filtrate with 2 mL of distilled water and 1 mL of Wage reagent (0.03% of FeCl3.6H2O with 0.3% sulphosalicylic acid) and measured its absorbance at 500 nm. Phytic acid mg/100g was calculated according to the calibration curve of standard.

2.6.3 Flavanoids

The extraction process was performed by macerating 10 g of sample with 100 mL of 80% aqueous methanol at room temperature for 5 to 10 mins. The contents were filtered with a Whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and dried until a constant weight was achieved. Analysis and calculation of the flavonoid percentage was conducted according to method described by (Boham and Kocipai-Abyazan, 1974).

$$\% \text{ Flavonoids} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

W_1 = Weight of empty crucible, W_2 = Weight of crucible + residue

2.6.4 Alkaloids

In this process, 5 g of the sample was weighed into a 250 mL beaker, followed by the addition of 200 mL of 10% acetic acid in ethanol (C_2H_5OH). After that, the mixture was kept at room temperature under dark condition. Then, the mixture was filtered with Whatman filter paper No. 42 and subsequently concentrated using a water bath until quarter of the original volume was obtained. Drops of concentrated NH_4OH was then added. Alkaloids percentage of samples was evaluated due to the method described by (Doss, 2009).

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

W_1 = Weight of empty filter paper, W_2 = Weight of filter paper + Alkaloid

2.6.5 Total tannin

The total tannin content was determined with the Follin Denis titrating method was as described by Kokke (1977). Approximately 100 mL of petroleum ether was added into 20 g of the crushed sample within a conical flask, before it was covered for 24 hrs. Then, filtration on the sample was conducted followed by standing of 15 mins, with the purpose of enabling the evaporation of petroleum ether. A total of 4 hrs were taken in order to re-extract the sample, where it was soaked in 100 mL of 10% of acetic acid in ethanol. After filtration of the sample, 25 mL of NH_4OH was added to the filtrate for the precipitation process performed on the alkaloids. In order to remove some of the remaining NH_4OH within the solution, heating process was performed on the alkaloids. A total of 5 mL of precipate was taken and added with 20 mL of ethanol. Using phenolphthalein as an indicator, the liquid was titrated with 0.1 M of NaOH until the end point.Calculation was made on the tannin content using % ($C_1V_1 = C_2V_2$) molarities.

2.6.5 Total phenols

The total phenols was determined via spectrophotometric method following Edeoga *et al.* (2005). Using 50 mL of diethylether $(CH_3CH_2)_2$, the fat-free sample was boiled followed by the transfer of 0.5 mL of the boiled extract into 50 mL of flask and added with 10 mL of distilled water. Then, 5 mL of concentrated amylalcohol and 2 mL of ammonium hydroxide solution were added ($CH_3(CH_2)_3CH_2OH$). The absorbance was measured at 505 nm.

$$\text{Conc. of Sample (mg/L)} = \frac{\text{Absorbance of sample} \times \text{Conc. of sample}}{\text{Absorbance of Standard}}$$

2.7 Statistical analysis

For each measurement, triplicates were performed. Results were expressed as mean±SEM and SPSS (version 21.0) was utilized to perform t-test at $p<0.05$.

3. Results and discussion

3.1 Proximate composition of O. tenuiflorum L. leaves and stems

Table 1 shows that the result of the proximate analysis (%) of leaves and stems of *O. tenuiflorum* L. Based on the result, there were significant differences between leaves and stems, which could be seen from the fat and crude protein content in the leaves. To illustrate this, the amount of fat and crude protein content in leaves was higher, in comparison to the amount within the stems. However, based on the results, the amount of the protein content within the leaves and stems was slightly lesser, compared to the protein content of *O. grastismium* leaves. This result was reported by (Idris *et al.*, 2011). In contrast, there was a higher fat content within *O. tenuiflorum* Leaves, compared to the amount of fat content within *O. grastismium* plant. This was reported by (Idris *et al.*, 2011). Nevertheless, due to the protein's function as enzymes, hormones, and antibodies, it holds a high importance (Rampal *et al.*, 2010). Furthermore, besides having its has an essential role in the formulation of the outer layer of skin, hair, teeth, and bones, it aids in the maintenance of the structure of blood vessels and other tissues (Rampal *et al.*, 2010). Based on the result of our finding, it had shown that the stem of *O. tenuiflorum* L. lacked of lipids. This can be observed from the contents of food within leafy vegetables with low lipid. This provides health benefits which help avoid obesity (Lintas and Cappelloni, 1992).

Table 1. Proximate composition of *Ocimum tenuiflorum* L. leaves and stems (% d.b.)

Parameters	Composition	
	Leaves	Stems
Crude Protein	12.69±0.61*	5.21±0.11
Crude Fibre	0.56±0.05	0.87±0.03*
Ash	0.3±0.05*	0.17±0.06
Moisture content	14.71±0.61*	9.41±0.71
Fat	0.04±0.0*	0.01±0.00
Carbohydrate	71.7±0.05	84.33±0.04*

Values are expressed as mean ±SEM. *Significantly different $p<0.05$.

The largest amount of the crude fiber content of the stem (0.87±0.03) was present significantly, in comparison to the amount of it within the leaves (0.56±0.05). However, based on the result obtained, the amount of crude fiber content of our study was lesser, compared to the amount of the ones within the crude fiber content of *Balanites aegyptica* (Rampal *et al.*, 2010) and *Ocimum grastismium* (Idris *et al.*, 2011). In fact, fiber content is highly essential for body. To illustrate this, the intake of fiber can lead to the decrease of serum cholesterol level for the requirement for insulin, hypertension, diabetes, breast cancer, and constipation (Ramulu and Rao, 2003). However, compared to leaves and flowers, *O. tenuiflorum* L. could be a valuable source of dietary fiber than leaf and flowers. There is a higher amount of value shown by the moisture content of *O. tenuiflorum* L., in comparison to the amount of value within the stems. Based on this result, it has been indicated that this plant is capable of a long term storage without getting spoiled (Bouba, 2012). Furthermore, the amount of moisture within the content of leaves and stems of *O. tenuiflorum* L. was higher, in comparison to the amount of moisture from the whole plant of *O. grastismum* that reported by (Idris *et al.*, 2011).

Moreover, the ash content of *O. tenuiflorum* L. leaves almost doubled when compared with stems. However, the amount of ash in the leaves of *O. tenuiflorum* L. leaves was 0.3±0.05, which displayed a significant difference from the stems (0.17±0.06) at $p<0.05$. The previous study of *O. grastismium* reported the result of the ash content that the amount within the stems and leaves were higher (Idris *et al.*, 2011). Ash is an inorganic compound left after the removal of water and all organic and inorganic matter, which is performed by heating in the presence of oxidizing agents, which provides the overall amount of minerals within food (Bouba, 2012).

3.2 Fatty acid profile of O. tenuiflorum L. leaves and stems

The results of fatty acid analysis of the stem and leaves of *O. tenuiflorum* L are displayed in Table 2. Based on the results, the butanoic acid showed the highest amount of fatty acid in stems and leaves, in comparison to other fatty acid compositions. However, the amount of saturated fatty acid in comparison to stems is slightly higher than the ones in the leaves. At this point, the results of saturated fatty acid profile in the leaves display the amount of methyl tetradecanoate in the stem. On the other hand, there was no significant difference shown by octadecanoic acid, tetracosanoic acid, docosanoic acid, eicosanoic acid, pentadeconoic acid, capronic acid, and octanoic acid. Out of all substances, the discovery of tricosanoic acid only occurred on leaves. Based on the previous study, the daily diet of capric acid (>300μM) is highly effective for the regulation of the secretion of insulin from pancreas β -cell (Nagasumi *et al.*, 2009). Besides, the presence of deanoic acid results to its usefulness in ester manufacturing to produce artificial fruit flavour and perfume (Anderson, 2008).

Table 2. Fatty acid profile of *Ocimum tenuiflorum* L. leaves and stems

Compound name	Chemical Compound	Leaves	Stems
Saturated			
Butanoic acid ME	C4:0	66.87±2.31	81.98±2.20*
Capronic acid	C6:0	0.94±0.58*	0.57±0.30
Octanoic acid ME	C8:0	2.49±0.4*	1.84±0.10
Decanoic acid ME	C10:0	0.15±0.02*	0.11±0.01
Undecanoic acid ME	C11:0	0.09±0.01*	0.03±0.05
Dodecanoic acid ME	C12:0	0.09±0.01*	-
Tridecanoic acid ME	C13:0	0.55±0.05*	0.10±0.01
Methyl tetradecanoate ME	C14:0	0.06±0.04	0.57±0.06*
Pentadecanoic acid ME	C15:0	0.10±0.01	0.01±0.00
Hexadecanoic acid ME	C16:0	1.70±0.23*	0.93±0.72
Heptadecanoic acid ME	C17:0	0.04±0.01*	0.02±0.01
Octadecanoic acid ME	C18:0	0.12±0.02	0.12±0.01
Eicosanoic acid ME	C20:0	0.1±0.02	0.07±0.01
Heneicosanoic acid ME	C21:0	0.03±0.01	0.04±0.01
Docosanoic acid ME	C22:0	0.14±0.01	-
Tricosanoic acid ME	C23:0	0.29±0.05	0.05±0.01
Tetracosanoic acid ME	C24:0	0.1±0.02	0.07±0.01
Unsaturated			
Methyl myristoleate ME	C14:1	0.10±0.02	0.02±0.01
9-Hexadecenoic acid ME	C16:1	0.03±0.03	0.02±0.01
Polyunsaturated			
Vaccenic acid	C 18:1n-7	0.38±0.07	0.34±0.04
Oleic acid	C 18:1n-9	0.34±0.05	0.22±0.01
γ Linolenic acid ME	C 18:3n-6	0.12±0.02	-
9,12,15-Octadecatrienoic acid	C 18:3n-3	3.47±0.50*	0.80±0.05
7,10,13-Eicosatrienoic acid	C20:3n-6	0.07±0.02*	0.02±0.01
Arachidonate acid ME	C 20:4n-6	0.15±0.07	0.04±0.01
Methyl11,14,1eicosatrienoate	C20:3n-3	0.04±0.01	-
Gondoic acid	C20:1n-9	-	0.04±0.01
Docosapentaenoic acid n3	C22:5n-3	0.07±0.02*	0.02±0.01
Dihomo-gamma-linolenic	C20:3n-6	0.45±0.02	0.11±0.01
Docosahexaenoic acid	C22:6n-3	0.38±0.07	0.34±0.04

Values are expressed as mean ±SEM. *Significantly different $p<0.05$.

Based on the result, there was no significant effect shown between the leaves and stems for the unsaturated fatty acid. The amount of polyunsaturated fatty acids compositions in the leaves was higher compared to the polyunsaturated fatty acids compositions in the stem. However, there was no significant effect shown by vaccenic acid, oleic acid, arachidonate acid, and docosahexaenoic acid. Based on the previous study, not only the long-term effects on glucose metabolism were displayed by eicosanoic and docosahexaenoic acids, they were also required as a precursor in the production of bio -diesel fuel (Anderson, 2008). In fact, oleic acid is a fatty acid which naturally exists within many kinds of animals, oils, and vegetable. This monounsaturated fat, besides having a relation with the decrease in low-density lipoprotein (LDL) cholesterol, it leads to the increase of high-density lipoprotein (HDL) cholesterol (Lunn *et al.*, 2000). Moreover, due to the positive effects provided by lipoproteins metabolism, linolenic acid was recommended for the regulation of diabetes and heart disease (Oguanobi *et al.*, 2012). Besides, linolenic acid is the most essential unsaturated omega- 6 fatty acid. Not only that, it is also a polyunsaturated fatty acid used for some prostaglandins and the biosynthesis of arachidonic acid (AA) (Nelson and Cox, 2005).

3.3 Amino acid profile of O. tenuiflorum L. leaves and stems

The essential and non-essential amino acid profile is displayed in Table 3. The level of hydroxyprolin within stems is higher compared to the leaves. However, there is a higher level of essential amino acid within leaves, in comparison to the ones within the stem. They consist of the amino acids within *O. tenuiflorum* leaves and stem (tryptophan and phenylalanine, leucine, isoleucine, methionine, valine, threonine, arginine, histidine, and lysine), and the daily intake for most of them is according to (Food and Agriculture Organization and

World Health Organization, 2004) the reference values. Furthermore, these amino acids function as the untreated materials so that many other cellular products will be synthesized such as pigments, enzymes, and hormones.

In addition, a number of these amino acids are important for cellular metabolism (Krishna, 2013). Based on the result of non-essential amino acid profile, the number of amino acids within the leaves is the highest compared to those within the stem. Besides, the presence of cysteine can only be observed from leaves. Based on previous studies, cysteine has a role in protecting the beta cells of pancreas from oxidative stress (Yanpallewar *et al.*, 2004). Additionally, previous studies have also found that arginine functions as anti-inflammatory for the proliferation of fibroblast cells among people who are diagnosed with diabetic wound healing (Algariri *et al.*, 2013). According to the most recent report, insulin synthesis and sensitivity can be enhanced through the daily intake of glutamine (Krishna, 2013).

Table 3. Amino acid profile of *Ocimum tenuiflorum* L. leaves and stems

Compound name	Essential amino acid	
	Leaves	Stems
Hydroxyprolin	0.56±0.08	1.44±0.04*
Histidine	2.13±0.06*	1.73±0.05
Isoleucine	4.15±0.03*	3.71±0.01
Leucine	7.1±0.02*	6.40±0.07
Lysine	5.85±0.12*	5.32±0.12
Methionine	0.90±0.50*	0.63±0.01
Phenylalanine	5.08±0.08*	3.93±0.03
Threonine	4.43±0.06*	3.75±0.02
Tryptophan	3.77±0.10*	2.86±0.02
Valine	5.36±0.04*	4.84±0.03
Non-Essential amino acid		
Alanine	5.45±0.17*	4.78±0.06
Arginine	5.08±0.23*	4.13±0.10
Aspartic acid	11.20±0.10*	9.82±0.63
Cysteine	0.05±0.00*	-
Glutamine	12.10±0.20*	12.70 ±0.60
Glycine	4.10±0.12	4.43±0.60
Proline	5.26±0.06*	4.75±0.03
Serine	4.71±0.20	4.51±0.2 0
α-Aminobutyric acid	10.61±0.4	21.12±0.06*
Ammonia	0.30±0.02	0.40±0.01*

Values are expressed as mean ±SEM. *Significantly different $p<0.05$.

3.4 Vitamin compositions of O. tenuiflorum L. leaves and stems

Based on the result of vitamin content presented in Table 4, there was a high content of water-soluble vitamins and fat-soluble vitamins in the leaves of *O. tenuiflorum* contain, compared to those in the stems. Due to the previous research, the presence of vitamins showed that the high benefits in management of diabetic mellitus. Regarding to previous report, Jäpelt *et al.* (2013) indicated that the vitamins E, B3, B12, and C, which are all free radical scavengers. As mentioned in previous reports, vitamin E showed the important role in protecting of the beta-cells of pancreas from oxidative stress (Wang *et al.*, 2012). Furthermore, it has been reported that vitamin C gives impact to the post-meal glucose level in type II diabetes (Dakhale *et al.*, 2011). Meanwhile, based on the recent report, the preserving beta-cells function can be enhanced by vitamin group B (B3, B12) which was effect on production of the insulin level in body (Patel *et al.*, 2012).

Table 4. Vitamin compositions of *Ocimum tenuiflorum* L. leaves and stems

Vitamins	Amount mg/100 g	
	Leaves	Stems
Vitamin E	1.07±0.04*	0.22±0.063
Vitamin B3	0.078±0.02*	0.054±0.02
Vitamin B12	0.043±0.05*	0.027±0.02
Vitamin C	0.0017±0.0006*	0.0010±0.000

Values are expressed as mean ±SEM. *Significantly different $p<0.05$.

3.5 Phytic acid, saponin of O. tenuiflorum L. leaves and stems.

Table 5 shows that the amount of phytic acid and saponin content of *O. tenuiflorum* L. leaves and stems. The phytic acid did not show any significant difference between the leaves and stems. Due to previous study reported that the percentage of phytic acid's proportion in dry matter of oilseeds, legume, and cereal, ranged from 10% to 30% g/kg. However, based on the previous research, the lowering of phytate should be done as much as possible. The acceptable amount of phytic acid in food contain was reported at 25 mg or less per 100 g of the containing food in order to obtain the best physical condition (Inuwa *et al.*, 2011). Moreover, there is a negative effect coming along with the excessive consumption of phytic acid in diet on mineral balance, due to the formation of insoluble complexes with essential mineral (Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+}) and the reduction of the bioavailability of these minerals (Fordyce *et al.*, 1987). In addition, it has also been shown that there is an interaction between phytate and the basic residues of proteins, which suppresses several digestive enzymes (Ekholm *et al.*, 2003).

The result of saponin content in Table 5 indicated that the amount of saponin content within the leaves is slightly higher compared to the saponin content within the stems. Saponin is generally known as secondary

compounds, which are acknowledged as surface active compounds and non-volatile (Manivannan *et al.*, 2015). Furthermore, due to their positive effects of saponins on human's body, research about the effect of that is considerable interest (Edet *et al.*, 2015) and the results of researcher indicated that consuming of saponins are necessary for healthy since saponins showed the effect on reducing the risk of heart disease by binding of that with plasma membrane and cholesterol (Ogunleye *et al.*, 2016).

Table 5. Phytic acid, Saponin of *Ocimum tenuiflorum* L. leaves and stems

Constituent	Leaves (µg/mL)	Stems (µg/mL)
Phytic acid	1.22±0.2	0.99±0.15
Saponin	0.23±0.03*	0.20±0.01

Values are expressed as mean ±SEM. *Significantly different $p<0.05$.

3.6 Phytochemical values of O. tenuiflorum L. leaves and stems

These non-nutrient plant chemical compounds or bioactive components are mostly known as phytoconstituents (phyto is Greek work which means plant) or phytochemicals. Their role is to protect plants against infestations by pests or microbial infections (Doughari, 2009). There has been separation and isolation occurring of phytochemicals from fruits, green tea, red wine, spices such as turmeric and vegetables like broccoli and onion, grapes and apples, and many other sources (Tiwari *et al.*, 2011).

Table 6. Phytochemical constituents on dry weight basis expressed as mg/100 g

Constituent	Leaves (mg/100 g)	Stems (mg/100 g)
Phenols	1.7±0.02*	0.77±0.03
Alkaloids	2.9±0.40*	1.80±0.30
Tannins	0.51±0.10*	0.16±0.02
Flavonoids	1.16±0.20*	0.51±0.10

Values are expressed as mean ±SEM. *Significantly different $p<0.05$.

The overall content of phenols, alkaloids, tannins, and flavonoids within *O. tenuiflorum* L. leaves and stems are displayed in Table 6. In comparison to the phytochemical in the stems, the amount of phytochemical in leaves is higher. Based on the result, the duplication of the phenol content in leaves (1.7±0.02) occurs more frequently compared to the stems (0.77±0.03) of *O. tenuiflorum* L. Nevertheless, compared to the stems, the amount of alkaloid, flavonoid, and tannin contents within leaves were almost in duplicate and triplicate. Based on the previous study, the toxicity of alkaloids can occur from their high concentration, especially when it exceeds the lethal dose of 20 mg/100 g (Inuwa *et al.*, 2011). On the other hand, compared to other active compounds, the low toxicity of flavonoids provides humans with alternatives in their diet (Brill *et al.*, 2012). According to the *in vitro* study that was reported by Adebajo *et al.*, 2009, anti-allergic, anti-inflammatory, and anti-diarrheal activity could be discovered within flavonoids. Furthermore, there are chemical components which are usually in the form of natural colour pigments. These components like polyphenolics, phenols, or phenolics (or polyphenol extracts) play a role in the colour of fruits (Shahidi and Naczk, 2006). The phenolic compounds mainly function in protect human body from herbivore predators and pathogens; they are utilized to manage pathogenic infections in human's body (Eghdami and Sadeghi, 2010). Meanwhile, the largest group of secondary chemical constituents are alkaloids, which mostly comprise of ammonia compounds (Tiwari *et al.*, 2011). Aside from the function of the nitrogenous compounds of alkaloids as the protector of plants against pathogens and herbivores, they are widely used as poisons, narcotics, stimulants, and pharmaceuticals as they have strong biological activities (Moreno *et al.*, 2006). Moreover, there is a wide distribution of tannin occurring among flora. They are the phenolic compounds of high molecular weight which can be discovered in the outer layers, stem, bark, and the root of plant tissue (Pawar, 2011). Flavonoids belong to an important group of polyphenols, which population is dominant amongst flora. Furthermore, there is more than one benzene ring in the structure of flavonoids (a range of C15 aromatic compounds). Apart from that, their use as antioxidants or free radical scavengers receives numerous supports from study reports (Doughari, 2009).

4. Conclusion

The potentials of *O. tenuiflorum* L. leaves as the abundant source of the important nutrients needed in daily diet were demonstrated in the results of this study. Besides, they have the potential as a useful supplement needed for managing degenerative diseases which are resulted from the generation of free radicals. Finally, based on previous studies, the level of antinutritional compounds in the leaves and stems of *O. tenuiflorum* L. was found to be within the safe level. Besides, provided that these medicinal plants are a source of useful drugs, it is important to consume them in moderate amount for the improvement of health conditions.

Conflict of Interest

The authors declare no conflict of interest.

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