The Pharmaceutical and Chemical Journal, 2017, 4(4):79-84

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Chemical Composition and Antioxidant Properties of Kenari (*Canarium indicum*) Nut

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Abstract Kenari (*Canarium indicum* L) belongs to Burseraceae family and grows abundantly in Indonesia especially in Eastern Indonesia. The kernel is often called kenari nut. The nut contains high amount of lipid and various chemical substances, such as protein, phenolic compound, and tocopherol. The chemical composition is affected by some factors, especially area of origin. The objective of this study was to determine chemical composition and antioxidant properties of kenari nut grown in Sangihe, Minahasa, and Maluku. The results showed that the highest component of kenari nut is lipids, which account for 66.27 %, 65.93%, and 66,59 % of the nut from Sangihe, Minahasa, and Maluku respectively. The dominant fatty acid were oleic, palmitic, stearic, and linoleic. The second compound of kenari nut is protein which accounted for 14.20 %, 13.49%, and 13.38 % of nut from Sangihe, Minahasa, and Maluku respectively. The dominant amino acids were glutamate, leucine, arginine, and aspartate. Glutamate content of nut from Sangihe, Minahasa, and Maluku were 30.11%, 25.30 %, and 25.43%, respectively. In addition to those major compounds, the nut also contained of antioxidant substances, i.e. phenolic compound and tocopherol. Antioxidative activities based on the DPPH values, were 61.3%, 60.2%, and 53.2 % for nut from Sangihe, Minahasa, and Maluku, respectively. Therefore, kenari nut may be used as functional food.

Keywords kenari nut, fatty acids, amino acid, antioxidant.

Introduction

Kenari is a tropical perennial tree nut that belongs to Burceraceae family, genus *Canarium* with 100 species that grows in Melanesia wet forest [1]. Several *Canarium* species that grows in Indonesia are *C. lamili*, *C. vulgare*, and *C. indicum* [2]. Among those species, *C. indicum (*L.) is the most prominent and grows abundantly in eastern Indonesia, such as in Maluku as well as in North Sulawesi. These plants are not normally cultivated. Usually they are planted as fencer or grew wildly in forest.

Tree nut such as almond, cashew, walnut, Brazil nut, hazelnut, pecan, macadamia, and kenari nut are plants rich in bioactive compounds which have high contribution in human health. The compounds can reduce the risk of generative diseases such as high cholesterol, high blood pressure, diabetes, and cataract [3;4]. In general, bioactive compounds in fruit or seed of tree nuts are phenolic compounds, carotenoid, phytosterols, and tocopherols. The concentration of these compounds mainly depends on the variety and the age of the fruit [5]. Phenolic substances in tree nuts generally contribute to the taste and flavour. Some of them also contribute to the colour. These phenolic substances are classified as flavonoids, phenolic acids, and tannins [6].

There are many kind of foods made from kenari in North Sulawesi (Manado). However, scientific evidence on functional properties and health benefits are very limited and needs to be explored. To support this health benefit claim, it is necessary to provide scientific evidence on the functional properties.

The purposes of this research were to study the chemical composition and bioactive compounds and their activities in kenari nuts.

Materials and Methods

Collection of plant Materials

The kenari nut used in this research is *Canarium indicum* collected from Minahasa, Sangihe, and Maluku.
The experiment was conducted in a complete randomized design (CRD), consists of three treatments, (A = Kenari from Sangihe; B = Kenari from Minahasa; C = Kenari from Maluku). The experiment was replicated four times. The parameters measured were proximate composition, fatty acids, amino acids, vitamin E, total phenolic, and antioxidant activity.

Proximate analysis

The moisture, ash, crude fat, and crude protein contents of kenari nut were determined according to the Association of Official Analytical Chemists [7]. The total carbohydrate content was obtained by difference.

Fatty acids analysis

Dried kenari nuts were soaked in hexane and lipids were extracted by soxhlet apparatus, followed by demethylation of fatty acids to FAME [8]. As much as 1.5 µL of FAME sample were injected to Gas Chromatography (GC) and the fatty acid composition was determined according to AOAC *Official Method* 963.22. [7]. The GC system consisted of capiler column HP-5 (*Cross linked* 5% *phenyl methyl silicone*, lenght: 30 m, diameter: 0.32 mm); fitted to FID (temperature of injector was 260°C and that of detector was 270°C). The carrier gas was helium (flow rate 10 mL/min.) with initial temperature 80°C and increased to 250°C at final stage.

Determination of amino acids

Amino acids and dipeptides were determined by high performance liquid chromatography (HPLC) with external amino acid standard. The kenari nut (100 mg) was ground and mixed with 4 mL of 50% (v/v) acetonitril and 50% (v/v) water. The mixture was homogenized for 1 hour, diluted to 10 ml with aquabides and filtered (0.45 µm hydrophilic membrane) before injected to HPLC.

HPLC condition

High performance liquid chromatography (HPLC) was used in determining the amino acids concentration in kenari nut. The Column in used was OmniSpher 5 C18 (4.6 mm x 150 mm). The mobile phase was a mixture of 1% TFA in water : acetonitril : ethyl acetate (86 : 12 : 2). The flow rate was 1.0 ml/min at 40 °C and the pressure was 100 bar. The sample volume injected to HPLC was 20 µL. The UV detector recorded the absorbance of the effluent at 210 nm.

Vitamin E analysis

Vitamine E analysis was conducted according to Wong, *et al* [9] with some modifications. Kenari nut (200 mg) was mixed with 5 mL toluene, 3.5 mL of 0.07% (b/v) 2,2 bipyridin in ethanol, and 0.5 mL of 0.2% (b/v) ferri chloride in ethanol. Methanol was added to make 10 mL mixture. The mixture was shaken for 1 min., and the absorbance was recorded at λ= 520 nm. The absorbance reading was plotted against standard curve of tocopherol (0 – 1.3 µg/mL) and calculated.

Total phenolic

Total phenolic was determined by the Folin-Ciocalteu method [10]. In brief, 100 mg sample were mixed with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of methanol. The mixture was shaken for 1 min, and then 1.5 mL of 15% Na_2CO_3 was added and the mixture was shaken again. Finally, the solution was brought up to 10 mL by adding

distilled water. The mixture was incubated at 50^{o}C for 20 min and centrifuged at 3,000 rpm for 10 min. The absorbance was recorded at 750 nm. Total phenolic was calculated using Gallic acid as a standard.

Radical scavenging activity by DPPH method

Radical scavenging activity was determined following the method of Obame et al. [11] with some modifications. Ground kenari nut were soaked in methanol and and filtered (filter paper Whatman no.1) and 200 μL filtrate was added to 1 mL of 500 μ*M* DPPH in ethanol (end concentration: 250 μ*M*). The mixture was shaken and left for 20 min. at room temperature in the dark. A mixture of blank solution was prepared as control.The absorbance reading was conducted at 517 nm and Radical scavenging activity was calculated using the following formula: Radical scavenging activity (%) = (C-A)/Cx100, where A is the difference absorbance reading between control and sample, and C is the absorbance reading of control.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and least significant differences (LSD) were calculated following a significant F test.

Results and Discussion

Proximate composition

Proximate composition of kenari sample are presented in Table 1. The major component in kenari nut is lipid, followed by protein and carbohydrate. The lipid content in kenari nut is not affected its origin, i.e., Sangihe, Minahasa, and Maluku, which are 66.27%, 65.93%, and 66.59%, respectively. The lipid content is similar to that of Brazil nut as well as almond seed [12]. Kenari nut is suitable as the source of edible oils as well as for high-lipid foods because of the high content of lipids.

Table 1: The proximate composition of Kenari nut collected from different area

No.	Components	Area of origin		
		Sangihe	**Minahasa**	**Maluku**
1	Water content (%)	5.16	6.08	5.68
2	Protein (%)	14.20	13.49	13.38
3	Lipids (%)	66.27	65,93	66.59
4	Ash (%)	3.32	3.41	3.37
5	Carbohydrate (%)	11.05	11.09	10.98

The second major component is protein. Like for lipids, the place of origin did not affect carbohydrate content which accounted for 11.05 %, 11.09 %, and 10.08 % of the nut from Sangihe, Minahasa, and Maluku, respectively.

Based on the content of lipid, protein, and carbohydrate, kenari nut is highly appropriate as foods ingredient. In general, tree nuts are the source of minerals. He and Xia reported that K, Ca and Mg were found abundantly in *C. album* L [13], which may be similar to *C. indicum* L. in this experiment. Commonly, kenari nut is the main raw material in producing halua (traditional food natively from Minahasa), cake topping, and others.

Fatty acids

Oleic is the main fatty acid in kenari nut oil, followed by palmitic, stearic, and linoleic acids (Table 2). The fatty acid composition was not affected by the area of origin.

Table 2: The composition of fatty acids in triacilglycerol extrated from Kenari nut, collected from different areas

No.	Fatty Acids	Area of origin		
	(%)	**Sangihe**	**Minahasa**	**Maluku**
1	Lauric	0.08	0.10	0.06
2	Myristic	0.17	0.11	0.14
3	Palmitic	25.94	25.27	26.34
4	Stearic	13.80	15.70	13.76

5	Oleic	44.96	44.42	44.93
6	Linoleic	13.78	13.75	13.82
7	Linolenic	0.57	0.65	0.54

The data indicated that triacilglycerol of kenari is dominated by unsaturated fatty acids. Such fatty acid can be easily oxidized to form peroxide and the final product gives the shorter carbon chains, which influenced the rancid odor. However, carotenoids in kenari inhibits the oxidation reaction in kenari oil [14], hence may inhibits the rancidity and off-flavour. The content of oleic acids in kenari nut harvested from different area was similar.

Amino acids

Amino acid composition of kenari nut is shown in Table 3. Fifteen amino acids were detected in *Canarium indicum* nuts, including seven essential amino acids, namely methionine, lysine, leucine, isoleucine, threonine, phenylalanine, and valine and eight non-essential amino acids.

Table 3: Amino acid composition (%) of Kenari nut Protein from different area

No.	Amino Acid (%)	Area of origin		
		Sangihe	**Minahasa**	**Maluku**
1	Aspartate	8.66	6.18	8.42
2	Glutamate	30.11	25.30	28.43
3	Serin	3.47	4.57	3.07
4	Glysine	2.86	4.08	2.16
5	Histidine	2.67	3.31	3.23
6	Arginin	8.54	8.64	8.73
7	Threonine	1.30	2.08	1.76
8	Alanine	5.88	4.14	5.21
9	Tyrosine	2.51	2.72	2.66
10	Valine	2.23	3.63	2.14
11	Methionine	1.15	0.85	1.42
12	Isoleusine	2.65	3.14	2.76
13	Leusine	13.64	16.06	14.04
14	Phenylalanine	5.46	5.95	5.22
15	Lysine	1.91	2.73	1.01

The concentration of Glutamate is the highest. This amino acid contributes to the flavour of kenari nut, which belongs to umami flavour. In addition, some amino acids also can act as antioxidant, such as glutamic acid, methionine, alanine, asparagine, valine, aspartic acid, serine, lysine, tryptophan, and histidine [15].

Phenolic compounds

The phenolic and flavonoid compounds of Kenari nut extracted by methanol are presented in Table 4. Total phenolic in *C. odontophyllum* Miq. extracted by ethyl acetate and buthanol ranged from 4 to 10 mg GAE/g, respectively [16]. Phenolic compounds in *C. indicum* L. extracted by methanol in this experiment are in the range of 7.4 to 8.8 mg GAE/g, showing similar polarity characteristic of these two species of kenari nut.

Table 4: Total phenolic content of Kenari nut from different area

No.	Area of origin	Total Phenolic (mg/g) *
1	Sangihe	8.8 ± 0.7^{a}
2	Minahasa	8.0 ± 0.5^{b}
3	Maluku	7.4 ± 0.6^{b}

* values followed by different letter are significantly different at $P \leq 0.05$.

Vitamin E

Vitamin E (tocopherol) is a lipid-soluble vitamin. Vitamin E content of kenari nut from Sangihe, Minahasa and Maluku are 439.36 ppm, 442.83 ppm, and 420.27 ppm, respectively (Table 5) while Azlan et al. reported that vitamin E in the kernel of *C. odontophyllum* Miq. was 120 ppm. This result showed that *C.* indicum L. is a better source of vitamin E compared to *C. odontophyllum* Miq [17]. However, vitamin E level is not significantly different among areas of origin.

Table 5: Vitamin E content of Kenari nut from different area

Area of origin	Vitamin E (ppm)
Sangihe	439.36 ± 68.25
Minahasa	442.81 ± 46.67
Maluku	420.27 ± 46.20

Antioxidant Activity

Kenari nut contains high antioxidative substance, including phenolic and flavonoid. The correlation coefficients (R^2) of the antioxidant activity with phenolic and vitamin E contents are 0.72 and 0.58, respectively. The correlations are positive where the higher the phenolic and vitamin E content, the higher the level of antioxidant activity. Table 6 shows the antioxidant activity of kenari nut, expressed by DPPH's value. DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical-scavenging activity. In DPPH radical scavenging assay, antioxidant react with DPPH (deep violet color), and convert it to yellow coloured α,α-diphenyl-β-picryl hydrazine [18]. In this research, BHT was used as the positive control. There are no significant effects of area of origin on the percentage of DPPH scavenging activity.

Table 6: Percentage of DPPH in three different kenari nut

No.	Area	DPPH (%)
1	Sangihe	61.3±12.7
2	Minahasa	60.2±5.3
3	Maluku	53.2±9.6

Conclusions

The composition of kenari nut was dominated by lipid (more than 65%), most of it was oleic acid. Glutamic acid is the main amino acid in protein. Antioxidant activity of kenari nut expressed by Radical-Scavenging activity of DPPH is in mutual accord with phenolic compound.

Acknowledgements

We acknowledge USAID – Texas A & M University for their funding support to the completion of this research.

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