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Antioxidative Phytochemicals and Anti-Cholinesterase Activity of Native Kembayau (*Canarium Odontophyllum*) Fruit of Sabah, Malaysian Borneo

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Abstract

Canarium odontophyllum, known locally as "Kembayau" or "Dabai", is a highly seasonal fruit which is popular among local people in Borneo Island. This study was conducted to determine the antioxidant activity, phytochemicals (total phenolic, total flavonoid, total anthocyanin and total carotenoid contents) and acetylcholinesterase inhibitor potential of extracts of the flesh and seed of this fruit. *C. odontophyllum* was collected in Beaufort, Sabah, Malaysian Borneo and subsequently freeze-dried and extracted using 80% methanol and distilled water. Antioxidant activities were analyzed using DPPH free radical scavenging, ABTS decolorization and FRAP (Ferric reducing / antioxidant power) assays. Anti-Alzheimer's potential was determined using acetylcholinesterase enzyme inhibition assay. The results showed that the total phenolic and flavonoid contents were higher in the flesh of *C. odontophyllum* with the values of 11.96 ± 0.05 mg gallic acid equivalent (GAE)/g and 10.11 ± 1.54 mg rutin equivalent (RU)/g, respectively. Total anthocyanin and carotenoid content were also higher in the flesh of the fruit with the values of 12.75 ± 0.28 mg/100g and 2.84 ± 0.11 mg/100g. The flesh of the fruit also showed higher antioxidant activity as assessed using DPPH, ABTS and FRAP assays. However, anti-cholinesterase activity was higher in the seed of *C. odontophyllum* which showed that other phytochemical content (besides phenolic and flavonoid) might be responsible to the observed effects. The same trend of phytochemicals, antioxidant and anti-cholinesterase activity was also observed in the distilled water extract. These findings suggested that *C. odontophyllum* is not only nutritious but also displayed potential pharmacological properties.

Keywords: *Canarium odontophyllum*; Phytochemicals; Antioxidants; Acetylcholinesterase inhibition activity

Introduction

There are increasing researches that focus on phytochemical compounds which display diverse pharmacological properties [1]. Since synthetic antioxidants such as butylated hydroxytoluene (BHT) have been shown to be carcinogenic, natural antioxidants from plants and vegetables are considered to be preferable [2]. Consumption of fruits and vegetables has been shown to lower the risk and incidence of certain types of cancer, cardiovascular diseases, and diabetes [3]. In addition, previous study has shown the ability of fruits and vegetables to act as anti-Alzheimer's agents [4].

The rich biodiversity in Borneo offers many edible underutilised fruits that are yet to be fully explored. One such underutilized fruit is *Canarium odontophyllum* (commonly called "Kembayau" in Sabah, Malaysia and "Dabai" in Sarawak, Malaysia), which contains many important nutrients and is rich with phytochemicals that have potential health benefits [5,6]. The unripe skin of the fruit is light yellow in colour and turns to dark purple/black when ripe (Figure 1). The flesh and the skin of *C. odontophyllum* are edible. The flesh is white-yellow in colour and the texture is hard when raw. Local people usually eat the fruit by pouring hot water into the fruits for 10 to 15 minutes to soften the flesh.

Figure 1: The ripe fruit of *Canarium odontophyllum*.

The flesh of the fruit of *C. odontophyllum* weighs about 7-10 g which constitutes almost 47% of the total weight; the seed of the fruit contribute the remainder of the fruit. The diameter of the fruit is about 7-10 cm, and has a hard single three-angled seed (Figure 2). The fruit is

Figure 2: The three-angled seed of *Canarium odontophyllum*.

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highly seasonal, only being available in November and December each year. The flesh of the fruit has a creamy and milky taste and people usually eat *C. odontophyllum* by adding sugar or salt to the fruit. The fruit can also be processed into pickle products. Nowadays the fruit is used in dishes of fried rice, mixed vegetables, *maki* (dried seaweed roll), desserts and salad sauce.

In terms of health, *C. odontophyllum* benefits humans with its high antioxidant capacity that is mainly contributed to the total phenolic compound in the fruits [7]. In addition, the fruit is also nutritious due to the high content of protein, carbohydrate and fat. Besides, it is also rich in minerals such as potassium, phosphorus, calcium and magnesium [6]. A previous study showed that other *Canarium* species have potent antimicrobial activity [8]. He and Xia [9] reported that some *Canarium* species are rich in oleic and linoleic acids which are beneficial to skin as they can reduce acne, act as anti-inflammatory agent as well as maintaining the moisture of the skin [10]. Koudou et al. [11] reported that the essential oil of other *Canarium* species can also act as a painkiller. In light of the promising activity of *Canarium* species, this study was conducted to investigate the phytochemicals, antioxidant and anti-cholinesterase potential of *C. odontophyllum* fruit collected in Sabah, Malaysian Borneo.

Materials and Methods

Plant materials and extraction

The fruit of *C. odontophyllum* was collected from Beaufort, Sabah, Malaysia during October to December 2011. The fruit was cleaned, weighed and separated into edible (flesh+skin) and inedible (seed) components. Small cut pieces were freeze-dried, and the freeze-dried samples ground into fine powder using a dry grinder. The ground samples were sieved to get uniform size, then kept in an air-tight container and stored in a freezer (-20°C) until further analysis.

Samples (0.1 g) were extracted for 2 h with 80% methanol or distilled water with a ratio of 1:50 at room temperature on an orbital shaker set at 200 rpm [12]. The mixture was then centrifuged at 1400xg for 20 min and the supernatant decanted into a 15ml vial. The pellet was re-extracted under identical conditions. The supernatants were combined and used for analysis of total antioxidant activity, total phenolic, total flavonoid, total anthocyanins contents and total carotenoid content, together with antioxidant and anti-cholinesterase activity.

Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent as adapted from Velioglu et al. [12] with slight modifications. Extract (300 µl) was mixed with 2.25 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. To this was added 2.25 ml of sodium carbonate (60 g/l) solution. After 90 min at room temperature, absorbance was measured at 725 nm using spectrophotometer. Standards of gallic acid in the concentration ranges from 0 to 100 µg/ml were run with the test samples, from which a standard curve was plotted. The results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content was determined by using colorimetric method described by Dewanto et al. [13] with slight modification. Briefly, 0.5 ml of extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.3 ml of 10% $AlCl_3.6H_2O$ solution was added, and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added and allowed to stand for another 5 min. The mixture was mixed well with vortex, and the absorbance measured immediately at 510 nm using spectrophotometer. Standards of rutin in the concentration ranges from 0 to 100 µg/ml were run with the test samples, from which a standard curve was plotted. The results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

Determination of total anthocyanin content

Total anthocyanin content was measured using a spectrophotometric pH differential protocol described by Giusti and Wrolstad [14] with slight modification. Briefly, 0.5 ml of the extract was mixed thoroughly with 3.5 ml 0.025 M potassium chloride buffer pH 1. The mixture was mixed with vortex and allowed to stand for 15 min. The absorbance was then measured at 515 and 700 nm against distilled water blank. The extract was then combined similarly with 0.025 M sodium acetate buffer pH 4.5 and the absorbance was measured at the same wavelength after being allowed to stand for 15 min. The total anthocyanin content was calculated using the following equation:

$$\text{Total anthocyanin content } (\text{mg}/100\text{ g of dried sample}) = A \times MW \times DF \times 1000/(\varepsilon \times C)$$

where A is absorbance=(A_{515}–A_{700}) pH 1.0–(A_{515}–A_{700}) pH4.5; MW is molecular weight for cyanidin-3-glucoside=449.2; DF is the dilution factor of the samples, ε is the molar absorbtivity of cyanidin-3-glucoside=26900; C is the concentration of the buffer in mg/mL. The results were expressed as mg of cyanidin-3-glucoside equivalents in 100 g of dried sample (mg c-3-gE/100 g dried sample).

Determination of total carotenoid content

Total carotenoid content was measured by using the method of Hess et al. [15] with slight modification. In brief, 300 µl sample was added to 300 µl distilled water. The mixture was mixed with 1.2 ml n-hexane of the hexane layer. The mixture was centrifuged for 5 minutes at 4°C and absorbance was measured at 350 nm in a spectrophotometer. Standards of β-carotene in the concentration ranges from 0 to 100 µg/ml were run with the test samples, from which a standard curve was plotted. The results were expressed as mg of β-carotene in 100 g of dried sample (mg BC/100 g dried sample).

DPPH free-radical scavenging assay

The scavenging activity of the extract was measured by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical model and a method adapted from Magalhaes et al. [16]. An aliquot (300 µl) of samples or control (80% methanol or distilled water) was mixed with 3.0 ml of 500 µM (DPPH) in absolute ethanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in the dark. Absorbance of the mixture was measured spectrophotometrically at 517 nm, and the free-radical scavenging activity was calculated as follows:

$$\text{Scavenging effect } (\%) = [1 - \{\text{absorbance of sample}/\text{absorbance ofcontrol}\}] \times 100$$

FRAP (Ferric reducing/antioxidant power) assay

This procedure was conducted according to Benzie and Strain [17] with slight modification. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio prior to use and heated to 37°C in a water bath. A total of 3.0 ml FRAP reagent was added to a test tube and a blank reading was taken at 593 nm using spectrophotometer. A total of 100 µl of selected plant extracts and 300

µl of distilled water were added to the cuvette. A second reading at 593 nm was performed after 90 min of incubation at 37°C in water bath right after the addition of the sample to the FRAP reagent. The changes in absorbance after 90 min from initial blank reading were compared with standard curve derived from standard of Fe (II) concentrations in the range 0 and 1000 µg/ml. A standard curve was then plotted. The final result was expressed as the concentration of antioxidant having a ferric reducing ability in 1 gram of sample (µM/g).

ABTS decolorization assay

The ABTS decolorization assay was carried out according to the method describe by Re et al. [18] with slight modification. Working ABTS solution (7mM) and potassium persulfate (2.45 mM) were added into a beaker, and the mixture allowed to stand 15 hours in the dark to generate an ABTS free radical cation solution. The mixture was diluted with 80% methanol or distilled water in order to obtain absorbance of 0.7 ± 0.2 units at 734 nm. To 2 ml of this working ABTS free radical cation solution was added 200 µL of methanolic or distilled water test solution, the mixture vortexed for 45 seconds, and the resulting absorbance value read at 734nm using microtiter plate reader. Standards of ascorbic acid in the concentration range from 0 to 100 µg/ml were run with the test samples, from which a standard curve was plotted. The final result was expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC/g).

Anti-cholinesterase inhibition assay

The anti-cholinesterase inhibition assay was performed according to a previously reported method [19]. Fruit extract sample diluted in phosphate buffer 200 mM pH 7.7 in a total volume of 250 µl was preincubated. To this solution, 80 µl of DTNB (3.96 mg of DTNB and 1.5 mg sodium bicarbonate dissolved in 10 ml phosphate buffer pH 7.7) and 10 µl acetylcholinesterase (AChE; 2 U/ml) were added to the mixture. The mixture was incubated for 5 minutes at 25°C, after which 15 µl of substrate (10.85 mg acetylthiocholine iodide in 5 ml phosphate buffer) were added and the whole incubated for 5 minutes. Intensity of the yellow colour was measured by using microwell plate reader at 412 nm. The percent of inhibition was calculated by using the formula below.

$$\%\ \text{inhibition} = \frac{\text{control absorbance} - \text{sample / tested absorbance}}{\text{control absorbance}} \times 100\%$$

Statistical analyses

All experiments were carried out in 3 replicates in 3 independent experiments. The results were presented as mean ± standard deviation (SD) using Prism 5 software. The data were statistically analysed by one-way ANOVA and Duncan post-hoc test, with a level of statistical significance set at $p \leq 0.05$. Correlation analysis was performed by Pearson's Test.

Results and Discussion

Total phenolic content

The reduction of phosphomolybdic acid to molybdenum blue by the hydroxyl (-OH) groups of phenolic compounds in the presence of an alkaline medium is the principle used in this method. The blue coloured complex can be detected spectrophotometrically at a wavelength of 760 nm [20]. The total phenolic content of *C. odontophyllum* was higher in the flesh than in the seed (Table 1). The results of this study are in line with those of previous studies which also reported that the edible parts of *C. odontophyllum* (i.e peel and pulp) exhibit high phenolic content as compared to the seed part of the fruit [21,22]. Ellagic acid and vanilic acid are among the phenolic acids that are reported to be present in C. *odontophyllum* [23]. High polarity of organic solvent in 80% methanolic extract proved to be more efficient to extract phenolic compounds in the sample rather than mono component solvent, thus explaining the reason of why methanolic extract yields higher phenolic content than in aqueous extract in the present study [24].

Total flavonoid content

A previous study has demonstrated that the fruits of *C. odontophyllum* contain flavonoids, namely catechin and epicatechin. These flavonoids might contribute to the antioxidant activity in the fruit [23]. The present study showed that the total flavonoid content is higher in the flesh of *C. odontophyllum* compared to the seed part in both 80% methanol and aqueous extract (Table 1). Prasad et al. [21] also reported higher flavonoid content in both peel and pulp of *C. odontophyllum* rather than seed when extracted with ethyl acetate, n-butanol and water which is also similar to the present study. Among the two extracts, the 80% methanol extract always showed higher flavonoid content than the aqueous extract. This is in keeping with the observation that a mixture of two solvents producing a more polar medium facilitates extraction of more phytochemical compound [24].

Total anthocyanin content

Monomeric anthocyanins undergo a reversible structural transformation as a function of pH where the coloured oxonium form exists at pH 1.0 and the colourless hemiketal form exists at pH 4.5. Thus, the difference in absorbance of the pigment at 520 nm is proportional to the concentration of pigment [25]. Anthocyanins are usually associated with the colour of the fruits, being particularly responsible for the pink, red, blue and purple colours in plants [26]. Higher anthocyanin content was detected in the flesh part of *C. odontophyllum* as compared to its

Sample	Total Phenolics[1] (mg GAE/g)	Total Flavonoids[2] (mg RE/g)	Total Anthocyanins[3] (mg C-3-GE/100g)	Total Carotenoids[4] (mg BC/100 g)
80% methanol				
Flesh	11.96 ± 0.05^{a}	10.11 ± 1.54^{a}	12.75 ± 0.28^{a}	2.84 ± 0.11^{a}
Seed	5.54 ± 0.19^{b}	3.84 ± 0.05^{b,c}	0.82 ± 0.01^{c}	0.66 ± 0.09^{c}
Aqueous				
Flesh	5.72 ± 0.10^{b}	5.04 ± 0.19^{b}	9.06 ± 0.03^{b}	1.56 ± 0.12^{b}
Seed	3.94 ± 0.12^{c}	2.78 ± 0.06^{c}	0.53 ± 0.07^{c}	0.33 ± 0.03^{d}

Values are presented as mean ± SD (n = 3) which, with different letters (within column), are significantly different at $p < 0.05$.
[1]Total phenolic content was expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).
[2]Total flavonoid content was expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).
[3]Total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalents in 100 g of dried sample (mg C-3-GE/100 g dried sample).
[4]Total carotenoid content was expressed as mg of β-Carotene in 100 g of dried sample (mg BC/100 g dried sample).

Table 1: Content of total phenolics, total flavonoids, total anthocyanins and total carotenoids in extracts of fruit of *C. odontophyllum*.

seed in both extracts (Table 1). The flesh part of *C. odonthophyllum* which is dark purple in colour might be contributed mostly by the anthocyanin as suggested in a previous study [23].

Total carotenoid content

Epidemiological studies show that carotenoid-rich foods protect against cancer, heart disease and diabetes [27]. The present study indicated higher total carotenoid content in the flesh part of *C. odontophyllum* as compared to the seed part. Carotenoid synonymously known to gives colour to the fruits. Hence, this is clearly explained why the flesh (outer part) have more carotenoid contents as compared to its seed (Table 1). Previously, carotenoids profiles of peel, pulp and seed of the fruit and their related antioxidant capacities showed that the peel and pulp was enriched with all-trans-β-carotene, when compared with the seed part of *C. odontophyllum* [28], which also supported the findings of the present study.

Scavenging activity on 2,2-diphenyl-2-picrylhydrazyl radical

The process of either electron or hydrogen donation cause the reduction of purple colour of DPPH [29]. The ability of the antioxidant to decolorize the purple DPPH colour indicates the ability of the sample to act as donor to the DPPH free radicals [30]. The scavenging activity of the sample towards the free radicals can be measured spectrophotometrically at 518 nm.

In both extracts, the flesh of *C. odontophyllum* displayed higher scavenging effects as compared to the seed parts (Table 2). There were significant differences on the scavenging free radical activity in the flesh and seed part for both extracts ($p<0.05$). DPPH free radical scavenging activity has been reported to show high correlation with inhibition capacity towards lipid peroxidation process [31]. Higher scavenging activity in flesh of *C. odontophyllum* was also reported in a previous study by Azrina et al. [22], whilst Wei et al. [32] reported that *Canarium album Raeusch* fruit displayed stronger DPPH radical-scavenging ability than ascorbic acid.

Ferric reduction based on FRAP assay

Ferric Reducing Antioxidant Power (FRAP) method is one assay to determine the antioxidant capacity in samples which utilise Single Electron Transfer mechanism. In this study, the Fe^{3+}-tripyridyltriazine (TPTZ) complex is reduced to the Fe^{2+}-TPTZ-complex by an antioxidant sample, this latter complex possessing an intense colour in an acidic environment [17].

Samples	DPPH Assay[1] (%)	FRAP Assay[2] (mM Fe^{2+}/Liter)	ABTS Assay[3] (mg AEAC/g)
80% methanol			
Flesh	88.14 ± 1.42 [a]	30.52 ± 0.54[a]	46.71 ± 0.98[a]
Seed	80.84 ± 0.78[b]	17.00 ± 0.79[c]	41.52 ± 0.36[b]
Aqueous			
Flesh	77.50 ± 0.36[c]	26.43 ± 0.76[b]	43.29 ± 0.89[b]
Seed	73.39 ± 0.15[d]	13.52 ± 0.21[d]	30.38 ± 0.08[c]

Values are presented as mean ± SD (n = 3) which, with different letters (within column), are significantly different at P<0.05.
[1]DPPH free radical scavenging activity was expressed as % of scavenging.
[2]FRAP was expressed as μM ferric reduction to ferrous in 1 g of dry sample.
[3]ABTS free radical scavenging activity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dry sample.

Table 2: Antioxidant properties of extracts of different parts of *C. odontophyllum*, assessed by three different assays.

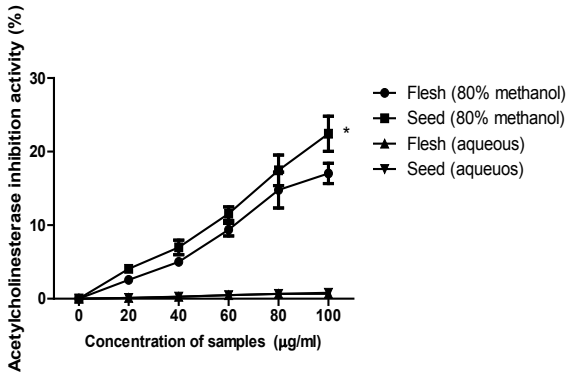

Figure 3: The anti-cholinesterase activity (%) of different parts of 80% methanol and aqueous extracts of the fruit of *C. odontophyllum* (n=3) (* significant at p< 0.05 as compared to the aqueous value at the same concentration).

The FRAP activity of the fruit of *C. odontophyllum* was higher in the flesh compared to the seed. The same trend was also observed in the aqueous extract (Table 2). This finding was similar to that of a previous study by Prasad et al. [21] which demonstrated that peel exhibited higher reducing power as compared to the seed. Azrina et al. [22] also reported higher reducing ability of the skin and flesh of *C. odontophyllum* as compared to the seed part. The flesh part of the *C. odontophyllum* have acceptable reducing power which might act as primary and secondary antioxidant [33].

ABTS scavenging assay

The principle of ABTS decolorization assay is quite similar to that of the DPPH assay in that it involves the scavenging activity of extracts against free radicals, but ABTS salt must be generated by enzymatic or chemical reaction [34]. For both extracts, the higher scavenging activity was found in the flesh part of the fruit of *C. odontophyllum* (Table 2). These findings support those of Prasad et al. [28] which also demonstrated the skin and pulp of the *C. odontophyllum* to have higher ABTS scavenging activity than the seed part.

Anti-cholinesterase activity

Alzheimer's disease is a form of dementia characterized by loss of central cholinergic neurons associated with a marked reduction in content of acetylcholinesterase (AChE), the enzyme responsible for the termination of nerve impulse transmission at cholinergic synapses. Consequently, one therapeutic approach to treatment of Alzheimer's disease is use of anti-cholinesterase drugs. Measurement of AChE activity is based on the yellow colour produced from thiocholine (product from acetylthiocholine) when it reacts with dithiobisnitrobenzoate ion [35,36]. The ability of the plant extracts to inhibit AChE can then be determined. The present study showed that only the 80% methanol extracts of the flesh and seed of *C. odontophyllum* displayed anti-cholinesterase activity when tested at 0-100 μg/ml (Figure 3). The 80% methanol extract inhibited AChE activity in a concentration-dependent manner such that a concentration of 100 μg/ml, the flesh and seed extracts displayed anti-cholinesterase activity of 22.4% and 18.6%, respectively. However, distilled water extracts of the flesh and seed of *C. odontophyllums* showed no or low anti-cholinesterase activity (less than 1%) as compared to the 80% methanol extracts. A previous study by Mogana et al. [37] showed the presence of scopoletin compound isolated in the leaves part of *Canarium patentinervium* Miq. (Burseraceae Kunth.) which inhibits the acetylcholinesterase enzyme moderately.

Relation between phytochemicals contents, antioxidant activity and anticholinesterase activity

Correlation analysis was performed to investigate the correlation between phytochemicals and antioxidant activity as well as antioxidant activity with anti-cholinesterase activity. The results demonstrated a strong positive correlation between the antioxidants assays with the total phenolics, flavonoids and carotenoids contents across the samples tested. The FRAP assay data displayed positive correlation with the phenolic, flavonoid, anthocyanin and carotenoid content with the r values of 0.827, 0.887, 0.987 and 0.962 respectively, while the ABTS data and the total phenolic, flavonoid, anthocyanin and carotenoid content also showed strong positive correlation with the r values of 0.756, 0.773, 0.778 and 0.818 respectively. The DPPH data also showed positive correlation with phenolic, flavonoid and carotenoid content with r values of 0.944, 0.909 and 0.818 with only a moderate positive correlation with anthocyanin content (r=0.693).

The results of this study were in agreement with earlier literature by Kim et al. [38] which showed that the phenolic content in the plum showed positive correlation with antioxidant activity of the samples. Anthocyanins, which are suspected to be concentrated in the purplish-blue peel might also contribute to the antioxidant activity in the fruit of *C. odontophyllum* [22].

The anti-cholinesterase activity also showed moderately positive correlation with antioxidant activity in the samples by FRAP and ABTS assays with r values of 0.403 and 0.639 respectively. Meanwhile, anti-cholinesterase inhibition activity in the present study showed a strongly positive correlation with DPPH assay with an r value of 0.913. Previous work by Amoo et al. [39] indicated positive relationship between antioxidant activity and AChE inhibitory properties of some selected long-term stored African medicinal plants, whilst the study by Jo et al. [40] also reported positive correlation between acetylcholinesterase and antioxidant activity of seed and pericarp of *Camellia sinensis* L.

Conclusion

The current study provides evidence that the flesh of the fruit of *C. odontophyllum* has potential as a natural antioxidant and an acetylcholinesterase inhibition agent with high content of phenolics, flavonoids, anthocyanins and carotenoids. Besides nutritional benefits, *C. odontophyllum* also displays pharmacological properties which might offer potent application in food and pharmaceuticals formulations.

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References

1. Steinmetz KA, Potter JD (1996) Vegetables, fruits, cancer prevention: A review. J Am Med Assoc 96: 1027-1039.
2. Habib HS, Saha S (2010) Burden of non-communicable disease: Global overview Diabetes and metabolic syndrome. Clin Res Rev 4: 41-47.
3. Ommen GS, Goodman GE, Thomquist MD, Barnes J, Cullen MR, et al. (1996) Effects of a combination of β-carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 3: 1150-1155.
4. Gill MA (2010) Anti-Alzheimer's Diet. Science Daily, United States of America.
5. Chew LY, Prasad NK, Amin I, Azrina A, Lau CY (2011) Nutritional composition and antioxidant properties of *Canarium odontophyllum* Miq. (dabai) fruits. J Food Comp Anal 24: 670-677.
6. Voon BH, Kueh HS (1999) The nutritional value of indigenous fruits and vegetables in Sarawak. Asia Pac J Clin Nutr 8: 24-31.
7. Shakirin FH, Prasad KN, Ismail A, Yuon LC, Azlan A (2010) Antioxidant capacity of underutilized Malaysian *Canarium odontophyllum* (Dabai) Miq. Fruit. J Food Comp Ana 23: 777-781.
8. Obame LC, Koudouz J, Kumukungui BS, Bassole IHN, Edou P, et al. (2007) Afr J Biotechnol 6: 2319-2323.
9. He Z, Xia W (2007) Nutritional composition of the kernels from *Canarium album* L. Food Chem 102: 808-811.
10. Letawe C, Boone M, Pierard GE (1998) Digital image analysis of the effect of topically applied linoleic acid on acne microcomedones. Clin Exp Dermatol 23: 56-58.
11. Koudou J, Abena AA., Ngaissona P, Bessie JM (2005) Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*. Fitoterapia 76: 700-703.
12. Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agr Food Chem 46: 4113-4117.
13. Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agr Food Chem 50: 3010-3014.
14. Giusti MM, Wrolstad RE (2003) Characterization and measurement of anthocyanins by UV-visible spectroscopy. In Current protocols in food analytical chemistry. (2nd Edn). John Wiley and Sons.
15. Hess D, Keller HE, Oberlin B, Boufanti R, Schuep W (1991) Simultaneous determination of retinol, tocopherols, carotenes & lycopene in plasma by means of high-performance liquid chromatography on reversed phase. Int J Vitam Nutr Res 61: 232-238.
16. Magalhaes LM, Segundo MA, Reis S, Lima JLFC (2006) Automatic method for determination of total antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl assay. Anal Chim Acta 558: 310-318.
17. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP Assay. Anal Biochem 239: 70-76.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biol Med 26: 1231-1237.
19. Atta-ur-Rahman, Choudary MI, Thomsen WJ (2005) Bioassay Techniques for Drug development. Harwood Academic Publishers.
20. Eberhardt MV, Lee CY, Liu RH (2000) Nutrition: Antioxidant activity of fresh apples. Nature 405: 903-904.
21. Prasad KN, Chew LY, Khoo HE, Kong KW, Azlan A, et al. (2010) Antioxidant capacities of peel, pulp and seed fractions of *Canarium odontophyllum* Miq Fruit. J Biomed Biotechnol 8.
22. Azrina A, Nurul Nadiah MN, Amin I (2010) Antioxidant properties of methanolic extract of *Canarium odontophyllum* fruit. Int Food Res J 17: 319-326.
23. Chew LY, Prasad KN, Amin I, Azrina A, Lau CY (2012) *Canarium odontophyllum* Miq.: an underutilized fruit for human nutrition and sustainable diets. Food and Agriculture Organisation of the United Nations (FAO).
24. Hasbay AI, Cetin HI, Yener ME, Bayindirh A (2007) Subcritical (carbon dioxide and ethanol) extraction of polyphenols from apple and peach pomaces and determination of antioxidant activities of extracts. J Supercrit Fluid 43: 55-63.
25. Wrolstad RE, Culbertson JD, Cornwell CJ, Mattick LR (1982) Detection of adulteration in blackberry juice concentrates and wines. J Assoc Off Anal Chem 65: 1417-1423.
26. Mezza G, Maniati E (1993) Anthocyanins, In: Fruits, Vegetables & Grains. CRC Press, Boca Raton, Florida,
27. Kritchevsky SB (1999) Beta-carotene, carotenoids and the prevention of coronary heart disease," J Nutr 129: 5-8.
28. Prasad KN, Chew LY, Khoo HE, Bao Y, Azlan A, et al. (2011) Carotenoids and antioxidant capacities from *Canarium odontophyllum* Miq fruits. Food Chem 124: 1549-1555.
29. Pokorný J (2001) Are natural antioxidants better – and safer – than synthetic antioxidants?," Eur J Lipd Sci Tecnhol 109: 629-642.

30. Brand-Williams W, Cuvelier ME, Benset C (1995) Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 28: 25-30.

31. Rekka E, Kourounakis PN (1991) Effect of hydroxyethyl rutosides and related compounds on lipid peroxidation and free radical scavenging activity of some structural aspects. J Pharm Pharmacol 43: 486-491.

32. Wei H, Peng W, Mao Y, Liu B (1999) Studies on chemical constituents in the fruit of *Canarium album Raeusch*. J Chin Mater Med 24: 421-423.

33. Yen GC, Chen HY (1995) Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 43: 27-32.

34. Arnao MB (2000) Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. Trends Food Sci Tech 11: 419-421.

35. Ellman GL, Courtney KD, Valentino AJ, Featherstone RM (1961) A new and rapid colourimetric determination of acetylcholinestrase activity. Biochem Pharmacol 7: 88-95.

36. Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, et al. (2007) Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J Ethnopharmacol 109: 359-363.

37. Mogana R, Teng-Jin K, Wiart C (2013) Anti-inflammatory, anticholinesterase and antioxidant potential of scopoletin isolated from *Canarium patentinervium* Miq. (Burseracea Kunth). J Evid Based Complementary Altern Med.

38. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY (2003) Quantification of polyphenolics and their antioxidant capacity in fresh plums. J Agric Food Chem 51: 6509-6515.

39. Amoo SO, Aremu AO, Moyo M, Staden VS (2012) Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. J Evid Based Complementary Altern Med 12: 87-96.

40. Jo YH, Yuk HG, Kim JH, Kim R, Lee SC (2012) Antioxidant, tyrosinase inhibitory and acetylcholinesterase inhibitory activities of green tea (*Camellia sinensis* L.) seed and its pericarp. Food Sci Biotechnol 12: 761-768.