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# Screening of antioxidant phytoextracts of *Canarium odontophyllum* (Miq.) leaves in vitro

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***ABSTRACT:*** *This is the first study to investigate for the antioxidant efficacy and to detect selected phytochemicals in leaf extracts of Canarium odontophyllum (Miq.). The aqueous, methanol and acetone extracts at 12.5 µg/ml, 25 µg/ml and 50 µg/ml, were screened for their in vitro antioxidant activity using FRAP assay with reference to ascorbic acid. Phytoconstituent screening test was conducted using the standard reagents. All the leaf extracts from Canarium odontophyllum were found to exhibit dose-dependent antioxidant potential but acetone extract displayed the highest antioxidant capacity of 89.49 ± 3.64µM $FeSO_4.7H_2O$, 181.46 ± 5.54 µM $FeSO_4.7H_2O$ and 355.26 ± 16.62 µM $FeSO_4.7H_2O$ at 12.5 µg/ml, 25 µg/ml and 50 µg/ml, respectively whereas aqueous extract has the lowest antioxidant power of 33.87 ± 0.97 µM $FeSO_4.7H_2O$ (12.5 µg/ml), 73.60 ± 2.78 µM $FeSO_4.7H_2O$ (25 µg/ml) and 140.29 ± 0.28 µM $FeSO_4.7H_2O$ (50 µg/ml). Methanol extract, on the other hand, exhibited moderate antioxidant activity which was significantly higher ($p< 0.05$) compared to aqueous extract but no significant difference to that of acetone extract. Despite its highest antioxidant potential with respect to aqueous and methanol extract, the standard agent (ascorbic acid) displayed almost twice the antioxidant power of the acetone extract. The phytoconstituents in the leaf extracts of Canarium odontophyllum were shown to be terpenoid, tannin and flavonoid.*

***CONCLUSION:*** *Our finding demonstrated for the first time that extract from Canarium odontophyllum leaves provide a natural source of antioxidant constituents for medical application and health benefits.*

***KEYWORD:*** *Canarium odontophyllum, Phytochemicals, Antioxidant, FRAP assay*

## I. INTRODUCTION

Free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) are associated with most chronic diseases such as cancer, cardiovascular disease, diabetes and many other health problems related to advancing age [1]. Therefore, great effort in the commercialization and utilization of plant sources as antioxidants are of increasing interest. This is done to minimize or to prevent the onset of the oxidation process by ROS, to destroy potential oxidants, to scavenge ROS and most importantly to improve the antioxidant level in the body [2-4]. Phytochemicals is the bioactive non-nutrient constituents that are derived from plants [5]. Plant-derived substances become of great interest owing to their versatile applications such as antimicrobial, anti-inflammatory, antioxidant, anti-cancer, anti-mutagenic, anti-tumor and liver protective properties [6]. A great number of effective antioxidants and antibacterial agents currently in use are derived from plants. Antioxidants have been detected in a large number of foods and agricultural products, including vegetables, fruits, cereal grains and plant extracts. Flavonoid, tannin and terpenoid have been shown to not only possess antioxidant potential, but also exhibit antimicrobial activity [7].

In this study, the modified version of the FRAP assay was employed in order to screen the antioxidant capacity of *Canarium odontophyllum* leaf extracts using 96-well microtiter plate [8, 9]. *Canarium odontophyllum* belongs to the family *Burseraceae* and is a tropical rain forest tree abundantly found in Sarawak, Malaysia. Fruit of *C. odontophyllum,* commonly known as "dabai" is consumed by the local community [10] due to its nutritional value related to longevity effect. The aim of the present work was to study the antioxidant capacities of aqueous, methanol and acetone extracts of *Canarium odontophyllum* leaves since various extracts from plant origin have been recognized to possess beneficial effects as natural antioxidants against free radicals in biological systems [11]. As such, the present data is hoped to provide some baseline information which will highlight the potential of the *Canarium odontophyllum* leaves as new source of natural antioxidant-rich agents with functional properties.

## II. MATERIALS AND METHODS

**Chemicals and Reagants :** Sodium acetate, dimethyl sulphoxide, glacial acetic acid and hydrochloric acid were from Ajax Finechem whereas iron (III) chloride 6-hydrate ($FeCl_3.6H_2O$) and iron (II) sulphate heptahydrate [$FeSO_4.7H_2O$] were bought from HmbG Chemical. Meanwhile, L-ascorbic acid and 2,4,6-tripyridyl-s-triazine (TPTZ) were from Sigma Chemical.

**Plant Material :** Fresh leaves of *Canarium odontophyllum* were obtained from Sarawak, Malaysia and was deposited at the Herbarium Universiti Kebangsaan Malaysia in Bangi, Malaysia with voucher specimen no. UKMB 40052. The leaves were oven-dried and grinded into powdered form using electric grinder.

- **Preparation of *Canarium odontophyllum* Leaf Extracts**

The crude extracts from *Canarium odontophyllum* leaves were prepared based on our previous report [12]. The mixture of 100g of the dried material in 500 ml acetone was shaken at 100 rpm for 24 h at 50°C prior to being filtered through Whatman No.1 filter paper. The residue was further extracted two times by adding 300 ml fresh solvent and all the filtrates were pooled together. The remaining residue was air-dried and the solvent from each filtrate was removed using rotary evaporator to form a pellet. Finally the pellet was pounded to dryness under hot air-dryer to remove the remaining solvent. The methanol extract was prepared by immersing 100 g of the dried material in 500 ml methanol and shaken at 100 rpm for 24 h at 50°C. The mixture was then filtered through Whatman No.1 filter paper. The residue was further extracted twice by adding 300 ml of fresh solvent each time, after then all the filtrate were combined together. The remaining residue were air-dried and further extracted with methanol. The solvent from the combined filtrate was evaporated using rotary evaporator until it formed a pellet and pounded to dryness. On the other hand, the aqueous extract was prepared by combining the supernatant and freeze-dried at -50°C under vacuum for 12 hrs. The yield of each extract was determined and stored at 4°C until further use. The formula in the estimation of the yield of crude extract was as follows :

$$\frac{\text{Weight of the crude extract (g)}}{\text{Weight of the dried leaf powder (g)}} \times 100\ \%$$

- **Phytochemical screening of *Canarium odontophyllum* Leaf Extracts**

Analysis of phytoconstituents of the crude extracts of aqueous and methanol extracts of *Canarium odontophyllum* was based on colour changes after addition of standard reagants [13].

- **Determination of Antioxidant Activity**

The antioxidant activity of aqueous and methanol extracts were determined by FRAP assay as described previously [14]. The extracts were first dissolved in DMSO with stock concentration of 100 mg/ml. Then, FRAP reagent was prepared freshly by mixing of acetate buffer (300 mM, pH 3.6), $FeCl_3$ (20 mM) and TPTZ (10 mM) solution with the ratio of 10:1:1 and was kept in dark at water bath, 37˚C. Further, serial dilution of extracts, $FeSO_4.7H_2O$ and ascorbic acid were carried out, in which $FeSO_4.7H_2O$ was used as a standard ranging from 100 - 1000 µM and ascorbic acid was used as a positive control. Then 50 µL of each concentration of extracts, $FeSO_4.7H_2O$ and ascorbic acid was added to a 96-well plate, followed by addition of 175 µL of the warmed FRAP reagent. The plate was then incubated at 37˚C for 5 minutes and absorbance was read at 595 nm by ELISA plate reader (Biorad), at least 3 experiments (n=3) was carried out for each extracts with triplicate to obtain average of absorbance reading. Finally, absorbance value of each extract was use to estimate the antioxidant capacity from the ability of extract to reduce the µM TPTZ-Fe(III) to µM TPTZ-Fe(II) by comparing to the standard curve (µM $FeSO_4.7H_2O$).

- **Statistical Analysis**

All the experiment was carried out in triplicates and the result was expressed as mean ± Standard Error of the mean. Data analysis was conducted using SPSS 21. One way ANOVA statistical test was used to compare the result between the extracts with significant level $p<0.05$.

## III. RESULT

The result of the percentage yield of *Canarium odontophyllum* leaves using three extraction solvents are presented in Table 1. In the extraction of *Canarium odontophyllum* leaves, distilled water produced the highest amount of yield (7.61%) followed by methanol (5.87%) and acetone (3.48%).

Figure 1 showed the result of the antioxidant capacity of the crude leaves extract using different solvents (distilled water, methanol, acetone) at three different concentrations (12.5 µg/ml, 25 µg/ml and 50 µg/ml. Out of the three extraction solvents employed, acetone was found to produce the highest antioxidant capacity, followed by methanol. The lowest antioxidant capacity was recorded by the aqueous extract. As depicted in this figure, antioxidant capacity or FRAP value was increased with the increased in the extracts concentration. At the lowest concentration of 12.5 µg/ml, FRAP value produced by acetone extract was the highest (89.49 µM $FeSO_4.7H_2O$) compared to methanol extract (88.76 µM $FeSO_4.7H_2O$) and aqueous extract (33.87 µM $FeSO_4.7H_2O$).

FRAP values of acetone, methanol and aqueous extracts at 50 µg/ml were 355.26, 281.15 and 140.29 µM $FeSO_4.7H_2O$, respectively. The results obtained indicated that acetone appeared to be the best solvent in the extraction of active compound with antioxidant property. There was a significant difference of FRAP values at 50 µg/ml of acetone extract (355.26 µM $FeSO_4.7H_2O$) compared to 25 µg/ml and 12.5 µg/ml of acetone extract. However, the FRAP values did not show any significant difference between 50 µg/ml of acetone extract and 50 µg/ml of methanol extract (281.15 µM $FeSO_4.7H_2O$). At 25 µg/ml, significant difference was observed between acetone extract and aqueous extract and not between methanol-acetone and methanol-aqueous extract. The same trend was observed at 12.5 µg/ml, whereby the significant difference was only observed between the acetone and aqueous extracts. The antioxidant activity of the extract was found to be significantly different ($p<0.05$) at all concentrations. In addition, the antioxidant capacity of the three extracts was also compared with the ascorbic acid (positive control) at all three concentrations. As shown in Figure 2, FRAP values of ascorbic acid were higher than acetone, methanol and aqueous extracts, at 152.81, 348.85 and 749.96 µM $FeSO_4.7H_2O$ at 12.5 µg/ml, 25 µg/ml and 50 µg/ml, respectively. However, there is no statistically significant difference of the FRAP values between the three extracts and ascorbic acid at 12.5 µg/ml and 50 µg/ml ($p>0.05$). On the other hand, significant difference ($p<0.05$) was obtained between ascorbic acid and all the three extracts at 25 µg/ml.
The result of phytoconstituent content of the *Canarium odontophyllum* leaf extracts was shown in Table 2. The phytoscreening assay clearly demonstrated that flavonoid, tannin and terpenoid were detected in all the extracts based on colour changes to orange, brownish-green and reddish brown, respectively. However, saponin and phenolic compound were only present in aqueous & methanol extract as observed from presence of froth and purple colour change, respectively. On the other hand, the content of alkaloid was not detected in all the extracts based on absence formation of blue black precipitate.

## IV. DISCUSSION

Dietary antioxidant such as polyphenolic compounds, vitamin E, vitamin C and carotenoids are believed to be the effective nutrients in the prevention of these oxidative stress-related diseases [15, 16]. This was supported by clinical trials and epidemiological studies that have established an inverse correlation between the intake of fruits and vegetables and the occurrence of those diseases [17].

*Canarium odontophyllum* or locally known as "dabai" is an indigenous to Borneo, Brunei, Indonesia and Philippines. *Canarium* is a large genus that has been identified belongs to Burseraceae family of the Sapindales order in the class of Eudicotyledoneae [18]. It consists of about 100 species and mostly found on Africa, Asia and Pacific Islands. In Malaysia, *Canarium odontophyllum* mainly found in tropical rain forest of Sarawak and is normally available from October to December [19, 20]. Plant from *Canarium* genus was proved to contain various biological activities such as antioxidant properties, antibacterial, antifungal, antitumor, anti-inflammatory, hepatoprotective, analgesic and anti-diabetic [21]. Previous study has been done on different solvent extraction of leaf, twig, stem, bark and also fruit of the plant which showed that *Canarium* genus is a potential source for natural antioxidant [11]. However, due to lack of promotion and as an economic potential which has not been fully explored therefore *Canarium odontophyllum* was classified as an underutilized fruit. The fruit is nutritious and has great potential to be exploited as nutraceutical due to its high nutrient and antioxidant properties.

In the present study, application of different solvents such as aqueous, methanol and acetone in the preliminary screening of antioxidant compounds from *Canarium odontophyllum* leaves was investigated. This study indicated that all the three extracts displayed antioxidant properties; acetone extract with the highest FRAP value compared to aqueous and methanol extract. However, comparison of FRAP values between the three extracts with ascorbic acid at 50 µg/ml did not show any significant differences statistically. This probably indicated that all the three extracts from *Canarium odontophyllum* leaves consist of potential antioxidant capacity as ascorbic acid. Our results were comparable with previous studies, which reported that the leaves from *Canarium album* and *Canarium patentinervium* contain antioxidant properties [22, 23]. In addition,

antioxidant properties has also been reported in the leaf extracts from Burseraceae family, such as *Commiphorasp* [24], *Dacryodes sp* [25], *Boswellia sp* [26], *Combretum* sp [27] and *Garugapinnata sp* [28].
Our finding was also in agreement with [29, 30] that methanol and aqueous extract of *Canarium odontophyllum* fruit contain high antioxidant capacity and methanol extract displayed higher antioxidant activity compared to aqueous extract. To our knowledge, most of previous studies were done on fruit (peel, pulp, seed and kernel) of *Canarium odontophyllum* with different extraction solvents [31], however no study has been done on the leaves of *Canarium odontophyllum.* Thus, our result demonstrated for the first time that the aqueous, methanol and acetone extracts from the leaves of *Canarium odontophyllum* consisted of phytonutrients with antioxidant properties. From our phytonutrient screening result, all the three extracts contain flavonoid, tannin and terpenoid. Only aqueous and methanol extract contain saponin and phenolic compound. This is mainly due to the polarity of solvent used, as distilled water and methanol have been shown as better solvents for the extraction of polar and phenolic compound compared to acetone, while the constituent of acetone extract was often associated with compound of high antioxidant properties [32]. Thus, the combination of different solvent was used in many studies in order to obtain compound with many biological activities.

## V. CONCLUSION

In conclusion, our finding demonstrated a preliminary result of the antioxidant activity and phytonutrient content in aqueous, methanol and acetone extract of *Canarium odontophyllum* leaves. However, FRAP is just one of the single electron transfer (SET) methods of determining the antioxidant activity hence, it does not justify completely that this plant has good antioxidant properties. Further work is necessary in view of also carrying out other SET and the hydrogen transfer method in order to confirm the antioxidant potential of the leaf from *Canarium odontophyllum*.

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## FIGURES AND LEGENDS

**Table 1 Percentage yield of *Canarium odontophyllum* leaves using various solvents**

| Percentage of yield (%) | Weight of extract (g) | Weight of powdered leaf sample (g) | Extraction solvent |
|---|---|---|---|
| 3.48 | 3.12 | 89.69 | Acetone |
| 5.87 | 4.63 | 78.92 | Methanol |
| 7.61 | 7.61 | 100 | Aqueous |

**Figure 1 Antioxidant capacities (FRAP values) of aqueous and methanol extracts of *Canarium odontophyllum* leaves at different concentrations, each point represents mean of triplicates ± SEM (*p<0.05). The wavelength of OD reading was at 595 nm at the y-axis**

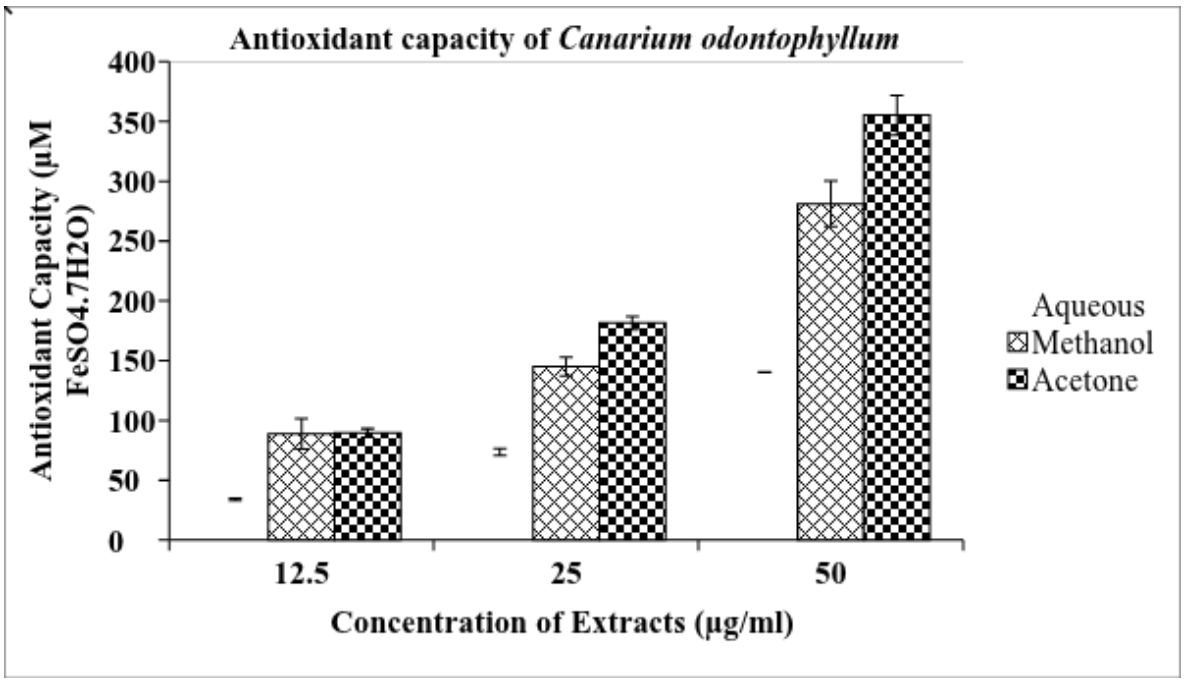

**Figure 2 Antioxidant capacities (FRAP values) of ascorbic acid at different concentration. Each point represents mean of triplicates ± SEM**

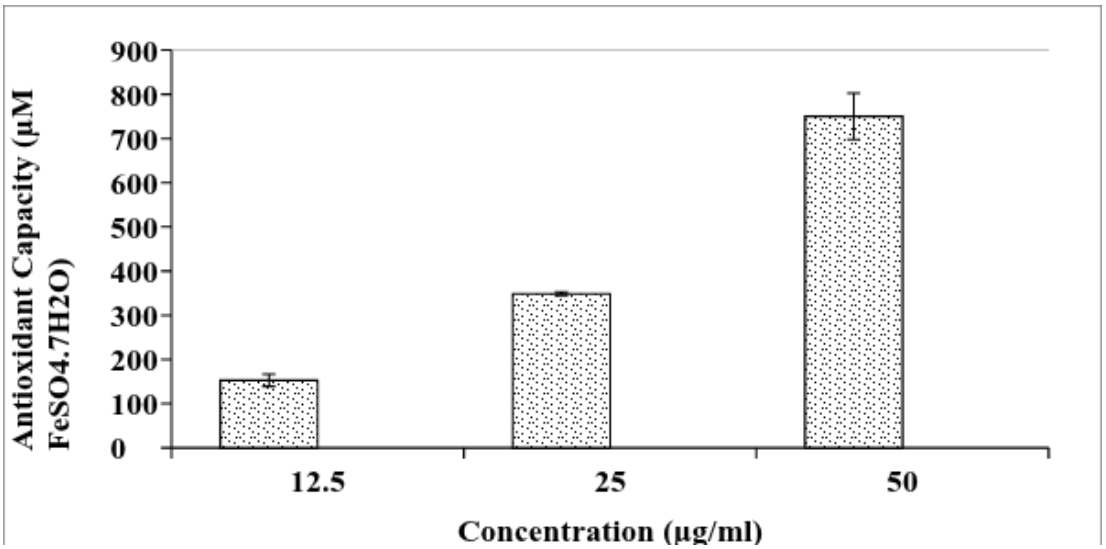


**Figure 3 Standard curve of $FeSO_4.7H_2O$ at concentration ranging from 100 to 1000 uM**

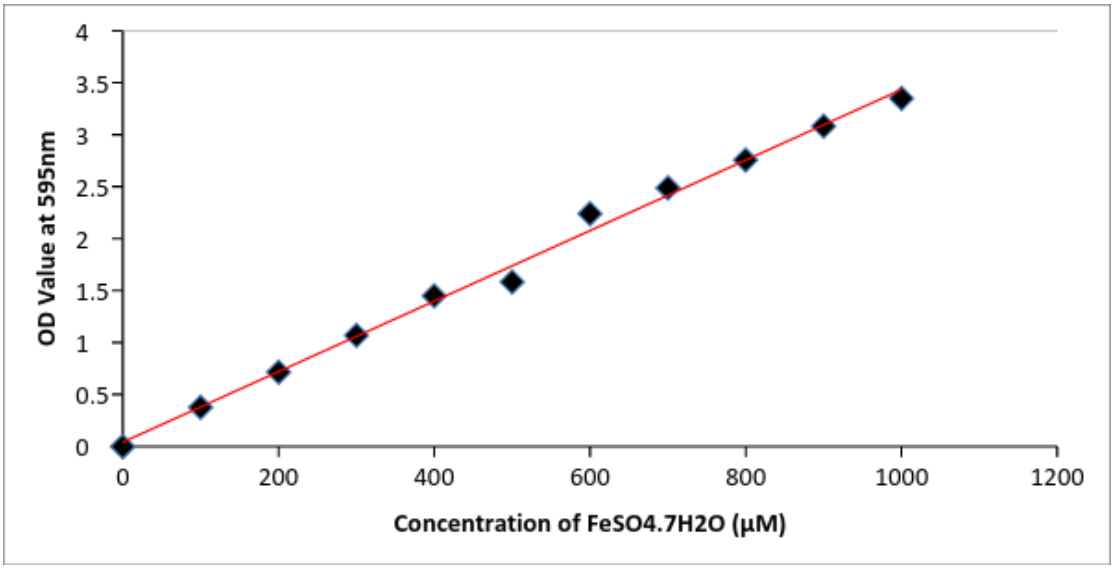


**Table 2 Phytoconstituent content in extracts of *C. odontophyllum* leaves**

| Acetone | Methanol | Aqueous | Phytoconstituent of *Canarium odontophyllum* leaves |
|---|---|---|---|
| + | + | + | Flavonoid |
| + | + | + | Tannin |
| - | - | - | Alkaloid |
| - | + | + | Saponin |
| + | + | + | Terpenoid |
| - | + | + | Phenolic compound |