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Leaves Extract from *Canarium odontophyllum* Miq. (Dabai) Exhibits Cytotoxic Activity against Human Colorectal Cancer Cell HCT 116

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Abstract

Colorectal cancer is the second most common cancer occurs in Malaysia after breast cancer. Bioactive compounds that reside in *Canarium odontophyllum* leaves could probably have the potential to being develop as an alternative cytotoxic agent against colorectal cancer. The objective of this study is to evaluate the cytotoxic effect of acetone, methanol and water extracts from the leaves of *C. odontophyllum* against human HCT 116 colorectal carcinoma cells using MTT assay. Result showed that the percentage yield of the extraction from *C. odontophyllum* leaves was the highest when distilled water was used as an extraction solvent followed by methanol extract and acetone extract. Phytochemical screening revealed the presence of flavonoid, tannin and terpenoid in the three extracts. All the extracts demonstrated cytotoxic effect after 24-hr treatment with acetone extract at IC_{50} value of 0.08 ± 0.003 mg/mL against HCT 116 cells compared to methanol and aqueous extracts with IC_{50} values of 0.10 ± 0.011 mg/mL and 0.40 ± 0.162 mg/mL, respectively. In conclusion, this preliminary study of the *C. odontophyllum* leaves extract against HCT116 cells provides an insight of its promising anticancer property.

Keywords: *Canarium odontophyllum; Burseraceae;* Extraction yield; HCT 116; Cytotoxicity; Phytochemical; Terpenoid

Introduction

Cancer is characterized by the uncontrolled growth of abnormal cell, which has the ability to invade other tissue and metastases to other parts of the body via blood and lymph [1]. Cancer has caused death to 7.6 million or 13% of the world population in 2008 [2]. Colon cancer or commonly known as colorectal cancer, is an uncontrolled cell growth forms in the tissue of the colon and most of them are adenocarcinomas. Colon cancer is the second most common cancer after breast cancer which represents 11.5% of the new cancer cases developed annually. Rectal cancer and colon cancer are genetically the same cancer and together, they're often referred to as colorectal cancers [3]. Approximately, about 95% of colon cancer can occurs in people with little or no genetic risk [4]. The common factors that contribute to the formation of this disease are diet and environment. Food derived heterocyclic amine carcinogens, polycyclic aromatic hydrocarbons and diet lack of fiber are the major risk factor for the formation of colon cancer [5]. Other risk factors include the demographic factor such as gender and lack of exercise [6].

Canarium odontophyllum which belongs to the Burseraceae family, have huge potential as an anticancer agent which will be used in this study to specifically fight colon cancer. *C. odontophyllum* fruit, especially the skin poses high content of antioxidant compound such as flavonoid, anthocyanins and phenolic compound [7]. Previous research [8,9] reported that the antioxidant activity of the fruit pulp contributed to the cholesterol lowering effect. The related study also proved the existence of the total phenolics and extractable condensed tannins in the leaves, twigs and stem bark of *Canarium album* [10]. However, work on the leaves extract of *C. odontophyllum* (Figure 1) against the human colorectal HCT116 cancer cell line has not been explored as yet.

Figure 1: Photograph showing the leaves from *Canarium odontophyllum.*

Materials and Methods

Plant materials

Leaves of *C. odontophyllum* were freshly imported from Kuching, Sarawak and were authenticated by Herbarium Universiti Kebangsaan Malaysia in Bangi, Malaysia with voucher specimen no. UKMB 40052. Only good quality of *C. odontophyllum* leaves with healthy physical structure were chosen and cleaned with tap water prior to extract preparation. The leaves were segmentally cut into small pieces and allowed to dry in oven at 45°C for 24 hours. The leaves were then weighed and this drying procedure was repeated until constant mass was obtained. The leaves were finally grinded into powder using an electrical blender before being kept in freezer at -20°C to prevent contamination.

Preparation of extracts

The dried powder was extracted using acetone, methanol and distilled water. The succession method was employed [11] on the

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extraction using acetone and methanol. In the ratio of 1:5, 89.69 g of powdered *C. odontophyllum* were soaked in 450 ml of acetone. The mixture was mixed well using magnetic stirrer for 24 hours at room temperature. The mixture was then filtered to separate the residue and the filtrate was collected. Meanwhile the residue was allowed to dry prior to addition with another 450 ml of acetone for the next filtration process. Both filtrates were then mixed and filtered using Whatman paper No. 43 prior to be subjected under reduced pressure using a rotary evaporator. The crude extract obtained was allowed to dry in fume hood in order to produce a dry acetone extract. The same procedure was repeated in the preparation of crude methanol extract by mixing the remaining residue with methanol in the ratio of 1:5. However, the preparation of aqueous extract involved maceration method whereby 100 g powdered leaves in 500 ml sterile distilled water were subjected to agitation using orbital shaker at 190 rpm for 24 hours. The mixture was then filtered and the filtrate obtained was centrifuged at 3000 rpm for 5 min. The supernatant collected was further filtered using Whatman paper No. 43 and the whole process for aqueous extraction was repeated using the remaining residue with distilled water in the ratio of 1:5. The supernatants were combined to be freeze-dried at -50°C under vacuum for 12 hours in order to produce a crystal-like crude aqueous extract. All the extracts were stored in sterile universal bottle at 4°C until further use.

Phytochemical screening of *C. odontophyllum* leaf extracts

The concentrated acetone, methanol and aqueous extracts of the leaves were subjected to qualitative chemical test for the identification of various active constituents [12]. For alkaloid identification test, 5 mg of each extract was dissolved in distilled water and was added with 3 drops of Wagner's reagent to form a blue-black precipitate reveal the positive test. For tannin identification test, 2 mg of each extract in 5 mL of distilled water were mixed with a few drops of 15% $FeCl_3$. The formation of blue black precipitate and brownish green precipitate indicated the presence of hydrolysable tannin and condensed tannin, respectively. For flavonoid identification test, about 2 mg of each extract was dissolved with 1M NaOH followed by drops of 0.6M HCl. The yellowish orange of NaOH that turns to colorless upon the addition of HCl confirmed that flavonoid was present. For terpenoid identification test, 2 mg of each extract was dissolved in 2 mL $CHCl_3$ followed by drops of H_2SO_4 Positive indication for the presence of terpenoid was determined by the formation of reddish brown. Saponin test was done by dissolving 5 mg of each extract with 5 mL distilled water. The mixture was then heated and shaked and the resulting formation of froth indicated positive result. For phenolic compound identification test, about 2 mg of each extract in 2 mL of distilled water followed by drops of 1% $FeCl_3$ to produce blue, black or green precipitate revealed the presence of phenolic compound.

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

Table 1: Percentage yield of various solvent extracts from *Canarium odontophyllum*.

Phytochemical	Extracts		
	Acetone	Methanol	Aqueous
Alkaloid	-	-	-
Saponin	-	+	+
Terpenoid	+	+	+
Tannin	+	+	+
Phenolic compound	-	+	+
Flavonoid	+	+	+

Table 2: The results of the phytochemical screening in acetone, methanol and aqueous extracts from *C. odontophyllum* leaves. (-) indicates absence of phytoconstituents ; (+) indicates the presence of phytoconstituents.

Evaluation of cytotoxic activity

The extracts which have been screened for the phytochemicals compound were evaluated for its cytotoxicity activity against human colorectal carcinoma cells HCT 116 using MTT assay [13]. The numbers of HCT 116 cells were counted using hemocytometer after subcultured process. The final concentration of the cells was multiplied until it reached 5 x 10^4cells/ml. The cells were seeded in the 96 well plates and were designed in triplicates for each treatment (n=3). The cells were then incubated for 24 hours with 5% of CO_2 at 37°C prior to treatment. The concentration of acetone, methanol and aqueous extracts used to treat the cells ranged from 0.0625 - 1.0 mg/ml. Menadione was used as a positive control in this cytotoxicity assay against HCT 116 cell lines at concentration ranging from 0.0625 - 1.0 mM. Cells with only McCoy's media solution were used as negative control. The blank comprised the treatment without cell was used in order to evaluate the MTT assay limitation. After 24 hours of incubation, cells treated with extract and blank treatment was added with MTT salt solution prior to the addition of DMSO solution. The cells viability and the blank were then subjected to ELISA reader at the wavelength of 570 nm in order to obtain the Optical Density value. The results were recorded as IC_{50} value which represented the concentration of the cytotoxic agent that inhibits the growth of cancer cells using the formula given below:

$$\%\ \text{Cell viability} = \frac{\text{Absorbance of treated sample (mean)}}{\text{Absorbance of control (mean)}} \times 100$$

The percentage of cell viability against the concentration of test compounds was plotted. The half maximal inhibition concentration (IC_{50}) was calculated based on the equation in the plotted graph.

Results

Extraction yield of *C. odontophyllum* leaves

(Table 1) showed the result of the percentage yield of the crude extract using acetone, methanol and distilled water. Out of the three extraction solvents employed, distilled water was found to produce the highest percentage yield of 7.61%. The acetone extract showed the lowest percentage (3.48%) of extraction yield whereas 5.87 % of crude extract was obtained when methanol was used as an extracting solvent. These findings demonstrated that distilled water appeared to be the best solvent in the leaf extraction from *C. odontophyllum* whereas acetone extracted the least yield.

Phytoconstituent screening of *C. odontophyllum* leaf extracts

Preliminary phytochemical investigation of acetone, methanol and aqueous extracts of leaves by tests tube reactions revealed that they contain terpenoid, tannin and flavonoid (Table 2). Alkaloid, however, was absent in all the leaves extracts of *C. odontophyllum* despite the presence of saponin and phenolic compound in the methanol and aqueous extracts. In other words, alkaloid, saponin and phenolic compounds were not detected in the acetone extract of *C. odontophyllum* leaves.

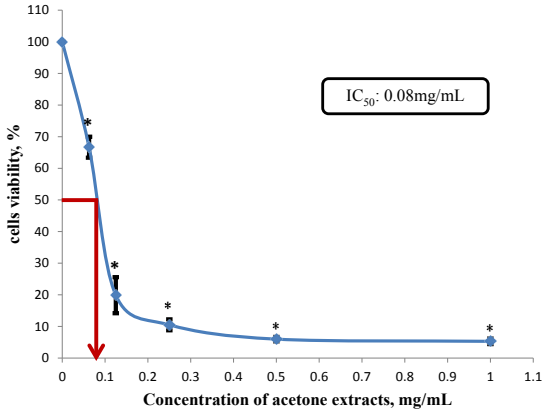
Cytotoxic activity of *C. odontophyllum* leaf extracts

(Table 3) showed the IC_{50} values of aqueous, methanol and acetone extracts against HCT 116 cancer cell lines. The IC_{50} for acetone extract was the lowest which is 0.08 ± 0.003 mg/mL (Figure 2) compared to

Cell Lines	IC_{50} of extracts (mg/mL)		
	Acetone	Methanol	Aqueous
HCT 116	0.08 ± 0.003[a]	0.10 ± 0.01[b]	0.40 ± 0.16

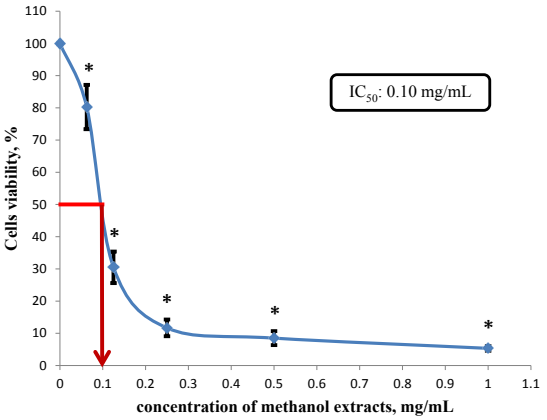
[a]Mean IC_{50} value significantly different from aqueous extract; p = 0.05
[b]Mean IC_{50} value significantly different from aqueous extract; p = 0.05
The data is presented as a mean of 3 replicates (n=3) ± SEM

Table 3: IC_{50} of of *C. odontophyllum* leaves after 24 hours of treatment against HCT 116 cells.



*significantly different compared to negative control; p = 0.05

Figure 2: Cytotoxic effect of acetone extract from leaves of *C. odontophyllum* on HCT116 cell viability at concentration range 0 - 1 mg/ml following 24 hours of treatment. Each point represents the mean of triplicates from 3 different experiments ± SEM. The negative control is the untreated cell.



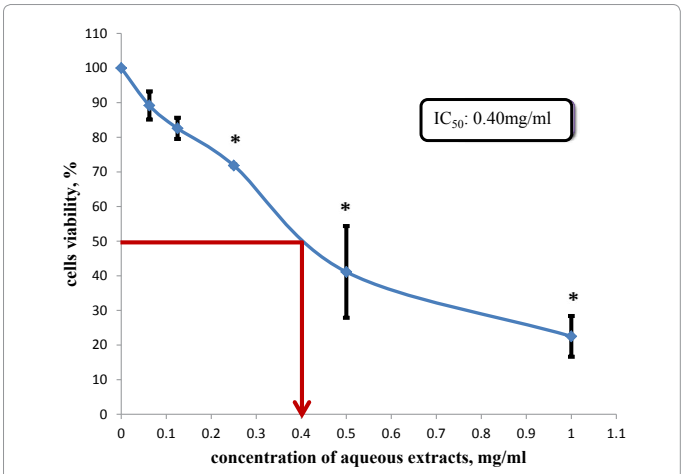
*significantly different compared to negative control; p = 0.05

Figure 3: Cytotoxic effect of methanol extract from leaves of *C. odontophyllum* on HCT116 cell viability at concentration range 0 - 1 mg/ml following 24 hours of treatment. Each point represents the mean of triplicates from 3 different experiments ± SEM. The negative control is the untreated cell.

methanol extract and aqueous extract at respectively, 0.10 ± 0.01 mg/mL (Figure 3) and 0.40 ± 0.16 mg/mL (Figure 4). It is interesting to note that the cytotoxic activity of acetone extract and methanol extract showed no significant difference. However, the aqueous extract showed a significantly ($p < 0.05$) weaker cytotoxic effect compared to both the acetone and methanol extracts. On the other hand, menadione as a positive control exhibited IC_{50} of 0.12 mM as illustrated in (Figure 5). These findings clearly demonstrated that despite the highest extractive potential of distilled water from the leaves of *C. odontophyllum*, the aqueous extract displayed the lowest cytotoxic activity against the HCT 116 cancer cell lines.

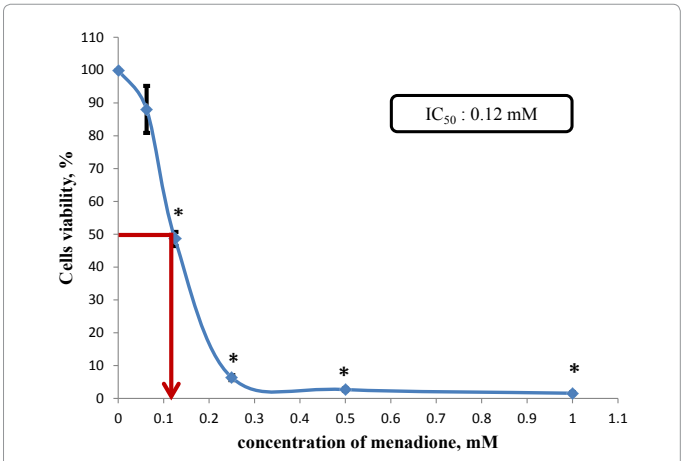
Discussion

This preliminary study aimed to evaluate the cytotoxicity activity of the *Canarium odontophyllum* or dabai leaves extracts against HCT 116 cell lines to become a benchmark for future cancer research. The powdered leaves of *C. odontophyllum* were extracted using three types of solvents namely acetone, methanol and distilled water. Aqueous extract showed the highest percentage of yield followed by methanol and acetone extracts. This is supported by [14] that distilled water was capable of producing the highest quantity of extract from the seeds *Agriophyllum pungens* followed by methanol and ethyl acetate. The polarity index for acetone and methanol were both 5.1 which is less than distilled water (1.0) hence, it can be deduced that the most polar solvent produced the highest yield from the leaf extract of *C. odontophyllum*. In



*significantly different compared to negative control; p = 0.05

Figure 4: Cytotoxic effect of aqueous extract from leaves of *C. odontophyllum* on HCT116 cell viability at concentration range 0 - 1 mg/ml following 24 hours of treatment. Each point represents the mean of triplicates from 3 different experiments ± SEM. The negative control is the untreated cell.



*significantly different compared to negative control; p = 0.05

Figure 5: Cytotoxic effect of menadione as positive control on HCT116 cell viability at concentration range 0-1 mg/ml following 24 hours of treatment. Each point represents the mean of triplicates from 3 different experiments ± SEM. The negative control is the untreated cell.

addition, the volatile characteristic of acetone and methanol at room temperature also affects the percentage yield of extracts [15].

Phytochemical screening result revealed the presence of terpenoid, tannin and flavonoid in all the three extracts studied. Fortunately, alkaloid was not detected in the leaves of *C. odontophyllum* which means that chewing the *C. odontophyllum* (dabai) leaves or drinking dabai tea can be considered safe for their consumption as chemopreventive supplement against colon cancer. The phytoconstituents contained in the leaf extracts of *C. odontophyllum* could well correlate for their cytotoxic activity as it was widely known that flavonoid, tannin and terpenoid played a protective role as antioxidants [16,17]. According to [18], the radical scavenging activities of the ethanol extract of leaves from *Canarium patentinervium* Miq. are mainly due to the presence of tannins and flavonoids.

The result of MTT assay showed that all the leaf extracts from *C. odontophyllum* in this study were capable of inhibiting the growth of HCT 116 cell line. The ethanol extracts from the leaves and bark of *Canarium patentinervium* Miq. also displayed growth inhibition against colon cancer cell line, HCT 116 [19]. Our finding showed that the acetone extract from *C. odontophyllum* leaves was the most cytotoxic whereas the aqueous extract exhibited significantly lower cytotoxic effect in comparison with the acetone and methanol extracts. The lower cytotoxic activity of methanol and aqueous extract in comparison to acetone extract may be due to the presence of saponin and phenolic compounds which might possibly act in antagonism to reduce the cytotoxic effect against HCT 116 cell line. This is in line with [20] that despite potentially valuable combination of phytochemicals in combating cancer drug resistance, the variety of phytocomponents can show opposing effects to produce antagonism. The lowest IC_{50} value displayed by acetone extract could be attributed to the absence of phenolic compounds that have been reported [21] capable of deactivate cytotoxic active compounds in the plant. Hence, the most promising cytotoxic activity against HCT 116 colon cancer cell lines was demonstrated by lowest yield of extract. Acetone and methanol extracts from the leaves of *Canarium odontophyllum* have the potential to be developed as anti-colon cancer agents. In conclusion, this preliminary finding on the cytotoxic effect of the leaf extracts from *C. odontophyllum* against HCT116 cell lines can pave way for future research in view of determining their mode of cancer cell death and isolation of the active compounds from this underutilized plant.

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