Phytochemical, Free Radical Scavenging and Cytotoxic Assay of Cucumis Melo L. Extract and β-Carotene

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***Abstract*—Deaths from cancer worldwide are estimated to continue rising. Free radicals are toxic to cellular components. It is known that they cause DNA damage, contribute to DNA instability and mutation, thus favor carcinogenesis. This research was conducted to determine the activity of *Cucumis melo* extract and β-carotene in anti-oxidative and cytotoxic potencies. The research was done by examining the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay. The cytotoxic potency was determined by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay on HeLa, HepG2 and NIH3T3 cell lines. β-carotene exhibited more active DPPH free radical scavenging activity compared with *C. melo* extract. *C. melo* extract showed more active anti-cancer both in HeLa (IC_{50}: 23.649 µg/mL) and HepG2 (IC_{50}: 110.403 µg/mL) cancer cells. *C. melo* extract (IC_{50}: 16,670.404 µg/mL) and β-carotene (IC_{50}: 50,645.994 µg/mL) had low cytotoxicity in NIH3T3 fibroblast. *C. melo* extract has lower antioxidant activity, but higher cytotoxic potency compared with β-carotene.**

***Index Terms*—antioxidant, free radical, melon, *cucumis melo*, β-carotene, cancer, cytotoxic.**

I. Introduction

As the world major public health problem, cancer-related deaths are projected to continue rising [1], [2], with an estimation of 12 million deaths per year expected by 2030 [3]. More than 700,000 new cancer cases per year occur in ASEAN countries, which is also expected to increase [4]. Cancer has a severe impact on individual and community, it leads to disability and death, with high-cost therapy [1], [5]. Cancer therapies include surgery [6], chemotherapy [7], radiotherapy [8], immunotherapy [9] and gene therapy [10], could have unwelcome side-effects [1], and possibly low cure rates [11].

Free radicals can cause DNA damage, instability and mutation, leading to damage of cellular components and/or carcinogenesis [3]. It has been reported that several anti-cancer subtances inhibit free radical production and oxidative DNA damage, hence inhibit tumor promotion [12].

There has been growing interest using herbs as new anti-cancer drugs. Plants contain a wide variety of chemicals that have potent biological effects, including anti-cancer activity [13]. Large number of plants and and pure isolated compounds contain potential anti-cancer activity [14]. The cucurbitacins provide great interest due to its various bioactivities [1]. Meanwhile muskmelons contain high vitamin A, vitamin C [15] and β-carotene [16], [17].

In this research, we performed determination of the phytochemical constituents of california cantaloupe or *Cucumismelo* L. (*C. melo*) extract, evaluation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging potency of *C. melo* extract and major constituent of β-carotene, and evaluation of cytotoxic potency of *C. melo* extract and β-carotene in HeLa, HepG2 and NIH3T3 cell lines.

II. Material and Method

A. Preparation of Extract

Five hundred grams of *C. melo* were purchased from traditional market located in Caringin, West Java, Indonesia. The fruits were washed with distilled water, ground and soaked in 70% destilated ethanol. After 24 hours, filtrate was collected and residue was soaked again in 70% ethanol for another 24 hours. These treatments were repeated until the filtrate became colorless. The

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filtrate was evaporated with a rotary evaporator at 40°C yielding 24.5 g extract. The extracts were stored at 4°C [18], [19].

B. Phytochemical Assay

C. melo extract was tested with phytochemical assay using modified Farnsworth method for flavonoid, phenolic, saponin, triterpenoid, steroid, terpenoid, tannin and alkaloid [20].

C. DPPH Free Radical Scavenging Activity Assay

Fifty µl *C. melo* extract and β-carotene (Sigma-Aldrich, St. Louis, MO) were introduced into a microplate followed by 200 µl of 0.077 mmol/l DPPH solution (Sigma-Aldrich). The mixtures was shaken vigorously and kept in the dark for 30 minutes at room temperature. DPPH free radical scavenging activity was determined with a microplate reader at 517 nm [18], [19]. The DPPH free radical scavenging activity of each sample was measured according to (1)

$$Scavenging\ \% = (Ac - As) / Ac \times 100. \quad (1)$$

As: sample absorbance
Ac: negative control absorbance (without sample)

D. Cytotoxic Asay

HeLa human cervical cancer cell line, HepG2 liver cancer cell line, NIH3T3 mouse fibroblast were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich), 100 U/mL penicillin (Sigma-Aldrich), and 100 µg/mL streptomycin (Sigma-Aldrich). Cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 [18], [19], [21]. After the cells reached 80% confluency, 5,000 cells were seeded in each well of 96-well plate. After 24 hours incubation, the cells were treated with *C. melo* extract or β-carotene at various concentrations (0, 1, 10, 100, 1000 µg/mL) for 24 hours. To determine cell viability, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega, Madison, WI, USA) was used. MTS was added to each well at a ratio of 1:5. The plate was incubated in 5% CO_2 at 37°C for 4 hours. Absorbance was measured at 490 nm on a microplate reader. The data were presented as number of cells, the percentage of viable cells (%), inhibition proliferation of growth cells (%). The data were analyzed using ANOVA and continued by Tukey post hoc test. We also calculated the median inhibitory concentration (IC_{50}) using Probit Analysis of IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, NY, USA) [22].

III. Result

A. Phytochemical Result

The phytochemical assay showed that extract of *C. melo* contained low terpenoids, tannins and phenols content (Table I)

B. DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity of the sample was marked by color change from dark purple to yellowish or pale yellow [21]. The DPPH free radical scavenging activity of *C. melo* extract or β-carotene was increased significantly in a concentration dependent manner (Table II). By adding higher concentration of *C. melo* extract or β-carotene, higher DPPH activity was obtained. Compared with *C. melo* extract, β-carotene showed higher DPPH activity.

C. Cytotoxic Activity

Number of viable HeLa and HepG2 cancer cells were decreased significantly in *C. melo* extract and β-carotene concentration dependent manners (Table III). By adding higher concentration of *C. melo* extract or β-carotene, lower number of viable cancer cell was obtained. Meanwhile, NIH3T3 cells were also decreased in *C. melo* extract and β-carotene concentration dependent manners, but not significant. The data of viable cancer cell number were then converted into percentage of viable cancer cell as shown in Table IV, so that the effect of *C. melo* extract and β-carotene on the cells could be demonstrated clearly. In order to decribe percentage of growth inhibition effect of *C. melo* extract and β-carotene on cancer cells, data shown in Table III were converted into Table V.

Concentration that could inhibit 50% cell growth (IC_{50}) of *C. melo* extract and β-carotene was calculated and shown in Table VI. Growth inhibition data in Table V were analyzed using probit analysis to obtain the IC_{50}. Both *C. melo* extract and β-carotene were not toxic in NIH3T3 cells since the calculated IC_{50} showed very high concentration.

TABLE I. The Result of Phytochemical Assay of *C. melo* Extract

Sample	**Compound content**							
	Tannins	Triterpenoids	Steroids	Terpenoids	Saponins	Alkaloids	Flavonoids	Phenols
C. melo extract	+	-	-	+	-	-	+	+

Additional information
++++ : very high content ++: moderate content - : undetected
+++ : high content +: low content

TABLE II. DPPH Free Radical Scavenging Activity of *C. melo* Extract and Beta-Carotene.

Samples	Concentration (µg/mL)						
	100	50	25	12.5	6.25	3.125	1.563
β-carotene	73.10±1.53^{f}	55.23±3.03^{e}	44.72±2.08^{d}	32.45±3.26^{c}	19.59±2.06^{b}	15.62±0.62^{ab}	12.49±0.94^{a}
C. melo extract	38.38±3.48^{e}	32.71±1.26^{d}	23.52±0.88^{c}	11.60±0.53^{b}	4.75±0.44^{a}	3.18±0.57^{a}	1.32±0.33^{a}

Data were presented as mean ± standard deviation. Different letters in the same row among concentrations of samples indicate significant differences (Tukey's HSD post hoc test). Each sample was performed in triplicate.

TABLE III. EFFECT *C. MELO* EXTRACT AND BETA-CAROTENE ON NUMBER OF CANCER CELL.

Samples	Concentration of treatment (µg/mL)				
	0	1	10	100	1000
C. melo extract					
HeLa	26,787±90^{e}	16,250±61^{d}	15,173±45^{c}	12,443±72^{b}	8,433±32^{a}
HepG2	22,085±260^{e}	17,317±32^{d}	13,235±132^{c}	12,008±20^{b}	81,28±10^{a}
NIH3T3	8,857±244^{c}	8,863±189^{c}	8,562±85^{c}	7,837±34^{b}	6,663±105^{a}
β-carotene					
HeLa	26,787±90^{e}	18,697±1,410^{d}	16,087±176^{c}	11,637±437^{b}	9,757±151^{a}
HepG2	22,085±260^{e}	17,272±195^{d}	13,768±161^{c}	13,188±79^{b}	9.203±53^{a}
NIH3T3	8,857±244^{d}	8,558±159^{cd}	8,195±56^{cd}	7,581±55^{b}	6,628±59^{a}

Data were presented as mean ± standard deviation. Different letters in the same row among concentrations of samples indicate significant differences (Tukey's HSD post hoc test). Each sample was performed in triplicate.

TABLE IV. EFFECT *C. MELO* EXTRACT AND BETA-CAROTENE ON PERCENTAGE OF VIABLE CANCER CELL.

Samples	Concentration of Treatment (µg/mL)				
	0	1	10	100	1000
C. melo extract					
HeLa	100.00±0.33^{e}	60.66±0.23^{d}	56.64±0.17^{c}	46.45±0.27^{b}	31.48±0.12^{a}
HepG2	100.00±1.18^{e}	78.41±0.14^{d}	59.93±0.60^{c}	54.37±0.09^{b}	36.80±0.05^{a}
NIH3T3	100.00±2.75^{c}	100.07±2.14^{c}	96.67±0.97^{c}	88.48±0.38^{b}	75.23±1.19^{a}
β-carotene					
HeLa	100.00±0.33^{e}	69.80±5.26^{d}	60.05±0.66^{c}	43.44±1.63^{b}	36.42±0.57^{a}
HepG2	100.00±1.18^{e}	78.21±0.88^{d}	62.34±0.73^{c}	59.72±0.36^{b}	41.67±0.24^{a}
NIH3T3	100.00±2.75^{d}	100.07±2.14^{cd}	96.67±0.97^{c}	88.48±0.38^{b}	75.23±1.19^{a}

Data were presented as mean ± standard deviation. Different letters in the same row among concentrations of samples indicate significant differences (Tukey's HSD post hoc test).

TABLE V. EFFECT *C. MELO* EXTRACT AND BETA-CAROTENE ON PERCENTAGE OF CANCER CELLS GROWTH INHIBITION.

Samples	Concentration of treatment (µg/mL)				
	0	1	10	100	1000
C. melo extract					
HeLa	0.00^{a}	39.34^{b}	43.36^{c}	53.55^{d}	68.52^{e}
HepG2	0.00^{a}	21.59^{b}	40.07^{c}	45.63^{d}	63.20^{e}
NIH3T3	0.00^{a}	-0.07^{a}	3.33^{a}	11.52^{b}	24.77^{c}
β-carotene					
HeLa	0.00^{a}	30.20^{b}	39.95^{c}	56.56^{d}	63.58^{e}
HepG2	0.00^{a}	21.79^{b}	37.66^{c}	40.28^{d}	58.33^{e}
NIH3T3	0.00^{a}	3.37^{ab}	7.47^{b}	15.26^{c}	25.16^{d}

Data were presented as mean ± standard deviation. Different letters in the same row among concentrations of samples indicate significant differences (Tukey's HSD post hoc test).

TABLE VI. THE IC_{50} OF *C. MELO* EXTRACT AND B-CAROTENE IN VARIOUS CANCER CELL LINES IN 24 HOURS TREATMENT.

Cell Type	IC_{50} (µg/mL)	
	β-carotene	*C. melo* extract
HeLa	51.760	23.649
HepG2	270.419	110.403
NIH3T3	50,645.994	16,670.404

IV. DISCUSSION

Our phytochemical result showed that *C.melo* contained low phenols and flavonoids. Although previous study showed that aqueous of *C. melo* extract contained high alkaloids, and flavonoids [23], a recent study has shown also that *C. melo* contain low bioactive constituents such as alkaloids and saponins [15]. The phenolic and flavonoid compounds in the plants could have various biological activity such as antioxidant activity [24], [25]. Meanwhile, activity of *C. melo* extract is particularly due to the presence of phenolic compounds especially flavonoids [26]. It has been reported that DPPH free radical scavenging activity increased along with increased concentration of *C. melo* extract and β-carotene [27]. We found DPPH free radical scavenging activity of 100 µg/mL*C. melo* extract was 38.38%. It was lower compared with previous study reporting that the activity was 55% [27]. This might be related with the source of *C. melo*. Compared with fruit, it has been reported that high antioxidant activity was found in leaf and stem extracts of *C. melo* [26]. In addition, *C. melo* seed extract had 75.59% DPPH free radical scavenging

activity at concentration of 300 µg/mL [28]. DPPH free radical scavenging activity of β-carotene was high, this result was validated with previous study reporting that β-carotene has potential antioxidant biological properties due to its chemical structure. β-carotene quenches singlet oxygen with a multiple higher efficiency than α-tocopherol [29]. β-carotene has antioxidant properties, is a precursor of Vitamin A, and imparts the orange color in some fruits and vegetables. This compound is the major carotenoid in *C. melo* [17].

Our data showed that *C.melo* extract and β-carotene had cytotoxic potency on cancer cells. This result is in accordance with many previous studies as follows. β-carotene have anti-neoplastic effects in some individuals [30]. β-carotene can be used in prevention and treatment of many cancer cells [31]. β-carotene increased the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ expression related with cell death [31]. β-carotene inhibited cell proliferation, arrested the cell cycle in different phases, and increased apoptosis. These findings indicate that the effect was cell type-dependent and that carotenoids are potential agents for biological interference with cancer [32].

Consumption of carotenoids is associated with reduced risk of chronic diseases, including cancer and vascular diseases [33]. β-carotene is associated with reduction in the risk of prostate cancer [34], HT-29 colon adenocarcinoma cancer cells [33] and AtT-20 mouse corticotroph tumor cell line [35]. β-carotene induced an increase of the cells in S and G2/M phases. β-carotene induced apoptosis, reduced expression of phosphorylated-Cx43 [35]. Carotenoids inhibited AtT-20 cell proliferation and colony formation, induced apoptosis and cell cycle arrest, and reduced adrenocorticotropic hormone (ACTH) secretion [36].

C. melo extract showed anti-cancer activity, this result is in accordance with previous study showing that *C. melo* reduced hyperplasia of the prostate and induces apoptotis [37]. A common component in melons, 3-methylthiopropionic acid ethyl ester (MTPE), was first identified as a volatile constituent of *C. melo* [38]. MTPE treatment for 4 days in RCM-1 human colon cancer cell line increased alkaline phosphatase (ALP) activity, indicated that MTPE possess potential anti-carcinogenic properties by inducing differentiation [38].

Cucurbitacin B (CuB) and CuE, which are obtained from the calyx melo of *C.melo* [1], significantly inhibited the growth of tumor *in vivo* and *in vitro* in a dose-dependent manner. Many anti-cancer agents arrest the cell cycle at the G1, S, or G2/M phase and then induce apoptotic cell death [1]. CuB had a significant inhibitory effect on proliferation of cervical cancer (HeLa), hepatoma (HepG2) and murine sarcoma (S180) cells in a dose-dependent manner [1]. CuE demonstrated cytotoxic activity against malignant glioblastoma multiforme 8401 cells and induced cell cycle G2/M arrest in these cells [39]. CuB is a natural anti-cancer agent isolated from the stem of *C. melo*. CuB inhibits signal transducer and activator of transcription 3 (STAT3) activation and the Raf/MEK/ERK pathway in K562 leukemia cells. CuA and CuE have significant anti-tumor activity [1], [26], [40]. In our current results, *C. melo* extract had more active cytotoxic potency compared with β-carotene, since *C. melo* constitutes many of potential compounds including β-carotene itself, CuA, CuB, CuE and MTPE, which each compound has anti-cancer activity.

In conclusion, *C. melo* extract has lower antioxidant activity, but higher cytotoxic potency compared with β-carotene in this in vitro study. Further mode of action test, preclinical and clinical studies should be pursued before pharmaceutical applications.

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