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Research Article

The antioxidant activity and cytotoxicity methanol extracts from cranberry plants

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ABSTRACT
The purpose of this study was to determine the total phenol content, antioxidant activity and cytotoxicity of methanol extracts from cranberry plants. The highest total phenol content of 17.1 mg/100 g, and antioxidant activity with IC_{50} = 23.8 mg/100 g. This situation shows that the total content of phenolic plant extracts examined correlated with DPPH activity. IC_{50} cytotoxicity of methanol extracts of each 75.11 µg/mL against Calu-6 cells, 177.53 from µg/mL against MCF-cells and 54.87 µg/mL against HCT- 116 cells. From the data obtained we can conclude that this plant has a quite high of total phenolic content and antioxidant activity. Correlation between total phenolics increased DPPH free radical scavenging and cytotoxic activities are quite good. The results of this study showed that cranberry plants can be used as the basis for the treatment of some diseases.
Key words: cranberry, methanol extracts, natural antioxidants, total phenolics content, activity of DPPH radical scavenger, cytotoxicity activity

INTRODUCTION

Experimental study of epidemiology, laboratory animal and human investigations indicate that consumption of fruits and vegetables are associated with lower risk of several diseases including cardiovascular disease and cancer[1,2,3,4,5]. These beneficial effects have been associated with bioactive properties of natural phenolic compounds. Many plants have antioxidant activity and is recommended for consumption[6,7].

The importance of phenolic compounds, especially flavonoids, are due to their ability to act as a recipient of a highly efficient free radical[8,9,10]. Therefore, several compounds as follows: isothiocyanate (vegetables), carotenoids including alpha-carotene, gamma carotene, beta-cryptoxanthin, zeatxanthin, luttein, lycopene (tomatoes), resveratrol (grapes and wine), ellagic acid (berries Various) , glutathione-S-transferase (garlic), diallyl sulfide (garlic), genestin (soybean), curcumin (turmeric), indole-3-carbinol, inositol, organosulfur compounds, sulforaphane, squalene, and terpenes active in cancer prevention[11].

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ), has also been widely used in food, however, are known to have few side effects[12]. Therefore, investigation of new natural resources as an antioxidant to increase the safety[13,14], and various types of antioxidants found in various plants, which does not cause a health risk for consumers researched[15,16,17].

The above-mentioned investigations have shown that increased consumption of fruits and vegetables significantly reduces the incidence of chronic diseases, like cancer, heart disease and other pathologies associated with aging[12]. Especially the antioxidant activity of caffeic acid and hydroxycinnamic acid compounds have been studied. Found that the antioxidant activity of these scavenging in the following order: rosmarinic> phenethyl ester of caffeic acid> caffeic acid tocopherol acid> chlorogenic> cidferulic acid> ferulic phenethyl ester> BHT[16].

This study attempted to determine the total phenolic content, antioxidant activity and cytotoxicity activity of methanol extract of cranberry plants. Cranberries are a group of evergreen dwarf shrubs or trailing vines in the subgenus *Oxycoccus* of the genus *Vaccinium*

(Family: *Ericaceae*). To conduct this research, we use several tests including the Folin-Ciocalteu assay, 1.1-diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH), and MTT (3 - (4,5-dimethylthiazol-2-yl) - 2.5-diphenyltetrazolium bromide) assay. Free radical scavenging assay, usually intended to determine the mechanism of antioxidants that inhibit lipid oxidation. It is understood that antioxidants, inhibitors of lipid peroxidation is not only important for food protection, but also, it is important for the defense of living cells against oxidative damage[1]. Generally, the methods used to determine free radical scavenging is 1.1-diphenyl-2-picrylhydrazyl - DPPH assay[18,19]. Compared with other methods, DPPH method is relatively short[18]. This study therefore using DPPH test.

MATERIALS & METHODS

Materials

Folin-Ciocalteu reagent, 1.1-diphenyl-2-picrylhydrazyl (DPPH), MTT (3 - (4, 5-dimethylthiazol-2-yl) -2.5-diphenyltetrazolium bromide), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid available from Sigma Chemical Co., St. Louis, MO, USA. All reagents, are pro analysis.

Methods

Samples preparation

Plants used were cranberries, including leaves and stems, which when cut into pieces, dried at 50^{o}C for five days. Sample extraction using 95% methanol at room temperature, and the extract obtained was concentrated using evaporators. Methanol extract obtained is then used for each determination of phenolic content, free radical scavenging activity of DPPH, and the cytotoxicity activity.

Determination of total phenolic content (TP)

Determination of total phenolic content (TP), using Folin-Ciocalteu test[20]. 5 mL of water, 0.5 to 1.0 mL sample, and 1.0 mL Folin-Ciocalteu reagent put into 25 mL volumetric flask, and left for 5-8 minutes at room temperature. Subsequently, 10 mL 7% sodium carbonate solution were added, and followed by the addition of water until the volume reached 25 mL. Mixture left at room temperature for 2 hours. Then the sample is filtered through a Whatman 0.45 m before the determination of TP. λ concentration using spectrophotometer at 640 nm. TP standardized content of ferulic acid and expressed as 100 mg/g ferulic acid (FAE). The range for this linearity test is determined as 0.5 to 5.0 mg/L FAE (R2 = 0.9990), giving an absorbance range of from 0.050 to 0.555 AU.

Determination of Free Radical DPPH scavenging

Each methanol extract at various concentrations (3.1, 6.3, 12.5, 25, and 50 mg/100 g) added to 1.5 × 10^{-4} M solution of DPPH in methanol and the reaction mixture shaken vigorously. Amount of DPPH which does not react determined at 520 nm, and free radical scavenging aktiviats obtained from the following equation: free radical scavenging activity (%) = ((OD control - OD sample) / control OD)) × 100. The antioxidant activity of plant extracts is expressed as IC_{50}, defined as the concentration (in mg/100g) of the extract required to inhibit the formation of DPPH radicals by 50%.

Determination of cytotoxicity

Used cell line: Calu-6 (human lung carcinoma), MCF-7 (human breast adenocarcinoma), and HCT-116 (human colon carcinoma). Tues grown in RPMI-1640 medium at 37^{o}C, 5% CO_2 incubator. Cell harvested, and counted (3 × 104 cells/mL), and transferred into 96-well plate and incubated for 24 hours before addition of test compounds. Dilutions of test samples prepared by dissolving compounds in DMSO followed by dilution with RPMI-1640 to give a final concentration of 25, 50, 100, 200, 400, and 800 ug/mL. 90 µL and 10 µL sample cell, incubated for 72 hours and added to solution of MTT (5 mg/mL dissolved in 1 mL of Phosphate Buffer Solution / PBS), a total of 10 µL into each well of 96-well plate as described by Tian *et.al*[21]. Well plates were incubated at 37^{o}C for 4 hours. From each well containing media, MTT is not tied and dead cells removed by aspiration, and 150 µL of DMSO added to each well. Then the optical density of samples was determined using ELISA reader at 540 nm Reader. As a positive control used distilled water and DMSO as solvent control. Cytotoxicity obtained by comparing the absorbance between sample and control. Those values are then used to calculate the concentration of extract required to grow 50% decrease (IC_{50}) in the growth of cells.

RESULTS AND DISCUSSION

Methanol extracts of total phenolic content was 17.1 mg/100g cranberry and total phenolic antioxidant BHT as standard is 1.5 mg/100 g (Figure 1). These results are comparable with the activity of DPPH free radical savenging[22]. Zhou and Yu[23] also reported that total phenolic

content of vegetable extracts is correlated with the activity of DPPH free radical scavenging, and showed that the amount of phenolics play an important role in the antioxidant activity of plant materials.

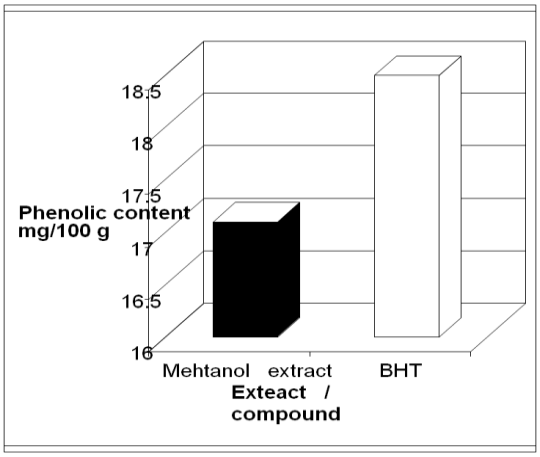

Figure 1. Total phenolic content of methanol extracts of cranberry and BHT

The result of the determination of DPPH radical activity scavengin cranberry plant extracts can be seen in Table 1. As can be seen, the methanol extracts had DPPH radical activity with IC_{50} value of 23.8 mg/100 g, whereas the activity of DPPH radical scavenging BHT have IC_{50} 11.3 mg/100 g. This indicates that the scavenging activity of plant extracts is lower than the scavenging activity of synthetic antioxidant BHT.

Table 1. Antioxidant activity of cranberry methanol extract and BHT

Sample	Concentrations, mg/100 g					
	3.1	6.3	12.5	25	50	IC50
Cranberry methanol extract	4.7	12.1	26.5	52.8	86.4	23.8
BHT	15.6	33.5	55.2	81.3	92.4	11.3

The result of the determination of the cytotoxicity test using three kinds of cell line showed that the methanol extract of cranberry possessed IC_{50} each values 75.11 µg/mL against Calu-6 cells, 177.53 of µg/mL against MCF-cells and 54.87 µg/mL against HCT-116 cells. (Table 2, Figure 2), whereas IC_{50} BHT values against all three cell line is 57.05 µg/ mL (Calu-6), 57.93 µg/ml (MCF-7) and 34.75 µg/mL (HCT-116) (Table 2 and Figure 3).

Table 2. Cytotoxicity activity of cranberry ethanol extract and BHT using a variety of cell line

Sample	IC_{50} (µg/mL)		
	Calu-6	MCF-7	HCT-116
Cranberry methanol extract	75.11	177.53	54.87
BHT	57.05	57.93	34.75

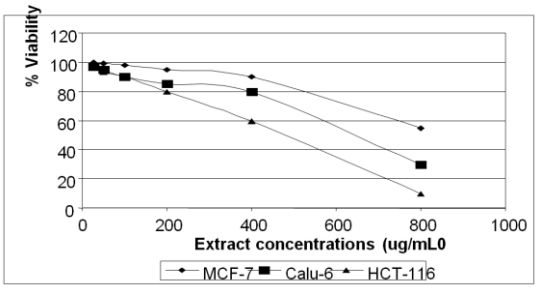

Figure 2. % cranberry methanol extract viability using cell line MCF-7, Calu-6 and HCT-116

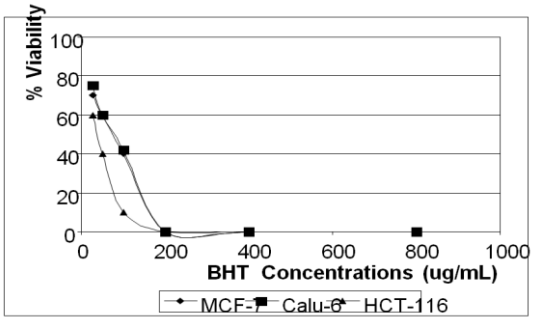

Figure 3. % BHT viability using MCF-7 cell line, Calu-6 and HCT-116

Increased consumption of fruits and vegetables significantly reduces the incidence of chronic diseases, like cancer, heart disease and other pathologies associated with aging [12,24]. Phytochemicals, phenolic compounds, especially natural products is suggested to be the bioactive compounds that have health benefits[25]. This situation points to the fact that phenolic compounds have antioxidant properties[26]. As a result, consumption of natural antioxidants is inversely related to several chronic diseases[26]. Antioxidants can delay or inhibit oxidation of lipids by inhibiting the initiation or propagation of oxidative chain reactions[22]. The antioxidant activity of phenolic compounds, mainly due to the nature of these redox compounds, which can play an important role in

absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or peroxide[27].

From this study the methanol extract of cranberry is also known to have radical scavenging activity of high DPPH (IC_{50} = 23.8 mg/100 g), this shows that the DPPH radical scavenging depends on the content and strong relationships between phenolics total content and activity level radical scavenging. This data is also supported by the results of research that has been reported by Lee et al.[6] who showed that extracts China traditional plants in addition to having a high total phenolic also has high scavenging DPPH radical activity.

Cai et al.[13] studied the content of phenolic compounds, antioxidant and anticancer activity of the 112 plant species from 50 families of traditional Chinese medicine plant, using a systematic method of extracts of the ABTS (2, 2-azinobis (3-ethylbenzthiazoline Acid-6-sulfonic acid) against drug crops. They found that TEAC (Trolox equivalent antioxidant capacity) values and total phenolic content for methanolic extracts of plants ranged from 46.7 to 17 323 Trolox equivalent (TE) / 100 g DW (dry weight) and from 0.22 to 50.3 g of gallic acid equivalent (GAE)/100 g DW.

Significant positive linear relationship between antioxidant activity and total phenolic content (R2 = 0.95 all) showed that the antioxidant phenolic compounds are the dominant components in the tested herbs. Major types of phenolic compounds from most plants tested were phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids.

TEAC and phenolic content of 32 spice extracts from 21 botanical families growing in Poland have also been investigated[26] and found that the total phenol content ranged from 0.07 to 15.2 mg GAE / 100 g DW. Similar analytical methods used for determination of total phenolics and free radical ABTS method also cited in the report. Phenolic acid compounds identified in the species being analyzed are caffeic, coumaric , ferulic and neochlorogenic, while the dominant compounds are flavonoids quercetin, luteolin, apigenin, kaempferol and isorhamnetin. Myricetin detected only in *Epilobium hirsutum* L. As indicated, this report also found that most of the spices studied have high levels of phenolics and also having high antioxidant activity. Dastmalchi et al.[29] in his research using the materials collected Moldavian balm from Iran. For the extraction of bioactive compounds used in seven solvents with different polarity (petroleum ether, dichloromethane, acetonitrile, ethyl acetate, methanol, n- butanol and water). The identification results show that hydroxylated cinnamic acid, and its derivatives and flavonoids found in the extract, rosmarinic acid is a component of the most widely identified.

Our results also showed that the association of high levels of phenolics with high antioxidant activity which affects anticancer activity of the plants studied. Methanol extract of cranberry in addition to having a high antioxidant ativities (IC_{50} = 23.8 mg/100 g) also have high anticancer activity against HCT-116 cells (IC_{50} = 54.87 μg/mL).

Cai et al.[13] stated that the prevention and treatment of cancer by using traditional Chinese medicines has risen high enough. Herbal medicine known to have high antioxidant activity and phenolic levels are significantly higher than vegetables and fruits. They concluded that the traditional Chinese medicinal plants associated with anticancer may have a potential source of natural antioxidants and can be beneficial chemopreventive compounds. Manosroi et al.[30] reported on the anti-proliferative activity of essential oil from 17 Thai medicinal plants against leukemia epidermal carcinoma cell line (KB) and murine cell line (P338) using the MTT assay. The results showed that the leaves and oil of Sweet Basil Guava (*Psidium guajava* L.) has an anti-proliferative activity against cell line KB and P388. Cho and Leung[14] has been studied in vitro and in vivo anti-tumor effects of *Astragalus membranaceus* (Fisch.) Bunge, commonly used as a medicinal plant of China, and proved able to restore T cell dysfunction in cancer patients. Of the five bioactive fractions isolated from *A. membranaceus* root, it is known that the fraction of AI (methanol fraction) is the best fraction of the five factions in connection with mitogenicity on murine splenocytes. Besides investigating the cytostatic effects of AI, activity on macrophage function, tumor necrosis factor production, induction of lymphokine-activated killer cells and tumor cell differentiation was also examined. Tumor macrophages and myeloid tumors found more sensitive to the cytostatic activity of the AI, whereas tumor fibroblasts and mouse Ehrlich ascites tumor seems relatively resistant. In addition, AI can effectively suppress the growth of syngeneic tumors in mice in vivo. The results showed that murine macrophages that dipertreatmen with AI can increase the cytostatic activity of MBL-2 tumor in vitro and in vivo.

Preincubation of mouse splenocytes with AI can induce activity in vitro lymphokine-activated on the cell line WEHI-164. In addition, the AI is able to induce the differentiation of both human monocytic cells and murine in vitro. AI can even restore the depressed mitogenic response in mice in vivo. Can be concluded that Astragalus

membranaceus can play both in vitro and in vivo anti-tumor effects, by activating anti-tumor immune mechanism for host. All the above results support the results of research conducted today is that cranberry plants extracted by methanol have antioxidant properties associated with anti-tumor properties.

CONCLUSIONS

Cranberry plants studied contain relatively high total phenolics and antioxidant activity. DPPH free radical scavenging activity increased with increasing levels of phenolics in cranberry extract. These results strongly suggested that cranberry plants extract is promising sources for total phenolics and antioxidant agents and can be used in addition to basic drugs in the treatment of some diseases.

As for anticancer plant further investigations such as components responsible for such activity, toxicity, and anti proliferations tests to cell-lines are required .

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