ANTIOXIDANT AND CYTOTOXICITY PROPERTIES OF ETHYL ACETATE FRACTIONS OF *Pandanus tectorius* FRUIT AGAINST HELA CELL LINES

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Abstract

Antioxidant agents play an important role in inhibiting free radical molecules activity thus preventing the cancer disease. This current study aimed to evaluate antioxidant and cytotoxicityproperties of ethyl acetate fractions of *Pandanustectorius* fruit extract on HeLa cell lines. The radical scavenging activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against human HeLa cell lines. The result showed that all ethyl acetate fractions of *P. tectorius*fruit had high antioxidant property. All of seven fractions of the ethyl acetate extract were observed based on the IC_{50} value and had high DPPH free radical scavenging activity with the IC_{50} value range from 0.3 to 2.4 mg/ml compare to positive control,quercetin(IC_{50}= 0.2 mg/ml). Based on the DPPH free radical scavenging activity (antioxidant property), three of seven fractions were chosen for the MTT assay to analyze their cytotoxic activity as anticancer potential agent against HeLa cell lines.From MTT result, one of tree fractions were showed cytotoxicity activity against HeLa cell lines with the IC_{50}value is 12 µg/ml (less than 30 µg/ml), while anothertwo fractions was found not cytotoxic against HeLa cell lines.It is clearly indicated that *P. tectorius*fruit has a very good potential as antioxidant and anticancer agent.

Keywords: *Pandanus tectorius*, DPPH, MTT assay, *HeLa* cell lines

INTRODUCTION

According to World Health Organization (WHO), cancer is a major cause of death worldwide which is about 8.2 million deaths in 2012. The main causes of cancer death are cancers of lung (1.59 million deaths), liver (745 000 deaths), stomach (723 000 deaths), colorectal (694 000 deaths), breast (521 000 deaths), oesophageal cancer (400 000 deaths) and cervical cancer (270 000 deaths) [1] . Radiotherapy, surgery and chemotherapy are such of cancer treatments that have been used to cure the cancer. However, most of these treatments had adverse effects. They are not only giving side effects to the patient, but also need more cost to treat the cancer with modern technology. Therefore, it is necessary to find the alternative treatment that based on the natural product, easy to obtain, affordable, cheap and does not or less have side effects to the patient.

A natural product is a substance that is produced by a living organism that can be found in nature. Plant is one of the natural products that have a lot of phytochemicals with various bioactivities like anti-inflammatory, antioxidant and anticancer activities [2]. It had been proof from some studies where extracts from natural products like vegetables, medical herbs and fruits have positive effect against cancer compared with fluent hormonal treatments or chemotherapy [3]. There are a lot of sources natural products such as from marine and terrestrial, animal, microbes and plants. As an example one of the natural products from mangrove plants is *Pandanustectorius.* According to the Zhang *et al.*, the fruit of *Pandanus tectorius* contain fifteen compounds which are ten phenolic compounds and five flavonoids [4]. Many phenolic

compounds have been reported to possess anti-inflammatory, antibacterial, antiviral, anticancer and potent antioxidant activity [5] .

Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing harm. Antioxidants are also known as "free radical scavengers". To prevent the types of free radical damage associated with cancer development, the increased levels of exogenous antioxidant in laboratory and animal studies had been shown. So, there are some studies recorded that, the risk of cancer development in human body can be lower by taking dietary antioxidant supplements. Many antioxidant substances have anti-carcinogenic properties and anticancer activity [6]. Thus, the antioxidant activity from *Pandanus tectorius* shows the potential of this plant as an anticancer.

METHODOLOGY

Pandanus tectorius fruit wascollected at the beach of BatuRakit in Kuala Terengganu, Terengganu, Malaysia on July and August 2015. Usually, the season of this fruit is on March until August. The collected fruit were separated into keys and core. They were minced and then kept in -80°C for two nights. After that, the sample was dried using freeze dry and grinded to be a sample powder. The sample was weighted and macerated in methanol solvent. The mixture was left at room temperature 24 hours. After 24 hours, the sample was filtered using filter funnel and filter paper. Then, the liquid part was evaporated using the rotary evaporator until they were become concentrated to be methanol extract. The residue was maceratedagain follow the samestep (5-7 times) or until the solvent part become colourless and evaporated to get methanol extract.All methanol extracts were combinied and weighed. After that, the methanol extract was continued to the fractionation process using water, hexane, and ethyl acetate by separating panel. The process of partition was carried based on the increasing of solvent polarity from non polar (hexane)to more polar solvent(ethyl acetate) with ratio 1:1 (v/v). Then, the hexane and ethyl acetate extracts from thepartitioning were evaporated by using rotary evaporator to become crude. Mean while, the water extract was lyophilized on the freeze drier [7] The fractionation of ethyl acetate extract was done by column chromatograpy using some solvents (hexane, chloroform, ethyl acetate, and methanol).

The quantitative antioxidant activity of the *Pandanus tectorius* fruit was determined using the DPPH free radical scavenging assay. Evaluation of radical scavenging activity of antioxidant in the plant against a chemically-synthesized radical was determined by using DPPH assay. The procedure of 96 well plate methods from Kanski *et al.* [8] was used in this assay. The cytotoxicity of *Pandanus tectorius* on cancer cells was done by MTT assay. This assay was performed according to modification of the methodology reported by Lee *et al.* [9] . The period of this method was about 5 days. For first day, seed cell was done where 5 ml of complete media were prepared. All the media from the cell culture flasks were removed and washed by 3 ml PBS for twice. 1 ml of trypsin was added and the cell culture were incubated about two minutes (for detach cell from flask). Then, 5 ml of complete media were added into flask. After that, 10 µl of cells in the media was stained with 10 µl of trypan blue and was removed into the haemocytometer for counting the viability HeLa cell to determine the volume of media needed in the cell culture. 30.67 ml volume of media was added into the petri dish that contains cells. Moreover, the cells were cultured in the 96-steriled well plate. Each wellwas loaded with 100 µl of cells that contain complete media and were incubated at 37 $^{\circ}$C with 5 % CO_2, 95 % air and complete humidity. For dilution of sample, 10 mg of fraction *Pandanustectorius* was mixed with 1 ml of DMSO in 1.5 ml appendorf tube. Then fold dilution by placing seven appendorf tubes. The first appendorf tube was added with 6 µl of sample and 994 µl of complete media. The first tubes were suspended first and 500 µl were transferred into other tube continuously until last.

For day two, all the media was removed from 96-well plate that contain cell culture and were replaced with the samples. Then, this 96-well plate was incubated about three days. After three days, the MTT assay were carried out in the dark where 40 mg of MTT powder was freshly prepared by dissolving 8 ml of PBS. 20 µl of MTT solution was

added into each well and were covered with aluminium foil then were incubated about four hours. After incubation, all media in the well were discharged and were replaced with 100 µl DMSO then were incubated about 10 minutes. After that, the absorbance was read by ELISA plate reader at 571 nm. Furthermore, the percentage of cell viability and cell death were measured according to the following formula.

$$\text{Cell viability (\%)} = \frac{\text{Absorbance sample}}{\text{Absorbance blank}} \text{ X } 100$$

$$\text{Cell death (\%)} = 100 - \text{cell viability}$$

The inhibition concentration for 50% (the IC_{50} value) was determined by using a graph of cell viability against concentration of stock. This IC_{50} were represented as the concentration that decreases 50% of the growth of cell.

RESULTS AND DISCUSSION

Antioxidant activity

Results on Figure 1 and 2 shows that all of seven fractions of the ethyl acetate extract have high DPPH free radical scavenging activity compare to standard quercetin (IC_{50} = 0.2 mg/ml), with the IC_{50}value range from 0.3 to 2.4 mg/ml. The DPPH free radical had been widely used to predict the antioxidant activities (the free radical-scavenging) because it is a stable free radical [10]. Duan *et al.* [11] stated that DPPH are used for determining the compound of the free radical scavenging activities. This study was conducted to determine antioxidant activity of ethyl acetate properties of *P.tectorius* fruit fractions (PFF). Some studies reported that ethyl acetate extract were usually rich with chemical constituents such as terpenoids, alkanoids, tannins, saponins, flavonoids and phenolics [12]. According to Praveen and Awang [13] ; Hermien *et al*. [14] , the major contributors to the antioxidant activity among all of these compounds is phenolics where it is semi polar compound. There was a correlation between phenolic compounds of *P. tectorius* fruit with free radical scavenging activity. The higher total phenolic compound means that the higher the antioxidant activity. Figure 1 and 2 showed the graph of the percentage of antioxidant (%) against concentration of the seven fraction of sample (mg/ml). They are observed based on the fifty percent inhibition concentration of the DPPH free radical scavenging activity (IC_{50}). This study used the standard quercetin as positive control, the IC_{50}value is 0.2 mg/ml. From the graph showed that the IC_{50} value range of all seven fractions of the ethyl acetate extract is between 0.3 to 2.4 mg/ml. The IC_{50} value of PFF and standard quercetin has only lower significant different which means that seven PFF have high antioxidant activity.

Anticancer potency

Anticancer potencyof *P. tectorius* was studied based on their cytotoxicity properties by MTT assay of ethyl acetate fractions of *P. tectorius* fruit extract against HeLa cell lines. There are three fractions (fraction 4, 6 and 7) from seven fractions that had been chosen to test their cytotoxicity property against HeLa cell lines. There are three fractions (fraction 4, 6 and 7) from seven fractions that had been chosen to test their cytotoxicity property against HeLa cell lines. The fractions were chosen based on the highest antioxidant activity. According these three fractions, only fraction 7 (Figure 3) was showed cytotoxic against HeLa cell lines with the IC_{50} value is 12µg/ml, while others fractions were not showed cytotoxic activity against HeLa cell lines (Figure 4 and 5). Fraction 7, 4 and 6 had been chosen from seven fractions based on the higher free radical scavenging activity. Furthermore, Andriani *et al.,* [15] states that the results obtained were interpreted as follows; the IC_{50} value less than 1.0 µg/ml is defined as highly toxic, the range between 1.0 to 10.0 µg/ml is toxic, the range from 10 to 30 µg/ml is moderately toxic, and the value more than 30 µg/ml is defines as non-toxic. Only the sample extract with the IC_{50} value lower than 30µg/ml is considered toxic and have the potential as anticancer agent.

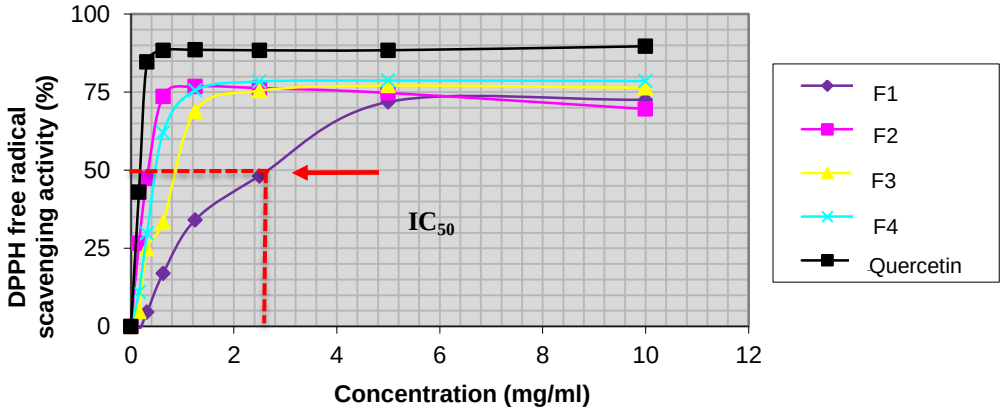

Figure 1. The percentage of DPPH free radical scavenging activity of four fractionsfrom etyl acetate extract of *P. tectorius* fruits with standard quercetin

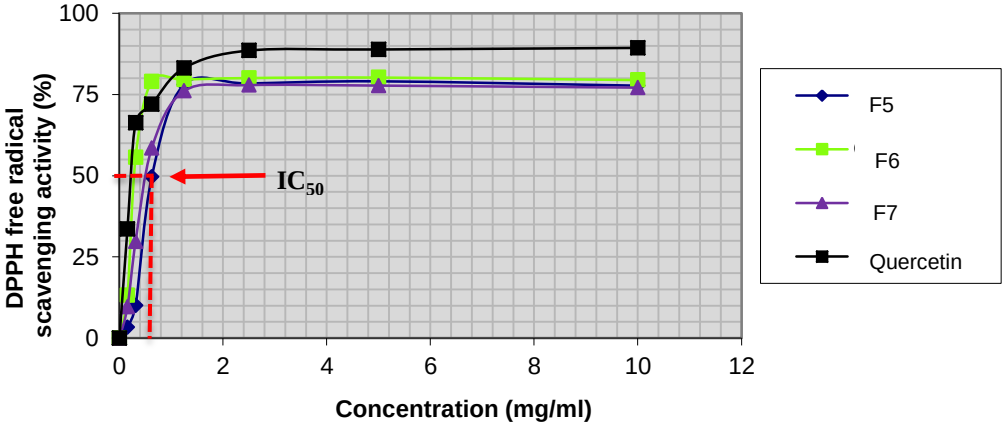

Figure 2. The percentage of DPPH free radical scavenging activity of three fractionsfrom etyl acetate extract of *P. tectorius* fruits with standard quercetin

Between these three fractions, only fraction 7 thas has the IC_{50} value lower than 30µg/ml which is 12µg/ml. Its means that, fraction 7 was toxic against HeLacell lines. The graph in the Figure 4 and 5 showed the data is above the IC_{50} value and means that both fractions are not toxic. Even though fraction 4 and 5 has higher antioxidant activity but they do have potential as anticancer agent against HeLa cell lines. Maybe it has benefits for other activity like antibacterial, anti-inflammatory and others. This can be identifying by further study of anticancer activity of *P.tectorius* on other cancer cell lines than HeLa cell lines (human cervical cancer).

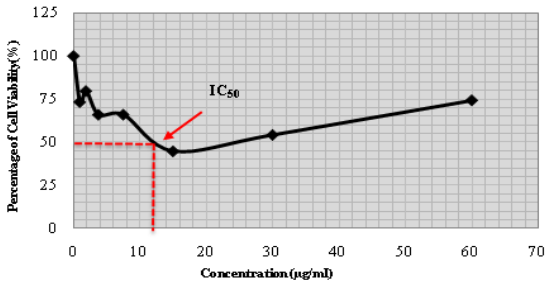

Figure 3.Cytotoxicity activity of fraction 7 against HeLa cell lines.

Moreover, Andriani*et al.* [16] reported that ethyl acetate extract from *P.tectorius* fruits showed no cytotoxicity activity against HeLa cell lines.

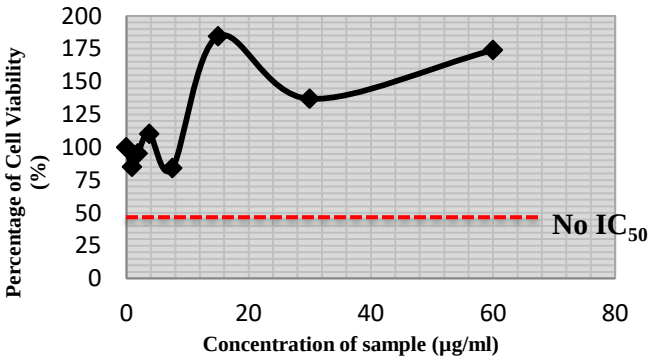

Figure 4.Cytotoxicity activity of fraction 4 against HeLa cell lines

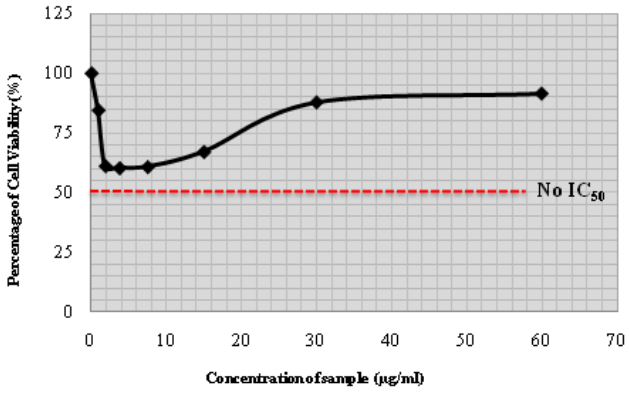

Figure 5.Cytotoxicity activity of fraction 6 against HeLa cell lines.

While in our present study, there was found one fraction from ethyl acetate extract that have shown cytotoxicity activity against HeLa cell lines. Antagonistic effect between chemical constituents in the extract could have had a responsible to this activity, hence the fraction (F7) showed high cytotoxic activity against HeLa cll lines. Further fractionation of the ethyl acetate extract would beinfluence to the fraction's activity and yielded higher activity than its extract. Antagonistic relationship among phytochemicals would be effect the efficacy of crude extracts was reported by Milugo*et al* [17] in 2013.

CONCLUSION

The results found that the natural chemical constituents derived from *P.tectorius*fruit extracts has very good potential use in the pharmaceutical industry as antioxidant and anticancer agents.

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