

## STUDY ON ANTIOXIDANT CAPACITY AND ANTICANCER ACTIVITY OF BISMILLAH LEAF (*VERNONIAAMYGDALINA*).

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### ABSTRACT

*Vernonia amygdalina* is commonly called bitter leaf because of its bitter taste. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. This study was carried out to evaluate antioxidant capacity of the methanol, chloroform and ethyl acetate extracts of *V. Amygdalina* and as antiproliferative agents in inhibiting the growth of human colon adenocarcinoma cell line (HT-29) and human breast adenocarcinoma cell line (MCF-7). It was found that ethyl acetate fraction have maximum amount of polyphenolics compounds (2.69 mg/g GAE in concentration 0.5 mg/mL); more effective than methanol and chloroform extract. This fraction also exhibited fairly good antioxidant activity with in both TBA (17.39% mg/g GAE) and FTC (12.65% mg/g GAE) methods. Antiproliferative study indicated that *V. Amygdalina* crude extract have great potential

as antiproliferative compounds against various cancer cell lines and can significantly inhibit the growth of MCF-7 and HT-29 cancer cells. The methanol *V. Amygdalina* extract has a broad spectrum of activity by inhibiting the growth of MCF-7 and HT-29 cancer cells.

**KEY WORDS :** *Vernonia amygdalina*, antioxidant activity, anti-proliferative activity, MCF-7 and HT-29 cancer cells.

## INTRODUCTION

*Vernonia amygdalina*, a member of the Asteraceae, is a small shrub that grows in the tropical Africa. The *V. Amygdalina* is commonly called bitter leaf because of its bitter taste. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. Many herbalist and naturopathic doctors recommend aqueous extracts for their patients as other treatment for emesis, nausea, diabetes, loss of appetite-induced ambrosia, dysentery and other gastrointestinal tract problems.

Through considerable previous study has been done with regard to antioxidants and polyphenols of *V. Amygdalina*. Very few reports are available with regard to antioxidants and polyphenols of *V. Amygdalina* and based on the results of antioxidant capacity of *V. Amygdalina* that was done previously. Thus, these studies evaluate the ability of possible anticancer activity of the various extracts and fractions of *V. Amygdalina* as anti-carcinogenic agent. As it has been reported the different antioxidant compounds exhibit differential scavenging activity on various reactive oxygen species (Wang and Jiao, 2000) and the reaction with hydroxyl radicals is non-specific, while reaction with other radicals is more specific (Singh et al., 2009).

Cancer is one of the major health problems in Malaysia and currently is the second leading cause of death worldwide. This disease has become increasingly important as a public health concern with the development and progress that has been achieved in this country. According to estimates, there are about 90-100,000 people in Malaysia living with cancer at any one time. The National Cancer Registry of Malaysia (NCR) records 21,773 Malaysians being diagnosed with cancer but estimates that almost 10,000 cases are unregistered every year. It is estimated that one in four Malaysians (1:4) will develop cancer by 75 years old. Increasing population and longer life spans contributes to rise of cancer. Less than 10% of cancers happen in children compared to over 50% in men and 35% in women aged 50 and above.

Cancer occurs more in females than males with a ratio of male to female (1:1.2). The cancer incidence out of 100,000 people are; 4,058 Malay males and 4,753 Malay females are diagnosed with cancer, 4,078 Chinese males and 4,422 Chinese females are diagnosed with cancer, and 629 Indian males and 1065 Indian females are diagnosed with cancer. Although considered the 3<sup>rd</sup> leading cause of premature death in Malaysia, only 30-40% of all deaths from cancer are medically certified, meaning there is no exact figure of people dying from

cancer. Findings show that 10.3% of Malaysians risk dying from the disease before 75 years old (Globocan, 2008). Cancer is becoming a leading cause of death due to avoidable risk factors like smoking and tobacco exposure, poor diet, alcohol, inadequate exercise or being overweight.

It is estimated that nearly 40% of all cancers are preventable, including colorectal, lung and cervical cancers, with smaller effects in breast and nasopharyngeal cancer. Improvement in early detection and treatment leads to better survival rates for people with cancer. Common cancers in Malaysia in which early diagnosis is possible and effective treatment is available include breast, nasopharyngeal and oral cavity cancers. Down-staging of cancer would automatically yield better results in cancer control. However, delay in diagnosis is common owing to factors such as frequent reliance on unorthodox medical remedies at initial presentation (Mellor and Zabedah, 1997).

For instance, the impact of infectious diseases has decreased with the advent of the widespread supply of clean water and sanitation facilities (Endriniet al., 2002). The use of chemotherapeutic drugs in cancer therapy involves the risk of life threatening host toxicity. The serious side effect and merely extend the patient's lifespan for a few years cause by conventional therapies for treating a cancer (Endrini et al., 2002). Cancer prevention and control may benefit from the potential that resides in alternatives therapies. There is an increasing demand to use alternative concepts or approaches for the prevention of cancer and an increasing attention and realization that chemotherapeutic agents act primarily by inducing cancer cell death through the mechanism of apoptosis.

However, there are many cancers that are intrinsically resistant to apoptosis, making it vital to develop novel drugs for combination chemotherapy (Merlin et al., 2010; Lowe and Lin, 2000). A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damages to normal cells. This ideal situation is achievable by inducing apoptosis in cancer cells. Chemopreventive agents comprise diverse groups of compounds with different mechanisms of action with ultimate ability to induce apoptosis. Understanding the modes of action of these compounds should be provided useful information for their possible applications in preventing cancer and perhaps as a agents therapy (David, 2004; Taraphdar et al., 2001). Through considerable previous study has been done with regard to antioxidants capacity of *V. Amygdalina*. Very few reports are available with regard to antioxidants and polyphenols of *V. Amygdalina* and based on the results of antioxidant capacity of *V.*

*Amygdalina* that was done previously. Thus, these studies evaluate the ability of possible anticancer activity of the various extracts and fractions of *V. Amygdalina* as anti-carcinogenic agent. As it has been reported the different antioxidant compounds exhibit differential scavenging activity on various reactive oxygen species (Wang and Jiao, 2000) and the reaction with hydroxyl radicals is non-specific, while reaction with other radicals is more specific (Singh et al., 2009). Because the elimination of cancer in the early stages is an integral part of chemoprevention, measuring anti-proliferative properties against cancer cells using common assay, such as the MTT assay, provide useful insight on the chemo-protective potential of natural extracts.

## MATERIALS AND METHODS

### Extraction Procedure

The method of Zakaria et al., (2007, 2010) with slightly modification was used. The leaves of *V. Amygdalina* will be collected from their natural habitat around the states of Terengganu, Malaysia. The leaves will be air-dried under shade at room temperature for 24-hour followed by the sequential soaking process (1:20; w/v) using methanol, chloroform and ethyl acetate for 24-hour at room temperature (24-25°C). The supernatants for each solvent were filtered through Whatman® No. 41 filter paper (pore size 20-25 µm) and was then concentrated under reduced pressure at 40°C. Finally, all the extracts were stored at -20°C until they were used for the analysis.

### Determination of Total Phenolic Compounds

Total phenolic content of leaves of *V. Amygdalina* extracts was determined using standard method of Folin-ciocalteu reagent introduced by Kahkonen et al. (1999) and I. Gulcin et al., (2004) with slightly modification using gallic acid as a standard phenolic compound. A total of 100 µL of extract solution (0.10 – 0.50 mg/mL) was added in test tube than 0.75 mL of Folin-ciocalteu reagent and left the mixture under room temperature for 5 minutes. After 5 minutes 0.75 mL sodium carbonate (60 g/L) was added and the mixture was allowed to stand at room temperature for 90 minutes. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Secoman, France). The concentration of total phenolic compounds in the extracts determined as microgram of gallic acid equivalent by using equation that was obtained from standard gallic acid graph.

### Antioxidant Assay

The antioxidant activities of *V. Amygdalina* leaf extracts were measured using ferric thiocyanate (FTC) and thiobarbituric acid (TBA). The FTC method was used to measure the amount of peroxide at the beginning of peroxidation while TBA method was used to measure free radicals present after peroxide oxidation.

### Ferric Thiocyanate(FTC) Method

The standard methods proposed by Kikuzaki and Nakatani (1993) with slightly modification was use for this study. A mixture of 4.0 mg of sample extract in 4.0 mL of absolute ethanol, 4.1 mL of 2.52% linolenic acid in absolute ethanol, 8.0 mL of 0.05 M phosphate buffer (pH 7.0), and 3.9 mL of distilled water was placed in test tube with a screw cap and then placed in dark oven at 40°C. To 0.1 mL of this solution were added 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely 3 minutes after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 532 nm every 24 hours until one day after the absorbance of the control reached its maximum. BHA, BHT and  $\alpha$ -tocopherol were used as positive controls and mixture without sample extract was used as the negative control.

### Thiobarbituric Acid (TBA) Method

The combination method Z.M. Zinet al., (2002) and Kikuzaki and Nakatani (1993) with slightly modification were followed. Approximately 1 mL of sample solution from FTC method was added with 2.0 mL of 20% trichloroacetic acid (TCA) and 2.0 mL of 0.67% thiobarbituric acid (TBA) in the test tube. The mixture was placed in water bath (95°C) for 10 minutes. After cooling the mixture was centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance on the final day of the FTC method. BHA, BHT and  $\alpha$ -tocopherol were used as positive controls and mixture without sample extract was used as the negative control.

### Free Radical Scavenging Activity

The free radical scavenging activity of the sample extract was measured in accordance to the standard method Shimada et al., (1992) with slightly modification. A total of 10 mg extracts were dissolved in 1.0 mL methanol and the solution added to a 1.0 mL DPPH solution at room temperature. The absorbance at 517 nm was measured utilizing UV-1601 Shimadzu spectrophotometer. The results were expressed as percentage of reduction of the initial DPPH absorption by test samples as follows:

$$\text{DPPH scavenging effect (\%)} = 100 - [(A_0 - A_1/A_0) \times 100]$$

Where  $A_0$  was the absorbance of the control reaction and  $A_1$  was the absorbance in the presence of the sample.

#### Anti-cancer study (MTT Assay)

All cell line cultures of the American Type Culture Collection (ATCC), namely MCF-7 (estrogen-dependent human breast adenocarcinoma) and HT-29 (human colon cancer) were used in the study. Cells were cultured in Roswell Park Memorial Institute 1640 supplemented with 10% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin using 25-cm<sup>2</sup> flasks, in 5% CO<sub>2</sub> incubator at 37°C. The viability of cells was determined with trypan blue reagent. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of  $1 \times 10^5$  cells/ml was prepared and was plated (100 µl/well) onto 96-well plates (NUNCTM, Denmark). Prior to addition of cells onto the plates, the stock solution was diluted with media, transferred onto the wells and sequentially added with cells in media to achieve the required starting concentration of 100 µg/ml in 1% DMSO. The 100 µg/ml extract in each well was serially diluted to achieve the concentration range of 100-12.5 µg/ml. The proliferative activity was determined using the MTT assay (3-[4, 5 - dimethylthiazol - 2-yl]-2,5-diphenyl tetrazolium bromide) (Abdullah Saniet al. 2004). The incubation period used was 72 hours. After solubilization of the purple formazan crystals was completed, the spectrophotometrical absorbance of the mammalian cell extract was measured using an ELISA reader at a wavelength of 550 nm. The cytotoxicity was recorded as the drug concentration causing 50% growth inhibition of the tumour cells (IC<sub>50</sub> value) using the formula given below:

$$\% \text{ cell viability} = \frac{\text{OD sample (mean)}}{\text{OD control (mean)}} \times 100\%$$

After the determination of the percentage of cytotoxicity, graphs were plotted against its respective concentrations. In all experiments, the antiproliferative assay was repeated three times with tamoxifen as the standard anti-tumour drug.

## RESULTS AND DISCUSSION

### Determination of Total Phenolic Compounds

Plant phenolics are the widest spread secondary metabolite in plant kingdom. These compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. Therefore, it is worthwhile to determine the total amount of phenolic content in the plant chosen for the study. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. However, this reagent does not measure the total phenols only; it also will react with any reducing substance. Perhaps there were other components that can react with the reagent such as ascorbic acid (Shahidi and Naczki, 1995). Besides, various phenolic compounds have different response to this assay (Singleton and Rossi, 1965). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. However, the measurement of colour changes after two hours storage could be used to determine the existence of phenol in samples. This may be due to the antioxidant properties of plant extract that react as reductant agent which is known as redox action.

**Table 1: Total phenolic content in ethyl acetate, chloroform, methanol and  $\alpha$ -tocopherol extracts of *V. Amygdalina*.**

Concentration (mg/mL)	Total phenolic content (mg/g) GAE			
	Ethyl acetate	Chloroform	Methanol	$\alpha$ -tocopherol
0.1	1.59	0.11	0.01	0.66
0.2	1.63	0.42	0.04	1.25
0.3	2.03	1.34	0.66	2.69
0.4	2.32	1.94	1.61	3.26
<b>0.5</b>	<b>2.69</b>	<b>2.25</b>	<b>2.05</b>	<b>4.28</b>

Javanmardiet al. (2003) stated that antioxidant activity of plant extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites, such as volatile oils, carotenoids, and vitamins, among others. The total phenolic content is expressed in gallic acid equivalence (GAE) with  $\alpha$ -tocopherol as positive control. Higher phenolic content was observed in higher extract concentration. At concentration 0.1 mg/mL all extracts show lowest phenolic content, whereby the highest phenolic content is observed in ethyl acetate extract, 1.59 mg/g and the lowest is in methanol extract, 0.01 mg/g. As the concentration of the extract increases, the phenolic content also increases. The highest phenolic content is recorded in 0.5 mg/mL concentration with  $\alpha$ -tocopherol extract shows



highest reading (4.28 mg/g), followed by ethyl acetate (2.69 mg/g), chloroform (2.25 mg/g) and the lowest is methanol extract (2.05 mg/g). The total phenolic contents of *V. Amygdalina* are shown in Table 1.

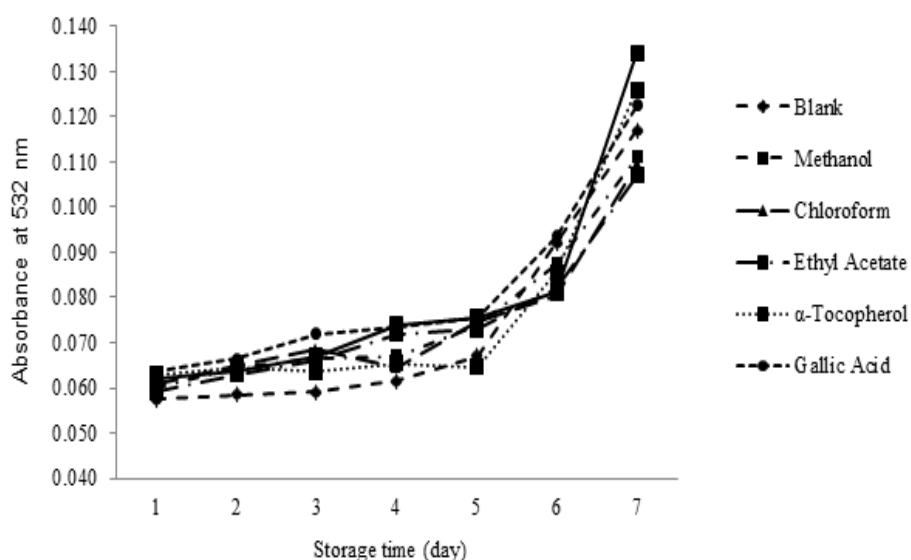
Based on the analysis, the phenolic content are increased by concentration of the solution. At concentration 0.5 mg/mL, all extract shows maximum amount of phenolic content, including  $\alpha$ -tocopherol. This indicates that at higher concentration, more phenols needed to inhibit the oxidation of Folin-Ciocalteu reagent. Total phenolic content of ethyl acetate extract (control) shows best value compared to chloroform and methanol extract. Methanol shows the least total phenolic content when using this Folin-Ciocalteu method. The total phenolic content increases with the concentration. Using this method, the *V. Amygdalina* extract in ethyl acetate displayed highest phenolic content; 2.693 mg/g, followed by chloroform extract and methanol extract.

### Ferric Thiocyanate Method

Antioxidant activity of crude extracts is measured using FTC and TBA methods. The ferric thiocyanate (FTC) method measures the amount of peroxide produced during the initial stages of lipid oxidation, in which peroxide reacts with ferrous chloride and form ferric ions. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate. This substance is red in colour. The darker the colour, the higher will be the absorbance (Huda-Faujanet al., 2009).

From the FTC analysis, it was found that the antioxidant activity of *V. Amygdalina*. It shows that all samples of crude extract had been oxidized when stored for seven days at 40-45°C. *V. Amygdalina* crude extract showed the lowest absorbance at the first day. Blank showed the lowest absorbance values at 0.0575, followed by ethyl acetate with absorbance at 0.0590, methanol (0.6000), chloroform (0.0610), ascorbic acid (0.0620),  $\alpha$ -tocopherol (0.0630) and lastly gallic acid (0.0635). Absorbance of each samples increase progressively by time of incubation. Lower absorbance values indicate higher antioxidant activities. Antioxidant activities are higher during the initial of the experiment than the end of the experiment.





**Figure 1: Antioxidant properties of crude extract *V. Amygdalina* determined with the FTC method**

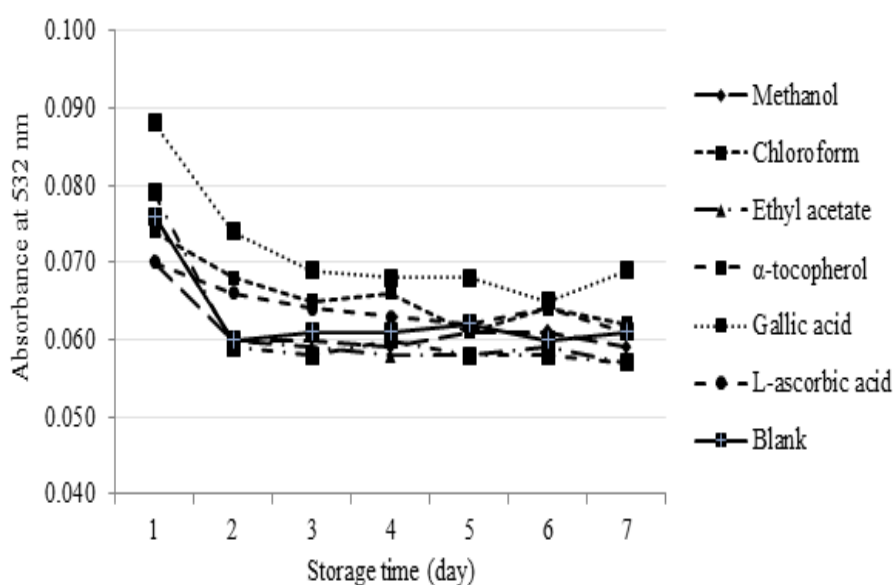
At the end of the experiment, all samples showed higher absorbance values than the day 1. Ethyl acetate extract showed the lowest absorbance value with 0.107, followed by chloroform (0.110), methanol (0.111), and blank (0.117). Compared to standards, gallic acid displays the highest absorbance (0.123), followed by ascorbic acid (0.094), and  $\alpha$ -tocopherol (0.092). Higher absorbance values showed lower antioxidant activities. Progressing days causes the antioxidant to reduce and this might be due to exposure to light. As antioxidant is easily damaged by light, it is crucial to ensure every procedure being carried out in a dark area. The percentage of inhibition in ascending order is ethyl acetate, followed by methanol and lastly chloroform. A higher percentage of inhibition, the higher antioxidant activity was recorded. After three days of storage, crude extract exhibited good effect in inhibiting linoleic acid oxidation from previous days. The percentage of inhibition increases from day 1 to day 3 and the subsequent measurements are in a scattered pattern with some higher and some lower. This might be probably due to handling error as incubation only proceeds up to third days

Figure 1 also depicts that the antioxidant activities also increased with the increasing concentration of *V. Amygdalina* crude extract. It is clearly shown that there is a positive correlation between total phenolic content and the antioxidant activities. The phenolic compounds may donate hydrogen and terminate the free radical reaction chain by changing it to the stable compounds (Amarowicz et al., 2000). None of the crude extract of *V. Amygdalina* showed absorbance values greater than the blank, the one without crude extracts

at the initial and end point of FTC method. This clearly indicates the presence of antioxidant activity in the blank. Ethyl acetate shows the strongest antioxidant activity compared to methanol and chloroform.

### Thiobarbituric Acid Method

FTC is used to measure the production of peroxide compound at the initial stage of oxidation while TBA test is used to measure the secondary product of are gradually decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by the TBA method (MohdZinet al., 2002). The TBA analyses of *V. Amygdalina* extract in seven days are shown in Figure 2. Mostly the absorbance readings are decrease by day until day seven, except for chloroform and gallic acid. Chloroform absorbance reduced from day one until day five, but increase dramatically at day six before undergo reduction at day seven. In contrast, gallic acid shows increasing absorbance from the first day until day five, before slightly reduced at day six. At day seven, all extract shows reduction in absorbance reading. The value is in range 0.057-0.065. Low absorbance correlate to high antioxidant activity.

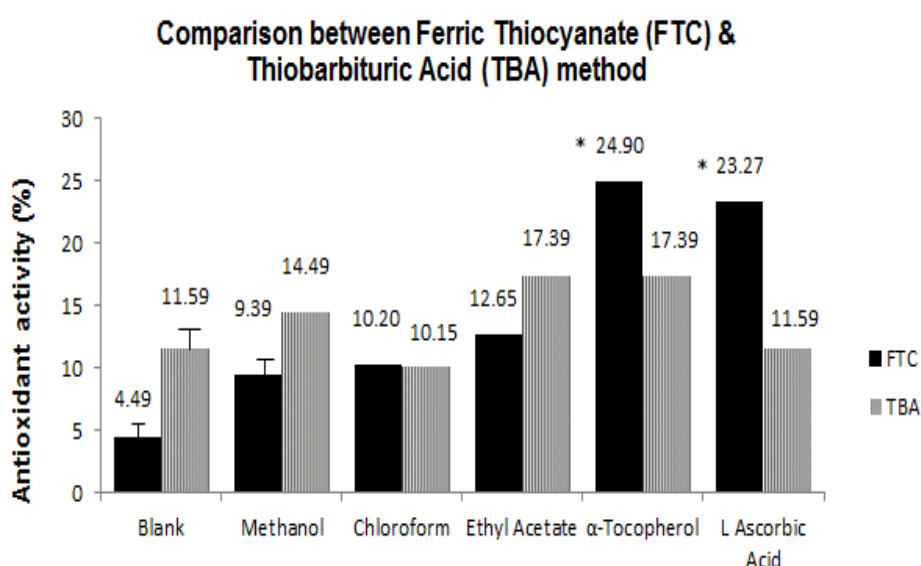


**Figure 2. Absorbance of antioxidant activities of *V. Amygdalina* as measured by the TBA method**

There are differences of the antioxidant activities trend between TCA and TBA method. TCA method shows increasing value of absorbance, while TBA shows increasing value on day two and falls prior to day seven. The total antioxidant activity of the FTC method is lower than the TBA method on day seven. Using FTC method, the ethyl acetate extract from the

vegetable of *Vignasinensis* displayed highest antioxidant activity followed by methanol extract and chloroform extract. However, using TBA ethyl acetate comes with the highest antioxidant activity as in FTC, followed by methanol and chloroform extract as least antioxidant activity. The results are inversely correlated between FTC and TBA.

From Figure 3, antioxidant activities of samples' extract from TBA method are higher than that of FTC method. In contrast, the antioxidant activities of standards ( $\alpha$ -tocopherol and ascorbic acid) seen to be higher from FTC compared to TBA method. This may indicate that the amount of peroxide in the initial stage of lipid peroxidation is greater than the amount of peroxide in the secondary stage as reported by Rahmatet al., (2003).



**Figure 3: A comparison between antioxidant activities of five extracts using ferric thiocyanate (FTC) & thiobarbituric acid (TBA) methods with  $\alpha$ -tocopherol and ascorbic acid as standards. Asterisk (\*) indicates significant difference at  $p < 0.05$**

However, the result negatively correlated to samples' extract. This also shows that the antioxidant activity detected with the FTC method was lower than that detected with the TBA method for samples' extract. It is highly possible that several compounds of different polarity may contribute to the antioxidative activity of *V. Amygdalina* extract (MohdZinet al., 2002). In addition, antioxidative activities observed in these plants could be the synergistic effect of more than two compounds that may present in the plant. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu and Foo, 1995).

### Anti-Cancer Study (MTT Assay)

The cytotoxic activity extract from methanol, ethyl acetate and chloroform extracts of *V. Amygdalina* in inhibiting the growth of human colon adenocarcinoma cell line (HT-29) and human breast adenocarcinoma cell line (MCF-7) were determined by using MTT assay. MTT is reduced from an insoluble purple formazan by mitochondrial dehydrogenase activity of viable tumor cells, into an insoluble colored formazan product, which can be measured spectrophotometrically after dissolution. Various cells were used to determine the relationship between the number of cells and amount of MTT formazan generated and between the amount of MTT formazan generated and the duration of cell incubation with MTT (Mossmann, 1983). Cell proliferative activities were measured by comparing the purple colour formations. Dead cells, on the other hand, do not form the purple formazan due to their lack of the enzyme (Wu et al. 2006). Under the experimental conditions of this study, the enzyme activity and number of formazan formed were proportional to the number of cells. Reduction in the number of cells by a particular agent (cytotoxicity) can generally be explained by cell killing and/or inhibition of cell proliferation. The  $IC_{50}$  value (the drug concentration caused 50% inhibition of tumor cells) was used as a parameter for cytotoxicity (Smit et al. 1995). According to Wall et al. (1987) any plant or food extract which has a  $IC_{50}$  value of below than 20  $\mu\text{g/mL}$  can be accepted as a potent cytotoxic extract.

The cytotoxicity activity of the methanol extracts of *V. Amygdalina* on the growth of various cancer (MCF-7 and HT29) in vitro was shown in Table 2. According to the National Cancer Institute standards, criteria crude extracts possessing an  $IC_{50}$  value less than 20  $\mu\text{g/mL}$  were considered active against the tested cancer cells (Chen et al. 1999; Geran et al. 1972). In this study, methanol, chloroform and ethyl acetate extracts *V. Amygdalina* of were evaluated for their cytotoxicity toward several cell lines. The degree of cytotoxicity was defined as a concentration that reduced the cell number to 50% as compared to the untreated ( $IC_{50}$ ). The  $IC_{50}$  value was determined quantitatively after staining the cells with crystal violet and quantitatively by using the MTT assay.

**Table 2: Cytotoxic effects of MCF-7 and HT29 cell lines by methanol, chloroform and ethyl acetate extracts of *V. Amygdalina*.**

<b><math>IC_{50}</math> Values (<math>\mu\text{g/mL}</math>)</b>		
<b>Extracts</b>	<b>MCF-7</b>	<b>HT-29</b>
Methanol	$2.50 \pm 0.02$	$1.20 \pm 0.10$
Chloroform	$5.80 \pm 0.08$	$2.20 \pm 0.35$
Ethyl Acetate	$8.50 \pm 0.01$	$4.60 \pm 0.50$

The cytotoxicity of methanol, chloroform and ethyl acetate extracts of *V. Amygdalina* against several different normal and cancerous cell lines range 2.50 to 1.20 µg/mL, 5.80 to 2.20 µg/mL and 8.50 to 4.60 µg/mL respectively (Table 2). HT-29 was found to be the most sensitive cell line towards *V. Amygdalina* methanol extract with the IC<sub>50</sub> value of 1.20 µg/mL followed by MCF-7 (2.50 µg/mL). The *V. Amygdalina* chloroform and ethyl acetate extracts also showed strong cytotoxic activity to HT-29 cell line with the IC<sub>50</sub> value of 2.20 and 4.60 µg/mL and followed by MCF-7 (4.60; 8.50 µg/mL).

In this study, *V. Amygdalina* extracts was shown to be cytotoxic towards various cancer (MCF-7 and HT29) cell lines and these findings were parallel with those obtained by Lim et al. 2007 and Mahattanataweeet al. 2006. The existence of methoxyl group (-OCH<sub>3</sub>) at C-1 position of red pitaya flesh extract was expected to show higher efficiencies for generation of singlet oxygen (<sup>1</sup>O<sub>2</sub>) and superoxide anion radical (O<sub>2</sub><sup>-</sup>) that supporting the capability of *V. Amygdalina* to be more toxic towards HT-29 and MCF-7 cell lines.

Besides that, *V. Amygdalina* extract also possesses high ability to generate reactive oxygen species (Rajendranet al. 2004) that makes red pitaya flesh extract capable to neutralize the toxicity that caused protein peroxidation, lipid peroxidation and DNA damage (Flora and Fergusen, 2005). From this study, we can conclude that *V. Amygdalina* extracts has a good potential to be used as a new cancer therapeutic agent.

## CONCLUSION AND SUGGESTION

The results expressed in this study are the first information on the antioxidant activities of *V. Amygdalina*. Total phenolic contents are influenced by the concentration of extract. Among all the fractions, the ethyl acetate fractions exhibited the highest total phenolic content comparing to methanol and chloroform fraction. The antioxidant capacity of *V. Amygdalina* measured by FTC and TBA method, the ethyl acetate extract from the vegetable of *V. Amygdalina* displayed highest antioxidant activity followed by methanol extract and chloroform extract. In this study, *V. Amygdalina* extracts was shown to be cytotoxic towards various cancer (MCF-7 and HT29) cell lines and these findings were parallel with those obtained by Lim et al. 2007 and Mahattanataweeet al. 2006. The existence of methoxyl group (-OCH<sub>3</sub>) at C-1 position of red pitaya flesh extract was expected to show higher efficiencies for generation of singlet oxygen (<sup>1</sup>O<sub>2</sub>) and superoxide anion radical (O<sub>2</sub><sup>-</sup>) that supporting the capability of *V. Amygdalina* to be more toxic towards HT-29 and MCF-7 cell lines. From this study, it is suggested that *V. Amygdalina* content high in phenolic compound and antioxidant

capacity in ethyl acetate extracts as compared to methanol and chloroform. extracts was shown to be cytotoxic towards various cancer (MCF-7 and HT29) cell lines and these findings were parallel with those obtained by Lim et al. 2007 and Mahattanataweeet al. 2006. We can conclude that *V. Amygdalina* extracts has a good potential to be used as a new cancer therapeutic agent.

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