5.0 DISCUSSION

5.1 Cytotoxicity activity of *A. auricula-judae*

Cells undergo death by two major mechanisms: necrosis, in which primary damage to the metabolic or membrane integrity of the cell occurs, or apoptosis, which is the process of programmed cell death through a tightly controlled program that plays important roles in many normal processes, ranging from fetal development to adult homeostasis (Reed *et al.,* 2001). During the past decade, the evidences gradually accumulated that many cancer chemotherapeutic agents kill the cancer cells by inducing apoptosis (Ren *et al.,* 2008). Thus, some mushrooms, which possess bioactive compounds to induce apoptosis in the cancer cells, were known to act as valuable source for novel chemotherapeutic reagents. According to that, much efforts has been directed towards searching for more compounds from mushrooms that influences cancer cells apoptosis (Reed *et al.,* 2001).

In this study, ethanol, methanol, dichloromethane, hot aqueous and polysaccharides extracts of *A. auricula-judae* were prepared and evaluated for their potential against antioxidant, cytotoxicity and anti HPV-16E6 activities. Firstly, these five extracts were chosen to evaluate inhibition activity against six cancer cells proliferation. The six cancer cells were namely human ovarian cancer cells (SKOV), human cervical cancer cells (CaSki), human breast cancer cells (MCF 7), human mouth epidermal cancer cells (KB), human intestinal colon cancer cells (HT 29) and human colon cancer cells (HCT 119). These cancer cells were chosen for this purpose, since they were easily subcultured, maintained and widely used because of their rapid reaction towards test agents (Leow, 2006). These cancer cells also were to have high proliferative activity (Leow, 2006). Initially, all the *A. auricula-judae* extract were tested at 20 μg/ml against all six cancer cells. Twenty microgram per milliliter concentration was used as a prescreening

concentration for the neutral red cytotoxicity test. If the inhibition percentage is more than 50% (ED_{50}) at the concentration below 20 µg/ml and then the mushroom extract was considered actively cytotoxic. ED_{50} values refer to the effective concentrations of extracts (µg/ml) that reduced the proliferation of cultures by 50%. Extracts found to be cytotoxically active in the prescreening test, should be retested with varying concentrations from each extract (Borenfreund and Puerner, 1986). However, mushroom extracts which show less than 50% inhibition at 20 µg/ml, were considered to be weak anticancer agent and no further test was required (Geran *et al.,* 1977).

NR assay was used in this study because it is most sensitive cytotoxicity assay showing statistically significant differences between the treated cells and the controls (George *et al.,* 2006). It is also very sensitive in detecting early toxicity. The assay is very useful as cytotoxicity indicator in cultures of primary hepatocytes and other cell lines (Fautz *et al.,* 1991; Morgan *et al.,* 1991). In this study, all the *A. auricula-judae* extracts produce inhibition percentage of less than 50% at 20 µg/ml indicating that they were not cytotoxically active against six cancer cells. This suggests that the evaluated extracts of *A. auricula-judae* did not contain bioactive compounds, which promote the cytotoxicity activity towards cancer cells. This finding in agreement with that of Chang *et al.,* (2004) who studied *A. auricula-judae* antiproliferative activity towards HepG2, human hepatoma cells. In this study, the Hep G2 cells treated with 100 µg/ml of *A. auricula-judae* for 48 hours. Based on this study, the inhibition percentage of the HepG2 cell growth is less than 50% (42%) which further suggesting that *A. auricula-judae* not active cytotoxically towards cancer cells (Chang *et al.,* 2004). Result also showed that these extracts were not cytotoxically active against normal lung epithelial cells (MRC5).

Although not considered cytotoxically active, the dichloromethane and hot aqueous extract from *A. auricula-judae* showed relatively higher inhibition percentages towards

SKOV, CaSki, and KB cancer cells as compared to the methanol, ethanol and polysaccharides extracts. The relative differences in initiation capacities are due to the presence of different compounds in the extracts. Dichloromethane solvent has been known to extract out non-polar compounds such as alkaloids, aglycones and volatile oils from mushrooms (Houghton and Raman, 1998). On the other hand, the hot aqueous extract has been known to contain polar compounds such as sugars, aminoacids and glycosides (Houghton and Raman, 1998).

In addition, numerous studies showed polysaccharides from mushrooms demonstrated antiproliferative activity towards cancer cells. *Pleurotus ostreatus* polysaccharide especially the high- molecular – weight ß- D- glucan preferentially exerts its antiproliferative effects towards HT-29 colon cancer cells and is significantly less effective towards normal human fibroblast (Lavi *et al.,* 2006). Similar to this study, *A. auricula-judae* also possess ultimately less inhibition percentage towards MRC5 normal cells. Not exceptional, the crude ethanol extract of *Pleurotus sajor-caju* possessed the strongest cytotoxicity with an ED_{50} value at 24.0 µg/ml towards human cervical epidermoid CaSki carcinoma cells (Sujatha*.,* 2005). This is followed by crude ethanol extract of *Pleurotus cystidiosus* with an ED_{50} value at 50.0 µg/ml towards HeLa cells (Sujatha*.,* 2005). At the same time, crude water extract of *Pleurotus hungarian* had an ED_{50} value at 63.5 µg/ml and 77.0 µg/ml towards HT29 cells and CaSki cells, respectively (Sujatha*.,* 2005). In another study, findings showed that polysaccharides extracted from *Trametes versicolor* significantly reduced proliferation of breast cancer cells by increasing p21 expression and decreasing cell-cycle protein cyclin D1 expression (Chow *et al.,* 2003).

Based on this study, it was clearly shown that ethanol, methanol, dichloromethane, hot aqueous and polysaccharides from *A. auricula-judae* were not active cytotoxically.

Briefly, anticancer mushrooms possess several types of steroids, terpenoides and nucleotides, which inhibited the growth of cancer cells (Sujatha*.,* 2005). However, in this study, *A. auricula-judae* is lack of compounds, which are cytotoxic. Further studies should be carried out to evaluate other bioactivities of *A. auricula-judae.*

5.2 Anti HPV-16E6 oncoprotein activity

The CaSki (cervical cancer) cell line contains HPV-16 DNA. The E6 oncogene of the HPV-16 is regularly expressed in cervical tumors and in the derived cell lines. The E6 oncoprotein via ubiquitin pathway specifically interacts with the p53 protein and directs its degradation (Scheffner *et al.*, 1990; Hoppe-Seyler and Butz, 1993). The persistent expression of E6 oncoprotein will interfere with host regulatory proteins and lead the host cell into a proliferate state required for malignant transformation (Arends *et al.,* 1998; Hall and Alexander, 2003). Therefore, screening for naturally occurring compounds with HPV-16E6 oncoprotein suppressing effect is crucial to take precautions to reduce the incidence of HPV-16E6 associated cervical cancer.

In this study, ethanol, methanol, dichloromethane, hot aqueous and polysaccharide extracts from *A. auricula-judae* were evaluated for anti HPV-16E6 activity. The two-step staining method with indirect (Horse Radish Peroxidase) HRP-DAB (diaminobenzidinetrahydrochloride) immunoperoxidase immunocytochemistry technique was applied. HRP can be attached to other proteins (DAB) either covalently or non covalently which can be performed using either one step or two step procedures. HRP oxidizes DAB to produce a brown end product that is highly insoluble in alcohol and other organic solvents (Mason *et al.,* 1981). Oxidation of DAB also causes polymerization resulting in the ability in increasing its staining intensity and electron density (Newman *et*

al., 1983). This test type was chosen because immunocytochemistry method easily done with the aid of several chemicals and it is able to produce consistent, sensitive and reliable results. During the test, monoclonal antibody HPV-16E6 diluted with sterile water in a ratio of 1:50. This correct dilution is very essential, as it will contribute to the quality of staining if they are prepared accurately and consistently. Dilution ranges must be compatible with the incubation time and temperature. In this study, the antibody incubated for an hour and thirty minutes in room temperature 32.7˚C. The increase in incubation time needs low antibody dilution titer (1:50) to obtain the optimal staining results. The low titer antibody must be incubated for longer time to reach equilibrium. Generally, equilibrium not reached during primary antibody incubations of less than 20 minutes. Consistent timing of this step is important. Inconsistent incubation time, can cause variations in overall stain quality. In a study by, Leow, (2006) used an hour for the HPV-18E6 monoclonal antibody titer (1:50), incubation at room temperature.

Untreated CaSki cells, incubated with anti HPV-16E6 monoclonal antibody, was used as the negative control for the test. This control slides incubated with the antibody showed very dark reddish brown stain. Simultaneously confirming the presence of HPV16 and abundant expression of the E6 oncoprotein. On the other hand, the negative control, which is not incubated with monoclonal antibody, showed no presence of reddish brown stain. This is supported by the principle of immunocytochemistry staining which states that the absence of specific primary antibody caused failure in detecting viral antigens (E6 oncoprotein) and hence no complex (reddish brown precipitate) being formed at the end of the staining process. The negative control without anti HPV16-E6 monoclonal antibody was used as an internal control in order to ensure the sensitivity of the immunocytochemistry staining method (Leow, 2006).

In the present study, the CaSki cells were treated with *A. auricula-judae* extracts at two concentrations; 25µg/ml and 100µg/ml. The appearance of reddish brown stain indicates the presence of HPV-16E6 oncoprotein in CaSki cells. The intensity of reddish brown stain directly correlated with the expression of HPV-16 E6 oncoprotein.

5.2.1 Anti HPV-16E6 oncoprotein activity of the *A. auricula-judae*

In this study, all the extracts especially hot aqueous from *A. auricula-judae* fresh fruitbodies revealed the strongest inhibition activity against HPV-16E6 oncoprotein in CaSki cells, increased with the increment in concentration of the mushrooms extracts. Based on the observation of reddish brown staining intensities, ethanol, methanol, dichloromethane and polysaccharides shows very strong (+4) and strong (+3) stages. This means their suppression level for anti HPV-16E6 in a very weak to weak level. However the hot aqueous extract showed staining and suppression in the moderate level. At the higher concentration, 100 µg/ml, hot aqueous extract showed very high suppression for HPV-16E6 activity. In other words, hot aqueous shows very good and better suppression from the rest. Thus it means that hot aqeous extract contains bioactive compounds which plays the antitumor role.

Other investigators have successfully experimented the expression of high risk HPV E6 oncoprotein. Leow (2006) evaluated plant extracts obtained from, *Zingiberaceae methanolic* extracts against Hela cells; the *Zingiber officianale*, *Zingiber montanum*, *Curcuma domestica*, *Curcuma mangga*, *Curcuma xanthorrhiza* and *Curcuma aeruginosa* found that remarkable suppressing effect against the E6 oncoprotein at 25 µg/ml, which is similar with hot aqueous extract of *A. auricula-judae*. Thus, this result indicates that, *A. auricula-judae* hot aqueous extract as good as that of *Zingiberaceae* methanolic extracts.

Thus, based on the overall results, especially hot aqueous extract from *A. auricula-judae* showed greater suppressing effect on the expression of HPV-16E6 oncoprotein in CaSki, cervical cancer cells. However, the difference of polarity among the ethanol, methanol, and dichloromethane solvents permits different group of bioactive compounds to be extracted from mushroom sample. Therefore, it would be beneficial if further investigation can be carry out to isolate and identify the specific active compounds which display high suppression effect against HPV-16E6 oncoprotein from these extracts.

5.3 Antioxidant capacity of *A. auricula-judae* extract on 1,1- diphenyl – 2- picry lhydrazyl (DPPH) free radicals

Generally, there are five types of antioxidant compounds, which can be categorized into five groups: 1) primary antioxidant compounds which mainly are phenolic substances, that terminate the free radical chain in lipid oxidation and function as electron donors, 2) oxygen scavengers compound that react with oxygen, and can thus remove it in a closed system, 3) secondary antioxidants compounds which function by decomposing the lipid hydroperoxides into stable end products, 4) enzymic antioxidants which function by removing dissolved/headspace O_2 or by removing highly oxidative species and 5) chelating agents which chelate metallic ions that promote lipid oxidation through a catalytic action (Kochhar and Rossell, 1990).

In this study, the capability of *A. auricula-judae* fresh fruitbodies extracts as a primary antioxidant were assessed by using DPPH scavenging activity. The scavenging activity of mushroom extracts was tested using methanol solution of the “stable” free radical, DPPH. Compared with other laboratory-generated free radicals such as the hydroxyl and superoxide anion, DPPH has the advantage of being unaffected by certain

side reactions, such as metal ion chelation and enzyme inhibition (Amarowicz *et al.,* 2004). The scavenging effect of DPPH radicals measured at 517 nm. The purple colour of freshly made DPPH solution, generally fades when antioxidant is present in the medium. Hence, the antioxidant molecules can scavenge DPPH radical (by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless / bleached product (i.e; 2, 2- diphenyl - 1 – hydrazine), resulting in a decrease in absorbance at 517 nm. Thus, the more rapidly absorbances decreases, the more significant the antioxidant activity of extract. This test also known as antioxidant activity, which commonly employed assay in antioxidant studies of specific compounds of extracts across a short time scale (Ferreira *et al.,* 2007).

In this study, extraction of chemical constituents in the fresh fruitbodies into antioxidants was very effective in hot aqueous extract of fresh *A. auricula-judae* with lowest EC_{50} value obtained at 87.66 mg/ml. This can be compared with the study by Kho *et al.,* (2009) which EC_{50} value obtained at 2.87 mg/ml for freeze dried fruitbodies of *A. auricula-judae*. The fresh fruitbodies of *A. auricula-judae* showed EC_{50} value at the higher concentration than the freeze dried fruitbodies of *A. auricula-judea,* which suggests that processing method of the *A. auricula-judae* effects its antioxidant capacity. In addition, EC_{50} value of other mushroom such as hot aqueous extract of *Boletus edulis* (22.2 mg/ml) and *Agaricus blazei* (7.6 mg/ml) much higher than *A. auricula-judae* hot aqueous extract (Tsai *et al.,* 2007). In another study by Lee and coworkers (2006), the hot water extract from *Hypsizigus marmoreus* achieved EC_{50} value which range from (13 -14) mg/ml.

When compared with other *Auricularia* sp specialty mushroom, the methanolic extract of *A. auricula-judae* with EC_{50} value of 94.07 mg/ml showed moderate antioxidant activity. This is because, methanol extracts of both *A. mesenterica* and *A. polytricha* showed an outstanding scavenging effect of 100% at 1.0 mg/ml, whereas *A. fuscosuccinea*

(brown strain) showed a scavenging effect of 95.4% at 3.0 mg/ml (Mau *et al.,* 2001). In addition, the methanolic extracts from *A. auricula-judae* possess EC_{50} value of 4.20 mg/ml which is much higher than the EC_{50} value obtained in this study (Chao *et al.*, 2001).

EC_{50} value of ethanolic extracts of *Agaricus blazei*, *Agrocybe cylindraceae* and *Boletus edulis* were 4.3, 4.6 and 4.7 mg/ml respectively (Tsai *et al*., 2007). In addition, the ethanolic extracts from *Hypsizigus marmoreus* (white mutant) was effective in antioxidant properties as evidenced by the EC_{50} value less than 10 mg/ml (Lee *et al.,*2008). The polysaccharides from *A. auricula-judae* gave EC_{50} value at a concentration of 129.07 mg/ml, which is higher concentration compared to the other extracts (Fig 4.10), indicating that polysaccharides was not a good radical scavenger. In accordance with other study, by Fan and coworkers (2006), the polysaccharides that derived from *A. auricula-judae* showed EC_{50} value at 100 mg/ml concentration.

Positive correlation between scavenging ability on DPPH radical and total phenolic content in extracts indicated that, moderate scavenging ability on DPPH radical would be due to phenolic compounds in extract of *A. auricula-judae* (Figure 4.12). Yen and coworkers (1993) also reported that significant correlation found between antioxidant activity with its total phenolic content of peanut hulls methanolic extract on DPPH radical. However, a study by Yu and coworkers (2002), found no correlation between scavenging activity and the total phenolic content in wheat extracts.

Therefore, extracts of *A. auricula-judae* were moderate free radicals inhibitors, acting possibly as primary antioxidants. The extracts from *A. auricula-judae*, particularly the peroxy radicals, which are the major propagator of the autooxidation chain of fat, thereby terminating the chain reaction (Gordon, 1990; Frankel, 1991; Shahidi & Wanasundara, 1992). Antioxidant activities of natural antioxidant have shown to be involved in termination of free radicals reaction and reducing power.

5.4 Ferric reducing antioxidant power (FRAP) of *A. auricula-judae* extracts

FRAP assay provides a reliable method to study the antioxidant activity of various compounds (Benzie & Strain, 1996). In this assay, the yellow colour of the test solution, which contained various concentrations of crude extracts with 0.2M phosphate buffer pH 6.6, 1% (w/v) solution of potassium ferricyanide, 10% (w/v) of trichloroacetic acid solution and 0.1% (w/v) solution of ferric chloride, changes to various shades of green and blue depending upon the reducing power of each extract (Oyaizu, 1986). The presence of reductants in different (i.e antioxidant) extracts causes the reduction of Fe^{3+} / ferricyanide complex to the ferrous form, which is subsequently measured at 700 nm (Oyaizu, 1986). Initially, this assay was designed to determine the antioxidant activity of plasma, it was also applied to other substrates such as tea (Benzie & Strain, 1999; Benzie & Szeto, 1999), wine (Benzie & Strain, 1999; Benzie & Szeto, 1999), coffee (Gonzalez *et al.,* 2005) cocoa beans (Othman *et al.,* 2007), vegetable and fruit extracts (Pellegrini *et al.,* 2003) and others.

According to this study, the data shows the hot aqueous extract of *A. auricula-judae* fresh fruitbodies contributes towards excellent reducing power capacity of 1.66 at 5 mg/ml. However, this reducing power value can be compared with other mushrooms such as, hot water extract of *Hypsizigus marmoreus* (1.01 nm) at 10 mg/ml (Lee *et al.,* 2008), *Agaricus blazei* (0.83 nm), *Agrocybe cylindracea* (0.86 nm) and *Boletus edulis* (1.15 nm) at 5 mg/ml (Tsai *et al.,* 2007). Hence, extract of hot aqueous expressed the highest FRAP value in terms of absorbance at 5 mg/ml. However, the reducing power value of BHA and ascorbic acid (positive control of FRAP assay), was relatively more pronounced than *A. auricula-judae* fresh fruitbodies extracts with 2.539 nm, 1.932 nm at 5 mg/ml respectively.

When comparing the FRAP value of methanolic extract of *A. auricula-judae* with other mushrooms methanolic extract, FRAP value of methanolic extract (0.59 nm at 5 mg/ml) was comparable with methanolic extracts of *Pleurotus cystidiosus* (1.00 nm at 10 mg/ml), *Pleurotus florida* (1.19 nm at 10 mg/ml) (Lin, 1999), two strains of winter mushrooms (0.52 and 0.65 at 10 mg/ml), *Auricularia sp* (0.67~0.82 at 5 mg/ml) and *A. polytricha* (silver ear) (0.32 at 5 mg/ml) (Chao, 2001). Hence, the comparison shows that the methanolic extract of *A. auricula-judae* fresh fruitbodies possess higher reducing power.

With regard to ethanolic extracts, the reducing power of *A. auricula-judae* were 0.57 nm at 5 mg/ml, 1.21 nm at 20 mg/ml which can be compared with *P. citrinopiletus* (1.03 nm at 5 mg/ml) (Huang, 2003), *A. bisporus* (0.76), *P.eryngii* (0.75), *P. ferulae* (0.70), *P. ostreatus* (0.61) at 20 mg/ml respectively (Lo, 2005). Hence, it can be seen that the reducing power of *A. auricula-judae* methanolic extract was lower than *P. citrinopiletus* and higher than those of *A. bisporus*, *P. eryngii*, *P. ferulae* and *P. ostreatus*.

5.5 Total phenolic content of *A. auricula-judae*

Phenolics can be defined as one of the major groups of non-essential dietary components that have been associated with the inhibition of atherosclerosis and cancer (Teissedre *et al.,* 1996; Williams & Iatropuolus, 1997). Furthermore, based on their scavenging abilities on free radicals and chelating abilities on ferrous ions, phenols possessed good antioxidant, antimutagenic and anticancer properties (Lotito *et al.,* 1998; Ahmad *et al.,* 1999). Besides that, studies also revealed that the antioxidant properties also may be related to the antioxidant vitamins, phenolics acids and micronutrient present in the plant extract (Hazeena *et al.,* 2002). Since mushrooms also contains a wide variety of free

radical scavenging molecules, beta-carotene, flavonoides and polyphenols, it is important to consider the effect of the total phenolic content on the antioxidant activity of mushrooms extracts (Liu *et al.,* 1997; Mau *et al.,* 2002).

Hot aqueous extract from *A. auricula-judae* fresh fruitbodies exhibited the highest total phenolic content, 56.89 mg GAE/g extracts but lower than the positive control, BHA and ascorbic acid. This is because the BHA and ascorbic acid consist of high concentration of phenolics as it is known as effective antioxidants (Madhavi *et al.,* 1996). When compared with other hot aqueous mushrooms, such as *H. marmoreus* (white mutant) with (10.01-13.14 mg GAE/g hot extract) (Lee *et al.,* 2008), *H. marmoreus* (normal strain) 19.2 mg GAE / g hot extract) (Lee *et al.,* 2006), *Agaricus blazei* (5.67 mg GAE/g hot extract), *Agaricus cylindracea* (5.80 mg GAE / g hot aqueous) and *Boletus edulis* (5.81 mg GAE/g hot extract) (Tsai *et al.,* 2007), the hot extract from *A. auricula-judae* showed higher value of total phenolic content.

In the meantime, other fresh fruitbodies extract of *A. auricula-judae* (ethanol, methanol, dichloromethane, and polysaccharides), seems to show lower value in total phenolic content compared with hot aqueous extract, containing (0.923 - 1.9) mg GAE/g extract. *Auricularia auricula-judae* methanolic extract shows low phenolic content when compared with other methanolic mushrooms extracts such as *Dictyophora indusiata* (16.28 mg GAE/g extract), *Griofola frondola* (12.31 mg GAE/g extract), *Hericium erinaceus* (12.05 mg GAE/g extract) (Mau *et al.,* 2002). However, the methanolic extracts was comparable with total phenolic content of apple juice (0.749 mg GAE / ml) and freeze dried longan flesh (1.60 mg GAE/g extract) (Soong & Barlow, 2004). At the same time, the polysaccharide from *A. auricula-judae* showed very less total phenolic content with (0.923 mg GAE/g extract). This extract can be compared with the total phenolic content of tomato juice (0.256 mg of GAE/ml) (Schlesier *et al.,* 2002).

5.6 Comparison of antioxidant activities between extracts

The hot aqueous extract functions as a very prominent extract of *A. auricula-judae* fresh fruitbodies, in terms of exhibiting the highest scavenging effect on DPPH radicals, highest total phenolic content and highest FRAP value (per gram of extract). Methanol, ethanol and dichloromethane extract of fresh fruitbodies exhibited moderate scavenging effect on DPPH radicals, good total phenolic content and good FRAP value after hot extract. Therefore, *A. auricula-judae* should be consumed boiled (or in soup form) of fruitbodies for protection against oxidative damage caused by free radicals which prevents the onset of many diseases such as cancer, rheumatoid arthritis, atherosclerosis and degenerative processes associated with aging. Mushroom soups have been widely consumed as a healthy dietary supplement in China.

Based on these antioxidant assays, it was thus suggested that phenolic compounds present in hot aqueous extract of *A. auricula-judae* contributes to the high scavenging effect on DPPH radicals, along with FRAP value. This could be due to the antioxidant mechanisms of phenolic compounds towards enhancing scavenging abilities on free radicals, and reducing power on ion Fe^{3+}.

Obviously, natural nutrients could be significantly lost during the thermal processing because most of the bioactive are relatively unstable with heat. The high temperature affects compound stability via chemical and enzymatic decomposition, losses by volatilization or thermal decomposition (Choi *et al*., 2006). However, in some cases, high temperature causes no change or may even have improved effects on the contents and activities of naturally occurring antioxidant. Moreover, the loss of natural antioxidant or heat labile nutrients can be minimized by an enhancement of an overall antioxidant activity

in plant foods due to their various chemical changes during the heat treatment (Choi *et al.,* 2006). There are studies, which revealed that higher heating temperature (121˚C) significantly enhanced the overall antioxidant activities of *Lentinus edodes* (Choi *et al.,* 2006). Eventually, this can be explained by the increased amount of antioxidant compounds, especially free polyphenolic compounds. It is also known that, many antioxidant compounds in plant materials are mainly present as a covalently bound form with insoluble polymers (Peleg *et al.,* 1991). Therefore, it was suggested that high temperature might disrupt cell wall and liberate high temperature antioxidant compounds from insoluble portion of mushroom, which increases the pool of bioaccessible antioxidant compounds (Wong *et al.,* 2007).

Besides that, ethanol and methanol extracts of *A. auricula-judae* fresh fruitbodies also possess a moderate DPPH radicals scavenging activity, total phenolic content and FRAP value besides hot aqueous extract. Subsequently, the methanol and ethanol solvent is very important to extract out polar components (sugars, aminoacids, and glycosides) (Kitzbeiger *et al.,* 2007). In addition, Kitzbeiger and co workers (2007) also quoted that most substances that show high antioxidant activity are polar components. Therefore, it was suggested to increase the extraction yield in order to extract out more antioxidant compounds (Table 4.1).

Moreover, this study also revealed that, the low antioxidant activity possessed by dichloromethane and polysaccharide extracts. Dichloromethane extract exhibited better in DPPH radicals scavenging effect and FRAP assay compared with the polysaccharide. This behavior of dichloromethane is probably due to the non polar characteristics of the solvent, resulting in the extraction of mainly non-polar components, with low antioxidant activity (alkaloides, aglycones and volatile oils) (Houghton & Raman, 1998). In comparison, polysaccharides extract showed low antioxidant values indicating that it is not a good

radical scavenger. This is due to the polysaccharide reacted more slowly with DPPH compared with other *A. auricula-judae* extracts at a slow rate (> 30 minutes) (Brand-Williams *et al.,* 1995).

6.0 CONCLUSION

The *A. auricula-judae*, fresh fruitbodies extracts possess moderate antioxidant activity when evaluated using different assays, namely, DPPH radical scavenging assay and reducing power assay. However, among the extracts, the hot water extract was most potent for antioxidant acitivty for *A. auricula-judae* as it contained high total phenolic content compared with methanol, ethanol, dichloromethane and polysaccharides extracts of fresh fruibodies. The various antioxidant mechanisms of the mushroom extracts may attribute to strong hydrogen donating ability and their effectiveness as moderate scavengers of superoxide and free radicals. Phenolic compounds seem to be the main components responsible for the antioxidant activity of all extracts of *A. auricula-judae*. Moreover, there is a moderate positive correlation between higher antioxidant activity and larger amount of total phenolic compounds for DPPH (r^2= 0.6243) and reducing power activity (r^2=0.5074).

The Neutral Red cytotoxicity assay indicates that, all the extracts from *A. auricula-judae* did not show active inhibition against cancer cells proliferations. This may be attributed to the absence of cytotoxic compound in *A. auricula-judae* fresh fruitbodies extracts. Further, all extracts inhibited the expression of HPV-16E6 oncoprotein at different degrees. Among the evaluated extracts, hot aqueous extract produced the best suppression activity against HPV-16E6 oncoprotein in CaSki cells.