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Full Length Research Paper

Antioxidant effect of *Zanthoxylum limonella* Alston.

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Zanthoxylum limonella **Alston. (Rutaceae) has been widely used as spice and folk medicine. Different part of this plant, such as ripe fruit was used as a condiment in curries, and its essential oil is quite useful for blood circulation and gastrointestinal tract. The bark was noted for its febrifugal, sudorific and diuretic properties. It has been used for treatment of fever caused by free radical production in the recticulo-endothelial system. The present work investigates the antioxidative potential of *Z. limonella* crude extracts and essential oil on cell-free and cell-based systems. 1,1-diphenyl-2-picrylhydrazyl (DPPH) and trolox equivalent antioxidant capacity (TEAC) assays showed the same ranking order of free radical scavenging activity, methanol extract of stems (SM) > dichloromethane extract of stems (SD) > essential oil of fruits (EO). However, leave extracts exhibited very low radical scavenging activity. Treatment with SD, SM, and EO significantly decreased the malondialdehyde (MDA) level of cell lysates obtained from pretreated prostate cancer cells, while glutathione (GSH) and catalase (CAT) levels increased. The result of the antioxidative potential of *Z. limonella* indicated that crude extracts, SD and SM, from stems and essential oil from fruits of *Z. limonella* exhibited the antioxidant activity possibly related to the regulation of CAT and GSH in prostate cancer cells.**

Key words: *Zanthoxylum limonella* Alston., antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH), trolox equivalent antioxidant capacity (TEAC), prostate cancer, lipid peroxidation, glutathione (GSH), catalase (CAT).

INTRODUCTION

The genus *Zanthoxylum*, Family Rutaceae consists of about 250 species of deciduous, evergreen trees and shrubs distributed worldwide around the tropical and subtropical areas. They have been cultivated in North America, South America, Africa, Asia, and Australia. In ancient period, *Zanthoxylum* has been extensively used as a folk medicine for different medical purposes such as stomach ache, toothache, intestinal worms, rheumatism, scabies, snakebites, fever and cholera (Pongboonrord, 1979). *Zanthoxylum limonella* Alston. is widely distributed in the northern part of Thailand and has been traditionally used in food, especially ripe fruits have been commercialized in local markets as a popular spice. Vitamin E has been found in the seed oil (Fish et al., 1975). The essential oil from the fruit affects the gastrointestinal system and initiates smooth muscle contraction by a non-specific mechanism (Ittipanichpong et al., 2002). *Z. limonella* oil has been used as a natural, eco-friendly, and biodegradable mosquito repellent. Antioxidant activity of *Zanthoxylum* has been described. The methanol extract from the fruits of Japanese pepper (*Z. piperitum* DC.) contains hyperoside (quercetin-3-*O*-galactoside), quercitrin (quercetin-3-*O*-rhamnoside) and a glycoprotein (ZPDC). It exhibits a significant antioxidant activity equal to that of α-tocopherol (Yamazaki et al., 2007). ZPDC glycoprotein consists of carbohydrate (18%) and protein (82%). It shows a strong scavenging activity against DPPH radical, superoxide anion, and hydroxyl radical in cell-free system (Lee and Lim, 2007). In addition, the anti-inflammatory activity of some *Zanthoxylum* due to reactive oxygen species (ROS) as a mediator has also been studied (Márquez et al., 2005). Plants in the *Zanthoxylum* genus contain a variety of

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active secondary metabolites, such as alkaloids, lignans, and coumarins, possibly attributed to the ROS in asthma, ulcers, rheumatism, and earache *via* the inflammatory processes (Roig, 1965). *Z. limonella* Alston. has been used in folk medicine for treatment of fever. It was previously found that fever increased the generation of free radicals by cell of the reticulo-endothelial system produced from lipid peroxidation, while decreases the cell antioxidant system (Riedel and Maulik, 1999; Zinchuk, 1999). Familial Mediterranian fever was also correlated with an increment of superoxide radical produced by neutrophils (Sarkisian et al., 1997).

Prostate cancer is the most prevalent type of internal malignancy found in men over the age of fifty. It is the second leading cause of cancer-related deaths among men in Western nations, especially African-American men (Jemal et al., 2007). The causes of prostate cancer are not completely understood. Age is found to be the strongest risk factor. Some epidemiologic studies have suggested that dietary fat closely associated with lipid peroxidation may be an important factor for prostate cancer (Vaca et al., 1988). Wang et al. (1995) suggested that dietary fat content can influence the tumor growth of androgen-sensitive, human prostatic adenocarcinoma cells (LNCaP cells) grown in nude mice. The highest prostate-specific antigen (PSA) levels were found in the high-fat diet group and the lowest in the low-fat group which indicated that prostate cancer progresses according to dietary fat. Extensive research has established a strong relationship between ROS generation and carcinogenesis including cancer progression. ROS can act as secondary messengers to monitor several signaling cascades. They can induce mutations and alter gene function resulting in carcinogenesis *via* oxidation processes (Halliwell, 1994). The mutation of p53 protein has been found associated with the progression of prostate cancer exhibiting various degree of aggressiveness (Navone et al., 1993). ROS played an essential role for migratory/invasiveness phenotypes of prostate cancer. Elimination of excessive ROS may be very effective method to decrease prostate cancer formation and metastasis. This method could be extended to other malignancies due to the strong relation between ROS and tumor formation. Several studies have investigated natural agents or chemopreventive agents from dietary substances to prevent and possible to cure cancer (Syed et al., 2008). Many reports mentions that some natural compounds and dietary agents such as selenium, vitamin E and D, lycopene, soy and isoflavone, low-fat diet, epigallocatechin-3-gallate (EGCG) from green tea, a few compounds from pomegranate reduce the possibility of prostate cancer, both growth and progression (Liao et al.,1995).

In this study, the primary anti-oxidative potential of *Z. limonella* crude extracts and essential oil was evaluated in cell-free system and further extended to prostate cancer cell lines.

MATERIALS AND METHODS

Plant materials

Fresh leaves, stems and fruits of *Z. limonella* Alston. were harvested in the mountain area of Phrae province, Thailand, during January to April 2007. A voucher specimen (BKF No.152276) was submitted to the Herbarium of the Royal Forest Department of Thailand.

Chemicals

1,1-diphenyl-2-picryl hydrazyl (DPPH), butylated hydroxyltoluene (BHT), 3,4,5-(dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT), 5,5'-Dithio (bis) nitrobenzoic acid (DTNB) were purchased from Sigma chemical Co. (St. Louis, MO, USA). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), and 2,2'-azinodi-3-ethylbenzthiazoline sulphonate (ABTS) were purchased from Fluka chemical Co. (Switzerland). All organic solvents used were of technical quality, except ethanol (analytical grade) that was purchased from Merck (Germany). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL.

Extraction procedure

Leaves and stems, 5 kg each, were air-dried and milled to coarse powder. Both powders were separately macerated exhaustively with dichloromethane (DC) and methanol (MeOH), respectively, for 4 weeks. The supernatants were filtered through Whatman No.1 filter paper. Solvents were evaporated from the filtrate by rotary vacuum evaporator (Buchi, R114, Switzerland). The residues were taken to dryness to obtain a viscous mass as the crude extract. The yield of the dichloromethane crude extracts of leave (LD) and stem (SD) were 5.49 and 0.66% dry weight, respectively. The methanol crude extracts of leaves (LM) and stem (SM) yields were also determined and found 6.52 and 4.62% dry weight, respectively.

Dried-ripe fruits of *Z. limonella* were blended by a domestic blender to get fine powdered. Essential oil (EO) was obtained by hydrodistillation using a Clevenger-type apparatus, resulting in light yellow oil with 11.63% yield. Essential oil was stored at 4°C until used.

DPPH assay

The DPPH• test is the conventional DPPH• capacity assay widely use for plant, food on natural product to screen and evaluate the free radical-scavenging effect. The radical form DPPH• with dark-blue color can be protonated by the antioxidant compounds and reduced to the more stable radical DPPH• with the yellow colored diphenylpicrylhydrazine and terminate radical chain reaction. DPPH• assay was able to perform both in thin layer chromatography (TLC)- and cuvette-DPPH radical scavenging assay.

TLC-DPPH radical scavenging assay

All crude extracts, LD, SD, LM, SM, and EO were spotted on to thin layer chromatography (TLC). TLC plates were developed with the appropriate solvent system for each extract and then air-dried for 20 min. The developed plates were sprayed by 0.2% DPPH in methanol and air-dried. The visible yellow spots were observed and photographed.

Cuvette-DPPH radical scavenging assay

Free radical scavenging activity on DPPH radical described by Sithisarn et al. (2006) was used to evaluate the scavenging activity of all extracts. Dimethylsulphoxide (DMSO) was used to dissolve the crude extracts. Serial dilutions of extracts were carried out to obtain a suitable concentration. A 250 μL of BHT or diluted extract was added to 250 μL DPPH ethanolic solution (2.4 mg in 100 ml ethanol). After incubation at ambient temperature for 20 min, the absorbance was monitored against ethanol as a blank at 520 nm by UV-spectrophotometer (Shimadzu, UV-160A, Tokyo, Japan). The percentage of scavenged DPPH was calculated as the percentage of inhibition using the following formula.

$$\% \text{ inhibition} = [(A_{blank}-A_{extract}) / A_{blank}] \times 100$$

where A_{blank} and $A_{extract}$ are the absorbance of blank and extract, respectively.

Ethanolic solution of butylated hydroxyltoluene (BHT) was used as positive control. DPPH, BHT, and reconstituted solution were freshly prepared. All extracts were performed in triplicate and concentration of extracts exhibited 50% inhibition (IC_{50}) obtained from dose response curve was averaged and used to compare the scavenging activity of each extract.

Trolox equivalent antioxidant capacity (TEAC) assay

TEAC assay was done according to Arts et al. (2004) with some modification. Briefly, ABTS and potassium persulphate solution were dissolved in deionized water to a concentration of 14 and 5 mM, respectively. The reaction mixture was left at ambient temperature for 12 to 16 h in a dark place to get the blue-green coloured $ABTS^{\bullet+}$ radical cation. The radical cation solution was diluted with deionized water to obtain an absorbance of 0.8 ± 0.05 at 734 nm. The water soluble vitamin E analogue, Trolox®, or various concentrations of extracts prepared by serial dilutions were added to the dilute radical cation solution. The decrease in absorbance after 1 min was spectrophotometically read at 734 nm. All of the assays were performed at least in triplicates. The $ABTS^{\bullet+}$ radical cation solution was freshly prepared daily. The decline in absorbance after 1 min caused by Trolox or extracts was plotted against concentration. The TEAC value was obtained from the ratio between the slopes of the linear plot and that of the Trolox.

Cell cultures

Human prostate adenocarcinoma cell lines DU-145 and PC-3 were a generous gift from Professor Thompson EW, Department of Surgery, St. Vincent's Institute, the University of Melbourne, Australia. The cancer cells were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin as monolayer in 55 cm^2 tissue culture dishes. Both cell cultures were maintained at 37°C in a tissue culture CO_2 incubator at a humidified atmosphere containing 5% CO_2 until 80% confluency and then subcultered twice a week

Lipid peroxidation determination

Whole tumor cell lysates were obtained by repeated freeze-thaw procedures according to the protocol previously described by Ohkawa et al. (1979). Cancer cells were trypsinized, harvested, washed with phosphate buffered saline (PBS), subjected to repeated freeze-thaw procedures in ice-cold bath, and centrifuged. The supernatant was collected and gently vortexed to get a uniform suspension. Homogenous lysates from cancer cells pretreated with various concentrations of crude extracts were used for lipid peroxidation measurement using thiobarbituric acid reactive substance (TBARS). For untreated cancer cells, lysates were combined with various concentrations of crude extract followed by the lipid peroxidation assayed using TBARS.

Thiobarbituric acids tested for malondialdehyde (MDA) were used as a lipid peroxidation following the method previously described by Ohkawa et al. (1979). Briefly, a 500 µL aliquot of cell lysates was combined with a reaction mixture containing 75 µl of 8.1% SDS, 565 µl of 20% acetic acid, and 565 µl of 0.8% thiobarbituric acid (TBA). The resulting mixture was vigorously mixed, incubated in a water bath at 95°C for 1 h, and cooled to room temperature with tap water. A 500 µl of *n*-butanol and pyridine (15:1, v/v) mixture was added to each sample, shaken vigorously, and centrifuged at 1200 g for 10 min. The supernatant fraction with the pink color of MDA-TBA complex was isolated and the absorbance at 532 nm was measured against mixture of *n*-butanol and pyridine as a blank. The same procedure was repeated with malonaldehyde bis(dimethyl acetal) as a positive control. The content of lipid peroxidation was expressed as nM MDA per mg protein by interpolation in a standard curve in water covering a concentration range of 0 to 200 mM. Protein concentration was estimated by Bradford's method. All samples were conducted independently in triplicate.

Catalase (CAT) assay

CAT catalyzes the decomposition of H_2O_2 molecules into water and oxygen. The reaction kinetics of CAT activity was conducted at 25°C using 50 mM phosphate buffer of pH 7.0 containing H_2O_2 as a substrate. After the 1-min incubation with cell lysates, the decrease of absorbance at 240 nm was monitored as the CAT activity to decompose hydrogen peroxide molecules. A molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$ for H_2O_2 was used.

Glutathione (GSH) assay

The total GSH contents of the cell lysates were estimated by colorimetric assay using the reaction between sulphydryl group of DTNB to produce a yellow-color of 5-thio-2-nitrobenzoic acid (TNB). TNB production is directly proportional to the concentration glutathione in cell lysates. All experiments were done in triplicate. Total glutathione was determined by measuring the absorbance 410 nm after 30 min incubation. The results were expressed in µmole per mg protein.

Statistical analysis

The statistic analysis of the obtained data was performed by SPSS (version 11.5) software for window. Data were statistically analyzed using one-way ANOVA and Turkey's multiple comparison testes in which the significance level was defined as $p < 0.05$.

RESULTS

DPPH assay

TLC chromatogram of crude extracts (Figure 1) was used for a qualitative screening of antioxidant activity. $DPPH^{\bullet}$ scavenging activity was visualized as yellow spots against purple background. LD extract gave no visualized

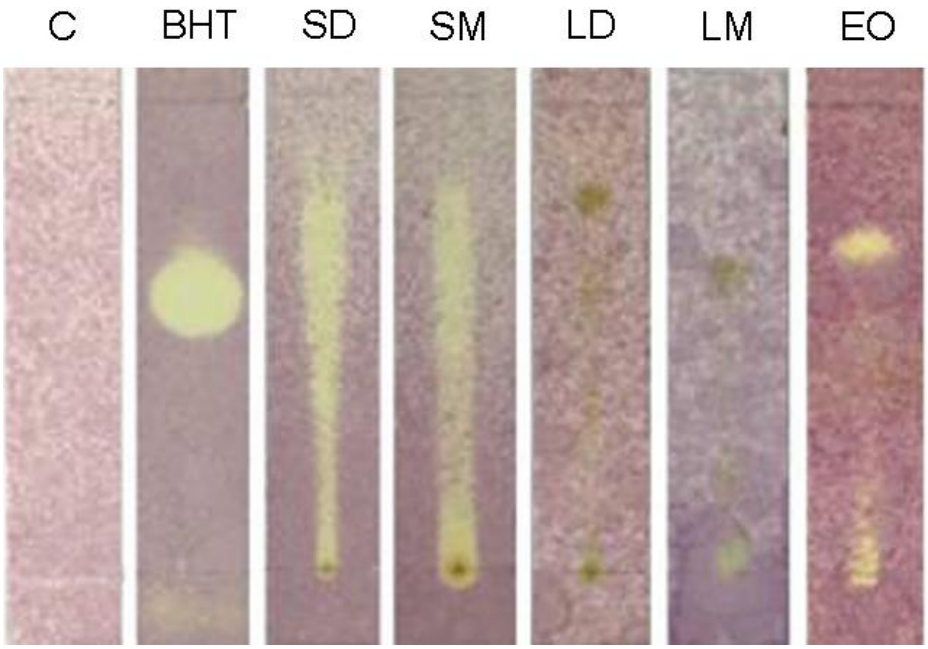

Figure 1. TLC plate sprayed with 0.2% DPPH$^{\bullet}$ solution in methanol, air dried, and visualized under visible light, and photographed under visible light. (C) control, (BHT) standard antioxidant butylated hydroxytoluene, (SD) dichloromethane extract of stem, (SM) methanol extract of stem, (LD) dichloromethane extracts of leave, (LM) methanol extract of leave, and (EO) essential oil.

spot on one-dimensional TLC analysis which means absence of antioxidant activity. LM extract showed a pale yellow area at the spotted point of extract which perhaps indicated the polar antioxidant in this crude extract. The last lane of EO slowly turned yellow after methanolic DPPH$^{\bullet}$ solution was sprayed, suggesting some degree of DPPH$^{\bullet}$ scavenging activity. The rapid development of yellow and long tail from SD and SM extracts showed the higher antioxidant activity than LM and EO extracts due to many antioxidant compounds present. The order of DPPH$^{\bullet}$ scavenging potency of the crude extracts from *Z. limonella* was as follow: Stem extracts > essential oil > leave extracts. Therefore, stem extracts and essential oil were further studied in prostate cancer cell lines.

The principle of quantitative analysis of DPPH antioxidant assay is based on a reaction in which a blue solution of stable free radical DPPH accepts an electron from a free radical scavenger, antioxidant compounds, and decolorizes. The amplitude of decolorization can be quantitatively determined by reading the absorbance. A large antioxidant capacity is revealed by low IC_{50} value (Table 1). IC_{50} of SD, SM, and EO are 54.63 $\pm$ 2.89, 117.47 $\pm$ 4.66, and 5,764.67 $\pm$ 6.45 μg/ml, respectively. As previously found in TLC chromatogram, SD and SM extracts still exhibited higher scavenging activity than EO. However, the IC_{50}'s of leave extracts, LD and LM was not determined due to the unability to decolorize the methanolic DPPH solution.

Trolox equivalent antioxidant capacity (TEAC) assay

Percentage inhibition of ABTS$^{\bullet+}$ absorbance as a function of the concentration for each crude extract was determined at 1-min time point. It was found that the percentage inhibition, from which the antioxidant activity could be inferred, varied in a dose-dependent fashion. TEAC values listed in Table 1 as μM Trolox/g crude extract is closely related to IC_{50} from DPPH assay with the same ranking order of free radical scavenging activity. Both values show that the crude extracts from stem have higher antioxidant activity than essential oil. TEAC values of SM, SD, and EO were 15.47 $\pm$ 0.34, 14.34 $\pm$ 0.31, and 7.05 $\pm$ 0.34 μM respectively.

Cell lysates from PC-3 and DU-145 at 24 h-pretreated with SD, SM, and EO were also tested by TEAC assay. In all treatments, TEAC values were found to increase with difference magnitude compare to the control (Figure 3).

Lipid peroxidation determination

In the present study, lipid peroxidation products, that is, MDA, were analyzed using TBAR assay, which consists of spectrophotometically measured color intensity of MDA-TBA complex. MDA-TBA complex nearly was absence in all extracts themselves, SD, SM, or EO after

Table 1. Antioxidative capacities of the several crude extract of *Z. limonella*.

Plant extract	50% DPPH scavenging activity (IC_{50}, μg ml^{-1})	Trolox equivalent antioxidant capacity (μM Trolox/g crude extract)
SM extract	54.6 ± 2.9	15.5 ± 0.3
SD extract	117.5 ± 4.7	14.3 ± 0.3
EO	5,764.7 ± 6.5	7.1 ± 0.3
BHT	19.7 ± 0.2	-

SM, Stem of methanol extract; SD, stem of dichloromethane extract; EO, essential oil of fruit.

been reacted with TBAR reagent. The MDA level of PC-3 untreated cell lysates slightly decreased, approximately 5 to 10% of control, by SM and EO. Moderately reduction of MDA, approximately 20% of control, was found by SD at 0.5 μg/ml. All extracts at 5 and 10 μg/ml seemed to exert no effect. In addition, all extracts at 0.5, 5 and 10 μg/ml exhibited no effect to MDA levels in DU-145 untreated cell lysates.

Cell lysates obtained from pretreated PC-3 and DU-145 with different concentrations: 0, 0.5 and 5 μg/ml of those three extracts was also used to test the antioxidant activity by TBAR assay. The concentrations of the extract at 0.5 and 5.0 μg/ml were previously proved not to influence cell growth via the proliferation assay (not shown). For PC-3 untreated cell lysates, MDA levels were significantly decreased by SD, SM and EO at dose 0.5 μg/ml at 78.80 ± 1.38, 88.89 ± 1.50, and 93.68 ± 2.67%, respectively. SM and EO expressed higher lipid peroxidation inhibition than SD at concentration of 5.0 μg/ml. Both concentrations of EO seemed to display no effect on the MDA level in DU-145 pretreated cell lysates. The same characteristic lipid peroxidation inhibitions of SD and SM were found in DU-145 and PC-3.

Catalase activity

CAT activity from cell lysates pretreated with SD and SM in both PC-3 and DU-145 increased significantly in a dose dependent manner. This was also found in cell lysates pretreated with EO in PC-3. The same magnitude of CAT elevation was observed in cell lysates pretreated with EO in DU-145 as shown in Figure 3.

Glutathione level

Reduced GSH levels of the cell lysates from PC-3 24 h-pretreated with SD and SM at concentration of 0.5 μg/ml seemed to significantly increase ($p < 0.05$) compared to the control group 111.97 ± 3.77 and 112.48 ± 3.23%, respectively. The increase percentage was lower when the concentration of SD and SM was increased 10-times, 104.83±4.85 and 104.20±3.11%, respectively. The same pattern of higher increase in reduced GSH could be observed in DU-145 pretreated cell lysates (Figure 3). GSH in PC-3 cell lysates pretreated with EO at 0.5 μg/ml was slightly decreased and significantly decreased with EO at 5 μg/ml.

DISCUSSION

Several compounds were isolated from *Z. limonella*. Somanabandhu et al. (1992) isolated five compounds from the bark of *Z. limonella*: Ubiquitous lupeol, alkaloid rutaecarpine and three coumarins, xanthoxyletin, osthol and scopoletin. In addition, there has been no report regarding antioxidant compound in *Z. limonella*. However, some of these compounds previously isolated from other plants were studied to possess antioxidant potential. Kim et al. (1997) reported that one of the components of *Artemisia iwayomogi*, scopoletin was unable to scavenge the $DPPH^{\bullet}$. In contrast, scopoletin was found to inhibit lipid peroxide and generation of superoxide and hydroxyl radicals. Kang et al. (1998) reported that the isolation of scopoletin from *Solanum lyratum* protects hepatocyte from CCl_4-induced toxicity by maintaining the GSH content, the activity of superoxide dismutase (SOD), and inhibiting the production of MDA as a result of its antioxidation and free radical-scavenging effect. Lupeol, a triterpene found in many fruits and vegetables was found to contain antioxidant, antimutagenic and antiimflammatory effect in *in vitro* and *in vivo* systems (Saleem et al., 2001; Geetha and Varalakshmi, 2001). Lupeol and its ester lupeol linoate effectively scavenge free radicals and reduce oxidative stress indices by enhancing the antioxidant capacity of the liver of cadmium treated rats (Sunitha et al., 2001).

Many reports have shown the antioxidant activity of extracts, synthetic, or natural compounds by DPPH and TEAC assays. These methods are based on similar redox reactions of stable free radicals and give information regarding the quality and the activity, both of which indicate the content to the radical scavenging compounds present in the tested sample. TLC chromatogram indicated that the extracts from fruits and stems exhibited a higher antioxidant activity than that of leaves. This has been attributed to terpenoids in the essential oil of fruit and coumarins and alkaloids in stem

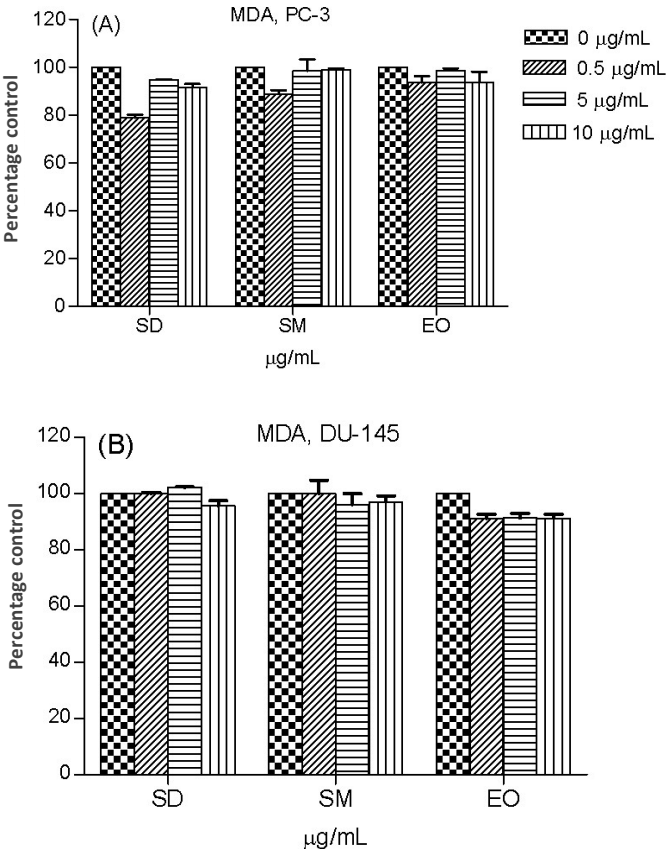

Figure 2. Change on MDA levels with SD, SM extract and EO in PC-3 (A) and DU-145 (B) cell lysates at 0 (control), 0.5, 5 and 10 μg/ml.

reported by Ittipanichpong et al. (2002) and Somanabandhu et al. (1992). The same ranking order of free radical scavenging activity among SD, SM, and EO was found, with a high correlation between TLC chromatogram, IC_{50} and TEAC values as shown in Figure 1 and Table 1. The data from DPPH and TEAC assays of crude extracts from *Z. limonella*, leaves, fruits, and stems revealed a wide range of antioxidant activity. Palasuwan et al. (2005) has reported a moderate degree of antioxidant activity of *Z. limonella* crude extract from seed. Since SD and SM showed a higher scavenger activity than EO, we extended the study to test the antioxidant capability on prostate cancer cell lines, PC-3 and DU-145.

In the erythrocytes of patients with prostate cancer, MDA levels have been found to be significantly higher (Aydin et al., 2006). It was previously mentioned that there is a higher oxidative stress in benign epithelium of prostate cancer patients than normal men. On the other hand, it is known that the generation of ROS is able to trigger many processes of lipid peroxidation, pro-carcinogenic processes, and protein damage. These cause considerable damages and disturb physiological functions of cellular essential components including lipid, protein and genetic materials. Several diseases were developed, especially many kinds of cancers and aging (Bandyopadhyay et al., 1999). Normally, the counteracting defense system to control excess free radicals was established intracellular as the antioxidant systems composed of enzymatic and nonenzymatic groups. The function of both is linked to each other and generates a balance between oxidative stress from ROS and antioxidant capacity to maintain an optimal health. Three important enzymes are involved as the effective antioxidants, superoxide dismutase (SOD), CAT, and glutathione peroxidase (GPX). SOD catalyses the conversion of $O_2^{\bullet-}$ to H_2O_2 and O_2, while CAT converts H_2O_2 to H_2O and O_2, and GPX reduces H_2O_2 to H_2O (Leewenburgh and Heinecke, 2001). Many compounds that function differently as nonenzymatic antioxidants include lipid-soluble and water-soluble compound, such as GSH, vitamin C, vitamin E, lipoic acid, uric acid, ubiquinol, etc. The action of antioxidants have been mentioned in four groups; chain breaking antioxidant, scavenging radicals, radical reduction, and chelating agent for transition metal catalysts (Pokorny et al., 2001). Many kinds of natural antioxidants such as vitamin E and lycopene are expected to play an important role for prostate cancer prevention and progression, especially when the patients are suffering and feel hopeless from conventional chemotherapy (Syed et al., 2007).

The information stated in Figure 2 showed quite low ability of SD, SM, and EO to change the MDA concentration in untreated cell lysates of PC-3 and DU-145. These results show that SD, SM, and EO had weak activity against MDA level in both cell lysates and can not provide direct protection against free radical to protect the cell membrane from the damage caused by lipid peroxidation. However, the indirect effect via intracellular antioxidant system might be possible.

Furthermore, the intracellular antioxidant system, GSH and CAT, was done in PC-3 and DU-145 pretreated with SD, SM, and EO at the concentration of 0, 0.5, and 5 μg/ml (Figure 3). SD and SM seemed to regulate GSH level and CAT activity, leading to the control of intracellular antioxidant power via an increasing of TEAC value. These observations may support the hypothesis that SM and SD might have antioxidant capacity by regulating the thiol-regulated cellular activity. GSH, the major low-molecular-mass thiol in the cytoplasm, acts as a free radical scavenger, trapping ROS that would otherwise interact with cellular thiols through an enzyme-catalysed reaction. GPX is used as an electron donor in the reduction of peroxidase, including lipid peroxides (Ghezzi, 2005).

On the other hand, EO has high possibility to regulate CAT activity more than GSH level in both cell lines. The intracellular antioxidant power of cell lines exposed to EO might largely depend on the CAT activity. CAT prevents the hydrogen peroxide from harming the cell itself. CAT is

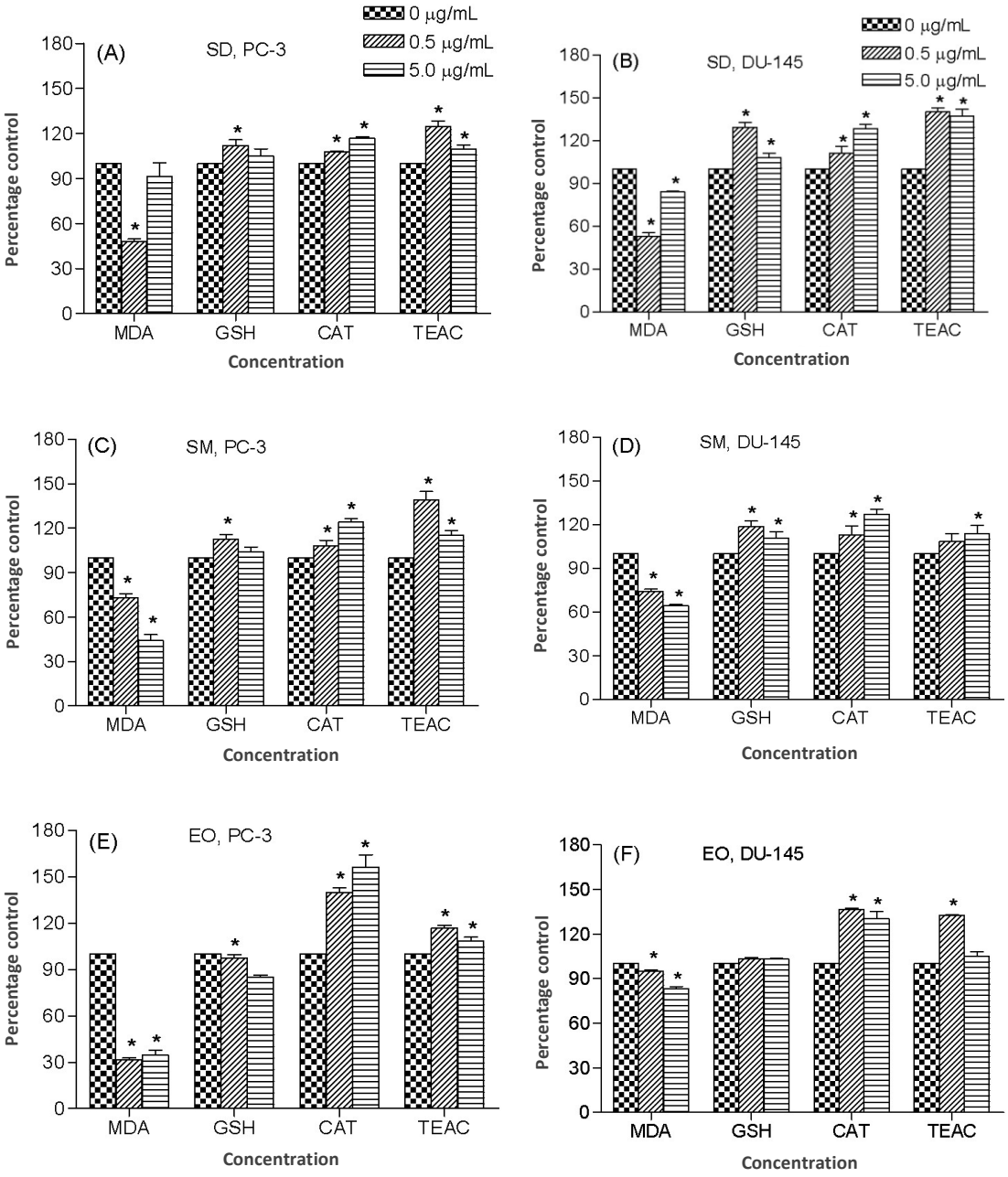

Figure 3. Change on MDA levels (ng μg^{-1} protein), GSH levels (mg mg^{-1} protein) and CAT (μmoles mg^{-1} protein min^{-1}), TEAC activities (μM Trolox mg^{-1} protein) with SD extract, SM extract and EO administration in PC-3 and DU-145 prostate cancer cells (A-F) at 0 (control), 0.5 and 5 μg/ml for 24 h.* p<0.05 versus untreated control as analyzed by one way ANOVA and Turkey's multiple-comparison test.

frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani et al., 1996). Gene expression of CAT and enzymes involved in thiol-regulated cellular activity is very interesting. Further investigation will determine gene expression in animal and human. The chemical analysis and compound isolation should be focused in searching effective natural compounds for the adjuvant therapy of prostate cancer.

Conclusion

The antioxidant capability of the extracts from *Z. limonella* was found in a wide range depended on various parts of plant. SD, SM, and EO expressed quite and interesting

indirect action via intracellular antioxidant content, GSH and CAT, to monitor the lipid peroxidation process.

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