嘉南藥理科技大學專題研究計畫成果報告

紅豆(Vigna angularis)抗氧化活性之探討

計畫類別：■個別型計畫　　□整合型計畫

計畫編號：CN90-FH-09

執行期間：90 年 1 月 1 日至 90 年 12 月 31 日

計畫主持人：周隆武

共同主持人：

計畫參與人員：

執行單位：食品系

中華民國　91　年　2　月　27　日

Evaluation of the antioxidant activities of azuki bean and hull (*Vigna angularis*)

Ming-Jiuan Wu*, Long-Wu Chou, Lisu Wang, Kuei-Jong Liang

Department of Food Health, Chia-Nan University of Pharmacy and Science, Tainan 717, Taiwan

* To whom correspondence should be addressed. Tel: +886-6-266-4911 ext 221; Fax: +886-6-266-6411. E-mail: imwu@mail.chna.edu.tw

Abstract

Increased production of reactive oxygen species have been involved in several human diseases, including cardiovascular diseases and cancers. Dietary antioxidants may be especially important in protecting against diseases associated with free radical damage to cellular DNA, lipids and proteins. In this study, the antioxidative effects of azuki beans and their hulls against lipid, DNA, deoxyribose and protein were investigated. The antioxidative effect is very system-dependent. We found that although neither of them was very strong antioxidant, azuki beans and their hulls exhibited fair extent on inhibition of peroxidation induced by Fenton reaction. Overall, azuki bean hulls had stronger antioxidative effect than azuki beans, and the mechanism underlying the activity may include the chelating ability of metals, reducing power, and scavenging effects of hydroxyl radical and free radicals.

Keywords: oxidative stress, Fenton reaction, antioxidant, azuki bean, scavenging effect, oxidative damage, DNA

Introduction

The oxidative deterioration of lipids in foods is responsible for rancidity with a subsequent decrease in dietary quality and safety caused by the formation of potentially toxic compounds (Moure et al., 2001). It is necessary to suppress lipid peroxidation in food in order to preserve flavor, color and nutritive value. The addition of antioxidants to foods is the most effective way for retarding the unpleasant flavor produced by lipid peroxidation. Antioxidants are also of interest to biologists and clinicians, because they may help to protect the human body against damage by reactive oxygen species (ROS) (Halliwell el al., 1995).

In food industry, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ), are widely used because they are effective and less expensive than natural antioxidants. Their safety issue, however, is highly debated, thus generates the need for searching substitute natural and safer sources of food antioxidants. Recently, much attention has focused on vitamins C, E and β-carotenoids (Frankel, 1996; Kamal-Eldin & Appelqvist, 1996; Mallet et al., 1994; Miller &Rice-Evans, 1997; Miller et al., 1996; Palozza & Krinsky, 1992; Tsuchihashi et al., 1995).

Several studies have analyzed the antioxidant potential of a wide variety of vegetables, including cacao beans (Sanbongi et al, 1998), potato, tomato, spinach, and legumes such as *Phaseolus vulgaris* (Ganthavorn & Hughes, 1997). Seeds, teas, and agricultural residues are also potential antioxidants ( ). Among the best investigated sources of natural antioxidants are the legume seeds (Ganthavorn & Hughes, 1997; Tsaliki et al., 1999; Miyase, 1999a; 1999b; Okada & Okada, 2000). Various potential antioxidant compounds have been isolated from legumes, many of them being polyphenolic compounds (Sandoval et al., 1997; Plumb et al., 1999; Moran et al.,

1997). Hulls also contain compounds with potentially natural antioxidants ( ). Active compounds were detected in hulls from peanut (Yen, Duh & Tsai, 1993; Yen & Duh, 1994; Yen & Duh, 1995; Duh & Yen, 1995 and 1997a; Xing & White, 1997), mung bean (Duh, Yen, Du & Yen, 1997) and buckwheat (Watanabe, Ohshita & Tsushida, 1997). These outer layers usually contain a greater amount of polyphenolic compounds, as expected from their protective function.

Azuki bean (*Vigna angularis*), also known as small red bean, is a legume cultivated throughout Asia and has been widely used in bakery and dessert for a long history. It contains saponins and isoflavonoids, which are widely distributed in leguminous seeds. In addition to dietary use, azuki bean was utilized to treat edema, beriberi and jaundice in traditional Chinese medicine.

The present study focused on the antioxidant activities of different extracts of azuki beans and their hulls against fatty acid, protein, and DNA. The scavenging effects on DPPH and Fe^{++} as well as reducing power will also be demonstrated.

Materials and Methods

Materials

Azuki beans (*Vigna angularis*) were purchased from local supermarket in Tainan, Taiwan. Azuki bean hulls were obtained from local farms in Tainan. Both were first dried and ground to powder then stored in plastic bottles at 4°C until used.

Chemicals

All chemicals were purchased from Sigma Co. (St. Louis, MO), and solvents were from Merck (Darmstadt, Germany). All of the reagents were prepared in deioinized water unless otherwise indicated.

Extraction

Azuki beans and their hulls were extracted with methanol, ethanol, acetone and water at the ratio of 50 ml per 5 grams in a shaking incubator at 37°C overnight. The extracts were filtered then concentrated *in vacuo* or lyopholized. The crude extracts were weighed and dissolved in original solvents then packaged in nitrogen and stored at -20°C until used.

Antioxidative activity in a hemoglobin-induced linoleic acid system

The antioxidative activity of all extracts was determined by a modified rapid photometric assay (Kuo *et al.*, 1999). 200 µl of solution which contained various amount of extract, 0.001 M linoleic acid emulsion, 25mM phosphate buffer, pH 6.5, and 0.003% hemoglobin was incubated at 37°C for 45 min. At the end, 5 ml of 0.6% HCl in ethanol was added to stop lipid oxidation. The peroxide value was then measured in triplicate by thiocyanate method by reading the absorbance at 480nm after coloring with 100µl of 0.02M $FeCl_2$ and 100µl of 30% ammonium thiocyanate.

Reducing power

50 µl of various concentrations of extract was mixed with 125 µl of 0.2M phosphate buffer, pH 6.5 and 125 µl of 1% potassium ferricyanide, then incubated at 50°C for 20 min. 125µl of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 x g for 10 min at room temperature. 375µl of the resulting supernatant was taken then mixed with 375 µl of ddH_2O and 75µl of 0.1% ferric chloride. The absorbance at 700nm was measured in triplicate. Increased absorbance indicated increased reducing power (Oyaizu, 1986).

DPPH scavenging effect (Shimada *et al.*, 1992)

Reaction was performed in 1.25 ml of methanol containing 0.2mM freshly made DPPH and various amount of extract. The reaction mixer was incubated at 37°C for

30 min, and the absorbance at 517nm was measured in triplicate.

Ferrious ion chelating effect (Dinis *et al.*, 1994)

Reaction mixture which contained 100µl of extract, 200µl of 0.5 mM ferrous chloride and 200 µl of 5 mM ferrozine was incubated at 37°C for 10 min. After adding 1.5 ml of ddH_2O to the mixture, the absorbance at 562 nm was measured. The lower the A562 indicated the stronger the chelating effect.

Antioxidative activity against double-stranded DNA in a H_2O_2-Fe^{+++}-ascorbate system

Plasmid relaxation reactions was used to evaluate the antioxidative activity against DNA (Kobayashi *et al.*, 1990). Reactions were performed in 10 µl of 10 mM (A) effect of water extract of azuki beans (B)effect of water extract of azuki bean hulls.

Tris-HCl buffer (pH 7.8) containing 200ng pUC18 double-stranded supercoiled DNA, 1mM hydrogen peroxide, 50µM ferric chloride, 100µM ascorbic acid and various amount of extracts. The reaction mixtures were incubated at 37°C for 30 min and then stopped by adding 1 µl of 0.5M EDTA. The samples were then separated on 0.7 % agarose gel electrophoresis followed by ethidium bromide staining and visualized under UV.

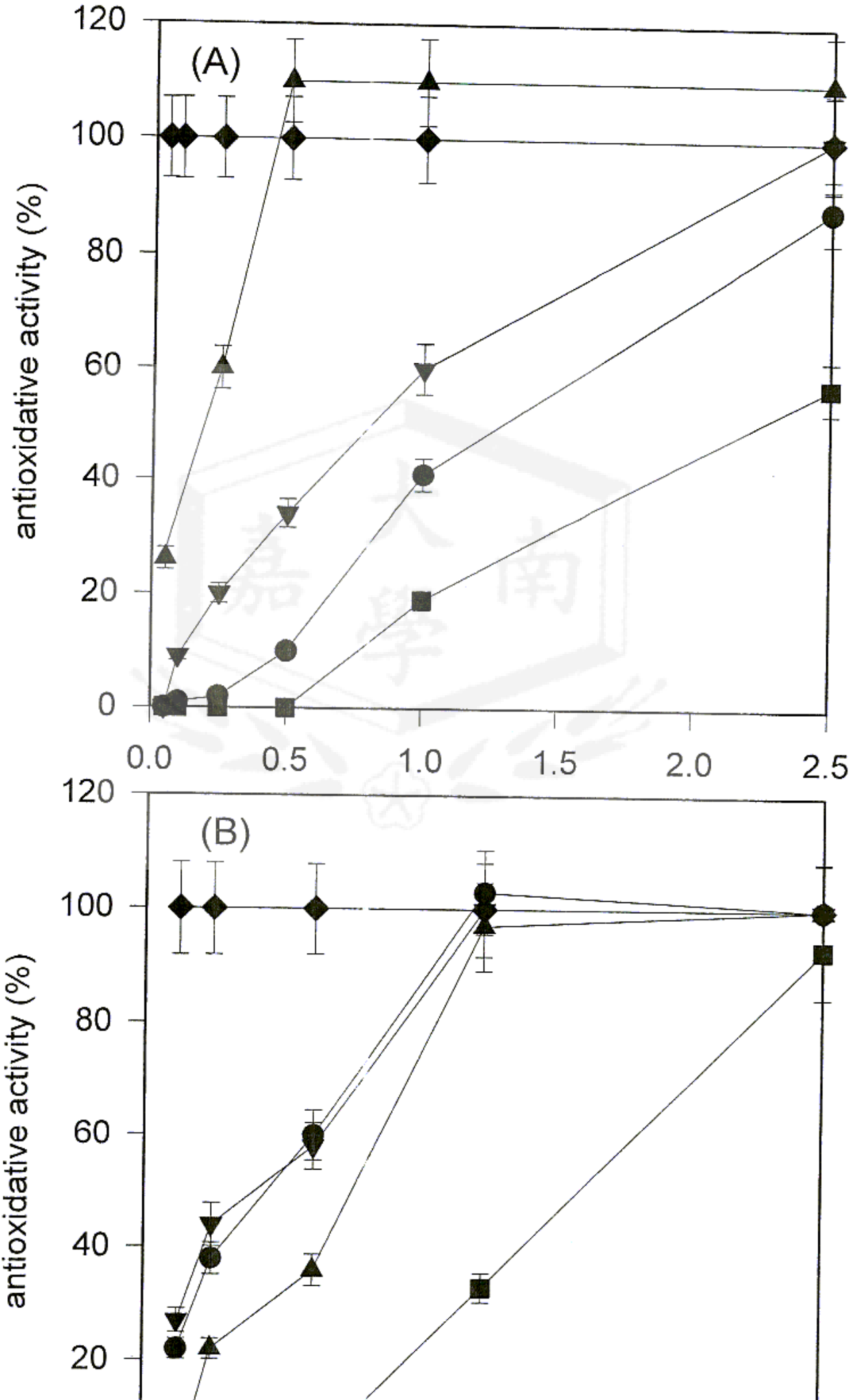
Antioxidative activity against protein in a H_2O_2-Fe^{+++}-ascorbate system (Reznick and Packer, 1994)

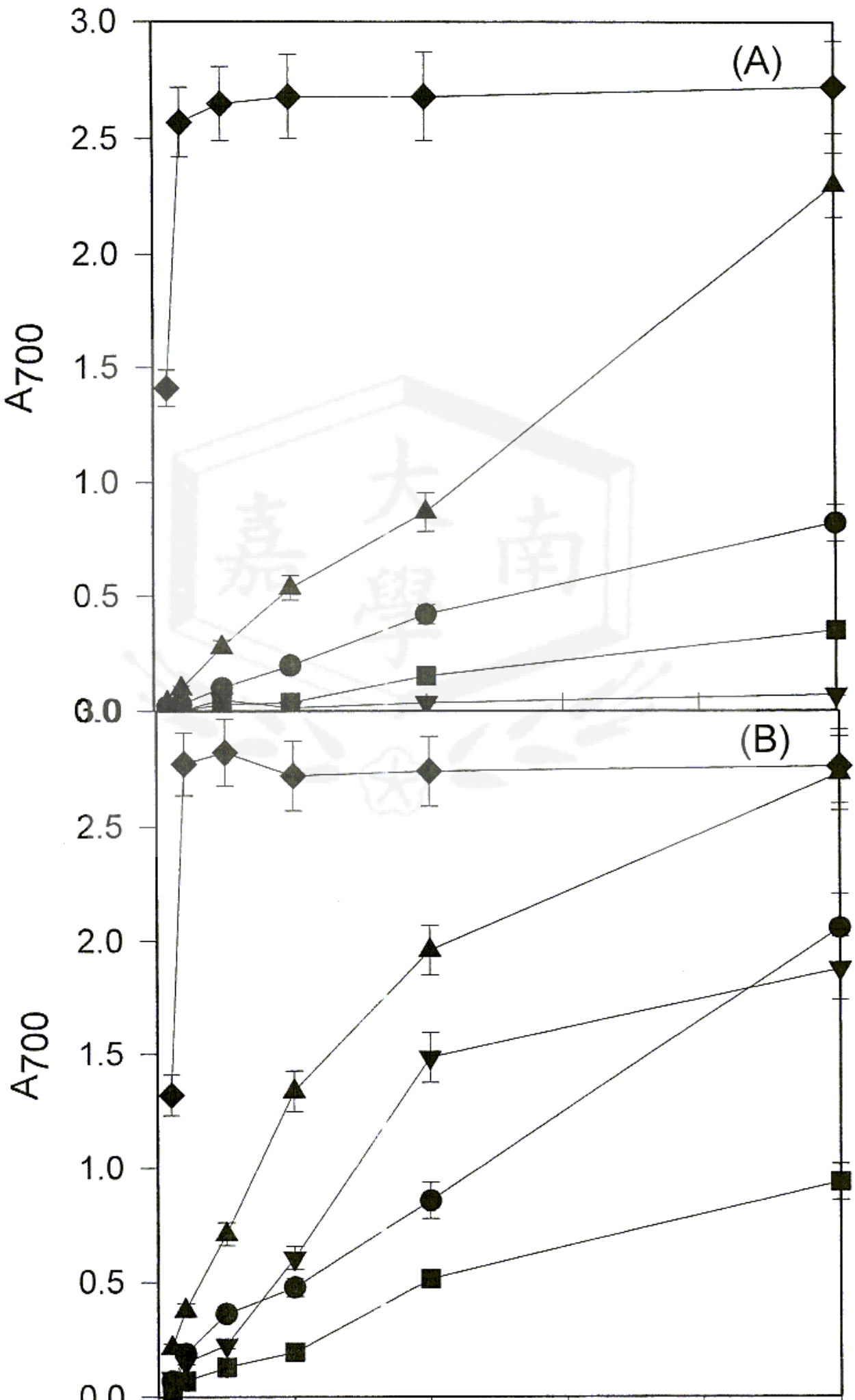
Reaction mixtures (500µl) contained various concentrations of extract; 1mg of bovine serum albumin;10mM phosphate buffer, pH 7.8; 2 mM H_2O_2; 0.5mM ferric chloride; and 0.5 mM ascorbate. After incubated at 37°C for 30 min, the degree of protein oxidation which is represented as the carbonyl content was measured by adding 500µl of 20mM 2,4-Dinitrophenylhydrazine (DNPH, prepared in 2.5N HCl).

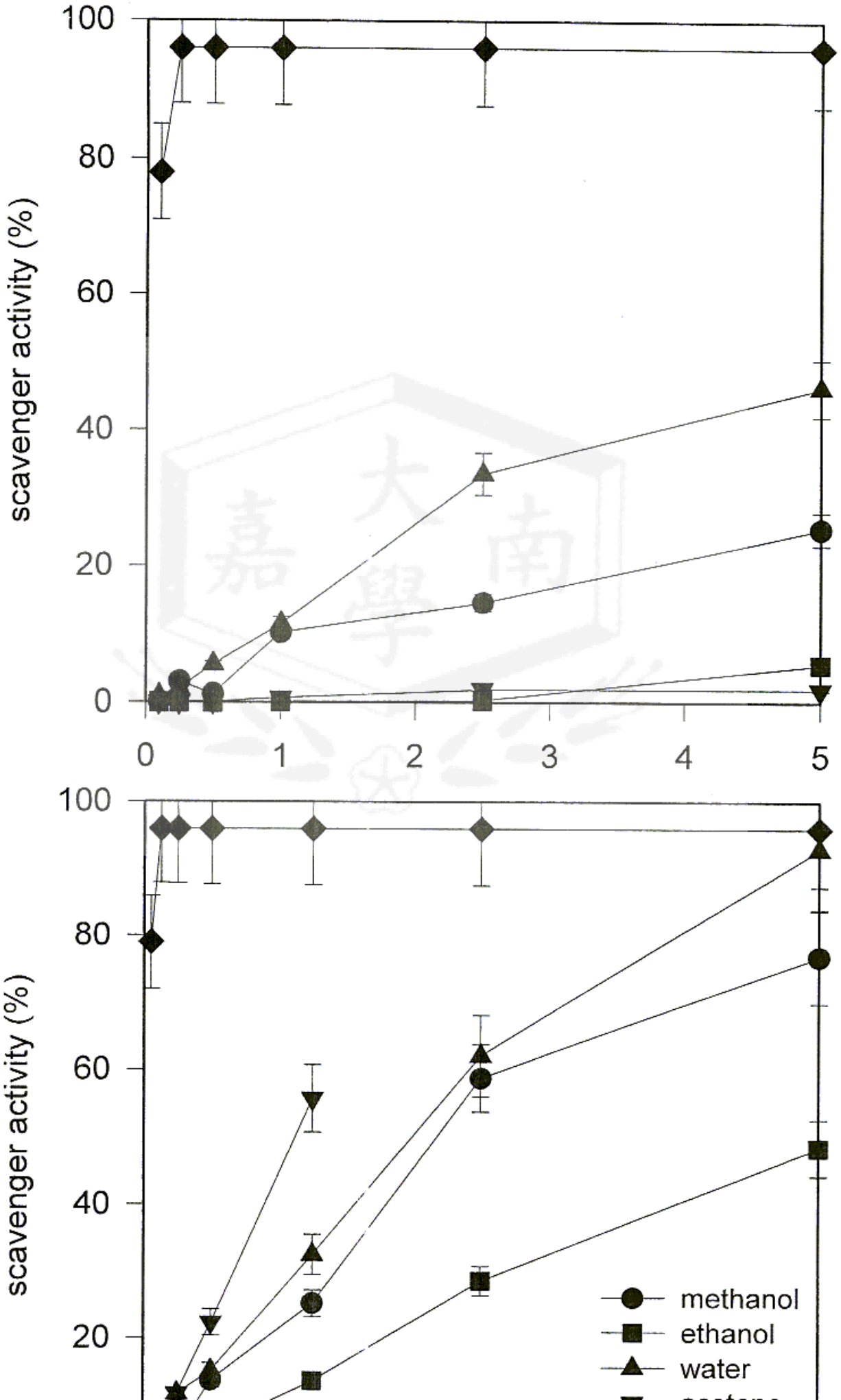
The reaction mixtures were then left for 1 hour of incubation at room temperature. Then, 500μl of 30% trichloroacetic acid was added, and left on ice for 10 min and centrifuged for 3 min to collect the protein precipicates. The pellets were washed 3 times with 1 ml of ethanol-ethyl acetate (1:1) (v/v) to remove the free DNPH. The final precipitates were dissolved in 1 ml of 6M guanidine hydrochloride solution (pH 2.3) and were left for 10 min at 37°C with general vortex mixing. Any insoluble materials were removed by additional centrifugation. The hydrazone was determined spectrophotometrically by its absorbance at 370 nm.

Mutagenicity assay

The mutagenicity assay was performed according to the Ames test (Maron and Ames, 1983). The histidine-requiring strain of Salmonella typhimurium TA98 was purchased from CRCC. The S9 mix ( ), was prepared from Sprague-Dawley male rats treated with Aroclor 1254, according to Ames *et al.* (1975). DMSO diluted various amounts of methanol extract of lotus leaves were added to the overnight-cultureed Salmonella typhimurium TA98 (0.1 ml) and S9 mix (0.5 ml) or phosphate buffer (0.1 ml) in place of S9 mix. The entire mixture was pre-incubated at 37°C for 20 min before molten top agar (2ml) was added; the mixture was poured on a minimum agar plate. The his+ revertant colonies were counted after incubating at 37°C for 48 hours. Assay of each sample was determined in triplicate plates per run and data presented are means ± SD of three determinations. At least two run of a single experiment were performed to validate the reproducibility. The mutagenicity is expressed as the number of revertants per plated, at a given concentration of each sample. In this mutagenicity testing, the result was recognized as positive when the number exceeds twice the number of spontaneous revertants (Ames et al., 1975).


(A)
antioxidative activity (%)
120
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
(B)
antioxidative activity (%)
120
100
80
60
40
20


(A)
(B)
A700
A700
3.0
2.5
2.0
1.5
1.0
0.5
0.0


scavenger activity (%)
100
80
60
40
20
0
0
1
2
3
4
5
scavenger activity (%)
100
80
60
40
20
methanol
ethanol
water
acetone