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Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides Sieb & zucc.*

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Abstract

This study was aimed to evaluate the antioxidative activity and mutagenic properties of the extracts from *Zanthoxylum ailanthoides Sieb & zucc*. The extracts of the plants were prepared as follows: 50% ethanolic extract of the stem (SE) and leaf (LE), and water extract of the stem (SW) and leaf (LW). The antioxidative activities, including α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging effect, reducing power, and antioxidative effect on Fe^{2+}/ascorbate-induced lipid peroxidation in a liposome model system, were studied in vitro. The results showed that all tested extracts had antioxidative characteristics, including the abilities of radical-scavenging, reducing, and lipid peroxidation inhibition. It was found that the antioxidative activities of all the extracts increased with the increase of their concentrations. No mutagenicity effect toward all tester strains (*Salmonella typhimurium* TA97, TA98, TA100, TA102, and TA1535) was found in the extracts of *Zanthoxylum ailanthoides Sieb & zucc.* by means of the Ames test. The results suggested that the *Zanthoxylum ailanthoides Sieb & zucc.* extracts were safe in genotoxicity and were antioxidants.

Keywords: *Zanthoxylum ailanthoides Sieb & zucc.*; Antioxidative activity; Mutagenesis

1. Introduction

Free radicals and other reactive oxygen species (ROS), such as superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), are an entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agents. Oxidative damage to crucial cellular molecules induced by ROS has been implicated as a possible factor in the etiology of several human diseases, including cancer, cardiovascular disease, and aging (Halliwell & Gutteridge, 1998). In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases (Kinsella, Frankel, German, & Kanner, 1993; Pryor, 1991; Terao & Piskula, 1997), and retard lipid oxidative rancidity in foods (Duthie, 1993). The most effective ones seem to be flavonoids and other phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits. Their metal-chelating capabilities and radical-scavenging properties, have enabled phenolic compounds to be thought of as effective free radical scavengers and inhibitors of lipid peroxidation (Bors & Saran, 1987; Miller, 1997; Terao & Piskula, 1997).

Zanthoxylum ailanthoides Sieb & zucc., also called Chhì-chhang or Tana, is a traditional Chinese herbal medicine, belonging to the Rutaceae family. In folk medicine, different tissues, including root, stem, leaves and fruits, of *Z. ailanthoides* have several health benefits, such as myocardium disorder attenuation, bone-injury alleviation, and cold resistance. Little information is available about its antioxidative activity, thus the water and ethanol extracts of *Z. ailanthoides* were used to

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explore antioxidative mechanism in this study. There are many extraction solvents available; for example, methanol is easy to work with in terms of low boiling point (temperature). However, the use of methanol in food material raises the issue of safety. The objectives of this study were to investigate the antioxidative activities of the various extracts from different parts of *Zanthoxylum ailanthoides Sieb & zucc.*, including stems with 50% ethanolic extract (SE), leaves with 50% ethanolic extract (LE), stems water extract (SW), and leaves water extract (LW). The selection of 50% ethanol was because our previous finding showed 50% ethanol was more effective for extracting various food antioxidants than were higher ethanol concentrations (e.g., 95% ethanol) (Chung, Chen, Hsu, Chang, & Chou, 2005). The antioxidative activities, including the radical-scavenging effect, reducing power and antioxidant effect on Fe^{2+}/ascorbate-induced lipid peroxidation in a liposome model system, were evaluated. In addition, results for the extracts were compared with those of α-tocopherol (α-Toc), a natural antioxidant commonly used in the food industry. For increasing of safety consciousness by consumers in food ingredients, the mutagenicity of the extracts from *Zanthoxylum ailanthoides* was also investigated.

2. Material and methods

2.1. Chemicals

α-Tocopherol (α-Toc), butylated hydroxytoluene (BHT), $FeCl_2$, $FeCl_3$, [4,4′-[3-(2-pyridinyl)-1,2,4-triazine-5,6-diyl] bisbenzenesulfonic acid] (ferrozine), trichloroacetic acid, α,α-diphenyl-β-pricryl-hydrazyl (DPPH), potassium ferricyanide, 2-aminoanthramine (2-AA), 2-aminofluoreene (2-AF), dimethyl sulfoxide (DMSO), 4-nitroquinoline-*N*-oxide (4-NQO), glucose-6-phosphate (G-6-P), β-nicotinamide-adenine dinucleotide phosphate (β-NADP), thiobarbituric acid (TBA), and trizma hydrochloride (Tris–HCl) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Phosphatidylcholine (PC) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). All other chemicals were reagent grade or purer.

2.2. Salmonella tester strains

The *Salmonella typhimurium* test strains TA97 (hisD6610/rfa/ΔuvrB/pKM101), TA98 (hisD3052/rfa/ΔuvrB/pKM101), TA100 (hisG46/rfa/ΔuvrB/pKM101), TA102 (hisG428/rfa/ΔuvrB/pKM101, pAQ1), and TA1535 (hisG46/rfa/ΔuvrB), were purchased from the Culture Collection and Research Center, Food Industry Research and Development Institution, Shinchu, Taiwan.

2.3. Preparation of the leaves water extract (LW), stems water extract (SW), leaves 50% ethanolic extract (LE), and stems 50% ethanolic extract (SE) from Zanthoxylum ailanthoides

Plants materials were obtained from the mountain area of Sinshe Township, Taichung, Taiwan. The plant samples, including stem and leaves, were washed and oven-dried at 45 °C overnight. Each 100 grammes of the dried plant tissues were extracted with 500 ml of distilled water at 100 °C or with 50% ethanol at 75 °C for 3 h and decocted three times. The decoctions were filtered, and then dried by a vacuum freeze-dryer. The extraction yields of the LE, SE, LW, and SW were 27.5%, 9.0%, 35.6% and 9.6%, respectively. The extracts were sealed in plastic bottles and stored at −70 °C until used.

2.4. Total phenolic content assay

Total phenol content was analyzed using the Folin-Ciocalteu reagent method (Sato et al., 1996). An aliquot of the extracts (0.5 ml, 0.625 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu's reagent and 0.05 ml of 10% Na_2CO_3, and then the absorbance was measured at 735 nm after a 1 h incubation at room temperature. Gallic acid was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per gramme of tested extracts.

2.5. Measurement of the DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the extracts from *Zanthoxylum ailanthoides* was measured according to the method of Chung, Chang, Chao, Lin, and Chou (2002). An aliquot of the extracts (0.1 ml, 0.08–10.0 mg/ml), or α-Toc (0.02–0.6 mg/ml) was mixed with the 100 mM Tris–HCl buffer (0.4 ml, pH 7.4), and then added to 0.5 ml of 500 μM DPPH in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The capability to scavenge DPPH radical was calculated by the following equation: scavenging effect (%) = [1 − (absorbance of sample at 517 nm/absorbance of control at 517 nm)] × 100%.

2.6. Measurement of reducing power

The reducing powers of the extracts from *Zanthoxylum ailanthoides* and α-Toc were determined according to the method of Yen and Chen (1995). The extracts (0.16–10.0 mg/ml) or α-Toc (0.02–0.6 mg/ml) were mixed with

an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50 °C for 20 min. An equal volume of 1% trichloroacetic acid was added to the mixture to stop the reaction, and then the mixture was centrifuged at 2790*g* for 10 min. The supernatant was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:0.2, and then the absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the absorbance values.

2.7. Measurement of Fe^{2+}-chelating ability

The Fe^{2+}-chelating ability was determined according to the method of Dinis, Madeira, and Almeida (1994). The Fe^{2+} was monitored by measuring the formation of ferrous iron–ferrozine complex. The extracts, or α-Toc (1.25–20.0 mg/ml) were mixed with 2 mM $FeCl_2$ and 5 mM ferrozine at a ratio of 10:1:2. The mixture was shaken and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. A lower absorbance of the reaction mixture indicated a higher Fe^{2+}-chelating ability. The capability to chelate the ferrous iron was calculated by the following equation: chelating effect (%) = [1 − (absorbance of sample at 562 nm/absorbance of control at 562 nm)] × 100%.

2.8. Measurement of antioxidative effect on liposome peroxidation

Liposome peroxidation was induced by Fe^{2+}-ascorbate and aquatinted by malondialdehyde–thiobarbituric acid (MDA–TBA) adduct according to the method described by Liao and Yin with slight modification (2000). Liposome (multi-lamellar vesicles) were prepared from 30 mg PC, 12 mg cholesterol, and 3 mg dicetyl phosphate at 4 °C. The solvents, chloroform and methanol, used for liposome preparation were removed and a lipid film was formed by rotary evaporation under N_2 flush. Then, the liposome was suspended with 10 ml of 50 mM sodium phosphate buffer (pH 7.2). A mixture containing 1 ml of liposome suspension, 0.6 ml of sodium phosphate buffer, 0.05 ml of 25 mM $FeCl_3$, 0.05 ml of 25 mM ascorbic acid, and 0.25 ml of the extracts (0.08–10.0 mg/ml) or α-Toc (0.01–5.0 mg/ml) was incubated for 1 h at 37 °C. After incubation, the solution was mixed with TBA (0.4% in 0.2 M HCl) and BHT (0.2% in 95% ethanol) at a ratio of 1:2:0.3, and then heated at 100 °C for 20 min. After cooling the mixture, an equal volume of *n*-butanol was added to extract the chromogen in the mixture. The absorbance of the *n*-butanol layer was measured spectrophotometrically at 532 nm. The capability to inhibit MDA formation was calculated by the following equation: inhibition effect (%) = [1 − (absorbance of sample at 532 nm/absorbance of control at 532 nm)] × 100%.

2.9. Preparation of culture medium

Nutrient broth was prepared by dissolving 25 g of Oxoid nutrient broth in 1 litre of water. Glucose minimal agar plate (MA plate) contained 1.5% agar, 0.02% $MgSO_4 \cdot 7H_2O$, 0.2% citric acid, 1% K_2HPO_4, 0.35% $NaHNH_4PO_4 \cdot 4H_2O$ and 2% glucose. Top agar contained 0.75% agar and 0.5% NaCl.

2.10. Toxicity test

Sample were prepared with 0.1 ml of 10 h-cultured test strain (approximately 10^8 cells/ml), 0.1 ml of the extracts (0.02–5 mg/plate), 0.1 ml phosphate buffer (0.2 M, pH 7.4), and 0.5 ml of S9 mix, or phosphate buffer. The serial dilutions were immediately made with phosphate buffer, and then 1 ml of the aliquot was mixed with 12 ml of nutrient agar. After incubation at 37 °C for 48 h, the number of colonies was counted. A toxicity effect was confirmed if the standard plate count of the tested compound was lower than that of the no-added-compound control.

2.11. Ames test

Mutagenicity was assayed by the standard Ames test (standard plate incorporation assay) (Maron & Ames, 1983; Mortelmans & Zeiger, 2000). A mixture containing 0.1 ml of the extracts (0.02–5 mg/plate), 0.5 ml of S9 mix or phosphate buffer, 0.2 ml of 0.5 mM histidine–biotin, and 0.1 ml of 10 h-cultured test strain (approximately 10^8 cells/ml), was added to a tube containing 2 ml of top agar. The tube was then gently vortexed and poured onto the MA plate. The extracts were tested with and without S9 mix, and triplicate plates were poured for each dose of extracts. Diagnostic mutagens, including 2-AF (10 μg/plate), 2-AA (20 μg/plate), and 4-NQO (1 μg/plate), were prepared by dissolving in DMSO and served as positive control chemicals. After incubation at 37 °C for 48 h, the number of revertant colonies was counted. A compound was considered a mutagen if there was a twofold increase in the number of revertants, compared with the number of spontaneous revertants (negative control) or a dose-related increase in the number of revertants for one or more strains.

2.12. Statistical analysis

All data were expressed as mean ± standard deviation. Analysis of variance was performed by the ANOVA procedures. Duncan's new multiple-range test was used to determine the difference of means, and $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Total phenol contents

The total phenolic contents of LE, SE, LW and SW were 21.4 ± 0.8, 24.3 ± 1.0, 50.3 ± 0.9 and 59.9 ± 1.6 mg gallic acid equivalent/g, respectively. It was noted that water extracts, including LW and SW, had significant higher total phenol contents than did 50% ethanolic extracts, LE and SE ($P < 0.05$). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (van Acker et al., 1996; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hertog, Hollman, & van de Putte, 1993). In this study, the total phenolic compounds of the various extracts from different tissues of *Zanthoxylum ailanthoides* were found to be in the ranges of 21.4–59.9 mg/g (as gallic acid), and they may cause the antioxidative activities of the *Zanthoxylum ailanthoides* extracts.

3.2. Scavenging effect on DPPH radical

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lai, Chou, & Chao, 2001; Lee, Hwang, Ha, Jeong, & Kim, 2003; Leong & Shui, 2002; Nagai, Inoue, Inoue, & Suzuki, 2003). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. Fig. 1 shows the dose–response curves of DPPH radical-scavenging activities of the four extracts from *Zanthoxylum ailanthoides*. It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. The scavenging effect was in the order: LW (67.0%) >SE (63.2%) ≈LE (62.1%) >SW (43.0%) at a concentration of 1.25 mg/ml ($P < 0.05$). The half-inhibition concentrations (IC_{50}) for DPPH radical-scavenging activity of LW, SE, LE and SW were 0.84 ± 0.05, 0.91 ± 0.06, 0.92 ± 0.06 and 1.12 ± 0.10 mg/ml, respectively. Based on the IC_{50} results, it was also shown that LW had the highest DPPH-scavenging activity, while SW showed the least activity. A comparison with the commercial antioxidant showed that the concentration needed to obtain 67.0% DPPH radical-scavenging activity for α-Toc was 0.24 mg/ml, which was about equal to the scavenging effect of LW at a concentration of 1.25 mg/ml. α-Toc had an IC_{50} value of 0.15 ± 0.01 mg/ml. In other words, to reach a similar extent of DPPH-scavenging effect, the concentrations required for *Zanthoxylum ailanthoides* extracts were significantly higher than that required for α-Toc. Although the DPPH radical-scavenging abilities of the extracts were significantly less than that of α-Toc, it was evident that the extracts did show the hydrogen-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

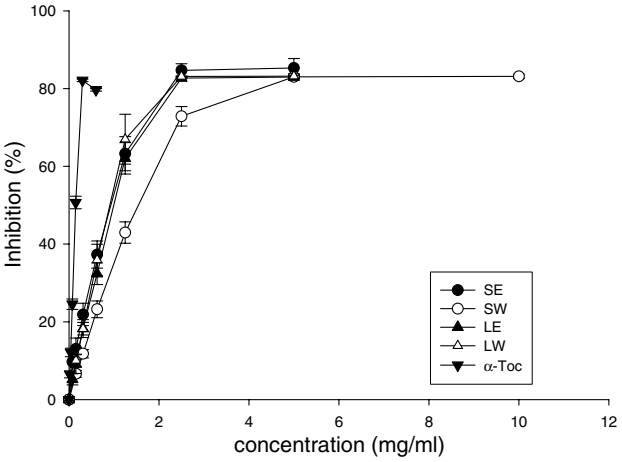

Fig. 1. Scavenging effects of the *Zanthoxylum ailanthoides* extracts on DPPH-radical. Each value represents a mean ± SD ($n = 6$). SE, 50% ethanolic extract of the stem; SW, water extract of the stem; LE, 50% ethanolic extract of the leaf; LW, water extract of the leaf; α-Toc, α-tocopherol.

3.3. Reducing power of Zanthoxylum ailanthoides extracts

In the reducing power assay, the presence of reductants (antioxidants) in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Fig. 2 shows the dose–response curves for the reducing powers of the extracts from *Zanthoxylum ailanthoides*. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. The extracts from *Zanthoxylum ailanthoides*, including LW, LE, SE, and SW, exhibited a good reducing power of 1.03, 0.92, 0.92, and 0.73 at 1.25 mg/ml, respectively. However, the reducing power of α-Toc at 0.3 mg/ml was 0.92. This indicates that to reach a similar extent of reducing power, the concentrations required for *Zanthoxylum ailanthoides* extracts were four to fivefold that required for α-Toc. Though the reductive abilities of the extracts were significantly less than that of α-Toc, it was evident that the extracts from *Zanthoxylum ailanthoides* did show reductive potential and could serve as electron donors, terminating the radical chain reaction (Yen & Chen, 1995).

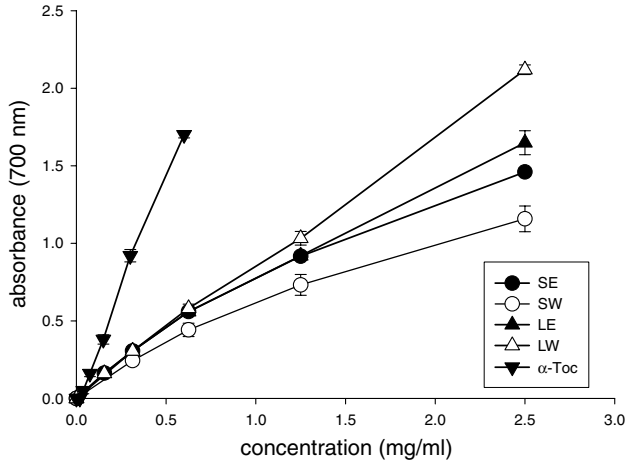

Fig. 2. Reducing powers of the *Zanthoxylum ailanthoides* extracts at different concentrations. Each value represents a mean ± SD ($n = 6$). SE, 50% ethanolic extract of the stem; SW, water extract of the stem; LE, 50% ethanolic extract of the leaf; LW, water extract of the leaf; α-Toc: α-tocopherol.

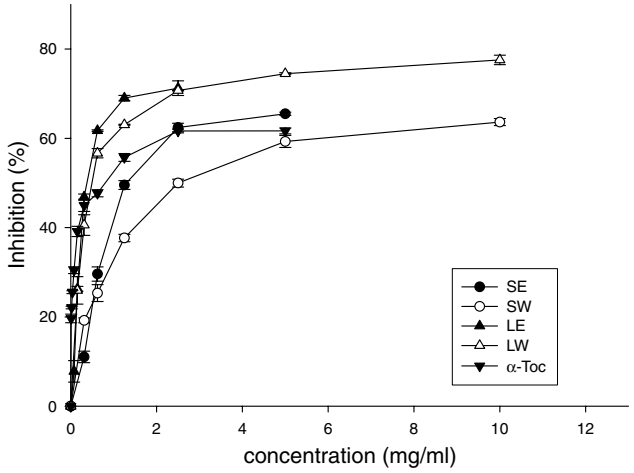

Fig. 3. Inhibition of the *Zanthoxylum ailanthoides* extracts on Fe^{2+}/ascorbic acid-induced lipid peroxidation in a liposome model system. Each value represents a mean ± SD ($n = 6$). SE, 50% ethanolic extract of the stem; SW, water extract of the stem; LE, 50% ethanolic extract of the leaf; LW, water extract of the leaf; α-Toc: α-tocopherol.

3.4. Fe^{2+}-chelating ability of *Zanthoxylum ailanthoides* extracts

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease (Halliwell & Gutteridge, 1990). Because Fe^{2+} also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe^{2+} concentration in Fenton reactions affords protection against oxidative damage. It was also found that the Fe^{2+}-chelating ability of all the extracts also increased with the increase of their concentrations (data not shown). However, all tested extracts exhibited low Fe^{2+}-chelating ability. The 20%-chelating concentrations for Fe^{2+}-chelating ability of SE, LE, SW and LW were 0.48 ± 0.04, 0.71 ± 0.04, 1.46 ± 0.01 and 1.47 ± 0.06 mg/ml, respectively.

3.5. Antioxidant effect on liposome peroxidation

Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction. It has been suggested to be an important event in cellular damage, which is strongly associated with aging, carcinogenesis and other diseases (Halliwell, Gutteridge, & Cross, 1992). In addition, lipid peroxidation also plays an important role in the deterioration of foods during storage (Duthie, 1993). Liposomes, artificial biomembranes, have been used extensively as a model system for in vitro lipid peroxidation studies (Duh, Du, & Yen, 1999; Yin & Cheng, 1998; Tsuda & Shiga, 1996). The antioxidative activity of the four *Zanthoxylum ailanthoides* extracts and α-Toc on MDA formation in Fe^{2+}/ascorbate-mediated lipid peroxidation in the liposome system are shown in Fig. 3. It was also found that the inhibitory effects on the lipid peroxidation of all the tested extracts were concentration-dependent. At the concentration of 1.25 mg/ml, the inhibitory effect of the four extracts followed the order LE ≈ LW > SE > SW ($P < 0.05$). The leaves extracts had a better inhibitory effect than the stem extracts at all concentrations. The IC_{50} values of LE, LW, SE, and SW were 0.37 ± 0.00, 0.47 ± 0.03, 1.32 ± 0.09, and 2.53 ± 0.07 mg/ml, respectively, and the values of LE and LW were even lower than that of α-Toc (0.75 ± 0.06 mg/ml, $P < 0.05$). In other words, the LE and LW exhibited significant higher lipid peroxidation inhibitory activity than that of α-Toc. The mechanism of the inhibitory effects by which the extracts protect against a lipid peroxidation may involve radical-scavenging and reducing capability.

3.6. Toxicity test

If chemicals tested for mutagenicity show toxicity (antimicrobial activity) against the tester strains, an erroneous result could be obtained when the *Salmonella typhimurium* test (Ames test) is employed (Maron & Ames, 1983). A preliminary toxic dose range experiment was performed to determine an appropriate dose range for the mutagenicity assay. Both SW and LW, at the tested concentrations (up to 5 mg/plate), did not show toxicity to the tester strains with or without the metabolic activator (S9 mix). However, in the SE or LE system, with or without S9 mix, an antimicrobial effect (toxicity) toward tester stains was detected when the concentration was above 0.31 mg/plate (data not shown). According to the results of the toxicity test, the highest concentrations of *Zanthoxylum ailanthoides Sieb & zucc.* extracts for the mutagenicity assay were selected as 5 mg/

Table 1
Mutagenicity of water extract from leaves of *Zanthoxylum ailanthoides Sieb & zucc.* toward *Salmonella typhimurium* TA97, TA98, TA100, TA102 and TA1535

Extracts	Revertants colonies (CFU/plate)[a]									
	TA97		TA98		TA100		TA102		TA1535	
	−S9[b]	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Control[c]	49 ± 17	93 ± 0	15 ± 4	18 ± 1	74 ± 19	82 ± 4	304 ± 18	343 ± 10	3 ± 2	7 ± 1
5 (mg/plate)	72 ± 7	91 ± 8	14 ± 3	15 ± 3	84 ± 16	99 ± 8	368 ± 3	388 ± 33	7 ± 3	6 ± 2
2.5 (mg/plate)	69 ± 16	93 ± 6	11 ± 3	15 ± 4	87 ± 5	85 ± 5	292 ± 36	371 ± 8	8 ± 2	8 ± 2
1.25 (mg/plate)	56 ± 8	75 ± 8	11 ± 5	13 ± 1	84 ± 24	90 ± 13	298 ± 12	361 ± 15	6 ± 2	6 ± 2
0.625 (mg/plate)	46 ± 9	81 ± 4	15 ± 2	14 ± 3	75 ± 14	88 ± 13	291 ± 14	345 ± 22	5 ± 4	5 ± 2
0.3125 (mg/plate)	53 ± 11	112 ± 10	18 ± 4	12 ± 2	85 ± 17	73 ± 12	290 ± 11	320 ± 11	6 ± 2	5 ± 2
2-AF[d] (10 μg/plate)	–	1205 ± 178	–	2509 ± 466		1514 ± 65	–	–	–	–
4-NQO[d] (1 μg/plate)	113 ± 8	–	45 ± 12	–	547 ± 34	–	2115 ± 236	–	–	–
2-AA[d] (20 μg/plate)	–	–	–	–	–	–	–	839 ± 126	11 ± 5	94 ± 27

[a] Date are Means ± SD of triplicates.
[b] S9 is a metabolic activation system consisting of the postmitochondrial fraction of the livers of rats.
[c] Negative control: without extract was treated with dideionize water; spontaneous revertants/plate.
[d] Positive controls: without S9: for strain TA97. TA98. TA100. TA102: 1 μg/plate 4-nitroquinoline-*N*-oxide (4-NQO) was used, for strain TA1535: 20 μg/plate 2-aminoanthracene (2-AA) was used; with S9 for strain TA97. TA98. TA100: 10 μg/plate 2-aminoflurene (2-AF) were used, and for strain TA102. TA1535: 20 μg/plate 2-AA were used, respectively.

plate for SW and LW, and 0.31 mg/plate for SE and LE. On the other hand, SE and LE exhibited strong antimcrobial activity toward *S. typhimurium* and this result led us to suppose that the antimicrobial potential of SE and LE were worthy of future study.

3.7. Mutagenicity test

The results of the Ames test conducted to determine the mutagenicities of SW, LW, SE and LE are shown in Tables 1–4, respectively. The numbers of revertants induced by the extracts, for all tester strains, were close to those of the negative control (spontaneous revertants, without extract) and were much lower than those of the positive control (with diagnostic mutagens). The results of the Ames test demonstrated that SW, LW, SE and LE did not have mutagenic effects within the tested dose range.

This study demonstrated that the extracts from *Zanthoxylum ailanthoides Sieb & zucc.* were safe in genotoxicity and showed antioxidant activity. The extracts had radical-scavenger effects, reductive capability for reducing Fe^{3+} to Fe^{2+}, and anti-lipid peroxidation activity in a liposome system. Further studies are needed to evaluate the in vivo antioxidative potential of these extracts in animal models and to isolate the antioxidative components in *Zanthoxylum ailanthoides Sieb & zucc.*

Table 2
Mutagenicity of water extract from stem of *Zanthoxylum ailanthoides Sieb & zucc.* toward *Salmonella typhimurium* TA97, TA98, TA100, TA102 and TA1535

Extracts	Revertants colonies (CFU/plate)[a]									
	TA97		TA98		TA100		TA102		TA1535	
	−S9[b]	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Control[c]	49 ± 17	93 ± 0	15 ± 4	18 ± 1	74 ± 19	82 ± 4	304 ± 18	343 ± 10	3 ± 2	7 ± 1
5 (mg/plate)	50 ± 30	120 ± 8	15 ± 4	18 ± 6	77 ± 8	80 ± 10	328 ± 30	359 ± 14	5 ± 3	6 ± 2
2.5 (mg/plate)	46 ± 15	128 ± 6	10 ± 3	19 ± 5	83 ± 4	93 ± 14	314 ± 25	362 ± 12	4 ± 1	6 ± 3
1.25 (mg/plate)	57 ± 21	135 ± 8	16 ± 2	16 ± 3	81 ± 13	83 ± 18	302 ± 44	355 ± 39	8 ± 0	7 ± 1
0.625 (mg/plate)	49 ± 22	143 ± 4	14 ± 2	20 ± 2	75 ± 5	95 ± 11	290 ± 26	329 ± 17	6 ± 2	5 ± 1
0.3125 (mg/plate)	65 ± 30	122 ± 10	12 ± 4	16 ± 4	72 ± 17	100 ± 8	272 ± 4	309 ± 14	8 ± 1	7 ± 2
2-AF[d] (10 μg/plate)	–	–	1205 ± 178	–	2509 ± 466	1514 ± 65	–	–	–	–
4-NQO[d] (1 μg/plate)	113 ± 8	–	45 ± 12	–	547 ± 34	–	2115 ± 236	–	–	–
2-AA[d] (20 μg/plate)	–	–	–	–	–	–	–	839 ± 126	11 ± 5	94 ± 27

[a] Date are Means ± SD of triplicates.
[b] S9 is a metabolic activation system consisting of the postmitochondrial fraction of the livers of rats.
[c] Negative control: without extract was treated with dideionize water; spontaneous revertants/plate.
[d] Positive controls: without S9: for strain TA97. TA98. TA100. TA102: 1 μg/plate 4-nitroquinoline-*N*-oxide (4-NQO) was used, for strain TA1535: 20 μg/plate 2-aminoanthracene (2-AA) was used; with S9 for strain TA97. TA98. TA100: 10 μg/plate 2-aminoflurene (2-AF) were used, and for strain TA102. TA1535: 20 μg/plate 2-AA were used, respectively.

Table 3
Mutagenicity of 50% ethanolic extract from leaves of *Zanthoxylum ailanthoides Sieb & zucc.* toward *Salmonella typhimurium* TA97, TA98, TA100, TA102 and TA1535

Extracts	Revertants colonies (CFU/plate)[a]									
	TA97		TA98		TA100		TA102		TA1535	
	−S9[b]	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Control[c]	29 ± 2	67 ± 12	10 ± 1	18 ± 3	82 ± 8	72 ± 5	219 ± 17	269 ± 13	7 ± 1	7 ± 2
0.3125 (mg/plate)	43 ± 5	63 ± 7	9 ± 2	11 ± 4	53 ± 10	81 ± 12	230 ± 12	294 ± 11	5 ± 2	4 ± 2
0.1563 (mg/plate)	45 ± 6	65 ± 10	10 ± 3	12 ± 2	73 ± 6	85 ± 7	231 ± 18	282 ± 15	8 ± 3	5 ± 2
0.0781 (mg/plate)	64 ± 12	72 ± 9	12 ± 3	13 ± 6	69 ± 3	83 ± 9	216 ± 9	258 ± 6	3 ± 1	9 ± 5
0.0361 (mg/plate)	65 ± 13	98 ± 16	11 ± 1	10 ± 1	76 ± 8	81 ± 4	206 ± 8	278 ± 4	10 ± 2	5 ± 3
0.0195 (mg/plate)	52 ± 9	99 ± 14	11 ± 4	12 ± 2	82 ± 11	86 ± 3	232 ± 8	268 ± 16	4 ± 2	7 ± 2
2-AF[d] (10 μg/plate)	–	1032 ± 143	–	2962 ± 800	930 ± 99	–	–	–	–	
4-NQO[d] (1 μg/plate)	77 ± 16	–	45 ± 12	–	1103 ± 61	–	1947 ± 221	–	–	–
2-AA[d] (20 μg/plate)	–	–	–	–	–	–	–	998 ± 28	7 ± 3	78 ± 4

[a] Date are Means ± SD of triplicates.
[b] S9 is a metabolic activation system consisting of the postmitochondrial fraction of the livers of rats.
[c] Negative control: without extract was treated with DMSO; spontaneous revertants/plate.
[d] Positive controls: without S9: for strain TA97. TA98. TA100. TA102: 1 μg/plate 4-nitroquinoline-*N*-oxide (4-NQO) was used, for strain TA1535: 20 μg/plate 2-aminoanthracene (2-AA) was used; with S9 for strain TA97. TA98. TA100: 10 μg/plate 2-aminoflurene (2-AF) were used, and for strain TA102. TA1535: 20 μg/plate 2-AA were used, respectively.

Table 4
Mutagenicity of 50% ethanolic extract from stem of *Zanthoxylum ailanthoides Sieb &zucc.* toward *Salmonella typhimurium* TA97, TA98, TA100, TA102 and TA1535

Extracts	Revertants colonies (CFU/plate)[a]									
	TA97		TA98		TA100		TA102		TA1535	
	−S9[b]	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Control[c]	29 ± 2	67 ± 12	10 ± 1	18 ± 3	82 ± 8	72 ± 5	219 ± 17	269 ± 13	7 ± 1	7 ± 2
0.3125 (mg/plate)	22 ± 3	73 ± 11	11 ± 1	10 ± 3	64 ± 6	92 ± 4	219 ± 4	271 ± 14	8 ± 3	4 ± 2
0.1563 (mg/plate)	32 ± 9	78 ± 13	9 ± 2	15 ± 4	63 ± 10	80 ± 3	204 ± 11	276 ± 4	4 ± 1	7 ± 4
0.0781 (mg/plate)	57 ± 5	96 ± 4	12 ± 4	18 ± 5	72 ± 9	85 ± 5	208 ± 4	254 ± 15	7 ± 0	6 ± 2
0.0361 (mg/plate)	57 ± 6	83 ± 4	10 ± 2	16 ± 4	63 ± 8	86 ± 2	227 ± 12	274 ± 24	6 ± 2	4 ± 2
0.0195 (mg/plate)	34 ± 9	89 ± 16	9 ± 1	17 ± 5	72 ± 13	88 ± 11	220 ± 19	270 ± 28	5 ± 1	6 ± 3
2-AF[d] (10 μg/plate)	–	1032 ± 143	–	2962 ± 800	930 ± 99	–	–	–	–	
4-NQO[d] (1 μg/plate)	77 ± 16	–	45 ± 12	–	1103 ± 61	–	1947 ± 221	–	–	–
2-AA[d] (20μg/plate)	–	–	–	–	–	–	–	998 ± 28	7 ± 3	78 ± 4

[a] Date are Means ± SD of triplicates.
[b] S9 is a metabolic activation system consisting of the postmitochondrial fraction of the livers of rats.
[c] Negative control: without extract was treated with DMSO; spontaneous revertants/plate.
[d] Positive controls: without S9: for strain TA97. TA98. TA100. TA102: 1 μg/plate 4-nitroquinoline-*N*-oxide (4-NQO) was used, for strain TA1535: 20 μg/plate 2-aminoanthracene (2-AA) was used; with S9 for strain TA97. TA98. TA100: 10 μg/plate 2-aminoflurene (2-AF) were used, and for strain TA102. TA1535: 20 μg/plate 2-AA were used, respectively.

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