Contributions of Major Components to the Antimutagenic Effect of Hsian-tsao (*Mesona procumbens* Hemsl.)

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Our aim was to determine the antimutagenic activity of various solvent extracts from an herb *Mesona procumbens* Hemsl, normally called Hsian-tsao in China. We also investigated the relationships between the special components in the water extract of Hsian-tsao (WEHT) and the antimutagenic activity. It was found that the extracts at 0−0.6 mg/plate from three solvents (water, methanol, and ethyl acetate) exhibited a dose-dependent antimutagenic effect against benzo[*a*]pyrene [B(a)P] and 2-amino-3-methylimidazo(4,5-*f*)quinoline (IQ), both are indirect mutagens in Salmonella tester strains TA98 and TA100. The WEHT from three different plantations revealed a similar inhibitory effect on the mutagenicity of IQ in TA 98 at 2.5−5.0 mg/plate. The inhibitory effect of WEHT on the mutagenicity of IQ correlates with their polyphenol and ascorbic acid contents but not with their chlorophyll contents. These findings suggest that the antimutagenicity activity of WEHT may be attributed mainly to their polyphenolic compounds and ascorbic acid.

Keywords: *Hsian-tsao; polyphenolic compounds; ascorbic acid; antimutagenic effect; 2-amino-3-methylimidazo(4,5-f)quinoline (IQ)*

INTRODUCTION

Human cancer is closely related to daily food. Numerous mutagens from some foods have been detected, causing remarkable concern about their actual involvement in carcinogenesis, whereas antimutagenic effects have also been observed in the same food (*1*). Concern about the role of diet in human cancer has prompted the search for compounds in common foods that may act as antimutagens. Some antimutagenic substances have been found from natural sources (*2*−*5*).

Mesona procumbens Hemsl, called Hsian-tsao in China, has been used as a folk medicine and as a beverage for many centuries. Kim et al. (*6*) reported that some of pharmaceutical effect of herbs could be related to their possible antioxidant activities. Moreover, some researchers reported that diverse compounds naturally occurring in food could exert an antimutagenic effect due to their antioxidant capacity. The water extracts and ethanolic extracts of Hsian-tsao have been demonstrated to exhibit the antioxidant activity (*7*). No studies, however, have been conducted to investigate the antimutagenic activity of Hsian-tsao. Hence, it is not clear whether Hsian-tsao contains antimutagenic activity or has any toxic side effects. Thus, the objectives of this paper were (i) to determine the antimutagenic activity of Hsian-tsao and (ii) to find the correlation between antimutagenic activity of Hsian-tsao and its major components.

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MATERIALS AND METHODS

Materials. The dried Hsian-tsao (HT) obtained from local oriental herbal stores in Taichung, Taiwan, was cut into pieces and ground into a fine powder in a mill. The powder (20 g) was extracted with boiling water (400 mL) for 30 min and filtered with filter paper. The filtrate was freeze-dried and weighed to determine the yield of soluble constituents. In addition, each HT powder (20 g) was extracted overnight with 400 mL of methanol or ethyl acetate, respectively. The extracts were filtered with filter paper. The filtrates were evaporated to dryness in vacuo and weighed to determine the yield of soluble constituents. The soluble materials prepared were dissolved with dimethyl sulfoxide (DMSO) or sterilized water and filtered with 0.45-μm Millipore filter paper. The filtrate was diluted, and each sample at an approximate quantity was used for the determination of mutagenicity, antimutagenicity, and chemical analyses.

Determination of Ascorbic Acid. Determination of ascorbic acid content was according to the method of Klein and Perry (*8*). Extracts at 0.05 g or dried HT powder at 0.5 g was extracted with 10 mL of 1% metaphosphoric acid. After being filtered, the filtrate (1 mL) was added to 9 mL of 2,6-dichloroindophenol (DIP), and the absorbance at 515 nm was read with a Hitach model 2000 spectrophotometer.

Determination of Chlorophyll. Determination of chlorophyll content was based on the method of Canjura and Schwartz (*9*). Extracts or dried HT powder (0.5 g) was extracted with 10 mL of acetone (or 5 mL of acetone for dried HT powder). The extraction was filtered with 0.45-μm Millipore filter paper. The filtrate was injected onto the HPLC column. HPLC was performed with a Hitachi Liquid Chromatograph (Hitachi, Ltd., Tokyo, Japan), consisting of a model L-6200 pump, a Rheodyne model 7125 syringe loading sample injector, a model L-4200 UV−vis detector set at 658 nm, and a model integrator. A Lichorspher RP-18 reversed-phase column (5 μm, 250 × 4 mm i.d., E. Merck, Darmstadt, Germany) was used for analysis. The volume injected was 10 μL. The elution solvents were A and B containing ethyl

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acetate−methanol−water (15:65:20, v/v/v) and ethyl acetate−methanol−water (61:30:10, v/v/v), respectively. The gradient elution program was set at 0.8 mL/min, and the gradient profiles were as follows: 0−3 min, 100% A; 3−6 min, 70% A; 6−8 min, 70−50% A; 8−9 min, 50−0% A; 9−24 min, 0% A; 24−28 min, 0−100% A; 28−36 min, 100% A.

To determine chlorophyll a, chlorophyll b, pheophytin a, and pheophytin b, the authentic samples of these compounds were prepared; dilutions were made so that the range of concentration correlated with the estimated content of chlorophyll a, chlorophyll b, pheophytin a, and pheophytin b in the samples. The contents of these compounds in the samples were calculated from the standard curve of chlorophyll a, chlorophyll b, pheophytin a, and pheophytin b. Triplicate samples were run for each set.

Determination of Tocopherols. Determination of tocopherol content was based on the method of Carpenter (*10*). Extracts of HT (0.5 g) or dried HT powder (1 g) were added to 6 mL of 6% pyrogallol in ethanol and 4 mL of 60% potassium hydroxide. The mixture was heated at 70 °C in a water bath for 20 min. After the mixture was heated, 15 mL of H_2O was added and then exrtacted with 15 mL of *n*-hexane. The layer of *n*-hexane phase was washed until its pH reached 7.0 and then dehydrated with anhydrous sodium sulfate. The solution prepared was filtered and evaporated to dryness in vacuo and then dissolved in 5 mL of *n*-hexane. After being filtered, the filtrate was injected onto the HPLC column. The chromatographic separation was performed on a Lichrosorb Si-60 column (5 μm, 250 × 4 mm i.d., E. Merck, Darmstadt, Germany) with a mobile phase of *n*-hexane−2-propanol−ethanol (100:0.3:0.2, v/v/v) at a flow rate 0.7 mL/min. A model L-4200 UV−vis detector was set at 295 nm.

Determination of β-Carotene. β-Carotene in the extract of HT was determined by the method of Kitada at al (*11*). Extracts at 0.2 g were added to 1.0% of 10 mL of pyrogallol [in methanol−dichloromethane (1:1, v/v)]. After being filtered, the filtrates were finally quantified to 10 mLwith glass-distilled water; the filtrates were filtered with 0.45-μm Millipore filter paper and injected onto the HPLC column. The HPLC was performed with a Hitachi liquid chromatograph, consisting of a model L-6200 pump, and a model L-4200 UV−vis detector set at 470 nm. A Lichrospher RP-18 (5 μm, 250 × 4 mm, i.d., E Merck, Darmstadt, Germany) was used for analyses. The mobile phase was acetone−methanol−acetonitrile (1:2:2, v/v/v) at a flow rate 0.7 mL/min.

Determination of Total Polyphenolic Compounds. The concentration of phenolic compounds was measured according to the method of Taga et al. (*12*) and calculated using gallic acid as standard. A sample (0.1 mL) was added to 2.0 mL of 2.0% Na_2CO_3. After 2 min, 50% Folin-Ciocalteu reagent (100 μL) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer.

Mutagenicity Assay. The mutagenicity of extracts was tested according to the Ames test with a 20-min first incubation at 37 °C (*13*). The histidine-requiring strains of *Salmonella typhimurium* TA98 and TA100 were kindly supplied by Dr. B. N. Ames (University of California, Berkeley). The S9 mix (Organ Teknika Co., Switzerland) was prepared from Sprague−Dawley male rats treated with Aroclor 1254. Diluted WEHT (0.1 mL) was added to the overnight cultured S. *typhimurium* TA98 or TA100 (0.1 mL) and S9 mix (0.5 mL) or phosphate buffer (0.1 mL) in place of the S9 mix. The entire mixture was incubated at 37 °C for 20 min before molten top agar (2 mL) was added; the mixture was counted after incubating at 37 °C for 48 h. Each sample was assayed in triplicate plates per run, and data presented are means ± SD of at least two experiments. To examine the toxic effect of WEHT on *S. typhimurium* TA98 and TA100, the mixtures after incubation were diluted with phosphate buffer, and the diluted mixtures were poured into nutrient agar plates that contained $MgCl_2$, KCl, glucose-6-phosphate, and NADP. The plates were incubated at 37 °C for 2 days, and the number of colonies was counted.

Antimutagenic Activity Assay. The antimutagenic effect of extract was assayed according to the Ames method except for the addition of mutagen before incubation (*13*). The mutagens used were NQNO (0.1 μg/plate for TA98 and 1.0 μg/plate for TA100); direct-acting mutagen, IQ (0.1 μg/plate for TA98 and 0.5 μg/plate for TA100); and B(a)P (5.0 μg/plate for TA98 and TA100), which required S9 mix for metabolic activation. Mutagen (0.1 mL) was added to the mixture of a strain (TA98 or TA100), and WEHT was added with the S9 mix for IQ and B(a)P or with phosphate buffer (0.1M, pH 7.4) for NQNO. The mutagenicity of each mutagen in the absence of WEHT is defined as 100%. A smaller percentage of revertants of the sample to the revertants of the control means a stronger antimutagenicity of the sample.

Statistical Analysis. Analysis of variance was performed by ANOVA procedures. Statistical differences at $P < 0.05$ were considered to be significant. The correlations between the antimutagenicity and the major components of Hsian-tsao were calculated as prescribed by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Yield of Various Solvent Extracts from Hsian-tsao. The extent of yields followed in the order of water extents (15.0%) > methanol extract (6.0%) > ethyl acetate extracts (2.7%). The color of water extracts of HT is deep brown; meanwhile, that of methanol and ethyl acetate extracts are green, indicating that the color of extracts of HT varies with various solvent extractions. This means that the components extracted with water may be different from that of the other two organic solvents.

Antimutagenicity of Hsian-tsao Extracts. Some phenolic compounds have been displayed to have antimicrobial activity. Yen and Lii (*14*) reported that if mutagenicity occurred in a tested sample, the results of antimutagenic assay would be influenced and confused thereafter due to increased or decreased numbers of revertants of TA98 and TA100. Therefore, the mutagenicity and toxicity of HT must be assayed before testing the antimutagenicity of HT. In the range of 0.2−0.6 mg/plate, no mutagenic activity and no toxicity in various solvent extracts of HT were observed toward TA98 or TA100 with and without S9 activation (data not shown). Hence, the dose with 0.2−0.6 mg/plate was selected for the antimutagenic assay. The antimutagenic activities of various solvent extracts from HT on IQ toward *S. typhimurium* TA98 and TA100 are shown in Table 1. The inhibitory effect of extracts at 0.2−0.6 mg/plate on the mutagenicity of IQ toward S. *typhimurium* TA98 and TA100 was dose-dependent. The water extracts of Hsian-tsao (WEHT) at 0.2−0.6 mg/plate showed a weakly inhibitory effect (20−27%) to IQ. Methanol extract and ethyl acetate extract at 0.6 mg/plate showed 76 and 64% inhibitory effect toward TA98 and 70 and 72% inhibitory effect toward TA100. Obviously, the inhibitory pattern of methanol extract may be similar to that of ethyl acetate extract but is significantly ($P < 0.05$) different from that of water extracts. This may be due to the different components between water extracts and both solvent extracts.

The antimutagenic activities of various solvents extracts from HT on B(a)P toward *S. typhimurium* TA98 and TA100 are shown in Table 2. The inhibitory effect of water extracts on the mutagenicity of B(a)P toward *S. typhimurium* TA98 and TA100 increased with increasing amounts of water extracts. A quantity of 0.6 mg/plate of water extracts was inhibited by 39 and 45% mutagenicity of B(a)P toward TA98 and TA100, respec-

Table 1. Inhibitory Effect of Various Solvent Extracts from Hsian-tsao on the Mutagenicity of IQ to *S. typhimurium* TA98 and TA100

sample[a]	dose (mg/plate)	his$^+$ revertants/plate[b] (% of inhibition)[c] TA98	TA100
water extracts	0.2	1829 ± 54 (20)	619 ± 47 (43)
	0.4	1825 ± 5 (20)	470 ± 24 (60)
	0.6	1681 ± 96 (27)	351 ± 28 (74)
	control[d]	2281 ± 149	997 ± 71
	spontaneous revertants[e]	45 ± 4	118 ± 7
methanol extracts	0.2	1137 ± 10 (48)	341 ± 29 (47)
	0.4	1019 ± 75 (54)	289 ± 22 (60)
	0.6	545 ± 21 (76)	246 ± 17 (70)
ethyl acetate extracts	0.2	1335 ± 102 (39)	344 ± 18 (46)
	0.4	880 ± 42 (60)	252 ± 21 (68)
	0.6	797 ± 107 (64)	240 ± 16 (72)
	control	2150 ± 24	534 ± 33
	spontaneous revertants	47 ± 4	122 ± 8

[a] Water extracts resuspended in water; methanol extracts and ethyl acetate extracts resuspended in DMSO; the concentration of IQ was 0.1 μg/plate for TA98 and 0.5 μg/plate for TA100. [b] Data are means ± SD of three plates. [c] Inhibition (%) = [1 − (no. of his$^+$ revertants in the presence of sample − no. of spontaneous revertants)/(no. of his$^+$ revertants in the absence of sample − no. of spontaneous revertants)] ×100. [d] The control was with mutagen but without Hsian-tsao extracts. [e] The number of spontaneous revertants was determined without Hsian-tsao extracts and mutagen.

Table 2. Inhibitory Effect of Various Solvent Extracts from Hsian-tsao on the Mutagenicity of B(a)P to *S. typhimurium* TA98 and TA100

sample[a]	dose (mg/plate)	his$^+$ revertants/plate[b] (% of inhibition)[c] TA98	TA100
water extracts	0.2	245 ± 29 (23)	389 ± 20 (19)
	0.4	236 ± 17 (27)	377 ± 32 (23)
	0.6	204 ± 6 (39)	304 ± 22 (45)
	control[d]	303 ± 10	451 ± 35
	spontaneous revertants[e]	50 ± 6	122 ± 9
methanol extracts	0.2	242 ± 11 (18)	212 ± 19 (72)
	0.4	183 ± 12 (42)	232 ± 7 (66)
	0.6	125 ± 14 (66)	215 ± 23 (71)
ethyl acetate extracts	0.2	203 ± 10 (34)	191 ± 13 (77)
	0.4	196 ± 10 (37)	207 ± 15 (73)
	0.6	165 ± 13 (49)	210 ± 15 (72)
	control	286 ± 15	473 ± 31
	spontaneous revertants	40 ± 5	108 ± 6

[a] Water extracts resuspended in water; methanol extracts and ethyl acetate extracts resuspended in DMSO; the concentration of B[a]P was 5.0 μg/plate for TA98 and TA100. [b] Data are means ± SD of three plates. [c] Inhibition (%) = [1 − (no. of his$^+$ revertants in the presence of sample − no. of spontaneous revertants)/(no. of his$^+$ revertants in the absence of sample − no. of spontaneous revertants)] ×100. [d] The control was with mutagen but without Hsian-tsao extracts. [e] The number of spontaneous revertants was determined without Hsian-tsao extracts and mutagen.

tively. As for the other two solvent extracts, methanol extracts at 0.6 mg/plate showed 66 and 71% inhibitory activity on B(a)P toward TA98 and TA100; meanwhile, ethyl acetate at 0.6 mg/plate showed 49% and 72% inhibitory effect toward TA98 and TA100. Thus, from the data presented, methanol extracts displayed the strongest inhibitory activity on the mutagenicity of B(a)P toward *S. typhimurium* TA98. However, ethyl acetate extracts showed the strongest inhibitory activity on the mutagenicity of B(a)P toward *S. typhimurium* TA100.

NQNO is a directing mutagen; however, various solvent extracts from HT show no inhibitory effect on the mutagenicity of NQNO toward *S. typhimurium* TA98 and TA100 (data not shown), indicating that these solvent extracts from HT definitely inhibited the mutagenicity of indirect mutagens such as IQ and B(a)P but not direct mutagens. This statement is in accordance with the results of many authors (*15*) that have established that all antimutagens, naturally occurring in food, cannot inhibit the mutagenicity of NQNO. According to the data obtained, various solvent extracts satisfactorily inhibited the mutagenicity of IQ and B(a)P toward *S. typhimurium* TA98 or TA100.

Table 3. pH, Yield, Contents of Ascorbic Acid and Polyphenolic Compounds in Hsian-tsao, and Its Water Extracts from Different Sources[a]

	water extracts[b]		ascorbic acid		polyphenolic compds	
sources	pH	yield (g)	water extracts[b] (mg/g)	Hsian-tsao[c] (μg/g)	water extracts (mg/g)	Hsian-tsao (mg/g)
Hua-lian	6.6$^{B\,d}$	3.0^C	6.6^A	130.7^A	238.7^A	193.5^B
Jia-yih	5.7^C	3.1^B	6.5^A	135.0^A	215.7^B	286.2^A
Guan-shi	7.2^A	3.2^A	3.2^B	113.0^A	144.3^C	254.8^A

[a] Values are means of three replicate analyses. [b] Hsian-tsao (20 g) was extracted with boiled water (400 mL) for 30 min, and the filtrate was freeze-dried. [c] Content in Hsian-tsao based on a wet-weight basis. [d] Data bearing different superscript letters in the same column were significantly different ($P < 0.05$).

According to the data presented, the extracts from various solvents demonstrated remarkable antimutagenic action against two indirect mutagens. From a toxicological point of view, however, WEHT is more safe and useful than the other two solvents extractions. Moreover, WEHT is used as processed drinks or as medicine. Hence, we focused on the use of the WEHT in the following study.

Table 4. Chlorophylls Content of Hsian-tsao and Its Water Extracts from Different Sources[a]

	chlorophyll a		chlorophyll b		pheophytin a		pheophytin b		total	
sources	water extracts[b] (μg/g)	Hsian-tsao[c] (μg/g)	water extracts (μg/g)	Hsian-tsao (μg/g)	water extracts (μg/g)	Hsian-tsao (μg/g)	water extracts (μg/g)	Hsian-tsao (μg/g)	water extracts (μg/g)	Hsian-tsao (μg/g)
Hua-lian	$5.1^{A\,d}$	187.3^{B}	2.3^{A}	90.5^{C}	4.7^{B}	37.1^{C}	0.9^{B}	2.5^{C}	12.9^{B}	317.5^{C}
Jia-yih	4.8^{A}	285.2^{B}	1.0^{A}	145.0^{B}	13.4^{A}	75.7^{B}	3.3^{A}	8.7^{A}	22.5^{A}	514.7^{B}
Guan-shi	4.8^{A}	726.6^{A}	2.4^{A}	364.3^{A}	6.5^{B}	108.2^{A}	0.4^{B}	4.7^{B}	14.1^{B}	1203.8^{A}

[a] Values are means of three replicate analyses. [b] Hsian-tsao (20 g) was extracted with boiled water (400 mL) for 30 min, and the filtrate was freeze-fried. The yields of water extracts of Hsian-tsao from Hua-lian, Jia-yih, and Guan-shi were 3.0, 3.1, and 3.2 g, respectively. [c] Content in Hsian-tsao based on a wet-weight basis. [d] Data bearing different superscript letters in the same column were significantly different ($P < 0.05$).

Table 5. Contents of β-Carotene and Tocopherols in Hsian-tsao from Different Sources[a]

	β-carotene	tocopherols (μg/g)[b]			
sources	(mg/g)[b]	α-	β-	γ-	δ-
Hua-lian	$0.21^{C\,c}$	51.5^{A}			
Jia-yih	0.29^{A}	14.6^{C}	4.4^{B}		33.2
Guan-shi	0.22^{B}	42.0^{B}	11.5^{A}		

[a] Values are means of three replicate analyses. [b] Content in Hsian-tsao based on a wet-weight basis. [c] Data bearing different superscript letters in the same column were significantly different ($P < 0.05$).

Yield and Major Components in Water Extracts of Hsian-tsao from Different Sources. In theory, the compositions of plants may change with climate, environment, methods of plantation, etc. Hence, investigation of the antimutagenic activity of the WEHT from different regions is needed. Table 3 shows the yields and pH of WEHT from different sources. Of the three samples tested, Guan-shi produced the highest yield (16.2%) followed by Jia-yih and Hua-lian. The pH were 6.6, 5.7, and 7.2 for Hua-lian, Jia-yih, and Guan-shi, respectively. These indicate that the cause of the different yields and pH among three different sources may be a result of plantation as well as different growing areas.

Table 3 also shows the contents of ascorbic acid and polyphenolic compounds in HT and water extracts of HT. As for the WEHT, the contents of ascorbic acid in WEHT were observed more in Hua-lian and Jia-yih, but there was no significant difference ($P > 0.05$) observed between them. As for HT, no significant difference in the contents of ascorbic acid was found among the three samples. As shown in Table 3, the greatest contents of polyphenolic compounds in water extracts were found in Hua-lian, followed by Jia-yih and Guan-shi. Meanwhile, as for Hsian-tsao, no significant difference in contents of phenolic compounds was found between Jia-yih and Guan-shi ($P > 0.05$), which were more than that of Hua-lian. The contents of phenolic compounds in water extracts of Guan-shi were significanly ($P < 0.05$) less than other sources. Laurena et al. (*16*) noted that the reduction of phenolic compounds in *Vigna unguiculata* L. correlated with the treatment of basic salt. Hence, we deem that the cause of reduction of phenolic compounds of Guan-shi may be a result of its higher pH value. In general, the contents of phenolic compounds in WEHT were different from that of HT, indicating that the differences may result from cultivar and water extraction. Yoshikawa et al. (*3*) reported that fractions, suggesting phenolic compounds, isolated from Japanese eggplant showed marked antimutagenic effect. Moreover, phenolic compounds of Japanese eggplant reduced mutation induced by heterocyclic amines (*17*). Luteolin, a phenolic compound isolated from plants, inhibited the mutagenicity of Trp-p-2. Therefore, the marked antimatagenic activity of WEHT may be concerned with its greater amount of phenolic compounds.

Table 4 shows the chlorophyll content in HT and in water extracts from different sources. Chlorophyll a content of water extracts from various sources varied from 4.8 to 5.1 μg/g and was not significant difference ($P > 0.05$) among the three samples. As for HT, no significant difference ($P > 0.05$) in the contents of chlorophyll a was found between Hua-lian and Jia-yih, but the chlorophyll a contents in both Hua-lian and Jia-yih are significantly ($P < 0.05$) less than that of Guan-shi. The amounts of pheophytin a and b in water extract of HT from Jia-yih was the greatest among the three samples. If chlorophyll is placed in an acid environment, it loses the Mg^{2+} ion from the phorbine ring and forms pheophytin. The pH of the water extracts of Jia-yia is 5.7, the lowest among the three samples, may explain the highest amounts of pheophytins a and b. Many researchers (*2*, *3*) reported that chlorophyll and its derivaties exhibited inhibitory effect on genetic toxicity.

Table 6. Effects of Hsian-tsao Extracts from Various Sources on the Mutagenicity of IQ toward *S. typhimurium* TA98[a]

sources	his$^+$ revertants/plates[b] (% of inhibition)[c]		
(mg/plate)	Jia-yih	Guan-shi	Hua-lian
0.2	$2443 \pm 224\ (9)^{B\,d}$	$2123 \pm 121\ (22)^{C}$	$2093 \pm 65(23)^{C}$
0.4	$1917 \pm 85(29)^{D}$	$2015 \pm 150\ (26)^{CD}$	$1236 \pm 34(55)^{F}$
0.6	$1489 \pm 51(46)^{E}$	$1591 \pm 46\ (42)^{E}$	$728 \pm 40(74)^{G}$
1.25	$537 \pm 62\ (81)^{H}$	$487 \pm 42\ (83)^{H}$	$139 \pm 6\ (96)^{I}$
2.5	$159 \pm 12(96)^{I}$	$147 \pm 18\ (96)^{I}$	$46 \pm 5\ (100)^{I}$
5.0	$56 \pm 6(100)^{I}$	$55 \pm 5(100)^{I}$	$35 \pm 3\ (100)^{I}$
control[e]	2694 ± 58^{A}		
spontaneous revertants[f]	45 ± 3		

[a] The concentration of IQ was 0.1 μg/plate for TA98. [b] Results are presented as means ± SD of three plates. [c] Inhibition (%) = [1 − (no. of his$^+$ revertants in the presence of Hsian-tsao extracts − no. of spontaneous revertants)/(no. of his$^+$ revertants in the absence of Hsian-tsao extracts − no. of spontaneous revertants)] × 100. [d] Data bearing different superscript letters in the same column were significantly different ($P < 0.05$). [e] The control was with mutagen but without Hsian-tsao extracts. [f] The number of spontaneous revertants was determined without Hsian-tsao extracts and mutagen.

Table 7. Correlation between Antimutagenicity toward *S. typhimurium* TA98 and Ascorbic Acid, Polyphenolic Compounds, and Chlorophylls Content of Hsian-tsao Water Extracts

	antimutagenicity[a]
ascorbic acid	0.430 [b] (0.248)[c]
polyphenolic compounds	0.756 (0.018)
chlorophyll a	0.302 (0.428)
chlorophyll b	0.222 (0.566)
pheophytin a	−0.543 (0.130)
pheophytin b	−0.227 (0.556)
total chlorophylls	−0.419 (0.260)

[a] Antimutagenicity of Hsian-tsao extracts from different sources against the mutagenicity of IQ toward *S. typhimurium* TA98; the concentration of IQ was 0.1 μg/plate for TA98. [b] Correlation coefficients. [c] Probability. Statistical correlation at $P < 0.05$ was considered to be significant.

Hence, in the present study, it is necessary to elucidate whether chlorophyll exhibits the antimutagenic effect or not.

Table 5 shows the contents of β-carotene and tocopherols in HT from different sources. The amount of β-carotene in Jia-yih was the greatest among the three samples, followed by Guan-shi and Hua-lian. Also, tocopherol existed in HT, and a significant difference ($P < 0.05$) in the tocopherol contents was observed among the samples. β-Carotene and tocopherol, however, were not detectable in the WEHT (data not shown). This may be a result of the hydrophobic properties of β-carotene and tocopherol so that β-carotene and tocopherol did not exist in WEHT. Therefore, we deem that the antimutagenic activity of WEHT could not attribute to β-carotene and tocopherol.

Correlation between Antimutagenicity and Major Components of Hsian-tsao Extracts. The effect of WEHT from various sources on the mutagenicity of IQ toward *S. typhimurium* TA98 is summarized in Table 6. In the range of 0.2−5.0 mg/plate, the inhibitory effect of water extracts of HT from various sources on the mutagenicity of IQ toward *S. typhimurium* TA98 was dose-dependent. Moreover, extracts greater than 2.5 mg/plate exhibited inhibitory effect by 90% and above.

Calculated coefficients of correlation between antimutagenicity and ascorbic acid, polyphenolic compounds, and chlorophylls content of WEHT are listed in Table 7. The antimutagenicity of extracts from HT on IQ toward TA98 was significantly correlated with their polyphenolic compounds ($r^2 = 0.756$, $P < 0.05$) and ascorbic acid ($r^2 = 0.430$, $P < 0.05$); however, it was not significantly correlated with total chlorophylls ($r^2 = -0.419$, $P < 0.05$). Apparently, polyphenolic compounds in the WEHT may contribute greatly to the antimutagenicity on IQ toward TA98. Huang et al. (*18*) indicated that some naturally occurring plant flavonoids inhibited the mutagenic activity of B(a)P. There was a positive correlation between antimutagenic activity and polyphenol content of dialyzates of vegetable and fruits (*17*). These investigations indicate that a large polyphenol content is closely related to the antimutagenic activity. In the present study, the results obtained clearly indicate that WEHT showed antimutagenic activity, and this function was concerned with the content of their phenolic compound and ascorbic acid. Further work is required to determine the mechanism involved in the anitmutagenic effect. In addition, more in vivo evidence and identification of antimutagenic components merit further investigation.

LITERATURE CITED

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