Separation, Characteristics, and Biological Activities of Phenolics in Areca Fruit

Chin-Kun Wang* and Wen-Hsiu Lee

Graduate Institute of Nutritional Science, Chung Shan Medical and Dental College, 113, Section 2, Ta-Chien Street, Taichung, Taiwan, Republic of China

Fresh unripe areca fruit is the main ingredient of betel quid in Taiwan. The phenolics in whole fresh areca fruit involved condensed tannins (92 mg/g of dry wt), hydrolyzable tannins (69 mg/g of dry wt), non-tannin flavans (84 mg/g of dry wt) and simple phenolics (56 mg/g of dry wt). For liquid chromatography, Sephadex LH-20 gels were used to separate the condensed tannin and noncondensed tannin phenol fractions from the crude phenolic extract of areca fruit. The condensed tannin phenol fraction was rich in condensed tannins and simple phenolics. The noncondensed tannin phenol fraction contained abundant non-tannin flavans (catechin, epicatechin). The crude phenolic extract of areca fruit and its two separated fractions exhibited marked antioxidative activity and an antimutagenic effect on 2-amino-3-methylimidazo[4,5-*f*]quinoline toward *Salmonella typhimurium* TA98 and TA100, did not induce chromosomal aberration, and increased the frequency of sister chromatid exchange (SCE) in CHO-K1 cells.

Keywords: *Phenolics; areca fruit; antioxidative activity; antimutagenicity; SCE*

INTRODUCTION

Betel quid is used as a masticatory and is very popular in Taiwan (Ko et al., 1992) owing to its physiological effects, including an increase in stamina and general well-being (Nieschulz, 1967; Hwang et al., 1992). However, oral submucous fibrosis (OSF), a chronic disabling disease (Paymaster, 1962), and a high incidence of oral cancer (Wenke et al., 1984) have been demonstrated by epidemiological studies to be closely associated with the habit of chewing betel quid (Kwan, 1976).

Betel quid used in India and other Southern Asian areas involves areca nut, slaked lime, betel leaf, tobacco, condiments, and sweetening agents. Areca nut is obtained from the fruit of the *Areca catechu* tree. The outer pericarp of the ripe fruit, which is orange-yellow, is removed to separate the nut (Bhosle et al., 1992). Areca nut contains polysaccharides, mineral, crude fiber, alkaloids, and some phenolics (Mujumder et al., 1979). The phenolics in areca nut are catechin dimers, leucocyanidin dimers, and leucopelargonidin (Mathew et al., 1969; Nonaka et al., 1981). The catechin and tannin extracts of areca nut inhibit the activity of collagenase (Scutt et al., 1987) and stimulate the synthesis of collagen (Canniff and Harvey, 1981). It seems that phenolics of areca nut are closely related to OSF. On the other hand, the phenolic extracts of areca nut are found to depress the formation of *N*-nitroso-L-proline in the human body (Stich et al., 1983). In Taiwan, betel quid is composed of fresh green areca fruit, slaked lime, and *Piper betle*. The phenolic composition in whole fresh areca fruit was complex and far different from areca nut used in other countries (Wang and Hwang, 1993).

The differences in biological activities of phenolics caused by different chemical structures have been reported recently (Okuda et al., 1991). It is necessary to understand the effect of phenolics in fresh unripe areca fruit on health. The objectives of this paper were to discuss the separation, characteristics, and biological activities of phenolics in whole fresh areca fruit used in Taiwan.

* Author to whom correspondence should be addressed (telephone 886-4-3896190 ext. 11351; fax 886-4-3890964; e-mail wck@mercury.csmc.edu.tw).

MATERIALS AND METHODS

Materials. Fresh unripe areca fruits were obtained from a farm in Nantou County, Taiwan. They were either used immediately or stored at 4 °C before use. Sephadex LH-20 was purchased from Pharmacia LKB Biotec. (Uppsala, Sweden). *Salmonella typhimurium* TA98 and TA100 were kindly provided by Prof. B. N. Ames (University of California, Berkeley, CA). Cinchonine sulfate, linoleic acid, and standard phenolic compounds ((+)-catechin, (−)-epicatechin, gallic acid) were the products of Sigma Co. (St. Louis, MO). 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) were purchased from Wako Pure Chemical Co. (Tokyo, Japan). Rat liver S9 mix, Aroclor 1254-induced microsomal fraction (protein content 42.1 mg/mL), was purchased from Organon Teknika Co.

Extraction of Crude Phenolics. A crude phenolic extract of whole fresh areca fruit was prepared according to the method described in our previous work (Wang and Hwang, 1993). In brief, fresh areca fruits were extracted with 80% aqueous acetone (1:10, w/v) in a Waring blender for 3 min, followed by soaking for 20 min before filtration. The filtrate, containing the extracted phenolics, was evaporated to dryness under reduced pressure, and the dry material was crude phenolic extract.

Isolation of Condensed Tannins by Adsorption on Sephadex LH-20. The isolation of condensed tannins was achieved according to the method of Strumeyer and Malin (1975). For column chromatography, Sephadex LH-20 gels were allowed to swell in 95% ethanol. The crude phenolic extract, dissolved in 95% ethanol, was applied to a column of Sephadex LH-20 (2.6 cm × 39 cm) equilibrated with 95% ethanol. The column was eluted with ethanol at a flow rate of 1.6 mL/min and 2.0-mL fractions were collected. The absorbance at 280 nm was determined with an Isco (Lincoln, Nebraska) spectrophotometer. The column was then eluted with 50% aqueous acetone at a flow rate of 1.6 mL/min. Fractions of 3.0 mL were collected, and the absorbance at 435 nm was determined. The fractions were assayed for tannins by the gelatin precipitation test (Strumeyer and Malin, 1975)

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and for different phenolic groups by the precipitation with formaldehyde and cinchonine (Peri and Pompei, 1971). Only the 50% acetone elute contained condensed tannins.

Estimation of Different Phenolic Groups. The contents of simple phenolics, non-tannin flavans, hydrolyzable and condensed tannins in the crude phenolic extract, condensed tannin, and noncondensed tannin phenol fractions were separately determined by the method of Peri and Pompei (1971) and expressed as milligrams of catechin equivalent per gram of sample. The condensed tannin and noncondensed tannin phenol fractions were separated from the crude phenolic extract by adsorption on Sephadex LH-20 as described above.

Relative Degree of Polymerization of Polyphenols. The relative degree of polymerization of polyphenols was determined according to the method of Butler et al. (1982).

HPLC Analysis of Phenolics. The method for the analysis of phenolics was modified from the method of Mueller-Harvey et al. (1987). In brief, the HPLC column was a 25 cm × 4 mm i.d. Lichrospher 100 RP-18 (5 μm) (Merck). Glacial acetic acid−water (5:95, v/v; solvent A) and methanol (solvent B) were used for gradient elution. A linear gradient was applied, staring with 100% solvent A and finishing with 100% solvent B over a 75-min period. The flow rate was kept at 0.8 mL/min, and the UV detector wavelength was set at 280 nm. Standard compounds were cochromatographed with test sample for tentative identification of phenolic compounds.

Antioxidative Activity. The antioxidative activity of each test sample was determined according to the method of Lingnert et al. (1979). Test sample, dissolved in methanol (200 μL), was mixed with 2 mL of linoleic acid emulsion (10 mM, in phosphate buffer, pH 6.5) in a test tube and placed in darkness at 37 °C. At the beginning and the end of 15-h incubation, 0.2 mL of mixture was separately mixed with 2 mL of methanol and 6 mL of 60% aqueous methanol. The antioxidative activity was determined by reading the absorbance at 234 nm of the experimental and control groups. The antioxidative activity (AOA) was expressed as the following. $AOA = [\Delta A_{234(c)} - \Delta A_{234}]/\Delta A_{234(c)}$, ΔA_{234} was the difference in absorbance at 234 nm between the beginning and the end of a 15-h incubation at 37 °C for the experimental group. $\Delta A_{234(c)}$ was the difference in absorbance at 234 nm between the beginning and the end of a 15-h incubation at 37 °C for the control group.

Mutagenicity and Antimutagenicity Assays. The mutagenic and antimutagenic effects of test sample were assayed according to the Ames test using *S. typhimurium* strains TA98 and TA100 (Maron and Ames, 1983). The mutagens used were IQ (0.01 μg/plate for TA98 and 0.5 μg/plate for TA100) and MNNG (10 μg/plate for TA98 and TA100). Each sample was added to overnight-cultured *S. typhimurium* TA98 or TA100 (0.1 mL), mutagen (0.1 mL), and S9 mix (0.5 mL). The entire mixture was preincubated at 37 °C for 20 min before molten top agar (2 mL) was added; the mixture was poured onto a minimal agar plate. The His^+ revertant colonies were counted after incubation at 37 °C for 48 h. Each sample was assayed using triplicate plates. The calculation of percent inhibition follows that described by Ong et al. (1986): inhibition (%) = [1 − (number of revertants in the presence of each sample/number of revertants in the absence of sample)] × 100%. The number of spontaneous revertants was determined without samples and IQ or MNNG.

Cell Culture. Chinese hamster overy K1 (CHO-K1) cells were grown in McCoy's 5A medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and PSN antibiotic mixture (Gibco).

Plating Efficiency Assay. A total of 100 cells/5 mL was incubated in a Petri dish (40-mm diameter) for 24 h at 37 °C in a humidified 5% CO_2 atmosphere; 0.1 mL of test sample was added to each culture and incubated for 24 h. New medium was added and the cells were incubated for 6−8 days. The medium was removed and the cells were treated with methanol. Methanol-treated cells were stained with 10% Geimsa for 5 min and counted (>50 cells/colony).

Chromosomal Aberration and SCE. Cells (2×10^5/10 mL) were prepared in a 25-cm^2 flask, and incubated for 24 h (37 °C, 5% CO_2). Then 0.2 mL of test sample and 0.1 mL of BrdUrd were added to each culture, and the resultant mix was incubated for 24 h. At 1.5 h before harvesting, 0.1 mL of a 10 μg/mL solution of colcemid was added to each culture. After gentle mixing, the cultures were returned to the incubator. At the end of the incubation period, each flask was shaken gently to dislodge mitotic cells and the cell suspension was transferred to capped 15-mL centrifuge tubes; 0.5 mL of trypsin−EDTA (37 °C, 3 min) was added to each flask to suspend the cells again. The cell suspension mixtures were centrifuged at 800 rpm for 10 min. The supernatant was discarded and the pellet of cells was resuspended in 8 mL of 0.075 M potassium chloride solution. The suspension was left for 7 min at room temperature and then the centrifugation process was repeated. In a dropwise manner, 8 mL of freshly made methanol−acetic acid (3:1) fixative was added and allowed to stand for 30 min at room temperature; the tube gently shaken to resuspend the cells. They were then centrifuged at 800 rpm for 10 min and the supernatant was discarded. The cells in the centrifuge tube were resuspended with 8 mL of fixative for 10 min. The centrifugation procedure was repeated twice more, each time discarding the old fixative and resuspending the cells in fresh fixative. After the final addition of fixative, the tubes were centrifuged as above, the fixative was discarded, and the cell button was resuspended in 3−4 drops of fixative. Slides were prepared by the air-drying technique and stained with 3% Giemsa for 10 min. Chromosomal aberration and SCE were measured by microscopic analysis.

Statistical Analysis. Results in this paper were obtained with Duncan's multiple range test (Duncan, 1955).

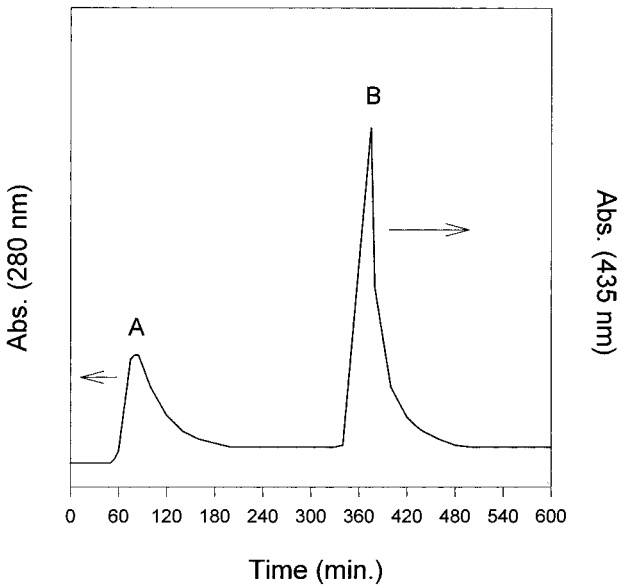

Figure 1. Fractionation of phenolics by adsorption on Sephadex LH-20, and elution with 95% ethanol followed by 50% aqueous acetone: peak A, eluted with 95% ethanol for 240 min; peak B, eluted with 50% aqueous acetone for 360 min.

RESULTS AND DISCUSSION

Fractionation of Phenolics by Adsorption on Sephadex LH-20. As illustrated by the chromatogram in Figure 1, crude phenolic extract, dissolved in 95% ethanol, was applied to a 2.6 cm × 39 cm column of Sephadex LH-20, and a single peak (A) was obtained by exhaustive washing with 95% ethanol. The residual substances, on the other hand, remained tightly adsorbed to the top of the column in 95% ethanol and could be eluted with 50% aqueous acetone to give peak B. Approximately 47% of the dry matter from the crude phenolic extract was found in peak A; 30% was obtained in peak B. Together they represent a total recovery of 77% of the material applied to the column. The total

Table 1. Yield and Relative Polymerization of Phenolics in Crude Phenolic Extract[a]

sample	yield (%)	rel polymerization (%)	1% gelatin precipitation
crude phenolic extract	100.0	211	+
peak A fraction	46.6	70	−
peak B fraction	29.4	174	+

[a] The peak A fraction and peak B fraction from areca fruit.

yield percentage was similar to that reported by Strumeyer and Malin (1975).

After removing the aqueous acetone, dissolving the residue in 95% ethanol, and repeating the adsorption−elution procedure, there was no 280-nm absorbing material eluted by 95% ethanol. With 50% aqueous acetone, a single peak was obtained which exhibited the ability to precipitate with 1% gelatin. The relative degree of polymerization of crude phenolic extract, peak A, and peak B were 211%, 70%, and 174%, respectively (Table 1). It revealed that most larger polyphenolics were in the peak B fraction, but smaller and non-gelatin-precipitable phenolics existed in the peak A fraction.

Estimation of Different Phenolic Groups. A well-differentiated distribution picture was obtained by applying the Folin−Ciocalteu method to the phenolic groups resulting from combined precipitations with both formaldehyde and cinchonine (Peri and Pompei, 1971). According to this procedure, the "total phenolics" value resulted from the sum of four groups of phenolic compounds: Condensed tannins were polymers or copolymers of catechin and leucoanthocyanins. Hydrolyzable tannins were polyesters of a sugar or related polyhydric alcohols and a phenolic carboxylic acid, usually gallic or ellagic acid. Non-tannin flavans included monomeric anthocyanins, catechins, and leucoanthocyanins. Simple phenolics were the derivatives of hydroxybenzoic and hydroxycinnamic acids. The crude phenolic extract of whole fresh areca fruit contained 92 mg/g of dry wt condensed tannins, 69 mg/g of dry wt hydrolyzable tannins, 84 mg/g of dry wt non-tannin flavans, and 56 mg/g of dry wt simple phenolics (Figure 2). Peak B contained abundant condensed tannins (121 mg/g of dry wt), simple phenolics (125 mg/g of dry wt), some hydrolyzable tannins, and non-tannin flavans. Peak A contained rich non-tannin flavans (59 mg/g of dry wt). Results showed that the condensed tannins were completely isolated from the crude phenolic extract in the peak B fraction by the Sephadex LH-20 column. However, most hydrolyzable tannins were in the peak B fraction, and few hydrolyzable tannins distributed in the peak A fraction. It is clear that peak B is a condensed tannin phenol fraction while peak A is a noncondensed tannin phenol fraction; most complex phenolics were in the condensed tannin phenol fraction.

HPLC Analysis of Phenolics. To understand the characteristics of phenolics in areca fruit, high-performance liquid chromatography was used to analyze the detailed composition of phenolics. As shown in Figure 3, crude phenolic extract contained catechin, epicatechin, and complex phenolics. Catechin and epicatechin were the main phenolics of the noncondensed tannin phenol fraction. However, the condensed tannin phenol fraction still contained complex phenolics. These results were similar to those for the estimation of different phenolic groups (Figure 2); only smaller simple phenolics (e.g., catechin and epicatechin) existed in the noncondensed tannin phenol fraction.

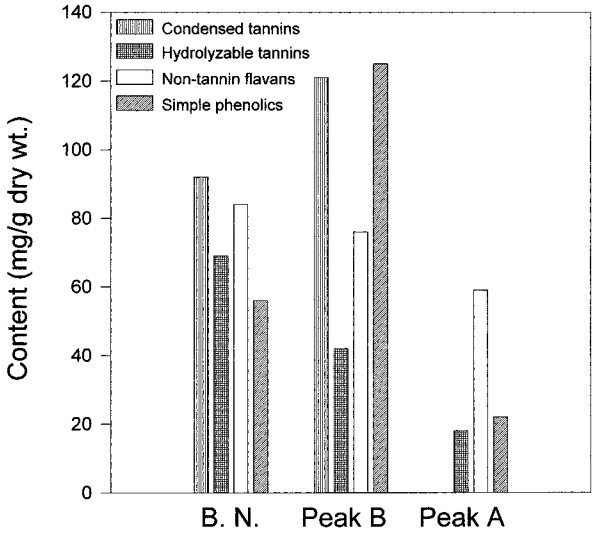

Figure 2. Contents of condensed tannins, hydrolyzable tannins, non-tannin flavans, and simple phenolics in crude phenolic extract, peak A fraction, and peak B fraction from areca fruit: B. N., crude phenolic extract from areca fruit; peak A, eluted with 95% ethanol; peak B, eluted with 50% aqueous acetone.

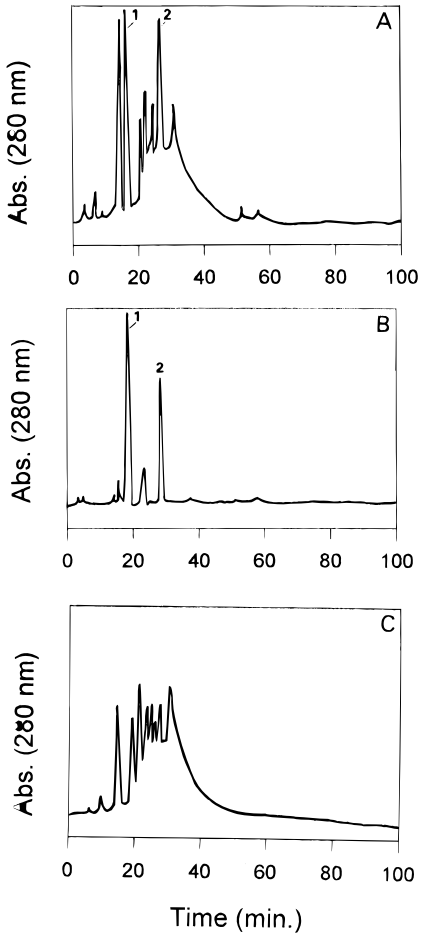

Figure 3. HPLC chromatograms of phenolic compounds in crude phenolic extract (A), noncondensed tannin phenol fraction (B), and condensed tannin phenol fraction (C) from areca fruit: peaks (1) catechin; (2) epicatechin.

Antioxidative Activity. Recent studies showed that many phenolic substances exhibited antioxidative activity, antimutagenicity, and antitumor activity (Wattenberg et al., 1980; Stich and Rosin, 1984). Owing to the close association among antioxidative activity and other

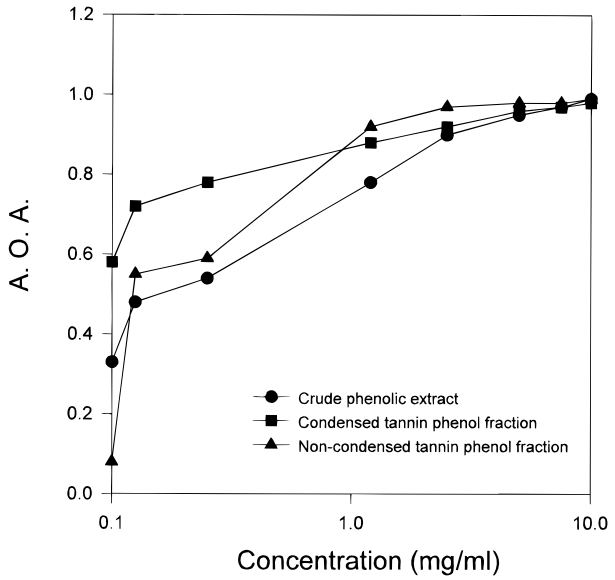

Figure 4. Antioxidative activities of crude phenolic extract, condensed tannin phenol fraction, and noncondensed tannin phenol fraction from areca fruit.

important biological activities, the antioxidative activity of phenolics in areca fruit is discussed first. The effects of crude phenolic extract, condensed tannin, and noncondensed tannin phenol fractions on the peroxidation of linoleic acid were shown in Figure 4. The oxidative activity of linoleic acid was markedly inhibited by the three samples. The antioxidative activity of the three samples increased with increasing concentration. The condensed tannin phenol fraction showed better antioxidative activity than the other fractions, below the concentration of 1 mg/mL. When the concentration of the three samples reached 10 mg/mL, antioxidative activity peaked at similar values for all three.

Mutagenicity and Antimutagenicity. A high incidence of oral cancer was closely associated with the habit of betel quid chewing (Boyle et al., 1990). The components or the metabolite of betel quid may be correlated with the oral cancer (Sundqvist et al., 1989). To understand the mutagenicity of phenolics in areca fruit, the mutagenic effect was assayed by the Ames test. As shown in Table 2, three test samples did not induce mutagenicity of *S. typhimurium* TA98 and TA100. For the strong antioxidative activities of phenolics in areca fruit, the antimutagenic activities on MNNG and IQ were also evaluated. At 4 mg/plate, three phenolic samples exhibited a weak inhibitory effect on MNNG toward TA100 (Table 3). But they showed a strong inhibitory activity on IQ toward TA98 and TA100 significantly (Table 4; $p < 0.05$), and the antimutagenic activity increased with increasing amount per plate. Nagabhushan and Bhide (1988) indicated that catechin showed antimutagenicity against environmental mutagens. As results show above, catechin and epicatechin were the main phenolic compounds of the noncondensed tannin phenol fraction. Therefore, it seems to explain the antimutagenic function. However, the antimutagenic function of the condensed tannin phenol fraction needs much more investigation.

Colony Formation Efficiency. The effect of phenolics in areca fruit on the colony formation in CHO-K1 cells was assayed. As shown in Figure 5, crude phenolic extract and condensed tannin and noncondensed tannin phenol fractions depressed, by 30%, colony formation at 10, 10, and 100 μg/mL, respectively.

Table 2. Mutagenic Activity of Crude Phenolic Extract, Condensed Tannin Phenol Fraction, and Noncondensed Tannin Phenol Fraction from Areca Fruit in *Salmonella typhimurium* TA98 and TA100

	His$^+$ revertant/plate[a]			
	TA98		TA100	
sample amt/plate (mg)	−S9	+S9	−S9	+S9
crude phenolic extract				
spontaneous revertants[b]	17 ± 1	43 ± 2	158 ± 8	173 ± 20
0.4	24 ± 2	nd[c]	nd	nd
1.0	21 ± 2	28 ± 2	161 ± 18	162 ± 34
1.5	15 ± 1	nd	nd	nd
2.0	nd	25 ± 1	187 ± 26	134 ± 31
4.0	nd	27 ± 1	196 ± 7	147 ± 25
condensed tannin phenol fraction				
spontaneous revertants	17 ± 1	31 ± 2	121 ± 13	181 ± 24
0.1	21 ± 3	nd	nd	nd
0.5	19 ± 6	nd	nd	nd
0.9	19 ± 4	nd	nd	nd
1.0	nd	26 ± 5	134 ± 11	170 ± 18
2.0	nd	21 ± 3	105 ± 10	146 ± 24
4.0	nd	24 ± 2	104 ± 19	112 ± 5
noncondensed tannin phenol fraction				
spontaneous revertants	17 ± 1	43 ± 2	173 ± 20	203 ± 5
1.0	21 ± 1	40 ± 3	127 ± 28	168 ± 21
2.0	13 ± 1	37 ± 4	193 ± 29	162 ± 21
4.0	25 ± 1	31 ± 9	165 ± 7	190 ± 4

[a] Means ±SD of three plates. [b] The number of spontaneous revertants was determined without samples. [c] nd, no data.

Table 3. Effect of Crude Phenolic Extract, Condensed Tannin Phenol Fraction, and Noncondensed Tannin Phenol Fraction from Areca Fruit on the Activity of MNNG toward *Salmonella typhimurium* Strains of TA98 and TA100 in the Absence of S9

	His$^+$ revertants/plate[a] (inhibition %)[b]	
sample amt/plate (mg)	TA98	TA100
control[c]	88 ± 12[a,e]	1594 ± 87[a]
crude phenolic extract		
0.1	95 ± 1[a] (−8.0)	nd
0.5	87 ± 4[a] (1.1)	nd
1.0	86 ± 4[a] (2.3)	1258 ± 83[b] (21.1)
2.0	nd	1297 ± 90[b] (18.6)
4.0	nd	1316 ± 99[b] (17.4)
condensed tannin phenol fraction		
0.1	100 ± 12[a] (−13.6)	nd
0.5	84 ± 4[a] (4.5)	nd
0.9	77 ± 12[a] (12.5)	nd
1.0	nd	1508 ± 53[a] (5.4)
2.0	nd	1526 ± 89[a] (4.3)
4.0	nd	1407 ± 75[b] (11.7)
noncondensed tannin phenol fraction		
1.0	88 ± 8[a] (0)	1493 ± 68[a] (6.3)
2.0	97 ± 11[a] (−10.2)	1340 ± 23[b] (15.9)
4.0	92 ± 2[a] (−4.5)	1359 ± 71[b] (14.7)
spontaneous revertants[d]	25 ± 8	168 ± 9

[a] Data are means ± SD of three plates. [b] Inhibition (%) = [1 − (number of revertants in the presence of each sample/number of revertants in the absence of sample)] × 100%. [c] The control number was determined without samples. [d] The number of spontaneous revertants was determined without samples and MNNG. [e] Data bearing different superscript letters in the same column were significantly different ($p < 0.05$). [f] nd, no data.

At a concentration of 1000 μg/mL, the three samples inhibited cell colony formation completely.

Chromosomal Aberration and SCE. Arecoline, a major alkaloid of betel nut, was found to have a weak chromosome-damaging effect in vivo, and the frequency of chromosomal aberrations in mouse bone marrow cells

Table 4. Effect of Crude Phenolic Extract, Condensed Tannin Phenol Fraction, and Noncondensed Tannin Phenol Fraction from Areca Fruit on the Activity of IQ toward *Salmonella typhimurium* Strains TA98 and TA100 in the Presence of S9

	His$^+$ revertants/plate[a] (inhibition %)[b]	
sample amt/plate (mg)	TA98	TA100
control[c]	2421 ± 334[a,e]	1039 ± 87[a]
crude phenolic extract		
0.01	2608 ± 418[a] (−7)	1304 ± 122[a] (−25)
0.10	2359 ± 151[a] (3)	887 ± 108[b] (15)
0.50	1055 ± 219[b] (56)	363 ± 34[c] (65)
condensed tannin phenol fraction		
0.01	1538 ± 278[c] (36)	1130 ± 129[a] (−9)
0.10	1945 ± 244[c] (20)	881 ± 59[b] (15)
0.50	1021 ± 28[b] (58)	311 ± 10[c] (70)
noncondensed tannin phenol fraction		
1.0	1048 ± 144[b] (57)	1177 ± 31[a] (−13)
2.0	566 ± 37[d] (77)	1051 ± 19[a] (−1)
4.0	315 ± 33[e] (87)	663 ± 54[d] (36)
spontaneous revertants[d]	35 ± 2	279 ± 2

[a] Data are means ± SD of three plates. [b] Inhibition (%) = [1 − (number of revertants in the presence of each sample/number of revertants in the absence of sample)] × 100%. [c] The control number was determined without samples. [d] The number of spontaneous revertants was determined without samples and IQ. [e] Data bearing different superscript letters in the same column were significantly different ($p < 0.05$).

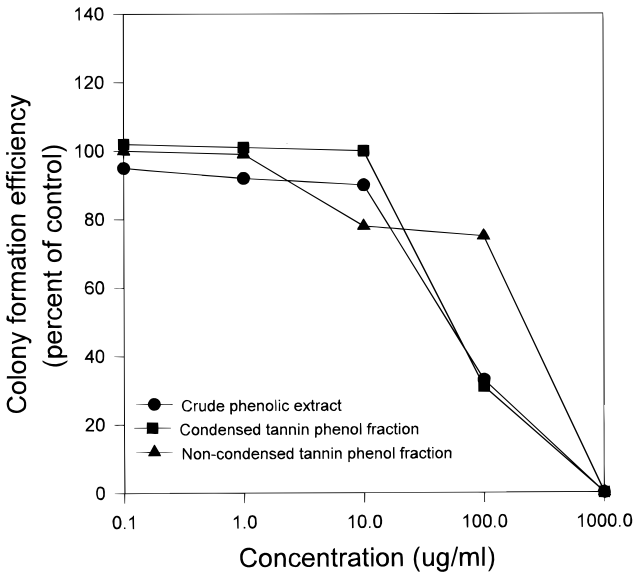

Figure 5. Colony formation efficiency of crude phenolic extract, condensed tannin phenol fraction, and noncondensed tannin phenol fraction from areca fruit in CHO-K1 cells.

showed a dose−response relationship (Panigrahi and Rao, 1982). In this study, there was no significant difference in the chromosomal aberrations in cells treated with three phenolic samples, by microscopic analysis. Cells prelabeled with BrdUrd were treated with the three samples for 24 h; no high frequencies of SCE were found (Table 5).

Conclusions. Our results indicate clearly that the phenolics of areca fruit show strong antioxidative activity, show antimutagenic activity on IQ toward *S. typhimurium* TA98 and TA100, do not induce chromosomal aberration, and increase the frequency of SCE in CHO-K1 cells. Results also show that the antimutagenic property of phenolics in areca fruit may be correlated with its antioxidative activity. Further research on the role of the antioxidative ability of the phenolics in the mutagenic process is required. The work also notes that the biological activities of phenolics in areca fruit maybe play an important role in the protection of health.

Table 5. Sister-Chromatid Exchange (SCE) Frequencies of CHO Cells Exposure to Crude Phenolic Extract, Condensed Tannin Phenol Fraction, and Noncondensed Tannin Phenol Fraction from Areca Fruit

	SCEs/metaphase[a]		
concn (μg/mL)	crude phenolic extract	condensed tannin phenol fraction	noncondensed tannin phenol fraction
control	9.42 ± 0.91[a,b]	9.42 ± 0.91[a]	9.42 ± 0.91[a]
0.1	8.21 ± 1.08[a]	7.78 ± 1.40[a]	9.50 ± 1.53[a]
10.0	8.09 ± 0.35[a]	7.61 ± 1.90[a]	nd[c]
100.0	nd	nd	7.93 ± 1.33[a]

[a] Data are means ± SD of three plates (30 cells/plate). [b] Data bearing different superscript letters in the same column were significantly different ($p < 0.05$). [c] nd, no data.

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