

MUTAGENICITY AND ANTIMUTAGENICITY OF WATER EXTRACTS FROM GAC FRUIT (*MOMORDICA COCHINCHINENSIS* SPRENG)

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ABSTRACT:

Background: Gac fruit (*Momordica cochinchinensis* Spreng) was used as food, colourant and medicine for a period of time. Moreover, several *in vitro* and *in vivo* studies showed that gac fruit contained many phytochemicals and exerted bioactive effects, including pro-vitamin A, antioxidant and antitumor activity. This study aimed to investigate the mutagenicity and the antimutagenicity against a standard mutagen; nitrite treated 1-aminopyrene (1-AP) of gac fruit.

Method: The water extracts from unripe pulp, half-ripe pulp, fully ripe pulp and aril of gac fruit were treated with and without nitrite and investigated for their mutagenicity using Ames test with modified pre-incubation method on *Salmonella typhimurium* strain TA98 and TA100 without enzymatic activation. The antimutagenicity against nitrite treated 1-AP of these fruit extracts was also evaluated.

Results: The results showed that none of them showed mutagenicity on both strains of *S. typhimurium* either with or without nitrite treatment. For the antimutagenicity, the extracts from various parts of gac fruit, especially the extracts from unripe pulp inhibited the mutagenicity of nitrite treated 1-AP with dose response manner on *S. typhimurium* strain TA98. According to the results from *S. typhimurium* strain TA100, the two higher amounts of the extracts from unripe pulp and half-ripe pulp showed weak to moderate degree of inhibition while the extracts from fully ripe pulp showed negligible effect. The extracts from aril also showed negligible effect at the study concentrations, although they tended to enhance the mutagenicity of nitrite treated 1-AP. Overall, the mutagenicity of nitrite treated 1-AP can be inhibited by the addition of the water extracts from gac fruit.

Conclusion: This study indicated that the water extracts from gac fruit showed no mutagenic activity and exhibited antimutagenic activity against nitrite treated 1-AP, especially from unripe pulp.

Keywords: *Momordica cochinchinensis* Spreng, Gac fruit, Mutagenicity, Antimutagenicity, Ames test

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INTRODUCTION

Gac fruit (*Momordica cochinchinensis* Spreng) belongs to Cucurbitaceae family and is indigenous to South and Southeast Asia. The fleshy gac fruit has green peel with short spines and become red at maturity with bright red aril inside [1]. Gac fruit is widely used in different purposes such as food, colorant and medicine. In Thailand, gac pulp is primarily consumed as a vegetable while gac aril is

prepared as a beverage. In Vietnam, gac aril is used as a colorant for cooking red sticky rice or xoi gac. In China, gac seed is used as a traditional medicine for treatment of skin disorders such as abscesses, bruises and sores [2-4]. Several studies have been reported that gac fruit contained high levels of polyunsaturated fatty acids and bioactive components such as vitamin E, flavonoids and carotenoids, especially β -carotene and lycopene, giving their antioxidant properties [5-8]. Due to high levels of β -carotene, fully ripe gac fruit has been used as a source of vitamin A to promote healthy

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vision and improve plasma levels of retinal, β -carotene and lycopene [9]. Interestingly, the extracts of gac fruit were found the anticancer properties. The aril extract induced apoptosis in human breast cancer cells (MCF-7) [10]. The water soluble proteins with MW of 35 kDa from water extract of gac seeds inhibited tumor growth and angiogenesis on Balb/C mice transplanted with the colon 20-26 adenocarcinoma and HepG2 cell lines [11]. Cochinin B; ribosome-inactivating proteins (RIPs) with MW of 28 kDa which was found in gac seeds exhibited strong antitumor activity on human cervical epithelial carcinoma (HeLa), human embryonic kidney (HEK293) and human lung cancer cells (NCI-H187) [12]. The gac seed extract also inhibited the proliferation and invasion of human breast cancer cells (ZR-75-30) [13]. However, the mutagenicity and antimutagenicity effects of gac fruit have not been reported. The aims of this study were to investigate the mutagenicity and antimutagenicity against standard mutagen nitrite treated 1-AP of water extracts from different parts of gac fruit using modified pre-incubation method of Ames test on *Salmonella typhimurium* strain TA98 and TA100 without enzymatic activation.

MATERIALS AND METHODS

Tester strains

S. typhimurium strain TA98 (to detect frameshift mutation) and TA100 (to detect base-pair substitute mutation) were kindly provided by Assoc. Prof. Keaw Kangsadalampai (Institute of Nutrition, Mahidol University). Both strains were confirmed their histidine dependent characteristic before using.

Chemical reagents

1-Aminopyrene (1-AP), ammonium sulfamate ($\text{NH}_2\text{SO}_3\text{NH}_4$), biotin, L-histidine, sodium nitrite (NaNO_2) and sodium dihydrogen phosphate (NaH_2PO_4) were obtained from Sigma-Aldrich (St. Louis, USA). Hydrochloric acid (HCl) was purchased from Normapur (Prolabo, Belgium). Acetonitrile was obtained from J.T. Baker (Phillipsburg, USA). Agar-Agar, magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and sodium chloride (NaCl) were obtained from Merck (Darmstadt, Germany). Dipotassium hydrogen phosphate anhydrous (K_2HPO_4), sodium ammonium hydrogen phosphate tetrahydrate ($\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$) and disodium hydrogen phosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) were obtained from Fluka (Buchs, Switzerland). Citric acid monohydrate ($\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$)

and D(+)-glucose were purchased from Analar (Poole, England). Oxoid nutrient broth No.2 was obtained from Oxoid Ltd. (Hampshire, England).

Sample preparation

Three kinds of gac fruit (*Momordica cochinchinensis* Spreng), including unripe, half-ripe and fully ripe fruits were purchased from the floating market in Samutprakan, Thailand during October-November 2014. After cleaning with tap water, unripe and half-ripe gac fruit were peeled off, cut into halves to remove the seeds and then chopped into small pieces. Aril and pulp of fully ripe gac fruit were separately scooped out and removed the seeds. Thus, the fruit samples were separately into four parts, including unripe pulp, half-ripe pulp, fully ripe pulp and aril. Each part of gac fruit (100 g wet weight) was blended with 200 ml of distilled water, in the ratio of 1:2 w/v and centrifuged at 4,500 rpm for 15 minutes. The supernatant of each fruit sample (1 ml of supernatant containing 0.5 g of fresh fruit sample) was collected in a tight protecting from light container at -20°C until required. The fruit sample extract was thawed and sterilized by filtration through a sterile 0.2 micron filter before assay.

Nitrite treatment procedure

For mutagenicity assay preparation, each fruit sample extract was treated with nitrite in an acidic condition. Briefly, an aliquot of each part of fruit sample extract (50, 100, 200 μl) was adjusted pH to 3.0-3.5 by 0.2 N hydrochloric acid and then mixed with 250 μl of 2 M sodium nitrite in a sterile tube with plastic cap to obtain the final volume of 1,000 μl . The mixture was incubated in a shaking water bath at 37°C for 4 hours before dipped into an ice bath for 1 minute to stop the reaction. The mixture was then added with 250 μl of 2 M ammonium sulfamate to decompose the residual nitrite and dipped into an ice bath for 10 minutes before mutagenic assay.

Standard mutagen model

Each concentration of 1-AP (0.06 $\mu\text{g}/\text{plate}$ for *S. typhimurium* strain TA98 and 0.12 $\mu\text{g}/\text{plate}$ for *S. typhimurium* strain TA100) was treated with nitrite according to the procedure described in 2.4. The nitrosated product obtained from this reaction was used as a standard mutagen or positive control of this study.

Mutagenicity assay

The mutagenicity of water extract from gac fruit was determined using the plate incorporation procedure of Maron and Ames [14] with modified

Table 1 Mutagenicity of water extracts of gac fruit with and without nitrite treatment on *S. typhimurium* strain TA98 and TA100 without enzymatic activation using Ames test

Amount of each part of gac fruit extract (µl)		His ⁺ revertants/plate ^a With nitrite treatment		His ⁺ revertants/plate ^a Without nitrite treatment	
		TA98	TA100	TA98	TA100
Unripe pulp	spontaneous	29 ± 6	112 ± 11	29 ± 6	112 ± 11
	50	42 ± 9	109 ± 9	31 ± 4	115 ± 12
	100	43 ± 6	110 ± 9	30 ± 10	85 ± 17
	200	46 ± 9	107 ± 13	35 ± 8	101 ± 8
Half-ripe pulp	spontaneous	37 ± 3	112 ± 8	37 ± 3	112 ± 8
	50	31 ± 5	116 ± 22	31 ± 8	115 ± 12
	100	30 ± 7	127 ± 15	29 ± 3	122 ± 15
	200	31 ± 11	123 ± 19	32 ± 6	113 ± 15
Fully ripe pulp	spontaneous	22 ± 5	106 ± 14	22 ± 5	106 ± 14
	50	21 ± 6	112 ± 14	18 ± 4	104 ± 10
	100	22 ± 3	114 ± 17	17 ± 4	106 ± 9
	200	17 ± 5	109 ± 9	19 ± 4	120 ± 11
Aril	spontaneous	20 ± 5	117 ± 4	20 ± 5	117 ± 4
	50	25 ± 6	155 ± 10	17 ± 6	118 ± 6
	100	38 ± 4	156 ± 7	19 ± 6	114 ± 7
	200	39 ± 15	134 ± 12	17 ± 6	105 ± 11

^aThe data were reported as means with standard deviations of triplicate plates from two experiments

method of Yahagi et al [15]. The pre-incubation of sample without enzymatic activation were tested on two strains of histidine dependent (His⁻) *Salmonella*, including *S. typhimurium* strain TA98 and TA100. For pre-incubation method, 100 µl of each nitrosated product from 2.4 was mixed with 500 µl of 0.5 M phosphate buffer (pH 7.4) and 100 µl of each bacterial strain in a sterile tube with plastic cap before shaken in a water bath at 37°C. After 20 minutes of pre-incubation, 2 ml of molten top agar (45°C) containing 0.5 mM of histidine and biotin was added. The mixture was carefully poured onto a minimal glucose agar plate after thoroughly mixed. The number of histidine (His⁺) revertants per plate was counted after turned plate upside down in an incubator at 37°C for 48 h.

For determining the mutagenicity of the extracts without nitrite treatment, the method was done as previously described in 2.4 but distilled water was placed instead of sodium nitrite and ammonium sulfamate.

Antimutagenicity assay

Antimutagenicity of water extract of gac fruit against nitrite treated 1-AP was investigated. Briefly, 100 µl of the reaction product of 1-AP treated with nitrite from 2.4 was mixed with various amounts (50, 100, 200 µl) of fruit sample extracts and made up to the volume of 300 µl by distilled water. The mutagenicity modification effect of the fruit extract against nitrite treated 1-AP was

evaluated according to the mutagenicity assay as described above.

DATA EVALUATION

The results were reported as means and standard deviations of the number of His⁺ revertants per plate. The mutagenicity of the gac extracts was determined as the number of His⁺ revertants from at least two concentrations of the gac extracts were higher than the number of spontaneous revertants with a dose-response relationship and the number of His⁺ revertants from at least one concentration of the extracts showed higher than two times of spontaneous revertants [16, 17].

The degree of inhibition (+) or enhancement (-) of mutagenic modification effect was classified into four levels as negligible (± <20%), weak (± 20-40%), moderate (± 40-60%) and strong (± >60%) [18, 19] by the following formula.

$$\% \text{ Modification} = \frac{A - B}{A - C} \times 100$$

A = Number of His⁺ revertants of nitrite treated 1-AP

B = Number of His⁺ revertants of nitrite treated 1-AP with gac extracts

C = Number of spontaneous revertants

RESULTS

The mutagenic activity of extracts from various

Table 2 Percentage of modification of water extracts of gac fruits on *S. typhimurium* strain TA98 and TA100 without enzymatic activation using Ames test

Amount of each part of gac fruit extract (µl)		TA98		TA100	
		His ⁺ revertants/plate ^a %modification		His ⁺ revertants/plate ^a % modification	
Unripe pulp	Spontaneous	29 ± 6	-	74 ± 6	-
	0 ^b	1,699 ± 51	-	841 ± 157	-
	50	1,442 ± 79	+ 15	788 ± 116	+ 7
	100	853 ± 26	+ 51	543 ± 115	+ 39
	200	701 ± 41	+ 60	503 ± 90	+ 44
Half-ripe pulp	Spontaneous	17 ± 4	-	112 ± 8	-
	0 ^b	1,337 ± 99	-	604 ± 30	-
	50	1,140 ± 49	+ 15	568 ± 26	+ 7
	100	892 ± 45	+ 34	469 ± 69	+ 28
	200	694 ± 55	+ 49	378 ± 29	+ 46
Fully ripe pulp	Spontaneous	22 ± 5	-	106 ± 14	-
	0 ^b	1,741 ± 69	-	921 ± 146	-
	50	1,580 ± 90	+ 9	1,098 ± 176	- 22
	100	1,484 ± 80	+ 15	842 ± 131	+ 10
	200	1,339 ± 65	+ 23	771 ± 126	+ 18
Aril	Spontaneous	35 ± 3	-	104 ± 4	-
	0 ^b	1,123 ± 90	-	1,069 ± 103	-
	50	1,008 ± 59	+ 11	1,063 ± 75	+ 1
	100	780 ± 78	+ 31	1,088 ± 90	- 2
	200	665 ± 118	+ 42	1,185 ± 61	- 12

^a The data were reported as means with standard deviations of triplicate plates from two experiments

^b No extract was added to the standard mutagen represented a positive control

+ or – indicates that the extracts inhibited or enhanced the mutagenicity of the model, respectively

parts of gac fruit, including unripe pulp, half-ripe pulp, fully ripe pulp and aril was shown in Table 1. They all showed no mutagenicity on both strains of *S. typhimurium* either with or without nitrite treatment. The mutagenic modification effect of these fruit extracts against a standard mutagen; nitrite treated 1-AP was shown in Table 2. The results showed that, all fruit extracts demonstrated weak to moderate inhibitory effects with dose response manner on *S. typhimurium* strain TA98. The extracts from unripe pulp, especially at the highest concentration tended to reduce the mutagenicity of nitrite treated 1-AP rather better than the other parts. According to the results from *S. typhimurium* strain TA100, weak to moderate degree of inhibition was observed only in the two higher amounts of the extracts from unripe and half-ripe pulp. Unlike, the extracts from fully ripe pulp slightly enhanced the mutagenicity of nitrite treated 1-AP at the lowest concentration but showed negligible effect in the two higher amounts. The enhancing trend was also found when the extract from aril part of gac fruit was evaluated.

DISCUSSION

Gac fruit contained several nutrients, including carbohydrate, protein, fat and fibre [20] as well as non-nutritive substances known as phytochemicals, some of which function as antioxidants and contributed to health benefit. Different kinds of phytochemicals were found in different parts of gac fruit. Aril contained the highest contents of carotenoids, especially lycopene and β-carotene. Ferulic acid and *p*-hydroxybenzoic acid were phenolic acids found in the highest amounts in unripe pulp. Myricetin was a flavonoid found in all parts with the highest amounts in unripe pulp. Apigenin; another flavonoid, was predominantly distributed in fully ripe pulp. From the previous study, the total phenolic and flavonoid contents in gac pulp tended to decrease by gac maturity from unripe to fully ripe fruit and consequently decreased in the antioxidant activity. Unlike, lycopene and β-carotene contents in gac pulp slightly increased during fruit development [5]. Thus, the finding whether edible parts of gac fruit have any mutagenicity or antimutagenicity against a mutagen

was important for consumer health.

In mutagenic activity assay, the extracts from various parts of gac fruit, including unripe pulp, half-ripe pulp, fully ripe pulp and aril showed no mutagenic activity. After treatment with nitrite, they also showed no mutagenicity effect by the study concentrations which implied that gac fruit did not produce the direct mutagen when reacted with nitrite. Many investigators reported that the direct-acting mutagens like *N*-nitroso compounds were the by products from the reaction of nitrite and the nitrosable mutagen precursors containing in many food products [21-24]. It suggested that gac fruit was rather safe for consumption.

This study further investigated antimutagenic activity of gac fruit. 1-AP treated with nitrite was used as a standard mutagen because it was a direct-acting mutagen which required no enzymatic activation [25, 26]. 1-AP represented a compound categorised in polycyclic aromatic hydrocarbons (PAHs) which were mainly contributed to air pollution causing mutation process [27]. In antimutagenic activity assay, all gac extracts had antimutagenic activity on *S. typhimurium* strain TA98 referred to the influence on frameshift-mediated mutagens. The highest antimutagenic activity of the extract from pulp was obtained from unripe stage (60% inhibition) and the effects tended to reduce by gac maturity. The phenolic antioxidants which were the main components in gac pulp may be involved in the antimutagenic effect. Previous studies reported that phenolic compounds had antimutagenic activity against many mutagens such as *N*-nitroso compounds; oxidative mutagen and smoke-induced mutagens [28, 29]. For *S. typhimurium* strain TA100, the extracts from unripe and half-ripe pulp showed weak to moderate antimutagenic activity. Unlike, the extracts from fully ripe pulp and aril had no antimutagenic activity. The mutagenic effects of the extracts from fully ripe pulp (22% enhancement) and the enhancing trend of the extracts from aril on mutagenic activity of nitrite treated 1-AP may be attributed to pro-oxidants at certain concentrations. Some natural antioxidants may exert toxic pro-oxidant activities by auto-oxidation and/or enzymatic activation [30]. As a result of previous reports, several fruits and vegetables containing natural antioxidants exhibited mutagenic activity toward *S. typhimurium* strain TA98 and TA100. Six samples (grapes, onions, peaches, raisins, raspberries and strawberries) from these fruits and

vegetables showed strong mutagenic activity [31]. Citrus fruit juices which contained many flavonoid compounds exhibited mutagenic activity after enzymatic hydrolysis [32]. Moreover, the water extracts of red grapes enhanced the mutagenic activity of hydrogen peroxide with dose dependent manner [33].

The reduction of His⁺ revertants of both strains of *S. typhimurium* induced by nitrite treated 1-AP suggested that gac fruit contained some bioactive compounds which inhibited the mutagenic activity of nitrite treated 1-AP. Further investigation still required on the isolation of the bioactive compounds from these fruit extracts and to understand the antimutagenic mechanism of which they may react against the mutagenicity of nitrite treated 1-AP.

CONCLUSION

This study indicated that the water extracts from gac fruit had no mutagenic activity either with or without nitrite treatment. Moreover, the presence of antimutagenic activity of these fruit extracts, especially from unripe pulp could be useful for the purpose of mutation chemoprevention.

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