ฤทธิ์ก่อกลายพันธุ์และฤทธิ์ต้านการกลายพันธุ์ของสารสกัดน้ำใบมะรุม

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บทคัดย่อ

มะรุมเป็นพืชที่ปลูกกันอย่างแพร่หลายในประเทศ ส่วนต่าง ๆ โดยเฉพาะใบถูกนำมาใช้ในการประกอบอาหารรวมถึงการแพทย์พื้นบ้าน การศึกษาในครั้งนี้ทำการทดสอบฤทธิ์ก่อกลายพันธุ์และฤทธิ์ต้านการกลายพันธุ์ของสารสกัดน้ำใบมะรุมโดยการทดสอบเอมส์ในแบคทีเรียซัลโมเนลลา ไทฟิมิวเรียม สายพันธุ์ TA98 และ TA100 ในระบบที่มีและไม่มีเอนไซม์เพื่อกระตุ้นการออกฤทธิ์ ผลการทดลองพบว่าสารสกัดใบมะรุมความเข้มข้น 0-5 mg/pl ไม่มีฤทธิ์ก่อกลายพันธุ์ในแบคทีเรียทั้ง 2 สายพันธุ์ แต่พบว่าสารสกัดใบมะรุมออกฤทธิ์ต้านการกลายพันธุ์ในแบคทีเรียสายพันธุ์ TA 100 เมื่อบ่มร่วมกับ AF-2 ในสภาวะที่ไม่มีเอนไซม์กระตุ้น โดยสารสกัดใบมะรุมสามารถลดจำนวนโคโลนีกลายพันธุ์แบบแปรผันตามความเข้มข้นในช่วง 0 - 5 mg/pl และมีนัยสำคัญที่ความเข้มข้นสูงสุด (p<0.05) โดยมีร้อยละของการยับยั้งเท่ากับ 51.33 ซึ่งกลไกในการออกฤทธิ์ต้านการกลายพันธุ์อาจเกี่ยวข้องกับการยับยั้งการเข้าสู่เซลล์หรือลบล้างฤทธิ์ของ AF-2 โดยตรง ด้วยเหตุนี้จึงเป็นที่น่าสนใจในการนำสารสกัดจากใบมะรุมไปทดสอบฤทธิ์ต้านการกลายพันธุ์ และฤทธิ์ต้านการเกิดมะเร็งในสัตว์ทดลองต่อไป

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Mutagenic and Antimutagenic Activities of Aqueous Extract of *Moringa oleifera* Leaves

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ABSTRACT

Moringa oleifera is a cosmopolitan tree that grows in many tropical countries and useful for its various nutritional and pharmacological applications. In this study, aqueous extract of *M. oleifera* leaves was evaluated for its mutagenic and antimutagenic activities by Salmonella/microsome mutagenicity assay (Ames test). Mutagenic assay in *Salmonella typhimurium* (TA 98 and TA 100 as tester strains) with and without metabolic activation showed that 0.2-5 mg/plate of *M. oleifera* extract did not induce the growth of His^+ revertant colonies in both strains, indicating the absence of mutagenicity. Therefore, antimutagenic activities of *M. oleifera* extract against the standard mutagens, aflatoxin B1 (AFB1) and 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) were tested in *S. typhimurium* TA100 strain. The number of His^+ revertants/plate of *S. typhimurium* after incubation of *M. oleifera* extract (1-5 mg/plate) with AFB1 (0.1 μg/plate) was not different from positive control, however, 5 mg/plate of the extract exhibited the significant inhibition of the His^+ revertant colonies (51.33% Inhibition) when incubated with AF-2 (0.01 μg/plate). It was clear from the present studies that aqueous extract *M. oleifera* was capable of antimutagenicity through the mechanism in which some active compounds contained might inhibit the mutagen penetration into *S. typhimurium* cells or neutralize the mutagenic potency prior to nucleus entering, thus protecting the hot-spot region of gene from mutation. Of the results obtained, the further studies will be forward to the investigations including antimutagenicity and cancer chemoprevention *in vivo*.

Keywords: *Moringa oleifera*, Mutagenicity, Antimutagenicity, and Ames test

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Introduction

Moringa oleifera (a member of Moringaceae) is a highly valued plant, distributed in many countries of the tropic and subtropics as food and herb. [1] In some parts of the world *M. oleifera* is referred to as the drumstick tree or the horse radish tree. In Thailand, immature pods, leaves, and fruits have been used as food ingredient, particularly, its leaves as a commercial food supplement. There has been an impressive range of medicinal uses with high nutritional value of its leaves, fruits, flowers, and immature pods. [2, 3] It has been reported that *Moringa* leaves are rich in *β*-carotene, protein, calcium, potassium, vitamin C and E, and polyphenols. [4, 5] Moreover, the biochemical and pharmacological investigations revealed its various properties including antioxidant, hypolipidemic, antiatherosclerotic, [6-8] antimicrobial activities, [9] protective activity of oxidative DNA damage [7] and myocardial destruction, [10] antitumor activities, [11] hypoglycemic potential, [12] and liver detoxification enhancement. [13, 14]

It is well understood that dietary factors play a critical role in human cancer and strong efforts have been made to identify protective substances in food. Therefore, searching for herbs or diets capable of antimutagenic and anticarcinogenic activities is the most interesting alternative for disease prevention. [15-19] It has been reported that *M. oleifera* drumstick shows chemopreventive activity against two stage model of DMBA-induced skin papillomagenesis by modulating carcinogen-metabolizing enzymes and antioxidant system.[20] To date, little is known about biological activity of *M. oleifera leaves* especially both antimutagenesis and anticarcinogenesis. As it is widely consumed as food ingredient, folk medicine, and food supplement. The present study was aimed to evaluate the mutagenic and antimutagenic activities of aqueous extract of *M. oleifera* leaves by Salmonella/microsome mutagenicity assay (Ames test) that is extensively used as *in vitro* standard method. [21-24] The obtained results will be the preliminarily scientific data for the further antimutagenic and anticarcinogenic studies.

Materials and Methods

Chemicals

All chemicals and solvents used were the analytical grade. The standard mutagens used including 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2), 2-aminoanthracene (2-AA) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan), and aflatoxin B1 (AFB1) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The tryptic soy broth, bacto agar was purchased from Difco Laboratories (Detroit, Michigan, USA). Nutrient broth no. 2 was purchased from Oxoid, Ltd. (Basingstoke and Hants, England).

Preparation of *M. oleifera* extract

The air-dried leaves of *M. oleifera* were kindly provided from Lampang Herb Conservation (Muang, Lampang, Thailand.). The dried leaves were grounded into powder and stored at 4 °C until extraction. 15 g of powdered leaves was extracted with 350 ml of hot water for 15 min. The liquid extract was filtered through Whatman no. 1 filter paper, and then the filtrate was lyophilized to obtain the powder of extract. The yellow-green powder of aqueous extract (% Yield = 28.12) was kept in amber glass at -20 °C until use.

Salmonella typhimurium preparation

Both TA98 and TA100 of *S. typhimurium* used as tester strains, and S9 mix (for enzymatically metabolic activation)

were kindly provided by Asst. Dr. Rawiwan Wongpoomchai, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. In brief, 20 µl of inoculum from the permanent culture was incubated in 10 ml of nutrient broth no. 2 at 37 °C for 14 hr, the bacterial concentration of an approximate 1.2 x 10^9 bacterial/ml was obtained.

Mutagenic assay

According to Maron and Ames, [25] the described method (preincubation technique) was performed to assay mutagenicity in both non-metabolic (-S9 mix) and metabolic (+S9 mix) activation of the extract. Briefly, 50 µl of solvent (negative control), 0.2-5 mg/plate of the extract, or standard mutagens as positive control, AF-2 or 2-AA, were added by 500 µl of phosphate buffer or S9 mix. The mixtures were then preincubated with 100 µl of overnight culture of TA98 or TA100 strains at 37 °C for 20 min before adding 2 ml of top agar containing 0.5 mM *L*-histidine/*D*-Biotin. The His^+ revertant colonies in all plates were analyzed after 37 °C incubation for 48 hr. Triplicate plates per run were assayed.

Antimutagenic assay

As mentioned in mutagenic assay, the preincubation technique was modified. In brief, the mixtures consisting of solvent alone (DMSO) or 1-5 mg/plate of extract in the presence of AFB1 (0.1 µg/plate) or AF-2 (0.01 µg/plate) were preincubated with overnight culture of TA100 strain with or without S9 mix at 37 °C for 20 min before 2 ml of top agar addition. The His^+ revertant colonies were analyzed after 37 °C incubation for 48 hr. Triplicate plates per run were assayed. The number of counted revertant colonies was subtracted by the spontaneous revertants before calculating the percentage inhibition.

Statistic analysis

Data obtained from three-independent experiments were expressed as mean±standard error of the mean (SEM). Analysis of variance was performed using ANOVA, and Bonferroni test with $p<0.05$ was considered as significant difference between control and treated groups.

Results and DISCUSSION

For the testing doses, killing effect, the sign of cytoxicity, was not observed in both TA98 and TA100 strains of *S. typhimurium*. As shown in Table 1, the number of TA98 His^+ revertants from plates treated with 0.2-5 mg/plate of extract was ranged from 40.00±7.94 – 43.33±4.60 (-S9 mix) and 32.50±0.87 – 40.00±0.58 (+S9 mix) that were not different from negative control, 37.33±4.50 (-S9 mix) and 54.00±13.91 (+S9 mix). Likewise, the similar results were found in *S. typhimurium* TA100 strain treated with the same concentration of the extract. In table 2, the revertant colonies of dosing plates with non-metabolic and metabolic activation was 113.33±5.59 – 145.00±17.64 and 143.50±18.76 – 152.50±20.50 compared with negative control 124.00±13.70 and 168.00±37.63, respectively. The results suggested that 0.2-5 mg/plate of *M. oleifera* leave extract was non-mutagenic in both strains of *S. typhimurium* either with or without metabolic activation. In consideration of antimutagenicity of *M. oleifera* extract in TA100 strain (Table 3), co-treatment of AF-2 and 1-5 mg/plate of extract in the absence of S9 mix showed an antimutagenic activity in dose-dependent manner, and highest dose treated exhibited significant reduction ($p<0.05$) of the revertant colonies (331.00±90.64, % Inhibition = 51.33±13.90)

in comparison with positive control (556.00±54.39). However, the revertant colonies observed from 1-5 mg/plate of extract co-treated with AFB1 in the presence of S9 mix were not different from positive control. In 2001, Rao and colleagues investigation exhibited the radioprotective effect of *M. oleifera* leave extract against γ-radiation induced chromosomal aberration in bone marrow cells and micronucleus formation in erythrocytes of mice. [26] Moreover, there are the investigations with the antioxidant assay of *M. oleifera* leaves extracted with different solvents, they indicated that the polyphenolic compounds contained in the extracts were involved in antioxidant properties. [6, 8] With regard to the observed results, it is possible that the antimutagenic activity might be from the polyphenolic compounds contained by mechanisms (1) inhibition of the mutagen penetration into *S. typhimurium* cells or (2) neutralization of mutagenic potency prior to nucleus entering, thus protecting the hot-spot region of gene from mutation. Of the present and previous studies including antioxidant, [6] anticlastogenicity, [26] protective effect of oxidative DNA damage, [7] and enhancement of detoxification, [13, 14] it is the interesting point for the further studies of antimutagenicity and cancer chemoprevention by *M. oleifera* leaves *in vivo*.

In conclusion, aqueous extract of *M. oleifera* showed non-mutagenic in both TA98 and TA100 strains of *S. typhimurium* and was able to inhibit the mutagenicity of AF-2 in non-metabolic activation condition.

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Table 1 Mutagenicity of aqueous extract of *M. oleifera* leaves in *S. typhimurium* TA98 strain with and without metabolic activation

	His^+ Revertants/plate	
	Metabolic activation	
Treatment	-	+
AF-2 (0.1 µg/plate)	257.00±24.67	N.A.
2-AA (0.5 µg/plate)	N.A.	446.50±61.50
Negative control	37.33±4.50	54.00±13.91
M. oleifera 0.2 mg/plate	40.00±7.94	40.00±0.58
M. oleifera 1 mg/plate	43.00±6.03	32.50±0.87
M. oleifera 5 mg/plate	43.33±4.60	35.00±0.58

Values expressed as Mean±SEM were obtained from three-independent experiments. No killing effect was observed.
2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) and 2-aminoanthracene (2-AA) were used as standard mutagens.
- = Non-metabolic activation (-S9 mix)
+ = Metabolic activation (+S9 mix)
N.A. = Not analyzed

Table 2 Mutagenicity of aqueous extract of *M. oleifera* leaves in *S. typhimurium* TA100 strain with and without metabolic activation

	His^+ Revertants/plate	
	Metabolic activation	
Treatment	-	+
AF-2 (0.01 µg/plate)	428.33±53.95	N.A.
2-AA (0.5 µg/plate)	N.A.	393.50±6.01
Negative control	124.00±13.70	168.00±37.63
M. oleifera 0.2 mg/plate	130.67±12.89	143.50±18.76
M. oleifera 1 mg/plate	145.00±17.64	152.50±20.50
M. oleifera 5 mg/plate	113.33±5.59	151.50±18.66

Values expressed as Mean±SEM were obtained from three-independent experiments. No killing effect was observed.
2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) and 2-aminoanthracene (2-AA) were used as standard mutagens.
- = Non-metabolic activation (-S9 mix)
+ = Metabolic activation (+S9 mix)
N.A. = Not analyzed

Table 3 Antimutagenic activities of aqueous extract of *M. oleifera* leaves towards the mutagenicities of AF-2 and AFB1 in *S. typhimurium* TA100 strain

	His^+ Revertants/plate	
	Metabolic activation	
Treatment	-	+
DMSO	80.50±3.48	N.A.
AF-2 (0.01 µg/plate)	556.00±54.39	N.A.
AF-2 (0.01 ug/plate) + *M. oleifera* 1 mg/plate	476.50±85.74	N.A.
AF-2 (0.01 ug/plate) + *M. oleifera* 5 mg/plate	331.00±90.64*	N.A.
DMSO	N.A.	117.50±5.38
AFB1 (0.1 µg/plate)	N.A.	827.00±39.84
AFB1 (0.1 ug/plate) + *M. oleifera* 1 mg/plate	N.A.	965.00±110.91
AFB1 (0.1 ug/plate) + *M. oleifera* 5 mg/plate	N.A.	1024.50±131.23

Values expressed as Mean±SEM were obtained from three-independent experiments.
*Significant difference in comparison with positive control (p<0.05)
2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) and aflatoxin B1 (AFB1) were used as standard mutagens.
- = Non-metabolic activation (-S9 mix)
+ = Metabolic activation (+S9 mix)
N.A. = Not analyzed