ANTIMUTAGENIC ACTIVITY OF *SESBANIA JAVANICA* MIQ. FLOWER DMSO EXTRACT AND ITS MAJOR FLAVONOID GLYCOSIDE

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Abstract. The antimutagenic activity of *Sesbania javanica* Miq. or Sano, an edible vegetable flower DMSO extract against aflatoxin B1 (AFB_1) benzo (a) pyrene [B(a)P], was evaluated by means of the Ames' test. The *Sesbania javanica* Miq. flower DMSO extract showed a strong inhibitory effect against AFB_1 and B(a)P mutagens. A search to isolate the major flavonoid in *Sesbania javanica* Miq. flower extract found the flavonol glycoside, Quercetin 3-2^G-rhamnosylrutinoside, which was confirmed by its physicochemical properties as a major constituent of the flower. Quercetin 3-2^G-rhamnosylrutinoside (207 μg/plate) also showed a strong inhibitory effect against AFB_1 and B(a)P with a more than 70% inhibition rate.

INTRODUCTION

Research suggests that a number of dietary components may be capable of increasing or decreasing cancer incidence (Wattenberg, 1983). Thus, manipulation of the diet may be a noninvasive approach to minimize cancer incidence (Liu *et al*, 1992). Increased consumption of fruits and vegetables has been found to be associated with a lower incidence of various types of cancer and lower cancer mortality rates in several human cohort and case-control studies for all common cancer sites (Ames *et al*, 1993; Willett, 1994). Intake of sufficient amounts of antimutagens and/or anticarcinogens is believed to confer a preventive effect against the development of human cancer (Ferguson, 1994). In animal experiments, vegetables common in human diets have been shown to have antimutagenic effects (Bingham, 1990; Wattenberg and Coccia, 1991; Vinitketkumnuen *et al*, 1994; Kusamran *et al*, 1998). Fruit and vegetables provide protection against mutagenicity and cytotoxicity which has been attributed to the various antioxidants contained in them (Ames, 1983; Gey, 1990; Cozzi *et al*, 1997). There is evidence indicating that free radicals cause oxidative damage to proteins, lipids, and nucleic acids. Therefore, antioxidants, which can neutralize free radicals, may be of importance in the prevention of several disease states. Fruits and vegetables contain a great variety of different antioxidant compounds. Most of the antioxidant capacity of fruit and vegetables comes from compounds other than vitamins C, E, β-carotene and flavonoid (Wang *et al*, 1996; Pietta, 2000; Yang *et al*, 2001).

Vegetables, such as Cha-om, Indian mulberry, cucumber, ivy gourd, lettuce, neem, sweet basil, lemon grass, sadao, and krachia daeng act as antimutagens against the mutagenicity of benzo (a) pyrene [B(a)P] and aflatoxin B_1 (AFB_1) (Viniketkumnuen *et al*, 1994; Kusamran *et al*, 1998; Nakahara *et al*, 2002). A wide variety of naturally occurring compounds or their mixtures, have been shown to inhibit mutagenicity and carcinogenicity cased by several chemicals. Humans can be exposed to AFB_1 or B(a)P by either ingestion or inhalation. AFB_1 is common in cultures and food products. Many agricultural products are vulnerable to attack by fungi (*Aspergillus flavus*, *Aspergillus parasiticus*, etc)

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which are able to produce toxic metabolites called mycotoxins. Among the various mycotoxins, AFB_1 has assumed significance due to its deleterious effects on humans, poultry and livestock. AFB_1 is primarily a hepatotoxin, and in some species, such as the rat and the rainbow trout, a hepatocarcinogen and mutagen. B(a)P is one of a group of compounds called polycyclic aromatic hydrocarbons (PAHs) which are found in the exhaust of motor vehicles and other gasoline and diesel engines, as well as in the emissions of coal-, oil-, and wood-burning stoves and furnaces, cigarette smoke, general soot and smoke of industrial, municipal, and domestic origin, and in cooked foods, especially charcoal-broiled foods, in incinerators, coke ovens, and in asphalt processing and use. It may be found in some foodstuffs and in some public and private drinking water supplies. It is a carcinogen, mutagen, tumorigen, neoplastigen and teratogen. Toxic response depends on how the molecule is metabolized, both mutagens require metabolic activation to DNA-reactive intermediates by cytochrome P450-mediated mixed-function oxidases (MFOs) in order to exert their carcinogenic action. Both these mutagens tested were chosen because they are the most frequently occurring in mutagens found in food and feed.

Sesbania javanica Miq. flower or "Sano" in Thai or "Phak hong hang" (northern Thai). The *Sesbania javanica* Miq. flower is a leguminous plant (Leguminosae) originally grown in Southeast Asia, especially in Thailand. The Sano (*Sesbania javanica* Miq.) flowers are harvested only in the rainy season. It is commonly consumed in Thailand. It has also been used for its diverse medicinal properties. In ancient Thai traditional medicine, it was used as an anti-inflammatory for insect bites, detoxification, intestinal abscess healing, stomach discomfort and to relieve internal fever and thirst.

Flavonoids are a diverse group of phytochemicals that are produced by various plants in high quantities. Natural flavonoids usually occur as glycosides (*eg* glucose, rhamnose, rhamnoglucosides, and rutinosides). Flavonoids have anti-bacterial, anti-viral, anti-inflammatory, anti-angional, analgesic, anti-allergic hepatoprotective, cytostatic, apoptotic, estrogenic, anti-estrogenic (Hodek *et al*, 2002), and antioxidant properties (Pietta, 2000). Their ability to scavenge hydroxyl redicals, superoxide anion radicals and lipid peroxyradicals highlights many of their flavonoid helth-promoting functions, which are important in the prevention of diseases associated with oxidative damage of membranes, proteins and DNA (Pietta, 2000; Ferguson, 2001) and the ability to modulate (inhibit or stimulate) several enzymes or cell receptors (Zhai *et al*. 1998; Omiecinski *et al*, 1999; Ciolino and Yeh, 1999).

In our study of the antimutagenic activity of DMSO extract of Sano (*Sesbania javanica* Miq), commonly used in the local diet, was observed. The major flavonoid constituent of the Sano (*Sesbania javanica* Miq.) flower was analyzed using a UV-diode array, ESI-MS, and ^1H, ^{13}C NMR. The quantitative values of this major flavonoid were determined.

MATERIALS AND METHODS

Plant material extraction

The *Sesbania javanica* Miq. flower was purchased in Bangkok. The petals were frozen at -70ºC and lyophilized. Freeze-dried petals were blended to powder. Ground, freeze-dried samples were extracted with DMSO and then centrifuged at 10,000*g* for 10 minutes to remove the flower debris and filtered through a 0.25 μm sterile membrane filter disc before being subjected it to antimutagenicity testing. The other 0.5 g ground freeze-dried sample was extracted with 10 ml of MeOH/DMSO (1:1) (Kawaii *et al*, 2000). The sample was mixed and centrifuged at 10,000*g* for 10 minutes to collect the extract solvent. These steps were repeated 3 times. The extracts were combined and MeOH was added to give a total volume of 20 ml.

Mutagens

AFB_1 and B(a)P were purchased from the Sigma Chemical (St Louis, USA). These mutagens were dissolved in DMSO. The AFB_1 concentration was 0.3 μg/plate for the *Salmonella* tester strains TA 98 and TA 100. The B(a)P concentrations were 10 μg/plate for the *Salmonella* tester strain TA 98 and 5.0 μg/plate for the *Salmonella* tester strain TA 100. AFB_1 and B(a)P are

potent mutagens and carcinogen agents. Precautions were taken for the proper handling and disposal of these chemicals.

Bacterial test strain

The *Salmonella typhimurium* TA 98 (frameshift) and TA 100 (base-pair substitution) strains which were histidine dependent due to a mutation of the histidine operon, were kindly provided by the research division of the National Cancer Institute, Bangkok, Thailand, and maintained as described by Maron and Ames (1983). The genotypes of the test strains were checked routinely for their histidine requirement.

S9 fraction and S9 mix

The method described by Matsushima *et al* (1976) was followed. Male Sprague-Dawley rats (from The Animal Center of Salaya, Mahidol University) were injected intraperitoneally with phenobarbital and β-naphthoflavone. Four days after injection, the rats were sacrificed, their livers were removed and minced in 0.15 M KCl, and then homogenized using a Potter-Elvehjem apparatus. The homogenate was centrifuged at 9,000*g* for 20 minutes in a refrigerated centrifuge, and the supernatant S9 fraction was decanted and immediately stored in cryogenic vials (Wheaton) of 1-2 ml, at -70ºC. The protein concentration of the S9 fraction was determined by the Lowry method (Lowry *et al*, 1951). The protein concentration required approximate 30 mg/ml. The S9 required for the preparation of the S9 mix was thawed at room temperature and placed in a container of crushed ice. The S9 mix was prepared as soon as the S9 had thawed. The components of the S9 mix were sodium phosphate-KCl buffer 13.2 ml (0.5M sodium phosphate, pH 7.4 15.15 ml, 1M KCl 2.5 ml added to DW 32.35 ml), 0.16M $MgCl_2$ 1.0 ml, 0.1M glucose-6-phosphate 1.0 ml, 0.1M NADP 0.8 ml, and S9 4.0 ml, mixed and kept in the ice box. The S9 mix was prepared freshly for each mutagenicity assay.

Antimutagenicity test

A modified plate incorporation procedure (Maron and Ames, 1983) was employed to determine the effect of the *Sesbania javanica* Miq. flower DMSO extracts on AFB_1 and B(a)P induced mutagenicity. In this study, *Sesbania javanica* Miq. flower DMSO extract was tested at 3 doses: 0.05, 0.5 and 5.0 mg/plate (100 μl). The major isolated compound was tested at 207 μg/plate in DMSO (100 μl). AFB_1 (0.3 μg/plate for TA 98 and TA 100) and B(a)P (10 μg/plate for TA 98; 5 μg/plate for TA 100) were used as standard mutagens. In brief, mutagen (0.1 ml) was distributed in sterilized capped tubes, and then 0.1 ml of tested *S. typhimurium* bacterial suspension from an overnight culture (1 x10^9 to 2 x 10^9 cells/ml) and 0.1 ml of *Sesbania javanica* Miq. flower DMSO extracts were added. Then, 0.5 ml of S9 mix was added. The entire mixture was pre-incubated (Matsushima *et al*, 1980), while shaking, at 37ºC for 20 minutes before 2 ml of molten top agar was added, then the mixture was poured onto a minimal medium agar plate. The plates were incubated at 37ºC for 48 hours, then the His^+ revertant colonies on each plate were counted. Each sample was assayed using duplicate plates. The data is presented as the mean ± standard error of the two independent assays. Plates without mutagens and without *Sesbania javanica* Miq. flower DMSO extracts were considered as negative controls and plates with mutagens as positive controls. The antimutagenicity effect is expressed as percentage of inhibition (% inhibition) =100 - (R_1/R_0 x 100), where R_1 is the number of His^+ revertant/plate of plates exposed to mutagens and *Sesbania javanica* Miq. flower extracts and the R_0 is the number of His^+ revertant/plate of the positive controls. The number of spontaneous revertants was substracted from the numerator and denominator. The mutagenicity of the mutagens (positive control) in the absence of *Sesbania javanica* Miq. flower DMSO extract was defined as 0% of the inhibition. The antimutagenic effect was considered moderate when the inhibitory effect of the *Sesbania javanica* Miq. flower DMSO extract was in the range of 25-40%, and strong when the inhibitory effect was >40%. An inhibitory effect <25% was considered weak, and was not recognized as a positive result.

Analytical HPLC was conducted on a SpectraSystem (P4000, AS3000, UV3000) liquid chromatography fitted with a C-18 ODS Hypersil (Thermo, Electron corporation, UK), re-

verse phase 4.6 x 250 mm (5 μm) column. HPLC analysis of the flavonoids was performed primarily according to the method of Owen *et al* (2000; 2003); 20 μl of *Sesbania javanica* Miq. flower extract was injected. Detection of flavonoids was carried out by means of a UV-detector set at an absorbance of 270 nm at room temperature. The mobile phase consisted of 2% acetic acid in water (B) and methanol (A) utilizing the following gradient over a total run time of 45 minutes: 95% B for 2 minutes, 75% B in 8 minutes, 60% B in 20 minutes, 50% B for 10 minutes, and 0% B until completion of the run. The flow rate of the mobile phase was 1 ml/minute.

Semi-preparative HPLC was conducted on the same SpectraSystem liquid chromatography fitted with a reverse-phase C-18 ODS Hypersil (Thermo, Electron corporation, UK), 25 cm (10 μm) column (internal diameter, 10 mm) similar to that used for analytical HPLC. The flow rate of the mobile phase was 3.0 ml/minute, with a 50 μl injection volume. Peaks eluting from the column were collected and pooled, then the solvent was removed by evaporation and lyophilization.

Acid hydrolysis

From each fraction collected by HPLC, 50 μl of the methanolic suspension were dried under a stream of nitrogen and 0.5 M H_2SO_4 in double distilled water (100 μl) was added and vertexed. The solutions were incubated at 100ºC for 1 hour. After incubation, double distilled water (1.0 ml) was added, passed through a Sep-Pak Vac 1-cc C-18 cartridge (Waters). The water elute (800 μl) was collected and identified by paper chromatography (Markham, 1982) using different sugars as standards.

Analytikjena-specord S100 spectrophotometer

The UV absorption data of these compounds in methanol were recorded on the analytikjena-specord S100 (Jena, Germany), by photo diode array was performed at a wavelength of 200-550 nm at the Department of Chemistry, Faculty of Science, Naresuan University.

Electrospray Ionization-Mass Spectrometry (ESI-MS)

The ESI mass spectra were recorded on a Bruker Esquire spectrometer (Bruker-Franzen Analytik) in methanol in the positive mode at the service division of the Department of Chemistry, Faculty of Science, Mahidol University.

Nuclear Magnetic Resonance Spectroscopy (NMR)

The NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer (Bruker Biospin AG) at the Department of Chemistry, Faculty of Science, Naresuan University. The ^1H and ^{13}C nuclear magnetic resonance spectra were recorded at 400.13 MHz and 100 MHz, respectively, at 25ºC using the standard technique. The compounds (ca. 4-8 mg) were dissolved in 0.4 ml methanol-d_4 (99.96%D) in 5 mm sample tubes. The following measurements were carried out: conventional 1D ^1H and ^{13}C. The chemical shifts (d) were reported in ppm relative to the solvent resonances. Methanol-d_4 was used as an internal secondary standard at δ_H 3.31 ppm for ^1H. The coupling constant (*J*) values were recorded in Hz; the δ_C = 49.05 ppm for ^{13}C.

Molecular modeling

As a guide for making the initial molecular formula and the NMR signal assignments, chemical shift predictions were made using the ChemDraw Ultra 6.0 (CambridgeSoft, Cambridge, MA).

RESULTS

The antimutagenicity test, the DMSO extract of *Sesbania javanica* Miq. flower strongly inhibited AFB_1 and B(a)P induced mutagenicity, by *S. typhimurium* tester strains TA98 and TA100 (Table 1). The relationship between the percentage of inhibition versus the extract concentration was analyzed by simple regression. Analyses were carried out using Microsoft Excel. The inhibitory effect of *Sesbania javanica* Miq. flower extract on AFB_1 ($r=0.894$; $p\leq0.01$) and B(a)P ($r=0.813$; $p\leq0.01$) for *Salmonella* TA 98 was obtained. The inhibitory effect of the *Sesbania javanica* Miq. flower extract on AFB_1 ($r=0.816$; $p\leq0.01$) and B(a)P ($r=0.803$; $p\leq0.01$) for *Salmonella* TA 100, increased with an increasing concentration of flower extract. The isolated major flavonoid (207 μg/plate) also possessed a strong antimutagenic effect (>70%) against AFB_1 and

Table 1
The antimutagenicity of *Sesbania javanica* Miq. flower DMSO extract on *S. typhimurium* TA98 and TA 100.

Amount of extract (mg)	Number of His$^+$ revertants/plate of *Salmonella* TA98				Number of His$^+$ revertants/plate of *Salmonella* TA100			
	AFB_1 0.3 μg (+S9)	(%) inhibition	B(a)P 10 μg (+S9)	(%) inhibition	AFB_1 0.3 μg (+S9)	(%) inhibition	B(a)P 5 μg (+S9)	(%) inhibition
0.05	278 ± 9[a]	58.7[b]	148 ± 9	60.4	350 ± 9	60.2	266 ± 6	62.1
0.5	238 ± 6	65.3	127 ± 4	67.4	288 ± 4	69.9	223 ± 7	71.6
5.0	201 ± 9	71.5	116 ± 10	71.1	255 ± 10	75	202 ± 10	76.2
Positive control	630 ± 9		328 ± 12		738 ± 12	-	548 ± 10	-
Spontaneous	30 ± 2		30 ± 2		94 ± 9	-	94 ± 9	-

The significance was tested by Student's *t*- test ($p<0.01$) *vs* control group.
[a] Values represent mean ± SD based on two experiments.
[b] The values in parenthesis are the inhibition rates (%).
Inhibition (%inhibition) = 100 - (R_1/R_0 x 100), where
R_1 is the number of His$^+$ revertant/plate of plates exposed to mutagens and *Sesbania javanica* Miq. flower extract
R_0 is the number of His$^+$ revertant /plate in the positive control.
The number of spontaneous revertants was substracted from the numerator and denominator.

Table 2
Antimutagenic effects of the major flavonol, Quercetin 3-2^G-rhamnosylrutinoside from the *Sesbania javanica* Miq. flower DMSO/MeOH (1:1) extract against AFB_1 and B(a)P on *S. typhimurium* TA98 and TA 100.

Quercetin 3 - 2^G-rhamnosyl rutinoside (μg/plate)	Number of His$^+$ revertants/plate of *S. typhimurium* TA98				Number of His$^+$ revertants/plate of *S. typhimurium* TA100			
	AFB_1 0.3 μg/plate (+S9)	(%) inhibition	B(a)P 10 μg/plate (+S9)	(%) inhibition	AFB_1 0.3 μg/plate (+S9)	(%) inhibition	B(a)P 10 μg/plate (+S9)	(%) inhibition
207	166±9[a]	76.1[b]	112±8	76.0	280±10	73.8	252±9	74.3
Positive control	596±10		369±8		824±14		729±10	
spontaneous	31±2		31±2		87±5		87±5	

The significance was tested by Student *t*- test ($p < 0.01$) *vs* control group.
[a]Values represent mean ± SD based on two experiments.
[b]The values in parenthesis are the inhibition rates (%).
Inhibition (%inhibition) = 100 - (R_1/R_0 x 100), where
R_1 is the number of His$^+$ revertant/plate of plates exposed to mutagens and *Sesbania javanica* Miq. flower extract
R_0 is the number of His$^+$ revertant /plate in the positive control.
The number of spontaneous revertants was substracted from the numerator and denominator.

B(a)P (Table 2).

In a search for the major flavonoid, the DMSO/MeOH (1:1) extract was reverse-phase high performance chromatographed over C-18 ODS Hypersil to yield the major compounds. The major compound was a flavonol glycoside isolated from the *Sesbania javanica* Miq. flower DMSO/MeOH: a pale-yellow amorphous pow-

der. On acid hydrolysis of this compound, glucose, and rhamnose were identified by paper chromatography (Markham, 1982). UV spectra of this compound in MeOH showed a λ_{max} of 357.81 and 256.67 nm as quercetin.

The physicochemical properties of this major compound were as follows: ESI-MS in the positive mode, showed a molecular ion of compound 1 at *m/z* 779.2 $(M + Na)^+$, with fragment ions 691.3, 413.2, and 301.1, which correspond to the formula $C_{33}H_{40}O_{20}$ *m/z* 756.2 $(M+H)^+$; λ_{max} in MeOH of 357.81 and 256.67 nm. ^1H NMR chemical shifts (CD_3OD) of aglycone were δ 6.18 (*d*, *J*=2.2 Hz, H-6), 6.38 (*d*, *J*= 2.2 Hz, H-8), 7.58 (*d*, *J* = 2.1 Hz, H-2'), 6.87 (*d*, *J*=8.9 Hz, H-5'), and 7.59 (*dd*, *J*= 2.2, 8.9, Hz, H-6'); 3-glucosyl were δ 5.58 (*d*, *J*= 7.7 Hz, H-1), 3.64 (*dd*, *J*= 7.7, 9.3 Hz, H-2), 3.53 (*t*, *J*=9.4 Hz, H-3), 3.34 (*t*, *J*=9.3 Hz, H-4), 3.33 (*ddd*, *J*= 1.5, 5.6, 9.4 Hz, H-5), 3.81 (*dd, J*= 1.5, 11.5 Hz, H-6a), and 3.39 (*dd*, *J*= 5.6, 11.5 Hz, H-6b); 2"-rhamnosyl were δ 5.22 (*d*, *J*= 1.5 Hz, H-1), 4.00 (*dd*, *J*= 1.5, 3.4 Hz, H-2), 3.79 (*dd*, *J*= 3.4, 9.4 Hz, H-3), 3.26 (*t*, *J*= 9.4 Hz, H-4), 4.07 (*dq*, *J*= 6.1, 9.4 Hz, H-5), and 1.00 [*d*, *J*= 6.1 Hz, H-6 (CH_3)]; 6"-rhamnosyl were δ 4.49 (*d*, *J*= 1.7 Hz, H-1), 3.58 (*dd*, *J*= 1.7, 3.4 Hz, H-2), 3.48 (*dd*, *J* = 3.4, 9.4 Hz, H-3), 3.22 (*t*, *J*= 9.5 Hz, H-4), 3.41 (*dq*, *J*= 6.3, 9.5 Hz, H-5), and 1.07 [*d*, *J*= 6.3 Hz, H-6 (CH3)]; ^{13}C NMR chemical shift (CD_3OD) aglycone were δ 158.94 (C-2), 134.44 (C-3), 179.29 (C-4), 163.15 (C-5), 99.74 (C-6), 165.57 (C-7), 94.69 (C-8), 158.45 (C-9), 105.93 (C-10), 123.45 (C-1'), 117.44 (C-2'), 145.91 (C-3'), 149.54 (C-4'), 116.06 (C-5'), and 123.56 (C-6'); 3-glucosyl were δ 100.50 (C-1), 80.06 (C-2), 78.93 (C-3), 71.89,

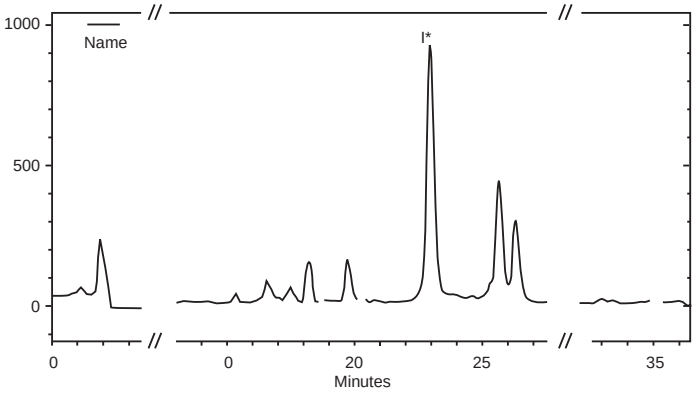


Fig 1–RP-HPLC chromatogram of DMSO/MeOH (1:1) extract of *Sesbania javanica* Miq. flower. I* is a major compound.

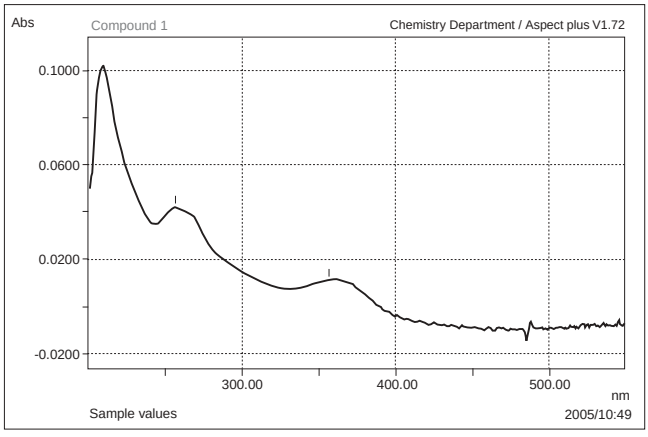
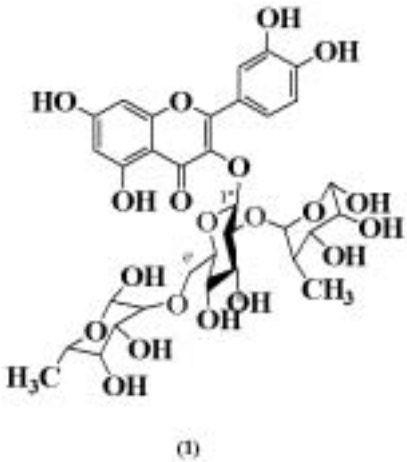


Fig 2–UV-visible spectra of compound I*.



(1)

Fig 3–Structure of the major flavonol glycoside (I*) isolated from the *Sesbania javanica* Miq. ('Sano') flower, Quercetin 3-2^G-rhamnosylrutinoside.

77.10 (C-5), and 68.30 (C-6); 2"-rhamnosyl were δ 102.64 (C-1), 72.41 (C-2), 72.31 (C-3), 74.07 (C-4), 69.96 (C-5), and 17.51 (C-6); and 6"-rhamnosyl were δ 102.26 (C-1), 72.15 (C-2), 72.28 (C-3), 73.88 (C-4), 69.71 (C-5), and 17.81(C-6). Thus, the major compound was identified as quercetin 3-2^{G}-rhamnosylrutinoside, a known compound. In the quantitation of this compound, 3.16 g/kg of dried weight was calculated from the collection steps.

DISCUSSION

The ^{1}H NMR spectrum in methanol-*d4* of this major compound showed the signals: δ 6.18 (*d*, *J*= 2.0 Hz, H-6), 6.38 (*d*, *J*= 2.0 Hz, H-8), 7.58 (*d*, *J*=2.1 Hz, H-2'), 6.87 (*d*, *J*=8.9 Hz, H-5'), and 7.59 (*dd*, *J*= 2.2, 8.9, Hz, H-6'), and the UV data λ_{max} in MeOH of 357.81 and 256.67 nm for quercetin. The anomeric protons in this major compounds at d 5.58 (*d*, *J*= 7.7 Hz, H-1 of glucose), 5.22 (*d*, *J*= 1.5 Hz, H-1 of 2"-rhamnose), and 4.49 (*d*, *J*= 1.5 Hz, H-1 of 6"-rhamnose) were characteristic of glucose and rhamnose. The configuration of these sugars at C-1 was determined as β for glucose and α for both rhamnoses from the coupling constants. This major flavonol glycoside, quercetin 3-2^{G}-rhamnosylrutinoside, has been reported in the petal of *Clitoria ternatea* L. and the ^{1}H NMR and ^{13}C NMR spectrum data were identical as those reported by Kazuma *et al* (2003).

Mutagenic assays, such as the *S. typhimurium* test, have been widely used to assess the antimutagenic and anticarcinogenic activities of various compounds. The aim of the present study was to evaluate the antimutagenic effects of *Sesbania javanica* Miq. flower DMSO extract and isolate the major flavonoid against AFB_1 and B(a)P using the Ames test. The results of this study demonstrate that *Sesbania javanica* Miq. flower DMSO extract and quercetin 3-2^{G}-rhamnosylrutinoside are capable of inhibiting the mutagenicity of $AFB_1$and B(a)P. These results indicate that the *Sesbania javanica* Miq. flower contains antimutagens inhibiting the mutagenicities of $AFB_1$and B(a)P. These results concur with the study of Nakahara *et al* (2002) with 80% methanol extract.

Most chemical carcinogens, such as AFB_1, B(a)P and PAHs, require metabolic activation to DNA-reactive intermediates by cytochrome P450-mediated mixed -function oxidases (MFOs), undergoings epoxidation of their aromatic ring, in order to exert their carcinogenic action (Dipple *et al*, 1984). These epoxide intermediates as an electrophilic metabolites covalent binding to cellular DNA, leading to adduct formation, are considered to be critical to the initiation of carcinogenesis. The mechanism by which the antimutagens in *Sesbania javanica* Miq. flower DMSO extract and the major isolated flavonol glycoside inhibited the mutagenicities of AFB_1 and B(a)P are not known. The constituents in the DMSO crude extract and quercetin 3-2^{G}-rhamnosylrutinoside may interact with some certain enzymes to inhibit activity which necessary for the activation of these chemical mutagens in the liver (cytochrome P450 enzymes). Many studies have shown that quercetin has antimutagenic effects against AFB_1 and B(a)P (Buening *et al*, 1981; Huang *et al*, 1983; Ogawa *et al*, 1985; Francis *et al*, 1989; Choi *et al*, 1994). Many papers have reportes the activity of flavonoids as inhibitors of enzymes involved in biotransformation of precarcinogens. These compounds are now seen as potential chemopreventive agents (Elangovan *et al*, 1994; Mooni *et al*, 1998; Zhai *et al*, 1998; Ciolino and Yeh, 1999).

A variety of naturally occurring compounds have been shown to inhibit the mutagenicity or carcinogenicity. We report the antimutagenic effects of *Sesbania javanica* Miq. flower DMSO extract and its major flavonol glycoside, quercetin 3-2^{G}-rhamnosylrutinoside against AFB_1 and B(a)P mutagens. This is an edible vegetable flower commonly found in Thai food.

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