Desmutagenic and antimutagenic potential of phenolics from *Khaya grandifoliola* (C.DC.), *Meliaceae*

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Background and objectives
Antimutagenic or protective effects have been attributed to many classes of phytocompounds, mainly flavonoids and phenolic compounds, present in foods. Anticancer, antioxidant and anti-inflammatory activities of *Khaya* spp. have been reported, but their desmutagenic and antimutagenic activities were not studied. The aim of this study was to identify the phenolic contents of *Khaya grandifoliola* and correlate the desmutagenic and antimutagenic activities of these compounds.

Materials and methods
Desmutagenic and antimutagenic activities of specimen extracts of *K. grandifoliola* leaves and flowers were evaluated by measuring the inhibition of *Salmonella typhimurium* TA100 His^+ revertants induced by ethyl methanesulphonate and ribose lysine. The phenolic contents of *K. grandifoliola* leaf extracts were determined using column and paper chromatography. Spectroscopic analysis UV, ^1H NMR, ^{13}C NMR and electrospray ionization were applied to identify the isolated compounds.

Results and conclusion
Five phenolic compounds were isolated for the first time from *K. grandifoliola* leaves. These compounds were identified as quercetin 3-*O*-rhamnoglucoside (rutin), quercetin 3-*O*-rhamnoside, quercetin 3-*O*-glucoside, quercetin and 6-methoxycoumarin-7-*O* arabinofuranoside. The alcoholic extracts of both leaves and flowers (total and successive) of *K. grandifoliola*, rutin and quercetin rhamnoside isolated from the leaves, exhibited desmutagenic and antimutagenic activity against ethyl methanesulphonate-induced and ribose lysine-induced reversion.

Keywords:
ethyl methanesulphonate, *Khaya grandifoliola*, *Meliaceae*, ribose lysine, rutin, *Salmonella typhimurium* TA100 (His^-), scopoletin 7-*O*-α-arabinofuranoside

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Introduction

Plants of the family *Meliaceae* Juss are trees, shrubs or rarely herbs that may be laticiferous (rarely, with a milky juice exuding from the bark). Plants of this family are distributed in tropical, subtropical and occasionally warm temperate regions. The genus *Khaya* comprises seven species native to tropical Africa and Madagascar. Limonoids are heavily oxygenated modified triterpenes dominant in the plants of this family and exhibit anticancer activity and antifeedant activity against insects. Compounds other than limonoids were isolated from *Khaya grandifoliola* (African Mahogany), which include catechin from the bark seed [1] and steroid hormone from the bark [2], whereas rutin and quercetin flavonoids were isolated from *Khaya senegalensis* leaves [3]. Antimalarial [4], schistosomicidal [5], hypoglycaemia and hypocholesterolaemic [6] activities were reported for *K. grandifoliola* stem bark. As the mutagens are involved in the initiation and promotion of several human diseases including cancer, the significance of novel bioactive phytocompounds in counteracting the promutagenic and carcinogenic effects are important. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are called antimutagens. Numerous studies have been carried out to identify compounds that might protect humans against DNA damage and its consequences. The antimutagenic and anticarcinogenic properties of a wide variety of dietary constituents and plant secondary metabolites have been reported [7–9]. Natural antimutagens from edible and medicinal plants are of particular importance, as they may be useful for human cancer prevention and have no undesirable xenobiotic effects on living organisms [9,10]. Anticancer [11], antioxidant [12], and anti-inflammatory [13] activities of *Khaya* spp. have been reported, but desmutagenic and antimutagenic activities were not studied. Directly assaying potential carcinogens by testing their ability to form tumours in animals is difficult and expensive. In addition to causing tumours in animal cells, most carcinogens are mutagens [12,14,15]. Hence, the objective of this work was the evaluation of the desmutagenic and antimutagenic potential of the plant.

Experimental

Ultraviolet (UV) spectra were obtained on a Shimadzu UV 240, Shimadzu Corporation (Tokyo, Japan) spectrometer. NMR Jeol ECA spectrometer, Jeol Corporation (Tokyo, Japan) 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR were used with DMSO-d6 as the solvent. All chemical shifts (δ) are given in ppm units with reference to TMS as an internal standard and the coupling constants (*J*) are given in Hz. Paper chromatography (Whatman No. 1 and 3 mm; Whatman, International Ltd, Maidstone, England, UK), mass spectrometer, electrospray ionization mass spectrometry (ESI-MS), and Thermo Finnigan, Finnigan Corporation, California, USA (ion trap) were used.

Materials and methods

Plant material

Fresh leaves and flowers of *K. grandifoliola* (C.DC.), *Meliaceae*, grown in Egypt were collected from Giza Zoo (Giza, Egypt). They were confirmed by Dr Mohamed El-Gebaly, a plant taxonomist.

Preparation of successive extracts

Air-dried powdered leaves (600 g) and flowers (100 g) were successively and separately extracted with petroleum ether, chloroform, ethyl acetate and 95% ethanol in a Soxhlet apparatus. The solvents were evaporated to dryness under reduced pressure at 40°C; the yield of leaf extracts was 25.2, 24.3, 0.3, and 60 g, respectively, whereas those of flowers were 2.5, 1.5, 1.7 and 10 g, respectively.

Preparation of 90% ethanol extracts

The crude extract was prepared by percolating the air-dried powdered leaves (500 g) and flowers (50 g) with 90% ethanol till exhaustion. The filtered percolate, in each case, was evaporated to dryness under vacuum at 40°C to yield 129 and 8 g, respectively.

Paper chromatography

Ethanol extracts from successive extractions were subjected to two-dimensional paper chromatography for the detection of flavonoids using Whatman No. 1 and solvent systems (s_1) *n*-butanol : acetic acid : water (3 : 1 : 1 v/v) and (s_2) acetic acid : water (15 : 85 v/v) for development. Chromatograms were examined under UV light (365 nm) before and after exposure to ammonia vapour and spraying with $AlCl_3$ solution.

Isolation of phenolics

Successive ethanol extracts of *K. grandifoliola* leaves (60 g) were fractionated on a reversed-phase polyamide column, with the gradient solvent system starting from 100% water to 100% methanol. The fractions were purified on subcolumns of Sephadex (LH-20) using butanol saturated with water as the eluent to yield compound I (20 mg), whereas compound V (14 mg) was isolated from the 30% (methanol/water) fraction, purified on a silica gel column using gradient elution from petroleum ether to chloroform, and then a column of Sephadex (LH-20) with methanol as eluent to yield yellow needle crystals. Compounds II, III and IV (6, 4 and 15 mg, respectively) were isolated from the ethyl acetate extract using preparative paper chromatography and were purified on columns of Sephadex (LH-20). Spots were detected in each fraction, their R_f values in systems s_1 and s_2 and their colours being recorded in Table 1. Purified compounds were subjected to UV spectral analysis and ^{1}H NMR, ^{13}C NMR determinations. Spectroscopic UV data of these compounds were compared with the published data [16] and are represented in Table 2. The NMR assignments are showed in Tables 3 and 4. Compound III was identified after acidic hydrolysis. The compound was completely dissolved in 6% aqueous HCl (5 ml) and minimal methanol. The solution was heated on a

Table 1 Chromatographic properties of compounds I, II, III and IV

Compound	R_f (s_1)	R_f (s_2)	UV	UV/ ammonia	$AlCl_3$
I	0.58	0.74	Purple	Yellow	Yellow
II	0.77	0.57	Purple	Yellow	Yellow
III	0.58	0.44	Purple	Yellow	Yellow
IV	0.96	0.02	Yellw	Yellow	Yellow

UV, ultraviolet; s_1, *n*-butanol : acetic acid : water (3 : 1 : 1 v/v); s_2, acetic acid : water (15 : 85 v/v).

Table 2 UV ($\lambda_{max}^{methanol}$) spectral data of compounds I, II, III and IV

Compound	Methanol (nm)	NaOMe (nm)	$AlCl_3$ (nm)	$AlCl_3$/HCl (nm)	NaOAc (nm)	NaOAc/H_3BO_3 (nm)
I	257, 266 (sh),	273, 327, 410	275, 304 (sh), 435	271, 300 (sh), 357, 402	273, 320, 413	263, 294 (sh), 380
II	359, 256, 339, 351	271, 326, 395	275, 305, 331, 432	269, 299 (s), 355, 395	271, 316, 339, 377	261, 308, 368
III	255, 269 (sh), 294,	274, 330, 409	272, 321 (sh), 449	264, 300, 339, 361, 422	254, 274, 329, 385	259, 295, 386
IV	371, 255, 270 (sh), 302 (sh), 371	247.8, 277 (sh), 333, 418	229 (sh), 271, 449	229 (sh), 265, 303, 357, 426	257, 276, 325, 385	260, 387, 461

UV, ultraviolet.

Table 3 ^{1}H NMR of compounds I, II, and IV in DMSO

Carbon number	Compound I δ ^{1}H (ppm) (J in Hz)	Compound II δ ^{1}H (ppm) (*J* in Hz)	Compound IV δ ^{1}H (ppm) (*J* in Hz)
6	6.15 (d, *J*=1.5, ^{1}H)	6.13 (d, *J*=1.5, ^{1}H)	6.21 (d, *J*=1.5, ^{1}H)
8	6.35 (d, *J*=1.5, ^{1}H)	6.32 (d, *J*=1.5, ^{1}H)	6.47 (d, *J*=1.1, ^{1}H)
2′, 6′	7.51 (m, ^{2}H)	7.25 (m, ^{2}H)	7.64 (d,d, *J*=2.2, 8.4, ^{1}H-6′); 7.74 (d, *J*=2.2, ^{1}H, 2′)
5′	6.81 (d, *J*=8.4, ^{1}H)	6.82 (d, *J*=8.4, ^{1}H)	6.95 (d, *J*=8.4, ^{1}H)
Glucose			
1″	5.29 (d, *J*=6.8, ^{1}H)	–	–
2″		–	–
3″		–	–
4″	fx13.2–3.8	–	–
5″		–	–
6″		–	–
Rhamnose			
1‴	4.34 (s, ^{1}H)	5.21 (s, ^{1}H)	–
2‴			–
3‴	fx23–4	fx33.9–4.7	–
4‴			–
5‴			–
6‴	0.96 (d, *J*=6.0, ^{3}H)	0.78 (d, *J*=6.1, ^{3}H)	–

Table 4 ^{13}C NMR of compounds I, II, and IV in DMSO, δ ^{13}C (ppm)

Carbon number	Compound I δ ^{13}C (ppm)	Compound II δ ^{13}C (ppm)	Compound IV δ ^{13}C (ppm)
2	157.11	157.66	146.60
3	133.81	134.63	135.59
4	177.87	177.72	175.67
5	161.73	161.77	160.56
6	99.3	99.50	98.12
7	164.68	167.68	163.98
8	94.11	94.47	93.25
9	156.94	157.66	156.01
10	104.44	105.58	102.78
1′	122.10	121.60	121.81
2′, 6′	115.75, 121.67	115.9, 121.15	114.87, 119.83
3′	145.28	145.79	144.94
4′	148.96	148.80	147.58
5′	116.77	116.09	115.47
Glucose			
1″	101.70	–	–
2″	73.0	–	–
3″	76.9	–	–
4″	68.8	–	–
5″	77.3	–	–
6″	60.8	–	–
Rhamnose			
1‴	101.26	102.29	–
2‴	70.8	70.73	–
3‴	71.0	70.57	–
4‴	72.3	71.14	–
5‴	70.5	70.85	–
6‴	18.25	18.02	–

steam bath for 45 min, then cooled and extracted by shaking with ether to yield the aglycon in the ethereal layer after drying on anhydrous sodium sulphate and the sugar moiety in the aqueous layer [16].

Table 5 ^{1}H NMR, ^{13}C NMR of compound V in MeOD

C/H	^{1}H, δ H (ppm)	^{13}C, δ C (ppm)
2		162.8
3	6.2 (^{1}H, d, $J_{3,4}$=9.15)	111.2
4	7.8 (^{1}H, d, $J_{4,3}$=9.15)	144.9
5	6.7 (^{1}H, s)	108
6		145.8
7		149.3
8	7.1 (^{1}H, s)	102
9		114.8
10		150
Arabinose		
1′	5.2	109
2′		71.0
3′	3–4	71.2
4′fx4		72.1
5′		62.8
OCH3′	3.9	55.5

Inhibition of mutagen-Induced revertants in *salmonella typhimurium*

Desmutagenic and antimutagenic activities of leaves and flowers of *K. grandifoliola* extracts were detected using the bacterial strain *Salmonella typhimurium* TA100 (His^{-}). The concentration of each mutagen used was as follows: 25 µl in 100 µl DMSO, ethyl methanesulphonate (EMS); ribose lysine (RL), 0.5 mol/l in water [17,18]. Ascorbic acid was used as the reference desmutagenic and antimutagenic compound [19].

Desmutagenic activity

Various amounts of the ethanolic extracts (total and successive) of *K. grandifoliola* (leaves and flowers), compound I (rutin), compound II (quercetin-3-*O*-α-L-rhamnopyranoside), ascorbic acid (as the reference drug)

and the mutagen were mixed with sterile distilled water (1 ml final volume) containing 100 μmol phosphate buffer (pH 7.4). The mixture was incubated at 37°C for 30 min, and 100 ml of a 24-h bacterial culture of TA100 His^- strain (10^8 cells) and 2 ml of molten top agar (45°C) were poured with the mixture onto minimal glucose agar plates. The plates were incubated once more for ½ h at 37°C. The number of His^+-induced revertants was scored after incubation for 48 h at 37°C.

Antimutagenic activity

Various concentrations of ethanolic extract (total and successive), compound I, compound II and ascorbic acid (as the reference drug) were added to sterile distilled water (1 ml final volume) containing 100 μl of the 24-h culture of the TA100 His^- test strain and 100 μmol of phosphate buffer pH 7.4. After incubation at 37°C for ½ h, cells were collected by centrifugation, washed twice with phosphate buffer to remove the antimutagen (tested compounds) and finally suspended in 1 ml of the buffer. After addition of the mutagen and 2 ml of soft agar, the mixture was poured onto minimal glucose agar plates. After incubation for 48 h at 37°C, revertant colonies (His^+) were counted.

Desmutagenic and antimutagenic activities were calculated as the percentage of decrease in induced revertants according to Amara-Mokrane and colleagues [10,20] after subtraction of the corresponding spontaneous reversion according to the equation: %inhibition = $100–(N/N_0 \times 100)$, where N is the revertant/plate induced by the mutagen in the presence of increasing amounts of the tested material and N_0 is the reversion induced in the control (Tables 6 and 7).

Results and discussion

Identification of isolated compounds

Compound I

Compound I was expected to be quercetin-3-*O*-glycoside on the basis of its chromatographic properties (R_f values, colour under UV/NH_3 and $AlCl_3$) [16] (Table 1). UV spectra (Table 2) show a bathochromic shift in band I (51 nm), on addition of NaOMe, accompanied by an increase in the intensity, which was an evidence of free 4′-OH. The bathochromic shift in band II (16 nm), on addition of NaOAc, referred to a free 7-OH. A bathochromic shift of 43 nm in band I in the presence of $AlCl_3$/HCl confirms the presence of 5-OH. In addition, a bathochromic shift of 21 nm in band I in the presence of NaOAc/H_3BO_3 and a hypsochromic shift of more than 21 nm of band I in $AlCl_3$/HCl spectrum relative to band I in the spectrum of $AlCl_3$ confirmed the presence of *ortho*-dihydroxyl groups in the B ring [21]. The ^1H, ^{13}C NMR spectra (Tables 3 and 4) of compound I revealed the presence of glucose, rhamnose and quercetin. The ^1H NMR spectrum showed an overlapping signal at δ 7.51 for H-2′, 6′ and a doublet at d 6.81 (J = 8.4 Hz) for H-5′ due to *ortho*-coupling with H-6′ and two doublets of two aromatic protons at d 6.35 and 6.15 (J = 1.5 Hz), each proton assigned for H-8 and H-6, respectively.

A β-D-rutinoside moiety at C-3 was deduced from the downfield signal of C-3 to 133.81 ppm and the

Table 6 Desmutagenic and antimutagenic potential of ethanolic extracts, compound I and compound II isolated from *Khaya grandifoliola* using EMS-induced and RL-induced revertants

Sample	Concentration (μg/plate)	Desmutagenic %inhibition		Antimutagenic %inhibition	
		EMS	RL	EMS	RL
Leaves					
Total ethanolic extract	100	100	0	75	0
	250	100	0	80	0
	500	100	0	86	0
	1000	100	57	90	0
Ethanol (successive)	100	88	0	85	0
	250	100	0	86	0
	500	100	60	97	20
	1000	100	80	100	30
Flower					
Total ethanolic extract	100	100	100	95	0
	250	100	100	97	0
	500	100	100	98	67
	1000	100	100	100	87
Ethanol (successive)	100	43	0	97	0
	250	100	0	100	0
	500	100	100	100	0
	1000	100	100	100	80
Rutin	25	88.5	0	54	0
	50	97	0	58	0
	100	100	0	77	34
Quercetin rhamnoside	25	90	0	79	0
	50	97	43	91	0
	100	100	55	95	0
Ascorbic acid	25	96	57	67	0
	50	97	86	99	0
	100	100	87	100	0

EMS, ethyl methanesulphonate; RL, ribose lysine.

Table 7 % Potency of compound I and compound II as desmutagenic and antimutagenic agents using EMS-induced revertants relative to ascorbic acid as the reference drug

Compound	Concentration (μg/plate)	Desmutagenic % potency	Antimutagenic % potency
Rutin	25	92.18	80.59
	50	100	58.58
	100	100	77.00
Quercetin rhamnoside	25	93.75	117.91
	50	100	91.91
	100	100	95.00

EMS, ethyl methanesulphonate.

two anomeric carbons appeared at 101.70 and 101.26 ppm, together with two anomeric proton signals at δ 5.29 (d, *J* = 6.8 Hz) and δ 4.34 (singlet) and a doublet of three protons at δ 0.96 (*J* = 6.0 Hz) for Me-6‴. A 1‴→6‴ interglycosidic linkage was followed from the characteristic up-field location of H-1‴ as a singlet at δ 4.34. Therefore, compound I was identified as rutin [quercetin-3-*O*-α-L-rhamnopyranosyl-(1‴→6‴)-*O*-β-D-glucopyranoside]. Rutin was previously isolated from *K. senegalensis* (A. Juss) leaves [3], but this is the first report for its isolation from the *K. grandifoliola*.

Compound II

The UV spectra of compound II (Table 2) show a bathochromic shift (44 nm) in band I with an increase in the intensity relative to that of MeOH upon addition of NaOMe, indicating free 4′-OH. It also showed a characteristic bathochromic shift in band II (15 nm) on addition of NaOAc attributed to a free 7-OH group. In contrast, there was a bathochromic shift of 44 nm in band I in the presence of $AlCl_3$/HCl, confirming the presence of 5-OH. In addition, a bathochromic shift of 17 nm in band I in the presence of NaOAc/H_3BO_3 and a hypsochromic shift about 37 nm of band I in $AlCl_3$/HCl spectrum relative to band I in the spectrum of $AlCl_3$ confirmed the presence of *ortho*-dihydroxyl groups in the B ring [21].

^{1}H, ^{13}C NMR spectra of compound II (Tables 3 and 4) revealed the presence of quercetin and rhamnose. The ^{1}H NMR spectrum showed an overlapping signal at δ 7.25 for H-2′, 6′ and a doublet at δ 6.81 (*J* = 8.4 Hz) for H-5′ due to *ortho*-coupling with H-6′. Two doublets at δ 6.13 and 6.32 ppm assigned H-6 and H-8, respectively. The downfield signal of C-3 in ^{13}C NMR to 134.63 ppm confirmed the flavonol structure. The anomeric carbon of rhamnose appeared at 102.29 ppm, with the anomeric proton appearing as a doublet of small *J* value at δ 5.21 ppm, whereas protons of the methyl group showed a doublet with *J* = 6 Hz at δ 1.19 ppm appearing in ^{13}C NMR at 18.02 ppm. Hence, compound II was identified as quercetin- 3-*O*-α-L-rhamnopyranoside.

Compound III

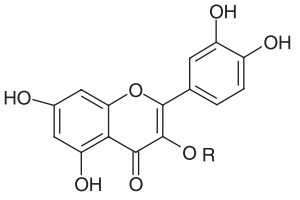
UV spectra (Table 2) show a bathochromic shift in band I (40 nm), on addition of NaOMe, with no decrease in the intensity, which was an evidence of free 4′-OH. In addition, a characteristic bathochromic shift in band II (20 nm) on addition of NaOAc referred to a free 7-OH group confirmed by a new peak appearing at 330 nm upon addition of NaOMe. A bathochromic shift of 31 nm in band I in the presence of $AlCl_3$/HCl confirmed the presence of 5-OH. In addition, a bathochromic shift of 15 nm in band I in the presence of NaOAc/H_3BO_3 and a hypsochromic shift of more than 27 nm of band I in $AlCl_3$/HCl spectrum relative to band I in the spectrum of $AlCl_3$ confirmed the presence of *ortho*-dihydroxyl groups in the B ring [21]. The compound was expected to be quercetin-3-*O*-glycoside on the basis of its chromatographic properties. The sugar moiety was determined after complete acid hydrolysis to yield glucose in the aqueous phase and quercetin in the organic phase (copaper chromatography with authentics using Aniline phthalate reagent for sugars and $AlCl_3$ for the aglycone). Hence, compound III was identified as quercetin-3-*O*-α-L-glucopyranoside.

Compound IV

Compound IV was expected to be quercetin on the basis of its chromatographic properties. The bathochromic and hypsochromic shifts observed in the UV spectra (Table 2) were in good agreement with quercetin aglycone [16].

UV spectra show a bathochromic shift in band I (47 nm), on addition of NaOMe, accompanied by an increase in the intensity, which was an evidence of free 4′-OH. The bathochromic shift in band II (11 nm), on addition of NaOAc, referred to a free 7-OH. A bathochromic shift of 55 nm in band I in the presence of $AlCl_3$/HCl was observed, confirming the presence of 5-OH. In addition, a bathochromic shift of 16 nm in band I in the presence of NaOAc/H_3BO_3 and a hypsochromic shift of 23 nm in band I in the $AlCl_3$/HCl spectrum relative to band I in the spectrum of $AlCl_3$ confirmed the presence of *ortho*-dihydroxyl groups in the B ring [21].

The ^{1}H, ^{13}C NMR spectra (Tables 3 and 4) of compound IV revealed the presence of a flavonol structure with a downfield signal of C-3 in ^{13}C NMR to 135.59 ppm. The ^{1}H NMR spectrum showed a signal at δ 7.64 for H-6′ d,d, with *J* = 8.4 Hz, due to *ortho*-coupling with 5′, which appeared as a doublet at δ 6.95 ppm and (*J* = 2.2 Hz) due to *meta*-coupling with 2′, which appeared as a doublet at δ 8.4 ppm and two doublets of two aromatic protons at δ 6.47 and 6.21 (*J* = 1.5 Hz), each proton assigned for H-8 and H-6, respectively. Hence, compound IV was identified as quercetin.



Compound I: R = rutinoside; compound II: R = rhamnoside; compound III: R = glucoside; compound IV: R = H.

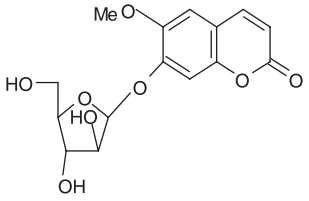
Compound V

Compound V appeared as a blue fluorescent spot on TLC (Silica gel $60GF_{254}$ precoated plates), turning yellow on exposure to ammonia vapour (R_f = 0.39; benzene : ethyl acetate, 7 : 3).

UV λ_{max}: 261, 288, 339, 363 nm (in methanol).

ESI-MS: (*m*/*z*, rel. int.) $C_{15}H_{16}O_8$ $[M+H]^{+1}$, 325.

The UV absorption bands at 288 and 339 nm could be attributed to the benzene and the pyrone rings, respectively. The NMR data (Table 5) shows signals corresponding to methoxy substituents in an aromatic system at δ 3.9 ppm, confirmed by ^{13}C NMR at 55.5 ppm. Signals corresponding to H-3 and H-4 appeared at 6.2 and 7.8 ppm, respectively, with $J_{3,4}$ = 9.15 Hz, confirming *ortho*-coupling. Two other singlets appeared at 6.7 and 7.1 ppm, corresponding to H-5 and H-8, respectively, confirming (with the ^{13}C NMR spectrum) the structure of 6,7-disubstituted coumarin. The signal appearing at 5.2 ppm in ^1H NMR with J = 1 Hz assigned the anomeric proton of the sugar moiety, which is identified by ^{13}C NMR as arabinose and confirmed by acid hydrolysis and cochromatography with authentic sugar. From these data, compound V was identified as 6-methoxycoumarin-7-*O*-arabinofuranoside (scopoletin 7-*O*-α-arabinofuranoside). This is the first isolation of this compound from the family *Meliaceae*.



6-Methoxycoumarin-7-*O*-α-arabinofuranoside.

Desmutagenic and antimutagenic potential

Desmutagenic and antimutagenic activities of specimen extracts of *K. grandifoliola* leaves and flowers were ascertained by measuring the inhibition of TA100 His^+ revertants induced by EMS and RL. The results in Table 6 showed that the desmutagenic and antimutagenic activity of flower extract is higher than that of leaf extract especially in case of EMS. Tests on EMS-induced reversion showed good activity of all the extract specimens. In contrast, desmutagenic and antimutagenic activities of RL were variable, whereas rutin and quercetin rhamnoside showed somewhat similar results in case of EMS and RL. The %potency of the two compounds was calculated relative to ascorbic acid as the reference drug [19]. Results illustrated in Table 7 show a higher %potency of quercetin rhamnoside than rutin as an antimutagenic agent in case of EMS-induced revertants. This phenomenon can be interpreted by the different action mechanisms of these mutagens, or presumably due to the selective activity of the antimutagen compound.

The antimutagenic factors are divided into two main classes according to differences in their modes of action: one is the desmutagen, which inhibits the formation of mutagens out of the cell or taking the mutagens into the cell, or inactivates or destroys mutagens directly or indirectly out of the cell, and pre-incubation treatment is designed to evaluate the desmutagenic effect. The other type is called a bioantimutagen, which suppresses the process of mutagenesis itself in the cell; for example, it eliminates radicals or increases DNA repair systems; other antimutagens exert this effect by acting as blocking agents [15]. Recent research has confirmed that plant flavonoids inhibit the mutagenicity induced by chemical mutagens [22].

Antimutagenic or protective effects have been attributed to many classes of phytocompounds, mainly flavonoids and phenolic compounds, present in food. However, such compounds have also been reported to exhibit a wide range of other biological activities such as antimicrobial, anti-inflammatory, antioxidant and free-radical scavenging [9,23]. In this study, rutin and quercetrin were the major flavonoids showing desmutagenic and antimutagenic activity similar to ascorbic acid. These compounds were reported for their antioxidant [24], anticancer [25] and anti-inflammatory [26] activities.

Conclusion

Alcoholic extracts of both leaves and flowers (total and successive) of *K. grandifoliola*, rutin and quercetin rhamnoside isolated from the leaves, exhibited desmutagenic and antimutagenic activity against EMS-and RL-induced reversion.

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Conflicts of interest

There are no conflicts of interest.

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