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ORIGINAL ARTICLE

Chemical constituents, anti-inflammatory, and antioxidant activities of *Anisotes trisulcus*

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Abstract *Anisotes trisulcus* (Forssk.) Nees. (family Acanthaceae) aerial part is used in folk medicine in the Arabian peninsula for treatment of hepatic conditions. It showed different activities such as antibacterial, hepatoprotective, and cytotoxicity. It is a rich source of alkaloids and is used as an antidiabetic, bronchodilator, hypotensive, and local anesthetic. To the best of our knowledge, there is no report on the phenolic constituents of *A. trisulcus*. Therefore, this study aims to identify the constituents and establish antioxidant and anti-inflammatory activities of the total methanolic extract and different fractions. One new benzoyl flavonol: 7,8,3′-trihydroxy-5-methoxy-4′-benzoyl flavonol (**5**), along with eight known compounds: α-amyrin (**1**), *β*-sitosterol (**2**), stigmasterol (**3**), (2*S*,3*S*,4*R*)-2[(2′*R*)-2′-hydroxytetracosanoyl amino]-octadecane-1,3,4-triol (**4**), allopateuletin (**6**), veratric acid (**7**), vanillic acid (**8**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**9**) were isolated from *A. trisulcus* aerial parts. Their structures were established by physical, chemical, and spectral data (UV, IR, MS, and 1D NMR), as well as comparison with authentic samples. The anti-inflammatory activity of the total methanolic extract and different fractions was evaluated using carrageenan-induced paw edema method at a dose of 400 mg/kg. Also, the antioxidant activity was determined using DPPH assay at concentrations 0.25, 0.5, and 1 mg/mL. The total MeOH extract and EtOAc fraction showed high antioxidant activity 75% and 68% (Conc. 1 mg/mL), respectively while, the *n*-hexane and EtOAc fractions exhibited significant anti-inflammatory effects.

1. Introduction

The genus *Anisotes* contains 23 species, which are represented by herbs, shrubs or sometimes climbers.[1–3] *Anisotes trisulcus* (Forssk.) Nees. (family Acanthaceae) is a stiff erect shrub with a height up to 3.5 m. It is monopodially branched carrying opposite decussate leaves at the nodes bearing bright orange–red colored tubular flowers.[2,3] *A. trisulcus* is known as Almodh, Madh, Moze, and Modaid in the Kingdom of Saudi Arabia (KSA).[1,3] It is used locally in several pharmaceutical forms to

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limit tobacco consumption and suppress appetite.[4] The plant was known to possess anti-bacterial, cytotoxic, bronchodilator, hypotensive, local anesthetic, antidiabetic, and hepato-protective activities.[1,5–7] It is also used as treatment for all hepatic conditions including hepatitis, jaundice, gallstone, and other hepatic problems.[1,5] Previous phytochemical study of *A. trisulcus* revealed the isolation and identification of several alkaloids such as 5-hydroxy vasentine, 7-hydroxy vasicine, 7-hydroxy vasicinone, anisotine, peganine, vasicinone, 5-methoxypeganine, and trisulcusine.[1,4,8] In continuation of our investigation, we report herein the isolation and structural elucidation of one new benzoyl flavonol: 7,8,3′-trihydroxy-5-methoxy-4′-benzoyl flavonol (**5**), along with eight known compounds: α-amyrin (**1**), *β*-sitosterol (**2**), stigmasterol (**3**), (2*S*,3*S*,4*R*)-2[(2′*R*)-2′-hydroxytetracosanoyl amino]-octadecane-1,3,4-triol (**4**), allopateuletin (**6**), veratric acid (**7**), vanillic acid (**8**), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**9**) from *A. trisulcus* aerial parts (Fig. 1). Their structures were established by various spectroscopic methods as well as comparison with authentic samples. The total methanolic (MeOH) extract and different fractions were evaluated for their antioxidant and anti-inflammatory activities.

2. Materials and methods

2.1. General procedures

Melting points were uncorrected and carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). Optical rotation was measured on a Perkin–Elmer Model 341 LC polarimeter. EI and FABMS were recorded on a Jeol the mass route JMS.600 H mass spectrometer. UV spectra were recorded in MeOH on a Shimadzu 1601 UV/VIS spectrophotometer. The IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Varian Oxford NMRYH-400 using DMSO-d_6, $CDCl_3$, and C_5D_5N as solvents. Column chromatographic separation was performed on silica gel 60 (0.04–0.063 mm, Merck), RP-18 (0.04–0.063 mm, Merck). TLC was performed on precoated TLC plates with SiO_2 60 F254 (0.2 mm, Merck). The used solvent systems for TLC analysis include *n*-hexane:EtOAc (95:5, S_1), *n*-hexane:EtOAc (90:10, S_2), *n*-hexane:EtOAc (80:20, S_3), *n*-hexane:EtOAc (70:30, S_4), $CHCl_3$:MeOH (95:5, S_5), $CHCl_3$:MeOH (90:10, S_6), $CHCl_3$:MeOH (80:20, S_7), and *n*-BuOH:acetone:formic acid:H_2O (60:17:8:15, S_8). Authentic sterols, flavonoids, and sugars were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University. The spots were detected by spraying with the following spray reagents: 1% $AlCl_3$ for flavonoids and *p*-anisaldehyde/H_2SO_4 for triterpenoids.[9] DPPH and carrageenan were purchased from Aldrich Co., USA.

2.2. Plant material

Aerial parts of *A. trisulcus* (Forssk.) Nees. were collected during the flowering season on March, 2005 from AL-Baha: Al-Abnaa escarpment (KSA). The plant was kindly identified by Dr. A.A. Fayed, Prof. of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher sample (AT-20114) was kept in the herbarium of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

2.3. Extraction and isolation

The air-dried powdered aerial parts of *A. trisulcus* (2.5 kg) were exhaustively extracted by cold percolation with MeOH (4 × 6 L). The alcoholic extract was concentrated under reduced pressure to get a viscous residue (270 g). This residue was suspended in 500 mL distilled H_2O and subjected to solvent fractionation using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH which were separately concentrated to give 60, 40, 20, and 70 g, respectively. The *n*-hexane fraction (60 g) was subjected to VLC using *n*-hexane:EtOAc gradient. Seven subfractions (HA-I:HA-VII) were obtained. Subfraction HA-II (8.5 g) was chromatographed

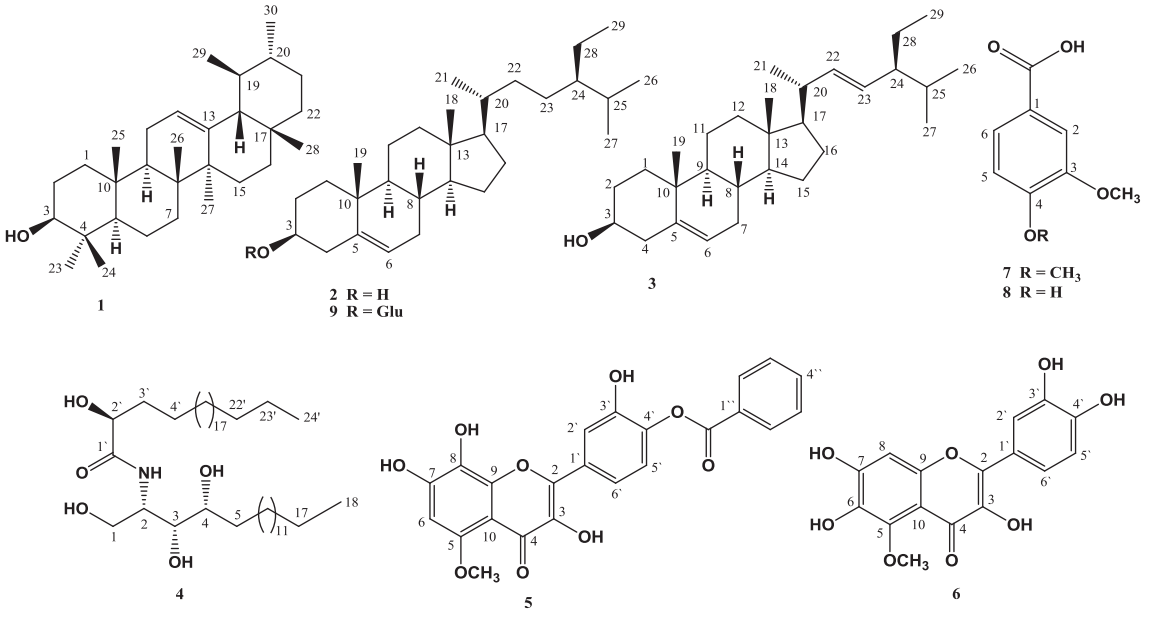

Figure 1 Structures of the isolated compounds.

on SiO_2 (250 g × 100 × 5 cm) using *n*-hexane:EtOAc gradient to give compounds **1** (50 mg, colorless needles), **2** (100 mg, white fine needles), and **3** (30 mg, white crystalline needles). Subfraction HA-V (5.5 g) was subjected to SiO_2 column (150 g × 50 × 5 cm) using *n*-hexane:EtOAc gradient elution to afford **4** (50 mg, white powder). The $CHCl_3$ fraction (40 g) was subjected to VLC using *n*-hexane:EtOAc gradient elution, five subfractions were obtained (CA-I:CA-V). Subfraction CA-II (8 g) was chromatographed on SiO_2 column (250 g × 100 × 5 cm) and eluted with *n*-hexane:EtOAc gradient. The fractions eluted with *n*-hexane:EtOAc (95:5) afforded compounds **5** (20 mg, yellow fine needles) and **6** (40 mg, fine yellow needles). The EtOAc fraction (20 g) was subjected to VLC with $CHCl_3$:MeOH gradient elution. Six subfractions were obtained EA-I:EA-V. Subfraction EA-II (4.5 g) was chromatographed on SiO_2 column chromatography (180 g × 200 × 5 cm) using $CHCl_3$:MeOH gradient elution. Fractions eluted with $CHCl_3$:MeOH (95:5) were subjected to SiO_2 column using $CHCl_3$:MeOH as an eluent to afford compounds **7** (50 mg, colorless crystals), **8** (100 mg, white amorphous powder), and **9** (300 mg, white amorphous powder). The other subfractions were subjected to further isolation and purification of their constituents but nothing significant was isolated.

(2S,3S,4R)-2[(2′R)-2′-hydroxytetracosanoyl amino]-octadecane-1,3,4-triol (**4**): It was isolated as white powder (50 mg, *n*-hexane); R_f 0.56, (S_4); $[\alpha]_D^{25}$ + 12.5 (C 0.1, $CHCl_3$); IR (KBr): γ_{max} 3580–3230 (OH or NH stretching), 1626 (amide carbonyl), 1554 (NH bending) cm^{-1}; ^1H NMR (C_5D_5N-d_5, 400 MHz): δ_H 4.51 (1H, dd, *J* = 11.2, 4.4 Hz, H-1A), 4.42 (1H, dd, *J* = 11.2, 4.4 Hz, H-1B), 5.09 (1H, m, H-2), 4.35 (1H, m, H-3), 4.29 (1H, m, H-4), 2.23 (1H, m, H-5A), 1.93 (1H, m, H-5B), 1.27–1.32 (24H, m, $(CH_2)_{12}$), 0.88 (6H, d, *J* = 6.7 Hz, H-18, 24′), 4.62 (1H, m, H-2′), 2.23 (2H, m, H-3′), 8.55 (1H, d, *J* = 9.0 Hz, N*H*), 6.12 (1H, brs, 1-OH), 6.59 (2H, brs, 3,4-O*H*), 7.53 (1H, brs, 2′-O*H*); ^{13}C NMR (C_5D_5N-d_5, 100 MHz): δ_C 62.2 (C-1), 53.1 (C-2), 76.9 (C-3), 73.1 (C-4), 34.3 (C-5), 29.6–32.2 $(CH_2)_n$, 14.29 (C-18, 24′), 175.8 (C-1′), 72.6 (C-2′), 35.8 (C-3′), 32.2 (C-22′), 23.0 (C-23′); (+) FABMS (rel.int.%): *m/z* 683 $[M+H]^+$ (35%), 340 (20%), 169 (30%), 120 (100%), 57 (35%); EIMS of FAME (fatty acid methyl ester)(rel. int.%): *m/z* 398 $[M]^+$ (15%), 380 $[M-H_2O]^+$ (5%), 367 $[M-CH_3O]^+$ (12%), 350 $[M-CH_3\text{-}OH]^+$ (5%), 337 $[M-CH_3COO]^+$ (10%), 228 (100%), 95 (70%), 69 (75%) and 55 (85%); EIMS of LCB (long chain base) (rel. int.%): *m/z* 317 $[M]^+$ (15%), 299 $[M-H_2O]^+$ (3%), 281 $[M-2H_2O]^+$(5%) and 263 $[M-3H_2O]^+$ (10%).

7,8,3′-Trihydroxy-5-methoxy-4′-benzoyl flavonol (**5**): It was isolated as yellow fine needles (20 mg, MeOH); R_f 0.66 (S_2); m.p. 236–238 °C; UV (MeOH): λ_{max} 270, 375; + NaOMe: 285, 390; + $AlCl_3$: 279, 415; + $AlCl_3$/HCl: 275, 395; + NaOAc: 282, 395; + NaOAc/H_3BO_3: 278, 385 nm; EIMS (rel. int.%): *m/z* 436 [M] + (4%), 331 $[M-benzoyl]^+$ (50%), 316 (12%), 300 $[M^+-(benzoyl+OCH_3)]$ (15%), 181 (15%), 172 (100%), 139 (24%), 134 (57%); ^1H and ^{13}C NMR spectral data: see Table 1.

Allopateuletin (**6**): It was isolated as fine yellow needles (40 mg, MeOH); R_f 0.66 (S_3); m.p. 245–247 °C; UV (MeOH): λ_{max} 265, 365; + NaOMe: 280, 415; + $AlCl_3$: 280, 395; + $AlCl_3$/HCl: 270, 385; + NaOAc: 280, 395; + NaOAc/H_3BO_3: 275, 400 nm; ^1H NMR (DMSO-d_6, 400 MHz): δ_H 6.61 (1H, s, H-8), 7.68 (1H, brs, H-2′), 7.29 (1H, d, *J* = 7.6 Hz, H-5′), 8.05 (1H, d, *J* = 7.6 Hz, H-6′), 10.81 (1H, s, 7-OH), 3.91 (3H, s, 5-OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C144.2 (C-2), 135.4 (C-3), 175.9 (C-4), 152.6 (C-5), 139.2 (C-6), 158.0 (C-7), 96.9 (C-8), 152.6 (C-9), 112.1 (C-10), 126.5 (C-1′), 114.7 (C-2′), 140.8 (C-3′), 141.8 (C-4′), 117.2 (C-5′), 121.7 (C-6′), 55.5 (5-OCH_3); EIMS (rel. int.%): *m/z* 332 $[M]^+$ (20%), 300 $[M-OCH_3]^+$ (20%), 181 (17%), 172 (100%), 139 (22%), 134 (50%).

Table 1 NMR data of compound **5** (DMSO-d_6, 400 and 100 MHz).

No.	δ_H [mult., *J*(Hz)]	δ_C (mult.)
2	–	143.5 (C)
3	–	135.3 (C)
4	–	174.3 (C)
5	–	152.9 (C)
6	6.10 s	93.4 (C)
7	–	157.8 (C)
8	–	131.8 (C)
9	–	156.2 (C)
10	–	108.8 (C)
1′	–	122.1 (C)
2′	7.66 brs	115.3 (CH)
3′	–	141.5 (C)
4′	–	139.8 (C)
5′	7.36 d (8.0)	117.7 (CH)
6′	8.06 d (8.0)	122.8 (CH)
7-OH	11.03 s	–
5-OCH_3	3.94 s	55.5 (CH_3)
1″	–	131.6 (C)
2″	7.33 m	127.7 (CH)
3″	7.27 m	127.5 (CH)
4″	7.17 t (7.6)	128.6 (CH)
5″	7.33 m	127.5 (CH)
6″	7.27 m	127.7 (CH)
7″	–	167.4 (C)

Veratric acid (**7**): It was isolated as colorless crystals (50 mg, MeOH); R_f 0.78 (S_6); m.p. 180–181 °C; IR (KBr): γ_{max} 2900 (C—H), 1671 (C═O), 1615, 1593, 1501 (aromaticity) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ_H 6.49 (1H, s, H-2), 6.83 (1H, d, *J* = 8.6 Hz, H-5), 7.43 (1H, dd, *J* = 7.6, 2.0 Hz, H-6), 3.80 (6H, s, 2 × OCH_3); ^1H NMR (DMSO-d_6, 100 MHz): δ_C 122.0 (C-1), 112.8 (C-2), 147.1 (C-3), 150.9 (C-4), 114.9 (C-5), 123.3 (C-6), 55.5 (2 × OCH_3), 167.2 (CO); EIMS (rel. int.%): *m/z* 182 $[M]^+$ (18%), 165 $[M-OH]^+$ (17%), 167 $[M-CH_3]^+$ (100%), 151 $[M-OCH_3]^+$ (20%), 120 $[M-(OCH_3)_2]^+$ (5%).

Vanillic acid (**8**): It was isolated as white amorphous powder (100 mg, MeOH); R_f 0.66 (S_7); IR (KBr): γ_{max} 3485 (OH), 1680 (C═O), 1500–1000 (aromaticity) cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ_H 7.38 (1H, brs, H-2), 6.82 (1H, d, *J* = 8.4 Hz, H-5), 7.42 (1H, dd, *J* = 8.4, 2.0 Hz, H-6), 3.78 (3H, s, 3-OCH_3), 9.86 (1H, brs, 4-OH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C 121.7 (C-1), 112.7 (C-2), 147.2 (C-3), 151.0 (C-4), 115.0 (C-5), 123.5 (C-6), 167.3 (C-3), 55.5 (3-OCH_3); EIMS (rel. int.%): *m/z* 168 $[M]^+$ (10%), 167 $[M-H]^+$ (100%), 153 $[M-CH_3]^+$ (70%), 151 $[M-OH]^+$ (20%), 124 $[M-COO]^+$ (27%), 107 $[M-(CH_3+COO)]^+$.

β-Sitosterol-3-O-*β*-D-glucopyranoside (**9**): It was isolated as white amorphous powder (300 mg, MeOH); R_f 0.75 (S_7); ^1H NMR (C_5D_5N, 400 MHz): δ_H 3.98 (1H, m, H-3), 5.37 (1H, brs, H-6), 0.68 (3H, s, H-18), 0.95 (3H, s, H-19), 0.84–0.99

Table 2 Results of antioxidant activity.

Extract	% Antioxidant activity (Conc. mg/mL)		
	0.25	0.5	1
DPPH	–	–	–
Quercetin	100	100	100
Total MeOH	51	67	75
n-Hexane	19	22	28
$CHCl_3$	35	36	38
EtOAc	55	63	68

(Other-C$\underline{H}_3$) 5.08 (1H, d, J = 7.2 Hz, H-1′) 4.06–5.10 (m, other sugar protons); ^{13}C NMR (C_5D_5N, 100 MHz): δ_C 38.0 (C-1), 30.7 (C-2), 79.1 (C-3), 40.4 (C-4), 141.4 (C-5), 122.4 (C-6), 32.7 (C-7), 32.5 (C-8), 50.8 (C-9), 37.4 (C-10), 21.8 (C-11), 39.8 (C-12), 43.0 (C-13), 57.3 (C-14), 25.0 (C-15), 29.0 (C-16), 56.7 (C-17), 12.5 (C-18), 19.7 (C-19), 36.9 (C-20), 19.9 (C-21), 34.8 (C-22), 26.8 (C-23), 46.5 (C-24), 29.9 (C-25), 19.5 (C-26), 20.5 (C-27), 25.0 (C-28), 12.6 (C-29), 103.06, 78.6 (C-2′), 79.0 (C-3′), 72.3 (C-4′), 75.8 (C-5′), 63.3 (C-6′).

2.4. Antioxidant activity

The antioxidant activity was determined as previously outlined by the decrease in the absorption of each concentration (0.25, 0.5, and 1 mg/mL) in DPPH solution (4 mg was dissolved in HPLC MeOH 50 mL to obtain a concentration of 80 μg/mL) monitored at 517 nm using a spectrophotometer.[10–12] The absorbance of DPPH in MeOH was measured after 2 min. The antioxidant activity of total MeOH extract and different fractions was measured in relation to quercetin (as a reference antioxidant) set as 100% antioxidant activity. Determinations were performed in triplicate. The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity} = 100 \times \left(1 - \frac{\text{absorbance with compound}}{\text{absorbance of the blank}}\right)$$

2.5. Pharmacological study

2.5.1. Animals

Adult male albino rats (120–150 g body weight) were used. All animal procedures were conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines and Institutional Ethical Committee Approval was obtained. The study protocol was approved by the Animal Ethics Committee of Assiut University. The animals were housed under standardized environmental conditions in the pre-clinical Animal House, Pharmacology Department, Faculty of Medicine, Assuit University. The animals were fed with standard diet and free access to water. They were kept at 24–28 °C, 60–70% relative humidity, 12 h day and night cycle for 1 week to acclimatize to the environmental conditions.

2.5.2. Anti-inflammatory activity

The anti-inflammatory activity of the total MeOH extract and different fractions was evaluated in adult albino rats (120–140 g body weight) by carrageenan-induced rat hind paw edema method according to the published procedures.[13] In this test, male albino rats were divided into six groups (six animals each). The first group was kept as negative control, injected intraperitoneally with 2% tween 80 in normal saline, while the second group was injected with indomethacin (reference group) at a dose of 8 mg/kg. The other groups were intraperitoneally injected with the total MeOH extract and different fractions at a dose of 400 mg/kg body weight. Foot paw edema was induced by injecting 0.1 mL of 1% carrageenin subcutaneously into the sub-planter portion of the right hind paw of each rat. The paws thicknesses were measured after injection of the phlogistic agent (0 h) and at 1, 2, 3, 4, and 5 h after the injection of carrageenin. The results of the measurements of percentage of anti-inflammatory activity are listed in Table 3. The percentage of edema inhibition (% of change) was calculated.

2.6. Statistical analysis

Data were analyzed using student's "*t*" test and the values were expressed as mean ± S.E. (n = 6 animals).

3. Results and discussion

Compound **5** was isolated as yellow fine needles. It gave positive tests for flavonoids.[14,15] The EIMS spectrum showed a molecular ion peak at m/z 436 $[M]^+$, consistent with molecular formula $C_{23}H_{16}O_9$. The fragment ion peaks at m/z 331 $[M-\text{benzoyl}]^+$ and 300 $[M^+-(\text{benzoyl}+OCH_3)]^+$ indicated that **5** is a flavonoid with benzoyl moiety. Its UV spectrum showed absorption bands at λ_{max} 270 and 375 nm, indicating its flavonol nature.[16] The bathochromic shift of bands I and II with $AlCl_3$ and NaOAc suggested the presence of hydroxy

Table 3 Results of anti-inflammatory activity.

Group	Dose (mg/kg)	Paw volume (as % of carrageenan group)				
		1 h	2 h	3 h	4 h	5 h
Carrageenan	–	100 ± 0.06	100 ± 0.09	100 ± 0.05	100 ± 0.11	100 ± 0.10
Carrageenan + indomethacin	8	34.43 ± 0.11*	61.75 ± 0.09*	84.66 ± 0.10*	91.82 ± 0.22*	93.17 ± 0.19
Carrageenan + *n*-hexane	400	28.16 ± 0.13*	56.84 ± 0.16*	79.19 ± 0.07*	90.41 ± 0.09*	90.49 ± 0.11
Carrageenan + $CHCl_3$	400	20.67 ± 0.25*	49.28 ± 0.19*	51.84 ± 0.08*	52.56 ± 0.10*	52.30 ± 0.14*
Carrageenan + EtOAc	400	56.93 ± 0.16	87.53 ± 0.23	90.67 ± 0.20	90.84 ± 0.19	91.18 ± 0.17
Total MeOH	400	37.83 ± 0.20	65.71 ± 0.12	81.47 ± 0.15	87.55 ± 0.18	92.10 ± 0.23

Each value represents the mean ± S.E.M., n = 6.
* Significant different from carrageenan only group at $P < 0.01$.

groups at C-3 and C-7, respectively. The hypsochromic shift in band II upon adding HCl indicated the presence of an *ortho*-dihydroxy group in ring A, which was confirmed by the bathochromic shift with $NaOAc/H_3BO_3$. The absence of band I shift with NaOMe indicated that the C-4′ hydroxy group may be absent or blocked.[16] The ^1H NMR spectrum (Table 1) displayed several aromatic protons; four of them were attributed to the flavonoidal skeleton and other protons for a mono-substituted benzene moiety. The proton signals at δ_H 8.06 (d, J = 8 Hz, H-6′), 7.66 (brs, H-2′), and 7.36 (d, J = 8 Hz, H-5′) indicated the presence of 1,2,4-*tri*-substituted benzene of ring B.[15] In addition, the resonated proton at δ_H 6.10 was assigned to H-6.[16] The two singlets at δ_H 3.94 and 11.03 were attributed to methoxy and 7-OH groups, respectively.[17] The signals at δ_H 7.17 (t, J = 7.6 Hz, H-4″) and 7.27–7.33 (m, other benzoyl protons) were assigned to a mono-substituted benzene moiety, which was further confirmed by the fragment ion peak at m/z 331 $[M\text{-benzoyl}]^+$. The ^{13}C NMR spectrum (Table 1) exhibited 23 carbon resonances, fifteen for the flavonoidal skeleton and seven for benzoyl moiety at δ_C 127.7 (C-2″, 6″), 127.5 (C-3″, 5″), 128.6 (C-4″), 131.6 (C-1″), and 167.4 (C-7″).[18] The benzoyl moiety and methoxy group could be attached to C-4′ and C-5, respectively based on the UV spectral data with different ionizing and complexing reagents. This was further confirmed by the fragment ion peaks at m/z 134 and 181 indicating the presence of two hydroxy and a methoxy group in ring A agrees with Retro Diels Alder fragmentation pattern.[18–20] By comparison of the NMR spectral data of **5** with previously reported data, **5** was identified as 7,8,3′-trihydroxy-5-methoxy-4′-benzoyl flavonol and considered as a new compound.[18–20]

The other isolated compounds were identified as α-amyrin (**1**),[9] *β*-sitosterol (**2**),[21] stigmasterol (**3**),[21] (2S, 3S, 4R)-2[(2′R)-2′-hydroxytetracosanoyl amino] octadecane 1,3,4-triol (**4**),[22,23] allopateuletin (**6**),[24] veratric acid (**7**),[25] vanillic acid (**8**),[26,27] and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**9**)[28] by comparison of their physical and spectral data with those in the literature. This is the first report for the isolation of compounds **1**–**3** from the plant, **8** and **9** from the genus, and **4**, **6**, and **7** from the family. The total MeOH extract and EtOAc fraction showed high antioxidant activity of 75% and 68%, respectively (Table 2). The obtained results could be attributed to the presence of phenolic compounds (flavonoids and phenolic acids). Anti-inflammatory activity of total MeOH extract and different fractions was measured against carrageenan-induced hind paw edema method. The *n*-hexane and $CHCl_3$ fractions gave potent anti-inflammatory activity compared with indomethacin (Table 3). These results may be attributed to their high content of flavonoids, phenolic compounds, sterols, and their corresponding.

4. Conflict of interest

None.

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