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Phytoconstituents of *Adenanthera pavonina* Linn from the bark extracts

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Abstract

Background: *Adenanthera pavonina* L. is an important medicinal plant and its barks are used in traditional medicine for treating different diseases. Therefore, a phytochemical investigation was carried out to isolate and identify secondary metabolites from its barks.

Results: Seven compounds namely ethyl 3,3-dimethyl-13-hydroxytridecanoate (1), stigmasta-5,22-dien-3β-ol (2), tert.butyl tridecanoate (3), 6-α-hydroxy stigmast-20(21)-en-3-one (4) of dichloromethane extract and 18-(2′, 3′-dihydroxyphenyl)nonadec-17-en-2-ol (5), 1-(N-propyl amino)-2-henecosanone (6), and stigmast–5(6), 20(21)-diene-3-one (7) were isolated from the barks of *Adenanthera pavonina* Linn. Of these compounds, 1, 4, 5, 6, and 7 appear new. The structures of these compounds were elucidated by spectroscopic techniques, mainly by NMR.

Conclusions: Five new and two known compounds have been isolated and characterized from the bark of *A. pavonina*. The isolated compounds could be a potential template for the synthesis and development of new lead compounds with interesting pharmacological properties.

Keywords: *Adenanthera pavonina* , Bark, Dichloromethane extract, Ethyl acetate extract, Chromatography

1 Background

Adenanthera pavonina L (Bengali: Rakta kambal) is an erect medium-sized tree (6–15 m tall and up to 45-cm diameter) with dark brown to grayish bark belongs to the family Leguminosae. The plant is native to the Asian continent and mostly found in Africa, Pacific and Caribbean regions [1]. It is also indigenous to India and Bangladesh particularly in the South-eastern region [2]. Different parts of this plant have been used in traditional medicine for the treatment of various diseases. The bark and leaves are used as a remedy for diarrhea, gout, hematuria, hematemesis, and chronic rheumatism [1–5]. The anti-inflammatory, analgesic, antioxidant, cytotoxic, anti-diarrheal, acute toxicity, antibacterial, antifungal, and blood pressure-reducing activities of the bark, leaf, and seed extracts and its isolated compounds have been reported [6–13]. Previous phytochemical investigation reported the presence of many bioactive compounds like robinetin, chalcone, butin and flavanol ampelopsin, stigmasterol glucosides, oleanolic acid, echinocystic acid, and sapogenins from the leaves and seeds of the plant [6, 14–19]. Very few compounds like as stigmasterol glucosides, oleanolic acid, and echinocystic acid have been reported from the bark part [15, 20]. Our previous in vitro studies have shown that the bark extracts of the plant possessed significant pharmacological activities [11–13]. Hence, our aim is to isolate bioactive compounds from this plant part.

2 Methods

2.1 Chemicals

n-Hexane, dichloromethane, chloroform, ethyl acetate, and methanol (Merck Germany) were used for solvent extraction. The laboratory grade petroleum ether (bp. 40–60 °C) was collected from fuel petrol by fractional distillation. Silica gel 60 H (E Merck, 7731), silica gel 60 (0.063–0.200 mm), vanillin, and sulfuric acid were from Merck, Germany.

2.2 General experimental procedures

Melting points were recorded by using an electro-thermal melting point apparatus (Stuart Scientific SMP3, UK) and OptiMelt (MPA100, Stanford Research Systems, USA). IR

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spectra were recorded using Nicolet iS10 FT-IR spectrometer by potassium bromide (KBr) pellets. ^{1}H NMR, ^{13}C NMR, DEPT 135 spectra, and attached proton test (APT) spectra were recorded in $CDCl_3$, CD_3OD, and mixture of $CDCl_3$ and CD_3OD with a 300-MHz NMR Spectrometer (Varian MERCURY-VX-300). Chemical shifts are presented in δ (ppm) using tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) are expressed in Hertz (Hz). Mass spectra were recorded by infusion into the ESI source using CH_3OH/CH_3OD as a solvent with a LC-ESI-MS/MS-System TSQ Quantum Ultra AM Finnigan and Triple Quadrupole MS with Mikro-HPLC (Surveyor plus). Pre-coated glass plates of silica gel (Keiselgel 60, F254, Merck KGaA, Darmstadt, Germany) were used for TLC analysis. The TLC spots were observed under long and short wavelength UV light (Fisher Scientific LCF-445) at 366 and 254 nm and the plates were sprayed with vanillin-sulfuric acid solution.

2.3 Plant material

The barks of *Adenanthera pavonina* were collected from the capital city Dhaka of Bangladesh. The plant was authenticated by Dr. Sardar Nasir Uddin of Bangladesh National Herbarium, Dhaka, and a voucher specimen (accession number-34196) was deposited in the Herbarium.

2.4 Extraction and isolation

The barks were air dried and ground into a powder. The powder (2.25 kg) was extracted successively with petroleum ether (b.p. 40–60 °C) (3 × 4 L), dichloromethane (3 × 2.5 L), ethyl acetate (3 × 2.5 L), and methanol (3 × 2.5 L) at room temperature for 72 h of each. The extracts were then concentrated in vacuo by rotary evaporator (Bucho, R-15v). A yellowish brown (4.42 g), greenish brown (8.0 g), reddish brown (5.7 g), and maroon (65.1 g) color extracts were obtained from the petroleum ether, dichloromethane, ethylacetate, and methanol extracts, respectively. Based on previous pharmacological investigation [11, 12], the crude dichloromethane (DCM) extract was selected for isolation of compounds. Therefore, the dichloromethane extract was run on TLC before extensive chromatographic separation, the solvent system that gave the best resolution in EtOAc:Petroleum ether (1:9). Seven spots (R_f 0.93, 0.89, 0.84, 0.68, 0.48, 0.34, and 0.18) were observed with tailing. After TLC study, the extract was loaded on a vacuum liquid chromatograhy (VLC) and the column was packed with silica gel (60 H). The column was eluted successively (according to their polarity index) with hexane, dichloromethane, ethyl acetate, and methanol by different polarity ratios at 200 mL of each. A total of 10 fractions were obtained. The fraction 1 (49 mg) and fraction 2 (6 mg) gave the same R_f value (0.8) in 100% petroleum ether (pet.ether). Therefore, fraction 1 and 2 were pooled together and obtained a pure brown needle-shaped crystal (18 mg, m.p. 115^0 C, R_f 0.8) of compound **1** (ethyl 3,3-dimethyl-13-hydroxytridecanoate) by re-crystallization method with hot methanol. Fraction 6 (1.49 g) eluted successively with pet.ether, DCM, EtOAc, and MeOH by different solvent ratio through glass chromatographic column (90 cm × 8 cm, internal diameter) to obtain 10 fractions (6A to 6J). Fraction 6E (220 mg) was further eluted with 10% dichloromethane in petroleum ether to give 22 mg of compound **2** (stigmasta-5,22-dien-3β-ol). The eighth fraction, 6H (90 mg) was subjected to again column chromatography (CC) over silica gel 60 and eluted with mixtures of EtOAc: pet.ether (1:9) which furnished 6 mg of compound **3** (tert.butyl tridecanoate). Fraction 6I (170 mg) was then separated by 10% pet.ether in chloroform by preparative thin layer chromatography (PTLC) method and yielded 10 mg of compound **4** (6-α-hydroxy stigmast-20(21)-en-3-one).

According to the previous biological studies on the different extracts of the barks [11, 12], the ethyl acetate extract had been considered for further chromatographic separation, The crude extract was checked on TLC before undergoing extensive chromatographic separation. The best resolution was observed in EtOAc:pet.ether (1:9) and found seven distinct spots with tailing. Then, the EtOAc extract (3.1 g) was started for separation by column chromatography by using pet-ether-DCM, DCM, DCM-EtOAc, EtOAc, and MeOH as solvent systems according to their polarity. This procedure gave 6 fractions. The fractions 1 (10 mg) and 3 (7 mg) checked on TLC with 100% pet.ether and pet.ether-DCM (9:1) and were obtained as pure compounds and labeled as compounds **5** (18-(2′, 3′-dihydroxyphenyl)nonadec-17-en-2-ol) and **6** (1-(N-propyl amino)-2-henecosanone), respectively. The fraction 4 of EtOAc extract (26 mg) was further eluted with pet-ether-DCM and DCM solvent systems by column chromatography and yielded two sub-fraction 4A (6 mg) and 4B (14 mg). The fraction 4B was further purified by PTLC method (solvent system, pet.ether: $CHCl_3$ = 4:1) which resulted in the isolation 3 mg of compound **7** (stigmast–5(6), 20(21)-diene-3-one).

3 Results

3.1 Characteristic data of compounds

3.1.1 Ethyl 3,3-dimethyl-13-hydroxytridecanoate (1)

Brown needle-shaped crystal (18 mg). Soluble in pet.ether. mp. 115 °C. R_f = 0.80 (100% pet.ether). ^{1}H NMR ($CDCl_3$ 400 MHz): δ 4.05 (q, *J* = 7.17 Hz, 2H, H-14), 3.33 (dt, 2H, H-13), 1.98 (s, 2H, H-2), 1.54 (m, 2H, H-12), 1.2–1.34 (m, 16H, H-4,5,6,7,8,9,10,11), 0.81 (t, 3H, H-15), 0.79 (s, 3H, H-16, 17), and 1.62 (br s, 1H, 13-OH). ESI-MS (Negative ion): m/z 283.7 (M^+ – 2H). Calculated for $C_{17}H_{34}O_3$ = 286.2508.

3.1.2 Stigmasta-5,22-dien-3β-ol (2)

White needle-shaped crystal (22 mg). Soluble in $CHCl_3$, mp 164 °C. R_f = 0.53 (pet.Ether: CH_2Cl_2 = 1:19). IR (KBr):

3676 (br,-OH str), 2960, 2852 (C-H str), 1558, 1601 (C-H bend), 1051, 960, 800 cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz): δ 5.35 (d, 1H, H-6), 5.15 (dd, 1H, J = 8.52 and 15.02 Hz, H-22), 5.03 (dd, 1H, J = 8.52 and 15.02 Hz, H-23), 3.51 (m,1H, H-3), 2.27 (m, 1H, H-20), 2.23 (m, 1H, H-24), 2.22 (m, 2H, H-4), 1.85 (m, 2×1H, H-25,7), 1.54 (m, 1H), 1.53 (m, 3×2H, H-15, 16, 17), 1.44 (m, 3×1H, 6×2H, H-2, 8, 9, 11, 12, 14), 1.25 (m, 2×2H, H-1, 28), 1.08 (d, 3H, H-21), 1.00 (s, 3H, H-19), 0.84 (d, 3H, J = 6.12 Hz, H-27), 0.82 (t, 3H, J = 6.98 Hz, H-29), 0.69 (s, 3H, H-18), 0.68 (d, 3H, J = 6.07 Hz, H-26). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 140.79 (C-5), 138.32 (C-22), 129.33 (C-23), 121.72 (C-6), 71.83 (C-3), 56.91 (C-14), 56.02 (C-17), 51.27 (C-24), 50.21 (C-9), 42.35 (C-13), 42.26 (C-4), 40.48 (C-20), 39.73 (C-12), 37.30 (C-1), 36.55 (C-10), 31.94 (C-25,8), 31.71 (C-7,2), 28.93 (C-16), 25.42 (C-28), 24.39 (C-15), 21.23 (C-26,11), 21.10 (C-21), 19.41 (C-19), 19.01 (C-27), 12.25 (C-29), 12.07 (C-18). ESI-MS (positive ion): at m/z 413.6 (M^+ +H). Calculated for $C_{29}H_{48}O$ = 412.3705 [21].

3.1.3 tert.butyl tridecanoate (3)

White solid (6 mg). Soluble in $CHCl_3$. mp 75 °C. R_f 0.77 in 100% DCM. IR (KBr):1707 (C=Ostrc), 2919 and 2843 (aliphatic C-H strc), 1464 and 1377 (aliphatic C-H bending), 1194 and 1096 (C-O strc) cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz): δ 2.34 (t, 2H, J = 7.40 Hz, H-2), 1.62 (m, 2H, H-3), 1.60 (m, 2H, H-4), 1.25 (s, 3×3H, H-15,16, 17), 1.24 (m, 8×2H, H-5, 6, 7, 8, 9, 10, 11, 12), 0.87 (t, 3H, J = 6.80 Hz, H-13). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 178.30 (C-1), 76.72 (C-14), 33.78 (C-2), 31.96 (C-3), 29.73 (C-4, 5), 29.47 (C-6, 7), 29.39 (C-15, 16, 17), 29.27 (C-8,9), 29.10 (C-10), 24.74 (C-11), 22.72 (C-12), 14.13 (C-13). ESI-MS (positive ion): at m/z 271.3 (M^+ +H). Calculated for $C_{17}H_{34}O_2$ = 270.2559. On the basis of these spectral data, compound **3** was identified as tert.butyl tridecanoate (http://www.nmrdb.org) (Fig. 1).

3.1.4 6-α-hydroxy stigmast-20(21)-en-3-one (4)

Brown semi-solid (10 mg). Soluble in $CHCl_3$. R_f = 0.52 (pet.ether: $CHCl_3$ = 1:95). IR (KBr): 3450 (O-H str), 2940, 2869 (C-H str), 1686 (C=O str), 1641 (C=C str), 1034 (C-O str), 983, and 883 cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz): δ. 4.73 (br, s, 1H, H-21b), 4.60 (br, s, 1H, H-21a), 4.15 (distorted t, 1H, H-6), 3.65 (distorted t, 2H, H-2), 3.18 (br, 2H, H-4), 2.99 (distorted t, 2H, H-1), 2.71 (m, 1H, H-17), 2.27 (m, 1H, H-5), 2.18 (m, 1H, H-24), 1.95 (m,2H, H-11), 1.67 (m, 2H, H-22), 1.60 (m,1H, H-25), 1.52 (m, 2×2H, H-15,16), 1.37 (m, 2H, H-28), 1.24–1.36 (m, 2×2H, 2×1H, H-12, 7, 8, 9), 1.27 (m, 2H, H-23), 1.24 (m,1H, H-14), 0.96 (d, 3H, J = 7.0 Hz, H-26), 0.94 (d, 3H, J = 7.0 Hz, H-27), 0.87 (distorted t, 3H, H-29), 0.81 (s, 3H, H-18), 0.74 (s, 3H, H-19). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 180.3 (C-3), 150.4 (C-20), 109.7 (C-21), 79.1 (C-6), 56.3 (C-13), 56.0 (C-17), 55.4 (C-9), 46.9 (C-4), 42.5 (C-14), 40.5 (C-22), 38.9 (C-10), 37.3 (C-5,8), 37.0 (C-1), 34.4 (C-2, 23), 32.2 (C-12), 31.9 (C-7), 29.4 (C-16), 27.4 (C-24), 24.9 (C-15, 28), 22.7 (C-11), 20.9 (C-27), 19.4 (C-26), 14.1 (C-29), 18.3 (C-18), 14.7 (C-19). On the basis of these spectral data, compound **4** was identified as 6-α-hydroxy stigmast-20(21)-en-3-one (Fig. 1).

3.1.5 18-(2', 3'-dihydroxyphenyl)nonadec-17-en-2-ol (5)

White crystal (10 mg). Soluble in $CHCl_3$, mp. 106–108 °C. R_f = 0.85 (pet.ether). IR (KBr): 3425 (O-H str), 2924 and 2852 (C-H aliphatic str.), 1558 and 1543 (C=C, aliphatic), 1506 (C=C, aromatic) cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz): δ 8.08 (d,1H, J = 8.0 Hz, H-4′), 7.60 (d, 1H, J = 7.2 Hz, H-6′), 7.47 (dd, 1H, J = 8.0 and 7.2 Hz, H- 5′), 5.42 (distorted triplet,1H, H-17), 3.9 (m, 1H, H-2), 2.35 (distorted triplet, 2H, H-16), 1.24–1.53 (m, 13×2H, H-3-15), 2.03 (s, 3H, H-19), 0.86 (d, 3H, J = 7.92 Hz, H-1). ESI-MS (positive ion) m/z 413.2 (M^++Na) and GC-MS m/z 391 (M^++H).

3.1.6 1-(N-propyl amino)-2-henecosanone (6)

White waxy solid (7 mg). Soluble in $CHCl_3$. mp 67 °C. R_f 0.58 (in pet.ether: DCM = 9:1). IR (KBr): 2916 and 2848 (aliphatic C-H str.), 1701 (C=O str) cm^{-1}. ^{1}H NMR ($CDCl_3$ 400 MHz): δ 4.04 (s, br, 2H, H-1), 2.34 (t, 2H, J = 7.4 Hz, H-3, 1′), 1.63 (m, 2H, H-2′), 1.24 (m, 34H, H-4 to 20), 0.86 (t, 3H, J = 6.28 Hz, H-21, 3′), 2.16 (br, N-H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 204 (C-2), 67.5 (C-1), 57.6 (C-1′), 33.65 (C-3), 33.65, 31.95, 29.45, 29.37, 29.09, 24.43, 22.71 (C-4 to 20), 24.43 (C-2′),14.13 (C-21, 3′).

3.1.7 Stigmast–5(6), 20(21)-diene-3-one (7)

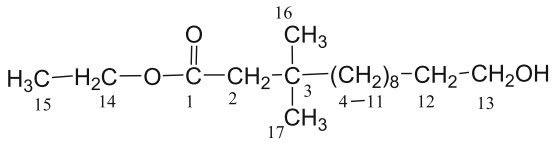
Brown semi-solid (3 mg). Soluble in $CHCl_3$. R_f = 0.51 (pet.ether: $CHCl_3$ = 1:4). ^{1}H NMR ($CDCl_3$ 400 MHz): δ 5.35 (distorted triplet, 1H, H-6), 4.72 (br, s, 1H, H-21a), 4.59 (br, s, 1H, H-21b), 3.65 (s,2H, H-4), 3.17 (distorted triplet, 2H, H-2), 2.99 (distorted triplet,2H, H-1), 2.98 (m, 1H, H-17), 2.27 (m, 1H, H-24), 1.68 (m, 2H, H-7), 1.63 (m, 1H, H-25), 1.60 (distorted triplet, 2H, H-22), 1.51 (m, 2×2H, H-15, 16), 1.36 (m, 2×2H, H-23, 28), 1.24 (m, 3×1H, 2× 2H, H-8, 9, 11, 12, 14), 0.96 (d, 3H, J = 6.8 Hz, H-26), 0.94 (d, 3H, J = 6.8 Hz, H-27), 0.86 (t, 3H, J = 6.9 Hz, H-29), 0.81 (s, 3H, H-19), 0.74 (s, 3H, H-18).

4 Discussion

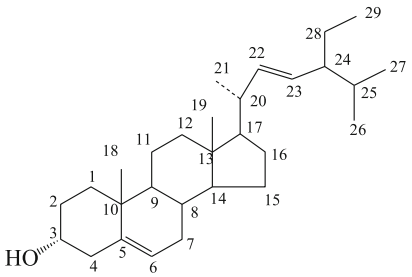
4.1 Characterization of compounds

4.1.1 Compound 1

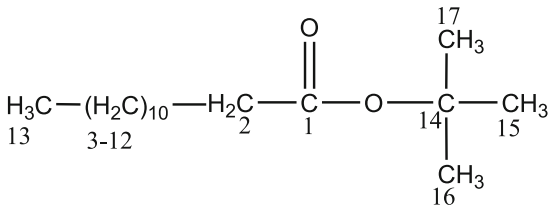
The ^{1}H-NMR data (Table 1) of compound **1** showed a triplet at δ 0.81 (H-15) and a quartet at δ 4.05 (J = 7.17 Hz, H-14). It indicates the presence of O-CH_2-CH_3 group. The peak at δ 1.62 (br, s, -OH) and a distorted triplet at δ 3.33 (H-13) is responsible for a primary hydroxyl group. The long chain of this compound is confirmed by a multiplet at δ 1.20–1.34 whose intensity indicates eight CH_2 group. A



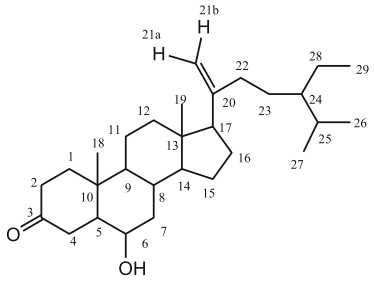
Ethyl 3,3-dimethyl-13-hydroxy tridecanoate (**1**)



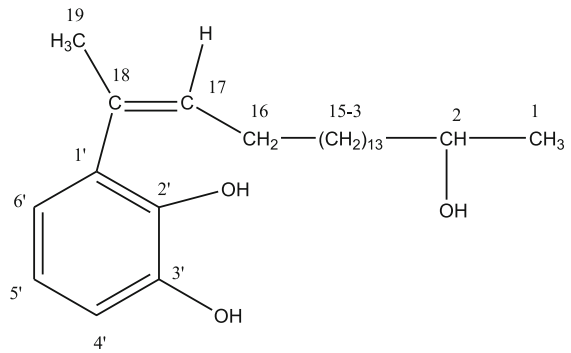
Stigmasta-5,22-dien-3 -ol (**2**)



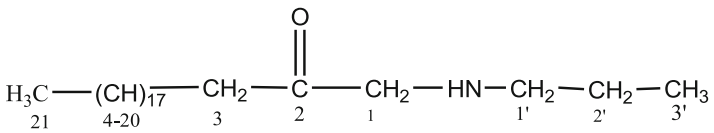
Tert butyl tridecanoate (**3**)



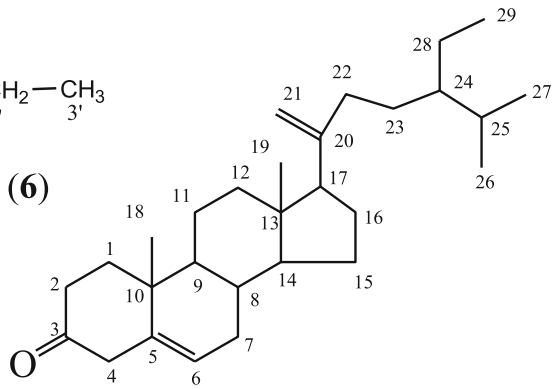
6- α -hydroxy stigmast-20(21)-en-3-one (**4**)



18-(2' ,3' -Dihydroxyphenyl)nonadec-17-en-2-ol (**5**)



1-(N-propyl amino)-2-henecosanone (**6**)



Sigmast-5(6), 20(21)-diene-3-one (**7**)

Fig. 1 Isolated compounds from the bark of *Adenanthera pavonina* Linn.

tertiary carbon substituted by two methyl groups into the chain which gave a peak at δ 0.79 (s, H-16,17). The ESI-MS spectrum showed peak at m/z 283.7 in the negative ion mood (M^+-2H). Thus, the molecular ion peak will be at m/z 286 and the molecular formula of the compound is $C_{17}H_{34}O_3$. On the basis of ^{1}H-NMR and mass spectral data,

Table 1 ^{1}H and ^{13}C NMR δ (ppm) data of compounds 1, 4–7

Carbon no.	1	4		5	6		7
	^{1}H δ (ppm)	^{1}H δ (ppm)	^{13}C δ (ppm)	^{1}H δ (ppm)	^{1}H δ (ppm)	^{13}C δ (ppm)	^{1}H δ (ppm)
1	-	2.99 (distorted t, 2H)	37.0	0.86 (d, 3H, *J* = 7.92 Hz)	4.04 (s, br, 2H)	67.5	2.99 (distorted triplet,2H)
2	1.98 (s, 2H)	3.65 (distorted t, 2H)	34.4	3.87 (m,1H)		204	3.17 (distorted triplet,2H, H-2)
3			180.3	1.24–1.53 (m,13×2H)	2.34 (t, 2H, *J* = 7.4 Hz)	33.65	
4	1.2–1.34 (m, 16H)	3.18 (br, 2H)	46.9		1.24 (m, 34H)	22.71, 24.43 29.09, 29.37, 29.45, 31.95, 33.65,	3.65 (s,2H)
5		2.27 (m, 1H)	37.3				
6		4.15 (m, 1H)	79.1				5.35 (distorted triplet, 1H)
7		1.24–1.36 (m, 2×2H, 1H)	31.9				
8			37.3				1.68 (m, 2H)
9			55.4				1.24 (m, 1H)
10			38.9				
11		1.95 (m,2H)	22.7				1.24 (m, 2H)
12	1.54 (m, 2H)	1.24 (m, 2H)	32.2				1.24 (m, 2H)
13	3.33 (distorted triplet, 2H) 1.62 (s, br, OH)		56.3				
14	4.05 (q, *J* = 7.17 Hz, 2H)	1.24 (m,1H)	42.5				1.24 (m, 1H)
15	0.81 (t, 3H)	1.52 (m, 2H)	24.9				1.51 (m, 2H)
16	0.79 (s, 3H)	1.52 (m, 2H)	29.4	2.35 (distorted triplet,2H)			1.51 (m, 2H)
17	0.79 (s, 3H)	2.71 (m, 1H)	56.0	5.42 (distorted triplet,1H)			2.98 (m, 1H)
18		0.81 (s,3H)	18.3				0.74 (s, 3H)
19		0.74 (s,3H)	14.7	2.03 (s,3H)			0.81 (s,3H)
20			150.4				
21		a 4.60 (br, s, 1H)	109.7		0.86 (t, 3H, *J* = 6.28 Hz)	14.13	4.72 (br, s, 1H,)
		b 4.73 (br, s, 1H)					4.59 (br,s, 1H)
22		1.67 (m, 2H)	40.5				1.60 (distorted triplet, 2H)
23		1.27 (m,2H)	27.4				1.36 (m, 2H)
24		2.18 (m, 1H)	34.4				2.27 (m, 1H)
25		1.60 (m,1H)	29.4				1.63 (m, 1H)
26		0.96 (d, 3H, *J* = 7.0 Hz)	19.4				0.94 (d, 3H, *J* = 9 Hz)
27		0.94 (d, 3H, *J* = 7.0 Hz)	20.9				0.94 (d, 3H, *J* = 9 Hz)
28		1.37 (m, 2H)	24.9				1.36 (m, 2H)
29		0.87 (distorted t, 3H)	14.1				0.86 (t, 3H, *J* = 6.9 Hz)
1′					2.34 (t, 2H, *J* = 7.4 Hz) 2.16 (br, -N-H proton)	57.6	
2′					1.63 (m, 2H)	24.43	
3′					0.86 (t, 3H, *J* = 6.28 Hz)	14.13	
4′				8.08 (d,1H, *J* = 8.0 Hz)			
5′				7.47 (dd,1H, *J* = 8.0 and 7.2 Hz)			
6′				7.60 (d,1H, *J* = 7.2 Hz)			

we can assign the structure of compound **1** as ethyl 3,3-dimethyl-13-hydroxy tridecanoate, which appears to be new.

4.1.2 Compound 2

Compound **2** was identified as stigmasta-5, 22-dien-3β-ol by comparing its spectral data with those published for this compound [21] (Additional file 1).

4.1.3 Compound 3

Compound **3** was identified as tert.butyl tridecanoate by comparing its spectral data with those reported for this compound (http://www.nmrdb.org) (Additional file 1).

4.1.4 Compound 4

The IR spectrum of compound **4** showed an absorption band at 3676 cm^{-1} indicated a hydroxyl group (-OH) and the band at 1685 is responsible for C=O bond. The sharp absorption band at 2940 and 2869 cm^{-1} were demonstrative of aliphatic C-H stretching. The bands at 983 and 883 cm^{-1} were demonstrative for the steroidal nature [22]. ^{1}H-NMR data (Table 1) of **4** showed two singlets at δ 0.74 and 0.81 (2×CH_3, C-18, 19) of 3H proton intensity of each and two doublets found at δ 0.94 and 0.96 (2×CH_3, C-26,27) of 3H proton. Moreover, 3H distorted triplet found at δ 0.87 (1×CH_3, C-29) is typical steroidal signal [22]. The distorted triplet for single proton at δ 4.15 is suggested for an oxymethine proton flanked by one methylene groups of cyclohexane ring system of a steroidal compound. The oxymethine proton may be attached to C-1, C-2, C-6, or C-12. If the oxymethine proton is attached to C-1, C-2, or C-12, it will find a triplet, but oxymethine proton showed a broad multiplet at δ 4.15 due to its β-axial orientation [23]. So, the oxymethine proton is at C-6. The spectrum displayed signals at δ 4.73 and 4.65 (1H, each, s, br) attributable to an exomethylene protons [24]. The presence of the double bonds at C-20 in this structure received support from ^{13}C-NMR data (Table 1) at δ 150.79 for C-20 and δ 109.2 for C-21. The presence of a keto group (C-3) and a hydroxyl group (C-6) is also confirmed by the ^{13}C-NMR at δ 180.3 for C-3 and δ 79.1 for C-6. Moreover, it responded to the Salkowsky and Liebermann-Burchard [25] color tests to exhibit its steroidal nature. On the basis of spectral data, we can assign the structure of compound **4** as 6-α-hydroxy stigmast-20(21)-en-3-one. The structure of **4** was confirmed on the basis of the comparison of their data with lupeol [24] and 12α-Hydroxystigmast-4-en-3-one [23].

4.1.5 Compound 5

The IR spectrum of compound **5** was assigned for the presence of hydroxyl group (-OH) at 3425 cm^{-1}. The band at 1506 cm^{-1} is responsible for double bond stretching of aromatic carbon, whereas the band at 1660 cm^{-1} indicated the aliphatic C=C stretching. From the ^{1}H-NMR data (Table 1), a doublet of doublet at δ 7.47 (J = 8.0 and 7.2 Hz, H-5′) and two doublets at δ 7.60 (J = 7.2 Hz, H-6′) and 8.08 (J = 8.0 Hz, H-4′) indicated the presence of a trisubstituted benzene ring. A distorted triplet at δ 5.42 indicated the presence of a single olefinic proton at H-17. The absorption band at δ 3.87 (m) indicated a H-2 proton which is attached to the oxygen (-O-) atom of hydroxyl group. Whereas =C-CH_3 group is shown by the singlet at δ 2.03 of 3H. It also showed 3H intensity at δ 0.86 (d). A distorted triplet found at δ 2.35 of H-16 and multiplets of 26 H (13×CH_2) are confirmed at δ 1.24–1.53. The ESI-MS (positive ion) m/z 413.2 is corresponding to (M^++Na) and GC-MS m/z 391 for (M^++H) which will be at 390. Thus, the molecular formula of compound **5** is $C_{25}H_{42}O_3$. On the basis of IR, ^{1}H-NMR, and mass spectral data, we have assigned the structure of compound **5** as 18-(2′, 3′–dihydroxyphenyl)nonadec-17-en-2-ol.

4.1.6 Compound 6

Strong IR absorption band at 1701 cm^{-1} clearly indicated the presence of a keto (-CO) group. On the other hand, absorption band at 3411 cm^{-1} indicated N-H stretching and C-N bond confirmed at 1652 and 1635 cm^{-1}. A broad peak at δ 2.16 in the ^{1}H-NMR data (Table 1) assigned for N-H proton. The ^{13}C-NMR also showed the peak at δ 57.6 indicated that amino-substituted carbon is there. The C-1 is flanked by a keto group as well as by NH group is indicated by the ^{1}H-NMR value at δ 4.04 (s, br) and ^{13}C-NMR value at δ 67.5. The n-propyl substituent of NH is indicated by the peaks at δ 0.8 (t, 3H), 1.63 (m, 2H), and 2.34 (t, 2H). The other broad absorptions at δ 1.24 corresponding to 34 H indicated the 17-methylene groups in the long chain. The ^{13}C-NMR data (Table 1) assumed 24 carbon atoms and therefore the molecular formula can be calculated as $C_{24}H_{49}NO$. On the basis of spectral data, the **6** assigned for 1-(N-propyl amino)-2-henecosanone.

4.1.7 Compound 7

^{1}H-NMR data (Table 1) of compound **7** showed a typical steroidal type compound [22]. Signals which observed two singlets at δ 0.74 and 0.81 (2×CH_3, C-18,19 of 3H proton of each), two doublets at δ 0.94 (2×CH_3, C-26, 27), and a 3H-distorted triplet at δ 0.86 (1×CH_3, C-29). On the other hand, the distorted triplet at δ 5.35 is suggestive of an alkene proton of cyclohexane ring system of a steroidal compound [23]. The spectrum attributable to an exomethylene protons at δ 4.72 and 4.59 (1H, each, br.s) [24]. The ^{1}H-NMR for H-2 at δ 3.17 and H-4 at δ 3.65 (s) indicated that the keto group is present in C-3 position. We could not do any ^{13}C-NMR and mass spectra due to the isolation of a very small amount of **7**. But the ^{1}H-NMR spectra is almost similar to that for

compound **4**. Moreover, it responded to the Salkowsky and Liebermann-Burchard color tests [25] of steroidal compounds. Therefore, ^{1}H-NMR spectral data of compound **7** is suggested as stigmast-5(6), 20(21)-diene-3-one by comparison with **4** which molecular formula is $C_{29}H_{46}O$. The structure of **7** is attained only by the removal of water from **4**, that results in a C=C double bond at C-5 and C-6.

5 Conclusions

Adenanthera pavonina Linn. has been reported for its various pharmacological activities in the field of traditional medicines. Present investigation has unfolded its seven compounds from the bark extract especially dichloromethane and ethyl acetate extract for the first time. The compounds were isolated through chromatographic methods and their structures were established by extensive spectroscopic techniques, particularly NMR. This investigation may open up future research in the field of synthetic chemistry to synthesis new series of compounds with immense medicinal importance.

6 Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s43088-019-0013-0.

Additional file 1: Figure S1. ^{1}H-NMR spectrum of the compound 1. **Figure S2.** ESI-MS (Negative ion) spectrum of the compound 1. **Figure S3.** IR spectrum of the compound 2. **Figure S4.** ^{1}H-NMR spectrum of the compound 2. **Figure S5.** ^{13}C-NMR spectrum of the compound 2. **Figure S6.** ESIMS spectrum of the compound 2. **Figure S7.** IR spectrum of the compound 3. **Figure S8.** ^{1}H-NMR spectrum of the compound 3. **Figure S9.** ^{13}C-NMR spectrum of the compound 3. **Figure S10.** IR spectrum of the compound 4. **Figure S11.** ^{1}H-NMR spectrum of the compound 4. **Figure S12.** ^{13}C-NMR spectrum of the compound 4. **Figure S13.** IR spectrum of the compound 5. **Figure S14.** ^{1}H-NMR spectrum of the compound 5. **Figure S15.** ESI-MS (positive ion) spectrum of the compound 5. **Figure S16.** GC-MS spectrum of the compound 5. **Figure S17.** IR spectrum of the compound 6. **Figure S18.** ^{1}H-NMR spectrum of the compound 6. **Figure S19.** ^{13}C-NMR spectrum of the compound 6. **Figure S20.** ^{1}H-NMR spectrum of the compound 7. **Table S1.** ^{1}H-NMR of compound 2 and Comparison of ^{13}C-NMR data of compound 2 with those of the published data [19]. **Table S2.** ^{13}C-NMR and 1H-NMR data of compound 3.

Abbreviations

APT: Attached proton test; br.s: Broad singlet; CC: Column chromatography; DCM: Dichloromethane; DEPT: Distortionless enhancement by polarization transfer; HPLC: High-performance liquid chromatography; IR: Infrared spectroscopy; LC-ESI-MS: Liquid chromatography-electron spray ionization–Mass spectroscopy; NMR: Nuclear magnetic resonance; PTLC: Preparative thin layer chromatography; VLC: Vacuum liquid chromatography

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Authors' contributions

AR designed and carried out the chromatographic separation and isolated the compounds. MMS participated in the characterization of compounds and drafted the manuscript. MAH supervised the experiment. MA participated in the elucidation of structures of compounds and supervised the study. CMH elucidated the structure of the isolated compounds and checked themanuscript. All authors read and approved the final manuscript.

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