SUPPLEMENTARY MATERIAL

Chemical composition, antioxidant and antibacterial properties of *Pteranthus dichotomus* from Algerian Sahara

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The phytochemical study of ethyl acetate and *n*-butanol extracts of *Pteranthus dichotomus* Forssk. led to the isolation and identification of eleven compounds, including three glycolipids **1–3**, one lignan **4**, three flavonoids **5–7** and four phytosterols **8–11**. Structures of the isolated compounds have been elucidated by analysis of 1D and 2D NMR data, and mass spectrometry EI-MS and ESI-MS and by comparison with literature data. Furthermore, the ethyl acetate and *n*-butanol extracts were examined for their antioxidant and antibacterial activities. The results showed that both extracts (**PDAC** and **PDBU**) had a moderate antioxidant activity (IC₅₀ = 375.514 µg/mL and 691.333 µg/mL) respectively.

Keywords: Caryophyllaceae, *Pteranthus dichotomus*, glycolipids, flavonoids, NMR, ESI, antioxidant activity, antibacterial activity.

Experimental details

1. General experimental procedures

UV spectra were measured on a Kontron UVS900 lite, Uvikon 941 spectrophotometer (Bio Tek instruments, USA). IR spectra were obtained using a Shimadzu model IR-470 spectrometer (Shimadzu, Ontario, Canada). ^1H and ^{13}C NMR spectra were recorded in CD_3OD and CDCl_3 using a Bruker Avance II spectrometer (Bruker, France) equipped with a cryoprobe at 500 MHz for ^1H and 125 MHz for ^{13}C . Complete assignments were performed on the basis of 2D experiments (COSY, HSQC and HMBC). Positive and negative ESI mass spectra were produced on ion trap Bruker Esquire. EI-MS were recorded using Maldi micro MX, micromasse walter. ESI-MS spectra were performed on a Bruker Micromass Q-TOF. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (Perkin-Elmer, USA). Analytical TLC was performed in silica gel plates Kieselgel 60 F₂₅₄ (Merck, Darmstadt, Germany) and developed by spraying with 50 % sulfuric acid reagent followed by heating. Column chromatography (CC) was performed using Merck Kieselgel 60 (70–230 mesh), Polyamide SC6, Sephadex LH-20 and lobar LiChroprep RP-8 (40–68 μm ; Merck). The antioxidant activity was evaluated using spectrophotometer (Vis-7220G & UV-9200-UK).

2. Plant material

Pteranthus dichotomus Forssk. was collected in May 2010 from the region of Biskra (Algerian Sahara) and was identified by Prof. Bachir Oudjehih of the agronomic department of Batna University where a voucher specimen has been deposited (No.710 / LCCE).

3. Extraction, fractionation and isolation

The powdered aerial parts of *Pteranthus dichotomus* (600 g) were macerated in $\text{MeOH-H}_2\text{O}$ (80:20) (6 Liters $\times$ 2) for 3 days at room temperature. Filtration and evaporation of the extract gave an aqueous-alcoholic solution. The aqueous phase was extracted successively with petroleum ether, ethyl acetate and *n*-butanol (each 300 mL $\times$ 3). The organic phases were dried with Na_2SO_4 . After drying, filtration and evaporation the following extracts were obtained: petroleum ether **PDPE** (4 g), ethyl acetate **PDAC** (6.5 g), and *n*-butanol **PDBU** (15 g). 6 g of ethyl acetate extract (**PDAC**) were separated on vacuum liquid chromatography VLC (50 mm $\times$ 50 mm; fractions of 100 mL) using silica gel as stationary phase. The elution was carried out with a mixture of petroleum ether–EtOAc and then EtOAc–MeOH to give 10 fractions Fr₁ to Fr₁₀. Fractions Fr₁₊₂ (57 mg) were chromatographed on CC of silica gel eluting with petroleum ether–EtOAc (100:0 to 60:40) to yield 4 mg of compound **10** and 7 mg of a

mixture of two compounds **8** and **9**. Fraction Fr₄ (966 mg) was purified by silica gel column chromatography CC using CHCl₃–MeOH (100:0 to 70:30) and afforded 10 subfractions (Fr_{4.1} to Fr_{4.10}). Fr_{4.3} (44 mg) was purified by gel filtration on Sephadex LH-20 with CH₂Cl₂ as eluent to give 21.3 mg of compound **4**. Fraction Fr₈ (1200 mg) was filtered on Sephadex and provided 4 subfractions (Fr_{8.1} to Fr_{8.4}). Fr_{8.2} (800 mg) was further chromatographed on a silica gel CC eluting with CHCl₃–MeOH (100:0 to 70:30) to yield 10 subfractions (Fr_{8.2.1} to Fr_{8.2.10}). From fraction Fr_{8.2.5} (400 mg), 4.5 mg of pure compound **11** were obtained by precipitation in MeOH. Fr_{8.2.10} (70 mg) was subjected to CC of polyamide eluting with toluene–MeOH (100:0 to 80:20) and afforded two compounds **6** (4 mg) and **5** (3.2 mg).

The *n*-butanol extract **PDBU** (7.5 g) was submitted to vacuum liquid chromatography VLC (50 mm × 50 mm; fractions of 100 mL) on RP-8 using H₂O–MeOH (80:20 to 0:100) to obtain 14 fractions of 100 mL combined together into 6 total fractions. After repeated polyamide CCs eluted with a gradient of toluene–methanol on fraction Fr₂ (882 mg) followed by filtration on Sephadex LH-20 (methanol), 41 mg of compound **7** were isolated. Fractions Fr₄₊₅ (480 mg) presented two separated components and were chromatographed over silica gel CC using a gradient of CHCl₃–MeOH (100:0 to 70:30) as eluent to yield 8 subfractions (Fr_{(4+5).1} to Fr_{(4+5).8}). Fr_{(4+5).5} (154 mg) was purified by CC on silica gel eluting with CHCl₃–MeOH (100:0 to 80:20) and followed by filtration on Sephadex LH-20 CC using CHCl₃–MeOH (80/20) as eluent to afford three compounds **3** (14 mg), **2** (5 mg) and **1** (4 mg).

4. Spectroscopic data

1-*O*-palmitoyl-3-*O*-(6-sulfo- α -D-quinovopyranosyl)-glycerol (1**)**

White amorphous powder; mp: 143; $[\alpha]_D^{+47.1^\circ}$ (C = 0.035, MeOH); IR (KBr) $\nu_{\max}$: 3400, 1730, 1170, 1150, 1060, 1035, 771 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ_H (ppm): 4.05 (1H, m, H-3a), 3.39 (1H, m, H-2b), 4.07 (1H, m, H-2a), 4.20 (1H, dd, *J* = 5.9, 6.5 Hz, H-3a), 4.10 (1H, m, H-3b), 2.37 (1H, t, *J* = 7.6 Hz, H-2''), 1.61 (1H, m, H-3''), 1.20–1.40 (m, H-4''–H-15''), 0.90 (3H, t, *J* = 6.7 Hz, H-16''), 4.78 (1H, d, *J* = 3.8 Hz, H-1'), 4.48 (1H, m, H-2'), 3.37 (1H, t, *J* = 8.9 Hz, H-3'), 3.08 (1H, t, *J* = 6.3 Hz, H-4'), 4.07 (1H, d, *J* = 6.3 Hz, H-5'), 2.92 (1H, dd, *J* = 9.2, 8.2 Hz, H-6'b), 3.35 (1H, *J* = 1.5, 2.0 Hz, H-6'a); ¹³C NMR (500 MHz, CD₃OD) δ_C (ppm): 70.7 (C-1), 69.9 (C-2), 66.7 (C-3), 175.9 (C-1''), 35.1 (C-2''), 26.1 (C-3''), 23.9–33.2 (C-4''–C-15''), 14.6 (C-16''), 100.4 (C-1'), 73.8 (C-2'), 75.3 (C-3'), 75.1 (C-4'), 96.9 (C-5'), 54.9 (C-6'); ESI-MS (negative mode) *m/z* 555 [M-H]⁻ (C₂₅H₄₈O₁₁S).

1,2-di-*O*-palmitoyl-3-*O*-(6-sulfo- α -D-quinovopyranosyl)-glycerol (2)

White amorphous powder; mp: 122; $[\alpha]_D^{+48.0^\circ}$ (C = 0.15, CH₃Cl:MeOH = 5:1); IR (KBr) $\nu_{\max}$: 3420, 1736, 1121, 1028 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ_H (ppm): 4.10 (1H, dd, J = 6.4, 5.8 Hz, H1-a), 3.57 (1H, dd, J = 5.2, 5.4 Hz, H-2b), 5.31 (1H, m, H-2), 4.50 (1H, dd, J = 7.1, 7.0 Hz, H3-a), 4.12 (1H, dd, J = 3.0, 3.5 Hz, H3-b), 4.76 (1H, d, J = 3.6 Hz, H-1'), 3.40 (1H, dd, J = 3.5, 4.2 Hz, H-2'), 3.63 (1H, dd, J = 8.9, 2.3 Hz, H-3''), 3.09 (1H, t, J = 9.2 Hz, H-4'), 4.07 (1H, dd, J = 1.6, 1.6 Hz, H-5'), 2.93 (1H, dd, J = 9.3, 9.6 Hz, H-6'b), 3.36 (1H, m, H-6'a), 2.37 (1H, t, J = 7.6 Hz, H-2'', H-2'''), 1.61 (1H, m, H-3'', H-3'''), 1.20–1.40 (m, H-4''–H-15'', H-4'''–H-15'''), 0.90 (6H, t, J = 6.7 Hz, H-16'', H-16'''); ¹³C NMR (500 MHz, CD₃OD) δ_C (ppm): 69.6 (C-1), 71.8 (C-2), 66.6 (C-3), 175.07 (C-1''), 175.2 (C-1'''), 35.0 (C-2'', C-2'''), 26.1 (C-3'', C-3'''), 30.4–31.0 (C-4''–C-15'', C-4'''–C-15'''), 14.6 (C-16'', C-16'''), 100.4 (C-1'), 73.8 (C-2'), 75.2 (C-3'), 75.0 (C-4'), 96.9 (C-5'), 54.9 (C-6'); ESI-MS (negative mode) m/z 793 [M-H]⁻ (C₄₁ H₇₉O₁₂S).

Soyacerebroside I (3)

White amorphous powder; mp: 114; $[\alpha]_D^{+3.358^\circ}$ (C = 0.358, MeOH:CHCl₃ = 3:2); IR (KBr) $\nu_{\max}$: 3455, 2975, 1658, 1610, 1328, 1130, 1025 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ_H (ppm): 0.90 (6H, t, J = 6.7 Hz, H-16', H-18'), 1.20–1.35 (m, H-11–H-17, H-5'–H-15'), 1.42 (1H, m, H-4'), 1.58 (1H, ddd, J = 14.0, 8.4 Hz, H-3'a), 1.70 (1H, dq, J = 14.7 Hz, H-3'b), 1.97 (1H, m, H-10), 2.06 (1H, m, H-6), 2.07 (1H, m, H-7), 3.19 (1H, dd, J = 8.8, 7.7 Hz, H-2''), 3.28 (1H, m, H-4''), 3.31 (1H, m, H-5''), 3.36 (1H, t, J = 9.0 Hz, H-3'''), 3.67 (1H, dd, J = 12.0, 5.1 Hz, H-6'a), 3.71 (1H, dd, J = 10.0, 3.5 Hz, H-1a), 3.81 (1H, dd, J = 12.0, 1.4 Hz, H-6'b), 3.98 (1H, ddd, J = 7.5, 5.0, 3.5 Hz, H-2), 4.00 (1H, dd, J = 8.0, 3.5 Hz, H-2'), 4.10 (1H, dd, J = 10.0, 5.5 Hz, H-1b), 4.12 (1H, m, H-3), 4.27 (1H, d, J = 7.7 Hz, H-1''), 5.42 (2H, m, H-8, H-9), 5.47 (1H, dd, J = 15.0, 7.0 Hz, H-4), 5.73 (1H, m, H-5). ¹³C NMR (500 MHz, CD₃OD) δ_C (ppm): 14.3 (C-16' and C-18), 30.37–31.01 (m, C-11–C-16, C-6'–C-14'), 23.2 (C-15', C-17 and C-4'), 32.8 (C-10), 33.1 (C-7), 35.2 (C-3'), 53.9 (C-2), 62.1 (C-6''), 69.0 (C-1), 70.8 (C-4''), 72.4 (C-3), 72.6 (C-2'), 74.2 (C-2''), 77.1 (C-3'' and C-5''), 103.8 (C-1'), 129.8 (C-4), 130.0 (C-9), 131.6 (C-8), 134.2 (C-5), 177.2 (C-1'); ESI-MS (negative mode) m/z 712 [M-H]⁻, ESI-MS (positive mode) m/z 736 [M+Na]⁺ (C₄₀ H₇₅O₉N).

8-oxo-pinoresinol (4)

White powder ; mp: 110 °C; $[\alpha]_D -3.5^\circ$ (C = 0.20, MeOH); IR (KBr) $\nu_{\max}$: 3430, 1753, 1631, 1600, 1501, 1230 and 1021 cm^{-1} ; ^1H NMR (500MHz, CDCl_3) δ_H (ppm): 3.27 (1H, m, H-1), 5.36 (1H, dd, $J = 3.5, 3.8$ Hz, H-2), 4.07 (1H, dd, $J = 4.5, 4.5$ Hz, H-4b), 4.36 (1H, dd, $J = 6.6, 6.6$ Hz, H-4a), 3.27 (1H, m, H-5), 5.34 (1H, dd, $J = 3.5, 3.5$ Hz, H-6), 6.81 (1H, m, H-2'), 6.82 (1H, m, H-6'), 6.88 (1H, m, H-2''), 6.90 (1H, m, H-6''); ^{13}C NMR (500 MHz, CDCl_3) δ_C (ppm): 53.5 (C-1), 83.5 (C-2), 72.8 (C-4), 50.1 (C-5), 84.8 (C-6), 177.2 (C-8), 131.2 (C-1'), 107.9 (C-2'), 147.1 (C-3'), 146.2 (C-4'), 114.9 (C-5'), 118.6 (C-6'), 132.4 (C-1''), 108.2 (C-2''), 146.9 (C-3''), 145.6 (C-4''), 114.6 (C-5''), 118.2 (C-6'') and 55.6 (C-3', C-3''); ESI-MS (negative mode) m/z 371 $[\text{M-H}]^-$, ESI-MS (positive mode) m/z 395 $[\text{M}+\text{Na}]^+$ ($\text{C}_{20}\text{H}_{20}\text{O}_7$).

Quercetin (5)

Yellow powder; mp: 316.3°C; UV ($\text{AlCl}_3 + \text{MeOH}$) $\lambda_{\max}$: 272, 448 nm; IR (KBr) $\nu_{\max}$: 3500, 1662, 1614, 1512 cm^{-1} ; ^1H NMR (500MHz, CD_3OD) δ_H (ppm): 6.20 (1H, d, $J = 2.0$ Hz, H-6), 6.40 (1H, d, $J = 2.0$ Hz, H-8), 7.75 (1H, d, $J = 2.1$ Hz, H-2'), 6.9 (1H, d, $J = 8.5$ Hz, H-5'), 7.65 (1H, dd, $J = 8.5, 2.1$ Hz, H-6'); ^{13}C NMR (500 MHz, CD_3OD) δ_C (ppm): 148.2 (C-2), 137.2 (C-3), 177.5 (C-4), 162.6 (C-5), 99.4 (C-6), 165.7 (C-7), 94.6 (C-8), 158.4 (C-9), 104.7 (C-10), 124.3 (C-1'), 116.1 (C-2'), 146.3 (C-3'), 150.3 (C-4'), 116.2 (C-5'), 121.8 (C-6'); ESI-MS (negative mode) m/z 303 $[\text{M-H}]^-$, ESI-MS (positive mode) m/z 325 $[\text{M}+\text{Na}]^+$ ($\text{C}_{15}\text{H}_{10}\text{O}_7$).

Apigenin (6)

Yellow powder; mp : 347.5°C ; UV ($\text{AlCl}_3 + \text{MeOH}$) $\lambda_{\max}$: 341, 271 nm; IR (KBr) $\nu_{\max}$: 3340, 1656, 1610, 1508 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ_H (ppm): 6.21 (1H, s, H-6), 6.49 (1H, s, H-8), 6.59 (1H, s, H-3), 6.90 (1H, d, $J = 8.8$ Hz, H-3'), 6.90 (1H, d, $J = 8.8$ Hz, H-5'), 7.40 (1H, d, $J = 8.8$ Hz, H-2'), 7.40 (1H, d, $J = 8.8$ Hz, H-6'); ^{13}C NMR (500 MHz, CD_3OD) δ_C (ppm): 164.6 (C-2), 102.4 (C-3), 182.4 (C-4), 161.8 (C-5), 98.7 (C-6), 164.9 (C-7), 93.5 (C-8), 149.5 (C-9), 103.9 (C-10), 122.3 (C-1'), 127.5 (C-2'), 115.3 (C-3'), 158.0 (C-4'), 115.3 (C-5'), 127.5 (C-6'); ESI-MS (negative mode) m/z 371 $[\text{M-H}]^-$ ($\text{C}_{15}\text{H}_{10}\text{O}_5$).

Isovitexin (7)

Yellow powder; mp: 286; UV (MeOH) $\lambda_{\max}$: 334, 270, 302 nm; IR (KBr) $\nu_{\max}$: 3400, 1650, 1610, 1520 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ_H (ppm): 7.85 (2H, d, $J = 8.5$ Hz, H-3', H-5'), 6.94 (2H, d, $J = 8.4$ Hz, H-2', H-6'), 6.71 (1H, s, H-3), 6.42 (1H, s, H-8), 4.56 (1H, d, $J =$

9.8 Hz, H-1''); ^{13}C NMR (500 MHz, CD_3OD) δ_{C} (ppm): 163.3 (C-2), 102.6 (C-3), 181.7 (C-4), 160.6 (C-5), 108.9 (C-6), 163.3 (C-7), 93.7 (C-8), 156.3 (C-9), 102.9 (C-10), 121.0 (C-1'), 128.3 (C-2', C-6'), 70.5 (C-2), 116.0 (C-3', C-5'), 78.9 (C-3''), 161.3 (C-4), 70.2 (C-4''), 73.1 (C-1''), 81.3 (C-5''), 63.3 (C-6''); ESI-MS (positive mode) m/z 471 $[\text{M} + \text{K}]^+$, m/z 455 $[\text{M} + \text{Na}]^+$ ($\text{C}_{21}\text{H}_{20}\text{O}_{10}$).

Stigmat-7-en-3-ol (8)

White crystals; IR (KBr) ν_{max} : 3400, 2988, 2860, 1614 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ_{H} (ppm): 1.82 (1H, m, H-1a), 1.09 (1H, m, H-1b), 1.77 (1H, m, H-2a), 1.39 (1H, m, H-2b), 3.52 (1H, m, H-3), 1.70 (1H, m, H-4a), 1.22 (1H, m, H-4b), 1.40 (1H, m, H-5), 1.74 (1H, m, H-6a), 1.22 (1H, m, H-6b), 5.08 (1H, m, H-7), 1.65 (1H, m, H-9), 1.47 (1H, m, H-11), 1.98 (1H, m, H-12a), 1.18 (1H, m, H-12b), 1.18 (1H, m, H-14), 1.50 (1H, m, H-15a), 1.38 (1H, m, H-15b), 1.80 (1H, m, H-16a), 1.23 (1H, m, H-16b), 1.25 (1H, m, H-17), 0.47 (3H, s, H-18), 1.13 (3H, s, H-19), 1.95 (1H, m, H-20), 0.97 (3H, d, $J = 6.6$ Hz, H-21), 1.36 (1H, m, H-22a), 1.07 (1H, m, H-22b), 1.21 (1H, m, H-23), 1.60 (1H, m, H-24), 1.49 (1H, m, H-25), 0.75 (3H, d, $J = 6.6$ Hz, H-26), 0.75 (3H, d, $J = 6.6$ Hz, H-27), 1.42 (1H, m, H-28a), 1.18 (1H, m, H-28b), 0.74 (3H, t, $J = 7.4$ Hz, H-29); ^{13}C NMR (500 MHz, CDCl_3) δ_{C} (ppm): 37.4 (C-1), 31.7 (C-2), 71.3 (C-3), 38.2 (C-4), 40.4 (C-5), 29.9 (C-6), 117.7 (C-7), 139.8 (C-8), 49.6 (C-9), 34.2 (C-10), 21.7 (C-11), 39.7 (C-12), 43.5 (C-13), 55.4 (C-14), 23.2 (C-15), 28.7 (C-16), 56.1 (C-17), 12.3 (C-18), 13.3 (C-19), 41.1 (C-20), 18.9 (C-21), 33.9 (C-22), 26.0 (C-23), 45.9 (C-24), 32.1 (C-25), 21.6 (C-26), 19.2 (C-27), 25.6 (C-28), 12.5 (C-29); EI-MS m/z 414 $[\text{M}]^+$ ($\text{C}_{29}\text{H}_{50}\text{O}$).

Spinasterol (9)

White crystals; IR (KBr) ν_{max} : 3400, 2988, 2860, 1614 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ_{H} (ppm): 1.82 (1H, m, H-1a), 1.09 (1H, m, H-1b), 1.77 (1H, m, H-2a), 1.39 (1H, m, H-2b), 3.52 (1H, m, H-3), 1.70 (1H, m, H-4a), 1.22 (1H, m, H-4b), 1.40 (1H, m, H-5), 1.74 (1H, m, H-6a), 1.22 (1H, m, H-6b), 5.08 (1H, m, H-7), 1.65 (1H, m, H-9), 1.47 (1H, m, H-11), 1.98 (1H, m, H-12a), 1.18 (1H, m, H-12b), 1.18 (1H, m, H-14), 1.50 (1H, m, H-15a), 1.38 (1H, m, H-15b), 1.80 (1H, m, H-16a), 1.23 (1H, m, H-16b), 1.25 (1H, m, H-17), 0.47 (3H, s, H-18), 1.13 (3H, s, H-19), 1.95 (1H, m, H-20), 0.97 (3H, d, $J = 6.6$ Hz, H-21), 4.95 (1H, dd, $J = 15.1, 5.8$ Hz, H-22), 5.08 (1H, dd, $J = 15.1, 8.5$ Hz, H-23), 1.60 (1H, m, H-24), 1.49 (1H, m, H-25), 0.75 (3H, d, $J = 6.6$ Hz, H-26), 0.75 (3H, d, $J = 6.6$ Hz, H-27), 1.42 (1H, m, H-28a), 1.18 (1H, m,

H-28b), 0,74 (3H, t, $J = 7.4$ Hz, H-29); ^{13}C NMR (500 MHz, CDCl_3) δ_{C} (ppm): 37.4 (C-1), 31.7 (C-2), 71.3 (C-3), 38.2 (C-4), 40.4 (C-5), 29.9 (C-6), 117.7 (C-7), 139.8 (C-8), 49.6 (C-9), 34.2 (C-10), 21.7 (C-11), 39.7 (C-12), 43.5 (C-13), 55.4 (C-14), 23.2 (C-15), 28.7 (C-16), 56.1 (C-17), 12.3 (C-18), 13.3 (C-19), 41.1 (C-20), 21.3 (C-21), 138.4 (C-22), 192.6 (C-23), 51.5 (C-24), 32.1 (C-25), 21.6 (C-26), 19.2 (C-27), 25.6 (C-28), 12.5 (C-29); EI-MS m/z 412 $[\text{M}]^{+}$ ($\text{C}_{29}\text{H}_{48}\text{O}$).

β -Sitosterol (10)

White powder; mp: 136.4°C ; $[\alpha]_{\text{D}}^{-30}$ ($C = 0.8$, CHCl_3); IR (KBr) ν_{max} : 3398, 2943, 2863, 1639 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ_{H} (ppm): 1.90 (1H, m, H-1a), 1.13 (1H, m, H-1b), 1.88 (1H, m, H-2a), 1.56 (1H, m, H-2b), 3.58 (1H, m, H-3), 2.34 (1H, ddd, $J = 13.0, 5.0, 2.0$ Hz, H-4a), 2.30 (1H, t, $J = 10$ Hz, H-4b), 5.40 (1H, dd, $J = 5.2, 2.3$ Hz, H-6), 1.50 (1H, m, H-7), 2.03 (1H, m, H-8), 0.98 (1H, m, H-9), 1.55 (1H, m, H-11a), 1.50 (1H, m, H-11b), 2.03 (1H, m, H-12a), 1.21 (1H, m, H-12b), 1.04 (1H, m, H-14), 1.63 (1H, m, H-15a), 1.11 (1H, m, H-15b), 1.89 (1H, m, H-16a), 1.30 (1H, m, H-16b), 1.16 (1H, m, H-17), 0.74 (3H, s, H-18), 1.06 (3H, s, H-19), 1.40 (1H, m, H-20), 0.95 (3H, d, $J = 5.1$ Hz, H-21), 1.34 (1H, m, H-22a), 1.04 (1H, m, H-22b), 1.19 (2H, m, H-23), 0.97 (1H, d, $J = 6.9$ Hz, H-24), 1.71 (1H, m, H-25), 0.88 (3H, d, $J = 6.9$ Hz, H-26), 0.87 (3H, d, $J = 6.9$ Hz, H-27), 1.31 (3H, m, H-28), 0.89 (3H, t, $J = 12.0$ Hz, H-29); ^{13}C NMR (500 MHz, CDCl_3) δ_{C} (ppm): 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.0 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.0 (C-28), 12.0 (C-29); ESI-MS (negative mode) m/z 413 $[\text{M}-\text{H}]^-$; ESI-MS (positive mode) m/z 437 $[\text{M}+\text{Na}]^+$ ($\text{C}_{29}\text{H}_{50}\text{O}$).

β -Sitosterol-3-*O*-glucoside (11)

White powder; mp: 285.2°C ; $[\alpha]_{\text{D}}^{-41.5}$ ($C = 0.4$, MeOH); IR (KBr) ν_{max} : 3421, 2943, 1639 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ_{H} (ppm): 1.88 (1H, m, H-1a), 1.08 (1H, m, H-1b), 1.91 (1H, m, H-2a), 1.62 (1H, m, H-2b), 3.59 (1H, m, H-3), 2.42 (1H, d, $J = 10$ Hz, H-4a), 2.28 (1H, t, $J = 10$ Hz, H-4b), 5.37 (1H, d, $J = 5.0$ Hz, H-6), 1.47 (1H, m, H-7), 1.97 (1H, m, H-8), 0.96 (1H, m, H-9), 1.45 (1H, m, H-11a), 1.02 (1H, m, H-11b), 2.01 (1H, m, H-12a), 1.17 (1H, m, H-12b), 1.03 (1H, m, H-14), 1.59 (1H, m, H-15a), 1.11 (1H, m, H-15b), 1.84 (1H, m, H-16a), 1.29 (1H, m, H-16b), 1.14 (1H, m, H-17), 0.70 (3H, s, H-18), 1.03 (3H, s, H-19), 1.37 (1H,

m, H-20), 0.95 (3H, d, $J = 5.1$ Hz, H-21), 1.34 (1H, m, H-22a), 1.04 (1H, m, H-22b), 1.19 (2H, m, H-23), 0.93 (1H, m, H-24), 1.68 (1H, m, H-25), 0.94 (3H, d, $J = 5.3$ Hz, H-26), 0.83 (3H, d, $J = 6.9$ Hz, H-27), 1.26 (3H, m, H-28), 0.85 (3H, t, $J = 7.4$ Hz, H-29), 4.41 (1H, d, $J = 7.9$ Hz, H-1'), 3.23 (1H, t, $J = 7.9$ Hz, H-2'), 3.42 (1H, t, $J = 7.9$ Hz, H-3'), 3.42 (1H, m, H-4'), 3.29 (1H, m, H-5'), 3.84 (1H, d, $J = 10.0$ Hz, H-6a), 3.75 (1H, dd, $J = 10.0, 5.0$ Hz, H-6b); ^{13}C NMR (500 MHz, CD_3OD) δ_{C} (ppm): 37.6 (C-1), 29.9 (C-2), 79.5 (C-3), 39.0 (C-4), 140.7 (C-5), 122.4 (C-6), 32.3 (C-7), 32.3 (C-8), 50.6 (C-9), 37.1 (C-10), 21.2 (C-11), 40.8 (C-12), 42.7 (C-13), 57.2 (C-14), 24.6 (C-15), 28.6 (C-16), 56.4 (C-17), 12.1 (C-18), 19.5 (C-19), 36.5 (C-20), 18.9 (C-21), 34.3 (C-22), 26.4 (C-23), 46.2 (C-24), 29.5 (C-25), 19.9 (C-26), 19.2 (C-27), 23.4 (C-28), 12.3 (C-29), 101.5 (C-1'), 74.0 (C-2'), 76.9 (C-3'), 70.7 (C-4'), 76.3 (C-5'), 62.2 (C-6'); ESI-MS (negative mode) m/z 575 $[\text{M}-\text{H}]^-$, ESI-MS (positive mode) m/z 599 $[\text{M}+\text{Na}]^+$ ($\text{C}_{35}\text{H}_{60}\text{O}_6$).

5. Total phenol content

The concentration of total phenols in each plant extract (**PDBU** and **PDAC**) was determined with Folin–Ciocalteu reagent following the colorimetric method adapted by (Li et al. 2007). 200 μL of diluted sample were added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 μL of saturated sodium carbonate (75 %) were added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Measurements were carried out in triplicate and calculations were based on a calibration curve obtained with gallic acid (0–200 $\mu\text{g}/\text{mL}$). The levels of total phenols were expressed as micrograms of gallic acid equivalents per milligrams of extract (μg GAE ‘gallic acid equivalent’ /mg of extract).

6. Antioxidant activity

6.1. DPPH radical-scavenging assay

The anti-radical activity of EtOAc, *n*-butanol and fraction containing glucolipids of *P. dichothomus* was determined by DPPH according to the procedure described by (Mansouri et al., 2005) with some modifications. 25 μL of diluted samples were added to 975 μL DPPH solution (6×10^{-5} M) and vortexed. After incubation in the darkness at room temperature during 30 minutes, the absorbance was determined at 517 nm, using a spectrophotometer in a 10 mm quartz cuvette. Methanol was used to zero the spectrophotometer. For negative control, the absorbance of the DPPH radical without sample was measured. The experiments

were done in triplicate. Quercetin was used as reference compound. The free radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

The IC₅₀ value is the concentration of the sample that scavenges 50 % of the DPPH radical, The ratio [phenolic] (μg)/[DPPH] (μg) was plotted against the % of remaining DPPH to obtain the amount of sample necessary to decrease the initial DPPH concentration by 50 % (EC₅₀). The antiradical efficiency (AE) is calculated as follows:

$$\text{AE \%} = 1/\text{EC}_{50}$$

6.2.β-Carotene linoleic acid assay

The antioxidant activity of the examined plant extracts was evaluated using a β-carotene-linoleate model system following the method described by (Kartal et al. 2007). A stock solution of β-carotene/linoleic acid was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 mL of chloroform; the chloroform was subsequently evaporated using a vacuum evaporator. Then 25 μL of linoleic acid and 200 mg of Tween 40 were added, then, 100 ml of distilled water saturated with oxygen (30 min at 100 mL/min) were added with vigorous shaking. Aliquots (2.5 mL) of this reaction mixture were transferred to test tubes, and 350 μL portions of the extracts were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT, and a blank containing only 350 μL of methanol. After the incubation period, the absorbance was measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank. The absorbance of each sample was measured in different time intervals (0, 1h, 2h, 4h, 6h, 24h, 26h, 28h, and 48h). After 48 h, relative antioxidant activities (RAA %) of the extracts were calculated from the equation given below:

$$\text{RAA \%} = A_{\text{Sample}}/A_{\text{BHT}} \times 100$$

Where A_{BHT} is the absorbance of the positive control BHT and A_{Sample} is the absorbance of the extract.

7. Antibacterial activity assay

The antibacterial activity of the extracts (**PDBU** and **PDAC**) was evaluated *in vitro* by using disc-diffusion method (Sacchetti et al. 2005). Negative control was prepared using the DMSO which is employed to dissolve the samples. Each extract was subjected to serial dilution technique by using dimethyl sulphoxide as a solvent to give: 500 mg/mL, 250 mg/mL, 125 mg/mL and 65 mg/mL solutions. These different concentrations were tested against one Gram positive bacteria (*Staphylococcus aureus* ATCC 25923) and two Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) and two bacterial strains isolated from patient samples: *Klebsiella pneumoniae* BLSE and *Enterobacter* sp BLSE. Bacterial strains were cultured in Mueller Hinton. Whatman sterile filter paper discs (6 mm diameter) were impregnated with 5 µL of each dilution of *P. dichotomus* extracts and placed on the inoculated agar. The plates were incubated at 37 °C for 24 h and the inhibition zone diameter of the tested bacteria was determined to the nearest mm. Each experiment was replicated for three times. Gentamicin and ampicillin was used as reference drug controls.

8. Statistical analysis

The data were expressed as mean±SD. One-way ANOVA analysis of variance and Newman-Keuls Multiple Comparison test were carried out to determine significant differences ($p < 0.05$) between the means. The analyses were made using GraphPad Prism (Version 5.0).

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Figure S1. Absorbance change of β -carotene at 490 nm in the presence of *Pteranthus dichotomus* extracts, negative and positive control (MeOH, BHT).

Figure S2. Antioxidant activity percentage of *P. dichotomus* determined by β -carotene bleaching test and comparison with the reference (BHT).

Table S1. The concentration that scavenges 50 % of DPPH (IC50), antiradical efficiencies (AE) and efficient concentrations (EC50) determined by quercetin and *P. dichotomus* extracts.

Extracts/compound	IC 50 ($\mu\text{g mL}^{-1}$)	EC50 ($\mu\text{g}/\mu\text{g DPPH}$)	AE
7	358.888 $\pm$ 11.213	15.337 $\pm$ 0.479	0.065 $\pm$ 0.002
<i>PDBU</i>	375.514 $\pm$ 21.194	16.047 $\pm$ 0.903	0.062 $\pm$ 0.003
<i>PDAC</i>	691.333 $\pm$ 88.143	29.544 $\pm$ 3.766	0.034 $\pm$ 0.004
Fr_{(4+5),5}	912.667 $\pm$ 40.203	39.002 $\pm$ 1.718	0.029 $\pm$ 0.013
QUER	1.149 $\pm$ 0.0004	0.491 $\pm$ 0.0	15.274 $\pm$ 0.0

* Data are represented as mean $\pm$ SD (n = 3). Significant difference (p<0.05)

Table S2. Antimicrobial activity of *PDAC* and *PDBU* extracts expressed as diameter of the inhibition zones.

		<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i> BLSE	<i>Enterobacter sp</i> BLSE
<i>PDBU</i> (mg /mL)	500	10 $\pm$ 2.828	-	-	13.5 $\pm$ 0.707	-
	250	8 $\pm$ 1.414	-	-	8 $\pm$ 1.414	-
	125	-	-	-	-	-
	65	-	-	-	-	-
<i>PDAC</i> (mg /mL)	500	7 $\pm$ 0.0	-	10.67 $\pm$ 1.527	-	8.33 $\pm$ 0.577
	250	-	-	10 $\pm$ 1.732	-	7 $\pm$ 0.0
	125	-	-	9.67 $\pm$ 1.527	-	-
	65	-	-	8.33 $\pm$ 0.577	-	-
AMP (μg)	10	15	20	32	-	-
GEN (μg)	10	14	19	28	14	12

* Values are the average of three replicates. (-): No inhibition.

* AMP: ampicillin; GEN: gentamicin.

Compound 1:

1-*O*-palmitoyl-3-*O*-(6-sulfo- α -D-quinovopyranosyl)-glycerol (Diop et al. 2004).

Mass spectrum ESI-MS of compound 1 (negative mode)

^1H -NMR spectrum of compound 1 (500 MHz, MD_3OD)

^{13}C -NMR spectrum of compound 1 (500 MHz, CD_3OD)

Compound 2:

1,2-di-O-palmitoyl-3-O-(6-sulfo- α -D-quinovopyranosyl)-glycerol (Plouguerné et al. 2013)

Mass spectrum ESI-MS of compound **2** (negative mode)

^1H -NMR spectrum of compound **2** (500 MHz, MD_3OD)

^{13}C -NMR spectrum of compound **2** (125 MHz, CD_3OD)

Compound 3 : Soyacerebroside I (Voutquenne et al. 1999).

Mass spectra ESI-MS of compound **3** (positive and negative modes)

^1H -NMR spectrum of compound **3** (500 MHz, CD_3OD)

^{13}C -NMR spectrum of compound **3** (125 MHz, CD_3OD)

Compound 4: 8-oxo-pinoresinol (Kui-Wu et al. 2012).

Mass spectrum ESI-MS of compound 4 (positive and negative modes)

1H -NMR spectrum of compound 4 (500 MHz, $CDCl_3$)

^{13}C -NMR spectrum of compound 4 (125 MHz, $CDCl_3$)

Compound 5: Quercetin (Choi et al. 2006).

Mass spectrum ESI-MS of compound **5** (positive mode)

^1H -NMR spectrum of compound **5** (500 MHz, CD_3OD)

^{13}C -NMR spectrum of compound **5** (125 MHz, CD_3OD)

Compound 6: Apigenin (Benabdelaziz et al. 2014).

Mass spectrum ESI-MS of compound **6** (positive mode)

^1H -NMR spectrum of compound **6** (500 MHz, CD_3OD)

^{13}C -NMR spectrum of compound **6** (125 MHz, CD_3OD)

Compound 7: Isovitixine (Peng et al. 2005),

Compounds 8 and 9: a mixture of two phytosterols: Stigmat-7-en-3ol (Smith et al. 1975) and Spinasterol (Ragasa et al. 2005).

Mass spectrum EI-MS of compound 8 and 9

¹H-NMR spectrum of compound 8 and 9 (500 MHz, CDCl₃)

^{13}C -NMR spectrum of compound **8** and **9** (125 MHz, CDCl_3)

Compound 10 : β -Sitosterol (Haba et al. 2007).

Mass spectra ESI-MS of compound **10** (positive and negative modes)

¹H-NMR spectrum of compound **10** (500 MHz, CDCl₃)

¹³C-NMR spectrum of compound **10** (125 MHz, CDCl₃)

Compound 11: β -Sitosterol-3-O-glucoside (Burdi et al. 1991).

Mass spectra ESI-MS of compound **11** (negative and positive modes)

1H -NMR spectrum of compound **11** (500 MHz, CD_3OD)

^{13}C -NMR spectrum of compound **11** (125 MHz, CD_3OD)