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Oleanane-type saponins from the roots of *Parkia bicolor* A. Chev.

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Highlights

- ▶ Five undescribed oleanane-type saponins were isolated from *Parkia bicolor*.
- ▶ One undescribed cassane-type diterpene was isolated from *Parkia bicolor*.
- ▶ Their structures were elucidated by 1D-, 2D-NMR and HR-ESI-MS analyses.
- ▶ Their cytotoxicity against the chronic myeloid leukemia K562 cells was evaluated.

Abstract

Five undescribed triterpene-type saponins, parkibicolorosides A-E, a cassane-type diterpene, and a known trimethoxy benzene glucoside were isolated from the roots of *Parkia bicolor* A. Chev. Their structures were elucidated by different spectroscopic methods including 1D- and 2D-NMR experiments as well as HR-ESI-MS analysis. Their cytotoxic activity against the chronic myeloid leukemia (K562) cell line was evaluated. The monosaccharides saponins exhibited a moderate antiproliferative activity with IC₅₀ ranging from 48.49 ± 0.16 to 81.66 ± 0.17 μM.

Keywords: *Parkia bicolor*, Fabaceae, triterpenoid saponins, diterpene, cytotoxic activity.

1. Introduction

The pantropical genus *Parkia* belongs to the subfamily Mimosoideae of the family Fabaceae. It consists of about 35 species with centres of distribution in South America, Africa and South-east Asia [1,2]. The phytochemical investigation on some species of the genus *Parkia* showed the isolation of flavonoids [3,4], proanthocyanidin [5,6], and tannins [7]. *Parkia bicolor* A. Chev. is a tree up to 40 m in height, with alternate and bipennate leaves of 30-45 cm long, and fruit is a linear pod with 40 cm long, growing in Côte d'Ivoire, Guinea, Sierra Leone and to the east of Congo RD [8,9]. Several parts of *P. bicolor* are used in traditional medicine [9]. The stem barks are used in decoction against bad coughs in children, treatment of gynecological troubles disorders [9] whereas the pulverized bark is employed in wound healing [10]. The roots of *P. bicolor* are used against children measles, woman sterility and sexually transmitted diseases [9]. The antioxidant and antibacterial activities of leaf and roots extracts were reported [1,11,12]. Its seed oil composition [10] and gum polysaccharides were reported [2]. Lupeol, lichexanthone, gallic acid and methyl gallate were isolated from the ethyl acetate extract of its stem bark [12]. Phytochemical screening of *P. bicolor* revealed that the leaf and stem bark extracts contained saponin and tannin [1], but none, to the best of our knowledge, have reported on their composition. As part of our ongoing research on new bioactive compounds from Ivoirian medicinal plant, we have studied the roots bark of *P. bicolor*. The present paper describes the isolation and structure elucidation of five new triterpenoid saponins and cassan diterpenoid. Their cytotoxicity against the chronic myeloid leukemia K562 cells was evaluated. To our best knowledge, this study is the first report of saponins in *Parkia* genus.

2. Experimental

2.1. General experimental procedures

NMR spectra were acquired in CD₃OD on Bruker Avance DRX III 600 instruments (¹H at 600 MHz and ¹³C at 150 MHz). Standard pulse sequences and parameters were used to obtain 1D ¹H and ¹³C and 2D COSY, ROESY, TOCSY, HSQC-TOCSY, and HMBC spectra. HR-ESI-MS data were gained using a Micromass Q-TOF high-resolution mass spectrometer. Optical rotations were determined in MeOH by Perkin-Elmer 241 polarimeter. TLC was performed on precoated silicagel 60 F₂₅₄ Merck and compounds were visualized by spraying the dried plates with 50% H₂SO₄, followed by heating. CC was carried out on HP-20 resin (Sigma Aldrich). Flash chromatography was conducted on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace® cartridges (Silica gel or RP-C₁₈). HPLC was performed on a Dionex apparatus equipped with an ASI100, ultimate 3000 Pump, a diode array detector UVD 340S and a chromeleon software. A prepacked RP-C₁₈ column (Phenomenex 250 x 15 mm, Luna 5 μ) was used for semi-preparative HPLC. The eluting mobile phase consisted of H₂O with TFA (0.0025%) and CH₃CN with a flow rate of 5 mL/min and the chromatogram was monitored at 205, 210, 254, and 300 nm.

3. Plant material

The stem roots of *Parkia bicolor* Eng. were collected in August 2016 at Adiopodoumé (Abidjan) in the south-central Cote d'Ivoire and identified by National Center of Floristic of FHB University of Cocody and a voucher specimen (No 12006) has been deposited.

4. Extraction and isolation

Dried and powdered stem roots of *Parkia bicolor* (1 kg) was extracted with 80% aqueous MeOH (3 × 10 L) at room temperature. After filtration and evaporation procedures, MeOH 80% (23.6 g) extract was obtained. This extract was dissolved in H₂O (200 mL) and then loaded onto a Diaion HP-20 open column (40 cm × 4.3 cm; the volume of the column was 363 mL) and

sequentially eluted with mixtures of H₂O and MeOH (0, 25, 50, 75%, and finally 100% MeOH; 2 L of each solvent mixture) to provide fractions I-V, respectively. Fraction II (1 g) was purified by flash chromatography over RP-C₁₈ eluted by a gradient system of CH₃CN-H₂O (5-80%, in 40 min) to afford 85 sub-fractions f_{II-1}-f_{II-85}. Subfractions f_{II-11-13} (200 mg) was subjected to flash chromatography over silica gel eluted by a gradient system of CHCl₃-MeOH (9:1 - 7:3, in 15 min) to yield compound **7** (12 mg). Subfractions f_{II-29-38} (300 mg) was purified by flash chromatography over silica gel eluted by a gradient system of CHCl₃-MeOH-H₂O (7:3 - 7:3:0.5, in 15 min) and subfractions [46-57] were purified by semi-prep HPLC (50-65% CH₃CN, in 15 min) affording compound **4** (*R_t* 7.8 min, 3 mg). Fraction III (3 g) was subject to vacuum liquid chromatography over silica gel (7 × 5.5 cm) eluted successively with the solvent mixtures CHCl₃-MeOH-H₂O (8:2:0, 7:3:0, 7:3:0.5, 6:4:0.7, 5:5:1, v/v/v, each 500 mL) to give fractions III-A to III-E, respectively. Fraction III-A (600 mg) was purified by flash chromatography over silica gel eluted with a gradient of CHCl₃-MeOH (9:1 - 7:3) and subfractions [47-50] (36.6 mg) were purified by semi-prep HPLC using a gradient (5-50% CH₃CN, in 20 min) affording compound **5** (*R_t* 14.5 min, 2 mg). Fractions III-C and III-D was purified by flash chromatography over RP-18 eluted by a gradient system of CH₃CN-H₂O (40-60%, in 40 min) to give **1** (5 mg) and **2** (14 mg). Fraction IV (800 mg) was purified by flash chromatography over RP-C₁₈ eluted with a gradient of MeCN-H₂O (40-60%, in 20 min) and subfractions IV₆₅₋₇₉ (50 mg) were purified by semi-prep HPLC (40-65% CH₃CN, in 15 min) to give compound **3** (*R_t* 9.1 min, 4 mg). Subfractions IV₈₆₋₉₅ (40 mg) were purified by semi-prep HPLC using an isocratic elution (60% CH₃CN) to give compound **2** (*R_t* 5.5 min, 10 mg). Subfractions IV₁₅₂₋₁₅₄ (26 mg) were purified by semi-prep HPLC using an isocratic elution with 70% CH₃CN to yield compound **6** (*R_t* 11.7 min, 9 mg).

4.1. Parkibicoloroside A (**1**)

132 Yellowish, amorphous powder; $[\alpha]_D^{20} + 7$ (c 0.73, MeOH); ^1H and ^{13}C NMR of the aglycone
133 part, see Table 1; ^1H and ^{13}C NMR of the glycosidic part, see Table 2; HR-ESI-MS m/z
134 1345.6204 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{66}\text{H}_{98}\text{O}_{27}\text{Na}$, 1345.6193).

135 *4.2. Parkibicoloroside B (2)*

136 Yellowish, amorphous powder; $[\alpha]_D^{20} + 6$ (c 0.26, MeOH); ^1H and ^{13}C NMR of the aglycone
137 part, see Table 1; ^1H and ^{13}C NMR of the glycosidic part, see Table 2; HR-ESI-MS m/z
138 1507.6713 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{72}\text{H}_{108}\text{O}_{32}\text{Na}$, 1507.6721).

139 *4.3. Parkibicoloroside C (3)*

140 Yellowish, amorphous powder; $[\alpha]_D^{20} + 3$ (c 0.46, MeOH); ^1H and ^{13}C NMR of the aglycone
141 part, see Table 1; ^1H and ^{13}C NMR of the glycosidic part, see Table 2; HR-ESI-MS m/z
142 1391.6235 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{67}\text{H}_{100}\text{O}_{29}\text{Na}$, 1391.6248).

143 *4.4. Parkibicoloroside D (4)*

144 Yellowish, amorphous powder; $[\alpha]_D^{20} + 5$ (c 0.35, MeOH); ^1H and ^{13}C NMR of the aglycone
145 part, see Table 1; ^1H and ^{13}C NMR of the glycosidic part, see Table 2; HR-ESI-MS m/z
146 1537.6838 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{73}\text{H}_{110}\text{O}_{33}\text{Na}$, 1537.6827).

147 *4.5. Parkibicoloroside E (5)*

148 Yellowish, amorphous powder; $[\alpha]_D^{20} + 5$ (c 0.11, MeOH); ^1H and ^{13}C NMR of the aglycone
149 part, see Table 1; ^1H and ^{13}C NMR of the glycosidic part, see Table 2; HR-ESI-MS m/z
150 789.4411 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{41}\text{H}_{66}\text{O}_{13}\text{Na}$, 789.4401).

151 *4.6. 16-O-methyl-cass-13(15)ene-16,18-dioic acid (6).*

Colorless oil; $[\alpha]^{20}_{\text{D}} -90$ (*c* 0.58, MeOH); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) see Table 1; HR-ESI-MS m/z 371.2192 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{32}\text{O}_4\text{Na}$, 371.2198).

5. Acid hydrolysis

Acid hydrolysis was carried out to obtain the sugar residues of compounds **1-7**. An aliquot of the saponin-containing fraction (100 mg of fraction D) was treated with 2N TFA (trifluoroacetic acid, aqueous solution, 15 mL) at 90 °C for 6 h. After extraction with CH_2Cl_2 (10 mL x 3), the water-soluble layer was evaporated to dryness. The sample (55 mg) was purified by preparative Si-gel TLC ($\text{MeCOEt}:\text{iso-PrOH}:\text{Me}_2\text{CO}:\text{H}_2\text{O}$, 20:10:7:6) to afford rhamnose [2.5 mg, $R_f = 0.73$, $[\alpha]^{20}_{\text{D}} +11$ (*c* 0.21, H_2O)]; arabinose [2 mg, $R_f = 0.59$, $[\alpha]^{20}_{\text{D}} +43$ (*c* 0.17, H_2O)]; xylose [1.9 mg, $R_f = 0.52$, $[\alpha]^{20}_{\text{D}} +18$ (*c* 0.2, H_2O)]; and glucose [4 mg, $R_f = 0.48$, $[\alpha]^{20}_{\text{D}} +30$ (*c* 0.33, H_2O)].

6. Cytotoxicity bioassay by MTS

K562 cells (chronic myeloid leukemia) were trypsinized, harvested, and spread onto 96-well flat-bottom plates at a density of 1000 cells per well, and then incubated for 24 h in RPMI 1640 Medium supplemented with 10% fetal bovine serum and antibiotics. After culture, the cells were treated with compounds **1-7** for 72 h. The cell cultures were then analyzed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) according to the manufacturer's instructions (Promega Corporation, Charbonnières, France). Doxorubicin was used as positive control. MTS is bio-reduced by cells into a colored formazan product. Absorbance was analyzed at a wavelength of 540 nm with a Multiskan Ex microplate absorbance reader (Thermo Scientific, Paris, France). Percentage of cell growth was calculated as $100\% \times (\text{absorbance of the treated cells}) / (\text{absorbance of the negative control})$

cells). Control cells were treated with complete culture medium containing 0.2% DMSO. The values represent averages of three independent experiments.

7. Results and discussion

The 80% EtOH extract from the dried roots of *P. bicolor* was subjected to Diaion HP-20 resin column chromatography to give the saponin-containing fraction, which was subjected to further column chromatography to yield five previously undescribed oleanane-type saponins, named parkibicolorosides A-E (**1-5**), a cassane-type diterpene (**6**) and one known phenolic compounds (**7**) (Figure 1). Upon acid hydrolysis with 2N TFA, an aliquot of the saponin-containing fraction allowed the identification of four monosaccharides as D-glucose, D-xylose, L-arabinose and L-rhamnose by comparison with authentic samples (see Experimental Section).

Compound **1** was obtained as a yellowish, amorphous powder. Its molecular formula, $C_{66}H_{98}O_{27}$, was determined by the positive-ion HR-ESI-MS at m/z 1345.6204 $[M+Na]^+$ (calcd for $C_{66}H_{98}O_{27}Na$, 1345.6193). Extensive analysis of 1D and 2D NMR spectra (1H - 1H -COSY, ROESY, HSQC, and HMBC) indicated the presence of an oleanane skeleton. It is characterized by seven tertiary methyl groups at δ_H 0.84 (s, Me-26), 0.86 (s, Me-24), 0.96 (s, Me-29), 0.98 (s, Me-25), 1.07 (s, Me-23), 1.12 (s, Me-30), and 1.22 (s, Me-27), an olefinic proton signal at δ_H 5.33 (t, $J=3.7$ Hz, H-12), one oxymethine proton at δ_H 3.14 (dd, $J=11.5, 4.2$ Hz, H-3), and a methine proton at δ_H 2.99 (dd, $J=13.8, 4.3$ Hz, H-18), which were typical signals of the oleanolic acid skeleton [13]. However, the methylene protons at δ_H 1.78 (dd, $J=12.9, 11.9$ Hz, H-22ax) and 1.82 (dd, $J=12.9, 4.9$ Hz, H-22eq) showed spin-couplings in the 1H - 1H COSY spectrum with the hydroxymethine proton at δ_H 4.95 (dd, $J=11.9, 4.9$ Hz). This hydroxymethine proton showed HMBC correlations with δ_C 47.1 (C-17), 46.3 (C-19), 36.4 (C-22) and two methyl signals at δ_C 17.3 (C-30), 27.8 (C-29), indicating the presence of a hydroxyl group at C-21 (Figure 2). This was confirmed by the downfield shift of C-21 at δ_C 75.5. The β -configuration

198 of the C-21 hydroxyl group was evident by the large J value of H-21/H-22ax ($J=11.9$ Hz)
 199 characteristic of an axial proton, and from the ROESY correlations between H-21 and H₃-30 β -
 200 oriented. The unambiguous assignment of all ^1H and ^{13}C NMR signals of the aglycone of **1**
 201 (Table 1), identified as machaerinic acid ($3\beta,21\beta$ -dihydroxyolean-12-en-28-oic acid), using
 202 correlations observed in COSY, ROESY, HSQC, and HMBC spectra, was in full agreement
 203 with literature data [14-16]. The downfield shift of the C-21 carbon (δ_{C} 75.5, δ_{H} 4.95) and its
 204 neighboring atoms [δ_{C} 34.9 (C-20); δ_{C} 36.4, δ_{H} 1.78/1.82 (C-22)] pointed toward an attachment
 205 of an ester residue at position C-21. The ^1H NMR spectrum of **1** revealed signals for two
 206 doublets of a trans-disubstituted olefinic bond (δ_{H} 6.53, d, $J=16.1$ Hz, H-8'; 7.70, d, $J=16.1$ Hz,
 207 H-7') and five aromatic protons (δ_{H} 7.63, dd, 8.1, 2.3 Hz, H-2',6'; 7.43-7.44, m, 3H, H-3',4',5')
 208 which characterized the *E*-cinnamoyloxy group. The presence of this group was confirmed by
 209 the ^{13}C NMR spectrum [δ_{C} 134.3 (C-1'), 127.9 (C-2',6'), 128.6 (C-3',5'), 131.0 (C-4'), 144.9 (C-
 210 7'), 117.6 (C-8'), 166.9 (C-9') [14,17]. This cinnamoyl group was attached to the hydroxyl at C-
 211 21 as confirmed by the observation of HMBC correlation between H-21 and the carbonyl
 212 carbon at δ_{C} 166.9 (C-9'). The ^1H NMR spectrum of the sugar portion of compound **1** showed
 213 five anomeric signals at δ_{H} 4.43 (d, $J=7.2$ Hz), 4.38 (d, $J=7.8$ Hz), 4.68 (d, $J=7.4$ Hz), 4.74 (d,
 214 $J=7.8$ Hz), and 4.57 (d, $J=4.5$ Hz), which correlated with five anomeric carbon atom resonances
 215 at δ_{C} 104.3, 102.1, 104.4, 103.6, and 99.2, respectively in the HSQC spectrum (Table 2). The
 216 spin systems of the five monosaccharides were assigned starting from the anomeric protons by
 217 means of COSY, TOCSY, HSQC, and HMBC experiments (Table 2). The ^1H and ^{13}C NMR
 218 spectra of **1** indicated the presence of two hexoses units identified as β -glucopyranosyl at δ_{H}
 219 4.38 (glc) and 4.74 (glc'), characterized by their large $J>8$ Hz. The three other sugars units were
 220 pentoses, two of them were elucidated as β -xylopyranosyl units at δ_{H} 4.43 (xyl) and 4.68 (xyl'),
 221 and the last one as α -arabinopyranosyl unit at δ_{H} 4.57 (ara). Based on the coupling constants of
 222 anomeric protons and the chemical shifts of anomeric carbons, the anomeric configuration of

glucopyranose and xylopyranose was determined as β in 4C_1 conformation. The anomeric
 proton coupling constant of ara ($J=4.5$ Hz) indicated that it was present in the 1C_4 conformation
 [18,19] (Table 2) and its chemical shifts of anomeric carbons indicate an α -configuration. The
 deshielded signals of C-2 (δ_C 81.3) and C-4 (δ_C 77.1) of xyl, and of C-3 (δ_C 81.7) and C-4 (δ_C
 70.8) of xyl', indicated that the two xylopyranoses units were disubstituted. The sequencing of
 the glycoside chains was achieved by analysis of HMBC and ROESY experiments. In the
 HMBC spectrum, the anomeric proton signals at δ_H 4.57 (ara-H-1), 4.74 (glc'-H-1), 4.68 (xyl'-
 H-1), 4.38 (glc-H-1), and 4.43 (xyl-H-1) showed cross-peaks with the carbon signals at δ_C 70.8
 (xyl'-C-4), 81.7 (xyl'-C-3), 81.3 (xyl-C-2), 77.1 (xyl-C-4), and 89.3 (aglycone-C-3),
 respectively. These signals provided ample evidence to determine the linkages between the
 sugars, and the sugar and the aglycone. These linkages were also confirmed by ROESY
 correlations between aglycone-H-3/xyl-H-1, xyl-H-4/glc-H-1, xyl-H-2/xyl'-H-1, xyl'-H-3/glc'-
 H-1, and xyl'-H-4/ara-H-1. Based on all the foregoing evidence, compound **1** was elucidated as
 3-*O*-{ α -L-arabinopyranosyl-(1 \rightarrow 4)-[β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-xylopyranosyl-(1 \rightarrow 2)}-
 [β -D-glucopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranosyl-21-*O*-cinnamoyl-machaerinic acid.
 Compound **1** was named parkibicoloroside A after its plant origin (Fig. 1).

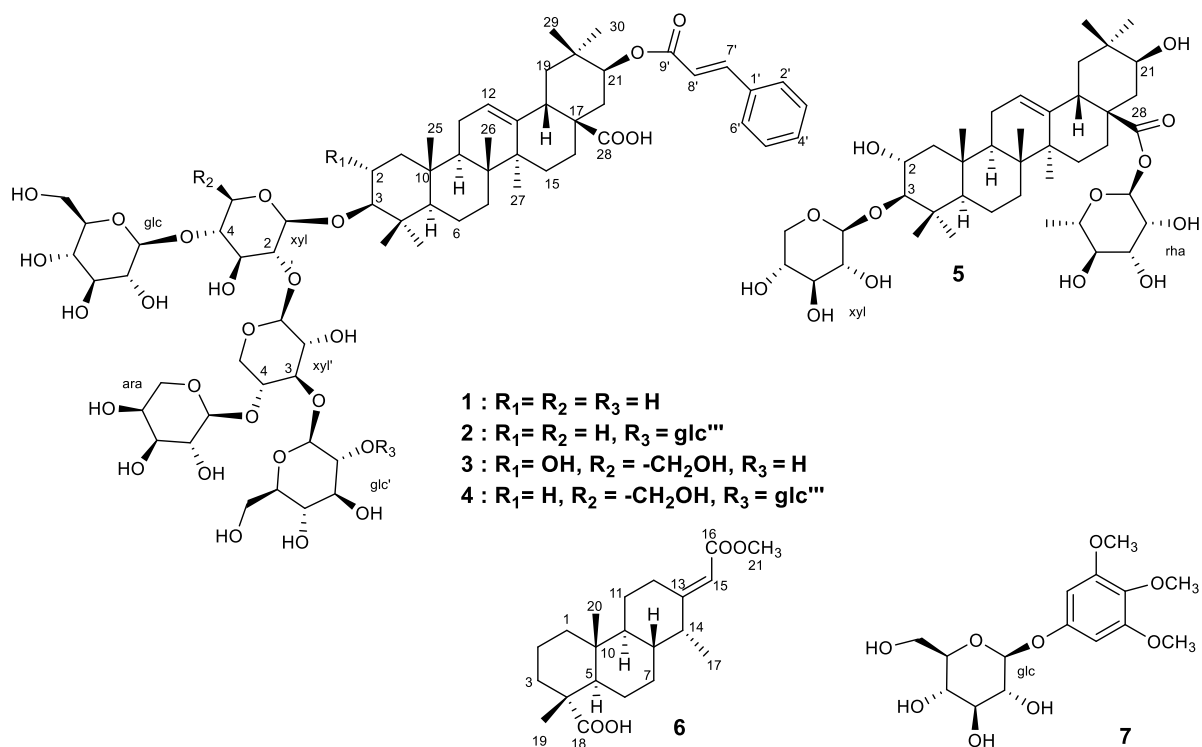


Figure 1. Structures of compound **1-7** isolated from *Parkia bicolor* roots.

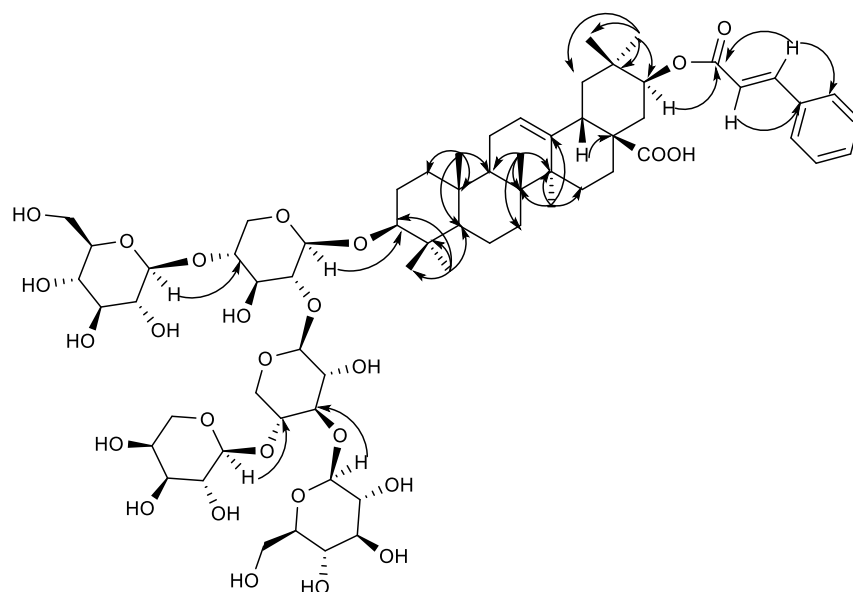


Figure 2. Key HMBC correlations for compound **1**.

Compound **2**, obtained as a yellowish, amorphous powder, displayed a molecular ion peak $[M+Na]^+$ at m/z 1507.6713 in the positive HR-ESI-MS, in accordance with an empirical

molecular formula of $C_{72}H_{108}O_{32}Na$ (calcd for $C_{72}H_{108}O_{32}Na$, 1507.6721), suggesting a supplementary hexose unit compared to **1**. The findings from the HR-ESI-MS analysis were confirmed by the NMR data, which displayed six additional carbons to the otherwise analogous resonances for the sapogenin 21-cinnamoyl-machaerinic acid with the xyl, ara and glc unit analogous to **1**. By extensive analysis of NMR spectra of **2** and in a comparison of the ^{13}C NMR signals for aglycone and cinnamoyl moieties of **2** with those of **1** (Tables 1 and 2), all signals due to the aglycone and cinnamoyl moiety at C-21 of **2** were in agreement with those in **1** (Table 1). The NMR data of the sugar part of **2** were very similar to those obtained from **1**, except for a significant downfield shift of C-2 (δ_C 83.2) of the glc' and the appearance of a set of additional signals, corresponding to a terminal β -D-glucopyranosyl group (glc'') in **2** which was attached at C-2 of glc' (Table 2). The linkage points of the sugar units to each other and to the aglycone were determined by following HMBC correlations: δ_H 4.44 (xyl-H-1) with δ_C 89.2 (aglycone-C-3), δ_H 4.48 (glc-H-1) with δ_C 76.4 (xyl-C-4), δ_H 4.71 (xyl'-H-1) with δ_C 81.6 (xyl-C-2), δ_H 4.87 (glc'-H-1) with δ_C 82.9 (xyl'-C-3), δ_H 4.56 (ara-H-1) with δ_C 70.8 (xyl'-C-4) and δ_H 4.66 (glc''-H-1) with δ_C 83.2 (glc'-C-2). Hence, compound **2** was established as 3-O-{ β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-(1 \rightarrow 3)-[α -L-arabinopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranosyl-(1 \rightarrow 2)}-[β -D-glucopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranosyl-21-O-cinnamoyl-machaerinic acid. Compound **2** was named parkibicoloroside B (Fig. 1).

Compound **3** was isolated as yellowish, amorphous powder with the elemental formula for $C_{67}H_{100}O_{29}$ (HR-ESI-MS m/z 1391.6235 $[M+Na]^+$; calcd for $C_{67}H_{100}O_{29}Na$, 1391.6248). When compared to the spectroscopic data of **1** and **2**, the sapogenin of **3** differed only by the presence of an additional hydroxyl group at position 2 (Table 1). This was corroborated by the downfield shift of C-2 (δ_C 66.5), its deshielded additional proton signal (δ_H 3.75), and the downfield shifts of the signals of the neighboring atoms [δ_C 46.1 (C-1), 95.1 (C-3), 40.3 (C-4),

270 37.4 (C-10)]. The α -configuration of the C-2 hydroxyl group was deduced from the large $J_{H-2,H-}$
 271 $_3$ value ($J=11.5$ Hz), characteristic of an axial proton, and confirmed by ROESY correlations
 272 between H-2 and H₃-25 β -oriented. The aglycone of **3** was identified as 2 α ,3 β ,21 β -trihydroxy-
 273 olean-12-en-28-oic acid. Full assignment of all ¹H and ¹³C NMR signals (Table 1) of using
 274 correlations observed in 2D NMR spectra, indicate the presence of the cinnamoyl unit at C-21
 275 as in compounds **1** and **2**, and was in perfect agreement with literature data for the genin
 276 2 α ,hydroxy-machaerinic acid [15,19]. The sugar part of **3** consists of five residues as evidenced
 277 by ¹H NMR spectrum which displayed five anomeric protons at δ_H 4.50 (d, $J=7.8$ Hz), 4.38 (d,
 278 $J=7.8$ Hz), 4.82 (d, $J=7.6$ Hz), 4.77 (d, $J=7.8$ Hz), and 4.57 (d, $J=4.8$ Hz), showing correlations
 279 in the HSQC spectrum to carbons at δ_C 103.2, 102.0, 103.6, 103.6, and 99.1, respectively (Table
 280 2). Severe overlap of some proton and carbon resonances requested the use of the HSQC-
 281 TOCSY experiment to map the spin systems. The detailed analysis of 1D and 2D NMR spectra
 282 led to the identification as in **1** of a 3,4-disubstituted β -D-xylopyranose (δ_{H-1} 4.82, xyl), a
 283 terminal α -L-arabinopyranose (δ_{H-1} 4.57, ara), and two terminal β -D-glucopyranose units (δ_{H-1}
 284 4.38 and 4.77, glc' and glc"). The NMR signals belonging to a 2,4-disubstituted β -D-
 285 glucopyranose unit were assigned starting from the anomeric proton at δ_H 4.50 (δ_{C-2} 79.8 and
 286 δ_{C-4} 78.4, glc) (Table 2). The sequencing of the glycoside chains was achieved by analysis of
 287 HMBC and ROESY experiments. HMBC correlations were observed between the anomeric
 288 proton signals of ara-H-1, glc"-H-1, xyl-H-1, glc'-H-1, and glc-H-1 with the carbon signals at
 289 δ_C 70.8 (xyl-C-4), 81.9 (xyl-C-3), 79.8 (glc-C-2), 78.4 (glc-C-4), and 95.1 (aglycone-C-3),
 290 respectively. These linkages were also confirmed by ROESY correlations between aglycone-
 291 H-3/glc-H-1, glc-H-4/glc'-H-1, glc-H-2/xyl-H-1, xyl-H-3/glc"-H-1, and xyl-H-4/ara-H-1.
 292 These findings led to the identification of compound **3** as 3-*O*-{ α -L-arabinopyranosyl-(1 \rightarrow 4)-
 293 [β -D-glucopyranosyl-(1 \rightarrow 3)]- β -D-xylopyranosyl-(1 \rightarrow 2)}-[β -D-glucopyranosyl-(1 \rightarrow 4)]- β -D-

glucopyranosyl-21-*O*-cinnamoyl-2 α -hydroxy-machaerinic acid. Compound **3** was named parkibicoloroside C (Fig. 1).

Compound **4** was isolated as an amorphous yellowish powder. The molecular formula was established as C₇₃H₁₁₀O₃₃ by HR-ESI-MS (m/z 1537.6838 [M+Na]⁺; calcd for C₇₃H₁₁₀O₃₃Na, 1537.6827). The resonances derived from the NMR spectra revealed the same aglycone as in compounds **1** and **2**. Extensive 2D NMR analysis (Table 2) showed that the glycosidic part of compounds **3** and **4** differed by the presence of one additional sugar in **4** which was identified as a terminal β -D-glucopyranose (glc'''). The HMBC correlations at δ_H 4.67 (d, $J=7.7$ Hz, glc'''-H-1)/ δ_C 83.2 (glc''-C-2), 4.87 (d, $J=7.8$ Hz, glc''-H-1)/ δ_C 82.7 (xyl-C-3), 4.57 (d, $J=4.5$ Hz, ara-H-1)/ δ_C 70.9 (xyl-C-4), 4.77 (d, $J=7.6$ Hz, xyl-H-1)/ δ_C 81.0 (glc-C-2), 4.46 (d, $J=7.5$ Hz, glc'-H-1)/ δ_C 78.3 (glc-C-4), and 4.48 (d, $J=7.7$ Hz, glc-H-1)/ δ_C 89.6 (aglycone-C-3) suggested the linkage of glc''' at the C-2 position of the glc''. This was confirmed by the ROESY cross-peak at δ_H 3.52 (glc''-H-2)/ δ_H 4.67 (glc'''-H-1). Thus, the structure of **4** was elucidated as 3-*O*-{ β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-(1 \rightarrow 3)-[α -L-arabinopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranosyl-(1 \rightarrow 2)}-[β -D-glucopyranosyl-(1 \rightarrow 4)]- β -D-glucopyranosyl-21-*O*-cinnamoyl-machaerinic acid. Compound **4** was named parkibicoloroside D (Fig. 1).

Compound **5** possessed the molecular formula of C₄₁H₆₆O₁₃ on the basis of its HR-ESI-MS at m/z 789.4411 [M+Na]⁺ (calcd C₄₁H₆₆O₁₃Na, 789.4401). Its NMR data of the aglycone moiety were similar to those of **3**, except for the absence of the cinnamoyl group at C-21 in **5**. This was confirmed by HMBC correlation between H-21/C-29, C-30 and the obvious upfield shift of H-21 (δ_H 3.69) (Table 1). The relative configuration of the aglycone moiety of **5** was established as being identical to that of **3** by analyzing their NMR data and ROESY interactions. The sugar part of **5** consists of two residues as evidenced by ¹H NMR spectrum which displayed

two anomeric protons at δ_{H} 4.28 and 5.95, showing correlations in the HSQC spectrum to carbons at δ_{C} 105.6 and 93.7, respectively (Table 2). An α -L-rhamnopyranose unit (rha) was identified by equatorial anomeric proton at δ_{H} 5.95 (d, $J=1.5$ Hz), the small coupling constant between $\text{H}_{\text{eq-2}}$ and $\text{H}_{\text{ax-3}}$ ($J=3.5$ Hz), the large coupling constants between $\text{H}_{\text{ax-3}}$ and $\text{H}_{\text{ax-4}}$ ($J=9.0$ Hz), and the coupling constant values of 6.2 Hz of methyl doublets at δ_{H} 1.26 (rha-H-6) (Table 2). The second monosaccharide unit was identified as a β -D-xylopyranose moiety δ_{H} 4.28 (d, $J=7.6$ Hz) by interpretation of 2D-NMR spectra. The cross-peak observed in the HMBC spectrum between xyl-H-1/aglycone-C-3 (δ_{C} 94.5) and rha-H-1/aglycone-C-28 (δ_{C} 177.4) indicated the points of attachment of the monosaccharides at the aglycone. Thus, compound **5** was concluded to be 3-*O*- β -D-xylopyranosyl-28-*O*- α -L-rhamnopyranosyl-2 α -hydroxy-machaerinic acid. Compound **5** was named parkibicoloroside E (Fig. 1).

Compound **6**, a colorless oil, possess a molecular formula $\text{C}_{21}\text{H}_{32}\text{O}_4$ determined based on the positive ion peak at m/z 371.2192 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{32}\text{O}_4\text{Na}$, 371.2198) in the HR-ESI-MS spectrum. Analysis of the ^1H NMR spectroscopic data of this compound (Table 1) indicated that the structure of **6** possessed three methyl groups at δ_{H} 0.77 (s), 0.94 (d, $J=6.7$ Hz), and 1.06 (s), an olefinic proton signal at δ_{H} 5.54 (s), and a methoxyl group at δ_{H} 3.55 (s). The ^{13}C NMR spectrum (Table 1) exhibited 21 signals, of which three methyl carbons at δ_{C} 13.2 (C-17), 13.3 (C-20), and 16.0 (C-19), an ester carbon at δ_{C} 166.9 (C-16), a carboxyl carbon at δ_{C} 181.0 (C-18), a methoxyl carbon at δ_{C} 49.9 (C-21), a trisubstituted double bond at δ_{C} 169.1 (C-13), 110.8 (C-15). These data suggested **6** to be an esterified cassane-type diterpene [20, 21]. Extensive analysis of 1D and 2D NMR spectra (^1H NMR, ^{13}C NMR, COSY, ROESY, HSQC, and HMBC) indicated that the NMR data of **6** were similar to those of 7-deoxycassane-16(18)dioic acid [21]. The HMBC spectrum showed long-range correlations from the Me-17 δ_{H} 0.94 (d, $J=6.7$ Hz) to the carbons at δ_{C} 41.0 (C-8), 45.0 (C-14) and 169.1 (C-13). The Me-19 showed HMBC correlations with C-4 (δ_{C} 47.2), C-5 (δ_{C} 49.4), C-3 (δ_{C} 36.9), and C-18 (δ_{C}

181.0) whereas Me-20 exhibited HMBC correlations with C-5, C-10 (δ_C 36.1), C-9 (δ_C 47.7), and C-1 (δ_C 38.5). The 1H - 1H -COSY correlations between H-12/H-11, H-11/H-9, H-9/H-8, H-8/H-14, H-14/H-20, H-8/H-7, H-7/H-6, H-6/H-5, H-1/H-2, and H-2/H-3 (Table 1) allowed the assignment of these proton signals. The olefinic proton at δ_H 5.54 (H-15) also showed long-range correlations to the carbons at δ_C 45.0 (C-14), 23.8 (C-12), a 169.1 (C-13), and δ_C 166.9 (C-16). In the HMBC spectrum, the methoxy signal at δ_H 3.55 gave a cross-peak with the carbonyl carbon C-16 which suggested it is located at C-16. The NOESY cross-peaks observed between Me-19/Me-20, Me-20/H-8, and H-8/H-14 confirmed their β -axial orientations whereas the NOESY correlations between H-5/H-9 and Me-17/H-9, indicated their α -axial orientations (Figure 3). The olefinic proton H-15 displayed a NOESY cross-peak with the methine proton H-14, indicating a *E*-configuration of the double bond. Compared to the literature data [21,22], the chemical shifts of C-12 (δ_C 23.8) and C-14 (δ_C 45.0) were in good agreement with a *E*-configuration. Therefore, **6** was elucidated as 16-*O*-methyl-cass-13(15)ene-16,18-dioic acid.

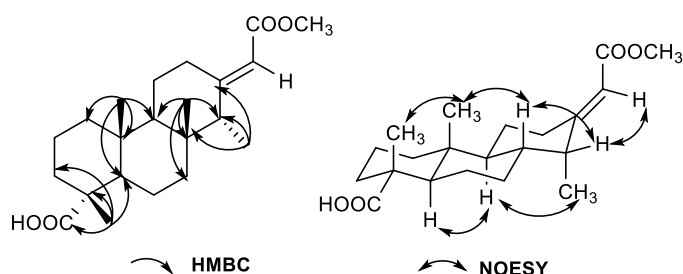


Figure 3. Key HMBC and NOESY correlations for compound **6**.

Compound **7** was identified as 3,4,5-trimethoxyphenyl-1-*O*- β -D-glucopyranoside [23].

The cytotoxic activity of compounds **1-7** was evaluated against K562 chronic myeloid leukemia cells. Compounds **5-7** were not active at the concentration tested (100 μ M). The monosaccharides saponins (**1-4**) exhibited a moderate antiproliferative activity with IC_{50} ranging from 48.49 ± 0.16 to 81.66 ± 0.17 μ M (Table 3), compared to the disaccharides saponin (**5**). The pentasaccharide (**1**, **3**) were slightly more active than the hexasaccharides (**2**, **4**)

saponins, and Parkibicoloroside C (**3**) with 2-hydroxy-machaerinic acid is less active than parkibicoloroside A (**1**), parkibicoloroside B (**2**), and parkibicoloroside D (**4**).

Table 1. NMR spectroscopic data of the aglycone moieties for compounds **1-5** (600 MHz, CD₃OD).

	1		2		3		4		5	
	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}
1	0.99	38.4	0.89	38.4	0.86	46.1	1.00	38.4	0.78	46.0
	1.63		1.53		2.03		1.68		1.90	
2	1.70, dd (12.7, 4.2)	25.8	1.60	25.8	3.75	66.5	1.71, dd (12.7, 4.2)	25.7	3.54	72.0
	1.81		1.71				1.97			
3	3.14, dd (11.5, 4.2)	89.3	3.03, dd (11.5, 4.3)	89.2	3.01, d (11.5)	95.1	3.18, dd (11.6, 4.2)	89.6	3.01, d (11.2)	94.5
4	-	39.2	-	39.2	-	40.3	-	39.1	-	40.1
5	0.80, brd (11.4)	55.7	0.68, brd (11.5)	55.7	0.96	55.3	0.81, brd (11.4)	55.7	0.77	55.2
6	1.44, td (11.0, 3.9)	17.9	1.33	17.9	1.46, td (11.0, 3.9)	18.0	1.44, td (12.6, 3.9)	17.9	1.36	17.9
	1.55		1.49		1.62		1.62		1.48	
7	1.35	32.6	1.24	32.6	1.36	32.5	1.34	32.7	1.23	32.5
	1.48		1.43		1.55		1.54		1.44	
8	-	38.9	-	39.0	-	39.2	-	38.9	-	39.3
9	1.60	47.7	1.50	47.8	1.68	47.7	1.63	47.8	1.53	47.6
10	-	36.5	-	36.5	-	37.4	-	36.5	-	37.4
11	1.90	23.2	1.82	23.2	1.99	23.2	1.96	23.2	1.86	23.3
12	5.33, t (3.7)	123.1	5.21, t (3.6)	123.0	5.33, t (3.5)	122.8	5.33, t (3.6)	123.0	5.39, t (3.5)	123.2
13	-	142.4	-	142.5	-	142.5	-	142.6	-	142.5
14	-	41.8	-	41.5	-	41.5	-	41.8	-	41.5
15	1.16, dt (13.8, 3.5)	27.4	1.08	27.4	1.15	27.1	1.14, dt (13.8, 3.5)	27.5	1.01	27.2
	1.76		1.72		1.78		1.67		1.63	
16	1.83	23.9	1.73	23.9	1.83	23.9	1.83	23.9	1.68	23.7
	2.14, td (13.4, 3.7)		2.01, td (13.5, 3.6)		2.14, td (13.4, 3.7)		2.12, td (13.5, 3.5)		2.08, td (13.6, 3.5)	
17	-	47.1	-	47.1	-	47.1	-	47.5	-	47.5
18	2.99, dd (13.8, 4.3)	40.9	2.89, dd (13.5, 4.4)	40.8	2.99, dd (13.8, 4.3)	40.8	3.00, dd (13.8, 4.3)	40.8	2.97, dd (14.0, 4.3)	41.1
19	1.34	46.3	1.22	46.4	1.32	46.6	1.35	46.4	1.05	46.2
	1.98		1.87		1.96		1.97		1.74	
20	-	34.9	-	34.9	-	34.9	-	35.0	-	35.8
21	4.95, dd (11.9, 4.9)	75.5	4.84, dd (11.6, 4.8)	75.5	4.95, dd (11.7, 5.0)	75.7	4.96, dd (11.6, 4.9)	75.6	3.69, dd (11.5, 4.7)	71.1
22	1.78, dd (12.9, 11.9)	36.4	1.78	36.5	1.78	36.4	1.78	36.5	1.57	39.7
	1.82, dd (12.9, 4.9)		1.82		1.82		1.82		1.72	
23	1.07, s	26.9	0.95, s	27.0	1.14, s	27.1	1.18, s	27.0	1.13, s	27.2
24	0.86, s	15.3	0.74, s	15.3	0.92, s	16.2	0.86, s	15.4	0.92, s	16.6
25	0.98, s	14.6	0.86, s	14.6	1.04, s	15.7	0.99, s	14.6	1.04, s	15.7
26	0.84, s	16.3	0.72, s	16.3	0.85, s	16.3	0.84, s	16.4	0.81, s	16.2
27	1.22, s	24.8	1.09, s	24.9	1.22, s	24.8	1.22, s	24.9	1.19, s	24.8
28		178.5		178.8		178.5		178.6		177.4
29	0.96, s	27.8	0.84, s	27.8	0.96, s	27.8	0.96, s	27.9	0.99, s	28.0
30	1.12, s	17.3	0.99, s	17.4	1.12, s	17.3	1.12, s	17.4	0.95, s	16.2
	C-21-O- cinn		C-21-O- cinn		C-21-O- cinn		C-21-O- cinn			
1'		134.3		134.3		134.3		134.3		
2'	7.63, dd (8.1, 2.3)	127.9	7.52, dd (8.2, 2.2)	127.9	7.63, dd (8.1, 2.3)	127.9	7.63	127.9		
3'	7.43	128.6	7.32	128.6	7.43	128.6	7.43	128.6		
4'	7.44	131.0	7.33	130.2	7.44	129.1	7.44	131.1		
5'	7.43	128.6	7.32	128.6	7.43	128.6	7.43	128.6		
6'	7.63, dd (8.1, 2.3)	127.9	7.52, dd (8.2, 2.2)	127.9	7.63, dd (8.1, 2.3)	127.9	7.63	127.9		
7'	7.70, d (16.1)	144.9	7.58, d (16.0)	144.9	7.70, d (16.1)	144.9	7.69, d (16.1)	144.9		
8'	6.53, d (16.1)	117.6	6.42, d (16.0)	117.6	6.54, d (16.1)	117.6	6.53, d (16.1)	117.7		
9'	-	166.9	-	166.9	-	166.9	-	166.9		

^a Overlapping ¹H NMR signals are reported without designated multiplicity.

Table 2. NMR spectroscopic data of the sugar moieties for compounds **1-5** (600 MHz, CD₃OD).

	1		2		3		4		5	
	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}
	xyl at C-3		xyl at C-3		glc at C-3		glc at C-3		xyl at C-3	
1	4.43, d (7.2)	104.3	4.44, d (7.1)	104.3	4.50, d (7.8)	103.2	4.48, d (7.7)	103.9	4.28, d (7.6)	105.6
2	3.52, m	81.3	3.48, t (9.3)	81.6	3.66, t (8.3)	79.8	3.54, t (7.9)	81.0	3.26, t (8.4)	73.9
3	3.70, t (8.5)	74.9	3.70, t (8.5)	74.9	3.81, t (8.5)	75.5	3.75, t (8.7)	75.6	3.36, t (8.5)	76.1
4	3.71, m	77.1	3.72, m	76.4	3.67, t (9.6)	78.4	3.63, t (9.5)	78.3	3.54, m	69.6
5	3.31, m	62.7	3.29, m	62.8	3.76, m	75.1	3.75, m	75.0	3.27, m	65.6
	4.01, dd (12.0, 4.6)		4.02, dd (12.1, 4.5)						3.91, dd (11.5, 5.5)	
6					3.90, m	60.1	3.78, m	60.7		
					3.90, m		3.93, dd (11.2, 4.1)			
	glc at xyl-C-4		glc at xyl-C-4		glc' at glc-C-4		glc' at glc-C-4		rha at C-28	
1	4.38, d (7.8)	102.1	4.48, d (7.8)	101.8	4.38, d (7.8)	102.0	4.46, d (7.5)	102.9	5.95, d (1.5)	93.7
2	3.22, t (8.5)	73.2	3.23, t (8.5)	73.1	3.23, dd (9.0, 8.0)	73.5	3.25, t (7.9)	73.4	3.77, dd (3.5, 1.5)	70.0
3	3.31	76.5	3.37, t (8.5)	76.4	3.36, t (9.1)	76.5	3.39, t (9.0)	76.4	3.69, dd (9.0, 3.5)	71.0
4	3.29, t (9.1)	70.1	3.29	70.1	3.33	70.1	3.33	69.9	3.46, t (9.8)	71.9
5	3.34	76.7	3.31	76.7	3.34	77.8	3.34	76.7	3.73	66.4
6	3.67	61.2	3.67, dd (11.8, 2.6)	61.2	3.68, m	61.0	3.68	61.0	1.26, d (6.2)	16.8
	3.89, dd (12.1, 2.1)		3.89		3.90		3.91			
	xyl'-at xyl-C-2		xyl'-at xyl-C-2		xyl-at glc-C-2		xyl-at glc''-C-2			
1	4.68, d (7.4)	104.4	4.71, d (7.6)	103.9	4.82, d (7.6)	103.6	4.77, d (7.6)	103.6		
2	3.50, t (8.5)	75.1	3.59, t (9.2)	74.8	3.51, t (8.5)	75.0	3.56, t (9.2)	74.9		
3	3.76, t (8.9)	81.7	3.70, t (9.2)	82.9	3.75, t (8.6)	81.9	3.71, t (9.3)	82.7		
4	3.86, m	70.8	3.85, m	70.8	3.84, m	70.8	3.85, m	70.9		
5	3.26, dd (11.8, 6.9)	63.1	3.26	63.1	3.25, dd (9.8, 9.6)	63.1	3.25	63.1		
	4.05, dd (11.9, 6.0)		4.04, dd (11.5, 5.0)		4.05, dd (11.9, 6.0)		4.05, dd (11.6, 6.0)			
	glc'-at xyl'-C-3		glc'-at xyl'-C-3		glc''-at xyl-C-3		glc''-at xyl-C-3			
1	4.74, d (7.8)	103.6	4.87, d (7.7)	102.0	4.77, d (7.8)	103.6	4.87, d (7.8)	102.6		
2	3.29, t (8.7)	74.1	3.51, t (8.7)	83.2	3.30	74.1	3.52	83.2		
3	3.38, t (9.0)	76.4	3.59, t (9.0)	76.0	3.39, t (9.0)	76.4	3.59, t (9.0)	76.1		
4	3.42, t (9.4)	69.3	3.49, t (9.0)	69.1	3.42, t (8.9)	69.4	3.48, t (8.7)	69.2		
5	3.73, m	76.7	3.25, m	76.6	3.28, m	76.7	3.28, m	76.6		
6	3.76	60.6	3.72	60.4	3.88	60.6	3.75	60.5		
	3.88, dd (12.5, 3.0)		3.88, dd (12.4, 3.0)		3.86, dd (12.4, 3.0)		3.87, m			
	ara at xyl'-C-4		ara at xyl'-C-4		ara at xyl-C-4		ara at xyl-C-4			
1	4.57, d (4.5)	99.2	4.56, d (4.4)	99.0	4.57, d (4.8)	99.1	4.57, d (4.5)	99.0		
2	3.74	69.4	3.74	69.4	3.75	69.2	3.74	69.4		
3	3.66, dd (8.9, 3.0)	71.9	3.66, dd (9.3, 3.0)	71.9	3.67, dd (8.9, 3.0)	71.8	3.67, dd (8.8, 3.2)	71.9		
4	3.92, m	65.7	3.92, m	65.6	3.92, m	65.7	3.92, m	65.7		
5	3.53, m	62.2	3.53, m	63.5	3.53, m	62.2	3.53, m	62.2		
	4.07, m		4.05, m		4.07, m		4.06, m			
			glc''-at glc'-C-2				glc'''-at glc''-C-2			
1			4.66, d (7.7)	104.7			4.67, d (7.7)	104.6		
2			3.31, t (8.3)	75.1			3.33, t (8.3)	75.0		
3			3.39, t (9.3)	76.0			3.35, t (9.3)	76.1		
4			3.43, t (9.3)	69.4			3.45, t (9.3)	69.4		
5			3.39, m	77.2			3.37, m	77.2		
6			3.77, dd (11.5, 4.7)	60.7			3.77	60.5		
			3.94, dd (12.2, 2.1)				3.89			

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371 ^a Overlapping ¹H NMR signals are reported without designated multiplicity.

Table 3. Cytotoxic activity of compounds **1-7** against K562 cells^a

	IC ₅₀ (μM)
Compounds	K562
1	48.49 ± 0.16
2	65.67 ± 0.18
3	81.66 ± 0.17
4	56.43 ± 0.18
5	>100 (18.5 ± 2.91) ^b
6	>100 (19.80 ± 3.41) ^b
7	>100 (15.10±2.25) ^b
Doxorubicin*	0.59 ± 0.04

^aResults are means ± SD of 3 independent experiments performed in duplicate.

^bPercent growth inhibition at 100 μM.

*used as standard.

8. Conclusion

In summary, seven compounds were isolated from the roots of *P. bicolor*, among them five previously undescribed oleanane-type saponins, a cassane-type diterpene, and a known trimethoxy benzene glucoside. Their structures were elucidated by different spectroscopic methods including 1D- and 2D-NMR experiments as well as HR-ESI-MS analysis. Their cytotoxic activity against the chronic myeloid leukemia (K562) cell line was evaluated and only the monodesmosidic saponins possessed a moderate activity.

In addition, from the chemotaxonomic point of view, this study represents a valuable contribution to the chemotaxonomic of leguminous, Fabaceae family and Mimosaceae subfamily, which is know to be a rich source of triterpenoid saponins [24, 25, 26] and cassane diterpenoid [27, 28, 29]. The aglycones were identified as machaerinic acid (**1**, **2** and **4**) and 2α, hydroxy-machaerinic acid (**3** and **5**). The sugar moiety linked at C-3 was either β-D-xylose (**1**, **2**) or β-D-glucose (**3** and **4**), substituted at C-2 and C-4 by a β-D-xylose and a β-D-glucose, respectively. The β-D-xylose at C-2 was substituted at C-3 and C-4 by an α-L-arabinose and a β-D-glucose. This study reports for the first time the occurrence of saponins and cassane-type diterpene in the *Parkia* genus.

Appendix A. Supplementary data

Supplementary data to this article can be found online

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