1 **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.

38 **Abstract**

39 Five undescribed triterpene-type saponins, parkibicolorosides A-E, a cassane-type diterpene,
40 and a known trimethoxy benzene glucoside were isolated from the roots of *Parkia bicolor* A.
41 Chev. Their structures were elucidated by different spectroscopic methods including 1D- and
42 2D-NMR experiments as well as HR-ESI-MS analysis. Their cytotoxic activity against the
43 chronic myeloid leukemia (K562) cell line was evaluated. The monosaccharides saponins
44 exhibited a moderate antiproliferative activity with IC_{50} ranging from 48.49 ± 0.16 to 81.66 ±
45 0.17 μM.

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

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62 **1. Introduction**

63 The pantropical genus *Parkia* belongs to the subfamily Mimosoideae of the family Fabaceae.
64 It consists of about 35 species with centres of distribution in South America, Africa and South-
65 east Asia [1,2] . The phytochemical investigation on some species of the genus *Parkia* showed
66 the isolation of flavonoids [3,4], proanthocyanidin [5,6], and tannins [7]. *Parkia bicolor* A.
67 Chev. is a tree up to 40 m in height, with alternate and bipennate leaves of 30-45 cm long, and
68 fruit is a linear pod with 40 cm long, growing in Côte d'Ivoire, Guinea, Sierra Leone and to the
69 east of Congo RD [8,9]. Several parts of *P. bicolor* are used in traditional medicine [9]. The
70 stem barks are used in decoction against bad coughs in children, treatment of gynecological
71 troubles disorders [9] whereas the pulverized bark is employed in wound healing [10]. The roots
72 of *P. bicolor* are used against children measles, woman sterility and sexually transmitted
73 diseases [9]. The antioxidant and antibacterial activities of leaf and roots extracts were reported
74 [1,11,12]. Its seed oil composition [10] and gum polysaccharides were reported [2]. Lupeol,
75 lichexanthone, gallic acid and methyl gallate were isolated from the ethyl acetate extract of its
76 stem bark [12]. Phytochemical screening of *P. bicolor* revealed that the leaf and stem bark
77 extracts contained saponin and tannin [1], but none, to the best of our knowledge, have reported
78 on their composition. As part of our ongoing research on new bioactive compounds from
79 Ivoirian medicinal plant, we have studied the roots bark of *P. bicolor*. The present paper
80 describes the isolation and structure elucidation of five new triterpenoid saponins and cassan
81 diterpenoid. Their cytotoxicity against the chronic myeloid leukemia K562 cells was evaluated.
82 To our best knowledge, this study is the first report of saponins in *Parkia* genus.

83 **2. Experimental**

84 *2.1. General experimental procedures*

85 NMR spectra were acquired in CD_3OD on Bruker Avance DRX III 600 instruments (^1H at 600
86 MHz and ^{13}C at 150 MHz). Standard pulse sequences and parameters were used to obtain 1D
87 ^1H and ^{13}C and 2D COSY, ROESY, TOCSY, HSQC-TOCSY, and HMBC spectra. HR-ESI-
88 MS data were gained using a Micromass Q-TOF high-resolution mass spectrometer. Optical
89 rotations were determined in MeOH by Perkin-Elmer 241 polarimeter. TLC was performed on
90 precoated silicagel 60 F_{254} Merck and compounds were visualized by spraying the dried plates
91 with 50% H_2SO_4, followed by heating. CC was carried out on HP-20 resin (Sigma Aldrich).
92 Flash chromatography was conducted on a Grace Reveleris system equipped with dual UV and
93 ELSD detection using Grace® cartridges (Silica gel or RP-C_{18}). HPLC was performed on a
94 Dionex apparatus equipped with an ASI100, ultimate 3000 Pump, a diode array detector UVD
95 340S and a chromeleon software. A prepacked RP-C_{18} column (Phenomenex 250 x 15 mm,
96 Luna 5 μ) was used for semi-preparative HPLC. The eluting mobile phase consisted of H_2O
97 with TFA (0.0025%) and CH_3CN with a flow rate of 5 mL/min and the chromatogram was
98 monitored at 205, 210, 254, and 300 nm.

99 **3. Plant material**

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

103 **4. Extraction and isolation**

104 Dried and powdered stem roots of *Parkia bicolor* (1 kg) was extracted with 80% aqueous
105 MeOH (3 × 10 L) at room temperature. After filtration and evaporation procedures, MeOH 80%
106 (23.6 g) extract was obtained. This extract was dissolved in H_2O (200 mL) and then loaded onto
107 a Diaion HP-20 open column (40 cm × 4.3 cm; the volume of the column was 363 mL) and

108 sequentially eluted with mixtures of H_2O and MeOH (0, 25, 50, 75%, and finally 100% MeOH;
109 2 L of each solvent mixture) to provide fractions I-V, respectively. Fraction II (1 g) was purified
110 by flash chromatography over RP-C_{18} eluted by a gradient system of CH_3CN-H_2O (5-80%, in
111 40 min) to afford 85 sub-fractions f_{II-1}-f_{II-85}. Subfractions $f_{II-11-13}$ (200 mg) was subjected to flash
112 chromatography over silica gel eluted by a gradient system of $CHCl_3$-MeOH (9:1 - 7:3, in 15
113 min) to yield compound **7** (12 mg). Subfractions $f_{II-29-38}$ (300 mg) was purified by flash
114 chromatography over silica gel eluted by a gradient system of $CHCl_3$-MeOH-H_2O (7:3 - 7:3:0.5,
115 in 15 min) and subfractions [46-57] were purified by semi-prep HPLC (50-65% CH_3CN, in 15
116 min) affording compound **4** (*Rt* 7.8 min, 3 mg). Fraction III (3 g) was subject to vacuum liquid
117 chromatography over silica gel (7 × 5.5 cm) eluted successively with the solvent mixtures
118 $CHCl_3$-MeOH-H_2O (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
119 III-A to III-E, respectively. Fraction III-A (600 mg) was purified by flash chromatography over
120 silica gel eluted with a gradient of $CHCl_3$-MeOH (9:1 - 7:3) and subfractions [47-50] (36.6 mg)
121 were purified by semi-prep HPLC using a gradient (5-50% CH_3CN, in 20 min) affording
122 compound **5** (*Rt* 14.5 min, 2 mg). Fractions III-C and III-D was purified by flash
123 chromatography over RP-18 eluted by a gradient system of CH_3CN-H_2O (40-60%, in 40 min)
124 to give **1** (5 mg) and **2** (14 mg). Fraction IV (800 mg) was purified by flash chromatography
125 over RP-C_{18} eluted with a gradient of MeCN-H_2O (40-60%, in 20 min) and subfractions IV_{65-}
126 $_{79}$ (50 mg) were purified by semi-prep HPLC (40-65% CH_3CN, in 15 min) to give compound **3**
127 (*Rt* 9.1 min, 4 mg). Subfractions IV_{86-95} (40 mg) were purified by semi-prep HPLC using an
128 isocratic elution (60% CH_3CN) to give compound **2** (*Rt* 5.5 min, 10 mg). Subfractions $IV_{152-154}$
129 (26 mg) were purified by semi-prep HPLC using an isocratic elution with 70% CH_3CN to yield
130 compound **6** (*Rt* 11.7 min, 9 mg).

131 *4.1. Parkibicoloroside A (**1**)*

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

135 *4.2. Parkibicoloroside B (**2**)*

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

139 *4.3. Parkibicoloroside C (**3**)*

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

143 *4.4. Parkibicoloroside D (**4**)*

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

147 *4.5. Parkibicoloroside E (**5**)*

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

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

152 Colorless oil; $[\alpha]^{20}_{D}$ -90 (*c* 0.58, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 153 150 MHz) see Table 1; HR-ESI-MS *m/z* 371.2192 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_4Na$, 371.2198).

154 **5. Acid hydrolysis**

155 Acid hydrolysis was carried out to obtain the sugar residues of compounds **1**-**7**. An aliquot of 156 the saponin-containing fraction (100 mg of fraction D) was treated with 2N TFA (trifluoroacetic 157 acid, aqueous solution, 15 mL) at 90 °C for 6 h. After extraction with CH_2Cl_2 (10 mL x 3), the 158 water-soluble layer was evaporated to dryness. The sample (55 mg) was purified by preparative 159 Si-gel TLC (MeCOEt:iso-PrOH:Me_2CO:H_2O, 20:10:7:6) to afford rhamnose [2.5 mg, R_f = 160 0.73, $[\alpha]^{20}_{D}$ +11 (*c* 0.21, H_2O)]; arabinose [2 mg, R_f = 0.59, $[\alpha]^{20}_{D}$ +43 (*c* 0.17, H_2O)]; xylose 161 [1.9 mg, R_f = 0.52, $[\alpha]^{20}_{D}$ +18 (*c* 0.2, H_2O)]; and glucose [4 mg, R_f = 0.48, $[\alpha]^{20}_{D}$ +30 (*c* 0.33, 162 H_2O)].

163 **6. Cytotoxicity bioassay by MTS**

164 K562 cells (chronic myeloid leukemia) were trypsinized, harvested, and spread onto 96-well 165 flat-bottom plates at a density of 1000 cells per well, and then incubated for 24 h in RPMI 1640 166 Medium supplemented with 10% fetal bovine serum and antibiotics. After culture, the cells 167 were treated with compounds **1**-**7** for 72 h. The cell cultures were then analyzed using 3-(4,5- 168 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner 169 salt (MTS) according to the manufacturer's instructions (Promega Corporation, Charbonnières, 170 France). Doxorubicin was used as positive control. MTS is bioreduced by cells into a colored 171 formazan product. Absorbance was analyzed at a wavelength of 540 nm with a Multiskan Ex 172 microplate absorbance reader (Thermo Scientific, Paris, France). Percentage of cell growth was 173 calculated as 100% × (absorbance of the treated cells) / (absorbance of the negative control

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

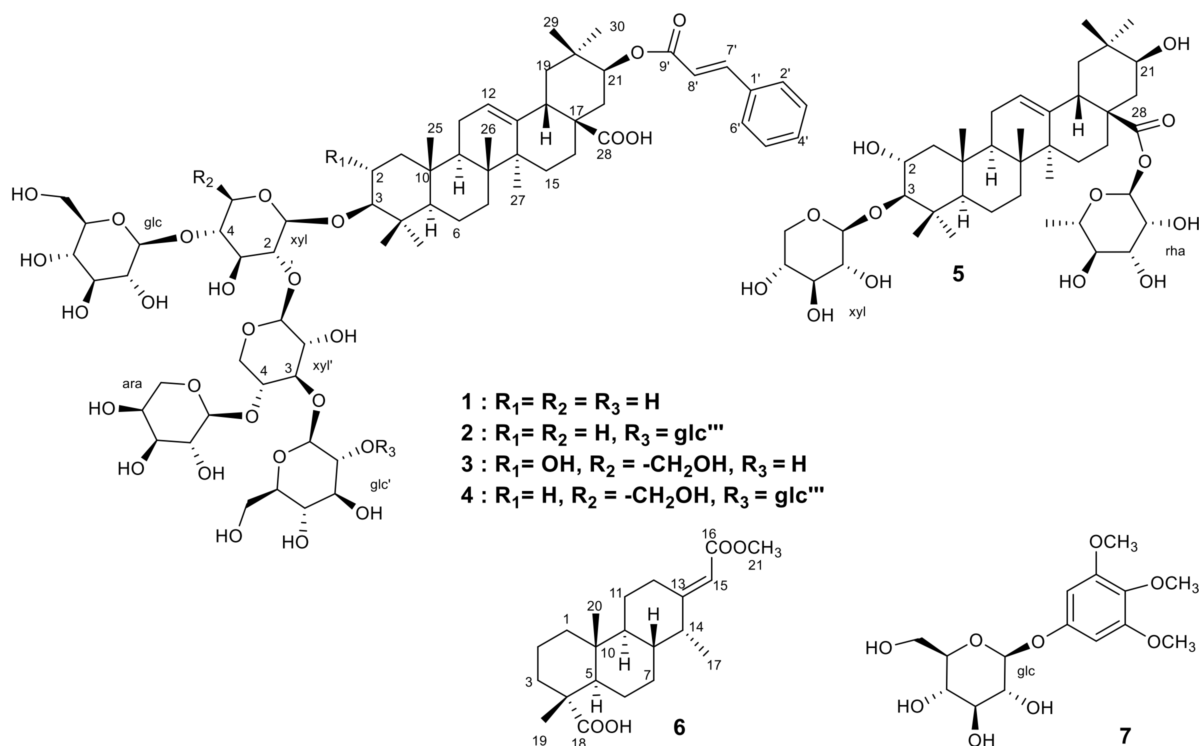
176 **7. Results and discussion**

177 The 80% EtOH extract from the dried roots of *P. bicolor* was subjected to Diaion HP-20 resin
178 column chromatography to give the saponin-containing fraction, which was subjected to further
179 column chromatography to yield five previously undescribed oleanane-type saponins, named
180 parkibicolorosides A-E (**1-5**), a cassane-type diterpene (**6**) and one known phenolic compounds
181 (**7**) (Figure 1). Upon acid hydrolysis with 2N TFA, an aliquot of the saponin-containing fraction
182 allowed the identification of four monosaccharides as D-glucose, D-xylose, L-arabinose and L-
183 rhamnose by comparison with authentic samples (see Experimental Section).

184 Compound **1** was obtained as a yellowish, amorphous powder. Its molecular formula,
185 $C_{66}H_{98}O_{27}$, was determined by the positive-ion HR-ESI-MS at *m/z* 1345.6204 $[M+Na]^+$ (calcd
186 for $C_{66}H_{98}O_{27}Na$, 1345.6193). Extensive analysis of 1D and 2D NMR spectra (^1H-^1H-COSY,
187 ROESY, HSQC, and HMBC) indicated the presence of an oleanane skeleton. It is characterized
188 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,
189 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
190 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
191 methine proton at δ_H 2.99 (dd, *J*=13.8, 4.3 Hz, H-18), which were typical signals of the oleanolic
192 acid skeleton [13]. However, the methylene protons at δ_H 1.78 (dd, *J*=12.9, 11.9 Hz, H-22ax)
193 and 1.82 (dd, *J*=12.9, 4.9 Hz, H-22eq) showed spin-couplings in the ^1H-^1H COSY spectrum
194 with the hydroxymethine proton at δ_H 4.95 (dd, *J*=11.9, 4.9 Hz). This hydroxymethine proton
195 showed HMBC correlations with δ_C 47.1 (C-17), 46.3 (C-19), 36.4 (C-22) and two methyl
196 signals at δ_C 17.3 (C-30), 27.8 (C-29), indicating the presence of a hydroxyl group at C-21
197 (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 H_3-30 *β*-
200 oriented. The unambiguous assignment of all ^{1}H and ^{13}C NMR signals of the aglycone of **1**
201 (Table 1), identified as machaerinic acid (3*β*,21*β*-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 ^{1}H 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'; 7.43-744, 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-l'), 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 ^{1}H 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 ^{1}H 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

223 glucopyranose and xylopyranose was determined as β in $^4C_1$ conformation. The anomeric
224 proton coupling constant of ara (J=4.5 Hz) indicated that it was present in the $^1C_4$ conformation
225 [18,19] (Table 2) and its chemical shifts of anomeric carbons indicate an α-configuration. The
226 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
227 70.8) of xyl', indicated that the two xylopyranoses units were disubstituted. The sequencing of
228 the glycoside chains was achieved by analysis of HMBC and ROESY experiments. In the
229 HMBC spectrum, the anomeric proton signals at δ_H 4.57 (ara-H-1), 4.74 (glc'-H-1), 4.68 (xyl'-
230 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
231 (xyl'-C-4), 81.7 (xyl'-C-3), 81.3 (xyl-C-2), 77.1 (xyl-C-4), and 89.3 (aglycone-C-3),
232 respectively. These signals provided ample evidence to determine the linkages between the
233 sugars, and the sugar and the aglycone. These linkages were also confirmed by ROESY
234 correlations between aglycone-H-3/xyl-H-1, xyl-H-4/glc-H-1, xyl-H-2/xyl'-H-1, xyl'-H-3/glc'-
235 H-1, and xyl'-H-4/ara-H-1. Based on all the foregoing evidence, compound **1** was elucidated as
236 3-*O*-{*α*-L-arabinopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-xylopyranosyl-(1→2)}-
237 [*β*-D-glucopyranosyl-(1→4)]-*β*-D-xylopyranosyl-21-*O*-cinnamoyl-machaerinic acid.
238 Compound **1** was named parkibicoloroside A after its plant origin (Fig. 1).



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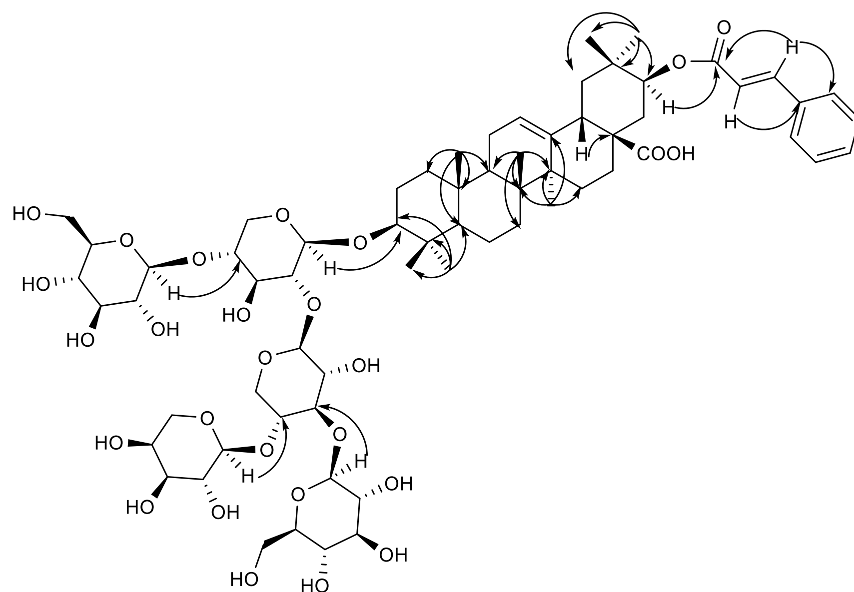
240 Figure 1. Structures of compound **1-7** isolated from *Parkia bicolor* roots.



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

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244 Compound **2**, obtained as a yellowish, amorphous powder, displayed a molecular ion

245 peak $[M+Na]^+$ at *m/z* 1507.6713 in the positive HR-ESI-MS, in accordance with an empirical

246 molecular formula of $C_{72}H_{108}O_{32}Na$ (calcd for $C_{72}H_{108}O_{32}Na$, 1507.6721), suggesting a
247 supplementary hexose unit compared to **1**. The findings from the HR-ESI-MS analysis were
248 confirmed by the NMR data, which displayed six additional carbons to the otherwise analogous
249 resonances for the sapogenin 21-cinnamoyl-machaerinic acid with the xyl, ara and glc unit
250 analogous to **1**. By extensive analysis of NMR spectra of **2** and in a comparison of the ^{13}C NMR
251 signals for aglycone and cinnamoyl moieties of **2** with those of **1** (Tables 1 and 2), all signals
252 due to the aglycone and cinnamoyl moiety at C-21 of **2** were in agreement with those in **1** (Table
253 1). The NMR data of the sugar part of **2** were very similar to those obtained from **1**, except for
254 a significant downfield shift of C-2 (δ_C 83.2) of the glc' and the appearance of a set of additional
255 signals, corresponding to a terminal *β*-D-glucopyranosyl group (glc'') in **2** which was attached
256 at C-2 of glc' (Table 2). The linkage points of the sugar units to each other and to the aglycone
257 were determined by following HMBC correlations: δ_H 4.44 (xyl-H-1) with δ_C 89.2 (aglycone-
258 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
259 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
260 (glc-''H-1) with δ_C 83.2 (glc'-C-2). Hence, compound **2** was established as 3-*O*-{*β*-D-
261 glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-[*α*-L-arabinopyranosyl-(1→4)]-*β*-D-
262 xylopyranosyl-(1→2)}-[*β*-D-glucopyranosyl-(1→4)]-*β*-D-xylopyranosyl-21-*O*-cinnamoyl-
263 machaerinic acid. Compound **2** was named parkibicoloroside B (Fig. 1).

264 Compound **3** was isolated as yellowish, amorphous powder with the elemental formula
265 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).
266 When compared to the spectroscopic data of **1** and **2**, the sapogenin of **3** differed only by the
267 presence of an additional hydroxyl group at position 2 (Table 1). This was corroborated by the
268 downfield shift of C-2 (δ_C 66.5), its deshielded additional proton signal (δ_H 3.75), and the
269 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 $J_{H\text{-}2,H\text{-}3}$

271 value (J=11.5 Hz), characteristic of an axial proton, and confirmed by ROESY correlations

272 between H-2 and H_3-25 β-oriented. The aglycone of **3** was identified as 2α,3β,21β-trihydroxy-

273 olean-12-en-28-oic acid. Full assignment of all ^{1}H and ^{13}C 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 2α,hydroxy-machaerinic acid [15,19]. The sugar part of **3** consists of five residues as evidenced

277 by ^{1}H 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 ($\delta_{H\text{-}1}$ 4.82, xyl), a

283 terminal α-L-arabinopyranose ($\delta_{H\text{-}1}$ 4.57, ara), and two terminal β-D-glucopyranose units ($\delta_{H\text{-}1}$

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 ($\delta_{C\text{-}2}$ 79.8 and

286 $\delta_{C\text{-}4}$ 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→4)-

293 [β-D-glucopyranosyl-(1→3)]-β-D-xylopyranosyl-(1→2)}-[β-D-glucopyranosyl-(1→4)]-β-D-

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

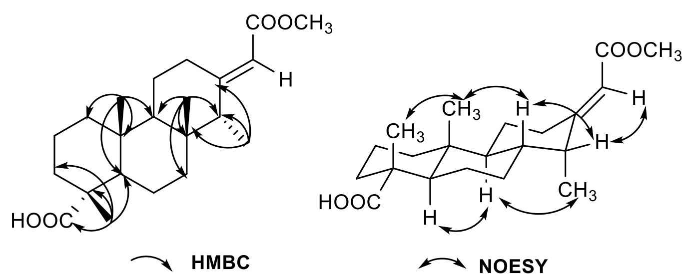
296 Compound **4** was isolated as an amorphous yellowish powder. The molecular formula
297 was established as $C_{73}H_{110}O_{33}$ by HR-ESI-MS (*m/z* 1537.6838 $[M+Na]^+$; calcd for
298 $C_{73}H_{110}O_{33}Na$, 1537.6827). The resonances derived from the NMR spectra revealed the same
299 aglycone as in compounds **1** and **2**. Extensive 2D NMR analysis (Table 2) showed that the
300 glycosidic part of compounds **3** and **4** differed by the presence of one additional sugar in **4**
301 which was identified as a terminal β-D-glucopyranose (glc'''). The HMBC correlations at δ_H
302 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),
303 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),
304 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
305 (aglycone-C-3) suggested the linkage of glc''' at the C-2 position of the glc''. This was confirmed
306 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**
307 was elucidated as 3-*O*-{β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-[α-L-
308 arabinopyranosyl-(1→4)]-β-D-xylopyranosyl-(1→2)}-[β-D-glucopyranosyl-(1→4)]-β-D-
309 glucopyranosyl-21-*O*-cinnamoyl-machaerinic acid. Compound **4** was named parkibicoloroside
310 D (Fig. 1).

311 Compound **5** possessed the molecular formula of $C_{41}H_{66}O_{13}$ on the basis of its HR-ESI-
312 MS at *m/z* 789.4411 $[M+Na]^+$ (calcd $C_{41}H_{66}O_{13}Na$, 789.4401). Its NMR data of the aglycone
313 moiety were similar to those of **3**, except for the absence of the cinnamoyl group at C-21 in **5**.
314 This was confirmed by HMBC correlation between H-21/C-29, C-30 and the obvious upfield
315 shift of H-21 (δ_H 3.69) (Table 1). The relative configuration of the aglycone moiety of **5** was
316 established as being identical to that of **3** by analyzing their NMR data and ROESY interactions.
317 The sugar part of **5** consists of two residues as evidenced by ^{1}H NMR spectrum which displayed

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

329 Compound **6**, a colorless oil, possess a molecular formula $C_{21}H_{32}O_4$ determined based
330 on the positive ion peak at *m/z* 371.2192 $[M+Na]^+$ (calcd for $C_{21}H_{32}O_4Na$, 371.2198) in the HR-
331 ESI-MS spectrum. Analysis of the ^1H NMR spectroscopic data of this compound (Table 1)
332 indicated that the structure of **6** possessed three methyl groups at δ_H 0.77 (s), 0.94 (d, *J*=6.7 Hz),
333 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
334 ^{13}C NMR spectrum (Table 1) exhibited 21 signals, of which three methyl carbons at δ_C 13.2 (C-
335 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
336 181.0 (C-18), a methoxyl carbon at δ_C 49.9 (C-21), a trisubstituted double bond at δ_C 169.1 (C-
337 13), 110.8 (C-15). These data suggested **6** to be an esterified cassane-type diterpene [20, 21].
338 Extensive analysis of 1D and 2D NMR spectra (^1H NMR, ^{13}C NMR, COSY, ROESY, HSQC,
339 and HMBC) indicated that the NMR data of **6** were similar to those of 7-deoxycassane-
340 16(18)dioic acid [21]. The HMBC spectrum showed long-range correlations from the Me-17
341 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-
342 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

343 181.0) whereas Me-20 exhibited HMBC correlations with C-5, C-10 (δ_C 36.1), C-9 (δ_C 47.7),
344 and C-1 (δ_C 38.5). The ^{1}H-^{1}H-COSY correlations between H-12/H-11, H-11/H-9, H-9/H-8, H-
345 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
346 assignment of these proton signals. The olefinic proton at δ_H 5.54 (H-15) also showed long-
347 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
348 (C-16). In the HMBC spectrum, the methoxy signal at δ_H 3.55 gave a cross-peak with the
349 carbonyl carbon C-16 which suggested it is located at C-16. The NOESY cross-peaks observed
350 between Me-19/Me-20, Me-20/H-8, and H-8/H-14 confirmed their *β*-axial orientations whereas
351 the NOESY correlations between H-5/H-9 and Me-17/H-9, indicated their *α*-axial orientations
352 (Figure 3). The olefinic proton H-15 displayed a NOESY cross-peak with the methine proton
353 H-14, indicating a *E*-configuration of the double bond. Compared to the literature data [21,22],
354 the chemicals shifts of C-12 (δ_C 23.8) and C-14 (δ_C 45.0) were in good agreement with a
355 *E*-configuration, Therefore, **6** was elucidated as 16-*O*-methyl-cass-13(15)ene-16,18-dioic acid.



356

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

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

359 The cytotoxic activity of compounds **1-7** was evaluated against K562 chronic myeloid leukemia
360 cells. Compounds **5-7** were not active at the concentration tested (100 µM). The
361 monosaccharides saponins (**1-4**) exhibited a moderate antiproliferative activity with IC_{50}
362 ranging from 48.49 ± 0.16 to 81.66 ± 0.17 µM (Table 3), compared to the disaccharides saponin
363 (**5**). The pentasaccharide (**1**, **3**) were slightly more actives that the hexasaccharides (**2**, **4**)

364 saponins, and Parkibicoloroside C (**3**) with 2-hydroxy-machaerinic acid is less active than

365 parkibicoloroside A (**1**), parkibicoloroside B (**2**), and parkibicoloroside D (**4**).

366 Table 1. NMR spectroscopic data of the aglycone moieties for compounds **1**-**5** (600 MHz, CD_3OD).

	1		**2**		**3**		**4**		**5**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J 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		

367

368 [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, CD_3OD).

	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			

[a] Overlapping ^{1}H NMR signals are reported without designated multiplicity.

372

373 **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

374 [a]Results are means ± SD of 3 independent experiments performed in duplicate.

375 [b]Percent growth inhibition at 100 µM.

376 *used as standard.

377

378 **8. Conclusion**

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

385 In addition, from the chemotaxonomic point of view, this study represents a valuable
386 contribution to the chemotaxonomic of leguminous, Fabaceae family and Mimosaceae
387 subfamily, which is know to be a rich source of triterpenoid saponins [24, 25, 26] and cassane
388 diterpenoid [27, 28, 29]. The aglycones were identified as machaerinic acid (**1**, **2** and **4**) and 2*α*,
389 hydroxy-machaerinic acid (**3** and **5**). The sugar moiety linked at C-3 was either *β*-D-xylose (**1**,
390 **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,
391 respectively. The *β*-D-xylose at C-2 was substituted at C-3 and C-4 by an *α*-L-arabinose and a
392 *β*-D-glucose. This study reports for the first time the occurrence of saponins and cassane-type
393 diterpene in the *Parkia* genus.

394

395 **Appendix A. Supplementary data**

396 Supplementary data to this article can be found online

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402 **Reference**

403 [1] Adaramola, T.F., Ariwaodo, J.O., Adeniji, K. A., 2012. Distribution, phytochemistry and
404 antioxydant properties of the genus *Parkia* R. br (Mimosaceae) in Nigeria. Int. J.
405 Pharmacognosy and Phytochem. Res. 172-173.
406 [2] Anderson, D.M.W., De Pinto, G.L., 1985. Gum polysaccharides from three *Parkia* species.
407 Phytochemistry 1, 77-79.
408 [3] Adewoye, R.O., Ajayi, O.O., 1988. Flavonols, flavones and tannins of *Parkia*
409 *clappertoniana*. J. Am. Leather Chem. Assoc. 83, 153-156.
410 [4] Lemmich, E., Adewunmi, C.O., Furu, P., kristensen, A., Larsen, L., Olsen, C.E., 1996. 5-
411 deoxyflavones from *Parkia clappertoniana*. Phytochemistry 42, 1011-1113.
412 [5] Adewoye, R.O., Ajayi, O.O., 1989. Anthocyanidins of *Parkia clappertoniana*. J. Soc.
413 Leather Tech. Chem. 73, 110-121.
414 [6] Tala, V.R.S., Da Silva, V.C., Rodrigues, C.M., Nkengfack, A.E., Dos Santos, L.C., Vilegas,
415 W., 2013. Characterization of Proanthocyanidins from *Parkia biglobosa* (Jacq.) G. Don.
416 (Fabaceae) by flow injection analysis electrospray ionization ion tandem mass
417 spectrometry. Molecules 18, 2803-2820.

418 [7] Tringali, C., Spatafora, C., Longo, O.D., 2000. Bioactive constituents of the bark *Parkia*
419 *biglobosa*. Fitoterapia 71, 118-125.
420 [8] Hopkins, H.C., 1983. The taxonomy, reproductive biology and economic potential of
421 *Parkia* (Leguminosae: Mimosoideae) in Africa and Madagascar. Bot. J. Linn. Soc. 87, 135-
422 167.
423 [9] Tchinda, A.T., 2008. *Parkia bicolor*, in: Louppe, D., Oteng-Amoako, A.A., Brink, M.
424 (Eds.), Plant resources of tropical Africa 7, timbers 1, Fondation PROTA. Wageningen, pp.
425 471-473.
426 [10] Aiyelaagbe, O.O., Ajaiyeoba, E.O., Ekundayo, O., 1996. Studies on the seed oils of *Parkia*
427 *biglobosa* and *Parkia bicolor*. Plant Foods Hum. Nutr. 49, 229-233.
428 [11] Ajaiyeoba, E.O., 2002. Phytochemical and antibacterial properties of *Parkia biglobosa*
429 and *Parkia bicolor* leaf extracts. Afr. J. Biomed. Res. Vol. 5; 125-129.
430 [12] Fotie, J., Nkengfack, A.E., Peter, M.G., Heydenreich2 and, M., Fomum, Z.T., 2004.
431 Chemical constituents of the ethyl acetate extracts of the stem bark and fruits of
432 *Dichrostachys cinerea* and the roots of *Parkia bicolor*. Bull. Chem. Soc. Ethiop. 18, 111-
433 115.
434 [13] Lehbili, M., Alabdul Magid, A., Kabouche, A., Voutquenne-Nazabadioko, L., Morjani,
435 H., Harakat, D., Kabouche, Z., 2018. Triterpenoid saponins from *Scabiosa stellata*
436 collected in North-eastern Algeria. Phytochemistry 150, 40-49.
437 [14] Delgado, M.C.C., Da Silva, M.S., Fo, R.B., 1984. 3*β*-hydroxy-21*β*-*E*-cinnamoyloxyolean-
438 12-en-28-oic acid, a triterpenoid from *Enterolobium contorstisiliquum.* Phytochemistry
439 23, 2289-2292.
440 [15] Mair, C.E., Grienke, U., Wilhelm, A., Urban, E., Zehl, M., Schmidtke, M., Rollinger, J.M.,
441 2018. Anti-Influenza Triterpene Saponins from the Bark of *Burkea africana*. J. Nat. Prod.
442 81, 515-523.

443 [16] Nihei, K., Ying, B.P., Murakami, T., Matsuda, H., Hashimoto, M., Kubo, I.,.
444 Pachyelasides A-D, novel molluscicidal triterpene saponins from *Pachyelasma*
445 *tessmannii*. J Agric Food Chem. 2005, 9, 53, 608-613.
446 [17] Alabdul Magid A., Lalun N., Long C., Borie N., Bobichon H., Moretti C., Lavaud C.,
447 2012. Triterpene saponins from *Antonia ovata* leaves. Phytochemistry 77, 268-274.
448 [18] Ono, M., Ochiai, T., Yasuda, S., Nishida, Y., Tanaka, T., Okawa, M., Kinjo, J.,
449 Yoshimitsu, H., Nohara, T., 2013. Five new nortriterpenoid glycosides from the bulbs of
450 *Scilla scilloides.* Chem. Pharm. Bull. 61, 592-598.
451 [19] Yokosuka, A., Kawakami, S., Haraguchi, M., Mimaki, Y., 2008. Stryphnosides A-F, six
452 new triterpene glycosides from the pericarps of *Stryphnodendron fissuratum*. Tetrahedron
453 64, 1474-1481.
454 [20] Chapman, G.T., Gilbert, J.N.T., Jaques, B., Mathieson, D.W., 1965. The structure of
455 dehydrocassamic acid. J. Chem. Soc. 60, 403-4066.
456 [21] Abad, A., Agulló, C., Arnó, M., Domingo, I. R., Zaragozá. R. J., 1990. ^{13}C Nuclear
457 Magnetic Resonance spectra of several Podocarpan and cassane triterpenoids. Magnetic
458 resonance in chemistry, 28, 529-532.
459 [22] Huang, X., Chen Z., Zhou, S., Huang, P., Zhuo Z., Zeng S., Wang, L., Wang, Y., Xu, C.,
460 Tian, H., 2018. Cassaine diterpenoids from the seeds of ErythrophleumBfordii and their
461 cytotoxic activities. Fitoterapia 127, 245–251.
462 [23] Hiroko, S., Yutaka, S., Motomu, O., Hiroko, T., 1988. Phenolic glucosides from
463 *Parabenzoin praecox*. Phytochemistry, 27, 644–646.
464 [24] Abdou, T., Turibio, K. T., Shota, U., Misa O., Ken-ichi K., Eunsang, K., Hiroyuki, M.,
465 Ibrahim, H., Ozgen, A., Çalis¸ k., Yoshihito, S., Bonaventure, T.N., 2017. Triterpene
466 saponins from the roots of *Acacia albida* Del. (Mimosaceae). Phytochemistry, 136, 31-38.

467 [25] Olivier, P. N., Dong, J., Cyril, A., Dominique, G., Dieudonne, E. P., Marie, C. K.,
468 Annelise, L., 2015. Triterpenoid saponins from *Albizia boromoensis* Aubre´v. & Pellegr.
469 Phytochem Lett., 11, 37-42.
470 [26] Abdou, T., Turibio, K., Tabopda, Narandulam, U., Ken-ichi, K., Eunsang, K., Hiroyuki,
471 M., Takuya, K., Yoshihito, S., Bonaventure, T.N., 2017. New triterpene saponins from the
472 roots of *Acacia macrostachya* (Mimosaceae). Bios Biotechnol Biochem., 81, 2261-2267.
473 [27] Landry, A.K., Joel, M.E.D., Martial, S., Timothe, A.O., Kicho, D.Y., Sebastien, K.O.,
474 Armand, P.K., Pascal, R., Pierre, C., 2014. Four new cassane diterpenoid amides from
475 *Erythrophleum suaveolens* [(Guill. et Perr.), Brenan]. Phytochem Lett., 10, 60-64.
476 [28] Ranjani, M., Makthala, R., Snehlata, S., Prem, P.Y., 2012. A review on cassane and
477 norcassane diterpenes and their pharmacological studies. Fitoterapia, 83, 272–280.
478 [29] Prem, P.Y., Ranjani, M., Jayanta, S., Ashish, A., Sanjeev, K.,
479 Sudhir, S., Srivastava, M.N., Ram, R., 2009. Cassane Diterpenes from *Caesalpinia bonduc*
480 Phytochemistry, 70, 256-261.