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## **Triterpene saponins from the roots of *Parkia bicolor* A. Chev**

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

2

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20

21 **Highlights**

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

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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<sub>50</sub> 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<sub>3</sub>OD on Bruker Avance DRX III 600 instruments (<sup>1</sup>H at 600  
86 MHz and <sup>13</sup>C at 150 MHz). Standard pulse sequences and parameters were used to obtain 1D  
87 <sup>1</sup>H and <sup>13</sup>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<sub>254</sub> Merck and compounds were visualized by spraying the dried plates  
91 with 50% H<sub>2</sub>SO<sub>4</sub>, 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<sub>18</sub>). 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<sub>18</sub> column (Phenomenex 250 x 15 mm,  
96 Luna 5 μ) was used for semi-preparative HPLC. The eluting mobile phase consisted of H<sub>2</sub>O  
97 with TFA (0.0025%) and CH<sub>3</sub>CN 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<sub>2</sub>O (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<sub>2</sub>O 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<sub>18</sub> eluted by a gradient system of CH<sub>3</sub>CN-H<sub>2</sub>O (5-80%, in  
111 40 min) to afford 85 sub-fractions f<sub>II-1</sub>-f<sub>II-85</sub>. Subfractions f<sub>II-11-13</sub> (200 mg) was subjected to flash  
112 chromatography over silica gel eluted by a gradient system of CHCl<sub>3</sub>-MeOH (9:1 - 7:3, in 15  
113 min) to yield compound **7** (12 mg). Subfractions f<sub>II-29-38</sub> (300 mg) was purified by flash  
114 chromatography over silica gel eluted by a gradient system of CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (7:3 - 7:3:0.5,  
115 in 15 min) and subfractions [46-57] were purified by semi-prep HPLC (50-65% CH<sub>3</sub>CN, 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<sub>3</sub>-MeOH-H<sub>2</sub>O (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<sub>3</sub>-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<sub>3</sub>CN, 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<sub>3</sub>CN-H<sub>2</sub>O (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<sub>18</sub> eluted with a gradient of MeCN-H<sub>2</sub>O (40-60%, in 20 min) and subfractions IV<sub>65-</sub>  
126 <sub>79</sub> (50 mg) were purified by semi-prep HPLC (40-65% CH<sub>3</sub>CN, in 15 min) to give compound **3**  
127 (*Rt* 9.1 min, 4 mg). Subfractions IV<sub>86-95</sub> (40 mg) were purified by semi-prep HPLC using an  
128 isocratic elution (60% CH<sub>3</sub>CN) to give compound **2** (*Rt* 5.5 min, 10 mg). Subfractions IV<sub>152-154</sub>  
129 (26 mg) were purified by semi-prep HPLC using an isocratic elution with 70% CH<sub>3</sub>CN to yield  
130 compound **6** (*Rt* 11.7 min, 9 mg).

131 *4.1. Parkibicoloroside A (I)*

132 Yellowish, amorphous powder;  $[\alpha]_D^{20} + 7$  (*c* 0.73, MeOH);  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the aglycone  
133 part, see Table 1;  $^1\text{H}$  and  $^{13}\text{C}$  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);  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the aglycone  
137 part, see Table 1;  $^1\text{H}$  and  $^{13}\text{C}$  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);  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the aglycone  
141 part, see Table 1;  $^1\text{H}$  and  $^{13}\text{C}$  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);  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the aglycone  
145 part, see Table 1;  $^1\text{H}$  and  $^{13}\text{C}$  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);  $^1\text{H}$  and  $^{13}\text{C}$  NMR of the aglycone  
149 part, see Table 1;  $^1\text{H}$  and  $^{13}\text{C}$  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).*

152 Colorless oil;  $[\alpha]_D^{20}$  -90 (*c* 0.58, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 600 MHz) and  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ,  
153 150 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).

## 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  $\text{CH}_2\text{Cl}_2$  (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 ( $\text{MeCOEt}:\text{iso-PrOH}:\text{Me}_2\text{CO}:\text{H}_2\text{O}$ , 20:10:7:6) to afford rhamnose [2.5 mg,  $R_f$  =  
160 0.73,  $[\alpha]_D^{20}$  +11 (*c* 0.21,  $\text{H}_2\text{O}$ )]; arabinose [2 mg,  $R_f$  = 0.59,  $[\alpha]_D^{20}$  +43 (*c* 0.17,  $\text{H}_2\text{O}$ )]; xylose  
161 [1.9 mg,  $R_f$  = 0.52,  $[\alpha]_D^{20}$  +18 (*c* 0.2,  $\text{H}_2\text{O}$ )]; and glucose [4 mg,  $R_f$  = 0.48,  $[\alpha]_D^{20}$  +30 (*c* 0.33,  
162  $\text{H}_2\text{O}$ )].

## 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 bio-reduced 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  $100\% \times (\text{absorbance of the treated cells}) / (\text{absorbance of the negative control})$

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

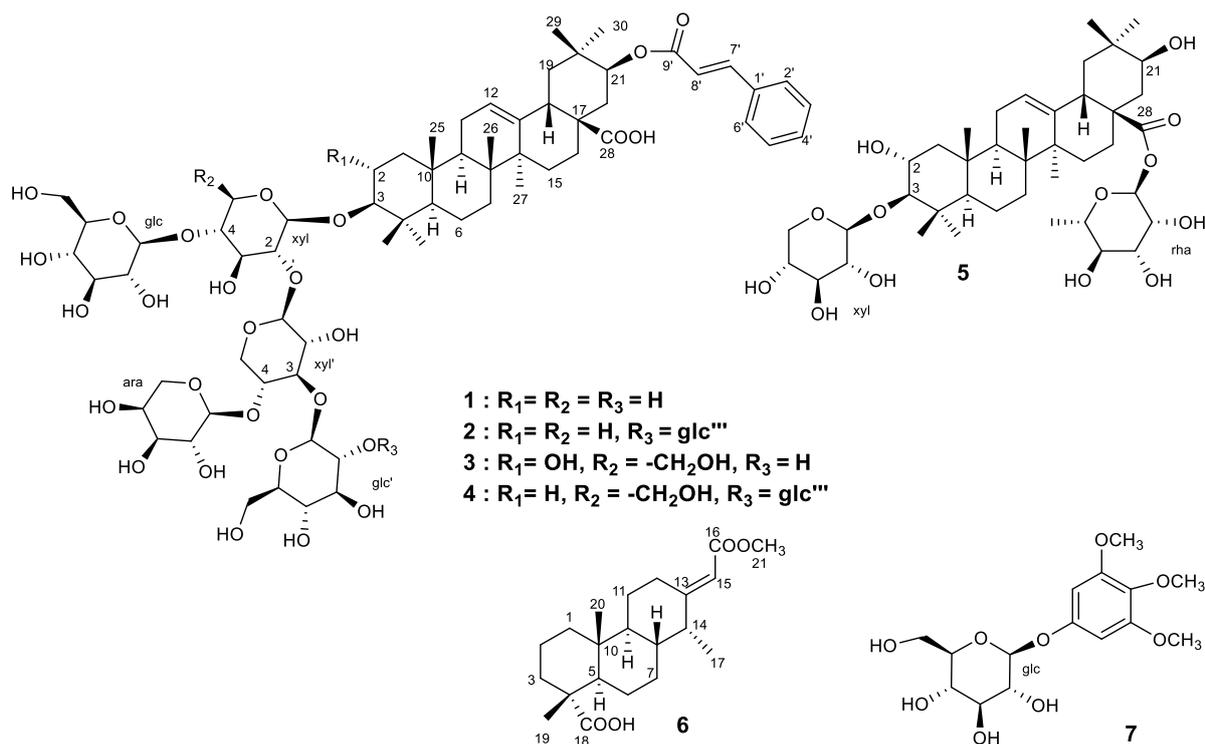
## 176 7. Results and discussion

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  $\delta_H$  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  $\delta_H$   
190 5.33 (t,  $J=3.7$  Hz, H-12), one oxymethine proton at  $\delta_H$  3.14 (dd,  $J=11.5, 4.2$  Hz, H-3), and a  
191 methine proton at  $\delta_H$  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  $\delta_H$  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  $\delta_H$  4.95 (dd,  $J=11.9, 4.9$  Hz). This hydroxymethine proton  
195 showed HMBC correlations with  $\delta_C$  47.1 (C-17), 46.3 (C-19), 36.4 (C-22) and two methyl  
196 signals at  $\delta_C$  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  $\delta_C$  75.5. The  $\beta$ -configuration

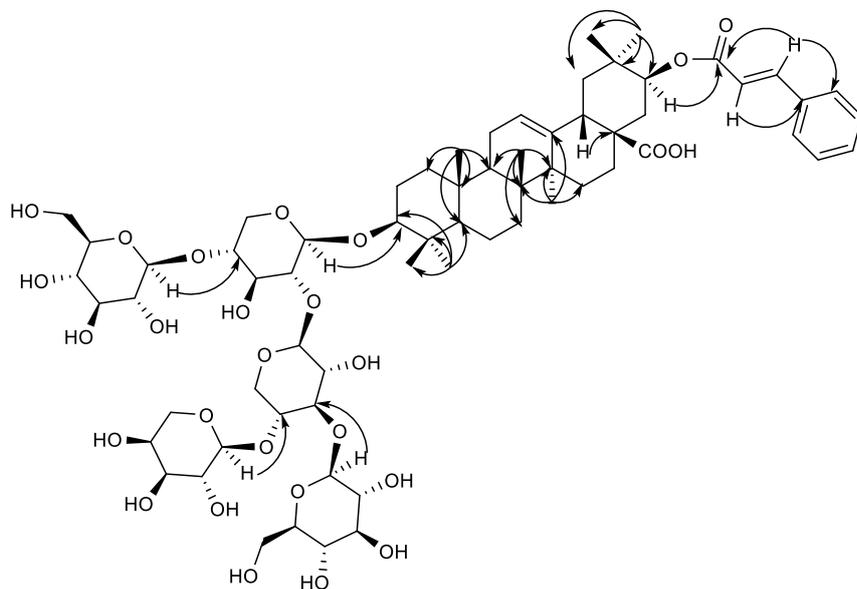
198 of the C-21 hydroxyl group was evident by the large  $J$  value of H-21/H-22ax ( $J=11.9$  Hz)  
199 characteristic of an axial proton, and from the ROESY correlations between H-21 and H<sub>3</sub>-30  $\beta$ -  
200 oriented. The unambiguous assignment of all <sup>1</sup>H and <sup>13</sup>C NMR 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 ( $\delta_C$  75.5,  $\delta_H$  4.95) and its  
204 neighboring atoms [ $\delta_C$  34.9 (C-20);  $\delta_C$  36.4,  $\delta_H$  1.78/1.82 (C-22)] pointed toward an attachment  
205 of an ester residue at position C-21. The <sup>1</sup>H NMR spectrum of **1** revealed signals for two  
206 doublets of a trans-disubstituted olefinic bond ( $\delta_H$  6.53, d,  $J=16.1$  Hz, H-8'; 7.70, d,  $J=16.1$  Hz,  
207 H-7') and five aromatic protons ( $\delta_H$  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 <sup>13</sup>C NMR spectrum [ $\delta_C$  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  $\delta_C$  166.9 (C-9'). The <sup>1</sup>H NMR spectrum of the sugar portion of compound **1** showed  
213 five anomeric signals at  $\delta_H$  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  $\delta_C$  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 <sup>1</sup>H and <sup>13</sup>C NMR  
218 spectra of **1** indicated the presence of two hexoses units identified as  $\beta$ -glucopyranosyl at  $\delta_H$   
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  $\beta$ -xylopyranosyl units at  $\delta_H$  4.43 (xyl) and 4.68 (xyl'),  
221 and the last one as  $\alpha$ -arabinopyranosyl unit at  $\delta_H$  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  $\beta$  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  $\alpha$ -configuration. The  
226 deshielded signals of C-2 ( $\delta_C$  81.3) and C-4 ( $\delta_C$  77.1) of xyl, and of C-3 ( $\delta_C$  81.7) and C-4 ( $\delta_C$   
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  $\delta_H$  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  $\delta_C$  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*-{ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 4)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)}-  
237 [ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\beta$ -D-xylopyranosyl-21-*O*-cinnamoyl-machaerinic acid.  
238 Compound **1** was named parkibicoloroside A after its plant origin (Fig. 1).



239

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



241

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

243

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 ( $\delta_C$  83.2) of the glc' and the appearance of a set of additional  
255 signals, corresponding to a terminal  $\beta$ -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:  $\delta_H$  4.44 (xyl-H-1) with  $\delta_C$  89.2 (aglycone-  
258 C-3),  $\delta_H$  4.48 (glc-H-1) with  $\delta_C$  76.4 (xyl-C-4),  $\delta_H$  4.71 (xyl'-H-1) with  $\delta_C$  81.6 (xyl-C-2),  $\delta_H$   
259 4.87 (glc'-H-1) with  $\delta_C$  82.9 (xyl'-C-3),  $\delta_H$  4.56 (ara-H-1) with  $\delta_C$  70.8 (xyl'-C-4) and  $\delta_H$  4.66  
260 (glc''-H-1) with  $\delta_C$  83.2 (glc'-C-2). Hence, compound **2** was established as 3-O- $\{\beta$ -D-  
261 glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 4)]- $\beta$ -D-  
262 xylopyranosyl-(1 $\rightarrow$ 2)}- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\beta$ -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 ( $\delta_C$  66.5), its deshielded additional proton signal ( $\delta_H$  3.75), and the  
269 downfield shifts of the signals of the neighboring atoms [ $\delta_C$  46.1 (C-1), 95.1 (C-3), 40.3 (C-4),

270 37.4 (C-10)]. The  $\alpha$ -configuration of the C-2 hydroxyl group was deduced from the large  $J_{\text{H-2,H-3}}$   
271 value ( $J=11.5$  Hz), characteristic of an axial proton, and confirmed by ROESY correlations  
272 between H-2 and H<sub>3-25</sub>  $\beta$ -oriented. The aglycone of **3** was identified as  $2\alpha,3\beta,21\beta$ -trihydroxy-  
273 olean-12-en-28-oic acid. Full assignment of all <sup>1</sup>H and <sup>13</sup>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  $2\alpha$ ,hydroxy-machaerinic acid [15,19]. The sugar part of **3** consists of five residues as evidenced  
277 by <sup>1</sup>H NMR spectrum which displayed five anomeric protons at  $\delta_{\text{H}}$  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  $\delta_{\text{C}}$  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  $\beta$ -D-xylopyranose ( $\delta_{\text{H-1}}$  4.82, xyl), a  
283 terminal  $\alpha$ -L-arabinopyranose ( $\delta_{\text{H-1}}$  4.57, ara), and two terminal  $\beta$ -D-glucopyranose units ( $\delta_{\text{H-1}}$   
284 4.38 and 4.77, glc' and glc"). The NMR signals belonging to a 2,4-disubstituted  $\beta$ -D-  
285 glucopyranose unit were assigned starting from the anomeric proton at  $\delta_{\text{H}}$  4.50 ( $\delta_{\text{C-2}}$  79.8 and  
286  $\delta_{\text{C-4}}$  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  $\delta_{\text{C}}$  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*-{ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 4)-  
293 [ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)}- [ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\beta$ -D-

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

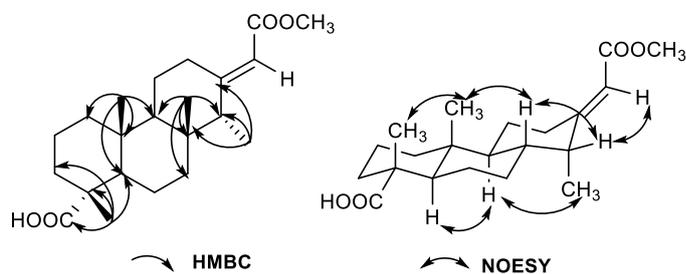
296 Compound **4** was isolated as an amorphous yellowish powder. The molecular formula  
297 was established as C<sub>73</sub>H<sub>110</sub>O<sub>33</sub> by HR-ESI-MS (*m/z* 1537.6838 [M+Na]<sup>+</sup>; calcd for  
298 C<sub>73</sub>H<sub>110</sub>O<sub>33</sub>Na, 1537.6827). The resonances derived 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  $\beta$ -D-glucopyranose (glc'''). The HMBC correlations at  $\delta_H$   
302 4.67 (d, *J*=7.7 Hz, glc'''-H-1)/ $\delta_C$  83.2 (glc''-C-2), 4.87 (d, *J*=7.8 Hz, glc''-H-1)/ $\delta_C$  82.7 (xyl-C-3),  
303 4.57 (d, *J*=4.5 Hz, ara-H-1)/ $\delta_C$  70.9 (xyl-C-4), 4.77 (d, *J*=7.6 Hz, xyl-H-1)/ $\delta_C$  81.0 (glc-C-2),  
304 4.46 (d, *J*=7.5 Hz, glc'-H-1)/ $\delta_C$  78.3 (glc-C-4), and 4.48 (d, *J*=7.7 Hz, glc-H-1)/  $\delta_C$  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  $\delta_H$  3.52 (glc''-H-2)/ $\delta_H$  4.67 (glc'''-H-1). Thus, the structure of **4**  
307 was elucidated as 3-*O*-{ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -L-  
308 arabinopyranosyl-(1 $\rightarrow$ 4)]- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)}-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\beta$ -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<sub>41</sub>H<sub>66</sub>O<sub>13</sub> on the basis of its HR-ESI-  
312 MS at *m/z* 789.4411 [M+Na]<sup>+</sup> (calcd C<sub>41</sub>H<sub>66</sub>O<sub>13</sub>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 ( $\delta_H$  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 <sup>1</sup>H NMR spectrum which displayed

318 two anomeric protons at  $\delta_{\text{H}}$  4.28 and 5.95, showing correlations in the HSQC spectrum to  
319 carbons at  $\delta_{\text{C}}$  105.6 and 93.7, respectively (Table 2). An  $\alpha$ -L-rhamnopyranose unit (rha) was  
320 identified by equatorial anomeric proton at  $\delta_{\text{H}}$  5.95 (d,  $J=1.5$  Hz), the small coupling constant  
321 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  $\text{H}_{\text{ax-4}}$  ( $J=9.0$   
322 Hz), and the coupling constant values of 6.2 Hz of methyl doublets at  $\delta_{\text{H}}$  1.26 (rha-H-6) (Table  
323 2). The second monosaccharide unit was identified as a  $\beta$ -D-xylopyranose moiety  $\delta_{\text{H}}$  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 ( $\delta_{\text{C}}$  94.5) and rha-H-1/aglycone-C-28 ( $\delta_{\text{C}}$  177.4)  
326 indicated the points of attachment of the monosaccharides at the aglycone. Thus, compound **5**  
327 was concluded to be 3-*O*- $\beta$ -D-xylopyranosyl-28-*O*- $\alpha$ -L-rhamnopyranosyl-2 $\alpha$ -hydroxy-  
328 machaerinic acid. Compound **5** was named parkibicoloroside E (Fig. 1).

329 Compound **6**, a colorless oil, possess a molecular formula  $\text{C}_{21}\text{H}_{32}\text{O}_4$  determined based  
330 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-  
331 ESI-MS spectrum. Analysis of the  $^1\text{H}$  NMR spectroscopic data of this compound (Table 1)  
332 indicated that the structure of **6** possessed three methyl groups at  $\delta_{\text{H}}$  0.77 (s), 0.94 (d,  $J=6.7$  Hz),  
333 and 1.06 (s), an olefinic proton signal at  $\delta_{\text{H}}$  5.54 (s), and a methoxyl group at  $\delta_{\text{H}}$  3.55 (s). The  
334  $^{13}\text{C}$  NMR spectrum (Table 1) exhibited 21 signals, of which three methyl carbons at  $\delta_{\text{C}}$  13.2 (C-  
335 17), 13.3 (C-20), and 16.0 (C-19), an ester carbon at  $\delta_{\text{C}}$  166.9 (C-16), a carboxyl carbon at  $\delta_{\text{C}}$   
336 181.0 (C-18), a methoxyl carbon at  $\delta_{\text{C}}$  49.9 (C-21), a trisubstituted double bond at  $\delta_{\text{C}}$  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 ( $^1\text{H}$  NMR,  $^{13}\text{C}$  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  $\delta_{\text{H}}$  0.94 (d,  $J=6.7$  Hz) to the carbons at  $\delta_{\text{C}}$  41.0 (C-8), 45.0 (C-14) and 169.1 (C-13). The Me-  
342 19 showed HMBC correlations with C-4 ( $\delta_{\text{C}}$  47.2), C-5 ( $\delta_{\text{C}}$  49.4), C-3 ( $\delta_{\text{C}}$  36.9), and C-18 ( $\delta_{\text{C}}$

343 181.0) whereas Me-20 exhibited HMBC correlations with C-5, C-10 ( $\delta_C$  36.1), C-9 ( $\delta_C$  47.7),  
 344 and C-1 ( $\delta_C$  38.5). The  $^1\text{H}$ - $^1\text{H}$ -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  $\delta_H$  5.54 (H-15) also showed long-  
 347 range correlations to the carbons at  $\delta_C$  45.0 (C-14), 23.8 (C-12), a 169.1 (C-13), and  $\delta_C$  166.9  
 348 (C-16). In the HMBC spectrum, the methoxy signal at  $\delta_H$  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  $\beta$ -axial orientations whereas  
 351 the NOESY correlations between H-5/H-9 and Me-17/H-9, indicated their  $\alpha$ -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 chemical shifts of C-12 ( $\delta_C$  23.8) and C-14 ( $\delta_C$  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.



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

358 Compound **7** was identified as 3,4,5-trimethoxyphenyl-1-*O*- $\beta$ -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  $\mu\text{M}$ ). The  
 361 monosaccharides saponins (**1-4**) exhibited a moderate antiproliferative activity with  $\text{IC}_{50}$   
 362 ranging from  $48.49 \pm 0.16$  to  $81.66 \pm 0.17$   $\mu\text{M}$  (Table 3), compared to the disaccharides saponin  
 363 (**5**). The pentasaccharide (**1**, **3**) were slightly more active than 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<sub>3</sub>OD).

	<b>1</b>		<b>2</b>		<b>3</b>		<b>4</b>		<b>5</b>	
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$								
<b>1</b>	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	
<b>2</b>	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			
<b>3</b>	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
<b>4</b>	-	39.2	-	39.2	-	40.3	-	39.1	-	40.1
<b>5</b>	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
<b>6</b>	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	
<b>7</b>	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	
<b>8</b>	-	38.9	-	39.0	-	39.2	-	38.9	-	39.3
<b>9</b>	1.60	47.7	1.50	47.8	1.68	47.7	1.63	47.8	1.53	47.6
<b>10</b>	-	36.5	-	36.5	-	37.4	-	36.5	-	37.4
<b>11</b>	1.90	23.2	1.82	23.2	1.99	23.2	1.96	23.2	1.86	23.3
<b>12</b>	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
<b>13</b>	-	142.4	-	142.5	-	142.5	-	142.6	-	142.5
<b>14</b>	-	41.8	-	41.5	-	41.5	-	41.8	-	41.5
<b>15</b>	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	
<b>16</b>	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)	
<b>17</b>	-	47.1	-	47.1	-	47.1	-	47.5	-	47.5
<b>18</b>	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
<b>19</b>	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	
<b>20</b>	-	34.9	-	34.9	-	34.9	-	35.0	-	35.8
<b>21</b>	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
<b>22</b>	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	
<b>23</b>	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
<b>24</b>	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
<b>25</b>	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
<b>26</b>	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
<b>27</b>	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
<b>28</b>		178.5		178.8		178.5		178.6		177.4
<b>29</b>	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
<b>30</b>	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
	<b>C-21-O- cinn</b>		<b>C-21-O- cinn</b>		<b>C-21-O- cinn</b>		<b>C-21-O- cinn</b>			
<b>1'</b>		134.3		134.3		134.3		134.3		
<b>2'</b>	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		
<b>3'</b>	7.43	128.6	7.32	128.6	7.43	128.6	7.43	128.6		
<b>4'</b>	7.44	131.0	7.33	130.2	7.44	129.1	7.44	131.1		
<b>5'</b>	7.43	128.6	7.32	128.6	7.43	128.6	7.43	128.6		
<b>6'</b>	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		
<b>7'</b>	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		
<b>8'</b>	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		
<b>9'</b>	-	166.9	-	166.9	-	166.9	-	166.9		

367

368 <sup>a</sup> Overlapping <sup>1</sup>H NMR signals are reported without designated multiplicity.

**Table 2.** NMR spectroscopic data of the sugar moieties for compounds **1-5** (600 MHz, CD<sub>3</sub>OD).

	<b>1</b>		<b>2</b>		<b>3</b>		<b>4</b>		<b>5</b>	
	$\delta_{\text{H}}$ m (J in Hz)	$\delta_{\text{C}}$								
	<b>xyl at C-3</b>		<b>xyl at C-3</b>		<b>glc at C-3</b>		<b>glc at C-3</b>		<b>xyl at C-3</b>	
<b>1</b>	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
<b>2</b>	3.52, m	<b>81.3</b>	3.48, t (9.3)	<b>81.6</b>	3.66, t (8.3)	<b>79.8</b>	3.54, t (7.9)	<b>81.0</b>	3.26, t (8.4)	<b>73.9</b>
<b>3</b>	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
<b>4</b>	3.71, m	<b>77.1</b>	3.72, m	<b>76.4</b>	3.67, t (9.6)	<b>78.4</b>	3.63, t (9.5)	<b>78.3</b>	3.54, m	<b>69.6</b>
<b>5</b>	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)	
<b>6</b>					3.90, m	60.1	3.78, m	60.7		
					3.90, m		3.93, dd (11.2, 4.1)			
	<b>glc at xyl-C-4</b>		<b>glc at xyl-C-4</b>		<b>glc' at glc-C-4</b>		<b>glc' at glc-C-4</b>		<b>rha at C-28</b>	
<b>1</b>	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
<b>2</b>	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
<b>3</b>	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
<b>4</b>	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
<b>5</b>	3.34	76.7	3.31	76.7	3.34	77.8	3.34	76.7	3.73	66.4
<b>6</b>	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			
	<b>xyl'-at xyl-C-2</b>		<b>xyl'-at xyl-C-2</b>		<b>xyl-at glc-C-2</b>		<b>xyl-at glc''-C-2</b>			
<b>1</b>	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		
<b>2</b>	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		
<b>3</b>	3.76, t (8.9)	<b>81.7</b>	3.70, t (9.2)	<b>82.9</b>	3.75, t (8.6)	<b>81.9</b>	3.71, t (9.3)	<b>82.7</b>		
<b>4</b>	3.86, m	<b>70.8</b>	3.85, m	<b>70.8</b>	3.84, m	<b>70.8</b>	3.85, m	<b>70.9</b>		
<b>5</b>	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)			
	<b>glc'-at xyl'-C-3</b>		<b>glc'-at xyl'-C-3</b>		<b>glc''-at xyl-C-3</b>		<b>glc''-at xyl-C-3</b>			
<b>1</b>	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		
<b>2</b>	3.29, t (8.7)	74.1	3.51, t (8.7)	83.2	3.30	74.1	3.52	83.2		
<b>3</b>	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		
<b>4</b>	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		
<b>5</b>	3.73, m	76.7	3.25, m	76.6	3.28, m	76.7	3.28, m	76.6		
<b>6</b>	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			
	<b>ara at xyl'-C-4</b>		<b>ara at xyl'-C-4</b>		<b>ara at xyl-C-4</b>		<b>ara at xyl-C-4</b>			
<b>1</b>	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		
<b>2</b>	3.74	69.4	3.74	69.4	3.75	69.2	3.74	69.4		
<b>3</b>	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		
<b>4</b>	3.92, m	65.7	3.92, m	65.6	3.92, m	65.7	3.92, m	65.7		
<b>5</b>	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			
			<b>glc''-at glc'-C-2</b>				<b>glc'''-at glc''-C-2</b>			
<b>1</b>			4.66, d (7.7)	104.7			4.67, d (7.7)	104.6		
<b>2</b>			3.31, t (8.3)	75.1			3.33, t (8.3)	75.0		
<b>3</b>			3.39, t (9.3)	76.0			3.35, t (9.3)	76.1		
<b>4</b>			3.43, t (9.3)	69.4			3.45, t (9.3)	69.4		
<b>5</b>			3.39, m	77.2			3.37, m	77.2		
<b>6</b>			3.77, dd (11.5, 4.7)	60.7			3.77	60.5		
			3.94, dd (12.2, 2.1)				3.89			

370

371

<sup>a</sup> Overlapping <sup>1</sup>H NMR signals are reported without designated multiplicity.

372

373 **Table 3.** Cytotoxic activity of compounds **1-7** against K562 cells<sup>a</sup>

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

374 <sup>a</sup>Results are means ± SD of 3 independent experiments performed in duplicate.

375 <sup>b</sup>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 2α,  
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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