



HAL
open science

Flavonoids from *Maytenus buchananii* as potential cholera chemotherapeutic agents

P.L.F. Tebou, J.-D.-D. Tamokou, D. Ngnokam, Laurence Voutquenne-Nazabadioko, J.-R. Kuate, P.K. Bag

► To cite this version:

P.L.F. Tebou, J.-D.-D. Tamokou, D. Ngnokam, Laurence Voutquenne-Nazabadioko, J.-R. Kuate, et al. Flavonoids from *Maytenus buchananii* as potential cholera chemotherapeutic agents. *South African Journal of Botany*, 2017, 109, pp.58-65. <10.1016/j.sajb.2016.12.019>. <hal-01996305>

HAL Id: hal-01996305

<https://univ-reims.hal.science/hal-01996305v1>

Submitted on 8 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

23 **Corresponding authors:**

24 *Doctor Jean-de-Dieu Tamokou, Laboratory of Microbiology and Antimicrobial Substances,
25 Department of Biochemistry, Faculty of Science, University of Dschang, PO. Box 67
26 Dschang, Cameroon, Tel: +237 677 000 897. E-mail: jtamokou@yahoo.fr

27 **Professor David Ngnokam, Laboratory of Environmental and Applied Chemistry,
28 Department of Chemistry, Faculty of Science, University of Dschang, PO. Box 67, Dschang,
29 Cameroon, Tel: +237 696 710 992. E-mail: dngnokam@yahoo.fr/ngnokam@univ-
30 dschang.org

31

32 **ABSTRACT**

33 *Maytenus buchananii* is worldwide distributed small evergreen tree traditionally used
34 as anti-tumor, anti-ulcer, anti-inflammatory, and as antimicrobial. The column
35 chromatography of the MeOH extract of *M. buchananii* leaves followed by purification of
36 different fractions led to the isolation of nine known flavonoids. Their structures were
37 elucidated on the basis of spectra analysis, and by comparison with those from the literature.
38 The antibacterial activity was assessed by performing minimum inhibitory concentration
39 (MIC), minimum bactericidal concentration (MBC) and the time-kill kinetic study while the
40 antioxidant activity was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and trolox
41 equivalent antioxidant capacity (TEAC) assays. All of the extracts/compounds showed
42 antibacterial activity against the strains of Gram-positive bacteria, *Staphylococcus aureus*, and
43 Gram-negative multi-drug resistance bacteria, *Vibrio cholerae* (causative agent of cholera)
44 and *Shigella flexneri* (causative agent of shigellosis). Compounds **2** (MIC = 32 to 128 µg/mL)
45 and **6** (MIC = 16 to 64 µg/mL) showed the largest antibacterial activities with the best MIC
46 (16 µg/mL) recorded with compound **6** on *Staphylococcus aureus*. The results of both DPPH

47 and TEAC assays found compounds **3** ($EC_{50} = 1.38 \mu\text{g/mL}$; $TEAC = 89.69 \mu\text{g/mL}$), **5** ($EC_{50} =$
48 $1.56 \mu\text{g/mL}$; $TEAC = 90.93 \mu\text{g/mL}$) and **6** ($EC_{50} = 1.42 \mu\text{g/mL}$; $TEAC = 89.76 \mu\text{g/mL}$) to
49 exhibit the most antioxidant activity. Results obtained from this study may help to exploit the
50 use of the *M. buchananii* leaf extracts and some of their flavonoid compounds as
51 pharmacological ingredients for promoting health, especially for cholera/shigellosis and
52 chronic diseases associated with oxidative stress.

53

54 *Keywords:* *Maytenus buchananii*; Celastraceae; leaf extract; phytochemical analysis;
55 flavonoids; antioxidant; antimicrobial; time-kill study.

56

57 **1. Introduction**

58 Acute watery diarrhea accounts for 80% of the cases (death account for 50%) in the
59 developing world (Tulloch and Richards, 1993). Among the diarrheal diseases, cholera is a
60 serious epidemic disease caused by the gram-negative bacterium *Vibrio cholerae* (Nair et al.,
61 1994). *Vibrio cholerae*, serotypes O1 and O139 has ability to produce an enterotoxin, cholera
62 toxin that is a major determinant of virulence for cholera. Among the other virulence factors,
63 ElTor hemolysin produced by *Vibrio cholerae* is also reportedly a potent toxin with both
64 enterotoxic and cytotoxic activities (Ichinose et al., 1987; Ramamurthy et al., 1993). Some
65 drugs such as racecadotril and loperamide used to treat the secretory diarrhea, have side
66 effects such as bronchospasm, vomiting and fever, and loperamide should not be administrated
67 to children below 6 years of age, patients with constipation, and intestinal obstruction (Roge
68 et al., 1993; Salazar et al., 2000). Emergence of multiply drug-resistant *Vibrio cholerae* is a
69 serious clinical problem in the treatment and containment of the disease, as reflected by the
70 increase in the fatality rate from 1% to 5.3% after the emergence of drug-resistant strains in
71 Guinea-Bissau during the 1996–1997 epidemic of cholera (Dalsgaard et al., 1999). The

72 increasing incidence of drug-resistant pathogens has drawn the attention of the pharmaceutical
73 and scientific communities towards studies on the potential antimicrobial activity of plant-
74 derived products, an untapped source of antimicrobial chemotypes, which are used in
75 traditional medicine in different countries. *Maytenus buchananii* (Loes.) R. Wilczek
76 belonging to the Celastraceae family is worldwide distributed small evergreen tree 2-12 m
77 high, with paired spines up to 2.8 mm long. It has been reported that plants of this genus are
78 widely used in folk medicine as anti-tumor, anti-asthmatic, anti-ulcer, treatment of stomach
79 problems, anti-inflammatory, analgesic and as antimicrobial (Ghazanfar, 1994; Muhammad et
80 al., 2000; Orabi et al., 2001; Al Haidari et al., 2002). The biological activities associated to
81 *Maytenus* species have been assigned to different classes of secondary metabolites such as
82 triterpenes (Sannomiya et al., 1998; El Tahir et al., 2001; Lindsey et al., 2006; Martucciello et
83 al., 2010), phenolics glucosides (Da Silva et al., 2008), alkaloids (Corsino et al., 1998a; Orabi
84 et al., 2001) and flavonoids (Vilegas et al., 1999; Lindsey et al., 2006; Dias et al., 2007).
85 Flavonoids, previously called bioflavonoids and included in aromatic compounds, are
86 important constituents of plants. The basic structural feature of flavonoid compounds is the 2-
87 phenylbenzopyrane or flavane nucleus, consisting of two benzene rings linked through a
88 heterocyclic pyrane ring. In total, there are 14 classes of flavonoids, differentiated on the basis
89 of the chemical nature and position of substituents on the different rings. For centuries,
90 preparations containing these compounds as the principal physiologically active constituents
91 have been used to treat human diseases such as infections associated with bacteria and those
92 related to oxidative stress. Many investigations revealed that flavonoids content contribute to
93 the antimicrobial (Araujo et al., 2011; Garcia et al., 2012; Djouossi et al., 2015) and
94 antioxidant (Pietta et al., 1998; Djouossi et al., 2015) activities of plants. In the course of our
95 continuing search for secondary metabolites of biological importance from Cameroonian

96 medicinal plants, we evaluate the antibacterial and antioxidant activities of extracts and
97 flavonoids from the leaves of *M. buchananii*.

98

99 **2. Materials and methods**

100 2.1. General

101 Optical rotations were measured on a Perkin-Elmer 341 polarimeter. ^1H and ^{13}C -NMR
102 spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoplatfom
103 (^1H at 600 MHz and ^{13}C at 150 MHz). 2D-NMR experiments were performed using standard
104 Bruker microprograms (Xwin-NMR version 2.1 software). Chemical shifts (δ) are reported in
105 parts per million (ppm) with the solvent signals as reference relative to TMS ($\delta=0$) as internal
106 standard, while the coupling constants (J values) are given in Hertz (Hz). The IR spectra were
107 recorded with a Shimadzu FT-IR-8400S spectrophotometer. UV spectra were determined as
108 methanol solution with a Cary 50 UV/VIS Spectrophotometer. HR-TOFESIMS experiments
109 were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an
110 electrospray source. The samples were introduced by direct infusion in a solution of MeOH at
111 a rate of 5 $\mu\text{L}/\text{min}$. Column chromatography (CC) was performed on silica gel 60 (70-230
112 mesh, Merck) and gel permeation on Sephadex LH-20 while TLC was carried out on silica
113 gel GF₂₅₄ pre-coated plates with detection accomplished by spraying with 50% H_2SO_4
114 followed by heating at 100 °C, or by visualizing with an UV lamp at 254 and 365 nm.

115

116 2.2. *Plant material*

117 The leaves of *Maytenus buchananii* was collected at Dschang, Menoua Division, West
118 Region of Cameroon, in March 2012. Authentication was done by Victor Nana, a botanist of
119 the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N^o
120 12659/SFR/CAM) is deposited.

121

122 2.3. Extraction and isolation

123 The dried and powdered plant material (4 Kg) was extracted two times (each for 24 h)
124 with 15 L MeOH at room temperature. The filtrate obtained was concentrated under reduced
125 pressure to yield a dark residue (540 g). This crude extract was fractionated with hexane,
126 EtOAc and *n*-BuOH, yielding after evaporation to dryness 58, 141 and 65 g of hexane, EtOAc
127 and *n*-BuOH fractions, respectively and 235 g of a brown gum.

128 A portion (50 g) of the hexane fraction was subjected to silica gel column chromatography
129 (CC) eluting with gradient mixtures of hexane-EtOAc (1:0; 9:1; 4:1; 7:3; 1:1; 3:7; and 0:1).
130 Fractions of 250 mL were collected and combined on the basis of their TLC profiles to give
131 four fractions noted FH1 to FH4. Fraction FH4 was purified by column chromatography over
132 silica gel with hexane-EtOAc (1:4) and fractions (25 mL each) were collected, yielding five
133 sub-fractions. Purification of sub-fraction [10-23] on silica gel column chromatography with
134 hexane-EtOAc (1:4) led to the isolation of compound **1** (13 mg).

135 130 g of the EtOAc fraction was submitted to silica gel column chromatography eluted
136 with gradient mixtures of hexane-EtOAc (1:1; 3:7; and 0:1) followed by EtOAc-MeOH (9:1;
137 4:1; and 3:2). Fractions of 250 mL were collected and combined on the basis of their TLC
138 profiles to give six fractions noted FE1 to FE6. Fraction FE2 was purified by column
139 chromatography over silica gel with hexane-EtOAc (1:1) and fractions (50 mL each) were
140 collected to give three sub-fractions. Sub-fraction [13-21] was subjected to Sephadex LH-20
141 column chromatography with MeOH to give compound **3** (12 mg). Fraction FE3 was also
142 purified by column chromatography over silica gel with hexane-EtOAc (3:7) and fractions (50
143 mL each) were collected to give six sub-fractions. Purification of sub-fractions [15-25] and
144 [36-51] afforded compounds **2** (32 mg) and **4** (36 mg) respectively. Fraction FE4 was
145 repeatedly purified by column chromatography over silica gel with hexane-EtOAc (1:4) and

146 fractions (75 mL each) were collected, yielding six sub-fractions. Purification of sub-fractions
147 [14-24] on silica gel with hexane-EtOAc (2:3) gave compound **7** (12 mg), while sub-fractions
148 [41-50] and [106-121] were passed through Sephadex LH-20 column chromatography using
149 MeOH and purified on silica gel column chromatography with hexane-EtOAc (3:7) and (1:9)
150 respectively to afford compounds **5** (14 mg) and **6** (24 mg) respectively.

151 50 g of the gum were repeatedly subjected to Sephadex LH-20 column chromatography
152 using MeOH to separate compounds (Extract) from the trail (Rest). This extract (35 g) was
153 purified by column chromatography over silica gel with EtOAc. Fractions of 100 mL were
154 collected and combined on the basis of their TLC profiles to give six fractions noted FG1 to
155 FG6. Fraction FG1 was purified on silica gel column chromatography eluted with hexane-
156 EtOAc (4:1) and fractions (25 mL each) were collected to give four sub-fractions. Purification
157 of sub-fraction [5] gave compound **8** (17 mg). Fraction F3G was purified over silica gel
158 column chromatography eluted with the mixture EtOAc-MeOH-H₂O (95-3-2) to give
159 compound **9** (20 mg).

160

161 *2.4. Structural identification of the isolated compounds*

162 Samples for NMR experiments were dissolved in CD₃OD on a BRUKER Avance DRX
163 600 Spectrometer (600MHz for ¹H and 150MHz for ¹³C). Column chromatography was
164 performed on silica gel 60 (70-230 mesh, Merck) and sephadex LH-20. Fractions were
165 monitored by TLC using Merck pre-coated silica gel sheets (60 F₂₅₄), and spots were
166 visualized under UV light (254 and 365 nm) and by spraying with 50% H₂SO₄ and heating at
167 100 °C. 1D and 2D-NMR experiments (COSY, TOCSY, ROESY, HSQC-*Jmod*, and HMBC)
168 were performed using standard Bruker pulse programs (XW in NMR version 2.1).

169 Quercetin-3-*O*- α -L-arabinopyranoside **1**: yellow powder. UV: 250, 279, 260, 375 nm. IR
170 ν_{\max} (KBr, cm⁻¹): 3289, 2995, 1660, 1505. ¹³C-NMR (CD₃OD, 150MHz) δ : 157.3 (C-2),

171 134.2 (C-3), 178.4 (C-4), 161.7 (C-5), 98.5 (C-6), 164.6 (C-7), 93.3 (C-8), 157.0 (C-9), 104.2
172 (C-10), 121.4 (C-1'), 116.0 (C-2'), 144.6 (C-3'), 148.6 (C-4'), 114.8 (C-5'), 121.6 (C-6') for
173 aglycone; 103.2 (C-1''), 71.5 (C-2''), 72.8 (C-3''), 67.7 (C-4''), 65.6 (C-5'') for sugar moiety.
174 ¹H-NMR (CD₃OD, 600MHz) δ: 6.23 (1H, d, *J* = 2.0 Hz, H-6), 6.42 (1H, d, *J* = 2.0 Hz, H-8),
175 7.77 (1H, d, *J* = 2.2 Hz, H-2'), 6.89 (1H, d, *J* = 8.5 Hz, H-5'), 7.60 (1H, dd, *J* = 2.2 and 8.5
176 Hz, H-6') for aglycone; 5.18 (1H, d, *J* = 6.6 Hz, H-1''), 3.92 (1H, dd, *J* = 6.6 and 8.4 Hz, H-
177 2''), 3.67 (1H, dd, *J* = 3.1 and 8.4 Hz, H-3''), 3.83 (1H, m, H-4''), 3.47 (1H, m, H-5''a), 3.86
178 (1H, m, H-5''b) for sugar moiety.

179 Epicatechin **2**: yellow powder. UV: 282 and 219 nm. IR ν_{\max} (KBr, cm⁻¹): 2600-3400
180 (broad), 1620, 1520, 1470, 1380, 1280, 1240. ¹³C-NMR (CD₃OD, 150 MHz) δ: 78.5 (C-2),
181 66.1 (C-3), 27.9 (C-4), 156.6 (C-5), 95.0 (C-6), 156.3 (C-7), 94.5 (C-8), 156.0 (C-9), 98.7 (C-
182 10), 130.9 (C-1'), 113.9 (C-2'), 144.4 (C-3'), 144.6 (C-4'), 114.5 (C-5'), 118.0 (C-6'). ¹H-
183 NMR (CD₃OD, 600 MHz) δ: 4.84 (1H, m, H-2), 4.19 (1H, m, H-3), 2.76 (1H, dd, *J* = 2.9 and
184 16.7 Hz, H-4a), 2.88 (1H, dd; *J* = 4.6 and 16.7 Hz, H-4b), 5.96 (1H, d, *J* = 2.3 Hz, H-6), 5.93
185 (1H, d, *J* = 2.3 Hz, H-8), 6.99 (1H, d, *J* = 1.8 Hz, H-2'), 6.78 (1H, d, *J* = 8.1 Hz, H-5'), 6.82
186 (1H, dd, *J* = 1.8 and 8.1 Hz, H-6').

187 Quercetin **3**: green powder. UV: 253, 278, 261, 370 nm. IR ν_{\max} (KBr, cm⁻¹): 1660, 1594,
188 1506, 1353, 1205. ¹³C-NMR (CD₃OD, 150 MHz) δ: 156.8 (C-2), 135.8 (C-3), 175.9 (C-4),
189 161.1 (C-5), 97.8 (C-6), 164.2 (C-7), 93.0 (C-8), 147.4 (C-9), 103.1 (C-10), 122.7 (C-1'),
190 114.8 (C-2'), 144.8 (C-3'), 146.6 (C-4'), 114.6 (C-5'), 120.2 (C-6'). ¹H-NMR (CD₃OD, 600
191 MHz) δ: 6.21 (1H, d, *J* = 2.0 Hz, H-6), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 7.76 (1H, d, *J* = 2.2 Hz,
192 H-2'), 6.91 (1H, d, *J* = 8.5 Hz, H-5'), 7.66 (1H, dd, *J* = 2.2 and 8.5 Hz, H-6').

193 Kaempferol-3-*O*- α -L-rhamnopyranoside **4**: yellow powder. UV: 255, 267, 372 nm. IR
194 ν_{\max} (KBr, cm⁻¹): 3290, 2981, 1663, 1592, 1457, 1351, 1210. ¹³C-NMR (CD₃OD, 150 MHz),
195 δ: 157.9 (C-2), 134.8 (C-3), 178.2 (C-4), 161.8 (C-5), 98.4 (C-6), 164.5 (C-7), 93.4 (C-8),

196 157.1 (C-9), 104.5 (C-10), 121.2 (C-1'), 130.5 (C-2'), 115.1 (C-3'), 160.2 (C-4'), 115.1 (C-
197 5'), 130.5 (C-6') for aglycone; 102.1 (C-1''), 70.5 (C-2''), 70.7 (C-3''), 71.8 (C-4''), 70.6 (C-
198 5''), 16.3 (C-6'') for sugar moiety. ¹H-NMR (CD₃OD, 600 MHz) δ: 6.22 (1H, d, *J* = 2.1 Hz, H-
199 6), 6.39 (1H, d, *J* = 2.1 Hz, H-8), 7.78 (2H, d, *J* = 6.8 Hz, H-2' and H-6'), 6.95 (2H, d, *J* = 6.8
200 Hz, H-3' and H-5') for aglycone; 5.40 (1H, d, *J* = 1.6 Hz, H-1''), 4.24 (1H, m, H-2''), 3.73
201 (1H, m, H-3''), 3.35 (1H, m, H-4''), 3.36 (1H, m, H-5''), 0.98 (3H, m, H-6'') for sugar moiety.

202 Quercetin-3-*O*-α-L-rhamnopyranoside **5**: yellow powder. UV: 257, 266, 274, 374 nm. IR
203 ν_{\max} (KBr, cm⁻¹): 3288, 2990, 1597, 1500, 1459, 1355, 1094. ¹³C-NMR (CD₃OD, 150 MHz)
204 δ: 157.9 (C-2), 134.8 (C-3), 178.2 (C-4), 161.8 (C-5), 98.4 (C-6), 164.5 (C-7), 93.3 (C-8),
205 157.1 (C-9), 104.5 (C-10), 121.4 (C-1'), 116.5 (C-2'), 145.0 (C-3'), 148.4 (C-4'), 114.7 (C-
206 5'), 121.6 (C-6') for aglycone; 102.1 (C-1''), 70.5 (C-2''), 70.7 (C-3''), 71.8 (C-4''), 70.6 (C-
207 5''), 16.3 (C-6'') for sugar moiety. ¹H-NMR (CD₃OD, 600 MHz) δ: 6.23 (1H, d, *J* = 2.1 Hz,
208 H-6), 6.39 (1H, d, *J* = 2.1 Hz, H-8), 7.36 (1H, d, *J* = 2.1 Hz, H-2'), 6.93 (1H, d, *J* = 8.3 Hz, H-
209 5'), 7.33 (1H, dd, *J* = 2.1 and 8.3 Hz, H-6') for aglycone; 5.37 (1H, d, *J* = 1.1 Hz, H-1''), 4.24
210 (1H, m, H-2''), 3.77 (1H, m, H-3''), 3.37 (1H, m, H-4''), 3.43 (1H, m, H-5''), 0.96 (3H, d, *J* =
211 6.2 Hz, H-6'') for sugar moiety.

212 Quercetin-3-*O*-β-D-glucopyranoside **6**: yellow powder. UV: 257, 263, 325, 429 nm. IR
213 ν_{\max} (KBr, cm⁻¹): 3286, 2993, 1660, 1597, 1455, 1350. ¹³C-NMR (CD₃OD, 150 MHz) δ:
214 156.9 (C-2), 134.3 (C-3), 178.0 (C-4), 161.2 (C-5), 98.6 (C-6), 164.6 (C-7), 93.4 (C-8), 156.7
215 (C-9), 104.3 (C-10), 121.4 (C-1'), 116.4 (C-2'), 144.8 (C-3'), 148.4 (C-4'), 114.9 (C-5'),
216 121.6 (C-6') for aglycone; 103.6 (C-1''), 71.7 (C-2''), 73.7 (C-3''), 68.6 (C-4''), 75.9 (C-5''),
217 60.3 (C-6'') for sugar moiety. ¹H-NMR (CD₃OD, 600 MHz) δ: 6.25 (1H, d, *J* = 2.0 Hz, H-6),
218 6.46 (1H, d, *J* = 2.0 Hz, H-8), 7.86 (1H, d, *J* = 2.3 Hz, H-2'), 6.91 (1H, d, *J* = 8.4 Hz, H-5'),
219 7.64 (1H, dd, *J* = 2.4 and 8.4 Hz, H-6') for aglycone; 5.30 (1H, d, *J* = 7.8 Hz, H-1''), 3.82

220 (1H, m, H-2''), 3.58 (1H, m, H-3''), 3.87 (1H, m, H-4''), 3.51 (1H, m, H-5''), 3.56 (1H, m, H-
221 6''a), 3.67 (1H, m, H-6''b) for moiety sugar.

222 Epigallocatechin **7**: yellow powder. UV: 282 and 219 nm. IR ν_{\max} (KBr, cm^{-1}): 2600-3400
223 (broad), 1620, 1520, 1470, 1380, 1280, 1240. ^{13}C -NMR (CD_3OD , 150 MHz) δ : 78.5 (C-2),
224 66.1 (C-3), 27.7 (C-4), 156.6 (C-5), 95.0 (C-6), 156.3 (C-7), 94.4 (C-8), 156.0 (C-9), 98.7 (C-
225 10), 130.1 (C-1'), 105.6 (C-2'), 145.3 (C-3'), 132.2 (C-4'), 145.3 (C-5'), 105.6 (C-6'). ^1H -
226 NMR (CD_3OD , 600 MHz), δ : 4.77 (1H, m, H-2), 4.19 (1H, m, H-3), 2.75 (1H, dd, $J = 2.9$ and
227 16.6 Hz, H-4a), 2.87 (1H, dd, $J = 4.6$ and 16.6 Hz, H-4b), 5.96 (1H, d, $J = 2.3$ Hz, H-6), 5.93
228 (1H, d, $J = 2.3$ Hz, H-8), 6.53 (2H, s, H-2' and H-6').

229 Kaempferol **8**: yellow powder. UV: 254, 260, 280, 372 nm. IR ν_{\max} (KBr, cm^{-1}): 2992,
230 1658, 1592, 1455, 1350, 1208, 1094. ^{13}C -NMR (CD_3OD , 150 MHz) δ : 158.3 (C-2), 137.2 (C-
231 3), 177.4 (C-4), 162.5 (C-5), 99.3 (C-6), 165.8 (C-7), 94.5 (C-8), 160.6 (C-9), 104.6 (C-10),
232 123.8 (C-1'), 130.7 (C-2'), 116.3 (C-3'), 148.1 (C-4'), 116.3 (C-5'), 130.7 (C-6'). ^1H -NMR
233 (CD_3OD , 600 MHz) δ : 6.26 (1H, d, $J = 2.0$ Hz, H-6), 6.45 (1H, d, $J = 2.0$ Hz, H-8), 8.12 (2H,
234 d, $J = 6.9$ Hz, H-2' and H-6'), 6.94 (2H, d, $J = 6.9$ Hz, H-3' and H-5').

235 Quercetin-3-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside] **9**: yellow powder.
236 UV: 254, 268, 310, 355 nm. IR ν_{\max} (KBr, cm^{-1}): 3288, 2987, 1598, 1504, 1460, 1348, 1209,
237 1092. ^{13}C -NMR (CD_3OD , 150 MHz) δ : 157.5 (C-2), 134.4 (C-3), 178.0 (C-4), 161.6 (C-5),
238 98.6 (C-6), 165.0 (C-7), 93.5 (C-8), 157.1 (C-9), 104.1 (C-10), 121.4 (C-1'), 116.5 (C-2'),
239 144.4 (C-3'), 148.6 (C-4'), 114.7 (C-5'), 121.6 (C-6') for aglycone; 104.6 (C-1''), 71.7 (C-2''),
240 73.7 (C-3''), 68.8 (C-4''), 73.9 (C-5''), 65.9 (C-6'') for glucosyl sugar, 100.5 (C-1'''), 70.7 (C-
241 2'''), 70.9 (C-3'''), 72.5 (C-4'''), 68.3 (C-5'''), 16.6 (C-6''') for rhamnosyl sugar. ^1H -NMR
242 (CD_3OD , 600 MHz) δ : 6.24 (1H, d, $J = 2.0$ Hz, H-6), 6.44 (1H, d, $J = 2.0$ Hz, H-8), 7.88 (1H,
243 d, $J = 2.2$ Hz, H-2'), 6.88 (1H, d, $J = 8.5$ Hz, H-5'), 7.63 (1H, dd, $J = 2.2$ and 8.5 Hz, H-6')
244 for aglycone; 5.10 (1H, d, $J = 7.8$ Hz, H-1''), 3.86 (1H, m, H-2''), 3.58 (1H, m, H-3''), 3.83

245 (1H, m, H-4”), 3.67 (1H, m, H-5”), 3.43 (1H, m, H-6”a), 3.77 (1H, m, H-6”b) for glucosyl
246 sugar, 4.54 (1H, d, $J = 1.4$ Hz, H-1”), 3.60 (1H, m, H-2”), 3.52 (1H, m, H-3”), 3.31 (1H, m,
247 H-4”), 3.55 (1H, m, H-5”), 0.95 (3H, m, H-6”) for rhamnosyl sugar.

248

249 2.5. Antimicrobial assay

250 2.5.1. Microorganisms

251 A total of six bacterial strains were tested for their susceptibility to compounds and
252 these strains were taken from our laboratory collection (kindly provided by Dr. T.
253 Ramamurthy, NICED, Kolkata). Among the clinical strains of *Vibrio cholerae* used in this
254 study, strains NB2 and SG24(1) belonged to O1 and O139 serotypes, respectively. These
255 strains were able to produce cholera toxin and hemolysin (Bag et al., 2008; Thakurta et al.,
256 2007). The other strains used in this study were *V. cholerae* non-O1, non-O139 (strains CO6
257 and PC2) (Bag et al., 2008); and *Shigella flexneri* (Acharyya et al., 2015). The *V. cholerae*
258 non-O1 and non-O139 strains, were positive for hemolysin production but negative for
259 cholera toxin production (Bag et al., 2008). The American Type Culture Collection (ATCC)
260 strain, *Staphylococcus aureus* ATCC 25923, was used for quality control. The bacterial
261 strains were maintained on agar slant at 4 °C and subcultured on a fresh appropriate agar
262 plates 24 h prior to any antibacterial test. The Mueller Hinton Agar (MHA) was used for the
263 activation of bacteria. The Mueller Hinton Broth (MHB) and nutrient agar (Hi-Media) were
264 used for the MIC and MBC determinations respectively.

265

266 2.5.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal 267 concentration (MBC)

268 MIC values were determined by a broth micro-dilution method as described earlier
269 (Nyaa et al., 2009) with slight modifications. Each test sample was dissolved in

270 dimethylsulfoxide (DMSO) and the solution was then added to Mueller Hinton Broth (MHB)
271 for bacteria to give a final concentration of 1024 µg/mL. This was serially diluted twofold to
272 obtain a concentration range of 0.50–1024 µg/mL. Then, 100 µL of each concentration was
273 added in each well (96-well microplate) containing 95 µL of MHB and 5 µL of inoculum for
274 final concentrations varying from 0.25–512 µg/mL. The inoculum was standardized at 1.5
275 $\times 10^6$ CFU/mL by adjusting the optical density to 0.1 at 600 nm using a JENWAY 6105
276 UV/Vis spectrophotometer. The final concentration of DMSO in each well was < 1%
277 [preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of the test organisms].
278 The negative control well consisted of 195 µL of MHB and 5 µL of the standard inoculum.
279 The plates were covered with sterile lids, then agitated to mix the contents of the wells using a
280 plate shaker and incubated at 35 °C for 24 h. The assay was repeated three times. The MIC
281 values of samples were determined by adding 50 µL of a 0.2 mg/mL *p*-iodonitrotetrazolium
282 violet solution followed by incubation at 35 °C for 30 min. Viable microorganisms reduced
283 the yellow dye to a pink color. MIC values were defined as the lowest sample concentrations
284 that prevented this change in color indicating a complete inhibition of microbial growth. For
285 the determination of MBC values, a portion of liquid (5 µL) from each well that showed no
286 growth of microorganism was plated on Mueller Hinton Agar and incubated at 35 °C for 24
287 h. The lowest concentrations that yielded no growth after this subculturing were taken as the
288 MBC values (Tamokou et al., 2011). Ciprofloxacin and ampicillin (Sigma-Aldrich,
289 Steinheim, Germany) were used as positive controls.

290

291 2.5.3. *The time-kill kinetic study (for antimicrobial drugs) against Vibrio cholerae SG24 (1)*

292 Time-kill dynamic assay was performed using broth microdilution method as
293 previously described (Avila et al., 1999) with minor modifications. Cultures of bacteria in
294 MHB (1×10^6 CFU/mL) were incubated separately at 37 °C for 0, 2, 4, 6, 10, and 24 hours in

295 the absence (control) and in the presence of the drug/extract at MIC and MBC of each sample.
296 Compounds **2**, **4**, **6** and ciprofloxacin were used in the time-kill dynamic experiment. The
297 final concentration of DMSO was 1%. A control sample was made using DMSO 1% and the
298 inoculum. At each incubation time point, liquids (50 μ L) were removed from the test solution
299 for ten-fold serial dilution. Thereafter, a 100 μ L liquid from each dilution was spread on the
300 surface of the MHA plates and incubated at 37 °C for 24h, and the number of CFU/mL was
301 counted. Experiments were carried out in triplicate. Time-kill curves were constructed by
302 plotting the surviving \log_{10} of number of CFU/mL against time (hours).

303

304 2.6. Antioxidant assay

305 2.6.1. DPPH free radical scavenging assay

306 The free radical scavenging activity of the MeOH extract as well as some of its
307 isolated compounds was performed according to described methods (Djouossi et al., 2015).
308 Briefly, the test samples, prior dissolved in DMSO (SIGMA) beforehand, were mixed with a
309 20 mg/L 2,2-diphenyl-1-picryl-hydrazyl (DPPH) methanol solution, to give final
310 concentrations of 1, 10, 20, 40, 80, 160, 320, 640 and 1280 μ g/mL. After 30 min at room
311 temperature, the absorbance values were measured at 517 nm and converted into percentage
312 of antioxidant activity. L-ascorbic acid was used as a standard control. The percentage of
313 decolouration of DPPH (%) was calculated as follows:

314

$$\text{\% decolouration of DPPH} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample}) \times 100}{\text{Absorbance of control}}$$

316

317 The radical scavenging percentages were plotted against the logarithmic values of the
318 concentration of test samples and a linear regression curve was established in order to

319 calculate the EC₅₀ (µg/mL), which is the amount of sample necessary to inhibit by 50% the
320 absorbance of free radical DPPH. All the analyses were carried out in triplicate.

321

322 2.6.2. Trolox equivalent antioxidant capacity (TEAC) assay.

323 The TEAC test was done as previously described (Rice-Evans and Miller, 1994) with
324 slight modifications. In a quartz cuvette, to 950 µL acetate buffer (pH =5.0, 100 mM), the
325 following were added: 20 µL laccase (1 mM stock solution), 20 µL test sample, 10 µL ABTS
326 (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) (74 mM stock solution). The laccase
327 were purified from *Sclerotinia sclerotiorum* according to the protocol described (Motet *al.*,
328 2012). The sample concentrations in the assay mixture were 400, 200, 100, 10 µg/mL for the
329 MeOH extract and 20 µg/mL for the isolated compounds. The content of the generated
330 ABTS^{•+} radical was measured at 420 nm after 230 s reaction time and was converted to gallic
331 acid equivalents by the use of a calibration curve (Pearson's correlation coefficient: $r = 0.996$)
332 constructed with 0, 4, 10, 14, 28, 56, 84 µM gallic acid standards rather than Trolox.
333 Experiments were done in triplicate.

334

335 2.7. Statistical analysis

336 Data were analyzed by one-way analysis of variance followed by Waller-Duncan Post
337 Hoc test. The experimental results were expressed as the mean ± Standard Deviation (SD).
338 Differences between groups were considered significant when $p < 0.05$. All analyses were
339 performed using the Statistical Package for Social Sciences (SPSS, version 12.0) software.

340

341 3. Results and discussion

342 3.1. Phytochemical analysis

343 The structures of the isolated compounds were established using spectroscopic analysis,
344 especially, NMR spectra in conjunction with 2D experiments, COSY, TOCSY, HSQC,
345 HMBC and direct comparison with published information. The nine compounds isolated from
346 the leaves of *M. buchananii* (Fig. 1) were identified as quercetin-3-*O*- α -L-arabinopyranoside
347 **1** (Kazuma et al., 2003), epicatechin **2** (Petrus et al., 2012), quercetin **3** (Hossain et al., 2006),
348 kaempferol-3-*O*- β -D-rhamnopyranoside **4** (Zhang et al., 2003), quercetin-3-*O*- α -L-
349 rhamnopyranoside **5** (Markam et al., 1978), quercetin-3-*O*- β -D-glucopyranoside **6** (Xiang et
350 al., 2008), epigallocatechin **7** (Zan et al., 2013), kaempferol **8** (Sarin et Sharma, 2012) and
351 quercetin-3-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside] **9** (Kazuma et al., 2000).
352 These compounds together with the extracts were tested for their antibacterial and antioxidant
353 activities and the results are reported in Table 1 and Fig. 2 - 4.

354

355 3.2. Antibacterial activity

356 The antibacterial activity of the MeOH, *n*-BuOH, EtOAc, and hexane extracts as well as
357 their isolated compounds were examined by microdilution susceptibility assay against six
358 bacterial strains selected on the basis of their relevance as human pathogens. The experiments
359 revealed that the extracts and isolated compounds exhibited variable MICs and significant
360 antimicrobial activity, depending on the microbial strains (Table 1). The MIC values of the
361 extracts ranged from 32 to 512 μ g/mL. No activity was noted with hexane extract on *Vibrio*
362 *cholerae* SG24 (1) and *Vibrio cholerae* NB2 at concentrations up to 512 μ g/mL while the most
363 sensitive bacterial strains were found to be *Shigella flexneri* and *Staphylococcus aureus*.
364 EtOAc extract (MIC = 32-256 μ g/mL) was the most active extract followed in decreasing
365 order by *n*-BuOH (MIC = 64-256 μ g/mL), MeOH (MIC = 128 - 512 μ g/mL) and hexane
366 (MIC = 512 - >512 μ g/mL) extracts. This observation suggests that the crude methanol extract
367 contains several antibacterial principles with different polarities. Phytochemicals are routinely

368 classified as antimicrobials on the basis of susceptibility tests that produce MIC in the range
369 of 100 to 1000 µg/mL (Simões et al., 2009). Activity is considered to be significant if MIC
370 values are below 100 µg/mL for crude extract and moderate when the MIC values vary from
371 100 to 625 µg/ml (Kueté, 2010). Therefore, the activities recorded with the *n*-BuOH fraction
372 on *Shigella flexneri* and *Staphylococcus aureus* and with EtOAc fraction on *Vibrio cholerae*
373 2, *Shigella flexneri* and *Staphylococcus aureus* can be considered as important.

374 The lowest MIC value of 16 µg/mL was recorded on *Staphylococcus aureus* with
375 compounds **6** and on *Escherichia coli* with compound **8**, whereas the lowest MBC value of 32
376 µg/mL was obtained on *Staphylococcus aureus* with compounds **6** and **2** and on *Shigella*
377 *flexneri* with compound **6**. However, the highest MIC value of 512 µg/mL was recorded on
378 *Vibrio cholerae* SG24 (1) and *Vibrio cholerae* CO6 with MeOH extract, and the highest MBC
379 value of 512 µg/mL was obtained on *Vibrio cholerae* SG24 (1) with the MeOH and EtOAc
380 extracts and on *Vibrio cholerae* CO6 and *Vibrio cholerae* NB2 with the MeOH extract. A
381 lower MBC/MIC (≤ 4) value signifies that a minimum amount of plant extracts/pure
382 compounds is used to kill the microbial species, whereas, a higher values signifies the use of
383 comparatively more amount of sample for the control of any microorganism (Djouossi et al.,
384 2015).

385 The strains of *V. cholerae* NB2, PC2 (Bag et al., 2008; Thakurta et al., 2007) and
386 *Shigella flexneri* (Acharyya et al., 2015) included in the present study were MDR clinical
387 isolates and these were resistant to commonly used drugs such as ampicillin, streptomycin,
388 tetracycline, nalidixic acid, furazolidone, *co*-trimoxazole, etc. However, these bacterial strains
389 were found to be sensitive to most of the tested samples, suggesting that their administration
390 may represent an alternative treatment against the *V. cholerae*, the causative agent of dreadful
391 disease cholera and *S. flexneri*, the causative agent of shigellosis. Taking into account the
392 medical importance of the tested bacteria, this result can be considered as promising in the

393 perspective of new antibacterial drugs development. Although flavonoid compounds have
394 been reported to possess antibacterial activity (Garcia et al., 2012; Djouossi et al., 2015), no
395 study has been reported on the activity of these compounds against these types of MDR
396 pathogenic strains.

397 With regard to the structure-activity relationship analysis, the eight flavonoids showed
398 different degrees of antibacterial activity. Compounds **2** (MIC = 32 to 128 µg/mL) and **6**
399 (MIC = 16 to 64 µg/mL) showed the largest antibacterial activities with the best MIC (16
400 µg/mL) recorded with compound **6** on *Staphylococcus aureus*. These observations show that
401 the sugar moieties and hydroxyl groups should be responsible for the difference in the
402 observed activity. The mechanism of the active compounds (**1-9**) is still to be studied;
403 nevertheless, their activity is probably due to their ability to complex with extracellular and
404 soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also
405 disrupt microbial membranes (Cowan, 1999). For example, (-)-epigallocatechin gallate inhibit
406 cytoplasmic membrane function, whereas the activity of quercetin has been at least partially
407 attributed to the inhibition of DNA gyrase (Cowan, 1999; Fowler et al., 2011).

408

409 3.3. The time-kill kinetic study

410 The time-kill kinetic study for compounds **2**, **4**, and **6** against *Vibrio cholerae* SG24
411 (1) (as a function of incubation time) is shown in Fig. 2. It can be noted that significant
412 reduction of the bacterial population is observed with the tested compounds and ciprofloxacin
413 at concentrations corresponding to their MIC, MBC and 2MBC values. At MBC/2MBC
414 values, all the bacterial population was completely killed after 6 h of incubation with
415 ciprofloxacin, compounds **2** and **6** while after 10 h of incubation, there was no observed
416 colony at MIC values with ciprofloxacin and compound **4**.

417

418 3.4. Antioxidant activity

419 Free-radical-scavenging activities of *M. buchananii* extracts and their isolated compounds
420 were assessed by DPPH \cdot and ABTS $\cdot+$. The results were expressed as gallic acid equivalent
421 antioxidant capacity of tested samples (Fig. 3) and as equivalent concentrations of test
422 samples scavenging 50% of DPPH radical (Fig. 4). Both DPPH \cdot and ABTS $\cdot+$ measure
423 reductions of radical solutions in the presence of a hydrogen-donating antioxidant. The results
424 of both assays found compounds **3** (EC₅₀ = 1.38 μ g/mL; TEAC= 89.69 μ g/mL), **5** (EC₅₀ =
425 1.56 μ g/mL; TEAC= 90.93 μ g/mL) and **6** (EC₅₀ = 1.42 μ g/mL; TEAC= 89.76 μ g/mL) to
426 exhibit the most activity and compound **7** (EC₅₀ = 107.56 μ g/mL; TEAC= 44.98 μ g/mL) to
427 exhibit the least activity. The results of the DPPH and ABTS free-radical-scavenging
428 activities are not in the same order for the extracts. This difference in the activity may be due
429 to the presence of potent molecule (s) in some extracts which is more capable of quenching
430 one particular radical than another. The free-radical activity of the extracts can be explained
431 by the presence of phenolic substances. Similarly, previous reports have shown phenolic
432 compounds to contribute significantly to the antioxidant activity of medicinal plants (Lim et
433 al., 2009; Zhao et al., 2010; Djouossi et al., 2015). Phenolic compounds such as flavonoids
434 are known to be potential antioxidant due to their ability to scavenge free radicals and active
435 oxygen species such as singlet oxygen, superoxide anion radical and hydroxyl radicals (Hall
436 and Cupett, 1997; Pietta et al., 1998).

437

438 **Conclusion**

439 Results obtained from this study may help to exploit the use of the *M. buchananii* leaf
440 extracts and some of their flavonoid contents as pharmacological ingredients for promoting
441 health, especially for cholera/shigellosis and chronic diseases associated with oxidative stress.

442

443 **Acknowledgements**

444 The authors gratefully acknowledge financial support from the research grant committees of
445 both the University of Dschang and the Cameroonian Ministry of Higher Education.

446

447 **References:**

448 Acharyya, S., Sarkar, P., Saha, D.R., Patra, A., Ramamurthy T., Bag. P.K., 2015. Intracellular
449 and membrane damaging activities of methyl gallate isolated from *Terminalia chebula*
450 against multi-drug resistant *Shigella species*. Journal of Medical Microbiology 64, 901-
451 909.

452 Al Haidari, R. Phytochemical and Biological Study of *Maytenus Forsskaoliana* (Sebsebe);
453 *Master Thesis*; KSU, Riyadh, KSA, 2002.

454 Araujo, M.G., Hilario, F., Nogueira, L.G., Vilegas, W., Santos, L.C., Bauab, T.M., 2011.
455 Chemical constituents of the methanolic extract of leaves of *Leiothrix spiralis* Ruhland
456 and their antimicrobial activity. Molecules 16, 10479–10490.

457 Avila, J.G., De Liverant, J.G., Martínez, A., Martínez, G., Muñoz, J.L., Arciniegas, A., De
458 Vivar, A.R., 1999. Mode of action of *Buddleja cordata* verbascoside against
459 *Staphylococcus aureus*. Journal of Ethnopharmacology 66, 75–78.

460 Bag, P. K., Bhowmik, P., Hajra, T. K., Ramamurthy, T., Sarkar, P., Majumder, M.,
461 Chowdhury, G., Das, S. C., 2008. Putative virulence traits and pathogenicity of *Vibrio*
462 *cholerae* non-O1, non-O139 isolated from surface waters in Kolkata, India. Applied and
463 Environmental Microbiology 74, 5635-5644.

464 Cordeiro, P.J.M., Vilegas, J.H.Y., Lanças, F.M., 1999. HRGC-MS Analysis of terpenoids
465 from *Maytenus ilicifolia* and *Maytenus aquifolium* ("Espinheira-santa"). Journal of the
466 Brazilian Chemical Society 10, 523-526.

467 Corsino, J., Bolzani, V.S., Pereira, A.M.S., França, S.C., Furlan, M., 1998. Bioactive
468 sesquiterpene pyridine alkaloids from *Maytenus aquifolium*. *Phytochemistry* 48, 137-
469 140.

470 Cowan, M.M., 1999. Plant product as antimicrobial agents. *Clinical Microbiology Reviews*
471 12, 564–582.

472 Da Silva, M.S., De Sousa, D.P., Medeiros, V.M., Folly, M.A.B., Tavares, J.F., Barbosa-Filho,
473 J.M., 2008. *Biochemical Systematics and Ecology* 36, 500-503.

474 Dalsgaard, A., Forslund, A., Bodhidatta, D., Serichantalergs, C., Pitarangsi, L., Pang, T.,
475 1999. A high proportion of *V. cholerae* isolated from children with diarrhoea in
476 Bangkok, Thailand are multiple antibiotic resistant and belong to heterogeneous non-
477 O1, non-O139 O-serotypes. *Epidemiology and Infection* 122, 217–26.

478 Dias, K.S., Marques, M. S., Menezes, I.A.C., Santos, T.C., Silva, A.B.L., Estevam, C.S.,
479 Sant’Ana, A.E.G., Pizza, C., Antonioli, A.R., Marçal, R. M., 2007. Antinociceptive
480 activity of *Maytenus rigida* stem bark. *Fitoterapia* 78, 460-464.

481 Djouossi, M.G., Tamokou, J.D.D., Ngnokam, D., Kuate, J.R., Taponjdjou, A.L., Harakat, D.,
482 Nazabadioko, L.V., 2015. Antimicrobial and antioxidant flavonoids from the leaves of
483 *Oncoba spinosa* Forssk. (Salicaceae). *BMC Complementary and Alternative Medicine*
484 15, 134.

485 El Tahir, A., Satti, G. M., Khalid, S. A., 2001. A novel antiplasmodial activity of pristemerin
486 isolated from *Maytenus senegalensis* (Lam). *Excell. Journal of the Saudi Chemical*
487 *Society* 5, 157-164.

488 Fowler, Z.L., Baron, C.M., Panepinto, J.C., Koffas, M.A., 2011. Melanization of flavonoids
489 by fungal and bacterial laccases. *Yeast* 28,181–188.

490 Garcia, A., Bocanegra-Garcia, V., Palma-Nicolas, J.P., Rivera, G., 2012. Recent advances in
491 antitubercular natural products. *European Journal of Medicinal Chemistry* 49,1–23.

492 Ghazanfar, S.A., "Handbook of Arabian Medicinal Plants", CRC Press, Boca Raton, 1994,
493 Pp.83.

494 Hall, C.A., Cuppett, S.L., 1997. Structure activities of natural antioxidants. In antioxidant
495 methodology *in vitro* concepts. Edited by Hudson BJL. London: Elsevier Applied
496 Science. 1–18.

497 Hossain, M.A., Islam, A., Jolly, Y.N., Kabir, M.J., 2006. A new flavonol glycoside from the
498 seeds of *Zea Mays*. Indian Journal of Chemistry 45, 1319-1321.

499 Ichinose, Y., Yamamoto, K., Nakasone, N., Tanabe, M.J., Takeda, T., Miwatani, T.,
500 1987. Enterotoxicity of ElTor-like haemolysin of non-O1 *Vibrio cholerae*. Infection and
501 Immunity 55, 1090–1093.

502 Kazuma, K., Noda, N., Suzuki, M., 2003. Malonylated flavonol glycosides from the petals of
503 *Clitoria ternatea*. Phytochemistry 62, 229-237.

504 Kazuma, K., Takahashi, T., Sato, K., Takeuchi, H., Matsumoto, T., Okuno, T., 2000.
505 Quinochalcones and flavonoids from fresh florets in different cultivars of *Carthamus*
506 *tinctorius* L. Bioscience Biotechnology Biochemistry 64, 1588-1599.

507 Kuete, V., 2010. Potential of Cameroonian plants and derived products against microbial
508 infections: a review. Planta Medica 76, 1479–1491.

509 Lim, T.Y., Lim, Y.Y., Yule, C.M., 2009. Evaluation of antioxidant, antibacterial and anti-
510 tyrosinase activities of four *Macaranga* species. Food Chemistry 114, 594–599.

511 Lindsey, K. L., Budesinsky, M., Kohout, L., Staden-van, J., 2006. Antibacterial activity of
512 maytenonic acid isolated from the root-bark of *Maytenus senegalensis*. South African
513 Journal of Botany 72, 473-477.

514 Markam, K.R., Ternai, B., Stanley, R., Geiger, H., Mabry, T.J., 1978. Carbon-13 NMR
515 studies of Flavonoids-III Naturally Occurring Flavonoids Glycosides and their
516 Acetylated Derivatives. Tetrahedron 34, 1389-1397.

517 Martucciello, S., Balestrieri, M. L., Felice, F., Estevam, C. S., Sant'Ana, A. E. G., Pizza, C.,
518 Piacente, S., 2010. Effects of triterpene derivatives from *Maytenus rigida* on VEGF-
519 induced Kaposi's sarcoma cell proliferation. *Chemico-Biological Interactions* 183, 450-
520 454.

521 Mot, A.C., Pârvu, M., Damian, G., Irimie, F.D., Darula, Z., Medzihradszky, K.F., Brem, B.,
522 Silaghi-Dumitrescu, R.A., 2012. "yellow" laccase with "blue" spectroscopic features,
523 from *Sclerotinia sclerotiorum*. *Process Biochemistry* 47, 968-975.

524 Muhammad, I., El-Sayed, K.A., Mossa, J.S., Al-Said, M.S., El-Feraly, F.S., Clark, A.M.,
525 Hufford, C.D., Oh, S. and Mayer, A.M.S., 2000. Bioactive 12-Oleanene Triterpene and
526 Secotriterpene Acids from *Maytenus undata*. *Journal of Natural Products* 63, 605-610.

527 Nair, G.B., Ramamurthy, T., Bhattacharya, S.K., Mukhopadhyay, A.K., Garg, S.,
528 Bhattacharya, M.K., 1994. Spread of *Vibrio cholerae* O139 Bengal in India. *The Journal*
529 *of Infectious Diseases* 169, 1029–1034.

530 Nyaa, Tankeu, B.L., Tapondjou, A.L., Barboni, L., Tamokou, J.D.D., Kuate, J.R., Tane, P.,
531 Park, H.J., 2009. NMR assignment and antimicrobial/antioxidant activities of 1 β -
532 hydroxyeuscaphic Acid from the Seeds of *Butyrospermum parkii*. *Natural Product*
533 *Sciences* 15, 76-82.

534 Orabi, K.Y., Al-Qasoumi, S.I., El-Olemy, M.M., Mossa, J.S., and Muhammad, I., 2001.
535 Dihydroagarofuran Alkaloid and Triterpenes from *Maytenus arbutifolia*.
536 *Phytochemistry*, 58, 475-480.

537 Petrus, A.J.A., Hemalatha, S.S., Suguna, G., 2012. Isolation and Characterization of the
538 Antioxidant Phenolic Metabolites of *Boerhaavia erecta* L. Leaves. *Journal of*
539 *Pharmaceutical Sciences and Research* 4, 1856-1561.

540 Pietta, P., Sionetti, P., Mauri, P., 1998. Antioxidant activity of selected medicinal plants.
541 *Journal of Agricultural and Food Chemistry* 46, 4487-4490.

542 Ramamurthy, T., Bag, P.K., Pal, A., Bhattacharya, S.K., Bhattacharya, M.K., Sen, D.,
543 1993. Virulence patterns of *V. cholerae* non-O1 isolated from hospitalized patients with
544 acute diarrhoea in Calcutta. *Indian Journal of Medical Microbiology* 39, 310–317.

545 Sarin, R., Sharma, P., 2012. Isolation and Characterization of Quercetin and Kaempferol *in*
546 *vivo* and *in vitro* from *Pedaliium murex*. *International Research Journal of Pharmacy* 3,
547 184-187.

548 Rice-Evans, C., Miller, N.J., 1994. Total antioxidant status in plasma and body fluids.
549 *Methods in Enzymology* 234, 279-293.

550 Rogé, J., Baumer, P., Bérard, H., Schwartz, J.C., Lecomte, J.M., 1993. The enkephalinase
551 inhibitor, acetorphan, in acute diarrhoea: a double-blind, controlled clinical trial versus
552 loperamide. *Scandinavian Journal of Gastroenterology* 28, 352–354.

553 Salazar, E., Santisteban, J., Chea, E., Gutierrez, M., 2000. Racecadotril in the treatment of
554 acute watery diarrhea. *New England Journal of Medicine* 343, 463–467.

555 Sannomiya, M., Vilegas, W., Rastrelli, L., Pizza, C., 1998. A flavonoid glycoside from
556 *Maytenus aquifolium*. *Phytochemistry* 49, 237-239.

557 Schaneberg, B.T., Green, D. K., Sneden, A. T., J. 2001. Dihydroagarofuransesquiterpene
558 alkaloids from *Maytenus putterlickoides*. *Journal of Natural Products* 64, 624-626.

559 Shirota, O., Tamemura, T., Morita, H., Takeya, K., Itokawa, H., 1996. Triterpenes from
560 Brazilian medicinal plant chuchuhausi (*Maytenus krukovii*). *Journal of Natural Products*
561 59, 1072-1075.

562 Simões, M., Bennett, R.N., Rosa, E.A., 2009. Understanding antimicrobial activities of
563 phytochemicals against multidrug resistant bacteria and biofilms. *Natural Product*
564 *Reports* 26, 746–757.

- 565 Tamokou, J.D.D., Kuate, J.R., Tene, M., Nwemeguela, K.T.J., Tane, P., 2011. The
566 antimicrobial activities of extract and compounds isolated from *Brillantaisia lamium*.
567 Iranian Journal of Medical Sciences 36, 24-31.
- 568 Thakurta, P., Bhowmik, P., Mukherjee, S., Hajra, T.K., Patra, A., Bag, P.K., 2007.
569 Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to
570 treat cholera and diarrhea in India. Journal of Ethnopharmacology 111 (3), 607-612.
- 571 Tullock, J., Richards L., 1993. Childhood diarrhoea and acute respiratory infections in
572 developing countries. The Medical Journal of Australia 159, 46–51.
- 573 Vilegas, W., Sanommiya, M., Rastrelli, L., Pizza, C., 1999. Isolation and structure elucidation
574 of two new flavonoids glycosides from the infusion of *Maytenus aquifolium* leaves.
575 Evaluation of the antiulcer activity of the infusion. Journal of Agricultural and Food
576 Chemistry 47, 403-406.
- 577 Xiang, L., Zhi, L., Xin-feng, Z., Li-juan, W., Yi-nan, Z., Chang-chun, Y., Guang-zhi, S.,
578 2008. Isolation and Characterization of Phenolic Compounds from the Leaves of *Salix*
579 *matsudana*. Molecules 13, 1530-1537.
- 580 Zan, X., Shang, M., Xu, F., Liang, J., Wang, X., Mikage, M., Cai, S., 2013. A-Type
581 proanthocyanidins from the stem of *Ephedra sinica* and their antimicrobial activities.
582 Molecules 18, 5172-5189.
- 583 Zhang, Z., Elsohly, H.N., Li, X-C., Khan, S. I., Broeldon, S. E. J., Rauli, R. E., Cihlar, R. L.,
584 Burandt, C., Walker, L.A., 2003. Phenolic compounds from *Nymphaea odorata*. Journal
585 of Natural Products 66, 548-550.
- 586 Zhao, H., Chen, W., Lu, J., Zhao, M., 2010. Phenolic profiles and antioxidant activities of
587 commercial beers. Food Chemistry 119, 1150–1158.

588

589 **Glossary**

590

- 591 $^{13}\text{C-NMR}$: thirteen Carbon Nuclear Magnetic Resonance
- 592 $^1\text{H NMR}$: Proton Nuclear Magnetic Resonance
- 593 2D NMR : Two-dimension Nuclear Magnetic Resonance
- 594 ABTS : 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
- 595 ATCC : American Type Culture Collection
- 596 CC : Column Chromatography
- 597 COSY : Correlation Spectroscopy
- 598 DMSO : Dimethylsulfoxide
- 599 DPPH : 1,1-diphenyl-2-picrylhydrazyl radical
- 600 EC_{50} : Concentration scavenging 50 % DPPH radicals
- 601 EtOAc : Ethyl acetate
- 602 HMBC : Heteronuclear Multiple Bond Connectivities
- 603 HR-EI-MS : High Resolution Electron Impact Mass Spectrometry
- 604 HR-TOFESIMS : High-resolution time of flight electrospray ionization mass spectrometry
- 605 HSQC : The Heteronuclear Single Quantum Coherence
- 606 IP : Institut Pasteur
- 607 IR : Infra-red
- 608 MBC : Minimum bactericidal concentration
- 609 MeOH : Methanol
- 610 MHA : Mueller Hinton agar
- 611 MHB : Mueller Hinton broth
- 612 MIC : Minimum inhibitory concentration
- 613 NA : Nutrient agar
- 614 $n\text{-BuOH}$: *n*-Butanol
- 615 NMR : Nuclear Magnetic Resonance

616 *Rf*: Retention factor
617 *ROESY*: Rotating-Frame NOE Spectroscopy
618 *SRF/CAM*: Section de réserve forestière du Cameroun
619 *TEAC*: Trolox equivalent antioxidant capacity
620 *TLC*: Thin Layer Chromatography
621 *TMS*: Tetramethylsilane
622 *TOCSY*: Total Correlation Spectroscopy
623 *UV*: Ultra-violet

624

625 **Figure Legends**

626 **Fig. 1.** Chemical structures of flavonoids isolated (**1-9**) from the leaves of
627 *Maytenusbuchananii*. **1**: quercetin-3-*O*- α -L-arabinopyranoside; **2**: epicatechin; **3**: quercetin; **4**: kaempferol-
628 3-*O*- β -D-rhamnopyranoside; **5**: quercetin-3-*O*- α -L-rhamnopyranoside; **6**: quercetin-3-*O*- β -D-glucopyranoside;
629 **7**: epigallocatechin; **8**: kaempferol and **9**: Quercetin-3-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside].

630 **Fig. 2.** Survival curves for *Vibrio cholerae* SG24 (1) cells exposed to the compounds **2, 4, 6,**
631 and ciprofloxacin. Control: MHB medium with DMSO 1% + inoculums.

632 **Fig. 3.** Gallic acid equivalent antioxidant capacity (TEAC; $\mu\text{g/mL}$) of tested samples.

633 Bars represent the mean \pm SD of three independent experiments carried out in triplicate. Letters a-i indicate
634 significant differences between samples according to one way ANOVA and Waller Duncan test; $p < 0.05$.

635 **Fig. 4.** Equivalent concentrations of test samples scavenging 50% of DPPH radical (EC_{50}).

636 Bars represent the mean \pm SD of three independent experiments carried out in triplicate. Letters a-h indicate
637 significant differences between samples according to one way ANOVA and Waller Duncan test; $p < 0.05$.

638

639 **Table 1**

640 Antibacterial activity (MIC and MBC in $\mu\text{g/mL}$) of extracts, isolated compounds and
 641 reference antibacterial drugs.

Extracts/ Compounds	Inhibition parameters	<i>Vibrio</i> <i>cholerae</i> SG24 (1)	<i>Vibrio</i> <i>cholerae</i> CO6	<i>Vibrio</i> <i>cholerae</i> NB2	<i>Vibrio</i> <i>cholerae</i> PC2	<i>Shigella</i> <i>flexneri</i> SDINT	<i>Staphylococcus</i> <i>aureus</i> ATCC 25923
MeOH extract	MIC	512	512	256	256	128	128
	MBC	>512	512	512	512	256	128
	MBC/MIC	/	1	2	2	2	1
<i>n</i> -BuOH extract	MIC	256	128	128	128	64	64
	MBC	256	256	256	256	64	64
	MBC/MIC	1	2	2	2	1	1
EtOAc extract	MIC	128	256	128	64	64	32
	MBC	128	512	128	64	64	64
	MBC/MIC	1	2	1	1	1	2
Hexane extract	MIC	>512	512	>512	512	512	512
	MBC	/	>512	/	>512	>512	>512
	MBC/MIC	/	/	/	/	/	/
1	MIC	256	128	256	256	128	64
	MBC	256	256	>256	256	128	64
	MBC/MIC	1	2	/	1	1	1
2	MIC	32	64	64	128	64	32
	MBC	64	64	128	256	128	32
	MBC/MIC	2	1	2	2	2	1
3	MIC	128	64	128	128	64	64
	MBC	256	128	128	128	128	64
	MBC/MIC	2	2	1	1	2	1
4	MIC	128	128	64	128	64	64
	MBC	256	128	128	256	64	128
	MBC/MIC	2	1	2	2	1	2
5	MIC	128	128	64	128	128	64
	MBC	256	256	128	128	256	128
	MBC/MIC	2	2	2	1	2	2
6	MIC	64	64	64	64	32	16
	MBC	128	128	128	64	32	32
	MBC/MIC	2	2	2	1	1	2
7	MIC	256	128	128	128	64	64
	MBC	>256	128	>256	256	64	64
	MBC/MIC	/	1	/	2	1	1

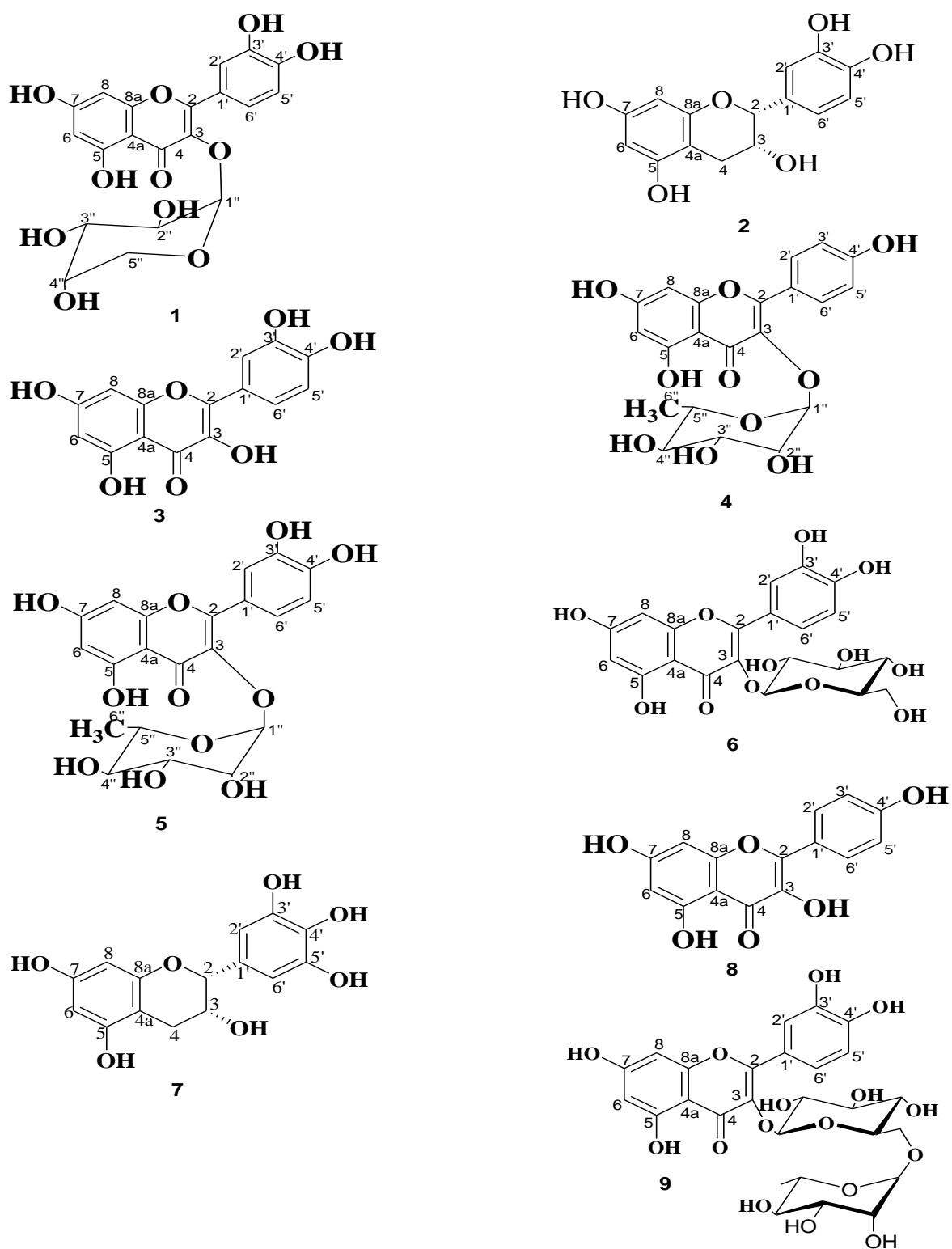
8	MIC	128	256	256	128	128	64
	MBC	256	256	>256	>256	256	128
	MBC/MIC	2	1	/	/	2	2
9	MIC	32	32	64	64	64	32
	MBC	64	32	64	128	64	32
	MBC/MIC	2	1	1	2	1	1
Ampicillin	MIC	16	16	>512	>512	>512	8
	MBC	16	16	>512	>512	>512	8
	MBC/MIC	1	1	/	/	/	1
Ciprofloxacin	MIC	8	8	16	16	16	2
	MBC	8	8	16	16	16	2
	MBC/MIC	1	1	1	1	1	1

642 /: not determined; MIC: Minimum Inhibitory Concentration; MBC Minimum Bactericidal Concentration.

643

644

645



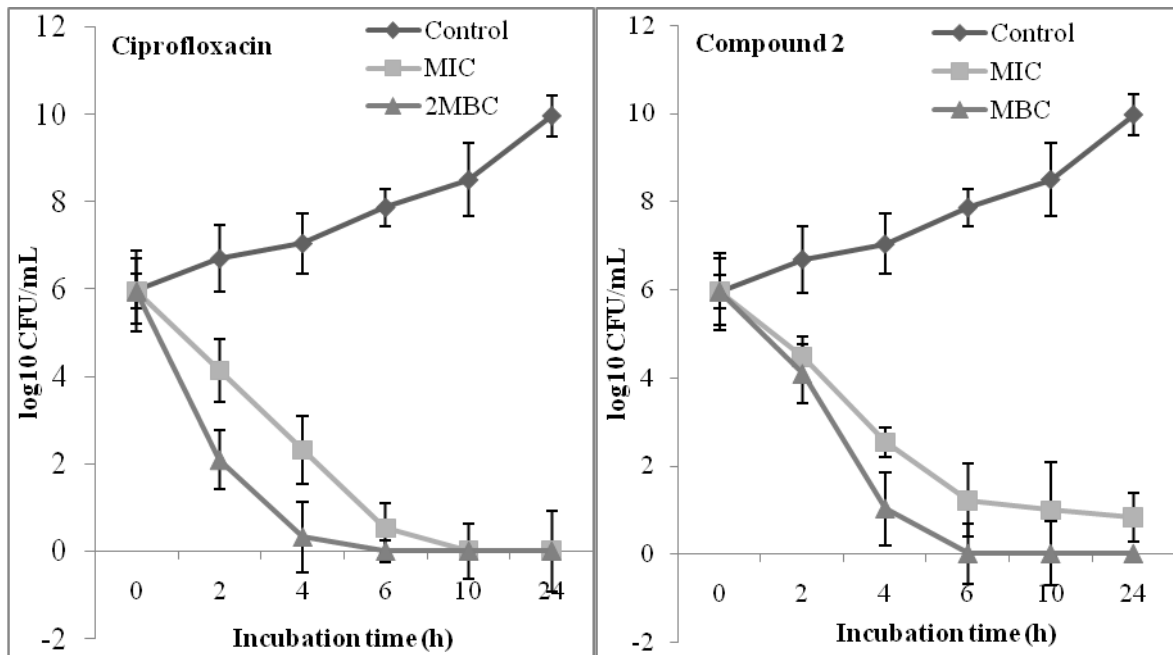
647
 648 **Fig. 1.** Chemical structures of flavonoids isolated (1-9) from the leaves of
 649 *Maytenusbuchananii*. **1:** quercetin-3-*O*- α -L-arabinopyranoside; **2:** epicatechin; **3:**quercetin; **4:**
 650 kaempferol-3-*O*- β -D-rhamnopyranoside; **5:** quercetrin-3-*O*- α -L-rhamnopyranoside; **6:**

651 quercetin-3-*O*- β -D-glucopyranoside; **7**: epigallocatechin; **8**: kaempferol and **9**: Quercetin-3-*O*-
652 [α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside].

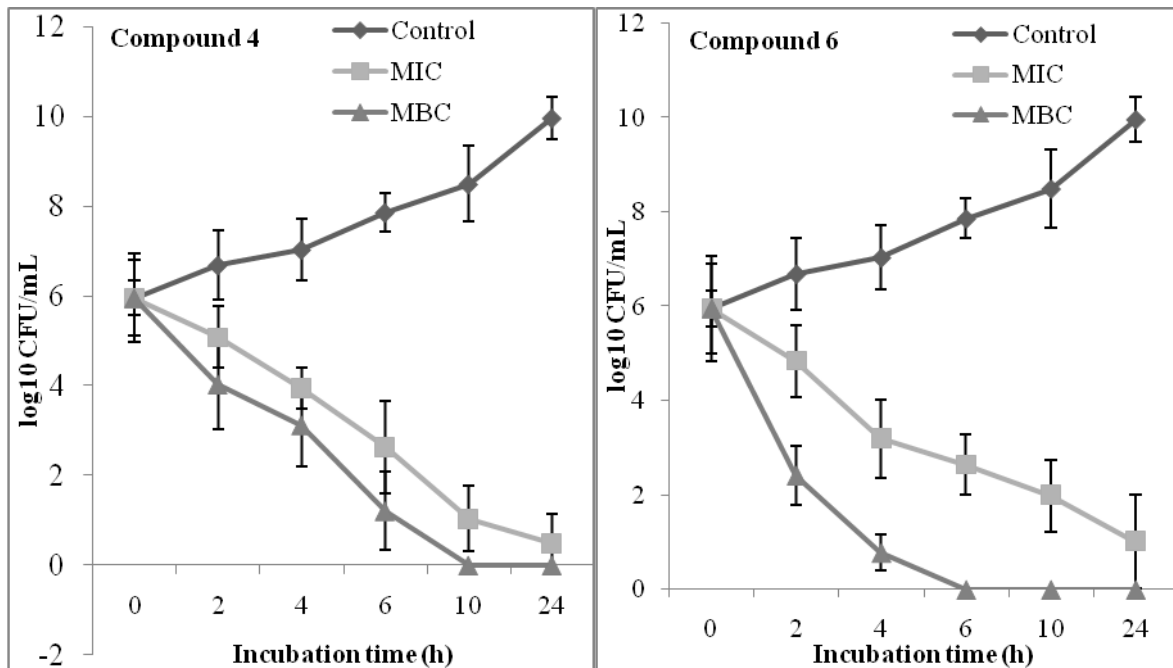
653

654

655



656

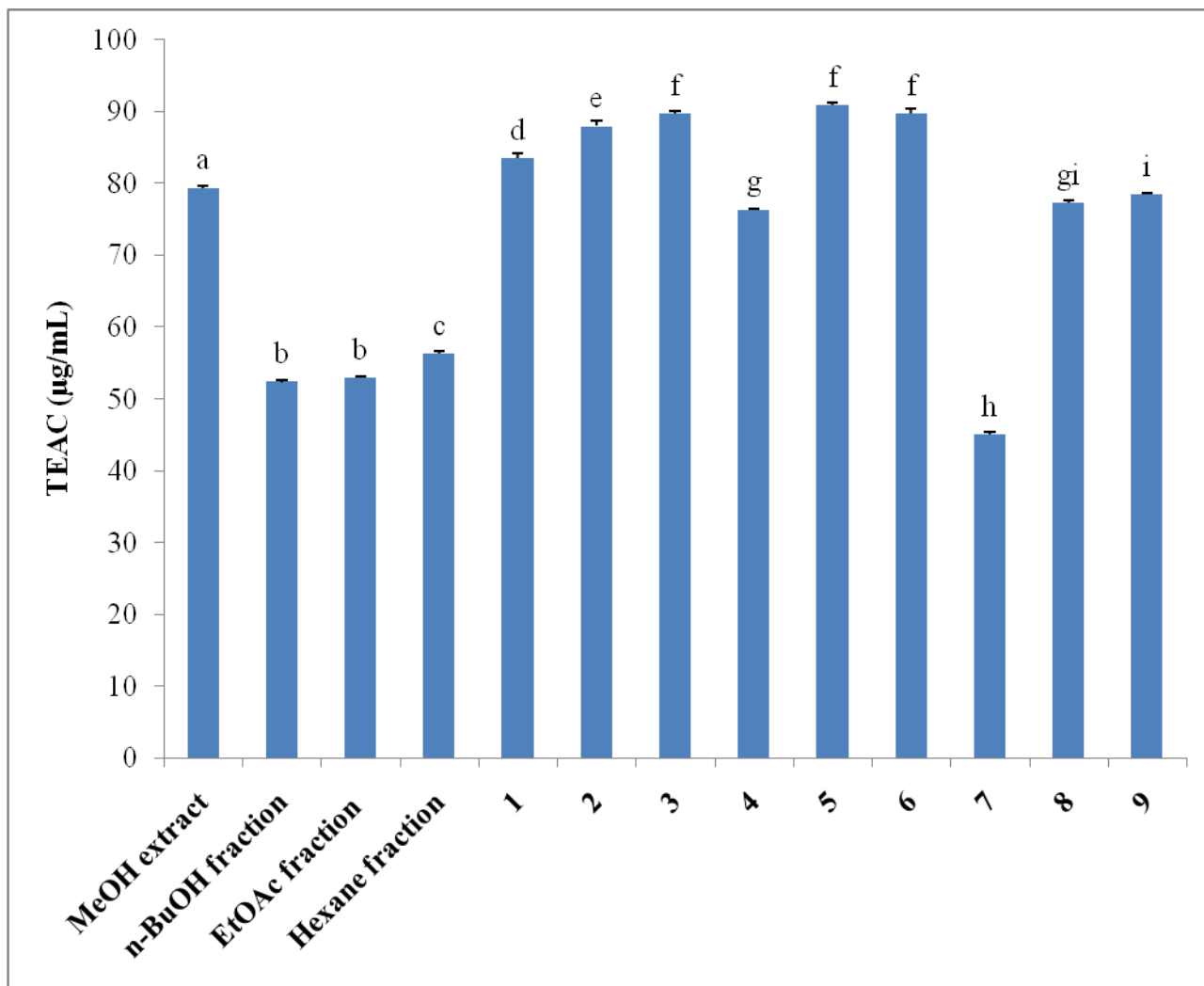


657

658

659 **Fig. 2.** Survival curves for *Vibrio cholerae* SG24 (1) cells exposed to the compounds 2, 4, 6,
660 and ciprofloxacin. Control: MHB medium with DMSO 1% + inoculums.

661



662

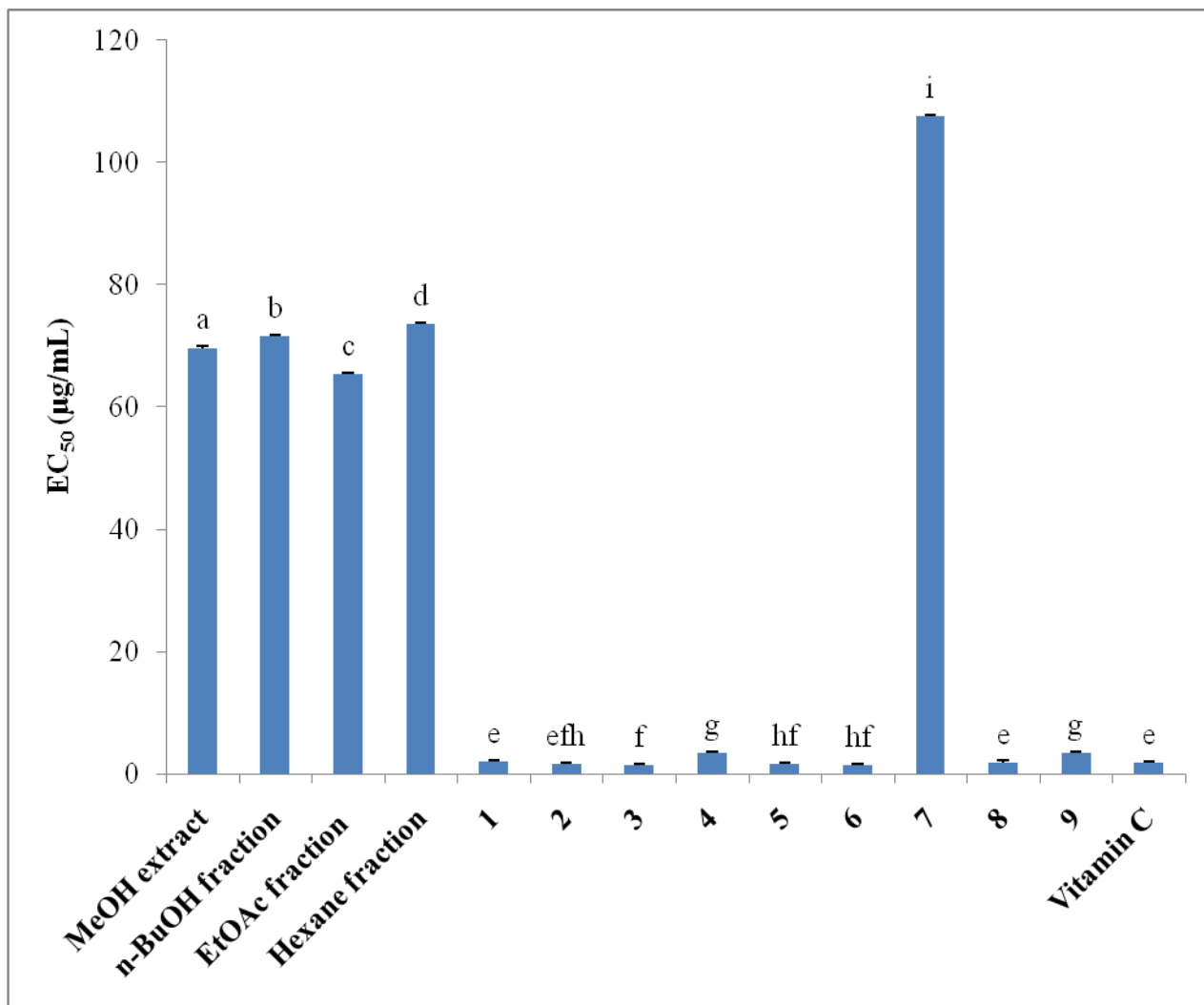
663 **Fig. 3.** Gallic acid equivalent antioxidant capacity (TEAC; µg/mL) of tested samples.

664 Bars represent the mean ± SD of three independent experiments carried out in triplicate.

665 Letters a-i indicate significant differences between samples according to one way ANOVA

666 and Waller Duncan test; $p < 0.05$.

667



668

669 **Fig. 4.** Equivalent concentrations of test samples scavenging 50% of DPPH radical (EC₅₀).

670 Bars represent the mean ± SD of three independent experiments carried out in triplicate.

671 Letters a-h indicate significant differences between samples according to one way ANOVA

672 and Waller Duncan test; p<0.05.

673

674