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1 **Antibacterial, antifungal and antioxidant activities of whole plant chemical**
2 **constituents of *Rumex abyssinicus*.**

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31

32

33 **Abstract**

34 **Background**

35 Antibiotic resistance has contributed to the burden of infectious diseases both in the hospital
36 and community setting, and represents a great threat to public health. Previous studies have
37 revealed the role of reactive oxygen species as intermediate mediators of tissue damage,
38 following antibiotherapies, indicating the need of associating antioxidants to these treatments.
39 Therefore, the present work was designed to study the antibacterial, antifungal and
40 antioxidant activities of extracts and compounds from *Rumex abyssinicus* Jacq.
41 (Polygonaceae), as well as to investigate the antibacterial mechanisms of action of the most
42 effective agents.

43 **Methods**

44 The plant extracts were prepared by maceration in organic solvents followed by column
45 chromatography of the EtOAc fraction and purification of different fractions which led to the
46 isolation and characterization of pure compounds. The antimicrobial activities of the
47 extracts/compounds and their combinations with ciprofloxacin and fuconazole were evaluated
48 using the broth microdilution method by determining the minimum inhibitory concentration
49 (MIC) and minimum microbicidal concentration (MMC). The effects of the extracts on the
50 bacterial cell membrane and microbial respiratory chain dehydrogenase enzyme activity were
51 determined by spectrophotometric methods. Antioxidant activity was evaluated using 1,1-
52 diphenyl-2-picrylhydrazyl (DPPH) and gallic acid equivalent antioxidant capacity (GAEAC)
53 assays.

54 **Results**

55 Chrysophanol (**1**), physcion (**2**), Ergosta-6,22-diene-3,5,8-triol (**3**), emodine (**4**), 6-
56 hydroxyemodin (citreorosein) (**5**), chrysophanein (**6**) and physcionin (**7**) were isolated from
57 EtOAc fraction of *R. abyssinicus* and displayed different degrees of antimicrobial activities

58 (MIC = 8 - 256 µg/mL). The MeOH extract and compounds **2** and **4** exhibited synergistic
59 effects with ciprofloxacin and fluconazole. Compounds **1**, **2** and the combined mixture of **6** +
60 **7** displayed the highest antioxidant activity (GAEAC = 83.38 – 106.03 µg/mL).

61 **Conclusion**

62 *R. abyssinicus* is a potential source of antibacterial, antifungal and antioxidant agents. The
63 antibacterial mechanisms of action of the MeOH extract and compound **2** are due to
64 disruption of the cytoplasmic membrane and inhibition of the microbial respiratory chain
65 dehydrogenase enzyme activity. To the best of our knowledge, this is the first report of test
66 samples and ciprofloxacin / fluconazole association against MDR strains. The observed
67 activity of the isolated compounds against bacteria and fungi including MDR strains deserves
68 further exploration.

69

70 **Keywords:** *Rumex abyssinicus*, antimicrobial, antioxidant, multiresistant strains, membrane
71 leakage, dehydrogenase activity.

72

73 **Background**

74 The increasing appearance of resistant pathogenic bacteria and fungi to synthetic
75 antimicrobial agents represents an alarming threat to public health. The most commonly
76 encountered antibiotic-resistant bacteria, methicillin-resistant *S. aureus* (MRSA),
77 vancomycin-resistant *Enterococci* (VRE), and penicillin and cephalosporin-resistant
78 *Streptococci* (PCRS) have contributed to the burden of infectious diseases both in the hospital
79 and community setting [1]. Majority of the classical antibiotics today sold in the market have
80 major disadvantages resulting from the side effects on patients and the developed multiple
81 drug resistances by the pathogenic microorganisms [2]. Hence, a growing interest in the
82 discovery of new natural antimicrobial agents has been observed, with the objective to combat

83 these resistant pathogens while avoiding or minimizing the undesirable consequences and side
84 effects related to the consumption of synthetic antibiotics [3]. Previous studies have
85 demonstrated detrimental side effects of bactericidal antibiotics such as quinolones,
86 aminoglycosides while, β -lactams caused mitochondrial dysfunction and reactive oxygen
87 species (ROS) overproduction in mammalian cells, leading to oxidative damage to DNA,
88 proteins, and membrane lipids [4]. Therefore, associating antioxidant with antibiotic therapy
89 seems to be a strategy to mitigate or prevent side effects.

90 Reactive oxygen species are oxygen-derived free radicals, metabolic products arising from
91 endoplasmic reticulum and mitochondria of various cells. Free radicals which are delivered as
92 a consequence of typical biochemical responses in the body are implicated in diabetes,
93 atherosclerosis, ageing, cancer, inflammation, immunosuppression, neurodegenerative
94 disorders and ischemic heart disease [5]. Free radicals are proven to be highly toxic to
95 pathogens and they are used as a means to prevent tissue colonisation by the microorganisms.
96 Thus, the production of free radicals is highly elevated during infection and this situation can
97 cause oxidative stress; which further complicates the patient's condition. Secondary
98 metabolites of plants such as flavonoids and terpenoids play an important role in the defense
99 against free radicals and pathogenic microorganisms [6]. Previous studies have shown that the
100 use of plant-derived medicines have increased tremendous interest in the search of alternative
101 antimicrobial and antioxidant agents because of the perception that they cause minimal
102 adverse effects and have a long history of use in folk medicine for the treatment of infectious
103 diseases and oxidative stress conditions [7-8]. However, the combination of antioxidant and
104 antimicrobial agents has gained wide acceptance within the pharmaceutical industries [9]. In
105 fact, combining two or more compounds could be more effective for the improvement of
106 antioxidant and antimicrobial activities and could offer a synergistic effect. The fact that
107 flavonoids, terpenoids and saponins can improve the susceptibility of some bacteria to certain

108 antibiotics have been demonstrated in many studies [10-11]. Natural products of higher plants
109 may possess a new source of antimicrobial and antioxidant agents with possibly novel
110 mechanisms of action [12]. Hence, three levels of interactions are involved: interaction with
111 the outer cellular components; interaction with the cytoplasmic membrane and interaction
112 with cytoplasmic constituents. Natural products can act with the bacterial cells at one level or
113 all three levels of interaction to produce their antimicrobial activities. Their systematic and
114 methodical screening may result in the discovery of novel active principles to overcome
115 resistance mechanisms in multidrug resistant microorganisms.

116 It is well documented that plants belonging to *Rumex* genus possess suitable medicinal
117 properties, which are based mainly on the presence of anthraquinones, flavonoids and
118 terpenoids [13]. *R. abyssinicus* Jacq. (Family: Polygonaceae) commonly known as Spinach
119 Rhubarb, is a large herbaceous perennial plant that grows up to 4 m in height. This plant is
120 mainly found in tropical Africa especially in the drier areas. *R. abyssinicus* is locally used as
121 astringent, purgative, taeniafuge, depurative and hemostatic [14]. The plant is also used in the
122 management of breast cancer, gonorrhoea, liver diseases, hypertension and hemorrhoids [14].
123 The fresh or dried plant is applied externally to treat cough, pneumonia, wounds, rheumatism,
124 sores and scabies [14]. An extract of rhizome is consumed to control mild forms of diabetes
125 and, with water, to cure stomach-ache [14]. The crude extracts of *R. abyssinicus* have been
126 shown to possess antibacterial [15-16], anticancer [16], antiviral [15], anti-inflammatory [15,
127 17], antioxidant [18], wound healing [17], antimalarial [19], diuretic and analgesic [20]
128 activities. Up to date, there has been no report on the antibacterial, antifungal and antioxidant
129 activities of compounds isolated from *R. abyssinicus*, although there is an ample
130 ethnobotanical claim for these properties. Therefore, the present work was designed to study
131 the antibacterial, antifungal and antioxidant activities of extracts and compounds from *R.*
132 *abyssinicus* as well as to investigate the mechanisms of antibacterial activity of the most

133 effective agents. Interactions of the methanol extract/compounds from *R. abyssinicus* and
134 antibiotics against bacterial and yeast species were also investigated.

135

136 **Methods**

137 **General experimental procedures**

138 **NMR analysis**

139 The ^1H and ^{13}C -NMR spectra were recorded on a Bruker Avance III 600 spectrometer
140 equipped with a cryo-platform (^1H at 600 MHz and ^{13}C at 150 MHz). 2D NMR experiments
141 were performed using standard Bruker microprograms (Xwin-NMR version 2.1 software). All
142 chemical shifts (δ) are reported in parts per million (ppm) with the solvent signal as reference
143 relative to TMS ($\delta = 0$) as internal standard, while the coupling constants (J) are given in
144 Hertz (Hz). Deuterated solvents, methanol (CD_3OD), dimethyl sulfoxide ($\text{DMSO-}d_6$), and
145 chloroform (CDCl_3) were used as solvents for the NMR experiments.

146 **Chromatographic methods**

147 Column chromatography was run on Merck silica gel (VWR, France) 60 (70–230
148 mesh) and gel permeation on Sephadex LH-20 (VWR, France), while TLC was carried
149 out on silica gel GF254 pre-coated plates and the spots were visualized by an UV lamp
150 multiband UV-254/365 nm (ModelUVGL-58 Upland CA 91786, U.S.A) followed by
151 spraying with 50% H_2SO_4 and then heating at 100 °C.

152 **Sample collection**

153 The whole plant of *Rumex abyssinicus* Jacq. was collected in February 2018 from the wild in
154 Dschang, western region of Cameroon. The botanical identification was carried out by Victor
155 Nana, a botanist of the National Herbarium of Cameroon, where a voucher specimen (N°
156 50551/HNC) has been deposited.

157 For the collection of plants, no specific permits were required for the described field studies.
158 For any locations/activities, no specific permissions were required. All locations of plant
159 collection were not privately-owned or protected in any way and the field studies did not
160 involve endangered or protected species.

161

162 **Extraction and fractionation**

163 The whole plant material of *R. abyssinicus* was air-dried at room temperature and ground into
164 fine powder. This dried powder (4.5 kg) was extracted at room temperature with methanol (3
165 × 20 L, 72 h) to yield 200 g of crude methanol extract after evaporation of solvent under
166 reduced pressure. A part of this crude extract (195 g) underwent a differential solubilization
167 with H₂O/EtOAc (300 mL/500 mL) followed by H₂O/*n*-BuOH (300 mL/500 mL). After
168 evaporation of each solvent under reduced pressure, we obtained 50 g of EtOAc and 18 g of
169 *n*-BuOH extracts respectively.

170

171 **Isolation of Compounds**

172 A part of the EtOAc fraction of *R. abyssinicus* (45 g) was subjected to silica gel column
173 chromatography eluted with *n*-hexane-EtOAc (95:5 → 80:20) followed by EtOAc-MeOH
174 (95:5 → 70:30) gradient graduated elution to yield seventy fractions of 400 mL each. These
175 were combined on the basis of TLC profiles to yield eight major fractions A-H (A: 1-3; B: 4-
176 10; C: 11-22; D: 23-28; E: 29-35; F: 36-44; G: 45-63; H: 64-70). Fraction A (4.0 g)
177 underwent column chromatography on silica gel with the *n*-hexane-EtOAc system (95:5) to
178 yield compounds **1** (15 mg) [21] and **2** (17 mg) [22]. Sephadex LH-20 gel column
179 chromatography of fraction C (1.9 g) led to two sub-fractions (C₁ and C₂). Purification of sub-
180 fraction C₁ (500 mg) by silica gel column chromatography (*n*-hexane-EtOAc, 90:10→80:20)
181 resulted in compound **3** (15 mg) [23]. The sub-fraction C₂ (300 mg), was purified on

182 Sephadex LH-20 gel column using MeOH as eluent to give compound **4** (40 mg) [22]. After
183 Sephadex LH-20 gel column using MeOH, fraction D (3.74 g) led to three sub-fractions D₁,
184 D₂ and D₃. Purification of D₃ (400 mg) sub-fraction by silica gel column chromatography
185 with *n*-hexane-EtOAc (85:15) gave compound **5** (11 mg) [24]. Recrystallization of fraction G
186 (5 g) afforded a mixture of two compounds **6** + **7** (10 mg) [25] which unfortunately, was not
187 separated by silica gel column chromatography method.

188

189 **Antimicrobial assay**

190 **Microorganisms**

191 Five bacteria and two yeasts were tested for their susceptibility to the studied samples. The
192 studied microorganisms were three Gram-positive (*Staphylococcus aureus* ATCC25923,
193 methicillin sensitive *S. aureus* MSSA01 and methicillin resistant *S. aureus* MRSA03) and
194 two Gram-negative (*Pseudomonas aeruginosa* ATCC27853, *Shigella flexneri* SDINT)
195 bacteria and two yeast strains of *Candida albicans* ATCC10231 and *Cryptococcus*
196 *neoformans* H99. These microorganisms were taken from our laboratory collection. The
197 bacterial and fungal species were maintained on agar slant at +4 °C and on nutrient agar (NA,
198 Conda, Madrid, Spain) and Sabouraud Dextrose Agar (SDA, Conda) slants respectively, prior
199 to any antimicrobial test.

200

201 **Determination of minimum inhibitory concentration (MIC) and minimum microbicidal** 202 **concentration (MMC).**

203 MIC and MMC values were determined as described earlier [26]. The test samples
204 were dissolved in dimethylsulfoxide (DMSO). The negative control well consisted of 195 µL
205 of MHB or SDB and 5 µL of the standard inoculum. The MICs were visually assessed and
206 were considered as the lowest sample concentration inhibiting the growth of the

207 microorganism. The lowest concentrations that showed no visual growth after the sub-
208 culturing were considered as the minimum microbial concentration (MMCs). Ciprofloxacin
209 (Sigma-Aldrich, Steinheim, Germany) and fluconazole (Merck, Darmstadt, Germany) were
210 used as positive controls for bacteria and yeasts, respectively. All tests were performed in
211 triplicate.

212 **Combined effect of antibiotics and MeOH extract, compounds 2 or 4**

213 The antimicrobial effects of a combination of samples (MeOH extract, compounds 2
214 and 4), which exhibited the highest antimicrobial activities, and antibiotics (ciprofloxacin and
215 fluconazole) were assessed by the checkerboard method as previously described [27]. The
216 inoculum was initially prepared as described above. The test microorganisms were inoculated
217 into a 96-well microtitre plates and a serial dilution of two antimicrobial agents: antibiotic and
218 MeOH extract of compound 2 or 4. Each well consisted of unique combination of test sample
219 and antibiotic concentrations. The plates were then incubated for 24 h at 37 °C. The analyses
220 were performed in triplicates. And the antimicrobial agents interactions were evaluated by
221 calculating the fractional inhibitory concentration (FIC) indices. The FIC is defined as
222 follows: MIC of antibiotic tested in combination/MIC of antibiotic tested alone + MIC of
223 extract/compound tested in combination/MIC of extract/compound tested alone. The FIC
224 index is interpreted as $FIC \leq 0.5$: synergistic effect, $0.5 < FIC \leq 1$: additive effect, $1 < FIC \leq 2$:
225 indifferent effect, and $FIC > 2.0$: antagonistic effect.

226 **Antibacterial mechanism studies**

227 *Cell membrane leakage assay*: The alteration of cell membrane of *P. aeruginosa* and *S.*
228 *flexneri* was evaluated by measuring the optical densities at 260 nm and 280 nm of
229 the bacterial suspensions in the presence and absence of MeOH extract and compound 2 using
230 the method described by Karsha and Lakshmi [28].

231 ***Evaluation of the sugar leakage through membrane of bacteria:*** 10 mL of the bacterial
232 suspension containing 10^8 CFU/mL were inoculated into MeOH extract or compound **2** at $\frac{1}{2}$
233 MIC, MIC and 2MIC and incubated at 37 °C under agitation at 150 rpm for 12 h. After
234 incubation, the mixture was centrifuged at 12,000 rpm and the supernatant was collected. The
235 concentration of reducing sugar was determined spectrophotometrically at 550 nm using 3-5
236 dinitro-salicylic acid (DNS) [29].

237 ***Assay of respiratory chain dehydrogenase enzyme activity in the bacteria***

238 Cellular bioenergetic is a domain with promising future in the development of novel
239 antimicrobials. Several studies have evaluated the bioenergetics of various bacterial
240 pathogens, which explain the abilities of electron donor and acceptor utilisation, and the
241 regulation of components of electron transport chain in bacteria. In this assay, the effect of the
242 most effective agents on respiratory chain dehydrogenase enzyme activity of pathogenic
243 bacteria as a test for mechanism of antibacterial action was performed. The dehydrogenase
244 activity assay was performed using 2,3,5- triphenyl tetrazolium chloride (TTC) as previously
245 described [30]. The TTC serves as the artificial electron acceptor and is reduced to red
246 coloured triphenyl formazan (TPF). The assay was carried out with 3 ml of nutrient broth-
247 glucose-TTC medium, supplemented with varying concentrations of MeOH extract or
248 compound **2** in 20 mL screw-capped test tubes. The TPF produced after each exposure period
249 (0, 30, 60 min) was extracted in 4 mL of amyl alcohol and determined spectrophotometrically
250 at 500 nm. The amount of formazan produced was determined from a standard dose-response
251 curve ($R^2 = 0.9983$). Dehydrogenase activity was expressed as the amount of TPF formed
252 (μg) per amount of dry cell weight of cell biomass (in mg). Data were expressed as the mean
253 \pm standard deviation.

254 **Antioxidant assay**

255 **Gallic acid equivalent antioxidant capacity (GEAC) assay**

256 The GEAC test was done as previously described [31] with slight modifications. In a
257 quartz cuvette, to 950 μ L acetate buffer (pH =5.0, 100 mM), the following were added: 20 μ L
258 laccase (1 mM stock solution), 20 μ L test sample, 10 μ L ABTS (2,2'-azinobis(3-
259 ethylbenzothiazoline-6-sulfonic acid) (74 mM stock solution). The purification of laccase
260 from *Sclerotinia sclerotiorum* was done according to the protocol described [32]. The sample
261 concentrations in the assay mixture were 800, 400, 200, 100, 10 μ g/mL for the extracts and
262 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56 μ g/mL for the isolated compounds. The content of
263 the generated ABTS^{•+} radical was measured at 420 nm after 240 s reaction time and was
264 converted to gallic acid equivalents by the use of a calibration curve (Pearson's correlation
265 coefficient: $r = 0.997$) constructed with 0, 4, 10, 14, 28, 56, 84 μ M gallic acid standards rather
266 than Trolox. Experiments were done in triplicate.

267 **Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay**

268 The free radical scavenging activity of extracts and compounds was evaluated according to
269 described methods [33]. The EC₅₀ (μ g/ml), which is the amount of sample necessary to inhibit
270 by 50% the absorbance of free radical DPPH was calculated [33]. Vitamin C was used as a
271 standard control. All the analyses were carried out in triplicate.

272

273 **Cytotoxicity assay**

274 Three male Wistar rats (*Rattus norvegicus*), aged 10 – 12 weeks and weighing 230 to 240 g
275 were used. These animals were bred in the animal house of the University of Dschang,
276 Cameroon. Efforts were also made to minimize animal suffering and to reduce the number of
277 animal used in the experiment. All the rats were anaesthetized via intraperitoneal injection of
278 the mixture of ketamine (50 mg/kg body weight, BW) and xylazine (10 mg/kg BW), in a
279 dose that is commonly used for operation purposes. Subsequently the unconscious animals
280 were decapitated swiftly and the whole blood (10 mL) was collected by cardiac puncture into

281 a conical tube containing Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant.
282 Erythrocytes were obtained by centrifugation at room temperature for 10 min at 1,000 x g and
283 were washed three times in PBS buffer [34]. The cytotoxicity was evaluated as previously
284 described [34].

285

286 **Statistical analysis**

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

291

292 **Results**

293 **Chemical composition**

294 A total of five pure compounds (**1** to **5**) and one mixture of two compounds (**6** and **7**) were
295 isolated from EtOAc fraction of *R. abyssinicus*. Based on their spectral data (¹H and ¹³C
296 NMR, ¹H-¹H COSY, HSQC, HMBC, and ROESY), their chemical structures as illustrated in
297 Fig. 1 were identified as follows: **1**: Chrysophanol; **2**: Physcion; **3**: Ergosta-6,22-diene-3,5,8-
298 triol; **4**: Emodine; **5**: 6-hydroxyemodin (Citreoersein); **6**: Chrysophanein; **7**: Physcionin.
299 Compounds **1**, **2** and **3** were derived from the EtOAc fraction while the remaining were
300 isolated from the methanolic extract.

301 **Chrysophanol**(**1**): Yellow powder ; (C₁₅H₁₀O₄) ; ¹H-NMR (600 MHz, CDCl₃) δ : 12.08 (s, 1-
302 OH), 11.97 (s, 8-OH) 7.84 (d, 7.5 Hz, H-5), 7.77 (br s, H-6), 7.69 (br s, H-4), 7.30 (d, 8.4Hz,
303 H-7), 7.12 (br s, H-2), 2.48 (s, -CH₃); ¹³C-NMR (150 MHz, CDCl₃) δ : 192.5 (C-9), 182.1 (C-
304 10), 162.7 (C-1), 162.4 (C-8), 149.3 (C-3), 137.0 (C-6), 133.6 (C-11), 133.2 (C-14), 124.5 (C-
305 7), 124.3 (C-2), 121.3 (C-4), 119.9 (C-5), 115.8 (C-12), 113.7 (C-13), 22.4 (-CH₃).

306 **Physcion (2)**: Yellow powder ; (C₁₆H₁₂O₅) ; ¹H-NMR (600 MHz, CDCl₃) δ: 12.34 (*s*, 1-OH),
307 12.15 (*s*, 8-OH), 7.65 (*br s*, H-5), 7.39 (*d*, 2.5 Hz, H-4), 7.10 (*br s*, H-7), 6.70 (*d*, 2.5 Hz, H-
308 2), 3.95 (*s*, OCH₃), 2.46 (*s*, -CH₃); ¹³C-NMR (150 MHz, CDCl₃) δ: 190.8 (C-9), 182.1 (C-10),
309 166.6 (C-3), 165.2 (C-1), 162.5 (C-8), 148.5 (C-6), 135.2 (C-14), 133.2 (C-11), 124.6 (C-7),
310 121.4 (C-5), 113.7 (C-12), 110.3 (C-13), 108.3 (C-4), 106.8 (C-2), 56.1(-OCH₃), 22.2 (-CH₃).

311 **Ergosta-6,22-diene-3,5,8-triol (3)**: White powder ; (C₂₈H₄₆O₃) ; ¹³C-NMR (150 MHz,
312 CDCl₃): δ 135.5 (C-6), 135.3 (C-22), 132.4 (C-23), 130.9 (C-7), 82.3 (C-5), 79.6 (C-8), 66.6
313 (C-3), 56.3 (C-17), 51.8 (C-14), 51.2 (C-9), 44.7 (C-13), 42.9 (C-24), 39.9 (C-20), 39.4 (C-
314 11), 37.1 (C-10), 37.0 (C-4), 34.8 (C-1), 33.2 (C-25), 30.2 (C-2), 28.8 (C-15) 23.5 (C-12),
315 21.0 (C-21), 20.8 (C-16), 20.1 (C-26), 19.8 (C-28), 18.3 (C-19), 17.7 (C-27), 13.0 (C-18).

316 **Emodin (4)**: Red powder; (C₁₅H₁₀O₅); ¹H-NMR (600 MHz, DMSO-*d*₆) δ: 12.1 (*s*, 3-OH),
317 12.0 (*s*, 8-OH) 7.48 (*d*, 0.7Hz, H-5), 7.16 (*d*, 0.7Hz, H-7) 7.11 (*d*, 2.4 Hz, H-4) 6.59 (*d*, 2.4Hz,
318 H-2), 2.41 (*s*, -CH₃); ¹³C-NMR (150 MHz, DMSO-*d*₆) δ: 190.2 (C-9), 181.9 (C-10), 166.1 (C-
319 1), 164.9 (C-3), 161.9 (C-8), 148.7 (C-6), 135.6 (C-14), 133.3 (C-11), 124.6 (C-7), 120.9 (C-
320 5), 113.8 (C-12), 109.4 (C-13), 109.3 (C-4), 108.4 (C-2), 21.9 (-CH₃).

321 **Citreorosein (5)**: Red powder; (C₁₅H₁₀O₆) ; ¹H-NMR (600 MHz, CD₃OD) δ: 7.28 (*br s*, H-2),
322 7.75 (*br s*, H-4), 7.20 (*br s*, H-5), 6.54 (*br s*, H-7), 4.70 (*s*, -OCH₂-); ¹³C-NMR (150 MHz,
323 CD₃OD) δ: 191.5 (C-9), 183.4 (C-10), 169.1 (C-8), 166.7 (C-6), 163.7 (C-1), 152.9 (C-3),
324 136.9 (C-11), 135.0 (C-14), 122.2 (C-2), 118.4 (C-4), 115.9 (C-13), 111.1 (C-5), 109.2 (C-7),
325 108.5 (C-12), 64.1 (-OCH₂-).

326 **Chrysophanein (6)**: Yellow powder ; (C₂₁H₂₀O₉) ; ¹H-NMR (600 MHz, DMSO-*d*₆) δ: 13.1
327 (*s*, 1-OH), 7.88 (*m*, H-5), 7.86 (*m*, H-6) 7.71 (*d*; 7.9Hz, H-7) 7.51 (*br s*, H-4) 7.21 (*br s*, H-2)
328 5.20–3.10 (Glu), 2.44 (*s*, 3-CH₃); ¹³C-NMR (150 MHz, DMSO-*d*₆) δ: 188.0 (C-9), 182.6 (C-
329 10), 162.2 (C-1), 158.7 (C-8), 148.1 (C-3), 136.4 (C-6), 135.2 (C-11), 132.6 (C-14), 124.5 (C-

330 2), 122.9 (C-7), 121.0 (C-5), 119.8 (C-4), 115.3 (C-12), 115.2 (C-13), 101.0 (C-1'), 77.8 (C-
331 5'), 77.0 (C-3'), 73.7 (C-2'), 70.0 (C-4'), 61.1 (C-6'), 21.9 (-CH₃).

332 **Physcionin (7)**: Yellow powder ; (C₂₂H₂₂O₁₀) ; ¹H-NMR (600 MHz, DMSO-*d*₆) δ: 12.8 (*s*, 1-
333 OH), 7.50 (*br s*, H-4), 7.37 (*d*, 2.3Hz, H-5) 7.19 (*d*, 2.3 Hz, H-7) 7.18 (*br s*, H-2), 5.20-3.10
334 (Glu), 3.97 (*s*, -OCH₃), 2.42 (*s*, -CH₃); ¹³C-NMR (150 MHz, DMSO-*d*₆) δ 186.9 (C-9), 182.4
335 (C-10), 165.2 (C-6), 162.1 (C-1), 161.2 (C-8), 147.6 (C-3), 135.1 (C-11), 132.5 (C-14), 124.7
336 (C-2), 119.7 (C-4), 114.96 (C-13), 114.95 (C-12), 107.9 (C-7), 106.9 (C-5), 101.1 (C-1'), 77.9
337 (C-5'), 77.1 (C-3'), 73.8 (C-2'), 70.3 (C-4'), 61.3 (C-6'), 56.6 (-OCH₃) 21.8 (-CH₃).

338

339 **Antimicrobial activity**

340 Analysis of inhibitory parameters revealed variability in antimicrobial activity within extracts
341 and isolated compounds, and within microbial strains tested (Table 1). Thus, all the three
342 organic extracts exhibited activity in all the microorganisms tested, with MIC and MMC
343 values varying between 32 and 256 µg/mL. The highest activity observed with the crude
344 extract (MIC = 32 µg/mL) was found against *C. neoformans*, and particularly the EtOAc
345 fraction against *S. flexneri*, *S. aureus* and *C. albicans*. This fraction was the most active with
346 MIC values between 32 and 64 µg/mL. Although in most cases the MMC values appeared to
347 be double the MICs, the methanolic and acetate extracts were found to be fungicidal against
348 *C. neoformans* at 32 µg/mL while at the same concentration the EtOAc fraction was
349 bactericidal against *S. flexneri*.

350 With regards to the isolated compounds, their MMC were either equal to or two times higher
351 than the corresponding MIC. The most active inhibited bacterial and fungal growth at a
352 concentration of 8 µg/mL, and in some cases their activity was comparable to that of the
353 reference drug. At this concentration of 8 µg/ml, the compounds **2** and **4** inhibited the growth
354 of *C. albicans* and *C. neoformans*, and also exhibited a fungicidal activity against these

355 strains. An inhibition of growth accompanied by microbicidal activity was noted with the
356 compound **4** as well as the mixture **6 + 7** against *C. neoformans* and *S. aureus*. However,
357 these compounds displayed bactericidal activity against *S. flexneri* at 16 µg/mL, i.e. twice
358 their MIC. Similar observations were noted on compound **2** against *P. aeruginosa*. All of the
359 strains tested were less sensitive to the compound **3**.

360 **Combined effect of the MeOH extract/compounds and antibiotics**

361 The results of the interaction study between the methanolic extract/compounds (**2** and **4**), and
362 ciprofloxacin in bacteria or fluconazole in yeasts are presented in Table 2. We found that the
363 effect of the association of the methanolic extract with this antibacterial and antifungal agents
364 was synergistic in nature whatever the microorganism tested. Nevertheless, the association
365 between compound **2** and ciprofloxacin exhibited an additive effect against MSSA01 and
366 MRSA03 strains. The interaction between compound **4** and fluconazole was also shown to be
367 additive with respect to the yeasts *C. albicans* and *C. neoformans*. An additive effect was also
368 observed against *C. albicans* when this compound was combined with ciprofloxacin.

369 **Mechanisms of Antibacterial Activity**

370 The study of the mechanism of action of the extracts compound **2** in comparison with the
371 reference antibiotic, ciprofloxacin was carried out by measuring on the one hand the optical
372 densities at 260 and 280 nm and on the other hand the appearance of reducing sugars in the
373 bacterial culture suspensions, and finally by measuring the activity of the respiratory chain
374 dehydrogenase enzyme activity. These results are illustrated in Fig. 2, 3 and 4, respectively.

375 ***Effect on the Cell membrane leakage***

376 It was noted that the methanolic extract and the compound **2** induced leakage of biological
377 material absorbing at 260 and 280 nm by *P. aeruginosa* (Fig. 2a and 2b) and *S. flexneri* (Fig.
378 2c and 2d), at concentrations equal to or two times their MIC. However, this effect of the
379 tested samples on membrane leakage was found to be concentration dependent in the

380 presence of both microorganisms. In the presence of *P. aeruginosa*, compound **2** displayed
381 higher leakage effect than the crude extract.

382 ***Effect on the sugar leakage of membrane from the bacteria.***

383 The study of the appearance of reducing sugar also revealed that the latter increased in the
384 bacterial culture suspensions as the concentration of the methanolic extract as well as that of
385 the compound **2** tested increased from $\frac{1}{2}$ CMI to 2 MIC through MIC (Fig. 3). The
386 appearance of reducing sugar was also more important in the presence of the product **2** as
387 compared to the *R. abyssinicus* extract.

388 ***Effect on the respiratory chain dehydrogenase enzyme activity in the bacteria.***

389 The effect of MeOH extract and compound **2** on respiratory chain dehydrogenase in *P.*
390 *aeruginosa* and *S. flexneri* expressed as average TriPhenyl Formazan (TPF) formed is
391 illustrated in Fig. 4. Generally, for a tested concentration of a compound and on a given
392 microorganism, we observed an increase in TPF released over time. However, as the
393 concentration of the agent tested increased, there was a significant decrease in the TPF formed
394 for each incubation time. This reduction was more important against *S. flexneri* both for the
395 crude extract and for the compound **2** tested.

396 **Antioxidant activity**

397 Regarding the antioxidant activity, the capacity of the extract or compounds to scavenge the
398 DPPH radical was determined and expressed in EC₅₀, followed by determination of the total
399 antioxidant capacity expressed in Gallic acid equivalent (GEAC). From these results
400 documented in Table 3, it was observed that compound **2** displayed a significant scavenging
401 potential against the DPPH radical (EC₅₀ = 3.08 ± 0.44 µg/mL), closed to that of the reference
402 vitamin C (1.81 ± 0.19 µg/mL). However, compounds **1** and **6 + 7** had an interesting
403 scavenging power against the DPPH radical with EC₅₀ values of 4.52 ± 0.36 µg/mL and 7.63

404 $\pm 1.27 \mu\text{g/mL}$, respectively compared to the MeOH ($62.11 \pm 0.39 \mu\text{g/mL}$), EtOAc ($72.29 \pm$
405 $0.71 \mu\text{g/mL}$) and *n*-BuOH ($76.54 \pm 0.78 \mu\text{g/mL}$) extracts.

406 In addition, compounds **1** and **2** displayed the highest total antioxidant capacity values of
407 $106.03 \pm 0.87 \mu\text{g/mL}$ and $104.87 \pm 1.43 \mu\text{g/mL}$, respectively. They were successively
408 followed by the mixture of compounds **6** + **7**, compound **5**, compound **4**, then the MeOH,
409 EtOAc and *n*-Bu-OH extracts with GEAC values of 83.38 ± 0.22 , 81.09 ± 0.93 , 79.54 ± 1.26 ,
410 73.23 ± 0.61 , 58.44 ± 0.38 and $40.46 \pm 0.74 \mu\text{g/mL}$, respectively.

411

412 **Cytotoxic activity**

413 The cytotoxic activity of extracts and isolated compounds from *R. abyssinicus* was studied by
414 assessing the haemolytic activity against red blood cells (RBCs) using Triton X-100 as a
415 positive control. We observed 100% lysis with the positive control, as compared to the
416 phosphate buffer saline (PBS) which showed no lysis of RBCs. Interestingly, none of the
417 tested extracts and compounds showed a loss of membrane integrity as a result of cell lysis at
418 concentrations up to $2048 \mu\text{g/mL}$ for the extracts and $256 \mu\text{g/mL}$ for the isolated compounds
419 (results not shown).

420

421 **Discussion**

422 The forthcome of resistant strains of bacteria and fungi against conventional antimicrobial
423 drugs as well as side effects associated to antibiotherapy has increased the search for natural
424 product as alternative ways to fight these organisms. Since earliest civilization, medicinal
425 plants have been utilized by medical practitioners to treat various health related problems, and
426 amongst these are bacterial and fungal infections [35]. Plant extracts and natural compounds
427 are effective in the treatment of infectious diseases while at the same time alleviating many of
428 the adverse effects associated with conventional antimicrobials [36]. This study assessed the

429 antimicrobial and antioxidant activities of *R. abyssinicus* extracts and its isolated compounds.
430 This plant is renowned for its multiple uses in herbal medicine to treat health issues involving
431 oxidative stress, as well as bacterial and fungal infections.

432 A bio-guided fractionation of this plant was then performed to identify the potential agents
433 endowed with antimicrobial and/or antioxidant activity. A total of five pure compounds (**1** to
434 **5**) and one mixture of two compounds (**6** and **7**) were isolated from EtOAc fraction of *R.*
435 *abyssinicus*. Compound **1**, identified as chrysophanol, was first reported from *Rheum*
436 *rhabarbarum*, a herbaceous perennial plant belonging to the Polygonaceae family [37], and it
437 has been found in various families, such as Polygonaceae, Rhamnaceae, Fabaceae, Liliaceae,
438 Asphodelaceae, Buphorbiaceae, Meliaceae, Podocarpaceae, Picramniaceae, and
439 Hemerocallidaceae [38-39]. Compound **2**, physcion, is a naturally occurring anthraquinone
440 derivative, and a major bioactive ingredient in the traditional Chinese medicine Radix and
441 *Rhizoma rhei* [40]. It is a dihydroxyanthraquinone or 9,10-
442 anthraquinone bearing hydroxy substituents at positions 1 and 8, a methoxy group at position
443 3, and a methyl group at position 6. Compound **3** was identified as ergosta-6,22-diene-3,5,8-
444 triol, a polyhydroxysterol that has been previously isolated from *Lentinus edodes* [23].
445 Compound **4**, emodine, is an anthraquinone derivative that was first reported in *Aspergillus*
446 *wentii*, a mycotoxin [41]. Compound **5** was identified as 6-hydroxyemodin (citreorosein),
447 reported in the *Rumex* genus for the first time by Ertürk et al. [42]. Compound **6**,
448 chrysophanein, is a chrysophanol glycoside that has been previously identified from leaves
449 and roots of *Aloe hijazensis* [43]. Finally compound **7** identified as physcionin, is distributed
450 in root of nearly all *Rheum* species [44].

451 All the three organic extracts exhibited activities against all the tested microorganisms, with
452 MIC and MMC values varying between 32 and 256 µg/mL. Previous studies have reported
453 the crude extract of *R. abyssinicus* to exhibit antibacterial [15-16], anticancer [16], antiviral

454 [15], anti-inflammatory [15, 17], antioxidant [18], wound healing [17], antimalarial [19],
455 diuretic and analgesic [20] activities.

456 However, the highest activity (MIC = 32 µg/mL) was found with the three extracts against
457 *C. neoformans*, and particularly the EtOAc fraction against *S. flexneri*, *S. aureus* and *C.*
458 *albicans*. This fraction was the most active with MIC values between 32 - 64 µg/mL.
459 However, although in most cases the MMC values appeared to be double the MICs, the
460 MeOH extract and EtOAc fraction were found to be fungicidal against *C. neoformans* at 32
461 µg/mL while at the same concentration the ethyl acetate fraction was bactericidal against *S.*
462 *flexneri*.

463 This study evaluated the antibacterial, antifungal and antioxidant activities of compounds
464 isolated from *R. abyssinicus*. Most of these compounds were found to exhibit microbicidal
465 effect with MMCs values that were either equal to or two times higher than the corresponding
466 MICs. Compounds **2** and **4** inhibited the growth of *C. albicans* and *C. neoformans* at
467 concentration of 8 µg/mL, and also exhibited a fungicidal activity against these strains.
468 Several studies have documented a variety of pharmacological properties of physcion
469 including laxative, hepatoprotective, antineoplastic, anti-inflammatory and anti-microbial
470 activities [40, 45]. Compound **4**, emodin, has also been shown to display antibacterial,
471 antifungal, antiparasitic, antioxidant, and antiviral activities [46]. The mixture of **6** + **7** also
472 displayed considerable antimicrobial activity with bactericidal effect against *S. flexneri* at 16
473 µg/mL while compound **3** was inactive against most of the tested strains.

474 The effect of the association of the MeOH extract with ciprofloxacin and fluconazole was
475 synergistic in nature irrespective of the microorganism tested. Compound **2** associated to
476 ciprofloxacin exhibited synergistic effect except on MSSA01 and MRSA03 strains. A
477 synergistic effect was also observed when compound **4** was combined with ciprofloxacin
478 against MSSA01 and MRSA03 strains. These results indicate an increased susceptibility

479 against the test antibiotics. Synergistic combinations have been shown to render the
480 microorganisms extremely susceptible to concentrations of both antimicrobial agents which
481 can be easily obtained or exceeded in the serum after administration of usual doses [47],
482 suggesting the need of exploring the combination potential of MeOH extract, compounds **2**
483 and **4** with reference antimicrobial drugs, to combat resistant strains. To the best of our
484 knowledge, this is the first report of test samples and ciprofloxacin / fluconazole association
485 against MDR strains.

486 This study also shows that MeOH extract and compound **2** induced leakage of biological
487 material absorbing at 260 and 280 nm, probably nucleic acids and proteins derivatives by *P.*
488 *aeruginosa* and *S. flexneri*, at concentrations equal to or two times their MIC. These
489 observations suggest the contribution of compound **2** and MeOH extract to the alteration of
490 microbial membrane, and the resulting leakage of intracellular material may lead to microbial
491 death, justifying their microbicidal effect. Similar mechanisms of bacterial death have been
492 reported in earlier studies [28]. The ability of the MeOH extract and compound **2** to alter
493 bacterial cell membrane was further demonstrated by increased reducing sugars in the culture
494 suspension as the concentration of both the tested MeOH extract and compound **2** raised from
495 $\frac{1}{2}$ MIC to 2 MIC through MIC. The appearance of reducing sugar was also more important in
496 the presence of the compound **2** as compared to the *R. abyssinicus* extract. These observations
497 have been documented with terpenoids from the leaves of *Tridax procumbens* Linn. against *E.*
498 *coli* [48]. In this study, the MeOH extract of *R. abyssinicus* and compound **2** were also found
499 to inhibit respiratory chain enzyme dehydrogenase of *P. aeruginosa* and *S. flexneri*. The
500 decrease in the activity of this enzyme, may contribute to the inhibition of microbial growth
501 and probably lead to death. Candidly, the inhibition of dehydrogenase activity in pathogenic
502 bacteria is indicative of a strong antimicrobial activity since inhibition of oxido-reductases
503 such as dehydrognases, affects respiration of the microbe.

504 The study of the antioxidant properties of *R. abyssinicus* extract and isolated compound
505 revealed the potential of the extract/isolated compounds to scavenge the DPPH radical, with
506 compound **2** identified with highest scavenging power against the DPPH radical. Compounds
507 **1, 4, 5** and **6 + 7** also displayed interesting scavenging power against the DPPH radical
508 compared to the MeOH, EtOAc and *n*-BuOH extracts. Overall, compounds **1** and **2** had the
509 highest total antioxidant capacity whereas the EtOAc and *n*-BuOH extracts were the least
510 active. These observations suggest that fractionation enhanced the antioxidant activities of
511 compounds **1, 2, 4, 5** and **6 + 7** and diluted those of the EtOAc and *n*-BuOH extracts.
512 Therefore, the presence of such compounds could be partially responsible for the antioxidant
513 activity (AOA) found in these plant extracts; the AOA depends on the method used,
514 reinforcing the concept that this plant extracts contain several antioxidant compounds that act
515 in different manners. The antimicrobial and antioxidant activities of compounds **1, 2** and **4** are
516 in accordance with the previous studies [19,29,40,46,49]. However, this is the first report on
517 the antibacterial, antifungal and antioxidant activities of compounds **3, 5 – 7** against free
518 radicals and pathogenic bacteria and fungi. The antioxidant activities of these compounds
519 coupled to their antimicrobial properties, may offer a therapeutic option for the treatment of
520 infectious diseases while simultaneously mitigating many of the side effects that are often
521 associated with conventional antimicrobials [36].

522

523 **Conclusions**

524 *R. abyssinicus* is a potential source of antibacterial, antifungal and antioxidant agents. Their
525 mechanism of antibacterial activity is due to disruption of the cytoplasmic membrane and
526 inhibition of the microbial respiratory chain dehydrogenase enzyme activity. Interestingly,
527 none of the tested extracts/compounds showed cytotoxic activity against normal cells;
528 highlighting their suitability and selectivity toward pathogenic bacteria and yeasts. The

529 MeOH extract and compounds **2** and **4** displayed synergistic effect with the ciprofloxacin and
530 fluconazole. The observed activity of the isolated compounds against bacteria and fungi
531 including MDR strains deserves further exploration.

532

533 **Abbreviations**

534 ¹³C-NMR: Carbon Thirteen Nuclear Magnetic Resonance; ¹H-NMR: Proton
535 Nuclear Magnetic Resonance; 2D NMR: Two-dimension Nuclear Magnetic
536 Resonance; ATCC: American Type Culture Collection; CC: Column
537 Chromatography; COSY: Correlation Spectroscopy; DMSO: Dimethylsulfoxide;
538 EtOAc: Ethyl acetate; HMBC: Heteronuclear Multiple Bond Connectivities;
539 HNC: Herbier National du Cameroun; HR-EI-MS: High Resolution Electron
540 Impact Mass Spectrometry; HR-TOFESIMS: High-Resolution Time of Flight
541 Electrospray Ionization Mass Spectrometry; HSQC: The Heteronuclear Single
542 Quantum Coherence; IR: Infra-red; MDR: Multi-Drug-Resistant; MeOH: Methanol; MHA:
543 Mueller Hinton agar; MHB: Mueller Hinton broth; MIC: Minimum inhibitory concentration;
544 MMC: Minimum Microbicidal Concentration; NA: Nutrient agar; *n*-BuOH: n-Butanol; NMR:
545 Nuclear Magnetic Resonance; Rf: Retention factor; TLC: Thin Layer Chromatography; TMS:
546 Tetramethylsilane; TOF-ESIMS: Time of Flight Electrospray Ionization Mass Spectrometry;
547 UV: Ultra-violet.

548

549 **Declarations**

550

551 **Ethics approval and consent to participate**

552 All the procedures and protocols involving animals and their care were followed in
553 conformity with the institutional guidelines and approved by the Cameroon National Ethical

554 Committee (Reg. No. FWA-IRB00001954) and in compliance with the ARRIVE guidelines.

555 **Consent for publication**

556 Not applicable.

557 **Availability of data and materials**

558 The datasets used and analyzed during the current study are available from the corresponding
559 author.

560 **Competing interests**

561 The authors declare that they have no competing interests.

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564 **Authors' contributions**

565 ICK, LDTF, AJN, CDJN and MDD contributed to the data collection and analysis. JDT
566 designated the study, did the biological assays and helped in manuscript writing and
567 editing. JDT, LVN and DN supervised and revised the manuscript critically for
568 important intellectual content. All authors read and agreed on the final
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576

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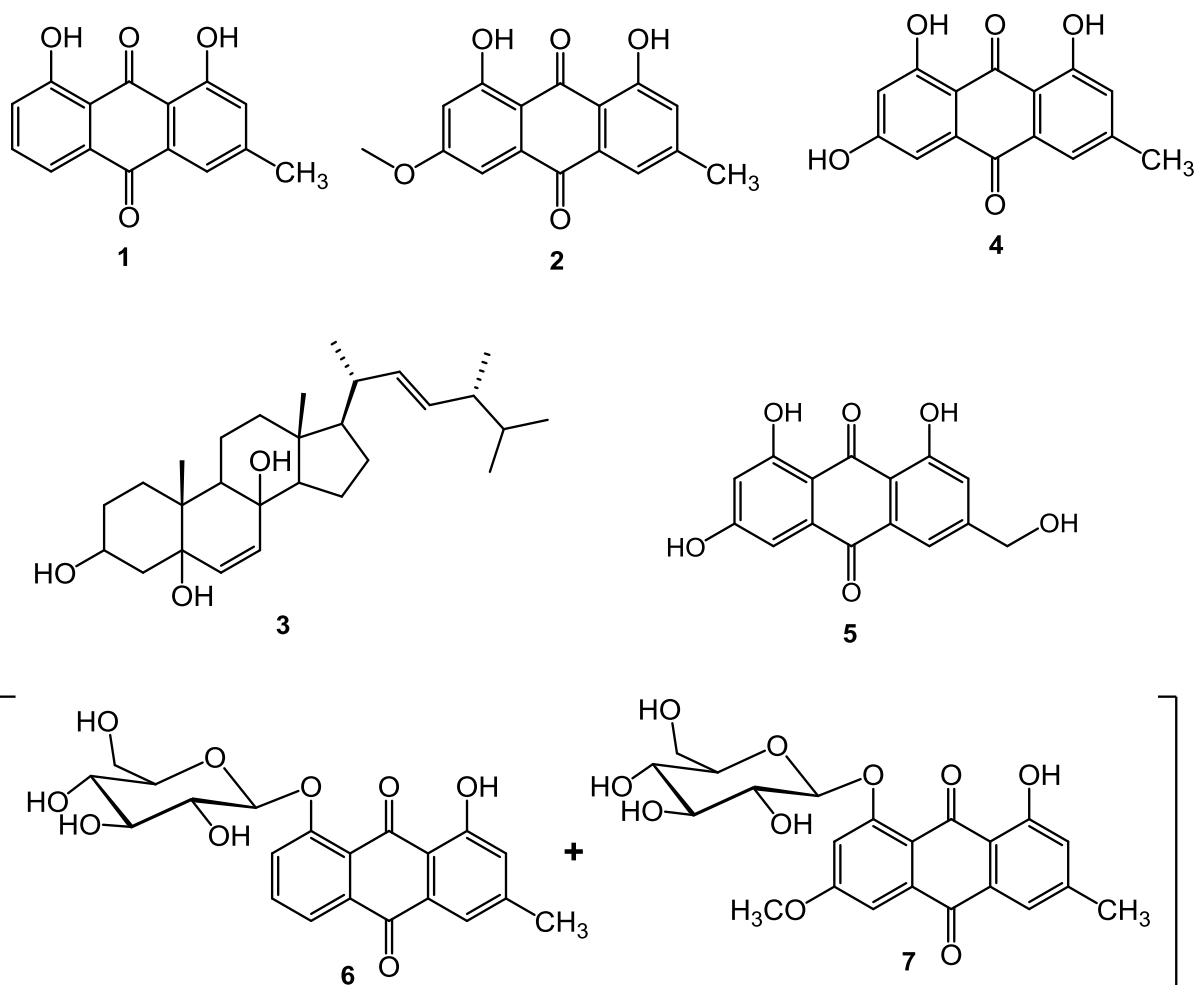
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714 **Figure Legends**



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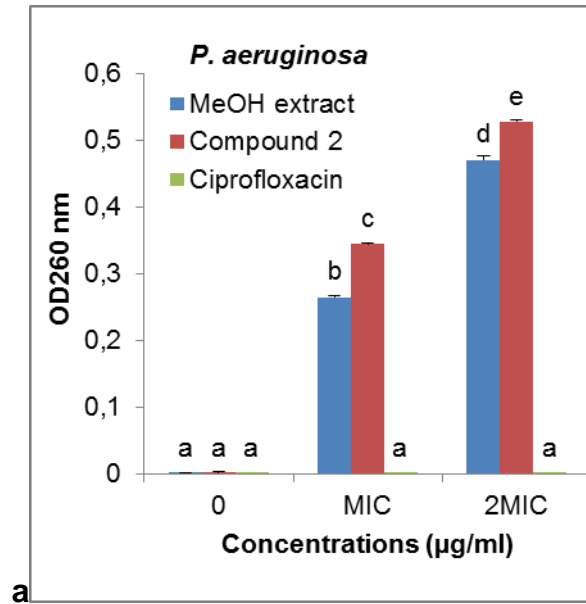
716 **Fig. 1** Chemical structures of compounds isolated from *R. abyssinicus* (1–7): 1:

717 Chrysophanol; 2: Physcion; 3: Ergosta-6,22-diene-3,5,8-triol; 4: Emodine; 5: 6-

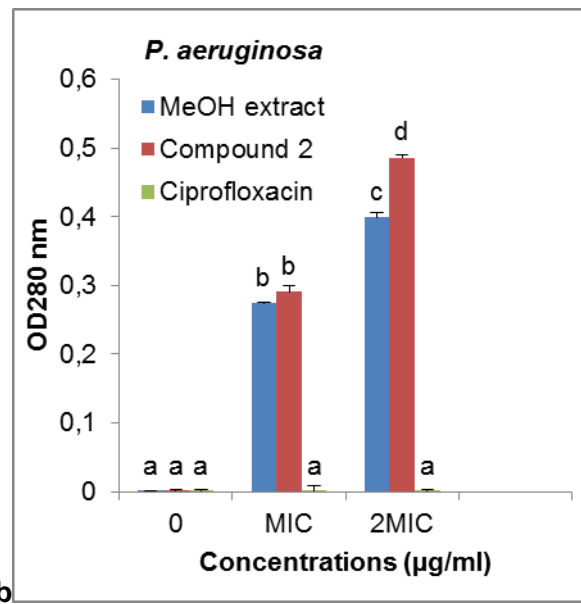
718 hydroxyemodin (Citreorosein); 6: Chrysophanein; 7: Physcionin

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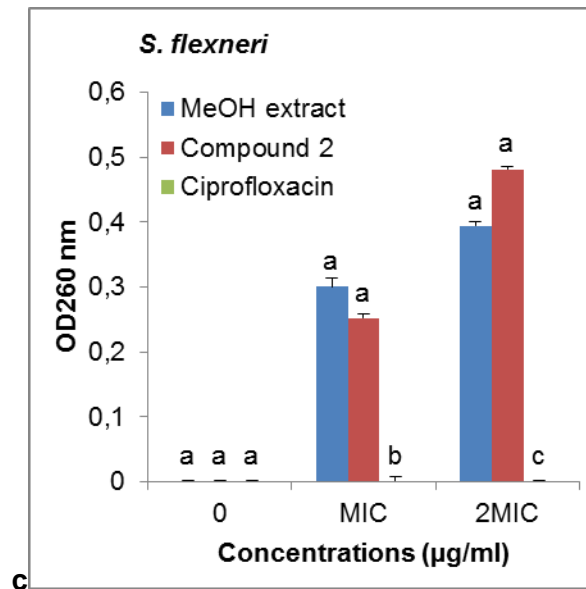
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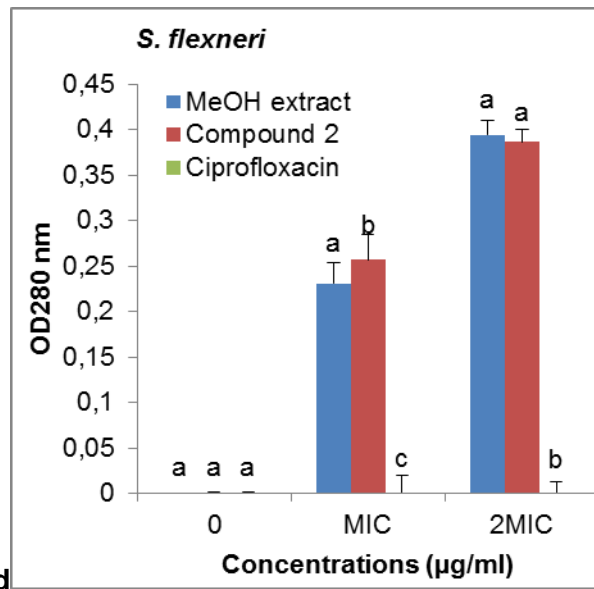
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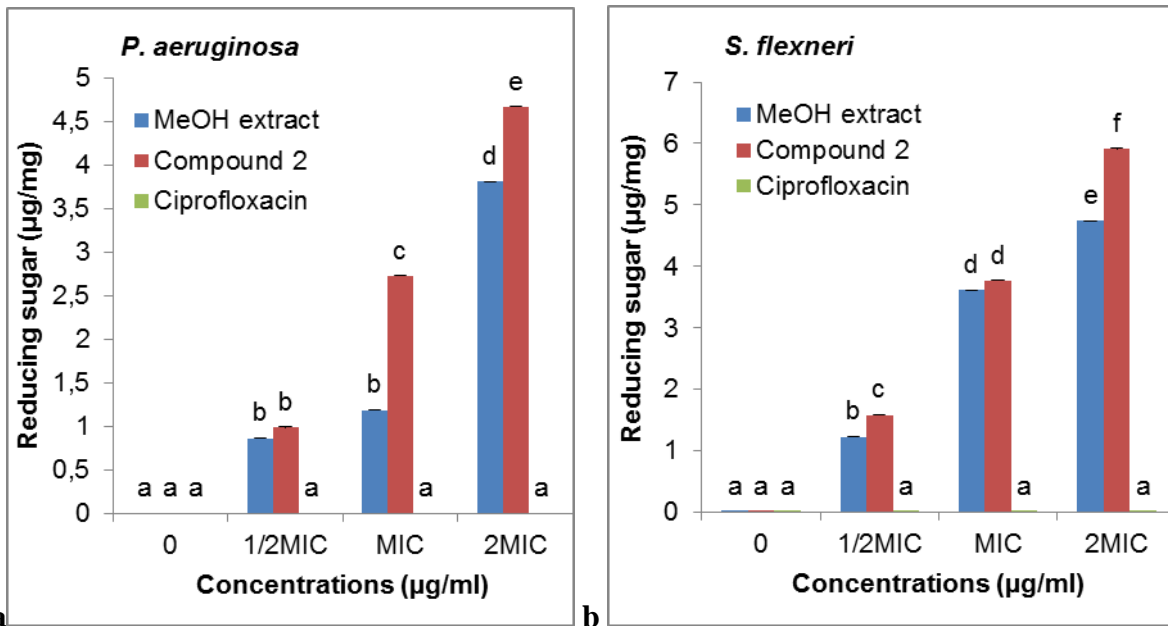
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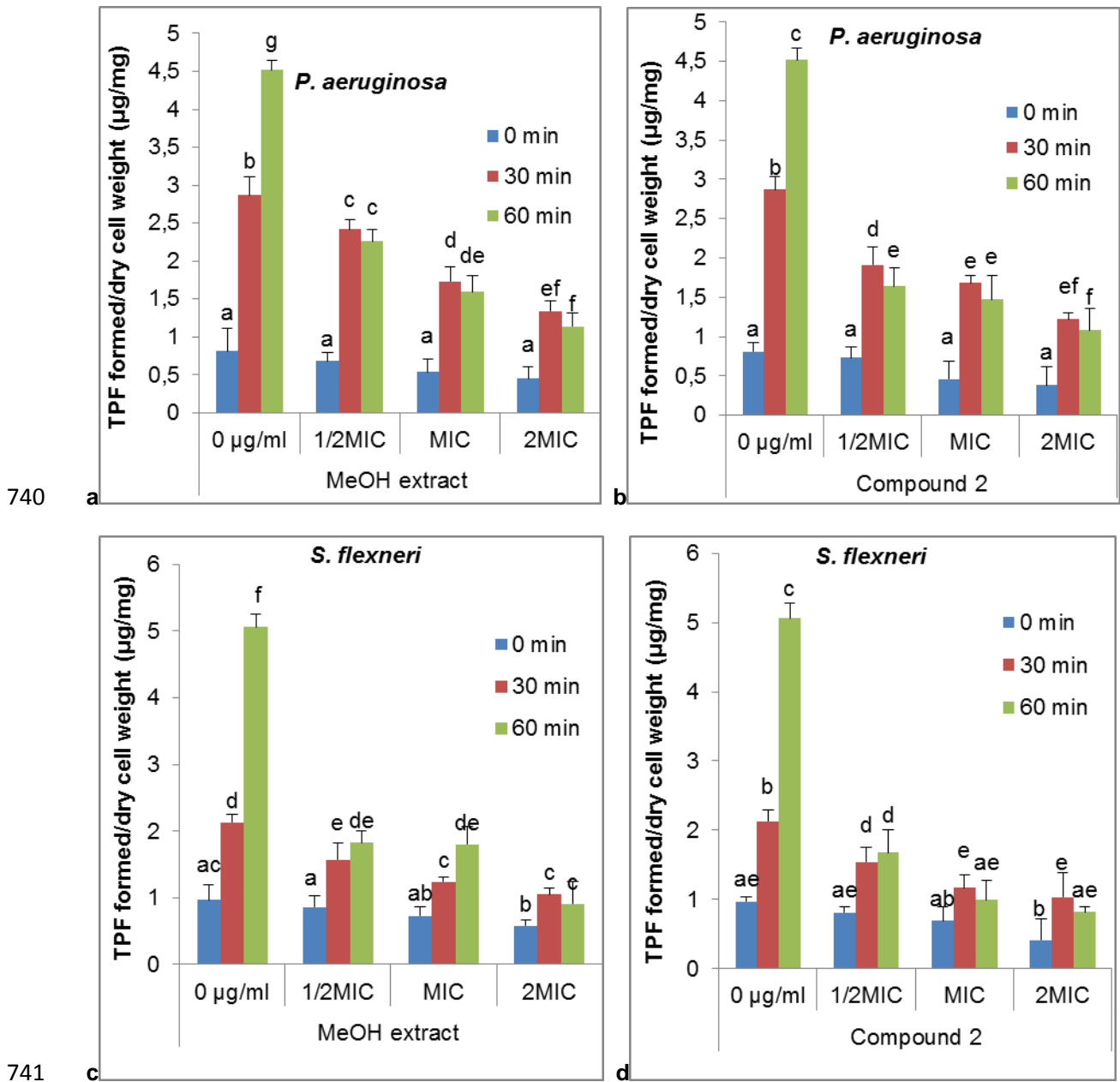
722 **Fig. 2** Appearance of 260 and 280 nm absorbing material in the filtrates of *P. aeruginosa* and
 723 *S. flexneri* in control suspensions and after treatment with the different concentrations
 724 of MeOH extract and compound 2.



725 **a**

726 **Fig. 3** Appearance of reducing sugar ($\mu\text{g/mg}$) in the filtrates of *P. aeruginosa* (a) and *S.*
 727 *flexneri* (b) for control suspensions and after treatment with the different
 728 concentrations of MeOH extract and compound 2.

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742 **Fig. 4** Effect of MeOH extract and compound 2 on respiratory chain dehydrogenase in *P.*
 743 *aeruginosa* and *S. flexneri*. TPF: TriPhenyl Formazan.

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746 **Table 1** Antimicrobial activity (MIC and MMC in $\mu\text{g/mL}$) of extracts and isolated
 747 compounds from *R. abyssinicus* as well as reference antimicrobial drugs.

Extracts/ Compounds	Inhibition parameters	<i>P.</i> <i>aeruginosa</i>	<i>S.</i> <i>flexneri</i>	<i>S.</i> <i>aureus</i>	<i>MSSA01</i>	<i>MRSA03</i>	<i>C.</i> <i>albicans</i>	<i>C.</i> <i>neoformans</i>
MeOH extract	MIC	64	128	64	64	64	64	32
	MMC	128	128	128	128	128	64	32
	MMC/MIC	2	1	2	2	2	1	1
EtOAc fraction	MIC	64	32	32	64	64	32	32
	MMC	128	32	64	64	64	64	32
	MMC/MIC	2	1	2	1	1	2	1
<i>n</i> -BuOH fraction	MIC	128	128	128	256	256	64	32
	MMC	256	256	128	256	256	64	64
	MMC/MIC	2	2	1	1	1	1	2
1	MIC	64	32	32	64	64	64	32
	MMC	128	32	64	64	128	64	32
	MMC/MIC	2	1	2	1	2	1	1
2	MIC	8	8	8	16	16	8	8
	MMC	16	16	8	32	32	8	8
	MMC/MIC	2	2	1	2	2	1	1
3	MIC	128	128	256	>256	>256	128	64
	MMC	256	>256	>256	>256	>256	>256	128
	MMC/MIC	2	/	/	/	/	/	2
4	MIC	16	8	8	32	32	8	8
	MMC	16	16	8	32	32	8	8
	MMC/MIC	1	2	1	1	1	1	1
5	MIC	32	32	32	128	128	16	16

	MMC	64	32	32	256	256	32	32
	MMC/MIC	2	1	1	2	2	2	2
6+7	MIC	16	8	8	16	16	16	8
	MMC	16	16	8	16	32	16	8
	MMC/MIC	1	2	1	1	2	1	1
Ref*	MIC	0.5	8	0.5	4	4	1	2
	MMC	0.5	8	0.5	4	4	1	2
	MMC/MIC	1	1	1	1	1	1	1

748 /: not determined; MIC: Minimum Inhibitory Concentration; MMC Minimum Microbicidal Concentration; *:

749 fluconazole for yeasts and ciprofloxacin for bacteria.

750 **Table 2** Interactions of the methanol extract/compounds from *R. abyssinicus* and antibiotics against bacterial and yeast species

Microorganisms	MeOH extract				Compound 2				Compound 4			
	FICA	FICE _x	FIC	Interpretation	FICA	FIC ₂	FIC	Interpretation	FICA	FIC ₅	FIC	Interpretation
<i>P. aeruginosa</i>	0.25	0.125	0.37	Synergistic	0.125	0.125	0.25	Synergistic	0.25	0.125	0.25	Synergistic
<i>S. flexneri</i>	0.125	0.125	0.25	Synergistic	0.031	0.25	0.281	Synergistic	0.0625	0.25	0.31	Synergistic
<i>S. aureus</i>	0.25	0.125	0.37	Synergistic	0.25	0.125	0.375	Synergistic	0.5	0.125	0.625	Additive
<i>MSSA01</i>	0.125	0.0625	0.18	Synergistic	0.0625	0.5	0.5625	Additive	0.25	0.25	0.5	Synergistic
<i>MRSA03</i>	0.125	0.125	0.25	Synergistic	0.0625	0.5	0.5625	Additive	0.25	0.125	0.375	Synergistic
<i>C. albicans</i>	0.125	0.0625	0.18	Synergistic	0.0625	0.0625	0.125	Synergistic	0.25	0.5	0.75	Additive
<i>C. neoformans</i>	0.0625	0.0625	0.125	Synergistic	0.0625	0.0625	0.125	Synergistic	0.25	0.5	0.75	Additive

751 FICA: MIC of antibiotic tested in combination/MIC of antibiotic tested alone; FICE_x: MIC of extract tested in combination/MIC of extract tested alone; FIC₂: MIC of
752 compound 2 tested in combination with antibiotic/ MIC of compound 2 tested alone; FIC₄: MIC of compound 4 tested in combination with antibiotic/ MIC of compound 4
753 tested alone; FIC: MIC of antibiotic tested in combination/MIC of antibiotic tested alone + MIC of extract/compound tested in combination/MIC of extract/compound tested
754 alone; antibiotics: ciprofloxacin for bacteria and fluconazole for yeasts.

755 **Table 3** Antioxidant activities of extracts and some isolated compounds from *R. abyssinicus*

Extracts/compounds	DPPH free radical scavenging activity (EC ₅₀ , µg/mL)	Gallic acid equivalent antioxidant capacity (GEAC, µg/mL)
MeOH extract	62.11 ± 0.39 ^a	73.23 ± 0.61 ^a
EtOAc fraction	72.29 ± 0.71 ^b	58.44 ± 0.38 ^b
<i>n</i> -BuOH fraction	76.54 ± 0.78 ^c	40.46 ± 0.74 ^c
1	4.52 ± 0.36 ^d	104.87 ± 1.43 ^d
2	3.08 ± 0.44 ^e	106.03 ± 0.87 ^d
4	10.69 ± 0.51 ^f	79.54 ± 1.26 ^e
5	9.88 ± 0.62 ^f	81.09 ± 0.93 ^e
6+7	7.63 ± 1.27 ^g	83.38 ± 0.22 ^f
Vitamin C	1.81 ± 0.19 ^h	NA

756 EC₅₀: Equivalent concentrations of test samples scavenging 50% of DPPH radical. Data represent the mean ± SD
 757 of three independent experiments carried out in triplicate. In the same column, values affected by different
 758 superscript letters (a-h) are significantly different according to one way ANOVA and Waller Duncan test; p <
 759 0.05.

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