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## **Antibacterial, antifungal and antioxidant activities of whole plant chemical constituents of *Rumex abyssinicus***

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

3  
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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,  $\beta$ -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  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra were recorded on a Bruker Avance III 600 spectrometer  
140 equipped with a cryo-platform ( $^1\text{H}$  at 600 MHz and  $^{13}\text{C}$  at 150 MHz). 2D NMR experiments  
141 were performed using standard Bruker microprograms (Xwin-NMR version 2.1 software). All  
142 chemical shifts ( $\delta$ ) 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 ( $\text{CD}_3\text{OD}$ ), dimethyl sulfoxide ( $\text{DMSO-}d_6$ ), and  
145 chloroform ( $\text{CDCl}_3$ ) 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%  $\text{H}_2\text{SO}_4$  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 H<sub>2</sub>O/EtOAc (300 mL/500 mL) followed by H<sub>2</sub>O/*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 (C<sub>1</sub> and C<sub>2</sub>). Purification of sub-  
180 fraction C<sub>1</sub> (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 C<sub>2</sub> (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 D<sub>1</sub>,  
184 D<sub>2</sub> and D<sub>3</sub>. Purification of D<sub>3</sub> (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 ( $\mu\text{g}$ ) 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  $\mu$ L acetate buffer (pH =5.0, 100 mM), the following were added: 20  $\mu$ L  
258 laccase (1 mM stock solution), 20  $\mu$ L test sample, 10  $\mu$ 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  $\mu$ g/mL for the extracts and  
262 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56  $\mu$ g/mL for the isolated compounds. The content of  
263 the generated ABTS<sup>•+</sup> 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  $\mu$ 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 EC<sub>50</sub> ( $\mu$ 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 (<sup>1</sup>H and <sup>13</sup>C  
296 NMR, <sup>1</sup>H-<sup>1</sup>H 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 ; (C<sub>15</sub>H<sub>10</sub>O<sub>4</sub>) ; <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$ : 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, -CH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$ : 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 (-CH<sub>3</sub>).

306 **Physcion (2)**: Yellow powder ; (C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>) ; <sup>1</sup>H-NMR (600 MHz, CDCl<sub>3</sub>) 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*, OCH<sub>3</sub>), 2.46 (*s*, -CH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, CDCl<sub>3</sub>) 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(-OCH<sub>3</sub>), 22.2 (-CH<sub>3</sub>).

311 **Ergosta-6,22-diene-3,5,8-triol (3)**: White powder ; (C<sub>28</sub>H<sub>46</sub>O<sub>3</sub>) ; <sup>13</sup>C-NMR (150 MHz,  
312 CDCl<sub>3</sub>): 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; (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>); <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) 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*, -CH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) 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 (-CH<sub>3</sub>).

321 **Citreorosein (5)**: Red powder; (C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>) ; <sup>1</sup>H-NMR (600 MHz, CD<sub>3</sub>OD) 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*, -OCH<sub>2</sub>-); <sup>13</sup>C-NMR (150 MHz,  
323 CD<sub>3</sub>OD) 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 (-OCH<sub>2</sub>-).

326 **Chrysophanein (6)**: Yellow powder ; (C<sub>21</sub>H<sub>20</sub>O<sub>9</sub>) ; <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) 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-CH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) 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 (-CH<sub>3</sub>).

332 **Physcionin (7)**: Yellow powder ; (C<sub>22</sub>H<sub>22</sub>O<sub>10</sub>) ; <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>) 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*, -OCH<sub>3</sub>), 2.42 (*s*, -CH<sub>3</sub>); <sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>) 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 (-OCH<sub>3</sub>) 21.8 (-CH<sub>3</sub>).

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 EC<sub>50</sub>, 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 (EC<sub>50</sub> = 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 EC<sub>50</sub> 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  $83.38 \pm 0.22$ ,  $81.09 \pm 0.93$ ,  $79.54 \pm 1.26$ ,  
410  $73.23 \pm 0.61$ ,  $58.44 \pm 0.38$  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 <sup>13</sup>C-NMR: Carbon Thirteen Nuclear Magnetic Resonance; <sup>1</sup>H-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

## 577 **References**

- 578 1. Dewal MB, Wani AS, Vidailac C, Oupický D, Rybak MJ, Firestine SM. Thieno[2,3-  
579 d]pyrimidinedione derivatives as antibacterial agents. *Eur J Med Chem.* 2012;51:145-53.
- 580 2. Nkomo LP. *In vitro* bioactivity of crude extracts of *Lippia javanica* on clinical isolates of  
581 *Helicobacter pylori*: Preliminary phytochemical screening. University of Fort Hare.  
582 Master' Thesis, University of Fort Hare, Faculty of Science & Agriculture; 2010. p. 97
- 583 3. Rojas J, Buitrago A. Essential oils and their products as antimicrobial agents: Progress and  
584 prospects. Chapter 13, 26p. In: *Therapeutic Medicinal Plants From Lab to Mark*; Edited  
585 By Marta C.T. Duarte, Mahendra Rai; 2015.
- 586 4. Kalghatgi S, Spina CS, Costello JC, Liesa M, Morones-Ramirez JR, Slomovic S, Molina A,  
587 Shirihai OS, Collins JJ. Bactericidal antibiotics induce mitochondrial dysfunction and  
588 oxidative damage in Mammalian cells. *Sci Transl Med.* 2013;5(192):192ra85-192ra85.
- 589 5. Chanda S, Kaneria M, Nair R. Antibacterial activity of *Psoralea corylifolia* L. seed and  
590 aerial parts with various extraction methods. *Res J Microbiol.* 2011;6(2):124.
- 591 6. Adeshina GO, Onaolapo JA, Ehinmidu JO, Odama LE. Phytochemical and antimicrobial  
592 studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria. *J*  
593 *Med Plants Res.* 2010;4(8):649-58.
- 594 7. Joubouhi C, Tamokou J-D-D, Ngnokam D, Voutquenne-Nazabadioko L, Kuate J-R.  
595 Iridoids from *Canthium subcordatum* iso-butanol fraction with potent biological  
596 activities. *BMC Compl Altern Med.* 2017;17(1):17.
- 597 8. Nzogong RT, Ndjateu FST, Ekom SE, Fosso J-AM, Awouafack MD, Tene M, et al.  
598 Antimicrobial and antioxidant activities of triterpenoid and phenolic derivatives from two  
599 Cameroonian Melastomataceae plants: *Dissotis senegambiensis* and *Amphiblemma*  
600 *monticola*. *BMC Compl Altern Med.* 2018;18(1):159.

- 601 9. Davidson PM, Branen AL. Food antimicrobials - an introduction. In Davidson PM, Sofos  
602 JN, Branen AL (Eds.), *Antimicrobial in food* (3rd ed.), Pp 1-10, New York, NY: CRC  
603 Press; 2005.
- 604 10. Sieniawska E, Swatko-Ossor M, Sawicki R, Skalicka-Woźniak K, Ginalska G. Natural  
605 terpenes influence the activity of antibiotics against isolated *Mycobacterium tuberculosis*.  
606 *Med Princ Pract* 2017;26(2):108-12.
- 607 11. Tagousop CN, Tamokou J-D-D, Kengne IC, Ngnokam D, Voutquenne-Nazabadioko L.  
608 Antimicrobial activities of saponins from *Melanthera elliptica* and their synergistic  
609 effects with antibiotics against pathogenic phenotypes. *Chem Cent J*. 2018;12(1):97.
- 610 12. Ahmad I, Aqil F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against  
611 ESβL-producing multidrug-resistant enteric bacteria. *Microbiol Res*. 2007;162(3):264-75.
- 612 13. Rao K, Ch S, David Banji HS, Mahesh V. A study on the nutraceuticals from the genus  
613 *Rumex*. *Hygeia J D Med*. 2011;3(1):76- 88.
- 614 14. Ken F (2019) Useful Tropical Plants Database [Online]. Available:  
615 <http://tropical.theferns.info/viewtropical.php?id=Rumex+abyssinicus> [Accessed 29-12-  
616 2019].
- 617 15. Getie M, Gebre-Mariam T, Rietz R, Höhne C, Huschka C, Schmidtke M, et al. Evaluation  
618 of the anti-microbial and anti-inflammatory activities of the medicinal plants *Dodonaea*  
619 *viscosa*, *Rumex nervosus* and *Rumex abyssinicus*. *Fitoterapia* 2003;74(1-2):139-43.
- 620 16. Tamokou J-D-D, Chouna JR, Fischer-Fodor E, Chereches G, Barbos O, Damian G, et al.  
621 Anticancer and antimicrobial activities of some antioxidant-rich Cameroonian medicinal  
622 plants. *PLoS One* 2013;8(2): e55880.
- 623 17. Mulisa E, Asres K, Engidawork E. Evaluation of wound healing and anti-inflammatory  
624 activity of the rhizomes of *Rumex abyssinicus* J.(Polygonaceae) in mice. *BMC Compl*  
625 *Altern Med*. 2015;15(1):341.

- 626 18. Mohammed SA, Panda RC, Madhan B, Demessie BA. Extraction of bio-active  
627 compounds from Ethiopian plant material *Rumex abyssinicus* (mekmeko) root—A study  
628 on kinetics, optimization, antioxidant and antibacterial activity. J Taiwan Inst Chem Eng.  
629 2017;75:228-39.
- 630 19. Muganga R, Angenot L, Tits M, Frederich M. Antiplasmodial and cytotoxic activities of  
631 Rwandan medicinal plants used in the treatment of malaria. J Ethnopharmacol.  
632 2010;128(1):52-7.
- 633 20. Mekonnen T, Urga K, Engidawork E. Evaluation of the diuretic and analgesic activities of  
634 the rhizomes of *Rumex abyssinicus* Jacq in mice. J Ethnopharmacol. 2010;127(2):433-9.
- 635 21. Guo S, Feng B, Zhu R, Ma J, Wang W. Preparative isolation of three anthraquinones from  
636 *Rumex japonicus* by high-speed counter-current chromatography. Molecules  
637 2011;16(2):1201-10.
- 638 22. Basu S, Ghosh A, Hazra B. Evaluation of the antibacterial activity of *Ventilago*  
639 *madraspatana* Gaertn., *Rubia cordifolia* Linn. and *Lantana camara* Linn.: isolation of  
640 emodin and physcion as active antibacterial agents. Phytother Res. 2005;19(10):888-94.
- 641 23. Rivera A, Benavides OL, Rios-Motta J. (22E)-Ergosta-6,22-diene-3 $\beta$ ,5 $\alpha$ ,8 $\alpha$ -triol: A new  
642 polyhydroxysterol isolated from *Lentinus edodes* (Shiitake). Nat Prod Res.  
643 2009;23(3):293-300.
- 644 24. Zhang C, Wang X, Zhang X, Zhang Y, Xiao H, Liang X. Bioassay-guided separation of  
645 citreorosein and other oestrogenic compounds from *Polygonum cuspidatum*. Phytother  
646 Res. 2009;23(5):740-1.
- 647 25. Kubo I, Murai Y, Soediro I, Soetarno S, Sastrodihardjo S. Cytotoxic anthraquinones from  
648 *Rheum pulmatum*. Phytochemistry 1992;31(3):1063-5.

- 649 26. Tamokou J-D-D, Tala MF, Wabo HK, Kuate JR, Tane P. Antimicrobial activities of  
650 methanol extract and compounds from stem bark of *Vismia rubescens*. J Ethnopharmacol.  
651 2009;124(3):571-5.
- 652 27. Climo MW, Patron RL, Archer GL. Combinations of vancomycin and  $\beta$ -lactams are  
653 synergistic against *Staphylococcus* with reduced susceptibilities to vancomycin.  
654 Antimicrob Agents Chemother. 1999;43(7):1747-53.
- 655 28. Karsha PV, Lakshmi OB. Antibacterial activity of black pepper (*Piper nigrum* Linn.) with  
656 special reference to its mode of action on bacteria. Indian J Nat Prod Resour. 2010;  
657 1(2):213-5.
- 658 29. Sathya-Bama S, Jayasurya Kingsley S, Sankaranarayanan S, Bama P. Antibacterial  
659 activity of different phytochemical extracts from the leaves of *T. procumbens* Linn.:  
660 identification and mode of action of the terpenoid compound as antibacterial. Int J Pharm  
661 Pharm Sci. 2012;4(1):557-64.
- 662 30. Kim K-J, Sung WS, Suh BK, Moon S-K, Choi J-S, Kim JG, et al. Antifungal activity and  
663 mode of action of silver nano-particles on *Candida albicans*. *Biometals* 2009;22(2):235-  
664 42.
- 665 31. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. *Methods*  
666 *Enzymol.* 1994;234:279-93.
- 667 32. Mot AC, Pârvu M, Damian G, Irimie FD, Darula Z, Medzihradsky KF, et al. “Yellow”  
668 laccase with “blue” spectroscopic features, from *Sclerotinia sclerotiorum*. *Process*  
669 *Biochemistry* 2012;47:968–75.
- 670 33. Djouossi MG, Tamokou J-D-D, Ngnokam D, Kuate J-R, Tapondjou LA, Harakat D, et al.  
671 Antimicrobial and antioxidant flavonoids from the leaves of *Oncoba spinosa* Forssk.  
672 (Salicaceae). *BMC Compl Altern Med.* 2015;15(1):134.

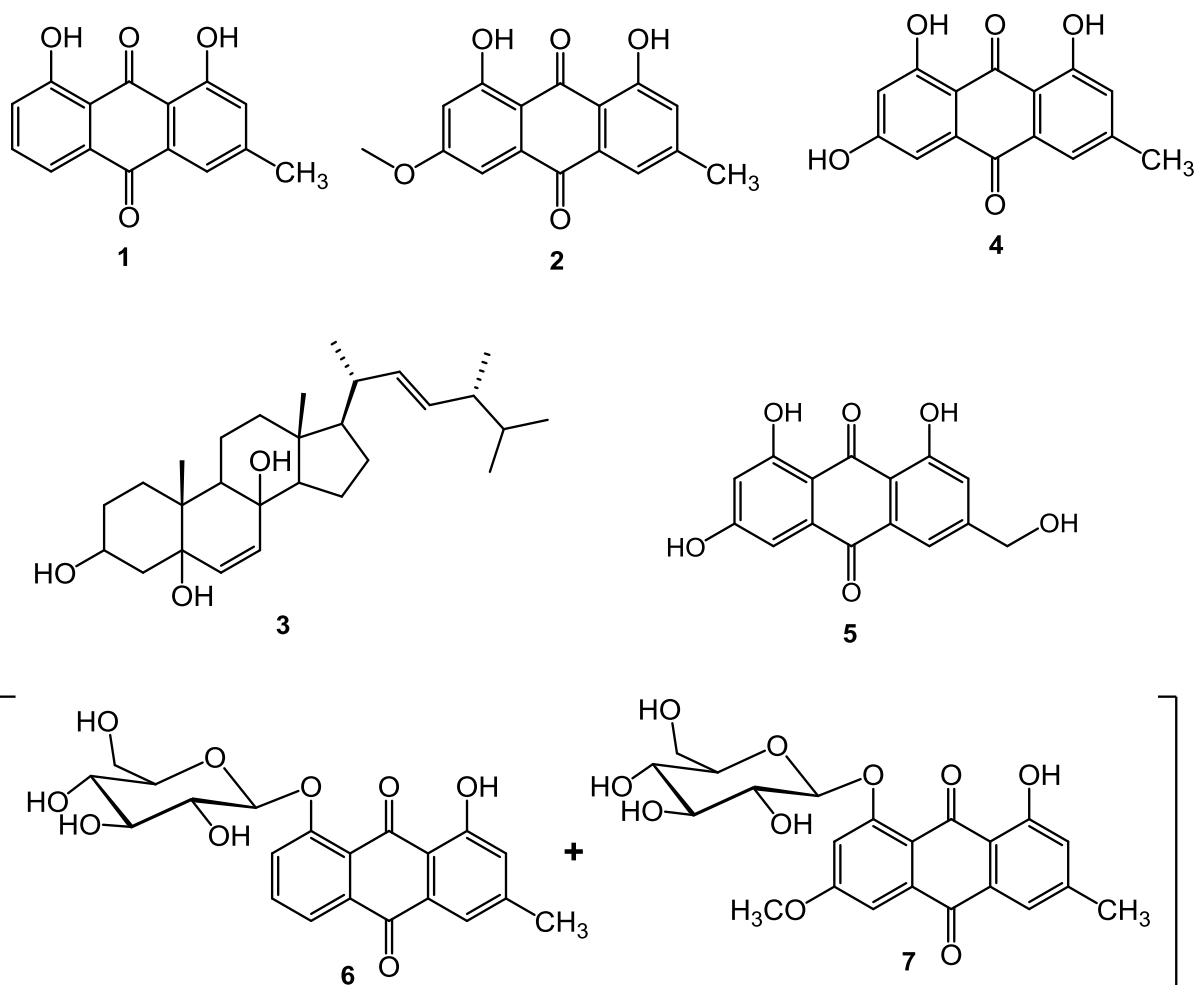
- 673 34. Situ H, Bobek LA. *In vitro* assessment of antifungal therapeutic potential of salivary  
674 histatin-5 two variants of histatin-5 and Salivary Mucin (MUC7) domain 1. *Antimicrob*  
675 *Agents Chemother.* 2000;44, 1485-93.
- 676 35. Tamokou J-D-D, Kuate JR, Gatsing D, Efouet AP, Njouendou AJ. Antidermatophytic  
677 and toxicological evaluations of dichloromethane-methanol extract, fractions and  
678 compounds isolated from *Coula edulis*. *Iran J Med Sci.* 2011;36(2):111-21.
- 679 36. Iwu M, Duncan AR, Okunji CO. New Antimicrobials of Plant Origin. Pp. 457–462. In:  
680 Janick J (ed.), *Perspectives on new crops and new uses*. ASHS Press, Alexandria, VA;  
681 1999.
- 682 37. Tutin F, Clewer HWB. XCIX.—The constituents of rhubarb. *J Chem Soc*  
683 *Trans.*1911;99(0):946-67.
- 684 38. Kuo Y-H, Lee P-H, Wein Y-S. Four New Compounds from the Seeds of *Cassia fistula*. *J*  
685 *Nat Prod.* 2002;65(8):1165-7.
- 686 39. Panichayupakaranant P, Sakunpak A, Sakunphueak A. Quantitative HPLC determination  
687 and extraction of anthraquinones in *Senna alata* leaves. *J Chromatogr Sci.*  
688 2009;47(3):197-200.
- 689 40. Agarwal S, Singh SS, Verma S, Kumar S. Antifungal activity of anthraquinone  
690 derivatives from *Rheum emodi*. *J Ethnopharmacol.* 2000;72(1-2):43-6.
- 691 41. Wells JM, Cole RJ, Kirksey JW. Emodin, a toxic metabolite of *Aspergillus wentii* isolated  
692 from weevil-damaged chestnuts. *Appl Microbiol.* 1975;30:26–8.
- 693 42. Ertürk S, Özbas M, Imre S. Anthraquinone pigments from *Rumex cristatus*. *ACTA Pharm*  
694 *Sci.* 2001;43(1):21-2.
- 695 43. Abd-Alla HI, Shaaban M, Shaaban KA, Abu-Gabal NS, Shalaby NM, Laatsch H. New  
696 bioactive compounds from *Aloe hijazensis*. *Nat Prod Res.* 2009;23(11):1035-49.

- 697 44. He J, Wang L, Guo H, Zhao H, Sun J. Chemistry, pharmacology and processing method  
698 of rhubarb (*Rheum* species): a review. *J Food Bioactives* 2019;8:42-50.
- 699 45. Pang M-J, Yang Z, Zhang X-L, Liu Z-F, Fan J, Zhang H-Y. Physcion, a naturally  
700 occurring anthraquinone derivative, induces apoptosis and autophagy in human  
701 nasopharyngeal carcinoma. *Acta Pharmacol Sin.* 2016;37(12):1623-40.
- 702 46. Izhaki I. Emodin – a secondary metabolite with multiple ecological functions in higher  
703 plants. *New Phytologist* 2002;155:205-17
- 704 47. Klastersky J, Cappel R, Daneau D. Clinical Significance of *in vitro* synergism between  
705 antibiotics in Gram-negative infections. *Antimicrob Agents Chemother.* 1972;2(6):470-5.
- 706 48. Bama SS, Kingsley SJ, Anan S, Bama P. Antibacterial activity of different phytochemical  
707 extracts from the leaves of *T. procumbens*: Identification and mode of action of the  
708 terpenoid compounds as antibacterials. *Int J Pharm Pharm Sci.* 2012;4(1):557-64.
- 709 49. Prateeksha, Yusuf MA, Singh BN, Sudheer S, Kharwar RN, Siddiqui S, et al.  
710 Chrysophanol: A natural anthraquinone with multifaceted biotherapeutic potential.  
711 *Biomolecules* 2019;9:68.

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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:

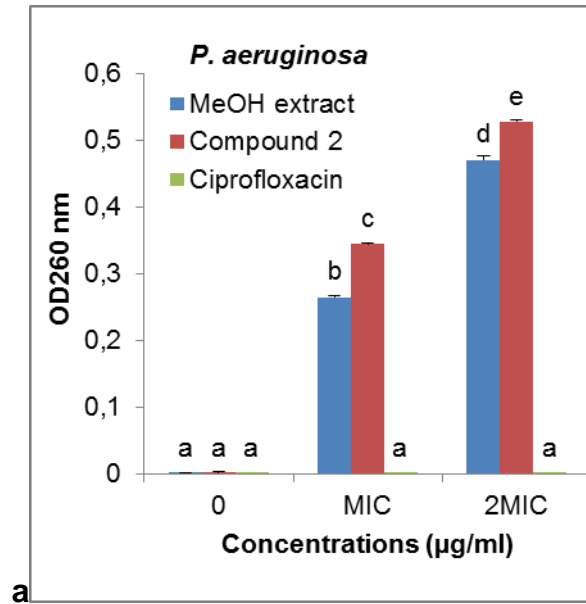
717 Chrysophanol; 2: Physcion; 3: Ergosta-6,22-diene-3,5,8-triol; 4: Emodine; 5:

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

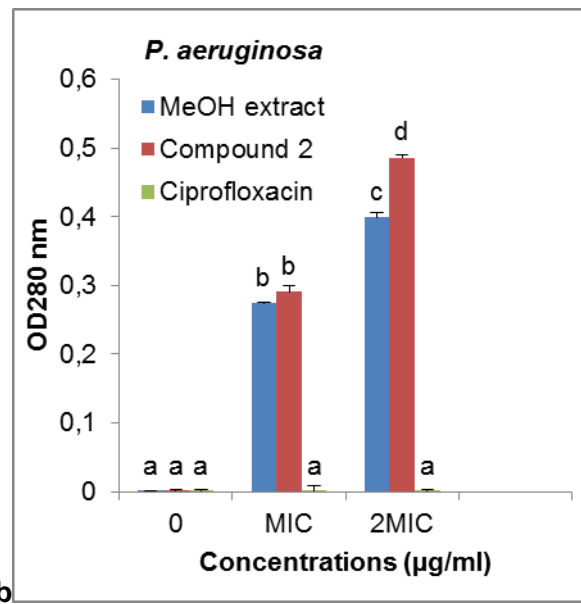
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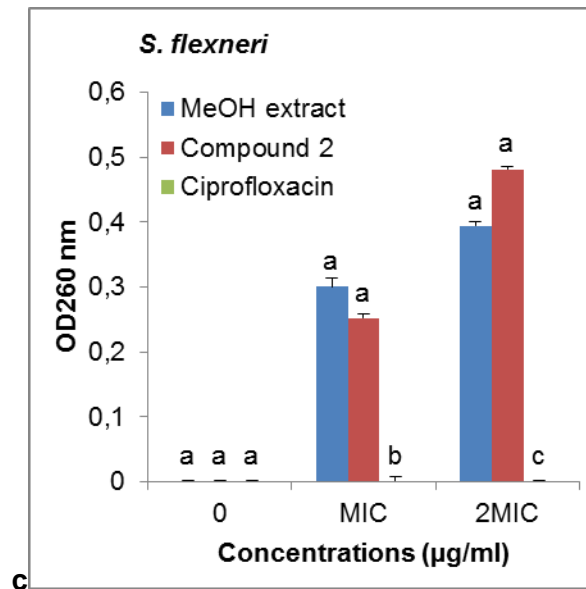
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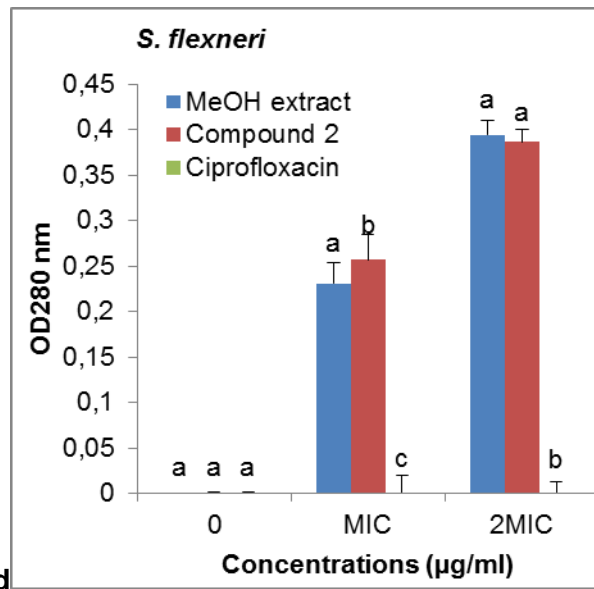
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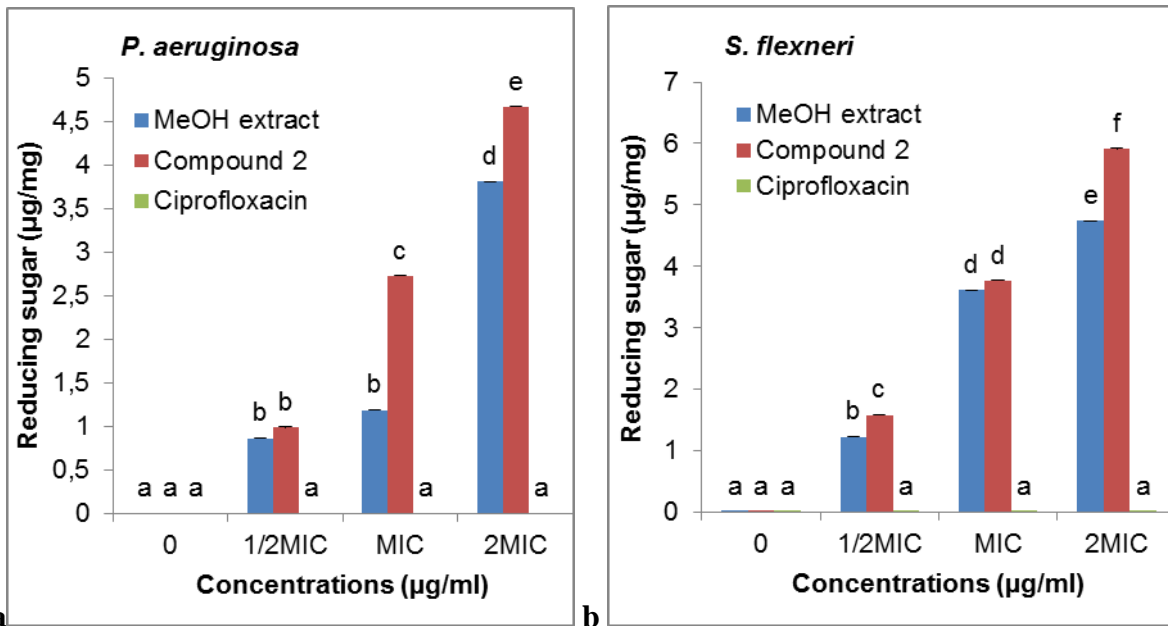
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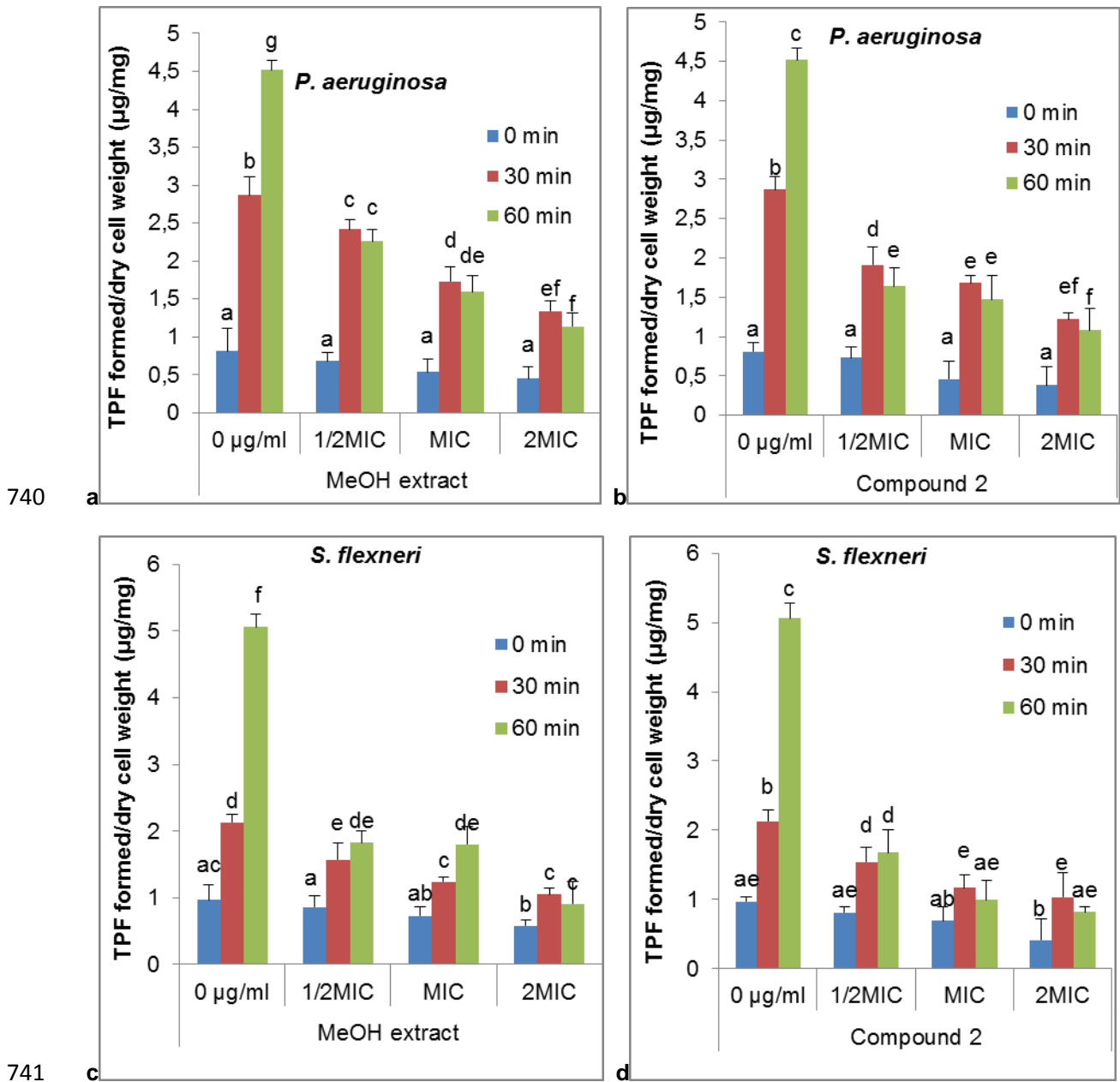
722 **Fig. 2** Appearance of 260 and 280 nm absorbing material in the filtrates of *P. aeruginosa* and  
 723 *S. flexneri* in control suspensions and after treatment with the different concentrations  
 724 of MeOH extract and compound 2.



725 **a**

726 **Fig. 3** Appearance of reducing sugar (µg/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
<b>1</b>	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
<b>2</b>	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
<b>3</b>	MIC	128	128	256	>256	>256	128	64
	MMC	256	>256	>256	>256	>256	>256	128
	MMC/MIC	2	/	/	/	/	/	2
<b>4</b>	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
<b>5</b>	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
<b>6+7</b>	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	FICEx	FIC	Interpretation	FICA	FIC2	FIC	Interpretation	FICA	FIC5	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; FICEx: 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 <sub>50</sub> , µg/mL)	Gallic acid equivalent antioxidant capacity (GEAC, µg/mL)
MeOH extract	62.11 ± 0.39 <sup>a</sup>	73.23 ± 0.61 <sup>a</sup>
EtOAc fraction	72.29 ± 0.71 <sup>b</sup>	58.44 ± 0.38 <sup>b</sup>
<i>n</i> -BuOH fraction	76.54 ± 0.78 <sup>c</sup>	40.46 ± 0.74 <sup>c</sup>
<b>1</b>	4.52 ± 0.36 <sup>d</sup>	104.87 ± 1.43 <sup>d</sup>
<b>2</b>	3.08 ± 0.44 <sup>e</sup>	106.03 ± 0.87 <sup>d</sup>
<b>4</b>	10.69 ± 0.51 <sup>f</sup>	79.54 ± 1.26 <sup>e</sup>
<b>5</b>	9.88 ± 0.62 <sup>f</sup>	81.09 ± 0.93 <sup>e</sup>
<b>6+7</b>	7.63 ± 1.27 <sup>g</sup>	83.38 ± 0.22 <sup>f</sup>
Vitamin C	1.81 ± 0.19 <sup>h</sup>	NA

756 EC<sub>50</sub>: 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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