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A new phenanthrene derivative from *Entada abyssinica* with antimicrobial and antioxidant properties

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Abstract: *Entada abyssinica* Steud. Ex A. Rich (Leguminosae) is a medicinal plant used traditionally for the treatment of infections. A phytochemical investigation of the methanol extract of *E. abyssinica* root bark led to the isolation of a new phenanthrene derivative named phenentada (**1**), together with seven known compounds (8 *S, 13 E*)-kolavic acid 15-methyl ester (**2**) and 8 *S*-kolavic acid 15-methyl ester (**3**) obtained as mixture, 8 *S*-kolavic acid 15-methyl ester (**3**), 8 *S*-kolavic acid 18-methyl ester (**4**), 13,14,15,16-tetranorclerod-3-ene-12,18-dioic acid (**5**), 1′,26′-bis-[(*S*)-2,3-dihydroxypropyl] hexacosanedioate (**6**), campesterol (**7**) and 3-*O*-*β*-D-glucopyranosylstigmasterol (**8**). Their structures were determined by NMR spectroscopy (1D and 2D), mass spectrometry (HRESIMS) and by comparison with previously reported data. The crude extract and some isolated compounds were evaluated for their *in vitro* antimicrobial activities by the microdilution method while, the antioxidant activity was evaluated by the DPPH methods. Regarding the antimicrobial activity, the crude extract showed significant inhibitory activities against bacteria strains (MIC 7.81–31.3 μg mL^{-1}) and yeasts (MIC 15.6–31.3 μg mL^{-1}) whereas all compounds tested exhibited significant activity against *Staphylococcus epidermidis*. Moreover, compounds **4**, **5** and **6** and the mixture **2/3** showed significant antimicrobial activity on *Candida parapsilosis* strain (MIC = 3.12 μg mL^{-1}), as well as selected antifungal property against *candida* pathogenic fungi strains. On the other hand, compounds (**1**) demonstrated the best bioactivities against *Candida albicans* and *Salmonella enterica* with MIC = 3.12 μg mL^{-1} while the mixture **2/3** appeared to have the highest inhibition on gram (+) bacteria strain *S. epidermidis* with MIC of 0.78 μg mL^{-1} and compound 5 (MIC = 1.56 μg mL^{-1}) against the gram (–) bacteria strain. Furthermore, the SC_{50} values measured by the antioxidant test for all samples varied between 47.21 and 52.44 μg mL^{-1} for DPPH. These results support the traditional uses of *E. abyssinica* in the management of several diseases including the claim in the skin disease treatment. Additionally, here is reported the first time isolation of a phenanthrene derivative in the Fabaceae family to the best of our knowledge.

Keywords: antimicrobial; antioxidant; *Entada abyssinica*; phenanthrene.

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1 Introduction

Infectious diseases are disorders caused by micro-organisms such as bacteria, viruses, fungi or parasites. Skin infections are very common throughout the world and vary from one region to another. Some skin infections can be attributed to poor hygienic conditions, which may be more prevalent in developing countries. It has been reported to make up to

42–65% of the total skin morbidity of children [1]. Health care systems around the world are faced with the challenge that bacteria are developing resistance to existing antimicrobial agents resulting in an increase in health care costs, morbidity and also mortality [2]. In addition, the oxygen radicals are key molecules in the pathogenesis of various infectious diseases [3]. Thus, it became urgent to search for new therapeutic agents with novel modes of action. In recent years, the uses of medicinal plants for the treatment of various diseases, including infectious diseases have really increased [4]. Our search for new as well as known secondary metabolites with antibacterial activities from natural sources led us to the phytochemical study of *Entada abyssinica*.

E. abyssinica Steud. Ex A. Rich is a small tree from the Fabaceae family which is widely spread in tropical Africa. Our attention was drawn to the fact that *E. abyssinica* is commonly used in Central Africa (Cameroon) for the traditional treatment of ailments such as coughs, rheumatism, skin infection, diarrhoea and fever [5, 6]. Consequently, this plants may contain various active antimicrobial compounds acting together synergistically in a multi-target fashion. Up to date previous chemical investigations of *E. abyssinica* reported the presence of secondary metabolites such as flavonoids, diterpenoids, triterpenoids, steroids, steroidal saponins, and fatty acids [7, 8]. In addition, some pharmacological properties of *E. abyssinica*, including anti-inflammatory, antimicrobial, antioxidant activities have already been reported as well as the antifungal activity of its methanol extract of root barks [7, 9, 10]. The focus of this study was consequently to isolate compounds possibly contributing to the antimicrobial activity, and which may therefore be promising precursors to be used for the development of novel antimicrobial drugs.

To neutralize free radicals and protect the body against oxidative damage, different antioxidants which are present in normal physiological conditions are able to counteract the production of reactive oxygen species. Free radicals are known to be the main cause of various diseases such as cancer and bacterial diseases. We describe herein the isolation, the structure elucidation, of one new phenanthrene derivative named: phenentada (**1**), together with seven known compounds (**2–8**) isolated from the methanol extract of root bark of *E. abyssinica*, as well as their *in vitro* antimicrobial and antioxidant activities.

2 Results and discussion

2.1 Phytochemical analysis

The methanol crude extract of *E. abyssinica* root bark were subjected repeatedly to column chromatography over silica gel to afford eight compounds (**1**–**8**, Figure 1). The known compounds (**2**–**8**) were identified by comparison of their spectroscopic data with those reported in literature. However, the structure elucidation of the new compound (**1**) is reported below.

Compound (**1**) was obtained as a white amorphous powder and was positive to the ferric chloride test indicating its phenolic nature. Its molecular formula $C_{17}H_{16}O_5$ was determined through the use of HR-ESI-MS which showed the protonated molecular ion pic at *m/z* = 301.1047 ($[M+H]^+$, $C_{17}H_{17}O_5^+$, calcd. *m/z* = 301.1076) and elementary analysis indicate 10° of unsaturation. The IR spectrum of (**1**) showed strong absorption bands associated with a hydroxyl (3382 cm^{-1}), together with the one of C–O (1023 cm^{-1}), while its UV spectrum showed maxima absorption bands at λ_{max} 224 and 280 nm characteristic of phenanthrene derivative [11].

Moreover, the ^1H NMR spectrum (Table 1) of (**1**) exhibited the signals of six aromatic protons among which the signal of one pair of *ortho*-coupled aromatic protons δ_H = 8.91 ppm (1H, d, *J* = 9.1 Hz, H-9) and δ_H = 8.30 ppm (1H, d, *J* = 9.1 Hz, H-10) [12] and four singlets at δ_H = 8.32 ppm (1H, s, H-4), 7.89 ppm (1H, s, H-5), 8.38 ppm (1H, s, H-8) and 7.76 ppm (1H, s, H-1) characteristics of a phenanthrene monosubstituted skeleton [13, 14]. It also showed a singlet of two protons at δ_H = 6.36 ppm (2H, *s*) suggesting a methylenedioxy moiety [15] and two singlets of three protons at δ_H = 4.05 ppm (3H, s, 6-OMe) and 4.24 ppm (3H, s, 7-OMe) indicating the presence of two methoxy groups. The decoupled ^{13}C NMR spectrum of (**1**) exhibited 17 carbon signals (Table 1) including those of two methoxy groups at δ_C = 56.8 ppm (6-OMe) and 57.8 ppm (7-OMe), one of a methylenedioxy unit at δ_C = 103.2 ppm (2-O-CH_2-O) [15, 16] and of 14 tertiary and quaternary aromatic carbons signals at δ_C = 148.9 ppm (C-2), 149.4 ppm (C-3), 105.1 ppm (C-4), 132.6 ppm (C4a), 132.9 ppm (C-4b), 109.2 ppm (C-5), 152.0 ppm (C-6), 158.7 ppm (C-7), 103.8 ppm (C-8), 124.6 ppm (C-8a), 119.8 ppm (C-9), 130.5 ppm (C-10), 120.4 ppm (C-10a) and 106.2 ppm (C-1) confirming the phenanthrene skeleton [13]. Moreover, extensive analysis of ^1H–^1H COSY, HSQC and HMBC spectra (Figure 2) led to the assignment of ^1H and ^{13}C resonances. The location of the methylenedioxy group was confirmed by the HMBC correlations observed between its protons at δ_H = 6.36 ppm and the *O*-bearing aromatic carbon at C-2 (δ_C = 148.9 ppm), this carbon is also having correlations with protons at δ_H = 7.79 ppm (H-1) and 8.32 ppm (H-4). The cross peak correlation observed between the methoxy protons at δ_H = 4.05 ppm (6-OMe) and 4.24 ppm (7-OMe) with carbons at δ_C = 152.0 ppm (C-6) and 158.7 ppm (C-7) respectively show that they are linked at C-6 and C-7. Consequently, the structure of (**1**) was elucidated as a new phenanthrene derivative named 2-(hydroxymethoxy)-

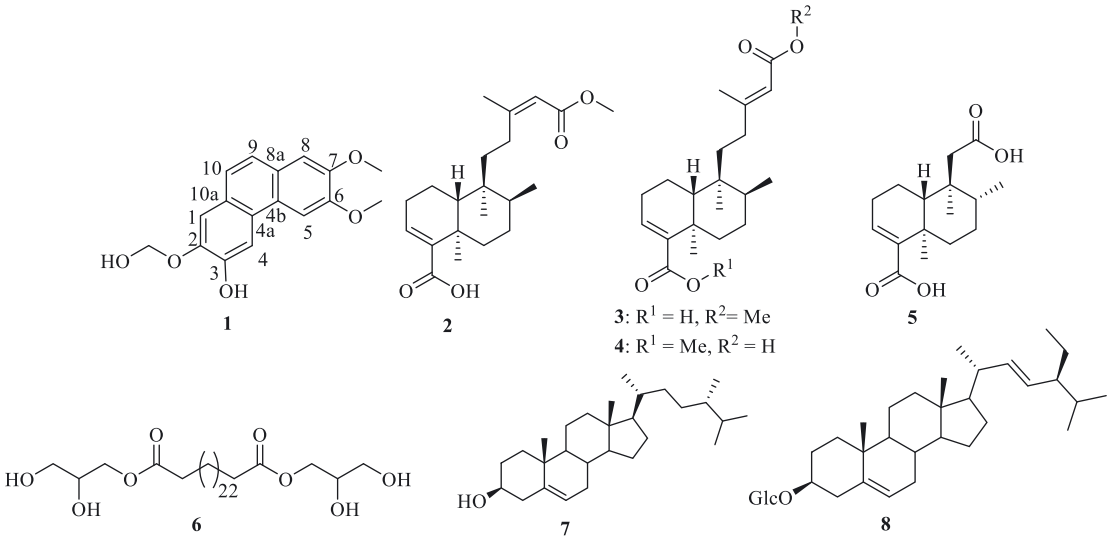

Figure 1: Structures of compounds **1**–**8** isolated from *E. abyssinica*.

Table 1: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR-spectroscopic data of compound **1** in DMSO-d_6

Position	δ_H (ppm)	δ_C (ppm)	HMQC	HMBC
1	7.76; s	106.2	CH	C-10, C-2, C-10a
2		149.4		
3		148.9		
4	8.32; s	105.1	CH	C-3, C-4b
4a		132.6		
4b		132.9		
5	7.89; s	109.2	CH	C-4a, C-7, C-6
6		152.0		
7		158.7		
8	8.38; s	103.8	CH	C-8a, C-7, C-6
8a		124.6		
9	8.91; d; *J* = 9.1 Hz	119.8	CH	C-4b, C-8a
10	8.30; d; *J* = 9.1 Hz	130.5	CH	C-8a, C-10a, C-1
10a		120.4		
2-O-CH_2-O	6.36; s	103.2	CH_2	C-2
6-OMe	4.05; s	56.8	CH_3	C-6
7-OMe	4.24; s	57.8	CH_3	C-7

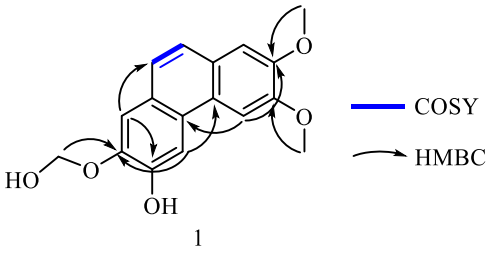

Figure 2: ^{1}H-^{1}H COSY and HMBC correlations observed in compound **1**.

6,7-dimethoxyphenanthren-3-ol to which we gave the trivial name phenentada.

The structures of known compounds were identified by comparison of their spectroscopic data with those reported in the literature as (8 *S, 13E*)-kolavic acid 15-methyl ester (**2**) and 8 *S*-kolavic acid 15-methyl ester (**3**) [6]; 8 *S*-kolavic acid 15-methyl ester (**3**) [7]; 8 *S*-kolavic acid 18-methyl ester (**4**) [6, 7]; 13,14,15,16-tetranorclerod-3-ene-12,18-dioic acid (**5**) [10]; 1′,26′-bis-[(*S*)-2,3-dihydroxypropyl] hexacosanedioate (**6**) [7]; campesterol (**7**) [17] and 3-O-*β*-D-glucopyranosyl-stigmasterol (**8**) [18] (Figure 1). Kolavic acid and derivates exhibit anti-inflammatory properties through various mechanisms contributing the anti-inflammatory properties of *E. abyssinica* [9, 19] and also inhibit *Trypanosoma brucei* [5], howewer, campesterol and derivates possess various pharmacological properties including cholesterol lowering effects, anti-inflammatory, antibacterial and antifungal activities and also have chemopreventive effects against cancer [18]. The identification of these compounds contributes to the verification of some therapeutic virtues of this species.

2.2 Antimicrobial activity

E. abyssinica is traditionally used in Cameroon to treat coughs, rheumatism, skin infection, diarrhoea and fever [5, 6]. The results of *in vitro* antimicrobial activities of the MeOH extract of root bark as well as compounds **1, 3**–**6** and the mixture of **2/3** against pathogenic bacteria and yeast are presented in Table 2. This table present the inhibition parameters (MIC, MBC and MBC/MIC ratio) of the crude

extract and tested compounds from *E. abyssinica*. The crude extract and isolates showed varying levels of antimicrobial properties against yeast and bacteria strains tested. Indeed, the antimicrobial activity of plant extracts can be classified as significant (MIC < 100 μg mL^{-1}), moderate (100 < MIC ≤ 625 μg mL^{-1}) and weak (MIC > 625 μg mL^{-1}) [20]. According to this classification, the inhibition potential of the methanol crude extract of *E. abyssinica* could be considered as significant against yeasts (MIC ranging from 15.6–31.3 μg mL^{-1}) and bacteria (MIC ranging from 7.81–31.3 μg mL^{-1}) except on *Salmonella enterica* strain. Furthermore, the gram (+) *S. epidermis* was the most sensitive with a MIC value of 7.81 μg mL^{-1} followed by *Candida albicans* (MIC = 15.6 μg mL^{-1}) and the *Candida parapsilosis* (MIC = 32.3 μg mL^{-1}). Additionally, the results obtained from the crude extract in this study were in line with those obtained by others authors in the literature. In fact, Harun et al. [21] demonstrated that the methanol extract of *Eleocharis spiralis* stem bark exhibited promising antifungal activity against three dermatophytes strains. While, Baidoo et al. and Dzoyem et al. [22, 23] showed antibacterial potential of methanol extract of *Epimyrma africana* root bark and *E. abyssinica* leaves respectively. According to the criteria used by Gatsing and Adoga [24], the antibacterial substance is considered as bactericidal, when the ratio MBC/MIC ≤ 4 and bacteriostatic when MBC/MIC > 4 [24]. Base on those criteria, the crude extract acted as good bactericide and fungicide against these pathogens suggesting that they could be potent candidates for the treatment of skin and other infectious diseases. Regarding pure compounds, antimicrobial cut-off points have been defined in the literature to enable the understanding of their effectiveness as follows: highly active (MIC < 1 μg mL^{-1}), significantly active (1 = MIC ≤ 10 μg mL^{-1}), moderately active (10 < MIC ≤ 100 mL^{-1}) and weakly active (100 < MIC ≤ 1000 μg mL^{-1}) [25]. Based on this cut-off, compounds **1**, **4** and the mixture of **2/3** showed significant activity against *C. albicans* with MIC ranging from 3.12–6.25 μg mL^{-1} while, compounds **4**, **5**, **6** and the mixture of **2/3** inhibited significantly *Candida parasilosis* with MIC values of 3.12 μg mL^{-1} each. The values of MIC of all the tested compounds in comparison to those of the crude extract against yeasts could justify his significant activity and

Table 2: MIC and MBC of extracts, isolated compounds and reference drugs from *E. abyssinica*.

Pathogen		Yeast		Gram (+)	Gram (–)			
Sample	**Strains**	***C. albicans***	***C. parasilosis***	***S. épidermidis***	***Pseudomonas aeruginosa***	***E. coli***	***S. typhi***	***S. enterica***
MeOH extract	MIC	15.6	31.3	7.81	15.6	15.6	31.3	ND
	MBC	31.25	62.5	15.6	ND	ND	ND	ND
	Ratio	2	2	2	—	—	—	—
1	MIC	3.12	12.5	1.56	3.12	3.12	6.25	3.12
	MBC	6.25	25	3.12	6.25	6.25	12.5	6.25
	Ratio	2	2	2	2	2	2	2
2/3	MIC	6.25	3.12	0.78	6.25	12.5	12.5	12.5
	MBC	12.5	6.25	1.56	12.5	25	25	ND
	Ratio	2	2	2	2	2	2	–
3	MIC	/	/	1.56	/	/	/	/
	MBC	/	/	6.25	/	/	/	/
	Ratio	—	—	4	—	—	—	—
4	MIC	6.25	3.12	1.56	3.12	12.5	6.25	6.25
	MBC	12.5	12.5	6.25	6.25	ND	12.5	12.5
	Ratio	2	4	4	2	–	2	2
5	MIC	12.5	3.12	1.56	12.5	1.56	6.25	12.5
	MBC	25	6.25	3.25	25	6.25	12.5	ND
	Ratio	2	2	2	2	4	2	–
6	MIC	12.5	3.12	1.56	6.25	12.5	12.5	3.12
	MBC	25	6.25	6.25	12.5	25	25	12.5
	Ratio	2	2	4	2	2	2	4
Ciprofloxacin	MIC	—	—	1.56	1.56	1.56	1.56	1.56
	MBC	—	—	1.56	1.56	1.56	1.56	1.56
	Ratio	—	—	1	1	1	1	1
Fluconazole	MIC	0.78	0.78	—	—	—	—	—
	MBC	0.78	1.56	—	—	—	—	—
	Ratio	1	2	—	—	—	—	—

ND, Not determined; /, MIC or MBC > 125; —, μg mL^{-1} not tested.

suggesting that many of them should proceed by synergism to enable the higher activity of the crude extract. Regarding the antibacterial test, all the screened compounds showed high to significant activity (MIC values between 0.78 and 1.56 μg mL^{-1}) against the gram (+) bacteria *S. epidermis* with the best activity observed to the mixture **2/3** (MIC = 0.78 μg mL^{-1}) compare to ciprofloxacin used as reference drug. Furthermore, compound **5** showed the best activity against the gram (–) bacteria *Escherichia coli* with MIC = 1.56 μg mL^{-1} while compound **1** showed a significant activity against all the selected gram (–) bacteria with MIC values ranging from 3.12–6.25 μg mL^{-1} and then confirm the antimicrobial potential of phenanthrene derivatives against a wide range of human pathogens [13]. All these compounds acted as bactericidal (MBC/MIC ≤ 4). Moreover, these results are in accordance with those obtained by Tchinda et al. [6] and Dzoyem et al. [6, 23] which have evaluated the antimicrobial activities of compounds **2, 3** and **4**, and which also assumed that the presence and position of ester and carboxylic groups in isolated diterpenoids played an important role in their antimicrobial activity.

2.3 Antioxidant activity

The *in vitro* antioxidant properties of crude extract and some of the isolated compounds along with standard drug (Gallic acid), using DPPH assays were reported in Table 3. Crude extract as well as compounds **1, 3**–**6** and the mixture of **2/3** exerted good scavenging capacities toward DPPH radical but not really significant with comparison to gallic acid. The scavenging capacity of each compound was similar except those of the compound **1** which had a SC_{50} of 4.72 ± 0.28 μg mL^{-1} which is similar to the gallic acid with a SC_{50} value of 2.86 ± 0.53 μg mL^{-1}. This compound belongs to the phenolic group which is well known for their antioxidant capacities due to the formation of stable radical when they give hydrogen to the radical DPPH [21, 26]. The scavenging capacity of the other compounds may be attributed to the presence of carboxyl group that classifies them as organic acids which can share their hydrogen with the radical DPPH. Relation between phenolic and organic acids with antioxidant capacities has been reported earlier [13, 21, 22, 27].

Table 3: Antioxidant activities of the MeOH extract some isolates and reference compound.

Samples	SC_{50} (μg mL^{-1})	Samples	SC_{50} (μg mL^{-1})
MeOH extract	47.8 ± 4.13	**4**	50.44 ± 2.05
1	4.72 ± 0.28	**5**	49.44 ± 9.76
2/3	48.05 ± 5.24	**6**	52.44 ± 3.32
3	52.21 ± 3.23	Gallic acid	2.86 ± 0.53

In conclusion, the phytochemical investigation of the MeOH extract from the root bark of *E. abyssinica* led to the isolation and characterization of eight compounds including one new phenanthrene derivative trivially name phenentada. It appears that the methanol extract, phenentada (**1**) and the mixture of **2/3** possess the most antifungal and antibacterial properties. Regarding the antioxidant activity, compound **1** exhibited an activity similar to that of gallic acid. In order to verify the synergetic and/or antagonism effect of different constituents in the mixture **2/3** which were more active than ciprofloxacin used as reference drug, we will focused our future investigation on isolation of compound **2** followed by evaluation of the antibacterial activity on the same strain. Additionally, these results highlight the traditional use of *E. abyssinica* in the treatment of infectious diseases, especially those caused by the tested microorganisms. Therefore, further studies should be conducted such as the evaluation of the toxicity of the extracts and the isolated compounds with the elucidation of their mechanism of action in order to propose a plant-based preparation for phytomedicine.

3 Experimental section

3.1 General experimental procedures

Gravitational column chromatography (CC) was performed using silica gel (Macherey-Nagel) (Si gel) 60 (70–230 mesh), 60 (240–400 mesh) while precoated Si gel 60 F254 (Merck) aluminum plates were used for thin layer chromatography (TLC). Si gel CC was eluted with *n*-hexane, to which EtOAc and MeOH were gradually added to increase the polarity while TLC plate was eluted with mixtures of solvents such as. TLC plates were sprayed with 50% diluted sulfuric acid and heated at about 96 °C or visualized under UV lamp at 254 and 365 nm. All the reagents were of analytical grade. The HR-ESI-MS spectra were performed with a spectrometer (QTOF Bruker, Germany) equipped with a HR-ESI source. The spectrometer operates in positive ion mode (mass range: 100–1500, with a scan rate of 1.00) with automatic gain control to provide high-accuracy mass measurements within 0.40 ppm deviation using Na formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200 °C. Nitrogen was used as sheath gas (10 L/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, Germany) UHPLC system consisting of LC pump, Diode Array Detector (DAD) (λ = 190–600 nm), auto-sampler (injection volume 10 μL) and column oven (40 °C). The separations were performed using a Synergi MAX-RP 100 A (50 × 2 mm, 2.5 μm particle size) with a H_2O (+0.1% HCOOH) (A)/acetonitrile (+0.1% HCOOH) (B) gradient (flow rate 500 μL/min, injection volume 5 μL). Samples were analyzed using a gradient program as follows: 95% A isocratic for 1.5 min, linear gradient to 100% B over 6 min, after 100% B isocratic for 2 min, the system returned to its initial

condition (90% A) within 1 min and was equilibrated for 1 min. Infra-Red (IR) spectroscopy was performed on an Alpha spectrometer (Bruker, MA, USA). The spectra were recorded from 4000 to 600 cm^{-1} in attenuated total reflectance (ATR) mode on a diamond crystal. The NMR experiments were performed on Bruker Avance 600 and Bruker Avance 500 spectrometers (Bruker, Belguim) in CD_3OD and in $CDCl_3$, DMSO-d_6, and Pyridine-d_5 respectively containing tetramethylsilane (TMS) as the internal standard. The resulting solution will be filtered and then concentrated by the rotary evaporator (Rotavapor (BUCHI) R II) to give the crude extract.

3.2 Collection and identification

The roots bark of *E. abyssinica* Steud. ex A. Rich were collected separately in October 2019 from Babadjou (Mbouda), in the Western Region of Cameroon. Identification was made by Mr. Victor Nana, a botanist from the National Herbarium of Cameroon in Yaoundé by comparison to the existing voucher specimen HNC 10672/SFR/CAM.

3.3 Extraction and isolation

The roots bark of *E. abyssinica* was chopped, air-dried and ground. The resulting powder (2 kg) was extracted by maceration using MeOH (100%) for 72 h and then filtered. The filtrate

The 40 g of the crude extract was assessed on silica gel column chromatography method (70–230 µm (Merck)) eluted with a gradient mixture of *n*-hexane-EtOAc (from 100/0 *v*/*v* to 0/100 *v*/*v*) followed by a gradient mixture of EtOAc/MeOH (from 100/0 *v*/*v* to 0/100 *v*/*v*). Collected fractions were pooled out according to their TLC profiles: F1 (14.7 g), F2 (2.4 g), F3 (10.6 g) and F4 (5.3 g). Fraction F1 (14.7 g) was subjected to column chromatography and eluted with hexane/EtOAc (9/1–7/3, *v*/*v*) to yield three subfractions (F1A – F1C). F1A was assessed on silica gel column chromatography eluted with hexane/EtOAc (7/3, *v*/*v*) to afford compound **1** (15 mg) and compound **2/3** (10 mg). From sub-fractions F1B and F1C, compound **3** (20 mg) precipitated as white powder and purified. Based on their TLC profiles, fractions F2 and F3 were combined and subjected to silica gel CC using a gradient elution of hexane/EtOAc (100:0–90:10, *v*/*v*) to afford four subfractions (F2,3A–F2,3D). Compound **4** (8 mg) was isolated from subfraction F2,3D after repeated silica gel and Sephadex column chromatography and compounds **5** (18 mg), **6** (12 mg), **7** (10 mg) and **8** (5 mg) were isolated from subfraction F2,3A. The chemical structure was elucidated by spectrometric method with comparison with literature data.

3.3.1 2-(Hydroxymethoxy)-6,7-dimethoxyphenanthren-3-ol (phenendata) (1): White powder. –UV (DMSO): 224 and 280 nm. –IR (DMSO): ν = 3382 (OH) and 1023 (C–O), 2920 cm^{-1}. $-^1$H NMR (600 MHz, DMSO-d_6): δ_H = 8.91 (*d*, *J* = 9.1 Hz, 1H, H-9), 8.30 (*d*, *J* = 9.1 Hz, 1H, H-10), 8.32 (*s*, 1H, H-4), 7.89 (*s*, 1H, H-5), 8.38 (*s*, 1H, H-8), 7.76 (*s*, 1H, H-1), 6.36 (*s*, 2H, 2-O-CH_2-O), 4.05 (*s*, 3H, 6-OMe), 4.24 (*s*, 3H, 7-OMe). $-^{13}$C NMR (150 MHz, DMSO-d_6): 56.8 (6-OMe), 57.8 (7-OMe), 103.2 (2-O-CH_2-O), 148.9 (C-2), 149.4 (C-3), 105.1 (C-4), 132.6 (C4a), 132.9 (C-4b), 109.2 (C-5), 152.0 (C-6), 158.7 (C-7), 103.8 (C-8), 124.6 (C-8a), 119.8 (C-9), 130.5 (C-10), 120.4 (C-10a) and 106.2 (C-1). –HRMS ((+)-ESI): *m*/*z* = 301.1047 (calcd. 301.1076 for $C_{17}H_{17}O_5$, $[M+H]^+$).

3.4 Antimicrobial activities assessment

3.4.1 Microorganisms: Crude extract and isolated compounds were tested for their antimicrobial activity against bacteria and yeast strains. The reference strains of microorganisms used in this study were obtained from BEI Resources and clinical isolates obtained from 'Centre Pasteur' of Cameroon. These microorganisms included two yeast viz *C. parapsilosis*, *C. albicans* and five bacterial species viz *Staphylococcus epidermidis* (Cocci Gram+), *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. typhi* (ATCC 19430) and *S. enterica* (NR4294) (Bacille Gram (–)) as previously reported.

3.4.1.1 Preparation of stock solutions of fungal extracts and reference drugs: The stock solutions were prepared by dissolving 2 mg of sample in 2 mL of 10% dimethylsulfoxide (DMSO) in water and 1 mg of the standard drug in 10 mL of 10% (DMSO). Fluconazol and ciproflaxacin obtained from Sigma Aldrich were used as positive control respectively for antifungal and antibacterial assays.

3.4.2 Antimicrobial assay: The estimation of the antimicrobial activities of the extracts and the isolated compounds were done using the broth microdilution method and the Minimum Inhibitory Concentration (MIC) of each sample was determined following the Clinical and Laboratory Standards Institute (CLSI) guidelines M27-A3 for yeast (CLSI, 2008) and M7-A10 for bacteria reported by Wouamba et al. [28]. Each sample was first tested in triplicate at a concentration of 500 µg/mL, and then samples showing inhibition were subsequently assessed for a cumulative concentration in order to determine their MIC. Briefly, 50 µL of Sabouraud Dextrose Broth (SDB) or Mueller Hinton Broth (MHB) were introduced in a 96-well microplate respectively for yeasts and bacteria. 50 µL of extract/compound concentrated at 1000 µg mL^{-1} were added to wells of the first line. A serial two-fold dilution was made by transferring 50 µL of the mixture of the first wells to the next one up to the last, final concentrations varying from 500 to 1.56 µg mL^{-1}. Then, 50 mL of an inoculum of 105 cells/mL for yeast and 106 cells/mL for bacteria were introduced in all the wells except those of the sterility control. Fluconazole and ciprofloxacin were used as positive control respectively for fungal and bacteria with a concentration varying from 100–0.78 µg mL^{-1}. The mixtures were incubated for 24 and 48 h for bacterial and fungal respectively. The lowest concentration of extract/compound that inhibited the visible growth of a microorganism was defined as minimum inhibitory concentration (MIC). The classification of criteria of the antibacterial activity of extract, fraction and compounds were based on the MIC threshold reported by Kuete [20]. The ratio MBC/MIC was calculated in order to determine the sample action method (bactericidal for a ratio ≤4) or (bacteriostatic for a ratio >4).

3.4.3 Antioxidant assays

3.4.3.1 DPPH free radical scavenging assay: Antiradical protocol is based on the decrease of the absorbance of the 2,2′-diphenyl-1-picrylhy-drazyl (DPPH) radical in the presence of anti-radical compounds measured at the wavelength of 517 nm. This was done according the method described by Brand-William and co-workers with slight modification in 1995 [29]. Samples (extracts and isolated compounds) and gallic acid, dissolved in methanol, were tested at the range of concentration between 100 and 1.56 µg mL^{-1} and 20–

0.78 µg mL^{-1} respectively in triplicate. A volume of 100 μL of the tested sample or standard was added in 900 µL of DPPH solution (50 mM). Methanol was used as blank. The scavenging capacity (SC) of the tested sample was calculated using the following formula: SC (%) = 100 × ($A0 - A1$)/$A0$, where $A0$ and $A1$ are the absorbance values for negative control (DPPH solution) and sample at 517 nm. The SC_{50} (scavenging capacity at 50%) values of sample and gallic acid were calculated by linear regression.

4 Supporting information

UV, IR, NMR and HR-ESI-MS spectra are given as supplementary material available online (https://doi.org/10.1515/znb-2021-0076).

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