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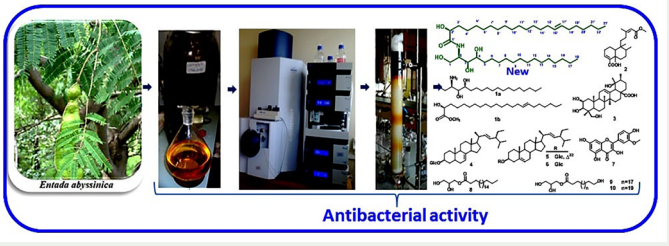
Chemical profiling by UHPLC-Q-TOF-HRESI-MS/MS and antibacterial properties of *Entada abyssinica* (Fabaceae) constituents

Larissa M. Magnibou[b], Steven C. N. Wouamba[a,c], Abel J. G. Yaya[b], Judith F. Mbougnia[d], Guy S. S. Njateng[d], Ghislain W. Fotso[c], Celine Henoumont[e], Sophie Laurent[e] and Talla Emmanuel[b]

[a]Laboratoire BONHEURS-EA 751, CY Cergy-Paris Université, Gennevilliers, France; [b]Department of Chemistry, Faculty of Science, University of Ngaoundéré, Ngaoundéré, Cameroon; [c]Department of Organic Chemistry, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon; [d]Faculty of Science, University of Dschang, Dschang, Cameroon; [e]Department of General, Organic and Biomedical Chemistry, University of Mons, Mons, Belgium

ABSTRACT

A rapid untargeted UHPLC-Q-TOF-ESI-MS/MS-Based metabolomic profiling of the medicinal plant *Entada abyssinica* was performed. A total of 18 metabolites were detected, of which 10 could not be identified. Based on this result, an extensive chemical investigation of the CH_2Cl_2-MeOH (1:1) extract of this plant was carried out, leading to the isolation of a new ceramide, named entadamide (**1**), together with nine known compounds: monomethyl kolavate (**2**), 24-hydroxytormentic acid (**3**) chondrillasterol (**4**), 3-*O*-*β*-D glucopyranosylstigmasterol (**5**), 3-*O*-*β*-D glucopyranosylsitosterol (**6**), quercetin 3′-methylether (**7**), 2,3-dihydroxypropyl icosanoate (**8**), 2,3-dihydroxypropyl 23-hydroxytricosanoate (**9**) and 2,3-dihydroxypropyl 24-hydroxytetracosanoate (**10**). Their structures were elucidated by the analyses of their spectroscopic and spectrometric data (1D and 2D NMR, and HRESI-MS) in comparison with those reported in the literature. Furthermore, the crude extract and some isolated compounds were tested against non-ciprofloxacin resistant strains *viz, Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Samonella thyphi* (ATCC 19430) and *Samonella enterica* (NR4294). The tested samples demonstrated significant activity against all the tested bacteria (MIC values: 3.12–12.5 µg/mL).



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CONTACT Steven C. N. Wouamba wouamba01s@yahoo.fr; Emmanuel Talla tallae2000@yahoo.fr
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1. Introduction

In Africa, alongside malaria, infections induced by bacterial pathogens are one of the major causes of mortality. Water-borne and enteric diseases come to the fore in Cameroon (Wouamba et al. 2020b; Nguiam et al. 2021; Tali et al. 2022). The access to conventional medicines being expensive for the Cameroonian indigenous population, they rely on medicinal plants for their primary health care (Kuete and Efferth 2010; Njanpa et al. 2021; Kemgni et al. 2021; Magnibou et al. 2022). *Entada abyssinica* Steud. ex A. Rich (Fabaceae), the subject of this study, is listed among these plants. It is a small to medium-sized, deciduous tree, 3–15 m high, with a flat, spreading crown; bark grey to reddish, slightly fissured, flaking off in irregular patches; slash pink with streaks of red; branchlets pendulous, glabrous or sometimes pubescent (Orwa et al. 2009; Magnibou et al. 2022). It is found throughout tropical Africa where it is used for the treatment of miscarriage and against fever (Dzoyem et al. 2017). A decoction of the bark is taken against cough, chronic bronchial engorgement, rheumatic and abdominal pains (Haile and Delenasaw 2007). In general people with low income such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common enteric infections (Fabricant and Farnsworth 2001). Bacterial infectious diseases caused by these multidrug resistant strains remain the leading cause of death. Thus people are turning their attention to alternative novel antimicrobial agents to combat such pathogens (Jain et al. 2011). Some pharmacological properties of *E. abyssinica* have been previously reported, including anti-inflammatory, antileshmanial, antimicrobial and antioxidant (Olajide and Alada 2001; Nyasse et al. 2004; Teke et al. 2011; Melong et al. 2014; Dzoyem et al. 2017; Magnibou et al. 2022; Mbougnia et al. 2022). Previous chemical investigations of *E. abyssinica* have reported the presence of secondary metabolites such as flavonoids, diterpenoids, triterpenoids, steroids, steroidal saponins, phenantrenes, cerebrosides, kolavic acid derivatives and fatty acids (Freiburghaus et al. 1998; Tchinda et al. 2007; Melong et al. 2014; Magnibou et al. 2022).

In our continuous search for secondary metabolites with biological importance from Cameroonian medicinal plants, we undertook the investigation of the CH_2Cl_2-MeOH (1:1) extract of the stem bark of *E. abyssinica*. Considering the vast potential of plants as sources of antimicrobial drugs, the objective of this research was to perform rapid, untargeted LC–MS/MS-based metabolomic profiling of this plant by LC-MS/MS, followed by isolation of the compounds and finally evaluation of their antibacterial activity. The final goal being the discovery of new molecules of interest for future generations.

2. Results and discussion

2.1. Qualitative determination of compounds contents of E. abyssinica using UHPLC(DAD)-Q-TOF-ESI-MS/MS

The present study, which contributes to phytochemical knowledge of the *Entada* genus, evaluated the chemical composition of *Entada abyssinica* stem bark and screened it for antibacterial activity.

Stem bark extract of *E. abyssinica* was extracted using the mixture of CH_2Cl_2/MeOH (1:1, v/v). It is worth noting that this solvent system was chosen because the CH_2Cl_2/MeOH (1:1, v/v) extract displayed the best antibacterial activity after micro-extraction compared to the hydroethanolic extract. The resulting crude extract was firstly subjected to liquid chromatography coupled to mass spectrometry (LC–MS/MS).

Figure S1 and Table 1 present the chromatographic profiles of the extract and the main peaks corresponding to compound detected by UHPLC-Q-TOF-(HR)ESI-MS/MS (positive ionisation mode). The blue curve represents the profile of the crude extract studied and the black curve represents the profile of the blank (see Figure S1). The superposition of these two chromatographic traces revealed the presence of several compounds. Eighteen of them showed good ionisation and fragmentation and therefore were detected. The putative identification of these compounds present in crude extract was performed according to the fragmentation profiles of the spectra generated in comparison with the data from the literature of compounds previously isolated from the *Entada* genus, and available in the databases (SciFinder, NIST/EPA/NIH Mass Spectral Library (NIST 14) and Mass Bank of North America (MoNA)) (Table 1., supplementary data). This strategy led to the identification of 8 compounds (**2**, **11**–**17**) among the 18 detected by LC-MS (see supplementary data, Figure S2). However, compound 17, diheptylphthalate, although isolated from this plant by Melong et al. (2014), could be a contaminant. In fact, phthalates are increasingly being considered as contaminants in plant extracts (Bianco et al. 2014; Venditti 2020; Thiemann 2021). The other compounds we have not identified were simply not found in the databases. They could be new classes of natural compounds present in the *Entada* genus in very low proportions.

Table 1. Metabolites assigned in *E. abyssinica* stem bark extract *via* UHPLC-Q-TOF-ESI-MS/MS in positive ion mode.

		$[M+Na]^+$ or $[M+H]^+$			Pseudo-molecular ion	
Nº	Tr (min)	Exp.	Calcd.	MS/MS	formula	Name of structure
1	0.31	198.0535	198.0525	60	$C_{10}H_9NO_2Na^+$	Acetic1H-Indole-3-acid (**11**)
2	1.58	226.1812	223.1802	125; 110	$C_{13}H_{24}NO_2^+$	2-methyl-N-(4-methylpentan-2-yl) hexa-2,4-dienamide (**12**)
3	1.68	246.1500	246.1489	60; 195	$C_{15}H_{20}NO_2^+$	2-(5-(tert-Butyl)-2-methyl-1H-indol-3-yl) acetic acid (**13**)
4	2.80	381.0590	381.0581	–	$C_{18}H_{14}O_8Na^+$	Not Identified
5	2.92	263.1651	263.1642	–	$C_{16}H_{23}O_3^+$	Not Identified
6	3.14	345. 2407	345.2400	–	$C_{20}H_{34}O_3\ Na^+$	Not Identified
7	3.28	316.2849	316.2846	288	$C_{18}H_{38}NO_3^+$	Not Identified
8	3.41	318.3008	318.3003	300	$C_{18}H_{40}NO_3^+$	Not Identified
9	3.80	303.1525	303.1567	246	$C_{16}H_{24}O_4Na^+$	parathyrsoidin G (**14**)
10	3.90	373.2355	373.2349	–	$C_{21}H_{34}O_4\ Na^+$	Not Identified
11	4.04	499.3286	499.3265	–	$C_{27}H_{47}O_8^+$	Not Identified
12	4.12	329.2457	329.2451	242; 236	$C_{20}H_{34}O_2Na^+$	Ethyl linolenate (**15**)
13	4.55	371.2155	371.2193	326; 350	$C_{21}H_{32}O_4Na^+$	Monomethyl kolavate (**2**)
14	4.87	353.2407	353.2087	224	$C_{21}H_{30}O_3Na^+$	Not Identified
15	5.06	325.2152	325.2138	258; 244	$C_{20}H_{30}O_2Na^+$	4-Epicommunic acid (**16**)
16	5.19	377.2674	377.2662	258	$C_{21}H_{38}O_4Na^+$	Not Identified
17	5.34	367.0433	367.0424	–	$C_{17}H_{12}O_8Na^+$	Not Identified
18	5.45	385.2363	385.2349	385	$C_{22}H_{34}O_4Na^+$	Diheptylphthalate (**17**)

While comparing the retarding factor, the experimental and calculated ion peak masses and the molecular formula, the result revealed that the CH_2Cl_2/MeOH (1:1, v/v) extract of *E. abyssinica* contained several secondary metabolites. This result paved the way for the isolation of secondary metabolites of this extract. This approach constitutes on the one hand an interesting lead towards the discovery of new compounds and on the other hand the evaluation of their antimicrobial activity could shed light on the ethno-pharmacological use of this plant.

2.2. Isolation and structure elucidation

After analysis of the LC/MS data, the crude extract (~30.5 g) was subjected to repeated silica gel and Sephadex LH-20 column chromatography (CC) and recrystallization to afford a previously undescribed ceramide named Entadamide [(**1**), 8.40 mg], together with nine known compounds *viz*, (8S, 13E)-kolavic acid 15-methyl ester [(**2**), 6 mg] (Tchinda et al. 2007), 24-hydroxytormentic acid [(**3**), 10.0 mg] (Houghton and Lian 1986), Chondrillasterol [(**4**), 7 mg] (Bergmann and McTigue 1948), mixture of 3-*O*-*β*-D glucopyranosylstigmasterol/3-*O*-*β*-D glucopyranosylsitosterol [(**5/6**), 15.0 mg] (Wouamba et al. 2020a), quercetin 3′-methylether [(**7**), 9.00 mg] (Fu et al. 2005), 2,3-dihydroxypropyl icosanoate [(**8**), 7.00 mg] (Rocchetti and O'Callaghan 2021), 2,3-dihydroxypropyl 23-hydroxytricosanoate [(**9**), 12.0 mg] and 2,3-dihydroxypropyl 24-hydroxytetracosanoate [(**10**), 8.00 mg] (Sharma et al. 2016) (Figure 1).

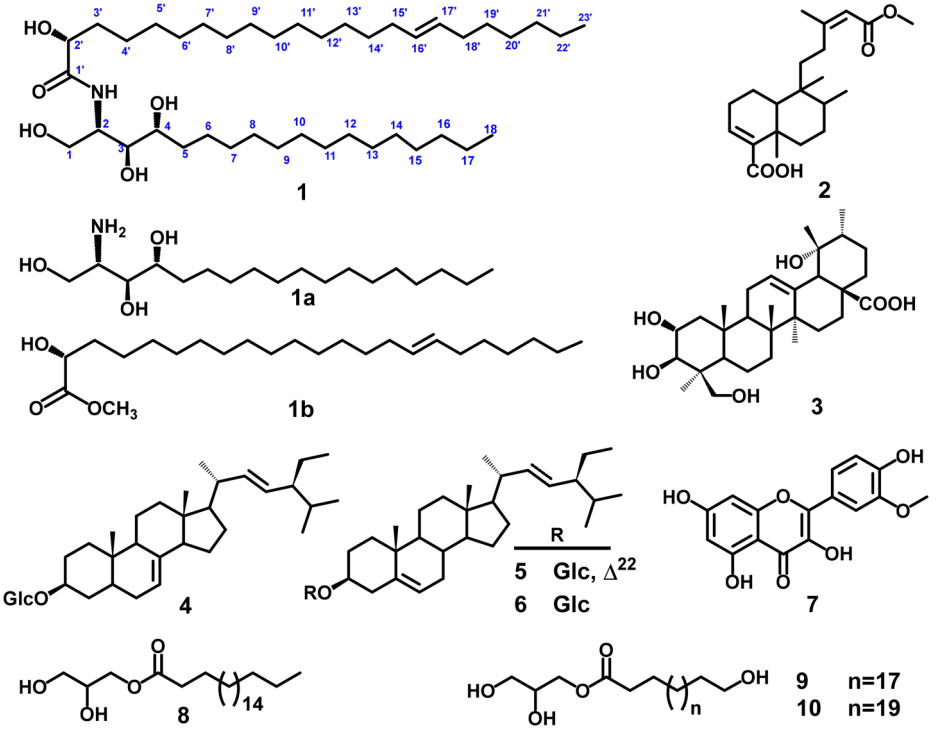

Figure 1. Structures of compounds **1**–**10** isolated from *E. abyssinica*.

The structures of the known compounds were identified by comparison of their spectroscopic and spectrometric data with those reported in the literature.

Compound **1** was obtained as a white powder. Its molecular formula, $C_{41}H_{81}NO_5$ was established from its HRESI-MS spectrum (Figure S3), showing the pseudo-molecular sodium adduct peak $[M+Na]^+$ at *m/z* 690.6024 ($C_{41}H_{81}NO_5Na^+$, calcd. 690.6007), indicating two degrees of unsaturation. Its IR spectrum (Figure S4) showed characteristic absorption bands for free OH groups (3329–3215cm^{-1}) and an amide group (1623 cm^{-1}) (Tsamo et al. 2021; Wonkam et al. 2020; Kamdoum et al. 2022). The structure of compound **1** was fully assigned after careful analyses of its ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC, tandem MS spectra and methanolysis reaction. Indeed ^{1}H-NMR spectrum (Figure S5, Table S1) of compound **1** showed the presence of an NH group at δ_H 8.60 (1H, d, $J=9.0$), two signals of one proton each in the form of two multiplets between δ_H 5.18–5.48 and 5.48–5.55 (2H, m, H-16′ and H-17′) suggesting the presence of olefinic protons in the structure of compound **1** (Wouamba et al. 2020a; Kamdoum et al. 2022). A broad signal centred at δ_H 1.52–1.20 (methylene protons), a distorted triplet at δ_H 0.88 (6H, t, $J=7.0$, H-18 and H-23′) (two terminal methyl groups) and two oxymethylene protons at δ_H 4.48 (dd, $J=10.7$, 5.0, H-1a) and 4.35 (dd, $J=10.7$, 4.8, H-1b), were indicative of a ceramide moiety (Simo et al. 2008; Fouatio Feudjou et al. 2022). This was supported by the presence of signals of a nitrogen-attached sp^3 carbon at δ_C 51.5 (C-2) and characteristic olefinic and oxymethine carbons at δ_C 130.6 (C-16′), 130.5 (C-17′), 76.5 (C-3), 72.2 (C-4), and 72.6 (C-2′). Further analysis of the ^{13}C NMR spectrum (Figure S6, Table S1) showed signals for oxymethylene carbon at δ_C 61.3 (C-1), and an amide carbonyl carbon at δ_C 175.0 (C-1′). In addition, conjunction of ^{13}C-NMR and HSQC spectra (Figure S6–S7) confirmed the signal of a nitrogen-attached sp^3 carbon at $\delta_{H/C}$ 5.14/51.5 (C-2), two diastereotopic protons of an oxymethylene at $\delta_{H/C}$ 4.35/61.3 (C-1) and 4.35/61.3 (C-1), as well as three oxymethine protons at $\delta_{H/C}$ 4.38/76.5 (C-3), 4.65/72.2 (C-4) and 4.31/72.6 (C-2′) respectively.

The HMBC correlations (Figure S8) of protons at δ_H 4.48/4.35 (H-1a/H-1b) to carbons δ_C 52.5 (C-2), and 76.5 (C-3); at δ_H 5.14 (H-2) to δ_C 61.3 (C-1), 76.3 (C-3), and 175.0 (C-1′); and at δ_H 4.31(H-2′) to δ_C 175.0 (C-1′), allowing us to locate the hydroxy groups at positions C-1, C-3, C-4, and C-2′, respectively and to conclude that, the two chains (acid and basic) are linked. Further important HMBC (Figure S8 and S10a) correlations were observed between δ_H 5.54 (H-16′ and H-17′) and δ_C 33.5 (C-18′), δ_H 1.97 (H-18′) and δ_C 31.8 (C-19′), and δ_H 1.97 (H-19′) with δ_C 31.8 (C-21′) and 14.0 (C-23′) suggesting that the double bond was located on the long fatty acid chain. Moreover, ^{1}H–^{1}H COSY correlations (Figures S9 and S10a) observed between H-1a/H-1b and H-2, H-2 and H-3, H-3 and H-4 allowed us to confirm the position of four hydroxy groups. All these evidences above, and by comparison with data of published related compounds, confirmed that **1** is a ceramide (Wouamba et al. 2020a; Kamdoum et al. 2022). The lengths of the fatty acid and sphingosine moiety as well as the position of double bond were deduced by the methanolysis using 0.9 N, HCl/MeOH, at 70 °C for 20 H (Simo et al. 2008) to yield sphingosine (**1a**) and fatty acid methyl ester (**1b**) (Figure S11). Specifically, the peak at at *m/z* 218.2 $[M_{LCB}+H]^+$ =318 corresponding to molecular formula $C_{18}H_{40}NO_3$ was attributable to the long chain base (**1a**) and do not contain degree of unsaturation (Figure S12).

Furthermore, this molecular formula of sphingosine suggested that the olefinic moiety is located in the long chain acide (LCA). The molecular formula of the methyl ester fatty acid ($C_{24}H_{46}O_3$) was deduced from the molecular formula of compound **1** by subtracting the molecular formula of the sphingosine moiety. The length of the long chain acid (LCA) was confirmed by the presence of some characteristic fragment ions are observed at *m/z* 389 $[CH_3(CH_2)_5CH=CH(CH_2)_{13}CH(OH)CONHNa]^+$,*m/z* 369 $[CH_3(CH_2)_5CH=CH(CH_2)_{13}CH(OH)CONH_4]^+$ and *m/z* 346 $[CH_3(CH_2)_5CH=CH(CH_2)_{13}CH(OH)Na]^+$. Futhermore the MS analysis showed the ions peak of the fragments from the allylic cleavages $[M+NH_4\text{-}C_6H_{13}]^+$ at *m/z* 600 and $[M+NH_4\text{-}C_8H_{14}]^+$ at *m/z* 571 confirming the $\Delta^{16'}$ location of the double bond (Figure S10b). The (*E*)-configuration was deduced from the chemical shift values at δ_C 33.8 and 33.5 of its allylic carbons C-15′ and C-18′ (Table S1). (Simo et al. 2008; Wouamba et al. 2020a; Tsamo et al. 2021). Generally, the stereochemistry of the olefinic functional group is assigned from the coupling constant values of $J=16$ Hz, and 8 Hz for *trans*- and *cis*-configurations, respectively. Nevertheless, if these coupling constant values are observed as multiplet, this could also be assigned by ^{13}C chemical shift values of allylic carbons, $\delta_C > 30$ for *trans*-configuration and $\delta_C \leq 27$ ppm for *cis*-configuration (Simo et al. 2008; Wouamba et al. 2020a; Tsamo et al. 2021; Kamdoum et al. 2022). Based on their biosynthetic evidence and as observed in all the naturally occurring sphingolipids, the absolute configurations of C-2 and C-3 were assigned theoretically as 2*S*, 3*S*, respectively (Wonkam et al. 2020; Kamdoum et al. 2022). The comparison of the data of compound **1** with those of previously reported compounds (Wouamba et al. 2020a; Wonkam et al. 2020; Kamdoum et al. 2022) further supported the theoretical assignment of absolute configurations *R* and *S* to C-4 and C-2′, respectively. From the spectroscopic analysis above, the structure of **1** was unambiguously determined as (2S,2′R,3S,4R,16E)-*N*-[2′-hydroxytricos-16-enoyl]-2-amino-octadecane-1,3,4-triol and trivially named entadamide.

Compound **2** was obtained as a white powder. Its molecular formula, $C_{41}H_{81}NO_5$ was established from its HRESI-MS spectrum (Figure S3), showing the pseudo-molecular sodium adduct peak $[M+Na]^+$ at *m/z* 690.6024 ($C_{41}H_{81}NO_5Na^+$, calcd. 690.6007), indicating two degrees of unsaturation. Its IR spectrum (Figure S4) showed characteristic absorption bands for free OH groups (3329–3215cm^{-1}) and an amide group (1623 cm^{-1}) (Tsamo et al. 2021; Wonkam et al. 2020; Kamdoum et al. 2022). The structure of compound **1** was fully assigned after careful analyses of its ^1H and ^{13}C NMR, ^1H-^1H COSY, HMQC, HMBC, tandem MS spectra

Compound **2** is a methyl ester isolated from the methanolic extract. Although its acidic (unmethylated) structural analogue was not detected by UHPLC-(DAD)-Q-TOF-HRESI-MS/MS analysis, this compound 2 could be an artefact. Indeed, compound 2 could be formed during the extraction process by derivatisation of its unmethylated analogue, as the methanol/dichloromethane mixture was used for extraction. However, we do not have sufficient evidence at this stage to conclude with certainty whether this is an artefact or a natural compound from *Entada abyssinica*. But it is interesting to note that this compound 2 was formerly isolated by Nyasse et al. (2004) from the pure methylene chloride extract of this plant. In addition, Tchinda et al. (2007) reported the isolation of compound (2) and its unmethylated analogue from the acetone extract of *E. abyssinica*.

2.3. Antibacterial assay

The crude extracts and some isolated compounds were evaluated for their antibacterial activity against *Pseudomonas aeruginosa* (ATCC 27853), Escher*ichia coli* (ATCC 25922), *Samonella thyphi* ATCC 19430) and *Samonella enterica* (NR4294) using the broth micro-dilution method as reported by Wouamba et al. (2020b) and ClinicalLaboratory Standard Institute (CLSI) 2008, 2015. The results obtained are shown in Table 2. According to Kuete et al. (2010), the activity of extracts is classified as significant when MIC < 100 µg/mL, as moderate when 100 < MIC <625 µg/mL and as weak when (MIC > 625 µg/mL). The (CH_2Cl_2/MeOH 1:1, v/v) stem bark crude extract demonstrated significant activity on the tested strains with MIC values ranging from 12.5 to 100 µg/mL. The ratio MBC/MIC values were globally less than 4 showing that the extract has bactericidal activity against all the tested strains (Marmonier, 1990) . From this extract, the obtained and evaluated compounds (**1, 2, 3, 7** and **8**) showed various degrees of antibacterial activities (Table 2). Except compound **1,** all the tested compounds (**2, 3, 7** and **8**) were active against all the tested bacteria. According to Kuete et al. (2010), the activity of pure compounds was classified as significant when (MIC < 10 µg/mL), as moderate when (10 < MIC <100 µg/mL) and as weak when (MIC > 100 µg/mL). Compound **3** showed significant activity against all the tested bacteria with MIC ranging from 1.56 to 6.25 µg/mL and was the most active compound. Its antibacterial activity against *E. coli* was comparable to that of the ciprofloxacin used as positive control. Compound **3**, is a derivative of tormentic acid, classified as an ursane-type pentacyclic triterpene. The antibacterial activity of tormentic acid and its hydroxy derivatives have been confirmed, both *in vitro* and *in vivo* (Olech et al. 2021). They have been shown to be potent agents against *Pseudomonas aeruginosa* (Ghosh et al. 2020). The mechanism of such activity involved increased depolarisation of the cytoplasmic membrane leading to bacterial cell disintegration (Olech et al. 2021). In addition, the anti-biofilm effect of tormentic acid against *P. aeruginosa* biofilm was revealed, and inhibition of biofilm formation was shown to be mediated by suppression of pyoverdine secretion, protease and swarming motility in *P. aeruginosa* (Ghosh et al. 2020). Compound **2** also exhibited significant activity against *P. aeruginosa* and *S. enterica* with MIC of 6.25 µg/mL and 3.12 µg/mL respectively; *w*hile compounds **7** and **8** were globally moderately active. The MBC/MIC ratio for the tested compounds

Table 2. MIC And MBC of the crude extract and compounds against the tested microbial strains.

	Microbial strains							
	P. aeruginosa		*E. coli*		*S. thyphi*		*S. enterica*	
	MIC and MBC (µg/mL)							
samples	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Compounds								
1	/	/	/	/	/	/	/	/
2	6.25	12.5	12.5	25	12.5	25	3.12	12.5
3	3.12	6.25	1.56	ND	6.25	12.5	6.25	12.5
7	6.25	12.5	12.5	25	12.5	25	12.5	ND
8	12.5	25	12.5	25	6.25	12.5	12.5	ND
Crude extract	12.5	50	50	ND	50	100	12.5	50
Ciprofloxacin	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56

varied between 1 and 4. According to Marmonier (1990), pure compounds exert two types of activities: a bacteriostatic (MBC/MIC ≥ 4) and bactericidal activity (MBC/MIC ≤ 4). So, globally, all the compounds exerted bactericidal activities against the tested bacteria. These results are in line with those obtained in previous studies (Magnibou et al. 2022).

The obtained results support the use of *E. abyssinica* in alternative medicine to treat diseases of bacterial origin with compound **3** being a possible promising drug candidate against bacterial infections.

3. Experimental (supplementary data)

3.6. Entadamide (1)

White powder; IR (KBr): 3329 cm^{-1}, 3215 cm^{-1} and 1623 cm^{-1}; HRESI-MS: $[M+Na]^+$ at *m/z* 371.2155 ($C_{41}H_{81}NO_5Na^+$, calcd. 371.2193); ^{1}H-NMR (600 MHz, C_5D_5N) & ^{13}C-NMR (150 MHz, C_5D_5N) see Table S1.

3.7. Monomethyl kolavate (2)

White powder; HRESI-MS: $[M+Na]^+$ at *m/z* 690.6024 ($C_{21}H_{32}O_4Na^+$, calcd. 690.6007); ^{1}H-NMR (600 MHz, C_3D_6O) δ_H 1.60–1.33 (m, 2H, H-1), 2.11–2.01 (m, 1H, H-2), 6.36 (1H; *s,* H-3), 1.67–1.42 (m, 2H, H-6), 1.63–1.38 (m, 2H, H-7), 3.45 (s, 1H, H-8), 1.14 (s, 1H, H-10), 1.19–1.21 (m, 2H, H-11), 1.97–2,10 (m, 2H, H-12), 5.54 (1H; *d; J* = 1,3Hz, H-14), 1.14 (3H; *s,* H-16), 0.79 (3H; *d; J* = 0,8Hz, H-17), 0.82 (3H; *s,*H-19), 1.58 (3H; *s,* H-20), 3.45 (3H; *s,* OCH3); ^{13}C-NMR (150 MHz, C_3D_6O) δ_C 17.9 (C-1), 26.8 (C-2), 136.5 (C-3), 142.4 (C-4), 37.5 (C-5), 37.6 (C-6), 25.2 (C-7), 34.9 (C-8), 37.5 (C-9), 45.0 (C-10), 29.5 (C-11), 34.0 (C-12), 161.2 (C-13), 115.0 (C-14), 166.9 (C-15), 16.9 (C-16), 14.3 (C-17), 166.9 (C-18), 19.8 (C-19), 20.9 (C-20),15-OMe(50,3)

4. Conclusion

From the total CH_2Cl_2-MeOH (1:1, v/v) stem bark extract of *Entada abyssinica*, this research allowed the rapid detection of 18 compounds by a metabolomic based on UHPLC-Q-TOF-ESI-MS/MS profiling. Using chromatographic, spectroscopic and spectrometric methods, we isolated and characterised 10 compounds (**1**–**10**) including entadamide (**1**), a new ceramide. MS fragmentation studies and the use of databases allowed us to identify 8 compounds (2, **11**–**17**) among those detected by LC-MS. Except compound **1**, all the tested samples demonstrated significant or moderate activities against the tested bacteria (MIC values: 3.12–12.5 µg/mL). Compound **3** showed significant activity against all the four bacteria with MIC ranging from 1.56 to 6.25 µg/mL and was identified as the most active among all followed by compound **2** which exhibited significant activities against *P. aeruginosa* and *S. enterica*. Compound **3** appears to be a potential candidate for drug discovery against bacterial infections. These results are in accordance with the use of *E. abyssinica* in folk medicine to treat diseases of bacteria origin. *E. abyssinica* may be useful for further investigation in view to develop Improved Traditional Medicines (ITM) to combat bacterial diseases.

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