

ANTIRADICAL, ANTIBACTERIAL ACTIVITIES AND *IN SILICO* MOLECULAR DOCKING OF ETHYL-*BD*-FRUCTOFURANOSIDE FROM HYDRO-ETHANOLIC CRUDE EXTRACT OF *ERYTHRINA EXCELSA* STEM BARK

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Abstract

The aim of the present study was to isolate and characterize compounds responsible of the antiradical and antibacterial activities in the hydro-ethanolic crude extract of *Erythrina excelsa* stem bark. Chemical investigation of this polar extract resulted in the isolation of a fructose derivative identified as ethyl- β -D-fructofuranoside (**3**) and an alkaloid designated L-(+)-Abrine (**4**); two compounds isolated here for the first time from the genus *Erythrina*, along with a mixture of sitosterol 3-O- β -D-glucopyranoside (**2**) and stigmasterol 3-O- β -D-glucopyranoside (**1**). Their structures were determined using spectroscopic methods (IR, 1 & 2D NMR) as well as HR-ESI-MS and literature data. The hydro-ethanolic extract and compound **3** were tested for antibacterial activity on *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium* and *Salmonella enterica*, and for antioxidant activity using free radical scavenging (DPPH) and Ferric Reducing Ability Power (FRAP). Compound **3** showed good antiradical potential (IC_{50} 21.11 ± 0.53 μ g/mL) and moderate antibacterial activity against all the tested bacteria with MIC of 200 μ g/mL. For the FRAP method, hydro-ethanolic extract showed weak activity with value of 425 mg GAE/g, while Ethyl- β -D-fructofuranoside (**3**) exhibited a very good reducing power (40 mg GAE/g). Ethyl- β -D-fructofuranoside (**3**) could, therefore, be responsible for the antiradical activity of the hydro-ethanolic crude extract.

Keywords. *Erythrina excelsa*; ethyl- β -D-fructofuranoside; L-(+)-abrine; antibacterial; antioxidant; molecular docking

Introduction

There are over 130 species of “coral tree” belonging to the genus *Erythrina*, which have been widely studied and are distributed in tropical and subtropical regions of the world (Hickey and King 1981; Pillay et al. 2001; De Luca et al. 2018). Studies performed on these plants have revealed that they are rich in various secondary metabolites which are mostly phenolic compounds and alkaloids (Majinda et al. 2005). In addition, most of the compounds isolated from *Erythrina* species have shown various activities such as antimicrobial, anti-inflammatory, antiplasmodial, antioxidant and anticancer (Majinda et al. 2005; Tueche et al. 2018; Yaya et al. 2020). *Erythrina excelsa* Baker is a medicinal plant used to treat various ailments. In some regions of Cameroon, the stem bark of *E. excelsa* is used against fever, stomachache, and skin diseases, for the treatment of ovarian cysts and uterine fibroids, to comfort menopausal complaints (Samy et al. 2008, Ngueu et al. 2016), against arthritis and catarrh (Jiofack et al. 2010; Ombuna et al. 2016). In Kenya, *E. excelsa* bark is used for gastrointestinal disorders, amoebiasis and as an antidote for snakebites (Ogut et al. 2012). Although many *Erythrina* species have gained considerable attention and have been intensively screened biologically and chemically, less is known about the biological potential as well as the chemicals content of *E. excelsa*. The available data on the chemical composition of *E. excelsa* reported the isolation of isoflavonoid, prenylated flavonoids, triterpenes and setroids (Kuate et al. 2014; Ngueu et al. 2016; Ombuna et al. 2016; Yaya et

al. 2020). Some of these isolated compounds from *E. excelsa* exhibited *in vitro* and *in vivo* estrogenic/anti-estrogenic activities, cytotoxic effects on breast (MCF-7), prostate (DU145, PC3), colon (HT-29) cancer cell lines and resistant cancer cells, and *in vitro* antioxidant potential (Kuate et al. 2014; Ngueu et al. 2016; Yaya et al. 2020). Infectious diseases are major causes of morbidity in the developing countries with about 50% of mortality rate (Ahmad et al. 2015). The antimicrobial resistance to antibiotics, uncontrolled urbanization, the unprecedented increase in population movements, and poverty in developing countries constitute fertile ground upon which new infections have flourished, while others, thought to have been eradicated, have reappeared (Usha et al. 2010). Those microbial infections contribute also to the development of oxidative stress which has been implicated in the pathogenesis of several non-communicable diseases (Finkel and Holbrook 2000; Li et al. 2007). The search for new molecules that can remedy all these problems is essential nowadays. The pharmacological and phytochemical study of plants from folk medicine could lead not only to the discovery of new antimicrobial compounds, but also to the valorization of local plant species through the investigation on their efficacy and safety. In this work, we reported for the first time, the isolation of ethyl- β -D-fructofuranoside (**3**) a fructose derivative and L-(+)-abrine (**4**) an acute toxic alkaloid in the genus *Erythrina* alongside with sitosterol 3-O- β -D-glucopyranoside (**2**) and stigmasterol 3-O- β -D-glucopyranoside (**1**). The structure of each compound was elucidated by extensive spectroscopic analyses. The hydro-ethanolic extract and the fructose derivative were tested for their antibacterial activity on *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium* and *Salmonella* sp. pl. and for their antioxidant potential using the DPPH free radical scavenging and the Ferric Reducing Ability Power (FRAP) methods.

Material and methods

GENERAL EXPERIMENTAL PROCEDURE

Melting points of the isolated compounds were recorded on an Electrothermal IA9000 Series digital melting point apparatus (Bibby scientific, Great Britain). The NMR data were recorded with a Bruker spectrometer with tetramethylsilane (TMS) as standard ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz). ESI accurate mass measurements were acquired using an Agilent 6220 time of flight spectrometer. Optical density was measured using an APADA V-1100 spectrophotometer. Column chromatography (CC) was performed with silica gel (Merck, 200–425 mesh particle size) as adsorbent and thin layer chromatography (TLC) on pre-coated silica gel on aluminum sheets (Merck KGaA).

SAMPLE COLLECTION AND IDENTIFICATION

The stem barks of *Erythrina excelsa* Baker (Fabaceae) were collected in December 2016 in Ngaoundere, Adamawa Region of Cameroon. The plant was identified with the help of a botanist from the National Herbarium of Cameroon by comparison to a known specimen recorded under voucher No. 62731/HNC.

EXTRACTION AND COMPOUNDS ISOLATION

The stem barks of *E. excelsa* were air-dried and powdered. Then, 1.03 kg of the powdered plant material was extracted with a mixture of ethanol-distilled water (80:20) for 72 h. The filtrate was concentrated using a Büchi rotavapor to yield 103.5 g of crude extract. To obtain other extracts, 78 g of the crude extract was dissolved in distilled water and subjected to a liquid-liquid partition using ethyl acetate and *n*-butanol to give the ethyl acetate extract (12 g) and the *n*-butanol extract (26 g). Hydro-ethanolic extract (25 g) was subjected to column chromatography on silica gel (200–425 mesh), using a gradient solvent system of hexane, hexane-ethyl acetate (EtOAc), EtOAc-methanol (MeOH) in increasing polarity. Compounds **1** and **2** were obtained as white powder at hexane-EtOAc (20:80), compound **3** was obtained at pure EtOAc as yellow oil. The *n*-butanolic extract (23 g) was also subjected to column chromatography on silica gel (200–425 mesh), using a gradient solvent system of CH₂Cl₂ and CH₂Cl₂-MeOH in increasing polarity. At the system CH₂Cl₂-MeOH (85:15), compound **4** was obtained as brown powder.

Ethyl-β-D-fructofuranoside (3): yellow oil. ¹H and ¹³C-NMR (500 and 125 MHz, MeOD) see Table 1. (+) – HR – ESI – MS *m/z*: 439.126 [2M + Na]⁺, C₈H₁₆O₆; (calcd: 208.21).

L-(+)-Abrine (4): brown amorphous powder. ¹H and ¹³C-NMR (500 and 125 MHz, MeOD) see Table 1. (+) – HR – ESI – MS *m/z*: 242.917 [M + Na + H]²⁺, C₁₂H₁₄N₂O₂; (calcd: 218.103).

Table 1. ¹H (500 MHz), ¹³C (125 MHz) NMR and HMBC data of ethyl-β-D fructofuranoside (**3**).

N°	δ _c (ppm)	δ _H (ppm)	HMBC
2	123.4	6.07 (1H, s)	C (3, 4a, 7a, 7)
3	106.0	–	–
4a	125.8	–	–
4	117.5	6.48 (1H, d, 8.43 Hz)	C (3/4a/5/7/7a)
5	120.8	6.01 (1H, t, 7.47 Hz)	C (4/7a)
6	118.2	5.93 (1H, t, 7.47 Hz)	C (4/4a/5/7)
7a	134.7	–	–
7	110.8	6.26 (1H, d, 8.43 Hz)	C (4a/6)
1'	24.3	2.12 ((1H, dd, 5.44 and 5.98 Hz) 2.20 (1H, dd, 5.44 and 5.98 Hz)	C (2/3/2'/3')
2'	62.3	2.57 (1H, t, 5.98 Hz)	C (3/1'/3'/1'')
3'	171.1	–	–
1'' H ₃ C-NH	30.9	1.37 (3H, s)	C-2'

ANTIOXIDANT ACTIVITY ASSESSMENT

The antioxidant activity of each extract and isolated compounds from the stem bark of *E. excelsa* was evaluated based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, and the ferric reducing power.

DPPH FREE RADICAL SCAVENGING ASSAY

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was determined using the method of Blois (1958) with slight modifications. Indeed, the standard solution of DPPH was prepared by dissolving 3 mg of DPPH in 75 mL of methanol (MeOH). This solution was 2-fold diluted with MeOH to obtain 225 mL of final solution. After preparing the different solutions, 2000 µL of the DPPH solution was added

into test tubes with 500 µL of pure compound (2.5, 5, 10, 25, 50 and 100 µg/L), extract (25, 50, 100, 250, 500 and 1000 µg/L), and catechin (2.5, 5, 10, 25, 50 and 100 µg/L) to a final volume of 2.5 mL per tube. All tests were carried out in triplicate in a dark room. The optical density was measured at a wavelength of 517 nm using a spectrophotometer of the brand Thermo-Fisher-Scientific: Evolution 300 UV-VIS, after 30 min of incubation.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

The ferric reducing antioxidant power (FRAP) assay is based on the reduction of the tripyridyltriazine ferric complex ($\text{Fe}^{3+}\text{TPTZ}$) to the tripyridyltriazine ferrous complex ($\text{Fe}^{2+}\text{-TPTZ}$) in the presence of an antioxidant. The chelation of ferrous ions was estimated using the method of Benzie and Strain (1996), slightly modified. Concretely, 2000 µL of FRAP solution was pipetted into different test tubes, followed by 75 µL of sample, gallic acid (used here as standard) at different concentrations. The tests were done in triplicate, and the mixture was incubated for 30 min in darkness. The optical density was measured at 593 nm using a spectrophotometer of the brand Thermo-Fisher-Scientific: Evolution 300 UV-VIS. The FRAP solution was prepared as follows: 14.1 mg of TPTZ was diluted in 9 mL HCl at 40 mM then ferric chloride (FeCl_3 at 20 mM) and acetate buffer 300 mM at pH 3.6 mixed in the ratio of 1:1:10 respectively to form the FRAP solution. The FRAP was expressed as mg/g gallic acid equivalents (GAEs/g).

ANTIMICROBIAL ACTIVITY

MICROORGANISMS

The following bacterial strains were used for the test: *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium* and *Salmonella enterica*. Isolates were obtained from the Yaounde Central Hospital, Cameroon and the reference strains from BEI resources and the American Type Culture Collection. The microorganisms were maintained on agar slope at 4 °C and sub-cultured for 24 h before use.

PREPARATION OF STOCK SOLUTIONS, EXTRACTS AND REFERENCE DRUGS

The stock solutions of crude extracts were prepared at 1 mg/ mL using 10% dimethyl sulfoxide (DMSO). Amoxicillin (Sigma Aldrich) was used as reference drug for bacteria. The stock solutions were filter-sterilized with a 0.20 µm syringe filter and stored at –20 °C until use.

ANTIMICROBIAL ASSAYS

For the estimation of the antimicrobial activities of extracts and isolated compounds, a broth dilution method was employed for minimum inhibitory concentration (MIC) determination following the Clinical and Laboratory Standards Institute (CLSI) guideline M7-A9 for bacteria (CLSI 2008). Each extract/compound was first tested in duplicate at 500 µg/mL and only samples showing inhibition were subsequently considered for MIC determination.

More specifically, 50 μ L of Mueller Hinton broth (MHB) was introduced in a 96-well microplate. 50 μ L of extract/compound dissolved DMSO 10% at a concentration of 1 mg/mL was added to wells of the first line. A serial twofold dilution was made by transferring 50 μ L of the mixture of the first wells to the next one up to the last, final concentrations varying from 500 to 31.25 μ g/mL. Then, 15 μ L of an inoculum of 1×10^6 cells/ mL for bacteria were introduced in all the wells except those of the sterility control. Amoxicillin was used as positive control. Each plate also contained a positive control, a negative control and a blank. Plates were incubated during 24 h. The lowest concentration of extract/compound that inhibited the visible growth of a microorganism was defined as minimum inhibitory concentration (MIC). Cut-off points for significant activity of extracts were as follow: very good (MIC < 62.5 μ g/mL), good (62.5 < MIC $\leq$ 125 μ g/mL), moderate (250 < MIC $\leq$ 500 μ g/mL) or weak (MIC > 500 μ g/mL).

STATISTICAL ANALYSIS

Each data was expressed as mean $\pm$ SD ($n = 3$). The analysis was done by ANOVA followed by Dunnett's multiple comparison test. The difference between standard and extracts concentrations was considered significant at $p < 0.05$. The graphical representation of the data was performed using the Graph Pad Prism 7.0 software (Microsoft, USA). The reducing power or SC₅₀ was determinate using the SPSS Statistic Software version 23.0.

MOLECULAR DOCKING STUDIES

PREPARATION OF LIGANDS

The 3D molecular structures of isolated compounds were generated using Chem3D 15.0 running a windows workstation with an Intel(R) Core (TM) i5-3340M processor. The 3D structures were then saved in .pdb format. They were imported to the workspace and preparation was done for docking studies.

PREPARATION OF ENZYMES AND MOLECULAR DOCKING ANALYSIS

To perform docking of compounds, the target protein was selected based on its function. 3D structures were obtained from protein data bank (<http://www.rcsb.org>) in .pdb format. The AutoDockTools (ADT) was used to prepare the ligand and receptor structures, add appropriate Gasteiger and Kollman charges, identify and modify ligand rotatable bonds. The potential binding sites of target were calculated using the Lamarckian GA (4.2) algorithm implemented in Autodock4. The population size, maximum number of evaluation (medium) and maximum number of generations were set at 150, 27 000 and 2 500 000 respectively. The search space of the simulation exploited in the docking studies was studied as a subset region of the active site containing the co-crystalized ligand. The water molecules were removed from the enzyme to decrease interactions between functional group of ligands and water molecules.

AutoDock program performs the research and evaluation of the different ligand configurations. It is possible to use several techniques to obtain the configurations (by simulated annealing, genetic algorithm or by Lamarckian genetic algorithm). A grid-based method was used to enhance the quick evaluation of the binding energy of conformations of the complexes formed. The grid boxes were centered using coordinates of a virtual center of mass atom for the enzymes. For each protein, the grid box was determined respectively in x, y and z dimension according to amino acids which formed active site. The affinity of the docked complexes was described using binding energy based on a semi empirical force field.

Results and discussion

CHARACTERIZATION OF THE ISOLATED COMPOUNDS

The crude hydro-ethanolic extract, and the *n*-butanol soluble fraction of *E. excelsa* stem barks, were subjected to successive silica gel column chromatography. This operation afforded stigmasterol 3-*O*- β -*D*-glucopyranoside (**1**, 15 mg) (Abdou et al. 2022; Matcheme et al. 2025), sitosterol 3-*O*- β -*D*-glucopyranoside (**2**, 15 mg) known as Daucosterol (Mbafor et al. 1996; Nyemb et al. 2018; Matcheme et al. 2025), and ethyl- β -*D*-fructofuranoside (**3**, 35 mg) (Zhen et al. 2009) from the crude extract. From the *n*-butanolic soluble fraction, a compound identified as *L*-(+)-abrine (**4**, 21 mg) (Figure 1), has been isolated. The structures of known steroids were elucidated and identified by comparison of their spectroscopic data with those reported in literature.

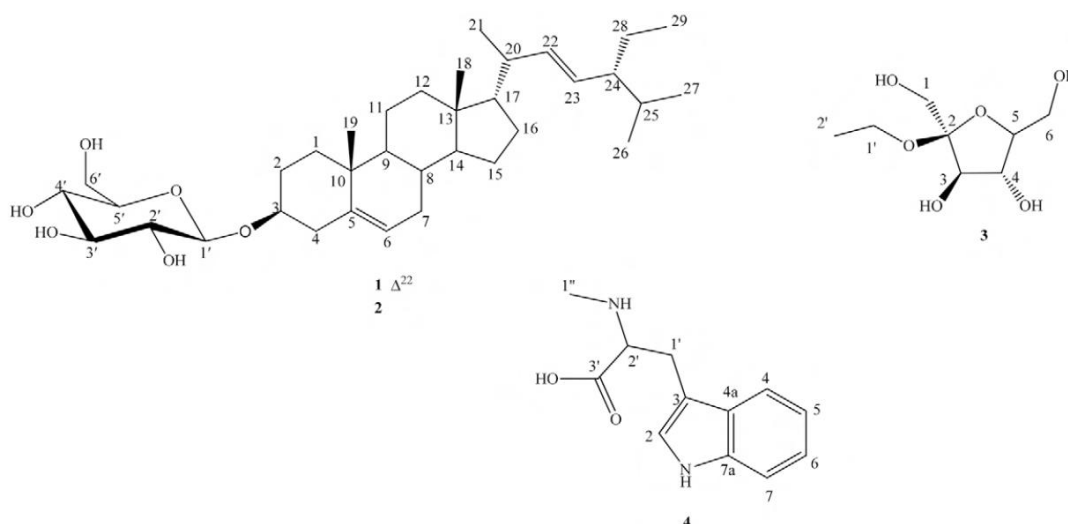


Figure 1. Chemical structures of compounds (**1–4**) isolated from stem bark of *E. excelsa* baker.

Compound **3** was isolated as yellow oil. Its molecular formula $C_8H_{16}O_6$, corresponding to one degree of unsaturation was deduced from the high-resolution electrospray ionization mass spectrum (HR-ESI-MS), which showed a *pseudo* molecular ion peak at m/z 439.126 $[2M + Na]^+$ (calcd: 208.21). The 1H NMR spectrum of compound **3** (Table 1) displayed signals for three oxymethine protons at δ_H 4.12 (1H, d, 7.35 Hz, H-3), 3.78 (1H, m, H-4), and 3.98 (1H, d, 7.35 Hz, H-5) suggesting the presence of no less

than three hydroxyl groups. Two oxymethylene protons at δ_H 3.55 (2H, m, 2H-1'), four diastereotopic oxymethylene protons at δ_H 3.58 (1H, m, H-6a) and 3.75 (1H, m, H-6b), and at δ_H 3.53 (1H, m, H-1a) and 3.69 (1H, m, H-1b) along with a signal of three methyl protons at δ_H 1.17 (3H, t, 7.05 Hz, H-2'), were also visible on this spectrum. In conjunction with the HSQC spectrum, the ^{13}C NMR spectrum (Table 1) indicated the presence of one methyl at δ_C 14.7 (C-2'); three oxymethylenes at δ_C 56.6 (C-1'), 60.5 (C-1) and 63.4 (C-6); three oxymethines at δ_C 76.8 (C-4), 81.7 (C-3) and 75.8 (C-5); along with one quaternary carbon highly oxidized at δ_C 103.9 (C-2). All those data seem like those of sugars. Correlations observed in its HMBC spectrum (Table 1, Figure 2) between proton H-1 (δ_H 3.53) with C-3 (δ_C 81.7) and C-2 (δ_C 103.9); H-3 (δ_H 4.12) with C-1 (δ_C 60.5) and C-2 (δ_C 103.9); H-5 (δ_H 3.98) with C-2 (δ_C 103.9) and C-4 (δ_C 76.8) and of H-6 (δ_H 3.58) with C-5 (δ_C 75.8) and C-4 (δ_C 76.8) allowed us to connect different carbons together. All these informations combined with COSY spectrum analysis allowed us to identify compound **3** as ethyl- β -D-fructofuranoside, a fructose derivative previously isolated from the rhizome of *Alisma orientalis* (Sam.) Juzep. (Zhen et al. 2009) and isolated here for the first time in the genus *Erythrina*.

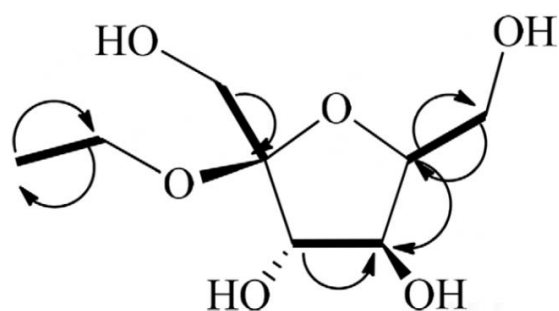


Figure 2. Important HMBC (curved arrow) and ^1H - ^1H COSY (straight arrow) correlations of ethyl- β -D-fructofuranoside (**3**).

Compound **4** was isolated as brown amorphous powder. Its molecular formula $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2$ was determined from the HR-ESI-MS which showed the *pseudo* molecular ion peak $[\text{M} + \text{Na} + \text{H}]^{2+}$ at m/z 242.917 (calcd: 218.103) revealing seven degrees of unsaturation. The ^1H NMR spectrum of compound **4** (Table 2) displayed signals for five aromatic protons: one at δ_H 6.07 (1H, s, H-2) four others in a AA'BB' coupling system at δ_H 6.48 (1H, d, 8.43 Hz, H-4), 6.01 (1H, dd, 7.27 and 8.43 Hz, H-5), 5.93 (1H, dd, 7.27 and 8.43 Hz, H-6) and 6.26 (1H, d, 8.43 Hz, H-7). Those signals suggest the presence of a *O*-disubstituted benzene ring. The same spectrum also displayed signals for two diastereotopic methylenic protons at δ_H 2.12 (1H, dd, 5.44 and 5.98 Hz, H-1'a) and 2.20 (1H, dd, 5.44 and 5.98 Hz, H-1'b); one azomethine proton at δ_H 2.57 (1H, t, 5.98 Hz, H-2') and three methyl protons at δ_H 1.37 (3H, s, H-1''). Aromatic protons of this compound are nearest to those of the indolic moiety of a bisindole alkaloid isolated from a marine fungus *Fusarium* sp. XBB-9 (Shaker et al. 2021). Its ^{13}C NMR spectrum (Table 2) in conjunction with its HSQC spectrum, indicated the presence of four indolic aromatic methines at δ_C 123.4 (C-2), 117.5 (C-4), 120.8 (C-5), 118.2 (C-6) and 110.8 (C-7); three quaternary indolic carbons at δ_C 106.0 (C-3), 125.8 (C-4a) and δ_C 134.7 (C-7a) (Shaker et al. 2021). The presence in this spectrum of an acid carbonyl signal at δ_C 171.1 (C-3'), a methylene carbon signal at δ_C 24.3 (C-1'), an azomethine carbon signal at δ_C 62.3 (C-2') and a methyl carbon linked to the nitrogen at δ_C 30.9 (C-1'') suggest that this compound is an amino acid. The analysis of COSY and HMBC spectra (Figure 3) of compound **4**, and literature data, allowed us to identify this compound as (2*S*)-3-(1*H*-indol-3-yl)-

2(methylamino) propanoic acid, known as *L*-(+)-abrine. This lethal alkaloid found in *Abrus precatorius* Linnaeus seeds, is an acute toxic alkaloid and chemical marker for abrin (Rudolph et al. 2009). This compound is reported here for the first time from this plant and from the genus *Erythrina*.

Table 2. ^1H (500 MHz), ^{13}C (125 MHz) NMR and HMBC data of *L*-(+)-abrine **4** in MeOD [δ (ppm), J (Hz)].

N°	δ_{C} (ppm)	δ_{H} (ppm)	HMBC
1	60.5	3.53 (1H, m) ; 3.69 (1H, m)	C (2,3)
2	103.9	—	—
3	81.7	4.12 (1H, d, 7.35 Hz	C (1, 2)
4	76.8	3.78 (1H, m)	C (5, 6)
5	75.8	3.98 (1H, d, 7.35 Hz)	C (2, 4)
6	63.4	3.58 (1H, m) ; 3.75 (1H, m)	C (5, 4)
1'	56.6	3.55 (1H ; m)	C-2'
2'	14.7	1.17 (3H, t, 7.05 Hz)	C-1'

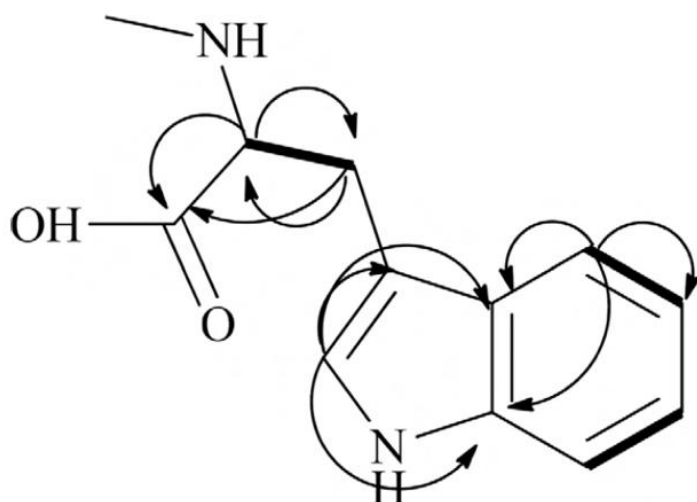


Figure 3. Important HMBC () and ^1H - ^1H COSY () correlations of *L*-(+)-abrine (**4**).

ANTIOXIDANT AND ANTIBACTERIAL RESULTS

Antioxidants play a crucial role in protecting cell tissues from free radicals, this come to protecting the organism against infections and degenerative diseases like diabetes, hypertension, and cancer. The antioxidant activity of the extracts indicates the presence of compounds that can interact with free radicals and act as electron donors (Tirado et al. 2017). The crude hydro-ethanolic extract and ethyl- β -D-fructofuranoside (**3**) were tested for their antioxidant potential using the DPPH free radical scavenging and FRAP methods. Table 3 presents the results obtained for the antiradical and ferric reducing potentials of hydro-ethanolic extract and ethyl- β -D-fructofuranoside (**3**).

DPPH is a common stable free radical and widely used for the radical scavenging assays. The principle of this method is based on the reduction of DPPH in solution by an hydrogen– donating compound, leading to the formation of the non-radical form DPPH-H, materialized by a color change from violet to pale yellow, and which can be spectrophotometrically monitored around 515 nm. After

a preliminary screening of all samples, those that showed 50% (or more) of inhibition of the DPPH free radical were diluted and their IC_{50} were calculated. As results obtained, the hydro-ethanolic extract of *E. excelsa* stem barks showed a weak activity for the DPPH free radical scavenging with IC_{50} value of 250.75 $\mu\text{g}/\text{mL}$ while ethyl- β -D-fructofuranoside (**3**) showed good activity for the same method with IC_{50} of 21.11 $\mu\text{g}/\text{mL}$. The fact that ethyl- β -D-fructofuranoside (**3**) is most active than extract from which it was isolated, could suggest that there is an antagonist effect between compounds in this tested extract. Some chemicals easily react with DPPH radicals by electron transfer or by donating H atoms (Talla et al. 2016). Phenolic compounds are the most reactive and important ones, because of the further stabilisation by resonance, of the resulting radical. Hydrogen atom abstraction contains reactions that form through electron transfer followed by or preceded by proton transfer and can be formally classified as hydrogen atom transfer reactions. The DPPH test, therefore gives an estimate of the total content of reductants present in the solution of plant extracts (Gulcin and Alwasel 2023). Thus, the weak activity of the hydro-ethanolic *E. excelsa* stem barks extract could demonstrate the poverty in phenolic compounds of this extract. However, it has been stated that complex molecules get in the way of each other more easily, and block access to DPPH radicals at low concentrations and strongly block the reaction at high concentrations. Additionally, methyl alcohol, which is generally used as a solvent for the DPPH method, strongly binds H atoms and inhibits hydrogen atom transfer processes (Gulcin and Alwasel 2023), leading to weak activities.

To avoid reactive oxygen species generation that could be associated with redox active metal catalysis, the main strategy is to involve chelating of the metal ions (Talla et al. 2016). The FRAP assay (Ferric Reducing Ability Power) evaluates total antioxidant power and is chosen to assess the presumable effects of medicinal plants. Ferrozine is a highly ferrous-stabilizing ligand such that, ferric ion in the presence of ferrozine easily oxidizes antioxidants and is itself reduced to Fe(II)-FZ , yielding a very high molar absorptivity and thus enhanced sensitivity for most antioxidants. From our results, hydro-ethanolic extract exhibited a weak chelating power with a value of 425 mgGAE/g , while ethyl- β -D-fructofuranoside (**3**) showed a very good chelating power, with a value of 40.0 mgGAE/g . The activity of the ethyl- β -D-fructofuranoside (**3**) was not significantly different from the one of gallic acid (37.5 mgGAE/g) used as reference. Generally, Chelating capacity is attributed to flavonoids and phenolic compounds with free hydroxyl group, which use their redox properties to chelate transition metals. Their oxygen atom and hydroxyl groups can chelate the Fe^{2+} ions. The good chelating power of ethyl- β -D-fructofuranoside (**3**) could therefore be due to the availability of hydrogen of its hydroxyl groups, which are not implicated in hydrogen bonds. The weak chelating power of the hydro-ethanolic extract of the *E. excelsa* stem bark could therefore suggest that this extract contains small amounts of ligands to compete with ferrozine and prevent the generation of hydroxyl radicals ($\text{HO}^{\bullet}$). This could be due to the fact that compounds present in this extract do not have enough free hydroxyl groups to chelate high amount of Fe^{2+} . In the body, the transition metals can catalyze the Fenton and Haber-weiss reactions, resulting in the generation of hydroxyl radicals. These transition metals can however be chelated by antioxidants, leading to the suppression of the $\text{HO}^{\bullet}$ generation and an inhibition of peroxidation of biological molecules (Chew et al. 2009). The results obtained in this study are similar to those we previously obtained (Yaya et al. 2020) when evaluating antioxidant potential of different extracts of the same plant.

The hydro-ethanolic extract and ethyl- β -D-fructofuranoside (**3**) were also evaluated for their antibacterial activity on *E. coli*, *S. typhi*, *S. typhimurium* and *S. enterica*. The samples were tested using the broth dilution method and ciprofloxacin as antibacterial drug reference. The results of the bioassay against the selected bacteria are presented as the values of the mean of three experiments $\pm$ S.D (Table 4).

Table 4. Antibacterial activities of EtOH-H₂O extract and ethyl- β -D-fructofuranoside (**3**).

Samples	Bacteria			<i>E. coli</i>			<i>S. typhi</i>			<i>S. typhimurium</i>			<i>S. enterica</i>		
				MIC	MBC	X	MIC	MBC	X	MIC	MBC	X	MIC	MBC	X
Compound 3	200	400	2	200	400	2	ND	ND	ND	200	>400	ND	200	>400	ND
EtOH-H ₂ O	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ciprofloxacin	1	4	4	0.5	2	4	0.5	2	4	1	4	4	1	4	4

MIC and MBC in $\mu\text{g/mL}$; X=MBC/MIC. Within a column, the MIC of samples with the same letter(s) are not significantly different ($p < 0.05$, Dunnett's test).

Results show that, the hydro-ethanolic extract did not show any bioactivity against any of the tested bacteria. Therefore, its MIC could not be determined. The tested compound, ethyl- β -D-fructofuranoside (**3**), showed moderate activity on *E. coli*, *S. typhi* and *S. enterica* with MICs of 200 $\mu\text{g/mL}$. Thus, all the tested bacteria were moderately sensitive to ethyl- β -D-fructofuranoside (**3**), except for *S. typhimurium*, which exhibited resistance to this compound. The above results could support the local uses of this plant part for the treatment of gastrointestinal disorders and amoebiasis, but are not in accordance with previous reports on antimicrobial assays of *E. excelsa* bark (Ombuna et al. 2016). Indeed, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) extracts of *E. excelsa* stem barks demonstrated antibacterial activity against *Staphylococcus aureus* ATCC 25923; hexane, dichloromethane and aqueous extracts had activity, against *Pseudomonas aeruginosa* ATCC 27853 (Ogutu et al. 2012). The study of Ogutu et al. (2012) was conducted using both microdilution method and disc diffusion method. The most potent extract was found to be DCM extract with a MIC of 0.163 mg/mL and a zone of inhibition of 7 mm against *S. aureus* ATCC 25923 (Ogutu et al. 2012). Another study, conducted by Ombuna and co-authors (2016) found that crude methanolic extract of *E. excelsa* stem barks was highly active against *S. aureus*, *Bacillus subtilis* and *E. coli* while EtOAc and DCM extracts were mild actives. No previous study was conducted against the tested bacteria for any extract of any part of this plant, except for *E. coli*. The difference in the activity of this plant against this bacterial strain observed in both studies, could be attributed to the difference in composition of the extracts used (crude MeOH extract against EtOH-H₂O extract); it could also be attributed to the composition of plant part that could be different from a geographic zone to another due to the climate. In contrast to the bioactivity observed in the crude hydro-ethanolic extract, the isolated compound **3**, namely ethyl- β -D-fructofuranoside was more active on tested bacteria. This observation may be attributed to the fact that, the amount of the active components in the crude extract could be diluted and fractionation may have increased their concentrations, hence reason for enhanced bioactivity against tested bacterial strains. There could also be a possibility of antagonism between various antibacterial compounds present in the crude extracts when grouped together, leading to a decrease in antimicrobial activity (Kuate et al. 2011). The activity observed for the ethyl- β -D-fructofuranoside (**3**), could be attributed to the presence in its structure, of many hydroxyl groups. This molecule has been reported to play bidirectional regulatory roles in immunity and anti-inflammation through the

regulation of nuclear factor- κ B (NF- κ B) signaling pathways (Niu et al. 2021). Stigmasterol 3-*O*- β -D-glucopyranoside (**1**) and daucosterol (**2**) were not considered for antimicrobial tests as their antimicrobial activities are already known (Lunga et al. 2014; Nyemb et al. 2018). L-(+)-abrine (**4**) has also not been evaluated because it is a well-known acute toxic alkaloid, used as chemical biomarker for abrin. Despite the presence of this toxic compound, the inactivity of the hydro-ethanolic extract toward the tested bacterial strains, shows that the toxicity of this compound has been annihilated probably by other compounds presents in the extract, through hydrogen bonds for example. Nevertheless, *E. excelsa* stem barks turn out to contain compounds with antimicrobial potential. It is the case of excelsaperoxide, isolated from the DCM-MeOH (1:1) extract, with a weak activity against *Enterobacter aerogenes* ATCC13048 (Kwamou et al. 2015). Other examples are: Glutinosalactone A from MeOH extract, with activities against *Aspergillus niger*, *Bacillus subtilis* ATCC 6051 and *S. aureus* ATCC 35844; Glutinosalactone B also isolated from MeOH extract, exhibited a high activity against *B. subtilis* and *C. albicans*; moreover, lupinifolin from DCM-hexane extract with mild activity against both *A. niger* and *C. albicans*. Stigmasterol and sitosterol had also been isolated in the same study and exhibited mild activity against *A. niger* (Ombuna et al. 2016).

Table 5. Molecular docking value of compounds **1–4** against *E. coli* (4XO8, 5L3J), *S. typhi* (4KR4), *S. typhimurium* (6IE9) and *S. enterica* (4IQN).

Receptors	Estimated ΔG Binding (kcal/mol)				
	1	2	3	4	L
4IQN	−5.9	−5.3	−3.4	−4.5	−3.3
4XO8	−6.2	−6.0	−5.7	−6.3	−6.6
5L3J	−8.3	−8.0	−4.9	−7.1	−7.2
6IE9	−6.9	−7.0	−4.4	−6.4	−9.6
4KR4	−8.3	−8.2	−5.4	−6.2	−7.1

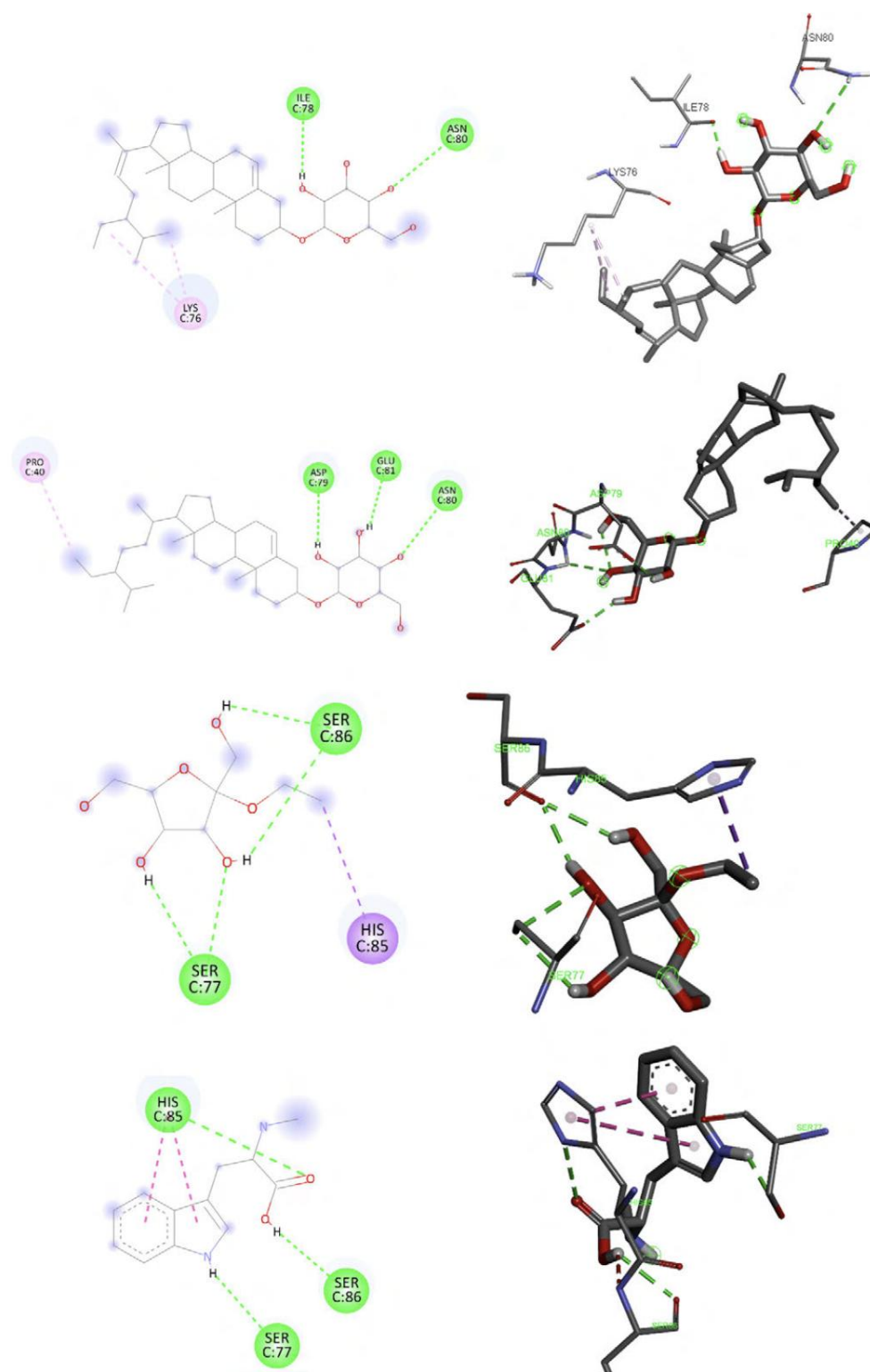


Figure 4. 2D And 3D binding interactions of compound **1** to **4** (from top right to bottom left) against 4IQN.

MOLECULAR DOCKING STUDY

Molecular docking study was performed to suggest the best candidates amongst the four isolated compounds based on their binding affinities.

The results of this study showed that all the tested compounds exhibited lower binding energy with 4IQN than the experimental ligand **L** (-3.3 kcal/mol). Therefore, the four compounds have the potential to inhibit the protein 4IQN. The best potential inhibitor is compound **1** with a binding energy of -5.9 kcal/mol. The affinity of the 4IQN-compound **1** complex (Figures 4 and 5) is demonstrated by two hydrogen bond interactions of two hydroxyl groups with the amino acid residues ILE-78 and ASN-80, and an alkyl interaction with the amino acid residue LYS-76. Compounds **3** and **4** have hydrogen bond interactions with the same amino acid residues, namely HIS-85, SER-86, and SER-77. Docking studies demonstrated that all the isolated compounds exhibited strong binding affinity against the bacterial lectin protein FimH of *E. coli* (4XO8), with binding energy values ranging from -5.7 to -6.3 kcal/mol; and the *E. coli* DNA gyrase B (5L3J) with binding energy values ranging from -5.7 to -6.3 kcal/mol. The docked pose clearly showed that, the drug molecules bind within the active sites of the FimH of *E. coli* (4XO8) and *E. coli* DNA gyrase B (5L3J) macromolecular structure (Figures 5 and 6). In contrast to the case of the protein 4IQN, no estimation of binding of the studied compounds with the protein 4XO8 yields a more stable result than the one of the experimental ligand **L** (-6.6 kcal/mol). However, compound **4** (-6.3 kcal/mol) and compound **1** (-6.2 kcal/mol) exhibited a binding energy comparable to the one of the experimental ligand. Compound **4** showed three hydrogen bond interactions between its carboxyl group and the amino acid residues ASP-140, PHE-1, and ASN-135 of this protein (Figure 5). Regarding the *E. coli* DNA gyrase B protein 5L3J, compounds **1** and **2** could be good inhibitors as they respectively showed binding energies of -8.3 kcal/mol and -8.0 kcal/mol compared to the experimental ligand (-7.2 kcal/mol). The best inhibitor was compound **1**. The affinity of the 5L3J-compound **1** complex (Figure 6) is manifested by three hydrogen bond interactions of three hydroxyl groups with the amino acid residues ASP-73, GLY-77, and TYR-165, and alkyl interactions with the amino acid residues VAL-97, VAL-93, and ILE-94. The estimations do not identify any of the four compounds as potential inhibitors of the protein 6IE9 as compared to the experimental ligand which had a binding energy of -9.6 kcal/mol. Finally, the activity of the four ligands on the 4KR4 protein is generally very similar to that on the 5L3J protein. Good inhibitors could be compounds **1** and **2** as they respectively present binding energies of -8.3 kcal/mol and -8.2 kcal/mol compared to the experimental ligand (-7.1 kcal/mol). The best inhibitor remains compound **1**. The affinity of the 4KR4-compound **1** complex (Figure 7) is manifested by three hydrogen bond interactions of three hydroxyl groups with the amino acid residues TYR-97, TYR-101, and SER-129, and alkyl interaction with the amino acid residue VAL-22.

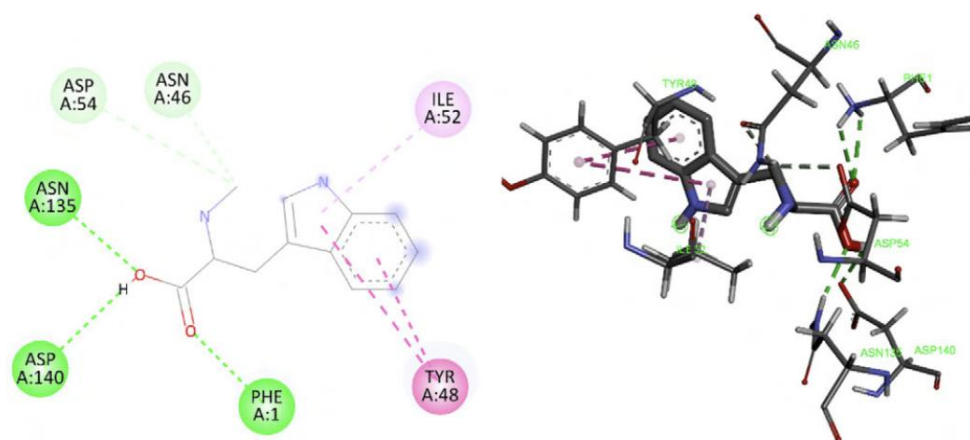


Figure 5. 2D And 3D binding interactions of compound **4** against 4XO8.

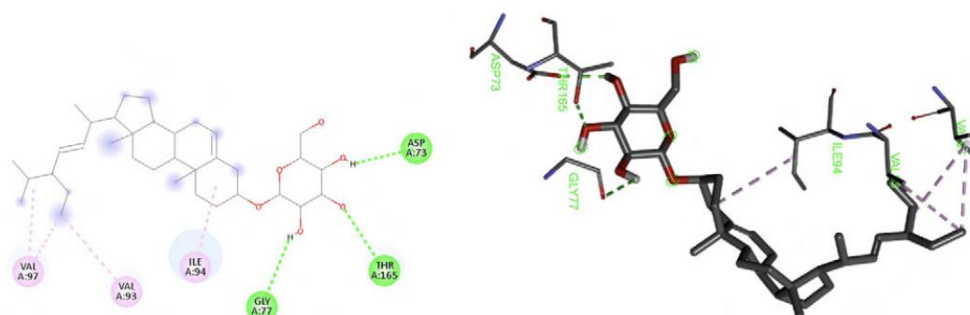


Figure 6. 2D And 3D binding interactions of compound **1** against 5L3J.

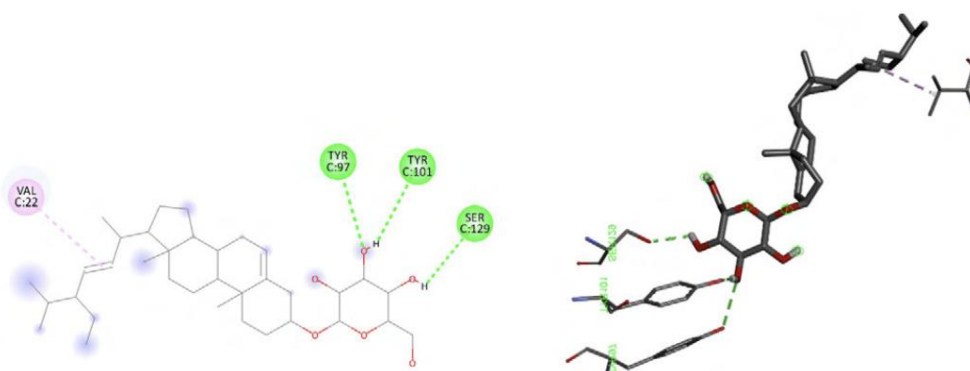


Figure 7. 2D And 3D binding interactions of compound **1** against 4KR4.

Conclusion

E. excelsa parts are used in Cameroon to treat various microbial infections. Considering numerous phytochemical works performed on plants from the genus *Erythrina*, present findings corroborate the importance of *Erythrina* species as sources of valuable secondary metabolites. Four compounds were isolated from the stem bark of *E. excelsa* amongst which Ethyl- β -D-fructofuranoside (**3**) and L-(+)-

Abrine (**4**) were isolated herein for the first time from the genus *Erythrina*. The *in vitro* antibacterial activity results showed that ethyl- β -D-fructofuranoside (**3**) exhibited moderate activities against *E. coli*, *S. typhi* and *S. enterica* with MICs of 200 μ g/mL. However, this compound showed good antiradical activity. Further investigations on others antioxidant mechanisms (*in vitro* and *in vivo*) are needed to clearly confirm its antioxidant potential. The docking results revealed that compounds **1** and **2** displayed better docking scores than the experimental ligand against *E. coli* DNA gyrase B (5L3J) and OmpF complex of *S. typhi*. The *in silico* studies suggest that compounds **1** and **2** could be considered as potential antimicrobial agents against *E. coli* and *S. typhi*. The findings of the present work support the potential use of *E. excelsa* in traditional medicine for the treatment of microbial diseases. Future work should prioritize a cell viability assay to support of the specificity of the antibacterial activity.

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Data availability statement

Data is contained within the article and supplementary material.

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