CYTOTOXIC FLAVONOIDS FROM *ERYTHRINA CAFFRA* THUNB

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ABSTRACT. *Erythrina caffra* is an important medicinal plant native to South Africa. Its stem bark was investigated for the flavonoid constituents and biological activity. Some isolated flavonoids, **3**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, **13**, **15** and **16** were found to be active against the human cervix carcinoma KB-3-1 cells with IC_{50} values in the micromolar range. Compounds **8**, **9**, **11**, **13** and **15** also showed weak to moderate antibacterial activity against some organisms using the disc diffusion assay at loadings of 62.5 μg/disc (**8**, **11**) and 125 μg/disc (**9**, **13**, **15**).

KEY WORDS: *Erythrina caffra*, Cytotoxicity, Antibacterial, Flavonoids, Fabaceae

INTRODUCTION

The genus *Erythrina* belongs to the family Fabaceae comprising over 110 species distributed in tropical and sub-tropical regions in both the southern and the northern hemispheres of the world as well as in some temperate regions of the world [1]. *Erythrina caffra*, the coast coral tree, is a tree native to south eastern Africa. It is widely appreciated for its warm red to scarlet-coloured flowers which appear from the cold winter months up to spring. It occurs in the warm and frost-free to light frost coastal regions of the Eastern Cape and Northern KwaZulu-Natal [2]. The bark of *E. caffra* is used to treat sores, tuberculosis, respiratory infections, wounds, abscesses, arthritis and toothache; infusions of the leaves are used as eardrops for earache; and decoctions of the roots are used for sprains [3]. Reports of phytochemical work on *E. caffra* are sporadic, with only a few secondary metabolites having been reported from different parts of the plant. In this paper we report antibacterial and cytotoxic flavonoids along with other compounds from *E. caffra*.

RESULTS AND DISCUSSION

Different extracts of *E. caffra* yielded a total of 29 compounds. The structure of these compounds were elucidated using ^1H, ^{13}C and 2D NMR data and by comparison with the literature values. These compounds included 20 flavonoids, 2 alkaloids, 3 terpenoids, 3 ferulic esters and 1 alcohol. The isolated compounds are *erycaffra* B **1**, erycaffra A **2** [4], lysisteisoflavanone **3** [5], erycaffra E **4** [6], erycaffra C **5** [4], alpinumisoflavone **6** [7], erysubin B **7** [8], derrone **8** [9], warangalone **9** [10], isoerysenegalensein E **10** [5], erysenegalensein E **11** [11], laburnetin **12** [12], 8-prenylerythrinin C or senegalensin **13** [13], erythrinin C **14** [14], isosenegalensein **15** [5], burttinone **16** [15], erycaffra D **17** [6], erycaffra F **18** [6], abyssinoneV-4'-methylether **19** [15], 8-oxo-erythrinine [16], 8-oxo-erythraline [17], oleanolic acid [18], β-amyrin [18], stigmasterol [19], octacosyl-E-ferulate [20], triacontyl-4-hydroxycinnamate [13], n-tetracosyl-p-coumarate [21] and octacosan-1-ol [22]. Only the structures of the flavonoids are reported in Figure 1.

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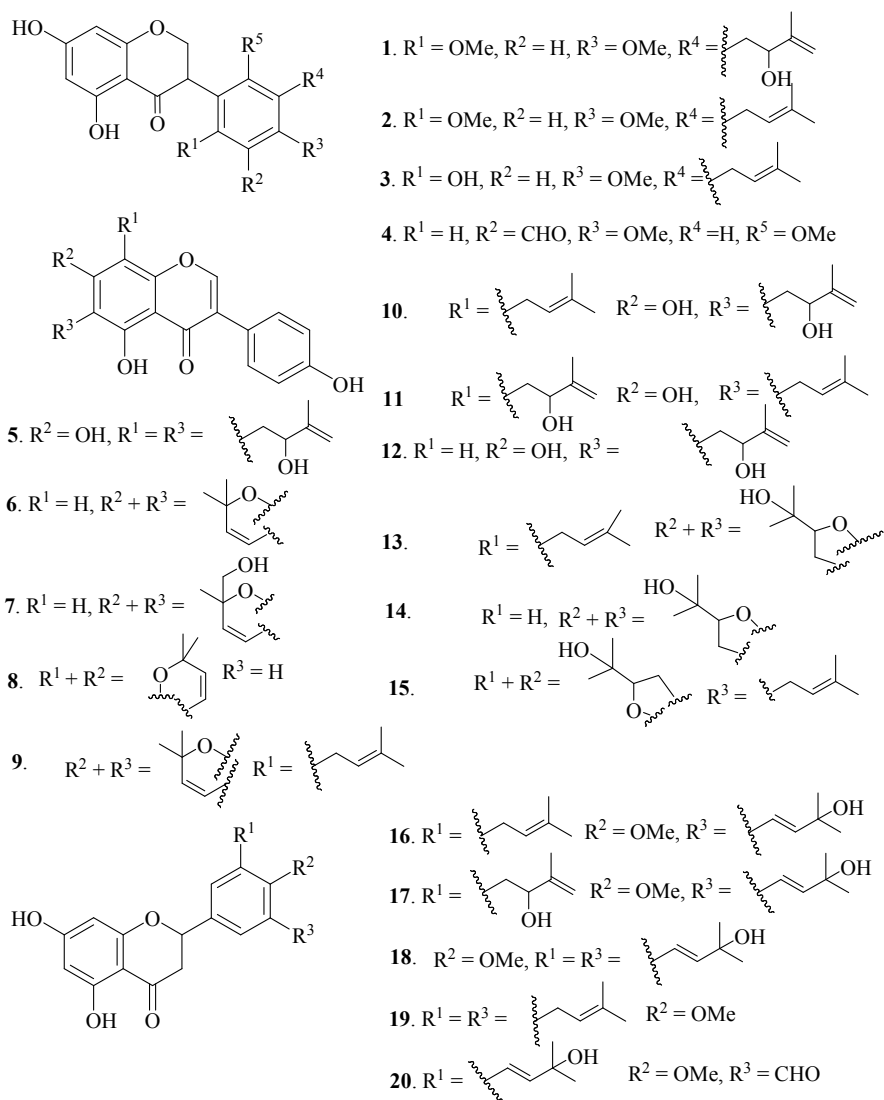*Corresponding author. E-mail: yibrazelalem@yahoo.com

Figure 1. Chemical structures of some isolated pure compounds from *E. caffra*.

Cytotoxicity test

Seventeen pure compounds and six extracts were evaluated for cytotoxicity. The method used for this cytotoxicity test was the cell-based resazurin cytotoxicity assay with fluorescence read-out. Human cervix carcinoma KB-3-1 cells were used and the reduction of the resazurin to resorufin was used as read out [23]. Of the seventeen pure isolates, compounds **3**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, **13**, **15** and **16** were found to be active against the human cervix carcinoma KB-3-1 cells with IC$_{50}$ values in the micromolar range (Table 1). Compounds **6**, **9**, **11**, **13**, **15** and **16**

exhibited greater cytotoxicity activity compared to the other compounds. Compounds **1**, **2**, **4**, **7**, **14**, and **17** did not exhibit cytotoxicity against the human cervix carcinoma KB-3-1 cells. Five of the seventeen compounds (**1**, **2**, **4**, **5** and **17**) were tested for the first time and only compound **5** exhibited greater cytotoxicity activity compared to the other four. On the other hand, compounds **3**, **8**, **9**, **10**, **11**, **12**, **13**, and **15** which exhibited cytotoxicity activity against the human cervix carcinoma KB-3-1 cells isolated and reported for the first time from *E. caffra*. Compounds **6** and **16** were previously isolated and reported from the stem bark of *E. caffra* [3]. Furthermore, compound **6** has been reported previously to exhibit significant cytotoxicity activity against KB cells with ED_{50} value of 4.13 µg/mL (12.3 µM) [7]. The cytotoxicity tests were also performed on the crude extracts and only the *n*-hexane and ethyl acetate extracts of the root bark were active against the human cervix carcinoma KB-3-1 cells at concentrations of 0.11 mg/mL and 0.06 mg/mL, respectively. However, other crude extracts were not active against the human cervix carcinoma KB-3-1 cells (Table 1). Finally it could be concluded that isoflavones exhibited a significant and higher cytotoxicity compared to other compounds, for instance, isoflavanones.

Table 1. Cytotoxicity data for some isolates and crude extracts from *E. caffra*.

Samples	Concentration	IC_{50} (µM)	Activity
1	0.1 mol/L	-	na
2	0.1 mol/L	-	na
3	0.1 mol/L	183	a
4	0.1 mol/L	-	na
5	0.1 mol/L	104	a
6	0.1 mol/L	71.4	a
7	0.1 mol/L	-	na
8	0.1 mol/L	230	a
9	0.1 mol/L	73.4	a
10	0.1 mol/L	99.0	a
11	0.1 mol/L	58.4	a
12	0.1 mol/L	250	a
13	0.1 mol/L	37.8	a
14	0.1 mol/L	-	na
15	0.1 mol/L	53.8	a
16	0.1 mol/L	58.8	a
17	0.1 mol/L	-	na
HS	0.25 mg/mL	-	na
EAS	0.25 mg/mL	-	na
MS	0.25 mg/mL	-	na
HR	0.11 mg/mL	-	a
EAR	0.06 mg/mL	-	a
MR	0.25 mg/mL	-	na

Key: HS: *n*-hexane extract of the stem bark; EAS: ethyl acetate extract of the stem bark; MS: methanol extract of the stem bark; HR: *n*-hexane extract of the root bark; EAR: ethyl acetate extract of the root bark and MR: methanol extract of the root; a: active; na: not active.

Antibacterial test

Seventeen pure compounds were evaluated for antibacterial activity, together with six crude extracts from the stem and root bark of *E. caffra*, using the disc diffusion assay (Table 2). The microorganisms for the test were collected from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) collection (German Collection of Microorganisms and Cell Cultures) (Braunschweig, Germany). The non-pathogenic model organisms, *Escherichia coli* (DSMZ

1058), *Pseudomonas agarici* (DSMZ 11810), *Bacillus subtilis* (DSMZ 704), *Micrococcus luteus* (DSMZ 1605), *Streptococcus ferus* (DSMZ 18308) and *Streptococcus minor* (DSMZ 17118) were used for the antibacterial test on pure compounds and crude extracts from *E. caffra*. According to the test result, only few isolated pure compounds and crude extracts exhibited weak to moderate inhibition (ZOI = 8-12 mm) against some of the bacteria. Most of the isolated pure compounds and crude extracts did not display any inhibition (ZOI = 0 mm) against the test organisms. Among the tested compounds, compound **8** exhibited an intermediate inhibition against *E. coli*, *B. subtilis*, *P. agarici* and *S. minor* whereas compounds **9** and **11** displayed an intermediate inhibition against *E. coli* and *S. minor*. Furthermore, compound **13** also showed some activity against *E. coli* and *B. subtilis* and compound **15** showed an intermediate inhibition against *E. coli*, *B. subtilis* and *P. agarici*. In previous work compound **6** had been evaluated for antibacterial activity using the disc diffusion method and did not exhibit significant inhibition against *E. coli* and *B. subtilis* [24]. In the microtitre bioassay method, however, compounds **6** and **16** are reported to exhibit significant antibacterial activity against *E. coli* and *B. subtilis* [3].

Table 2. Antibacterial activity test results for isolated pure compounds and crude extracts from *E. caffra* using the disc diffusion method.

Samples	Stock solution mg/mL	Loading dose μg	Type of bacteria used for tests					
			E. coli	*B. subtils*	*P. agarici*	*M. luteus*	*S. ferus*	*S. minor*
1	2	50	-	-	-	-	-	-
2	2	50	-	-	-	-	-	-
3	2	50	-	-	-	-	-	-
4	2	50	-	-	-	-	-	-
5	2	50	-	-	-	-	-	-
6	2	50	-	-	-	-	-	-
7	2.5	62.5	-	-	-	-	-	-
8	2.5	62.5	+	+	+	-	-	+
9	5	125	+	-	-	-	-	+
10	5	125	-	-	-	-	-	-
11	2.5	62.5	+	-	-	-	-	+
12	5	125	-	-	-	-	-	-
13	5	125	+	+	-	-	-	-
14	2.5	62.5	-	-	-	-	-	-
15	5	125	+	+	+	-	-	-
16	5	125	-	-	-	-	-	-
17	2.5	62.5	-	-	-	-	-	-
HS	5	125	-	-	-	-	-	-
EAS	4	100	-	-	-	-	-	-
MS	5	125	-	-	-	-	-	-
HR	5	125	-	-	-	-	-	-
EAR	5	125	+	+	-	-	-	+
MR	5	125	-	-	-	-	-	-
Amp	100	2.5	+++	+++	+++	+++	+++	+++

Key: **+**: intermediate (ZOI = 8-12 mm), **-**: resistant (ZOI = 0) and **+++**: strong activity (ZOI = 17-19 mm) of the standard (ampicillin) against the given microorganisms. HS: *n*-hexane extract of the stem bark; EAS: ethyl acetate extract of the stem bark; MS: methanol extract of stem bark; HR: *n*-hexane extract of the root bark; EAR: ethyl acetate extract of the root bark and MR: methanol extract of the root bark; Amp: ampicillin.

In Agar Overlay Technique compound **6** also exhibited significant antibacterial activities against *E. coli* and *B. Subtilis* [25]. It thus appears that the disc diffusion method is rather insensitive as evidenced by the lacking activity of compounds **6** and **16**, while these same compounds were active according to the other two methods. The problem could be that the compounds are not

diffusing well from the disc in to the agar and hence false-negative results are observed. The crude extracts of the stem and root bark of *E. caffra* were also evaluated for antibacterial test using disc diffusion method and only the crude ethyl acetate extract of the root bark exhibited an intermediate inhibition against *E. coli*, *B. subtilis*, and *S. minor*.

EXPERIMENTAL

General

^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC and HMBC of the compounds were recorded using Bruker Avance DPX 300, DRX 500 and DRX 600 MHz spectrometers using standard pulse sequences and referenced to residual solvent signals. HREI-MS was determined using GCT Premier Spectrometer. Column chromatography was carried out on silica gel 60 (0.040–0.063 mm, Merck) and Sephadex LH-20 as the stationary phases. Preparative TLC (0.5 mm thick) and analytical TLC were performed with precoated Merck silica gel 60 PF254+366. Visualisation of chromatograms were done under UV light (254 and 366 nm) and further by spraying vanillin-sulfuric acid spray followed by heating. The ultraviolet and visible (UV-Vis) spectra were measured on Spectro UV-Vis Double Beam PC8 scanning auto Cell UVD-3200, LABOMED, INC. Infrared (IR) absorptions were measured on a PerkinElmer FT-IR Spectrometer, Spectrum Two, PerkinElmer UATR Two. Optical rotation was determined by using an Autopol IV automatic polarimeter.

Plant material

Stem bark of *E. caffra* Thunb was collected from experimental garden, University of Botswana, Gaborone, Botswana in February 2011. The species was identified by Mr. M. Muzila and a voucher specimen, Ec-25-11, has been deposited at the University of Botswana Herbarium.

Extraction and isolation

The air-dried 600 g of stem bark of *E. caffra* was ground and extracted by soaking successively in *n*-hexane, EtOAc and MeOH each for 24 hours (three times with each solvent) and removal of the solvent under reduced pressure using a BUCHI flash evaporator to afford black gummy extracts of 5.0 g (for n-hexane), 29.5 g (for EtOAc) and 18.8 g (for MeOH).

The EtOAc extract (29.5 g) was fractionated by vacuum liquid chromatography (VLC) on silica gel (500 g), eluting with *n*-hexane containing increasing amounts of EtOAc (up to 100%), followed by EtOAc/MeOH by increasing the amounts of MeOH (up to 100%) to yield a total of 40 fractions of 200 mL each. Fractions that showed similar components on TLC analysis were combined together to give thirteen main fractions labelled A to M, respectively.

The combined fraction **C** from VLC (5-10) (3.0 g) was adsorbed onto 6.0 g of silica gel and applied on a column that was packed with 90.0 g of silica gel in *n*-hexane and eluted with *n*-hexane/EtOAc by increasing the amounts of EtOAc (up to 100%) followed by EtOAc/MeOH (up to 7:3) and yielded a total of 30 sub-fractions of 50 mL each. Of the 30 sub-fractions, sub-fractions 1-5 were combined together and passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 5 sub-fractions of 100 mL each. Of the five sub-fractions, sub-fraction 3 (1.5 g) was adsorbed onto 4.0 g of silica gel and applied on a column that was packed with 90.0 g of silica gel and eluted with *n*-hexane/chloroform solvent system by increasing amounts of CHCl$_3$ (up to 100%), followed by CHCl$_3$/MeOH with increasing amounts of MeOH (up to 100%) and afforded 3 pure compounds *triacontyl-4-hydroxycinnamate* (12.2 mg), *octacosyl-E-ferulate* (84.2 mg) and *octacosan-1-ol* (7.2 mg). Other components were long chain fatty acids and further work was not done.

The combined fraction **D** from VLC (11-12) (1.70 g) was passed through Sephadex LH-20 using MeOH/CHCl₃ (1:1) as the solvent system and yielded a total of 22 sub-fractions of 15 mL each. Based on TLC analysis these 22 sub-fractions were classified as 1-7 (a), 8-12 (b), 13-15 (c) and 16-22 (d). The sub-fraction (c) was applied on preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/CHCl₃ (7:2:1) and afforded the two pure compounds **19** (2.4 mg) and **9** (6.9 mg). The sub-fraction (d) was applied on the preparative TLC and eluted with the solvent system *n*-hexane/EtOAc /CHCl₃ (6:3:1) and afforded one pure compound **11** (7.3 mg). Other sub-fractions (a) and (b) gave long chain fatty compounds and did not show any interesting results on TLC and were thus discarded.

The combined fraction **E** from VLC (13–15) (2.0 g) was adsorbed onto 6.0 g of silica gel and applied on a column which was packed with 80.0 g of silica gel using a solvent n-hexane/EtOAc (7:3) system and eluted with *n*-hexane/EtOAc (7:3) solvent system by increasing the amounts of EtOAc (up to 100%), followed by EtOAc/MeOH by increasing the amounts of MeOH and yielded a total of 30 sub-fractions of 25 mL each. Of the 30 sub-fractions, sub-fraction 8 was pure and afforded an isoflavonoid **6** (88 mg). The sub-fractions 9-11 were combined together and applied onto a column which was packed with 60 g of silca gel and eluted with *n*-hexane/EtOAc (3:2) solvent system by increasing the amounts of EtOAc (up to 100%) and afforded the two pure compounds *oleanolic acid* (14.0 mg) and *β-amyrin* (2.8 mg). The sub-fractions 12-17 were combined together and applied onto a column which was packed with 30.0 g of silica gel and eluted with *n*-hexane/EtOAc (7:3) solvent system by increasing the amounts of EtOAc (up to 100%), followed by EtOAc/MeOH (7:3) system and yielded a total of 20 sub-fractions of 25 mL each. Of these 20 sub-fractions, sub-fractions 9-13 were combined together and applied on the preparative TLC and eluted with the solvent system DCM/*n*-hexane/MeOH (7:2:1) (2 developments) and afforded two prenylated isoflavonoids **1** (8.0 mg) and **2** (7.0 mg).

The combined fraction **F** from VLC (16–20) (4.0 g) was adsorbed onto 6.0 g of silica gel and applied onto a column which was packed with 120.0 g of silica gel and eluted with *n*-hexane/EtOAc (7:3) by increasing the amounts of EtOAc (up to 100%), followed by EtOAc/MeOH (7:3) and yielded a total of 39 sub-fractions of 25 mL each. These sub-fractions were combined together based on the TLC analysis to give a total of 5 main sub-fractions labelled (a) to (e), respectively. Of these 5 sub-fractions, sub-fraction (b) (3-6) was applied on preparative TLC and eluted with the solvent system *n*-hexane/EtOAc (4:1) and afforded one pure compound *n-tetracosyl-p-coumarate* (8 mg). The sub-fraction (c) (7-19) (2.0 g) was adsorbed onto 6.0 g of silica gel and applied onto a column which was packed with 80.0 g of silica gel and eluted with *n*-hexane/EtOAc (7:3) solvent system by increasing the amounts of EtOAc (up to 100%), followed by EtOAc/MeOH upto (7:3) and yielded a total of 25 sub-fractions of 25 mL each. Of these 25 sub-fractions, sub-fractions 9-11 were combined together and applied on preparative TLC plate and eluted with the solvent system DCM/*n*-hexane/MeOH (5:4.5:0.5) (2 developments) and afforded the two pure compounds **5** (7.5 mg) and **10** (14.8 mg). The sub-fraction (d) (20-30) was passed through Sephadex LH-20 using the solvent system CHCl₃/MeOH (1:1) and yielded a total of 15 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together in to 2 main sub-fractions labelled as 1-8 and 9-15. The first sub-fraction 1-8 contained long chains and chlorophyll and further work was not done. However, the sub-fractions 9-15 were combined together and applied on the preparative TLC plate and eluted with the solvent system DCM/*n*-hexane/MeOH (5:4:1) (3 developments) and afforded the three pure compounds **8** (3.2 mg), **12** (15.4 mg) and **3** (10.0 mg). The sub-fraction (e) (21-39) was not further analyzed due to lack of interesting components on TLC plate.

The combined fraction **G** from VLC (21-23) (3.0 g) was adsorbed onto 4.5 g of silica gel and applied onto a column which was packed with 100.0 g of silica gel and eluted with *n*-hexane/EtOAc (7:3) solvent system by increasing the amounts of EtOAc (up to 100%), followed

by EtOAc/MeOH (9:1) and yielded a total of 28 sub-fractions. These 28 sub-fractions were combined based on TLC analysis to give five main sub-fractions and classified as 1-6 (a), 7-10 (b), 11-13 (c), 14-16 (d) and 17-28 (e). Of these five main sub-fractions, sub-fraction (b) was applied on preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/CHCl$_3$ (5:4:1) (3 developments) and afforded one pure compound **17** (5.2 mg). The sub-fraction (c) was applied on preparative TLC plate and eluted with the solvent system *n*-hexane/CHCl$_3$/MeOH (5:4:1) (2 developments) and afforded one pure compound **13** (25 mg). The sub-fraction (d) was applied on the preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/CHCl$_3$ (5:4:1) (2 developments) and afforded the two pure compounds **15** (15.7 mg) and **16** (15.0 mg). The sub-fraction (e) did not show any interesting results on TLC plate and was thus discarded.

The combined fraction **H** from VLC (24-26) (2.80 g) was passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 21 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC plate were combined together to give 3 main sub-fractions. The sub-fractions 1-7 and 8-14 did not show any interesting results on TLC plate and were thus discarded. The sub-fractions 15-21 were combined together and applied on preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/CHCl$_3$/MeOH (5:3:1.5:0.5) (3 developments) and afforded three pure compounds **4** (4.9 mg), **14** (4.9 mg) and **7** (4.2 mg).

The combined fraction **I** from VLC (27-28) (2.00 g) was passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 13 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together to give 2 main sub-fractions. The sub-fractions 1-5 did not show any interesting results on TLC and were thus discarded. The sub-fractions 6-13 were combined and passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 10 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together to give 2 main sub-fractions. The sub-fractions 1-4 did not show any interesting results on TLC and were thus discarded. The sub-fractions from 5-10 were combined and applied on preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/DCM (4:4:2) (3 developments) and afforded the pure compound **20** (2.0 mg).

The combined fraction **J** from VLC (29-31) (1.10 g) was passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 20 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together to give 2 main sub-fractions. The sub-fractions 1-7 did not show any interesting results on TLC and were thus discarded. The sub-fractions 8-20 were combined and passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 10 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together to give 2 main sub-fractions. The sub-fractions 1-3 did not show any interesting results on TLC and were thus discarded. The sub-fractions from 4-10 were combined and applied on preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/CHCl$_3$/MeOH (5:3:1.5:0.5) (3 developments), which afforded the pure compound **18** (4.1 mg).

The combined fraction **K** (32-33) (1.0 g) was passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded a total of 15 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together to give 2 main sub-fractions. The sub-factions 1-7 did not show any interesting results on TLC and were thus discarded. The sub-fractions 8-15 were combined and passed through Sephadex LH-20 using the solvent system CHCl$_3$/MeOH (1:1) and yielded total of 8 sub-fractions of 10 mL each. Based on TLC analysis, the sub-fractions that showed similar components on TLC were combined together to give 2 main sub-fractions. The sub-factions 1-3 did not show any interesting results on TLC and were thus discarded. The sub-fractions from 4-

8 were combined together and applied on preparative TLC plate and eluted with the solvent system *n*-hexane/EtOAc/DCM (4:4:2) (2 developments) and afforded one pure alkaloid *8-oxo-erythrinine* (4.5 mg).

Cytotoxicity test

Cytotoxic activity of the isolates and crude extracts was done as described [23]. Briefly the KB-3-1 cells were cultivated as a monolayer in a DMEM (Dulbecco's Modified Eagel Medium) with glucose (4.5 g/L), L-glutamine, sodium pyruvate and phenol red, supplemented with 10% (KB-3-1) foetal bovine serum (FBS). The cells were maintained at 37 °C and 5.3% CO_2 humidified air. On the day before the test, the cells (70% confluence) were detached with trypsin-ethylenediamine tetraacetic acid (EDTA) solution (0.05%; 0.02% in DPBS (Dulbecco's Phosphate Buffered Saline) and placed in sterile 96-well plates in a density of 1000 cells in 100 µL medium per well. The dilution series of the compounds were prepared from stock solutions in DMSO of concentrations of 100 mM, 50 mM or 25 mM. The stock solutions were diluted with culture medium (10% FBS [KB-3-1]) down to pM range. The dilution prepared from stock solution was added to the wells. Each concentration was tested in six replicates. Dilution series were prepared by pipetting liquid from well to well. The control contained the same concentration of DMSO as the first dilution. After incubation for 72 h at 37 °C and 5.3% CO_2 humidified air, 30 µL of an aqueous resazurin solution (175 µM) was added to each well. The cells were incubated under the same conditions for 5 hours. Subsequently, the fluorescence was measured. The excitation was effected at a wave length of 530 nm, whereas the emission was recorded at a wavelength of 588 nm. The IC_{50} values were calculated as a sigmoidal dose response curve using GRAPHPAD PRISM 4.03. The IC_{50} values equal the drug concentration, at which vitality is 50%.

Antibacterial test

Simple antibacterial test for the isolated pure compounds and crude extracts from *Erythrina caffra* was done using disc diffusion method. In this method, a Petri dish with a nutrient broth agar was plated with 3 mL of a bacteria solution from a culture with an optical density of 0.2. The plated bacteria were incubated at 37 °C for 30 min. The sterilized paper discs were loaded with 25 µL of solution of pure compounds or extracts dissolved in DMSO. The discs were allowed to dry and then placed on seeded agar plate using sterilized forceps. The plates were then incubated at 37 °C for 24 hours, after which zones of inhibition, if any, were measured. For inactive compounds no zone of inhibition was observed. Concentration ranges from 2.0 to 5.0 mg/mL corresponding to loading doses of 50-125 µg/disc were used. The diameters of the zones of inhibition were measured in millimetres. Ampicillin (loading of 2.5 µg/disc or 25 µL of 100 µg/mL ampicillin solution) was used as a standard. The diameter of the zone of inhibition for ampicillin under these conditions ranged from 17-19 mm.

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