ISSN : 0974 - 7508 Volume 12 Issue 1

Natural Products

An Indian Journal

Full Paper

NPAIJ, 12(1), 2016 [012-020]

Chemical constituents from *Erythrina droogmansiana* (Fabaceae), radical scavenging and antibacterial potential of some extracts and compounds

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ABSTRACT

A new ceramide, droogmansiamide (**1**), was isolated from methanolic extract of roots wood of *Erythrina droogmansiana*, with eight known compounds namely 3-(3',4'-methylenedioxyphenyl)-2,3-epoxypropanol (2), erythrinasinate A (3), erythrinasinate B (4), abyssinone-IV-4'-methylether (5), erythrabyssin (6), phaseollidin (7), 4'-methoxylicoflavanone (8) and abyssinone-V-4'-methylether (9) respectively from methanolic extract of roots wood and EtOAc extract of roots bark of the same plant. Their structures were elucidated using spectroscopic methods (MS, NMR and IR) and by comparison with some data found in literature. Free radical scavenging (DPPH) and antibacterial potentials of extracts and compounds were also evaluated in this work. For radical scavenging, results showed that it is phaseollidin (7) which is responsible of radical scavenging potential in the ethyl acetate extract of roots barks with value of 1.31 mg/ml; for antibacterial, one of the tested compounds abyssinone-IV-4'-methylether (5) exhibited antibacterial activities against two strains: *Providencia stuartii*ATCC 29916 and *Enterobacter aerogenes* ATCC 13048 with MIC values of 25µg/ml.

KEYWORDS

Erythrina droogmansiana;
Ceramide;
Radical scavenging;
Antibacterial.

INTRODUCTION

The genus *Erythrina* (Leguminosae) has more than 110 species[1], growing in tropical regions of America, Africa and Asia. Pharmacological and phytochemical studies have been carried out on more than 80 species of this genus. These numerous studies have revealed that members of this genus are rich in a variety of secondary metabolites which are mostly phenolic and alkaloids[2]. In the same way,

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most of the compounds isolated from *Erythrina* species showed antimicrobial, anti-inflammatory, antiplasmodial, antioxidant and anticancer activities[2]. In this work, we reported isolation and structural elucidation of a new ceramide (1) alongside eight known compounds: 3-(3',4'-methylenedioxyphenyl)-2,3-epoxypropanol (2)[3], erythrinasinate A (3)[4], erythrinasinate B (4)[5], abyssinone-IV-4'-methylether (5)[6], erythrabyssin (6)[7], phaseollidin (7)[8], 4'-methoxylicoflavanone (8)[9] and abyssinone-V-4'-methylether (9)[10] from methanolic extract of roots wood and EtOAc extract of roots bark of *E. droogmansiana*. In addition, radical scavenging potential of methanolic, ethyl acetate extracts and of compounds 2, 5, 6, 7, 9 using DPPH as free radical, and antibacterial activity using Mueller Hinton Broth (MHB) by micro-dilution method[11] of compounds 1, 2 and 5 were also evaluated.

EXPERIMENTAL

General experimental procedure

Bruker spectrometer with tetramethylsilane (TMS) as standard (^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz)). Mass spectra (EI-MS) were performed on a JEOL MSRoute spectrometer. TLC was made out on pre-coated silica gel on aluminum sheets. Silica gel (E. Merck, 230-400 mesh) and Sephadex LH-20 were used for column chromatography. Optical density was read using an APADA V-1100 spectrophotometer; Electro-thermal 9100 apparatus was used for the melting point.

Plant material

The plant (roots barks and roots wood) was collected (stem bark and root bark) and identified with the help of a botanist of the National Herbarium of Cameroon by comparison to a known specimen deposited in the fore mentioned Herbarium under voucher number No.4261/SRFK. Extraction and isolation

Extraction of roots barks powder of the plant was carried out by maceration at room temperature for at least 48 hours with solvent renewal in order of increasing polarity (ethyl acetate and methanol) while the roots wood was percolated.

For roots wood, the concentrated crude methanol extract (60 g) obtained after evaporation of solvent was subjected to silica gel column chromatography using elucting solvents system hexane-EtOAc and EtOAc-MeOH with increasing polarity to give 5 fractions (S_1-S_5)which were regrouped based on their TLC profils. During elution of the main column, compounds 1 (30 mg) and 2 (15 mg) were obtained at hexane-EtOAc (65:35) and (90:10) respectively.

For root bark, 100 g of ethyl acetate extract was subjected to column chromatography, using a gradient solvent system of hexane, hexane-EtOAc, EtOAc-MeOH in increasing polarity to give 7 fractions (S_1-S_7) regrouped on the basis of TLC. S_2 (25g) was separated by successive column chromatography using hexane, hexane- EtOAc. This operation led to the isolation of compounds 3 (22 mg), 4 (15 mg), 5 (25 mg) and 6 (35 mg) 9 (200 mg) at hexane-EtOAc (95:05); hexane-EtOAc (90:10) and hexane-EtOAc (85:15) respectively. The purification of the fraction S'_3 (1g) obtained at Hexane-EtOAc (90:10) from the main column, through Sephadex LH 20 CC yielded 7 (80 mg) and 8 (35 mg).

Droogmansiamide (1)

White amorphous powder.

MP: 140-143°C

UV (MeOH-CH_2Cl_2): λ_{max} (nm) (log ε): 217 (1.99).

IR (KBr): 3736, 3334, 3219, 2920, 2850, 1620, 1541, 1465, 680 cm^{-1}

^{1}H NMR, ^{13}C NMR and HMBC: TABLE 1.

HR-EI-MS: 653.5894 (100) (calcd. 653.5893 for $C_{40}H_{79}NO_5$, $[M]^+$).

Biological activities

Free radical scavenging assay

The DPPH assay was carried out as described by Nyaa[12] with slight modifications. Briefly, a volume of 100µl of solution (extract or compound) was added to 1.9 ml of a methanolic solution of DPPH (50 mg/L). The absorbance of the reaction mixture was then recorded at 517 nm after 30 minutes in darkness. The assay was carried out in triplicate.

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The percentage inhibition was calculated using the formula:

$$IC_{50} = \frac{(DO\ dpph - DO\ sample)X\ 100}{DO\ dpph}$$

The concentration of the extract or compound that exhibits 50% of discoloration (IC50) was estimated.

Antibacterial assay

Microorganisms

Compounds were tested against a panel of microorganism including seven bacterial strains (*Enterobacter aerogenes* ATCC 13048, *Enterococcus faecalis*ATCC 10541,*Klebsiella pneumonia* ATCC 11296, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATCC 01, *Providencia stuartii*ATCC 29916 and *Staphylococcus aureus* ATCC 25922) and six isolates (*Escherichia coli*, *Shigellaflexeneri*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Klebsiella pneumonia* and *Staphylococcus aureus*). The isolates (microorganisms) were obtained from Centre Pasteur of Yaounde, Cameroon, while the reference strains were obtained from American Type Culture Collection (ATCC). The strains and isolates were grown at 35°C and maintained on nutrient agar. The bacterial cell suspension was prepared at 1.5×10^8 colony forming units per mL (CFU/ml) following the McFarland 0.5 turbidity standard.

Broth micro-dilution method

Minimum inhibitory concentrations (MICs) were determined using Mueller Hinton Broth (MHB) by micro dilution method[11]. A twofold serial dilution of the compounds (100–0.005 µg/ml) was performed in a total volume of 200 µl/well. A negative control (5%, v/v aqueous DMSO, medium and inoculum) and positive control (5%, v/v aqueous DMSO, medium, inoculum and water-soluble antibiotics) were included. Each well of 96-well sterile microplate received the test substance at the different concentrations and bacterial suspension (100 µl) in MHB. The plates were covered and incubated at 35$^{\alpha\%}$C for 18 h. Bacterial growth was monitored colorimetrically using *p*-iodonitrotetrazolium chloride (INT). Viable bacteria change the yellow dye of *p*-iodonitrotetrazolium violet to a pink color. MIC values are recorded as the lowest concentration of the substance that completely inhibited bacterial growth that is, the solution in the well remained clear after incubation with INT. Minimum bactericidal concentrations (MBCs) were determined by plating 10 µl from each negative well and from the positive growth control on Mueller Hinton Agar. MBCs were defined as the lowest concentration yielding negative subcultures. The experiments were performed in triplicate. Amoxicillin, ciprofloxacin and gentamicin at the concentration ranging between 128 and 0.062 µg/ml served as positive control.

RESULTS AND DISCUSSION

Methanolic extract of the roots wood and ethyl acetate extracts of barks of roots of *E. droogmansiana* were subjected to many VLC on silica gel. This treatment led to the isolation of nine compounds of various classes (Figure 1). The structures of these compounds were elucidated using spectroscopic methods (MS, NMR and IR) and by comparison with some data found in the literature. 2D NMR techniques (COSY, HSQC and HMBC) were also used.

Droogmansiamide (1) was obtained as white powder. The analysis of its spectra particularly the HR-EI-MS (*m/z* = 653.5894) and NMR corresponds to the molecular formula $C_{40}H_{79}NO_5$ comprising two insaturations.

The ^{1}HNMR spectrum indicated five characteristic signals of oxymethylene, oxymethines and methines related to nitrogen protons respectively at 3.8 (1H, dd, *J*= 5.5 and 10.5 Hz, H-1a); 3.72 (1H, d, *J* = 4.5 and 10.5 Hz, H-1b); 3.54 (1H, m, H-3); 3.53 (1H, dd, *J* = 2,5 and 5.5 Hz, H-4), 4,04 (1H, dd, *J* =3.6 and 7.2 Hz, H-2') and 4.10 (1H, q, *J* = 4.5 Hz, H-2). The same spectrum also showed at 5.41 (2H, m, H-19) a olefenic proton signal; at 0.88 (6H, t, *J* = 6.8 Hz, H-23 and H-16') a signal corresponding to methyl protons and several methylene protons between 1.27 and 1.36 ppm. This foregoing information suggests that the droogmansiamide could be a sphingolipid. The ^{13}CNMR spectrum indicated characteristic signals at 175.9 ppm corresponding to the

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TABLE 1 : ^{1}H (500 MHz), ^{13}C (125 MHz) NMR and HMBC data of compound 1 in $CDCl_3$-MeOH

Position	δ_C(in ppm)	δ_H(in ppm)	HMBC
1a b	61.0	3.80 (dd, *J* = 5.5 and 10.5 Hz) 3.72 (d, *J* =4.5 and 10.5 Hz)	2/3
2	51.5	4.10 (d, *J* = 4.5 Hz)	1/3/4/1’
3	75.5	3.54 br	1/2/4/5
4	72.2	3.53 (dd, *J* =2.5 and 5.5 Hz)	2/3/1
5	32.9	1.42	12/11
6	25.8	1.51 (ov) 1.42 (ov)	
7	29.8	1.27 (ov)	
8-10	29.5	1.27 (ov)	
11	29.7	1.27 (ov)	
12-17	29.6	1.27 (ov)	
18	31.8	1.27 (ov)	
19	130.6	5.41 (m)	21
20	129.7	5.41 (ov)	21
21	32.5	2.01 (ov)	15/16
22	32.9	1.69 (ov)	
23	22.6	1.26 (ov)	
24	13.9	0.88 (t, *J* = 6.8 Hz)	17/18/21
1’	175.9		
2’	71.9	4.04 (dd, *J* = 3.6 and 7.2 Hz)	1’/3’/14’
3’	34.3	1.69(ov) 1.81 (ov)	1’/2’/14’
4’	29.6	1.26 (ov)	
5’	29.3	1.26 (ov)	
6’-7’	29.5	1.26 (ov)	
8’-9’	29.6	1.26 (ov)	
10’-12’	29.7	1.26 (ov)	
13’	31.8	1.26 (ov)	
14’	25.2	1.42 (ov)	
15’	22.6	1.26 (ov)	
16’	13.9	0.88 (t, *J* = 6.8 Hz)	15’/14’/13’/12’

amide carbonyl, at 75.6; 72.3 and 71.9 ppm corresponding to oxymethines carbons, at 61.1 ppm corresponding to oxymethylene carbon at 51.6 ppm corresponding to a methine attached to nitrogen atom and at 130.7 and 129.7 of olefenic carbons. All the signals described above are characteristic of sphingolipids or more particularly of a ceramide (TABLE 1).

The HMBC spectrum showed the correlation (Figure 3: HMBC correlations) between H-19, H-24 and C-21 which indicate that olefenic proton and methyl protons are close. The length of the fatty chain was determined by the characteristic ions peaks (Figure 2: fragmentation peak) at: *m/z* 339 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_2]^+$, 383 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_2CHCH_2OH]^+$, 439 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_3CONHCHCH_2OH]^+$ and 225 $[CH_3(CH_2)_{13}(CHOH)]^+$. This spectra also confirmed the position of the double bond by ion peak at *m/z*= 83 $[CH_3(CH_2)_3(CH)_2]$. *Trans* bound was shown to be *trans* due to the presence of signals at 31.8 and 32.5 with are characteristic of *trans* ceramide[13-14]. The fragment at *m/z* = 439 which is not common to several ceramides confirmed the pro-

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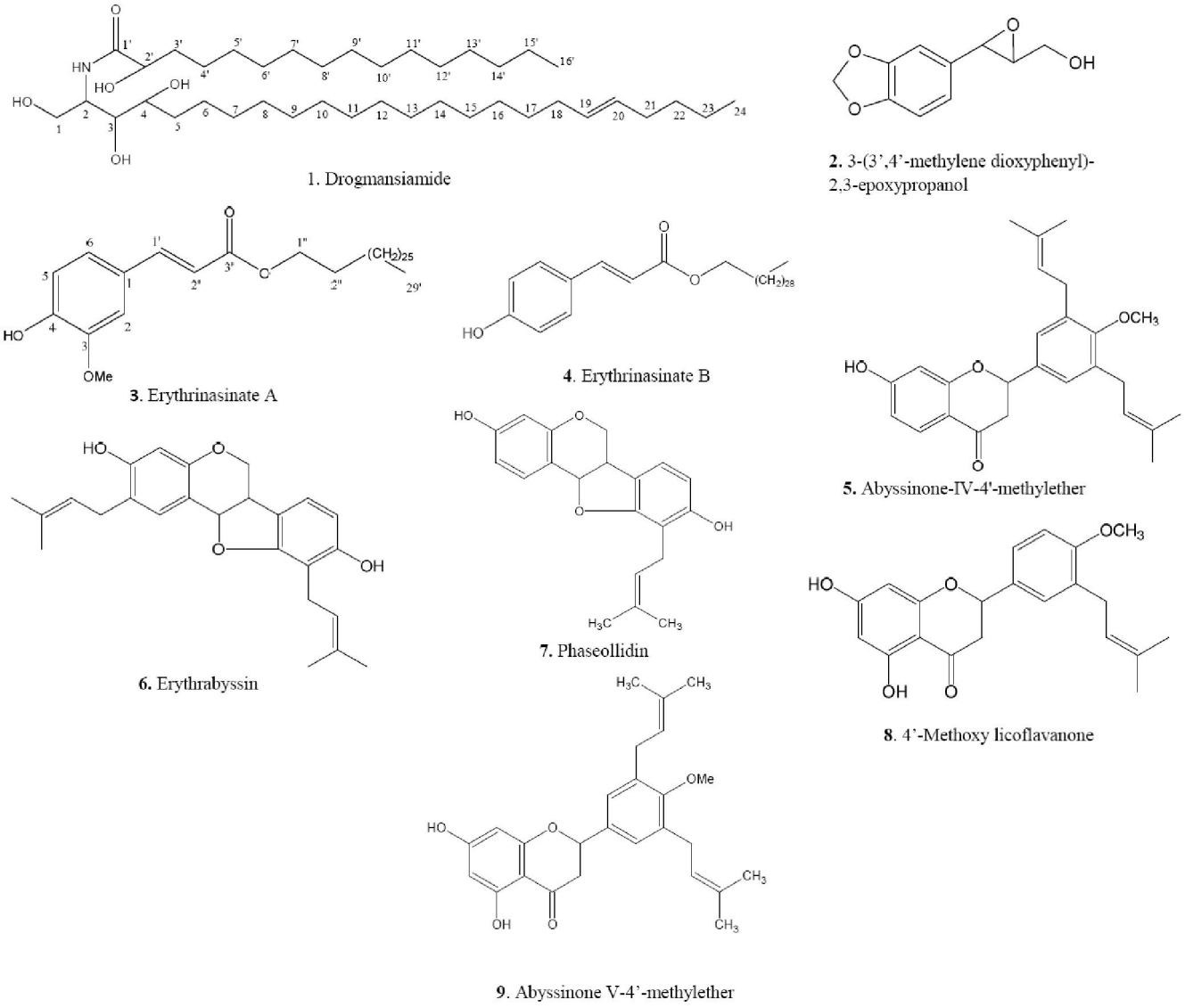

Figure 1 : Structures of compounds isolated from *Erythrina droogmansiana*

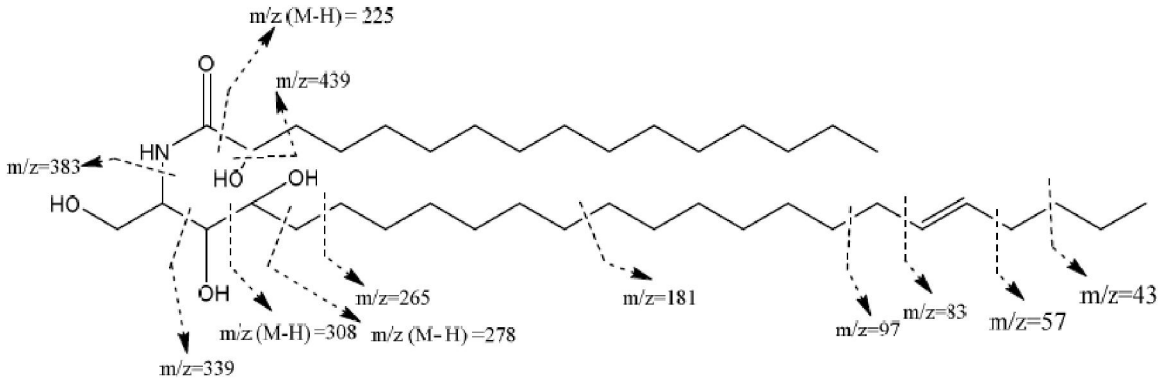

Figure 2 : Fragmentations of compound 1

posed. The structures of the other isolated compounds were established by comparison of their spectral data with those reported in literature.

The free radical scavenging potential of ethyl acetate extracts, methanolic extracts of roots barks and of stem barks, and of some isolated compounds (2, 5, 6, 7 and 9) was evaluated (TABLE 2). For extracts, ethyl acetate extract of roots barks was the

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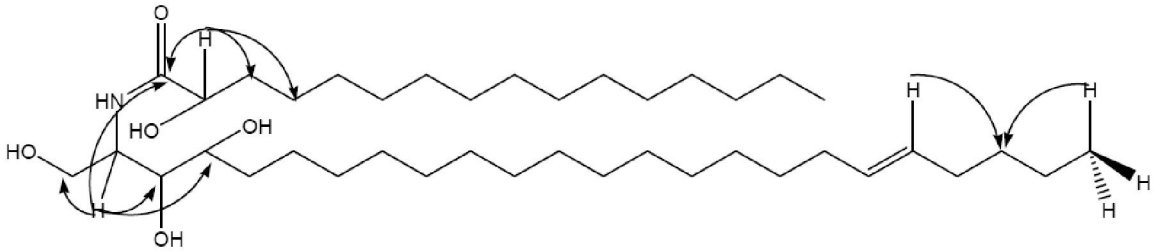

Figure 3 : HMBC correlations

TABLE 2 : Free radical scavenging data

Samples	CD_{50} (mg/ml)
Ascorbic Acid	0,063
EAExRB	1,30
EAExSB	1,62
MExRB	3,70
MExSB	4,33
Compound 2	3,31
Compound 5	2,18
Compound 6	1,31
Compound 7	1,30
Compound 9	14,32

EAExRB: ethyl acetate extract of roots bark; EAExSB: ethyl acetate extract of stem barks; MExRB: methanolic extract of roots barks; MExSB: methanolic extract of stem barks

most active with discoloration value of 1.30 mg/ml. For compounds, compound 7 is the most active with discoloration value of 1.31 mg/ml and its value is near of the value of its extract. Due to the fact that it has synergy in extract, we could say that compound 7 is the one which is the more responsible of this moderate free radical scavenging in the plant.

Compounds 1, 2 and 5 were tested for their antibacterial activity. Their MIC and MBC are shown in TABLES 3. Compounds 1 and 2 did not exhibit antibacterial activity against the tested bacteria. Compound 5 which showed activity, inhibited the tested bacteria with MICs ranging from 25-50 µg/ml for the isolates and Gram-negative, 25-100 µg/ml for the strains and Gram-positive. *E. aerogenes* ATCC 13048, *P. stuartii* ATCC 29916 and *S. aureus* were the most sensitive (MIC = 25 µg/ml). *E. faecalis* ATCC 10541 was the least sensitive (MIC = 100 µg/ml). The reference antibiotic compound ciprofloxacine exerted a higher inhibition on the tested bacterial (MIC = 0.125-128 µg/ml) than the compounds. However, the inhibitory activity of compound 5 against *E. aerogenes* ATCC 13048 and *P. stuartii* ATCC 29916 (MIC = 25 µg/ml) was better than that of ciprofloxacin (MIC = 32-128 µg/ml). The activity of pure compound 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)[15]. Compound 5 showed a moderate inhibitory activity against the bacteria tested. The MBC/MIC ratio for all the tested bacteriavaried between one (1) and four (4) for the compound 5. According to Marmonier (1990)[16], pure compounds exerted two types of activities: a bacteriostatic (MBC/MIC ≥ 4) and bactericidal activity (MBC/MIC ≤ 4).

Flavonoids have been reported to possess antibacterial properties[17-18]. MIC values of this class of secondary metabolites showed that Gram-negative and Gram-positive bacteria had a comparable susceptibility. This suggests that its mode of action is not related to the cell wall composition. Flavonoids may play a role in intercalation or hydrogen bonding with the stacking of nucleic acid bases and that this may explain the inhibitory action on DNA and RNA synthesis[19]. Flavonoids can also

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TABLE 3 : MIC and MBC (µg/ml) of compounds

Bacteria	Parameters	Compounds			Reference Antibiotic
		1	2	5	Ciprofloxacine
Gram-negative					
E. coli	MIC	—	—	—	1
	MBC	—	—	—	1
	MBC/MIC	—	—	—	1
E. aerogenes ATCC13048	MIC	—	—	25	32
	MBC	—	—	50	32
	MBC/MIC	—	—	2	1
K. pneumonia ATCC 11296	MIC	—	—	—	1
	MBC	—	—	—	1
	MBC/MIC	—	—	—	1
K. pneumonia	MIC	—	—	—	0.25
	MBC	—	—	—	0.25
	MBC/MIC	—	—	—	1
P. stuartiiATCC 29916	MIC	—	—	25	128
	MBC	—	—	100	128
	MBC/MIC	—	—	4	1
P. aeruginosa ATCC 27853	MIC	—	—	—	1
	MBC	—	—	—	16
	MBC/MIC	—	—	—	16
P. aeruginosa ATCC01	MIC	—	—	—	1
	MBC	—	—	—	32
	MBC/MIC	—	—	—	32
S. flexneri	MIC	—	—	50	0.25
	MBC	—	—	—	1
	MBC/MIC	—	—	—	4
S. paratyphi A	MIC	—	—	50	0.125
	MBC	—	—	—	0.5
	MBC/MIC	—	—	—	4
S. paratyphi B	MIC	—	—	50	0.5
	MBC	—	—	—	2
	MBC/MIC	—	—	—	4
Gram-positive					
E. faecalis ATCC 10541	MIC	—	—	100	4
	MBC	—	—	—	16
	MBC/MIC	—	—	—	4
S.aureusATCC 25922	MIC	—	—	50	8
	MBC	—	—	—	16
	MBC/MIC	—	—	—	2
S. aureus	MIC	—	—	25	8
	MBC	—	—	100	8
	MBC/MIC	—	—	4	1

—:>100 µg/ml for the compounds and >128 µg/ml for the reference antibiotic

reduce membrane fluidity of bacterial cells[20] and interfere with energy metabolism[21].

CONCLUSION

The present study showed that *Erythrina* genus is a rich in various classes of secondary metabolites. This study also showed that as literature mentioned it, flavonoids have much biological potential such as antimicrobial. It is for the first time to isolate ceramide skeleton on *Erythrina* genus.

ACKNOWLEDGEMENTS

The authors are grateful to the "Fonds National de la RechercheScientifique (FNRS) Belge" for a fellowship to TTA in the Laboratory of Pharmacognosy, University of Liege, Belgium and to the Third World Academy of Sciences (TWAS) for a fellowship offered to AJP at the Laboratory of Natural Product ISBN Karachi Pakistan.

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