Available online at www.ijpsdronline.com
International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(2): 160-163

Research Article ISSN 0975-248X

Antioxidant Activity of Compounds Isolated from the Root Woods of *Erythrina droogmansiana*

AJG Yaya[1], RD Feumba[2], T Emmanuel[3*], AT Tchinda[1], M Fredérich[5], J Oben[6], JT Mbafor[4]

[1]*Centre for Research on Medicinal Plants and Traditional Medicine (CRPMT), Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 6163, Yaounde, Cameroon*
[2]*Centre for Research on Food and Nutrition (CRAN), Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 6163, Yaounde, Cameroon*
[3]*Department of Chemistry, Faculty of Science, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon*
[4]*Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon*
[5]*Université de Liège, Laboratoire de Pharmacognosie, Centre Interfacultaire de Recherche sur le Médicament (CIRM), Département de Pharmacie, Université de Liège, B36, B-4000, Liège, Belgium*
[6]*Department of Biochemistry, Faculty of Science, University of Yaounde I, P.O. Box, 812, Yaounde, Cameroon*

ABSTRACT
The aim of this study was to isolate, to characterize secondary metabolites from methanolic extract of the root woods of *Erythrina droogmansiana* and to assess the antioxidant activity of the crude extract and isolated compounds. The phytochemical study led to the isolation of 3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol (1), asperphenamate (2) and three flavonoids namely genistein, diadzein and 4',5,7-trihydroxy-8-prenylisoflavone. These compounds were characterized using their ^{1}H NMR, ^{13}C NMR, HMBC, HSQC, COSY, mass spectral and the literature. To evaluate antioxidant activity of crude extract and isolated compounds, the radical scavenging (DPPH) and Ferric Reducing Ability Power (FRAP) were performed using ascorbic acid as standard. Compounds 1 and 2 showed moderate radical scavenging potential with IC_{50} value of 3.14 and 3.31 mg/ml respectively, and moderate reducing power ability with value of 0.14±0.01 mgAAE/mg and 0.21±0.01 mgAAE/mg respectively. The more active compound was genistein (3) with IC_{50} value of 1.96 mg/ml for the DPPH radical scavenging potential and 0.24±0.02 mgAAE/mg for its ability to reduce iron.

Keywords: *Erythrina droogmansiana*, isolation, asperphenamate, 3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol, antioxidant activities.

INTRODUCTION

The oxidative damage caused by reactive oxygen species (ROS) on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart disease, cancer and ageing. [1-3] Epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age-related diseases, such as coronary heart disease and cancer, which may be attributed to their antioxidant activity. [4-6] The fact that some synthetic antioxidants, such as BHT and BHA, need to be replaced with natural antioxidants, as they were found to be toxic and carcinogenic in animal Models. [7-8] Thus, it is important to identify new sources of safe and inexpensive antioxidants of natural origin.

***Corresponding author: Mr. T Emmanuel,**
Department of Chemistry, Faculty of Science, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon;
Tel.: +00 237 7486 3715; **E-mail:** tallae2000@yahoo.fr

The *Erythrina* genus is known as a source of various secondary metabolites with an abundance of flavonoids and alkaloids [9], but less is known on their biological activities. Some compounds isolated from several species of *Erythrina* have been reported to possess antiplasmodial [10], antimicrobial [11], radical scavenging [12] and anti-inflammatory [13-14] activities. *E. droogmansiana* is a tree of about 25 m height, widely distributed in Cameroon, Democratic Republic of Congo and Gabon. [15] To the best of our knowledge, this is the first report on the phytochemistry and antioxidant activity of *E. droogmansiana*. This study led to the isolation for the first time in this genus of 3-(3', 4'-methylenedioxyphenyl)-23 -and asperphenamate with three flavonoids previously isolated from others *Erythrina* species.

MATERIALS AND METHODS

General experimental procedures

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker spectrometer with trimethylsilane

(TMS) as standard. ESI-HRMS analyses were performed on a LTQ-Orbitrap XL hybrid mass spectrometer. TLC was carried out on coated silica gel on aluminum sheets. Silica gel (E. Merck, 230-400) was used for column chromatography. Optical density was read using an APADA V-1100 spectrophotometer.

Plant material

The plant (roots wood), selected on the basis of chemotaxonomy surveys, was collected at Nkomekoui (Centre Region of Cameroon) and identified by Mr. Nana Victor, a botanist of the National Herbarium Yaoundé (Cameroon), to voucher number No. 4261/SRFK.

Extraction and isolation

Powder of the air-dried roots wood (1.2 Kg) was macerated at room temperature for 48 hours with methanol and evaporated using a Rotavapor apparatus at 40°C. The concentrated crude methanol extract (60 g) obtained after evaporation of solvent was subjected to silica gel chromatography column using a gradient of hexane-EtOAc and EtOAc-MeOH with increasing polarity to give 5 series of fractions (S1-S5) which were regrouped based on their TLC profile. During elution of the main column, 3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol (1, 45 mg) [16], asperphenamate (2, 12 mg) [17], genistein (3, 65 mg) [18] and daidzein (4, 12 mg) [19] were obtained with hexane-EtOAc (9:1, 8.5:2.5, 6.5:3.5 and 5:5), respectively. S1 (5 g) was subjected to a Si gel CC using the mixture of hexane and ethyl acetate of increasing polarity to give asperphenamate (2, 5 mg) and 4',5,7-trihydroxy-8-prenylisoflavone (5, 10 mg) [20] at hexane-EtOAc (8.5:1.5).

Determination of antioxidant activity

The antioxidant activity of MExRW and four isolated compounds was assessed through the evaluation of the radical scavenging activity and reducing power. Solutions of pure compounds (1 mg/mL) and crude extract (3 mg/mL) used for these tests were obtained by dissolving samples in methanol.

Evaluation of radical scavenging activity

The DPPH assay was carried out as described by Nyaa [21] 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. The percentage inhibition was calculated using the formula:

$$IC_{50} = \frac{(Absorbance\,of\,DPPH - Absorbance\,of\,Sample)\,X100}{Absorbance\,of\,DPPH}$$

The concentration of the extract or compound that exhibits 50% inhibition (IC_{50}) was estimated.

Evaluation of reducing power

The reducing power of MEXRW and its chemical constituents was evaluated by determining their ability to reduce iron (III) to iron (II) as described by Oyaizu. [22] Briefly, an aliquot of 1 mL of sample solution (extract or compound) was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution. After 30 min of incubation at 50°C, 2.5 mL of a 10% trichloroacetic acid solution were added and the mixture was subjected to centrifugation at 3000 rpm for 10 min. Finally, 2.5 mL of the collected supernatant was mixed with distilled water (2.5 mL) and 0.5 mL of a solution of $FeCl_3$ (0.1%). After reading the absorbance at 700 nm, the reducing power was expressed as mg of ascorbic acid per mg of sample (mgAAE/mg).

RESULTS AND DISCUSSION

Phytochemistry study

Compound 1 was obtained as white powder. The presence in its ^{1}H NMR spectrum of an ABX system with signals at δ 6.77 (s, H-2'), at δ 6.80 (d, J = 1.3 Hz, H-5') and 6.85 (d, J = 1.6 Hz, H-6') suggested that this compound is a trisubstituted benzenic compound. The singlet at δ_H 5.95 (2H, s) suggested the presence of a methylenedioxy group. Apart from the above signals, others were observed at δ 3.05 (q, H-3), 3.87 (dd, J = 9.1, 3.8 Hz, H-1a), 4.23 (m, H-1b) and 4.71 (d, J = 4.4 Hz, H-3). The ^{1}H-^{1}H COSY spectrum showed clearly the connectivities H_2-3/H-2/H-1 attesting the presence of a propyl group. The HMBC spectrum showed the correlation between H-1, H-2 and C-4 indicating the attachment of the propyl group at C-4 of the aromatic ring. In the ^{13}C NMR spectrum, the signal at δ 101.1 confirmed the presence of the methylenedioxy group. Signals at δ 147.1 (C-4') and 147.5 (C-3') indicate that compound 1 is a benzodioxymethylene derivative. The signals at δ_C 85.8 (C-3), 71.7 (C-1), 54.3 (C-2) are predictive of the C-2/C-3 epoxide and the oxymethylene group (C-1). The HMBC spectrum also showed correlations between the protons at δ_H 5.95 (2H, s) and the carbons at δ_C 147.1 and 147.5 in agreement with the existence of the benzomethylenedioxy group. On the basis of all the NMR data, compound 1 was identified as 3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol previously isolated from *Artemisia vulgaris* L. However, it was not described. Only the ^{13}C NMR data of carbons 1, 2 and 3 were given. [8] This is therefore the first report of that compound in the *Erythrina* genus and its full description.

Compound 2 was isolated as a white powder. Its mass spectrum showed a molecular ion peak at *m/z* 507.22571 corresponding to the molecular formula $C_{32}H_{30}O_4N_2$. The presence in its ^{1}H NMR spectrum (Table 2) of four series of doublets at δ_H 2.91 (dd, J =13.8, 8.2 Hz, H-17a), 2.99 (dd, J = 13.8, 6.4 Hz, H-17b), 3.21 (1H, dd, J =13.8, 7.3 Hz, H-10a) and 3.29 (dd, J =13.8, 6.3 Hz, H-10b); and of two multiplets at 4.92 (q, J = 6.6 Hz, H-3) and at 4.63 (m, H-7) showed that compound 2 is a quasi-symmetrical molecule. Integration of signals between δ_H 7.21 and 7.71 suggested the presence of 20 aromatics protons from four different phenyls groups in this compound. The presence of two signals at δ_H 6.56 (d, H-2), and at 6.65 (d, H-8) which are not present in the HSQC spectrum but showing COSY and HMBC correlations (Table 2) suggested the presence of two secondary amide groups. In the same spectrum, the presence of another series of two doublets at δ_H 4.04 (dd, J =11.3, 4.4, H-6a) and at 4.54 (dd, J =11.3, 3.2, H-6b) confirmed the quasi-symmetrical nature of this compound. In its ^{13}C NMR spectrum, the presence of three signals at 167.2, 167.4 and 171.9 suggested that three carbonyl groups were present. The presence of three methylene groups respectively at δ_C 37.2 (C-17), 37.5 (C-10) and 65.4 (C-6) suggested that the latter is close to the carbonyl ester. Correlations observed in the COSY spectrum between H-17a (δ_H 2.91) and H-7 (δ_H 4.63), H-17b (δ_H 2.99) and H-7 (δ_H 4.29), H-10a (δ_H 3.2) and H-3 (δ_H 4.92), H-10b (δ_H 3.29) and H-3 (δ_H 4.92) and H-7 (δ_H 4.63) and methylene protons H-6a (δ_H 4.04) and H-6b (δ_H 4.54) showed the difference between the two parts of the molecule. HMBC correlations observed between H-10a (δ_H 3.29), H-10b (δ_H 3.21), H-3 (δ_H 4.63) and C-4 (δ_C 171.9); between H-2 (δ_H

6.56) and C-1 (δ_C 167.4) and C-4; and between H-8 (δ_H 6.65) and C-9 (δ_C 167.2) enabled the construction of the central part of the molecule which comprises two amides and one carbonyl ester groups. Moreover, correlations between H-6a (δ_H 4.04) and C-4, C-7 (δ_C 50.3), C-17 (δ_C 37.2) confirmed the quasidimeric nature of compound with the two moieties connected to the ester group (C-4). All these NMR data and those of literature led to the conclusion that compound 2 is asperphenamate which was firstly isolated from *Aspergillus flavus* [23], and then from *Piptadenia gonoacantha* [17] and here isolated for the first time in *Erythrina* genus.

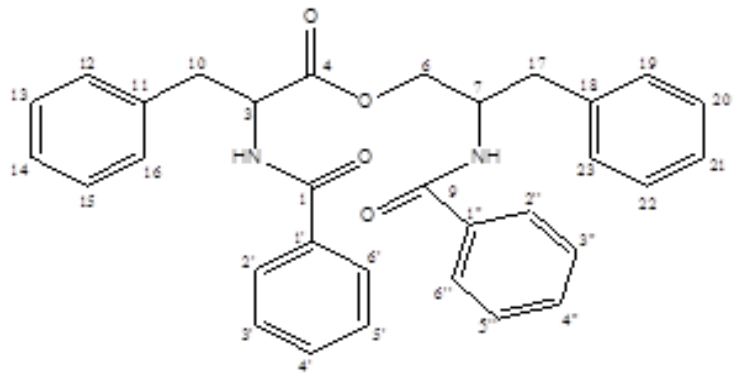
The others compounds isolated from MExRW were genistein (3) previously isolated from *E. indica* [10], daidzein (4) from *E. crista-galli* [11] and 4', 5, 7-trihydroxy-8-prenylisoflavone (5) from *E. variegata.* [12]

Table 1: ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) data of 3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol (1) in $CDCl_3$

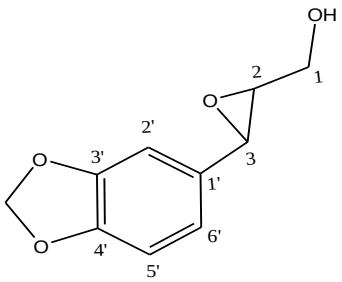
N° carbone	δ_C (mult.)	δ_H (mult. *J* in Hz)	HMBC	COSY
1a	71.7 (t)	4.23 (m)	C-1', C-2,C-3	1b, 2
b		3.87 (dd 9.1, 3.8)	C-1,C-1',C-2	1a, 2
2	54.3 (d)	3.05 (q)	C-1,C-1', C-3	1a, 1b, 3
3	85.8 (d)	4.71 (d, 4.4)	C-1,C-1',C-2,C-2' C-6'	2
1'	135.0 (s)	-	-	
2'	108.5 (d)	6.80 (d, 1.3)	C-1',C-2', C-3	6'
3'	147.1 (s)	-	-	
4'	147.5 (s)	-	-	
5'	106.2 (d)	6.77 (s)	C-1', C-5',	
6'	119.4 (d)	6.85 (d, 1.6)	C-3,C-3', C-4'	2'
-CH_2-O	101.1 (t)	5.95 (s)	C-4',C-5'	

Table 2: ^{13}C- and ^{1}H-NMR data of compound 2 in $CDCl_3$

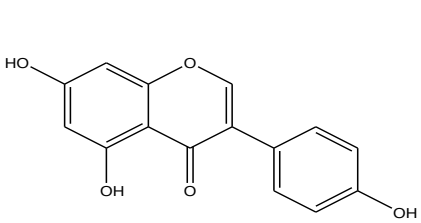
N° carbone	δ_C (mult.)	δ_H (mult., *J* in Hz)	HMBC	COSY
1	167.4 (s)	-	-	-
3	54.5 (d)	4.92 (q, 6.6)	C-1,C-4,C-10,C-11	2, 10a, 10b
4	171.9 (s)	-	-	-
6a	65.4 (t)	4.54 (dd 11.3, 3.2)	C-4,C-7,C-17	6b, 7
b		4.04 (dd 11.3, 4.4)	C-4,C-7,C-17	6a, 7
7	50.3 (d)	4.63 (m)	C-9, C-18	6a, 6b, 8, 17a, 17b
9	167.2 (s)	-	-	-
10a	37.5 (t)	3.29 (dd 13.8, 6.3)	C-3, C-4,C-12	2, 10b
b		3.21 (dd 13.8, 7.3)	C-3, C-4,C-12	2, 10a
11	135.7 (s)	-	-	-
12	128.9 (d)	7.25 (ov)		13
13	128.7 (d)	7.25 (ov)		12, 14
14	127 (d)	7.29 (ov)		13, 15
15	128.7 (d)	7.25 (ov)		14, 16
16	128.9 (d)	7.25 (ov)		15
17a	37.2 (t)	2.99 (dd 13.8, 6.4)	C-6,C-7,C-18,C-19	8, 17b
b		2.91 (dd 13.8, 8.2)	C-6,C-7, C-18, C-19	8, 17a
18	137.1 (s)	-	-	-
19	129.3 (d)	7.48 (s)		20
20	127 (d)	7.21 (ov)		19, 21
21	126.8 (d)	7.31 (ov)		20, 22
22	127 (d)	7.21 (ov)		21, 23
23	129.3 (d)	7.48 (s)		22
1'	133.3 (s)	-	-	-
2'	128.4 (d)	7.71 (d, 0.9)	C-1, C-3',C-4'	3'
3'	127.1 (d)	7.43 (tt 7.8, 1.3)		2', 4'
4'	132.4 (d)	7.50 (tt 7.8, 1.2)		3', 5'
5'	127.1 (d)	7.43 (tt 7.5, 1.3)		4', 6'
6'	128.4 (d)	7.71 (dd 8.2, 1.2)	C-1,C-4',C-5'	5'
1''	134.2 (s)	-	-	-
2''	127.4 (d)	7.66 (dd, 8.8, 1.3)	C-3'',C-4'', C-9	3''
3''	129.2 (d)	7.33 (ov)		2'', 4''
4''	131.4 (s)	7.43 (tt 7.5, 1.3)		3'', 5''
5''	129.2 (d)	7.33 (ov)		4'', 6''
6''	127.4 (d)	7.66 (dd, 8.8, 1.3)	C-4'',C-5'', C-9	5''



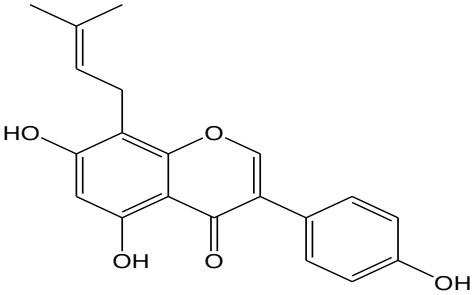
asperphenamate



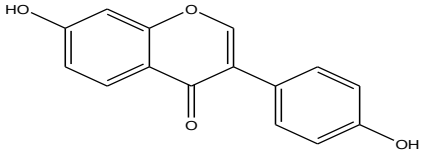
3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol



genistein



4',5,7-trihydroxy-8-prenylisoflavone



daidzein

Antioxidant activity

Antioxidant activities of MExRW and of compounds 1-3 and 5 were assessed using the DPPH scavenging antiradical and ferric reduction power methods. As reported in Table 3, MExRW exhibited moderately a DPPH free radical scavenging activity with an IC_{50} of 4.32 mg/ml. genistein 3 is the more active among the compounds isolated from this extract with IC_{50} of 1.96 mg/ml. Table 3 also represents the reducing power of MExRW and the isolated compounds. It can be noticed that as for reducing power, compound 3 possesses the highest reducing power with a value of 0.24 ± 0.02 mgAAE/mg and it is followed by compound 2 (0.21 ± 0.01 mgAAE/mg). It is worthy to mention that compound 1 is more powerful in reducing power than compound 1 and that is reverse concerning radical scavenging activity.

The reducing power is based on the ability of some products to create bonds with a metal. The little difference observed between the activity of compounds 1 and 3 could be due to the fact that compound 1 contains more oxygen atoms compared to compound 3. The isolation of 3-(3',4'-methelenedioxyphenyl)-2,3-epoxypropanol (1) and of asperphenamate (2) for the first time in the genus *Erythrina* shows that *Erythrina* plants are rich in various secondary metabolites.

Table 3: Antioxidant activities of MEXRW and its isolated compounds

Samples	DPPH radical scavenging activity (IC_{50} en mg/ml)	FRAP (mgAAE/mg)
Compound 1	3.31	0.21 ± 0.01
Compound 2	3.14	0.14 ± 0.02
Compound 3	1.96	0.24 ± 0.02
Compound 4	3.41	0.17 ± 0.01
MEXRW	4.32	0.08 ± 0.01
Ascorbic acid	0.06	-

ACKNOWLEDGEMENTS

The authors are grateful to the "Fonds National de la Recherche Scientifique (FNRS) Belge" for a fellowship to ATT.at the Laboratory of Pharmacognosy, University of Liege, Belgium.

REFERENCES

1. Hua-Bin L, Ka-Wing C, Chi-Chun W, King-Wai F, Feng C, Yue J. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007; 102: 771-776.
2. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239-247.
3. Madhavi DL, Deshpande SS, Salunkhe DK. Food antioxidants: Technological, toxicological. Health persp. 1996. New York: Marcel Dekker.
4. Eber-Hardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apples. Nature 2000; 405: 903-904.
5. Gey KF. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. Bioch Soc Trans. 1990; 18: 1041-1045.
6. Willett WC. Micronutrients and cancer risk. Amer J Clin Nutri. 1991; 53: 265S-269S.
7. Ito N, Hirose M, Fukushima S, Tsuda H, Shirai T, Tatematsu M. Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem Toxicol. 1986; 24: 1071-1082.
8. Safer AM, Al-Nughamish AJ. Hepatotoxicity induced by the antioxidant food additive butylated hydroxyl toluene (BTH) in rats: An electron microscopical study. Histol Histopath. 1999; 14: 391-406.
9. Majinda RTR, Cornelius CWW, Bernard FJ. Bioactive non-alkaloidal constituents from the genus *Erythrina*. Studies in Natural Products Chemistry 2005; 32: 821-853.
10. Yenesew A, Induli M, Derese S, Midiwo JO, Heydenriich M, Peter MG, Akala H, Wangui J, Liyala P, Waters MC. Antiplasmodial flavonoids from the stem bark of *Erythrina abyssinica*. Phytochemistry 2004; 65: 3029-3032.
11. Chacha M, Gomostang BM, Majinda RRT. Antimicrobial and radical scavenging flavonoids from the stem wood of *Erythrina latissima*. Phytochemistry 2005; 66: 99-104.
12. Rukachaisirikul T, Innok P, Aroonreck N, Boonamnuaylap W, Limrangsun S, Boonyon C, Woonjina U, Suksamrarn A. Antibacterial pterocarpans from *Erythrina subumbrans*. J Ethnopharmacol. 2007; 110: 171-175.
13. Sokeng SD, Talla E, Jeweldai V, Yaya AJG, Koube J, Dongmo F, Goulime M, Mbafor JT. Anti-inflammatory effect of abyssinone V-4'-methylether on acute and chronic inflammation models. Hygea. J. Drugs. Med. 2013; 5: 121-128.
14. Njamen D, Talla E, Mbafor JT, Fomum ZT, Kamanyi A, Mbanya JC, Cerdà-Nicilàs M, Giner RM, Recio MC, Rios JL. Anti-inflammatory activity of erycristagallin, a pterocarpene from *Erythrina mildbraedii*. Europ J Pharmacol. 2003; 468: 67-74.
15. Harris DJM. The vascular plants of the Dzanga-Sangha reserve, Central African Republic. National Botanic Garden of Belgium. 2002.
16. Xenia TT, Florecita de Guzman TVF. Anna Ma. Phytochemical analysis and hemodynamic actions of *Artemisia vulgaris* L. Clin Hemor Microcircul. 2000; 23:167-175.
17. Mário GC, Maritza ARC. Francisco EACJ, Acácio GC. Chemical constituents of *Piptadenia gonoacantha* (Mart.) J.F. Macbr (paujacaré). Acad Bras Ciênc 2010; 82: 561-567.
18. Nkengfack AE, Azebaze AGB, Waffo AK, Fomum ZT, Meyer M, van Heeden FR. Cytotoxic isoflavones from *Erythrina indica*. Phytochemistry 2001; 58: 1113-1120.
19. Flavia R, Maria LC, Daniela W, Fernando R, Timm A, Virginia M. Antimicrobila isoflavonoids from *Erythrina crista-galli* infected with *Phomopsis sp*. Z. Naturforsch. 2007; 164-168.
20. Mohammed ZR, Rahman MS, Kaisar A, Hossain A, Rashid MA. Bioactive isoflavones from *Erythrina variegata*. L. Turk J Pharm Sci. 2010; 7: 21-28.
21. Nyaa TLB, Barboni L, Tapondjou L, Tamokou J-D, Kuiate J-R, Tane P, Park HJ. NMR assignment and antimicrobial/antioxidant activities of 1β-hydroxyeuscaphic acid from seeds of *Butyrospermum parkii*. Nat Prod Sci. 2009; 15:76-82.
22. Oyaizu M. Studies of products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jap J Nutr.1986; 44: 307-315.
23. Clark AM, Hufford CD, Robertson LW. Two metabolites from *Aspergillus flavipes*. Lloydia. 1977; 40: 146-151.