Research Article

Antiprotozoal Activities of Methanolic Extracts and Three Diterpenoids Isolated from the Root Barks of *Erythrophleum ivorense* A. Chev. (Fabaceae)

Dibi Jacques Konan[1], Amon Diane Marina N'tamon[2], Kouassi Blaise Kouadio[3], Ahmont Landry Claude Kablan[4]*, Aka Faustin Kabran[5], Koffi Barthélemy Attioua[5], Janat Akhanovna Mamyrbékova-Békro[1], Yves-Alain Békro[1]

[1]Laboratoire de Chimie Bio-Organique et de Substances Naturelles (LCBOSN), UFR-SFA, Université NANGUI ABROGOUA, 02 BP 801 Abidjan 02, Côte d'Ivoire.
[2]Département de Chimie Analytique, Minérale et Générale, Technologie Alimentaire, UFR Sciences Pharmaceutiques et Biologiques, Université Félix HOUPHOUËT-BOIGNY, 06 BP 2256 Abidjan 06, Côte d'Ivoire.
[3]Université Jean Lorougnon GUEDE, Laboratoire de Sciences et Technologies de l'Environnement, UFR Environnement, Daloa, BP 150 Daloa, Côte d'Ivoire.
[4]Département de Mathématiques-Physique-Chimie, UPR de Chimie organique, UFR des Sciences Biologiques, Université Peleforo GON COULIBALY, BP 1328 Korhogo, Côte d'Ivoire.
[5]Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix HOUPHOUËT-BOIGNY, 22 BP 582 Abidjan 22, Côte d'Ivoire.

***Corresponding author's E-mail:** kablanahmont@yahoo.fr

Received: 05-07-2024; **Revised:** 30-09-2024; **Accepted:** 08-10-2024; **Published on:** 15-10-2024.

ABSTRACT

Cutaneous leihmaniasis and human African trypanosomiasis are two neglected tropical diseases. Leishmania and Trypanosomes species are protozoan parasites that affect millions of people worldwide. In the absence of adequate treatment, African trypanosomiasis and cutaneous leishmaniasis are fatal. Available drugs are severely limited by their toxicity, marginal efficacy, the need for parenteral administration and the spread of drug resistance. The genus Erythrophleum is rich in cassaine-type diterpenoid. These compounds have already shown leishmanicidal and trypanicidal activity. The aim of this study was to isolate cassane-type diterpenoids from *Erythrophleum ivorense* and evaluate them against against *Leishmania donovani* (promastigote) and *Trypanosoma brucei brucei* (Trymastigote). Three Cassaine-type diterpenoid amines namely 6α-hydroxy-norcassamine (1), Erythrophleguine (2), and nor-cassamine (3) were isolated from the methanolic extracts of the root barks; and their structures were carried out using NMR, UV and MS spectral. Cold methanolic root bark extract (CMex), Reflux methanolic root bark extract (RMex) and isolated compounds (1, 2, 3) showed moderate activities against *Leishmania donovani* (promastigote) and *Trypanosoma brucei brucei* (Trymastigote); comparing their values with those of the reference molecules (Pentamidine and Melarsopol).

Keywords: Fabaceae, *Erythrophleum ivorense*, Cassaine-type diterpenoid, antiprotozoal.

INTRODUCTION

Neglected tropical diseases (NTDs) are a diverse group of conditions that are mainly prevalent in tropical areas, where they thrive among people living in impoverished communities. They are caused by a variety of pathogens including viruses, bacteria, parasites, fungi and toxins, and are responsible for devastating health, social and economic consequences. Malaria has been considered a public health problem in many African countries for decades, due to inadequate treatment and the resistance developed by the parasite[1, 2]. Thus, the search for new antiprotozoal drugs remains a major challenge for all. Traditionally, African peoples have a custom of treating their ailments using natural substances, particularly medicinal plants[2, 3]. This is the case of plant species of *Erythrophleum ivorense*, which is exploited in traditional medicine to traite malaria, and as emetics, insect repellents, laxatives, anthelmintics, convulsions, smallpox, pain, swelling and wounds [4,5,6]. Concerning the chemical constuents of *E. ivorense*, somme studies have reported the presence of nitrogen compounds such as cassain, erythrophlein, coumarins, flavonoids, saponins, anthracenosides, cardiac glycosides, sterols, tannins and terpenoids[7,8,5,6]. As for its biological properties, many studies reported the antifungal, antioxidant, antischistosomal, antitrypanosomal, anticonvulsant, antibacterial, sedative, antigiardial, anti-inflammatory, antileishmanial, insecticidal and cytotoxic activities[9,10,11,12]. This study was therefore carried out to isolate amine-functional cassane-type diterpenoids from *E. ivorense* root barks and evaluate their antiprotozoal activities against (Promastigotes) and *Trypanosoma brucei brucei* (Trypomastigotes). To do this, Cold methanolic root bark extract (CMex), Reflux methanolic root bark extract (RMex) and isolated compounds were evaluated against *Leishmania donovani* (Promastigotes) and *Trypanosoma brucei brucei* (Trypomastigotes), using Pentamidine and Melarsopol as reference molecules.

MATERIALS AND METHODS

Plant material

The plant material consists of dried powder of E. ivorense root bark, collected in the Alepé region (Côte d'Ivoire). A specimen of the plant is registered under the number n°8 DIBI EI-2014 to the ''Centre National de Floristique'' of ''Université Félix HOUPHOUËT-BOIGNY'' (Côte d'Ivoire).

Phytochemical study

1. *General experimental procedures*

For the initial purifications, Grace silica cartridges of 24 g and 120 g were used for flash chromatography. Isolation of

compounds were carried out using HPLC-Preparative equipped with of an C18 analytical column. Agilent Scientific Instruments (Agilent 1260 Infinity HPLC chain coupled to an Agilent 6530 Q-TOF-MS) were used for spectral analyses. All solvents used were from Sigma-Aldrich. The measurement of the optical rotation was carried out at a temperature of 25°C. A Bruker AM-400 NMR spectrometer (400 MHz) was used for the NMR spectra; CD_3OD was used as a reference solvent.

2. Extraction and isolation of compounds 1, 2 and 3

The dried root bark of *E. ivorense* was pulverized to obtain 1000 g of powder, which was divided into two parts. The first one (500 g) was extracted at reflux with 1000 mL of methanol to give Reflux Methanolic root bark extract (RMex).

The second one (500 g) was extracted by cold maceration in methanol (500 mL) three times in a row. The solvents of the three collected extracts were then completely removed to give Cold methanolic extract of root bark of E. suaveolense (CMex). A part of CMex (25 g) was dissolved in the mixture water/methanol (9 :1), filtered on Whatman paper to afford 100 mL hydro-alcohol solution, which has been alkalinizer to pH 9 by ammoniaque (NH_4OH), acidified to pH 2 by sulfuric acid (H_2SO_4), then extracted four time with methylenchlorid (CH_2Cl_2, 4x1O0 mL). The pH of the acidic aqueous solution is brought to 9 then extracted four time with ethyl acetate (AcOEt, 4x100 mL) to give, after solvent evaporation, 5 g of extract. The ethyl acetate extract was first roughed up on a silica gel chromatographic column, then the fractions which presented a good profile were fractionated on a Sephadex® LH-20 column, finally purrified by the semi-preparative HPLC method. This purification allowed the isolation of three molecules. The first compound was obtained at a retention time of 11.36 min (**1**), the second at a retention time of 12.36 min (**2**) and the last at a retention time of 14.50 min (**3**).

Biological tests

1. In vitro antileishmanial test

The antileishmanial activities of extracts and pure molecules was evaluated in vitro against the L. donovani LV9 strain (MHOM/ET/1967/L82). The protocol was based on the tetrazolium dye, allowing measurement of mitochondrial dehydrogenase activity, and therefore determination of an EC50 value. This value corresponds to the 50% reduction in mitochondrial activity described by Abada et al[13]. Promastigotes were cultured in RPMI 1640 medium buffered with HEPES (25 mM). The medium was then supplemented with 40 mg/ml gentamicin and 10% fetal calf serum (FCS) at 27°C in the dark. Screening was performed in flat-bottomed 96-well plastic tissue culture plates and maintained at 27°C. Tested samples were dissolved in dimethyl sulphoxide and diluted with RPMI to reduce the final solvent concentration to less than 2% (v/v) and obtain different concentrations down to 0.08 mg/L. Each concentration was tested three times. Promastigote forms from a culture in the exponential growth phase (approximately 72 hours after culture) were captured. In each well, a volume of 195µL of the suspension of parasites at the promastigote stage corresponding to a density of 2.105 was used, then incubated in an oven at 27° C for 1 h before adding the drug. After this incubation, each test sample was added at a rate of 5 µL per well for 4 hours. Three wells were used for each concentration and the tests were performed three times. Evaluation of antileishmanial activity, expressed as EC_{50}, was carried out by two methods. The first method is a direct reading under the light microscope and by comparison with negative controls. This qualitative test assesses the vitality of the leishmania in each well on the basis of three criteria : (1) the number of parasites present in each well compared with the controls, (2) the mobility of the parasites and (3) the shape of the cells. The second method or Microculture Tetrazolium Assay (MTA) is a quantitative colorimetric test based on the use of tetrazolium salts. Promastigote stability was tested using the tetrazolium dye (MTT) colorimetric method. Results are expressed as an effective concentration (EC) inhibiting parasite growth by 50% (EC_{50}) after an incubation period of 3 days. The reference molecule used was pentamidine.

2. In vitro trypanocidal test

The leishmanicidal activity was tested at the Laboratoire de Chimiothérapie Antiparasitaire de la Faculté de Pharmacie de Châtenay-Malabry (Université Paris Saclay). The strain used was *Trypanosoma brucei brucei* strain GVR 35 (Glascow Veterinary Research). It was frozen in liquid nitrogen. The parasites were diluted in culture medium to obtain 200,000 trypomastigotes per ml. Circulating forms of the parasite were cultured in vitro without loss of infectivity for 24 hours at 37°C in an atmosphere containing 5% CO_2. The culture medium consisted of β-mercaptoethanol (0.2 mM): 1.4 µL; hypoxanthine: 1.36 mg; thymidine: 0.387 mg; sodium pyruvate: 22 mg; Hepes: 650 mg; glucose: 100 mg; $NaHCO_3$: 220 mg; depleted horse serum: 15 mL; gentamicin: 5 mg; "MEM (Minimum Essential Medium) non-essential amino acids": 1 mL; "MEM with Earle salts" and L-glutamine: qsp 100 mL. Parasites were distributed in a 200 µL 96-well plate at a rate of 2.105 per mL. Next, 5 µL of the appropriate dilution of the samples in DMSO was added, with each concentration tested in triplicate. The control wells received only DMSO (5 µL, i.e. 2.5%). After 24 hours of incubation, direct observation under a light microscope was used to estimate trypanosome viability. Experiments were performed in triplicate and repeated in triplicate. Test results were expressed as the lethal concentration killing all parasites in the wells after 24 hours (LC100, lethal concentration) after microscopic observation. The reference drug used was melarsoprol[14].

RESULTS AND DISCUSSION

Identification of compounds 1,2 and 3

Reflux extraction in methanol of 500 g of powdered root bark of *E. ivorense* gave 27.8 g of crude extract named RMex. Extraction by maceration in methanol of the same quantity of powdered root bark of *E. ivorense* gave 25.6 g of crude extract named CMex. The fractionnation of CMex by chromatography method gave three compounds which were identified as 6*α*-hydroxy-*nor*-cassamine (**1**; 5.2 mg)[15,16], erythrophleguine (**3**; 12.2 mg) [17,18] and *nor*-cassamine (**3**; 7,2 mg)[15,20] (**Figure 1**). The structures of these compounds were determined from their spectral data as follows.

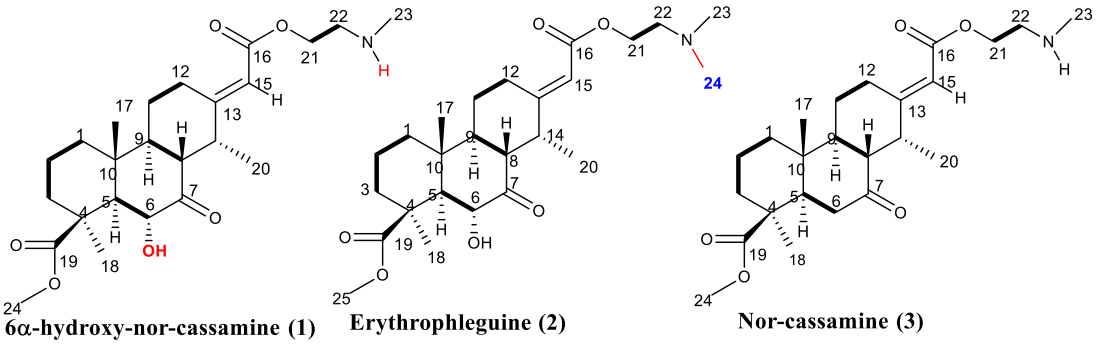

Figure 1: Cassane diterpenoids amines isolated from the root barks of *E. ivorense* (**1-3**).

Table 1: ^{1}H and ^{13}C NMR spectral data for compounds **1-3** (in CD_3OD)

	1		2		3	
	δ_H, m (*J* in Hz)	δc	δ_H, m (*J* in Hz)	δc	δ_H, m (*J* in Hz)	δc
1	1.04 m ; 1.78 m	39.7	1.03 m ; 1.81 *m*	39.9	1.09 m ; 1.94 m	40.1
2	1.38 m; 1.64 m	18.9	1.46 m ; 1.60 m	19.3	1.55 m ; 2.04 m	20.4
3	1.12 m; 2.11m	39.3	1.06 m ; 2.9 m	39.7	1.19 m ; 2.26 m	38.8
4		45.0	-	45.3	-	45.0
5	1.34 d (12.4)	58.3	1.32 d (12.0)	58.6	1.58 dd(3.4, 15.1)	54.7
6	4.46 dd (1.4, 13.6)	75.6	4.71 d (12.0)	75.9	2.65 m ; 2.95 m	41.5
7		209.8	-	210.2	-	211.9
8	2.38 dd (2.6, 12.8)	50.6	2.35 ; dd (11.6, 1.6)	51.4	2.36 dd; (3.9, 12.8)	54.7
9	1.66 m	46.5	1.65 m	47.1	1.78 dt (7.0, 17.0)	48.6
10		37.5	-	37.8	-	37.8
11	1.09 m ; 1.96 m	27.0	1.20 m ; 1.97 m	27.3	1.16 m; 2.06 m	28.3
12	2.00 m ; 3.70 m	23.6	2.00 m ; 3.73 m	23.8	2.09 m ; 3.78 m	25.1
13		165.9	-	164.9	-	167.3
14	2.89 m	39.7	2.91 m	39.5	3.01 m	40.1
15	5.72 s	111.5	5.70 s	113.3	5.81 s	112.8
16		167.8	-	166.7	-	169.5
17	0.80 s	12.8	0.83 s	12.3	0.84 s	12.5
18	1.33 s	30.7	1.40 s	31.6	1.19 s	28.5
19		177.7	-	177.4	-	178.6
20	1.05 d (6.9)	14.0	1.08 d (6,8)	15.2	1.11 d (6.8)	15.4
21	4.24 m	58.6	4.12 t (6.0)	61.5	4.36 m	60.0
22	3.20 m	47.8	2.57 t (6.0)	46.5	3.67 m	49.1
23	2.63 s	32.5	2.23 s	45.7	2.74 s	33.9
24	3.59 s	51.5	2.23 s	45.7	3.67 s	52.0
25	-	-	3.66 s	51.7	-	-

Compound 1, $[\alpha]_D^{25}$ -33.3, yellow color oil, soluble in methanol. High-resolution HR-ESI-MS mass spectrum shows the molecular ion at *m/z* 436.2714 $[M+H]^+$, which agrees with the molecular formula $C_{24}H_{37}NO_6$. The absorption bands at 1649, 1702 and 1718 cm^{-1}, on the IR spectrum, are characteristic of carbonyl functional groups. Study of ^{1}H NMR spectrum (**Table I**) shows signal resonance of proton carried by unsaturated carbons (C=C) at δH 5.72 (H-15, s), methyl groups at δH 1.33 (H-18, s) and 0.80 (H-20, s), one methoxyl at δH 3.59 (H-24, s) and a secondary amine (N-CH_3) which resonates at δH 2.63 (H-23). Analysis of the ^{13}C NMR spectral data (**Table I**) revealed the presence of

signals at 177.7 (C-19) for the ⍰⍰⍰-unsaturated ester and 209.8 (C-7) for the carbonyl of an oxo groupe and at δC 167.8 (C-16) for the carbonyl at position 16. The values of two visible carbons at δC 165.9 (C-13) and 111.5 (C-15) are attributable to olefinic carbons. Taken together, these spectral data allow us to identify **1** to the know compound 6α-hydroxy-*nor*-cassamine (**1**)[15] (**Figure 1**); it was isolated by Dade *et al.* (**2015**)[15] from the stem barks of *E. suaveolens* and and Ha *et al.* (**2017**)[21] from *E. fordii*.

Compound 2, $[\alpha]_D^{24}$ - 31.3, appeared as an colorless powder. The HR-ESIMS spectrum gave the molecular ion peak at m/z 450.2862 $[M+H]^+$ which corresponds to the chemical formula $C_{25}H_{39}NO_6$. The IR absorption bands at 3340, 1716, 1701, 1649 and 1258 cm^{-1} indicate the presence of OH (hydroxyl), -CO-O- (ester) and tertiary amine respectively. The presence of these functions is confirmed by ^1H and ^{13}C NMR spectral data. Indeed, proton resonances at δH 4.7 (H-6) and carbons signals at δC 75.9 (C-6), 210.2 (C-7) and 45.7 [(C-23) and (C-24)] confirme this. The compounds **2** and **1** have largely identical chemical shifts, except those at δH (2.33 and 3.66), and at δC 45.7 and 51.7) indicating the presence an N, N dimethyl group ($N(CH_3)_2$) on the carbon chain of compound **2;** this allowed it to be identified at the *erythrophleguine* (**2**) (**Figure 1**), a know cassanamine previously isolated from a *E. ivorense* and *E. suaveolens*[21].

Compound 3, $[\alpha]_D^{25}$ - 76.9, solid form. HR-ESIMS spectrum gives molecular ion peak at *m/z* 420.2735 $[M+H]^+$ which agrees with formula $C_{24}H_{37}NO_5$. Infrared absorption bands at 1726, 1701, 1702 and 1648 cm^{-1} are attribuable to carbonyl groups. The methyls (CH_3) and methylenes (CH_2) groups can also be observed at υ_{max} 2991; 2944 and 2931 cm^{-1}. Comparing NMR (proton and carbon) spectral data of compounds **3** and **1**, it seems that they are very similar, with one exception, that of the absence of the alcohol function signal in compound **3**. Based on this information, compound **3** was identified as a molecule known as nor-cassamine (**3**) (**Figure 1**). Indeed, nor-cassamine (**3**) is already known in several plant species of the Erythrophleum genus: *E. chlorostachys* stem bark[20] and *E. suaveolens* root bark[16].

Antileishmanial and antitrypanocidal activities

The antimalarial activities of crude methanolic extracts of *E. ivorense* root bark (CMex and RMex) and isolated compounds (**1,2,3**) were evaluated against *Leishmania donovani* (Promastigotes) and *Trypanosoma brucei brucei* (Trypomastigotes) parasites. Compared to the reference molecules (Pentamidine and Mélarsopol (Mel W), the observed activities are moderate overall (**Table II**). The highest activities are obtained with *6α*-hydroxy-norcassamine (**1**) with EC_{50} value of 87.36 ⍰M and LC_{50} value of 143.68 ⍰M against *Leishmania donovani* and *Trypanosoma brucei brucei* respectively. The results also show that cold and reflux methanolic extracts have the same effect on antimalarial activity.

Table 2: Antileishmanial and antitrypanocidal activities of extracts, isolated compounds (**1-3)** and reference drug against *Leishmania donovani* and *Trypanosoma brucei brucei*

Extracts and compounds tested	*Leishmania donovani* (Promastigote)		*Trypanosoma brucei brucei* (Trymastigote)	
	EC_{50} (mg/L)	**EC_{50} (M)**	**LC_{100} (mg/L)**	**LC_{100} (M)**
CMex	> 125	-	> 125	-
RMex	> 125	-	> 125	-
6α-hydroxy-norcassamine (**1**)	125	87.36	62.5	143.68
Erythrophleguine (**2**)	> 125	> 299,76	> 125	> 278.40
nor-cassamine (**3**)	90.9	219.95	62.5	149.16
Pentamidine	**2,6**	**7,7**	**-**	**-**
Mélarsopol (Mel W)	**-**	**-**	**0,2**	**0,4**

CMex: Cold methanol extract of root bark of *E. ivorense*; **RMex**: Reflux methanol extract of root bark of *E. ivorense*

CONCLUSION

In order to identify the constituents responsible for the antiprotozoal properties of *E. ivorense* roots, methanolic extracts of this organ were obtained by cold maceration (RMex) and reflux (RMex). The phytochemical study of the extract obtained by cold maceration in methanol (CMex) led to the isolation of three nitrogenous ditermpenoids : 6α-hydroxy-norcassamine (**1**), Erythrophleguine (**2**), and *nor*-cassamine (**3**). Antiprotozoal tests show moderate activity with the isolated molecules; the best being obtained with 6α-hydroxy-norcassamine (**1**). Although the observed activities are low, this study justifies the traditional use of *E. ivorense* as an antiprotozoal.

Declaration of Competing Interest

There is no conflict of interest for this work.

Acknowledgment: The authors wish to thank Dr Bories Christian and Prof. Loiseau M Philippe for antiprotozoal activities. This work was financially supported by PASRES (Programme d'Appui Stratégique à la Recherche

Scientifique) and ASCAD (Académie des Sciences, des Arts, des Cultures d'Afrique et des *Diasporas africaines*).

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Blum, J.A., Neumayr, A.L., Hatz, C.F. Human African trypanosomiasis in endemic populations and travellers. *European Journal of Clinical Microbiology & Infectious Diseases*, 2012;31(6):905–13.
2. Blum, J., Buffet, P., Visser, L., Harms, G., Bailey, M.S., Caumes, E. Leish Man recommendations for treatment of cutaneous and mucosal leishmaniasis in travelers, 2014. J. Travel. Med., 2014;21(2):116–29.
3. Clare, H., Karen, B., Ziyin W., Ross, M., Samuel, D., Jack, D.S., Richard J. W. *Molecular and Biochemical Parasitology,* 230, 2019, 24-36.
4. Cronund. A, Sandberg. F. New alkaloids from the bark of *Erythrophleum ivorense*, *ActaPharm. Suec*, 1971, 8 351-360.
5. Armah, F.A., Annan, K., Mensah, A.Y., Amponsah, I.K., Tocher, D.A., Habtemariam S. Erythroivorensin: A novel anti-inflammatory diterpene from the root-bark of *Erythrophleum ivorense* (A Chev). *Fitoterapia*, 2015;105:37- 42.
6. Maroyi, A**.** *Erythrophleum ivorense*: a synthesis and review of its chemistry, *pharmacology and medicinal potential, J. Pharm. Sci. & Res*, 2019;11(11):3675-3680.
7. Cronlund, A. Two new alkaloids from the bark of *Erythrophleum ivoirense*. *Acta Pharm. Suec*, 1973a;10:507–514.
8. Cronlund, A. New alkaloids from *Erythrophleum* species. *Planta Med*, 1973b;24:371-374.
9. Tsao, C.C., Shen, Y.C, Su, C.R, Li, C.Y, Liou, M.J, Dung, N.X, Wu, T.S. New diterpenoids and the bioactivity of *Erythrophleum fordii*. *Bioorg. Med. Chem*, 2008;16: 9867-9870.
10. Ngounou, F.N., Manfouo, R.N., Tapondjou, L.A., Lontsi, D., Kuete, V., Pénal V., Etoa, F.X, Dubois, M.A.L., Sondengam, B.L. Antimicrobial diterpenoid alkaloids from *Erythrophleum suaveolens* (Guill. & Perr.) Brenan. *Bull. Chem. Soc. Ethiop*, 2005;2:221-226.
11. Hung TM, Cuong TD, Kim JA. Cassaine diterpenpene alkaloids from Erythrophleum fordii and their anti-angiogenic effect. *Bioorg Med Chem Lett,* 2014a;24:168-172. https://doi.org/10.1016/j.bmcl.2013.11.048
12. Verotta, L, Aburjai, T., Rogers, C.B., Dorigo, P., Maragno, I., Fraccarollo, D., Santostasi, G., Gaion, R. M, Floreani, M., Carpenedo, F. Chemical and pharmacological characterization of *Erythrophleum lasianthum* alkaloids. *Planta Med*, 1995;61:271-274.
13. Abada, Z., Cojean S., Pomel, S., Ferrié, L., Akagah, B., Lormier, A.T. Synthesis and antiprotozoal activity of original porphyrin precursors and derivatives. *Eur. J. Med. Chem*, 2013;67(12):158-65.
14. Guerisson Bairy, Cyrille Oliver Ozzin-Kholy Zolipou and Romaric Nzoumbou-Boko. In vitro trypanocidal activity of extracts and compounds isolated from *Vitellaria paradoxa*. *BMC complement. med. Ther*, 2023;23:346-53. https://doi.org/10.1186/s12906-023-04175-6
15. Dade J.M, Kablan L.A., Say M., Okpekon T.A., Yapo Kicho D., Komlaga G., Boti J.B, Koffi A.P, Guei L.E, Djakouré L.A, Champy P. Cassane diterpenoids stem bark of *Erythrophleum suaveolens* [(Guill. et Perr.) Brenan]. *Phytochem. Lett.,* 2015;12:224-231.
16. Konan, J.D., Attioua, B.K., Kablan, C.L.A., Kabran, F.A., Koffi, P.A., Any-Grah, S.A., Sissouma, D., Seon-Meniel, B., LeBlanc, K., Jullian, J.-C., Beniddir, M.A. New cassane diterpenoids from the root bark of *Erythrophleum suaveolens. Phytochem. Lett*. 2019b;31:166-169.
17. Kablan L.A., Dade J.M.E.., Say M., Okpekon T.A., Yapo K.D., Ouffoue S.K., Koffi A.P., Retailleau P., Champy P. Four new cassane diterpenoid amides from*Erythrophleum suaveolens* [(Guill. etPerr.), Brenan]. *Phytochem. Lett.* 2014, 10, 60-64.
18. Qu, J, Wang JH., Li JB. Rapid structural determination of new trace cassaine-type diterpenoid amides in fractions from *Erythrophleum fordii* by liquid chromatography diode-array detection/electro spray ionizationt and emmass spectroscopy and liquid chromatography/nuclear magnetic resonance. *Rapid Commun Mass Spectrom*., 2007;21:2109-2119
19. Loder, J.W., Culvenor, C.C.J., Nearn, R.H., Russell, G.B., Stanton, D.W. Isolation of *nor*- cassamide and authentic *nor*-cassamidine from *Erythrophleum chlorostachys*. Structural revision of the *nor*-cassamidine, *nor*-cassamine, *nor*-cassamine, *nor*-erythrosuamine and deshydro-*nor*-erythrosuamine. *Tetrahedron Lett.,* 1972**;**50:5069-5072.
20. Ha, M.T. ; Tran, M.H. ; Phuong, T.T. ; Kim, J.A. ; Woo, M.H. ; Choi, J.S. ; Lee, S. ; Lee, J.H. ; Lee, H.K. ; Min, B.S**.** Cytotoxic and apoptosis-inducing activities against humanlung cancer celllines of cassaine diterpenoids from the bark of *Erythrophleum fordii. Bioorg. Med. Chem. Lett*., 2017;27: 2946 -2952.
21. Armah, F.A, Amponsah I.K, Mensah, A.Y., Dickson, R.A., Steenkamp, P.A, Madala N. and Adokoh, C.K. Leishmanicidal activity of the Root Bark of *Erythrophleum ivorense* (Fabaceae) and Identification of some of its compounds by Ultra Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry (UPLC-QTOF-MS/MS). J. Ethnopharmacol., 2017, 17, 378-8741.