ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 10:3 (2016) 287-293

records of natural products

Antiplasmodial Activity and Cytotoxicity of Isolated Compound from the Stem Bark of *Anthocleista liebrechtsiana*

Theodora K. Kowa[1,2], Denis Zofou[3], Roukayatou Mbouangouere[1], Michel F. Tala[1,4], Hippolyte K. Wabo[1], Ning-Hua Tan[4*], Vincent P.K. Titanji[3] and Pierre Tane[1*]

[1] *Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon*
[2] *Institute of Medical Research and Medicinal Plants Studies (IMPM), P.O. Box 6163, Yaounde, Cameroon*
[3] *Biotechnology Unit, University of Buea, P.O. Box 63, Buea, Cameroon*
[4] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, P.R. China*

(Received December 29, 2014; Revised December 29, 2014; Accepted March 28, 2015)

Abstract: One new cerebroside derivative, namely liebrechtsianoside A (**1**), along with five known compounds: tetracosanoic acid (**2**), swertiaperennin (**3**), decussatin (**4**), swertianin (**5**) and *β*-sitosterol glucoside (**6**) were isolated from the the stem bark of *Anthocleista liebrechtsiana*. Their structures were elucidated by interpretation of NMR and MS data, and by comparison of these data with those reported in literature. Compound **1** showed the highest antiplasmodial activity against Dd2 chloroquine-resistant strain of *Plasmodium falciparum*.

Keywords: *Anthocleista liebrechtsiana*; Loganiaceae; cerebroside; structure elucidation; antiplasmodial activity.

1. Introduction

Anthocleista liebrechtsiana De Wild & T. Durand. (Loganiaceae) is a small tree or shrub that occurs from East Ghana to Central African Republic and from Zambia to Namibia. About 14 species of the genus *Anthocleista* are found in Tropical Africa, Madagascar and Comores [1]. *A. liebrechtsiana* is used in Cameroonian traditional medicine for the treatment of fever [2]. In Congo its stem bark decoction is used in the treatment of hernia, while the root decoction is taken as a purgative and to treat stomach-ache, ovarian problems, venereal diseases, bronchitis and fever, and also to induce labour [1]. *A. liebrechtsiana* has also been reported for its use as skin infection phytomedicine, purgative, or antimicrobial [3]. Many species of the genus *Anthocleista* are used in the treatment of malaria in Nigeria and the scientific evidence for few ones has been demonstrated [4-6]. However, no information regarding previous investigations of *A. liebrechtsiana* as source of malaria drugs was found. Futhermore, no phytochemical study has been carried out on this species. But studies on other

* Corresponding author: E- Mail: ptane@yahoo.com; nhtan@mail.kib.ac.cn; Phone +237-33451735; +86-871-5223800.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 10/12/2015 EISSN:1307-6167

members of the genus revealed the presence of xanthones [7,8], alkaloids [9], steroids [7], terpenoids and benzopyrones [10]. As part of our ongoing search for new antiplasmodial substances from Cameroonian medicinal plants [11,12], the present work aims at isolating and evaluating potential antiplasmodial compounds from the *A. liebrechtsiana* stem bark from Cameroon.

2. Materials and Methods

2.1. Plant Material

The stem bark of *Anthocleista liebrechtsiana* was collected at Melong, in the Littoral Region of Cameroon in February 2011, and identified by Mr V. Nana (botanist). A voucher specimen (No 55963 HNC) has been deposited at the Cameroon National Herbarium, Yaoundé.

2.2. General Experimental Conditions

Melting points were determined using an Electrothermal IA 9000 Series digital melting point apparatus (Bibby scientific, Great Britain). IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer (Japan). UV spectra were recorded on a Shimadzu UV-160A spectrometer (Japan) in absolute ethanol (Scharlau) and alkaline ethanol. Optical rotations were obtained with a JASCO P-1020 digital polarimeter. 1D NMR spectra (^{1}H and ^{13}C NMR) and 2D NMR spectra (HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY) were measured on INOVA-600, Bruker HCT-500 or AV-600 spectrometers equipped with cryoprobe, with TMS as an internal reference. Chemical shifts (δ) were expressed in ppm with reference to TMS and coupling constants (J) were given in Hz. High resolution electronic impact (HREIMS); accurate mass measurements were recorded in positive mode on a Bruker instrument with a Bruker EI source. Silica gel 60 F_{254} (70-230; Merck; Darmstadt, Germany) was used for column chromatography with step gradients of *n*-hexane-EtOAc and EtOAc-MeOH as eluents. Precoated silica gel Kieselgel 60 F_{254} plates (0.25 mm thick) were used for TLC, and spots were detected by spraying with 50% H_2SO_4 followed by heating at 100 °C.

2.3. Extraction and Isolation

The air-dried and powdered stem bark (4 kg) of *A. liebrechtsiana* was exhaustively extracted with EtOH (20 L) for 72 h and then filtered. The filtrate was concentrated under vacuum at room temperature to afford 241 g of crude extract. A portion of this crude extract (231 g) was further extracted with acetone and *n*-BuOH successively, to give 65 g and 60 g of the respective extracts after removing the solvent under reduced pressure. The acetone-soluble portion (60 g) was subjected to column chromatography over silica gel, eluted with the mixture *n*-hexane-EtOAc of increasing polarity (from 100:0 to 0:100). Forty three fractions of 300 mL each were collected and combined on the basis of their TLC profiles to yield four major fractions labelled A-D.

Fraction A (18 g) was subjected to silica gel column chromatography eluted with a gradient of *n*-hexane-EtOAc, to afford compound **2** (10 mg). Fraction B (10.4 g) was further separated by silica gel column chromatography eluted with a step gradient of *n*-hexane-EtOAc to give compounds **3** (60 mg) and **4** (40 mg). Compound **5** (5 mg) crystallized from fraction C (14.5 g) and was filtered off. Fraction D (15.5 g) was chromatographed on a silica gel column using a step gradient of *n*-hexane-EtOAc to afford five sub-fractions (D_1-D_5). Sub-fraction D_1 (2.0 g) was first applied on a Sephadex LH-20 (CH_2Cl_2-MeOH 1:1), and then purified by chromatography over silica gel (CH_2Cl_2-EtOAc 70:30) to yield compounds **1** (6 mg) and **6** (20 mg).

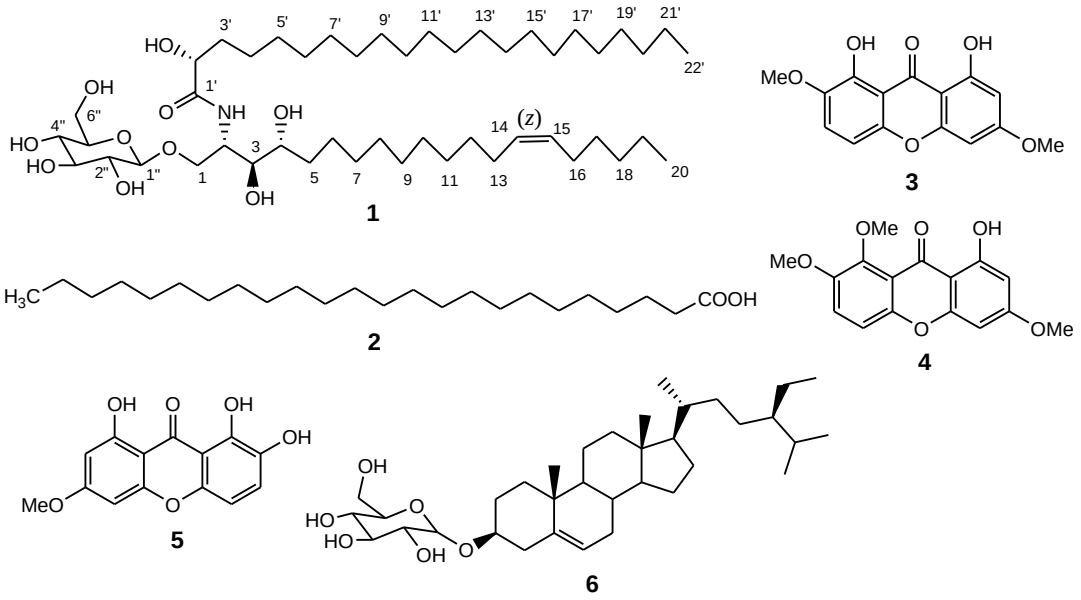

Figure 1. Structures of Compounds **1**-**6**.

*2.4. Hydrolysis and silylation of compound **1***

A solution of about 0.1 mg of **1** in 50 µL of MeOH and 50 µL of 1 M HCl was hydrolyzed at 80℃. After 4 h, the sample was taken to dryness at 0.1 mbar at room temperature, and the residue was derivatized with 50 mL of MSTFA (*N*-methyl-*N*-ethyldimethylsilyltrifluoroacetamide) at 40 °C for 1 h. GC-MS analysis showed three signals, with retention times of 9.86, 9.95 and 10.39 min. D-Glucose (R_t 9.86, 9.95, 10.39 min), D-galactose (R_t 9.29, 9.60, 9.91 min), and D-maltose (R_t 9.95 min) as standards were also hydrolyzed, silylated and analyzed in the same conditions.

2.5. In vitro Antiplasmodial and cytotoxicity assay

The culture of *Plasmodium falciparum* strains was performed by the method of Trager and Jensen as previously described with some modification [11]. Drug sensitivity assay was carried out using the parasite Lactate Dehydrogenase Assay, as previously reported [11]. The cytotoxicity of pure compounds was investigated against LLC-MK2 (ATCC, USA) monkey kidney epithelial cells according to the procedure described [11,13,14].

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was isolated as a white powder. Its molecular formula $C_{48}H_{93}NO_{10}$ was established on the basis of HREI-MS which showed a molecular ion peak $[M]^+$ at *m/z* 843.6778 (Calcd. 843.6799), in conjunction with NMR data. An IR absorption band at 3118 cm^{-1} indicated the presence of hydroxyl groups. The typical IR absorptions at ν_{max} 1622 cm^{-1} and 1535 cm^{-1} suggested an amide linkage, which was confirmed by a nitrogen-attached carbon signal at δ 49.9 and a carbonyl signal at δ 173.9 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **1** exhibited a doublet at δ 7.55 (J = 9.6 Hz) due to an NH-proton, a broad singlet peak at δ 1.21 (methylene protons), and a triplet at δ 0.83 (x $2CH_3$); all of which suggest the ceramide nature of the molecule [15]. This spectrum also showed two oxymethylene protons signals at δ 3.62 (dd, J = 4.2, 11.4 Hz, H-1a) and 3.79 (dd, J = 4.2, 11.4 Hz, H-1b) and three oxymethine signals at δ 3.84 (m, H-2′), 3.36 (m, H-3) and 3.32 (m, H-4). This assigment was further supported by four methine signals at δ 74.1 (C-3), 70.9 (C-2’), 70.5 (C-4)

and 69.1 (C-1) in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectra furthermore indicated two olefinic proton signals at δ 5.56 (m, H-14; δ_c 129,9) and 5.37 (m, H-15; δ_c 129.4), assignable to the presence of one double bond with a *cis* (*Z*) configuration. This was evidenced by the chemical shifts of the methylene carbons next to the olefinic carbons at δ 27.0 (C-13) and 26.8 (C-16) in **1** [15,16]. These data were characteristic of a sphingolipid skeleton [16,17]. The ^{1}H NMR spectrum of **1** also exhibited signals of a sugar unit with an anomeric proton at δ 4.12 (d, *J* = 7.8 Hz, H-1″), suggesting the glycosphingolipid or cerebroside nature of **1** [15]. In addition to the typical peaks of the sphingolipid backbone (δ 69.1, 74.1, 70.5 and 70.9), this spectrum also showed resonances of a glucopyranoside unit at δ 61.1, 70.0, 75.5, 76.5, 76.9 and 103.5 (Table 1). The presence of this later unit was confirmed by the EI-MS spectrum which gave a prominent ion peak at *m/z* 663 $[M - (C_6H_{12}O_6)]^+$ and further identified as *β*-glucopyranose by the anomeric proton at δ 4.12 (1H, d, *J* = 7.8 Hz, H-1″) and the chemical shifts in the ^{1}H and ^{13}C NMR spectra (Table 1).

The length of the fatty acid chain was determined by the characteristic fragmentation ions (Figure 2) at *m/z* 266 ($[CH_3(CH_2)_{18}\text{-}H]^+$), 308 ($[CH_3(CH_2)_{19}CHOH\text{-}3H]^+$), 339 ($[CH_3(CH_2)_{19}CHOHCO]^+$) in the EIMS. The length of the long chain amino base was also determined by the characteristic fragmentation ions at *m/z* 517 ($[M\text{-}CH_3(CH_2)_{16}(CHOH)_2CHO]^+$), 223 ($[CH_3(CH_2)_{13}CH{=}CH\text{-}H]^+$) in the EIMS [15]. The position of the hydroxyl groups was confirmed by the mass fragmentation pattern (Figure 2 b), the ^{1}H-^{1}H COSY, and the HMBC spectra (Figure 2a).

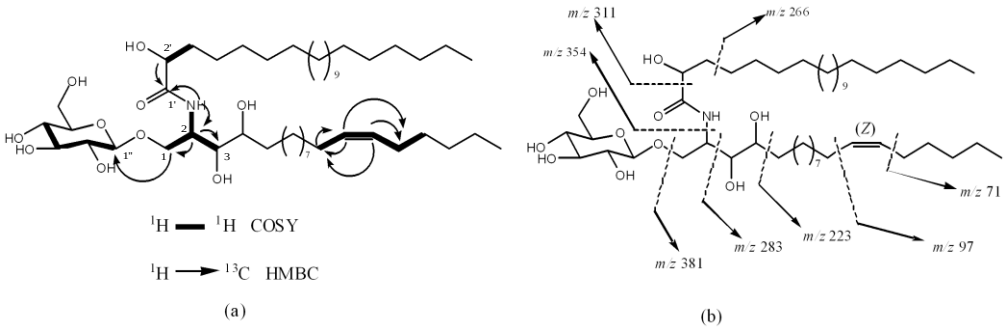

Figure 2. (a) Important ^{1}H-^{1}H COSY and HMBC correlations for liebrechtsianoside A (**1**); (b) Mass fragmentation pattern of compound liebrechtsianoside A (**1**).

Cross peaks was observed in ^{1}H-^{1}H COSY between the amide proton (NH) and H-2, the latter which in turn, showed coupling with H-1a, H-1b, and H-3 (Figure 2a) suggested this structural feature was in place. Similarly H-2' showed COSY correlations with H-3' and in the HMBC spectrum (Table 1, Figure 2a), ^{1}H–^{13}C long-range correlations were observed between H-2′ (δ 3.84) and C-1′ (δ 173.9), between the NH proton and C-1′ and C-2 (δ 49.9), between H-2 (δ 4.09) and C-1 (δ 69.9). These results confirmed that three of the four hydroxyl groups were present at C-3, C-4 and C-2'. The position of the double bond on the long-chaine base was determined by the HMBC correlations from H-13 (δ 1.92) to C-14 (δ 129.4), from H-14 (δ 5.56) to C-13 (δ 27.0) and C-16 (δ 26.8), as well as from H-15 (δ 5.37) to C-13 (δ 27.0). It was further supported by the EI-MS spectrum which displayed two ion fragments at *m/z* 71 $[C_5H_{11}]^+$ and 97 $[C_7H_{13}]^+$. The typical fragment ion at *m/z* 741 formed by elimination of hexene from $[M\text{-}H_2O]^+$ through Mc Lafferty rearrangement [18], further supported the position of the double bond in the long-chain base.

Concerning glucose unit, the relative configuration of the anomeric center was determined as *β* with respect to the large coupling constant (*J* = 7.8 Hz) of the anomeric proton. In order to determine the absolute configuration of this sugar moiety, **1** was sequentialy hydrolysed and silylated. The GC-MS analysis of the resulting sample showed three retention times at 9.86, 9.95 and 10.39 attributable to isomeric *α*/*β*-pyranone/furanone, compared with those of an authentic silylated D-glucose. Therefore, the absolute configuration of the *β*-glucose moiety was deduced to be D.

The stereochemistry of the sphingosine moiety was determined by comparison of the NMR data of cerebroside described by Tomoki et al. [19]. On the basis of this evidence, the structure of **1** is

suggested to be 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*,14*Z*)-2-(2′*R*-hydroxydocosanylamino)-14-icosene-1,3,4-triol, namely liebrechtsianoside A

Table 1. ^{1}H (600 MHz), ^{13}C (150 MHz) NMR data of compound **1** in DMSO-d_6, [δ (PPm), *J* (Hz)]

Position	δ_H	δ_C
NH	7.55 (1H, *d*, *J* = 9.6)	
1	3.79 (1H, *dd*, *J* = 4.2; 11.4)	69.9
	3.62 (1H, *dd*, *J* = 4.2; 11.4)	
2	4.09 (1H, *m*)	49.9
3	3.36 (1H, *m*)	74.1
4	3.32 (1H, *m*)	70.5
5	1.96 (1H, *m*); 1.53 (1H, *m*)	31.4
6	1.96 (1H, *m*); 1.53 (1H, *m*)	31.8
7-12, 4′-21′, 17-19	1.21 (2H, *brs*)	29.3-28.8
13	1.92 (2H, *m*)	27.0
14	5.56 (1H, *m*)	129.9
15	5.37 (1H, *m*)	129.4
16	1.96 (2H, *m*)	26.8
20, 22′	0.83 (6H, *t*, *J* = 9.8)	14.1
1′	-	173.9
2′	3.84 (1H, *m*)	70.9
3′	1.57 (1H, *m*); 1.40 (1H, *m*)	34.4
Glucosyl		
1″	4.12 (1H, *d*, *J* = 7.8)	103.5
2″	2.89 (1H, *m*)	73.5
3″	3.13 (1H, *m*)	76.5
4″	3.03 (1H, *m*)	70.0
5″	3.11 (1H, *m*)	76.9
6″	3.65 (1H, *dd*, *J* = 10.2, 6.0); 3.41 (1H, *dd*, *J* = 10.2, 6.0)	61.1

The other compounds were identified on the basis of their NMR spectra and the comparison of their data with those obtained to the literature as: tetracosanoic acid (**2**) [20], swertiaperennin (**3**) [7,8], decussatin (**4**) [7,8], swertianin (**5**) [4,7] and sitosterol 3-*O*-*β*-D-glucopyranoside (**6**) [7].

*Liebrechtsianoside A **(1)**:* Colorless powder; MP. 182-183 °C; $[\alpha]_D^{25}$ = + 56.66 (*c* = 0.002, DMSO); UV (MeOH): λ_{max} (log ε): 202 (3.94); IR ν_{max} (KBr): = 3118, 2918, 2850, 1622, 1535, 1463 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS: See Figure 2; HREIMS: *m/z* 843.6778 (calcd. 843.6799 for $C_{48}H_{93}NO_{10}$).

3.2. In vitro antiplasmodial and cytotoxic activity

The acetone extract, all the fractions obtained and four of the isolated compounds, liebrechtsianoside A (**1**), 1,8-dihydroxy-3,7-dimethoxyxanthone (**3**), 1-hydroxy-3,7,8-trimethoxyxanthone (**4**) and 1,7,8-trihydroxy-3-methoxyxanthone (**5**) were tested for their anti-malarial activity and cytotoxicity against mammalian cell-line, namely the LLC-MK2 Monkey kidney epithelial cell-line. The acetone extract showed a weak activity but the fractionation process potentiated the antimalarial profile very significantly, with three of the fractions exhibiting IC_{50} below 20 µg/mL. Among the purified compounds, liebrechtsianoside A (**1**) exhibited a highly significant activity, whereas the three others were only weakly active against the multidrug resistant Dd2 strain of *P. falciparum*. Further work is urgently required on liebrechtsianoside A, and this necessitates a diversity of approaches. For example, the investigation of its effects in living system, together with the elucidation of its targets in the malaria parasite should be undertaken to fully evaluate its suitability as drug candidate. Compound 1,7,8-trihydroxy-3-methoxyxanthone (**5**) was shown to possess weak antimalarial activity in mice, with a drug concentration of 50 µg/mL displaying 42% growth inhibition on *P. perghei*. Both 1-hydroxy-3,7,8-trimethoxyxanthone (**4**) and 1,8-dihydroxy-3,7-

dimethoxyxanthone (**3**) were previously isolated from *Anthocleista vogeli* [4] . From the same authors, the petroleum ether extract of *A. vogelii* displayed good antiplasmodial activity against the *P. berghei* parasite in mice. The present work therefore confirms the ability of *Anthocleista* genus to serve as source of anti-malarial substances. The weak activity observed with 1,8-dihydroxy-3,7-dimethoxyxanthone (**3**), 1-hydroxy-3,7,8-trimethoxyxanthone (**4**) and 1,7,8-trihydroxy-3-methoxyxanthone (**5**) could be improved on exploiting other drug development approaches as activity-guided structure modification, and combination studies.

Table 2. Antiplasmodial and cytotoxic activities of compounds and acetone extract from the stem bark of *A. liebrechtsiana.*

Substances	***Dd2 Plasmodium falciparum***		***CC_{50} on LLC-MK2 cells (µg/mL)*** **[SI]**
	IC_{50} (µg/mL)	**IC_{99} (µg/mL)**	
Extracts			
Acetone	30.83 ± 1.56	82.28 ± 12.26	-
Fraction A	30.99 ± 2.09		-
Fraction B	15.60 ± 5.66	526.73 ± 21.02	-
Fraction C	16.78 ± 2.45	43.47 ± 4.56	-
Fraction D	14.79 ± 2.12	31.43 ± 1.06	-
Pure compounds			
(**1**)	1.06 ± 0.32	6.43 ± 1.21	70.03± 4.21 [66.06]
(**3**)	17.05 ± 4.02	30.60 ± 1.09	30.5 ± 1.34 [1.78]
(**4**)	20.96 ± 5.34	32.58 ± 0.89	> 100 [-]
(**5**)	22.74 ± 3.67	36.03 ± 3.20	> 100 [-]
Chloroquine	0.100 ± 0.042	0.820 ± 0.010	-

IC_{50}: Drug concentration causing 50% inhibition of parasite growth and multiplication; IC_{99}: Drug concentration clearing almost completely the parasite growth and multiplication; CC_{50}: Cytotoxic concentration causing 50% inhibition of mammalian cell growth and multiplication; SI: Selectivity Index = CC_{50}/IC_{50}. IC_{50} and CC_{50} values are Means and standard deviations obtained from six different replicate tests. Dd_2 is a chloroquine resistant strain of *P. falciparum*

Acknowledgments

We are grateful to the University of Dschang for financing some consumables used in this work. We gratefully acknowledge the financial support from International Foundation for Science (IFS), Stockholm, Sweden, and the Organization for the Prohibition of Chemical Weapons, The Hague, Netherlands, IFS-OPCW, Grants No F/4901-1 and No F/5122-1 awarded to Drs Wabo and Zofou, respectively. We also gratefully acknowledge the financial support from the Chinese Academy of Sciences (CAS), through a travel grant to M.F.T. at the Kunming Institute of Botany, China.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] G. H. Schmelzer and A. Gurib-Fakim (2008). *Ressources végétales de l'Afrique tropicale 11 (1). Bois d'œuvre 1*, Fondation PROTA/Backhuys Publishers/CTA. Wageningen, Pays Bas. p105.

[2] J. E. Adjanohoun, N. Aboubakar, K. Dramane, M. E. Ebot, J.A. Ekpere, E. G. Enoworock, D. Focho, Z. O. Gbile, A. Kamanyi, K. J. Kamsu, A. Keita, K. T. Mben, C. N. Mbi, A. L. Mbiele, I. L. Mbome, N. K. Mubini, W. L Nancy, B. Nkongmeneck, B. Satabi, A., Sofowora, V. Tamze and C. K. Wirmum (1996). Traditional Medicinal Pharmacopoeia: Contribution to ethnobotanical and floristics studies in Cameroon, CNPMS (Centre National de Production de Manuels Scolaires), Porto- Novo, p 273.

[3] D. Olokudejo, A. B. Kadiri and V. A. Travih (2007). An ethnobotanical survey of herbal markets and medicinal plants in Lagos state of Nigeria, *Ethnobot. Leaflets* **12**, 851-865.
[4] S. A. C. Alaribe, A. B. H. Coker, O. F. Shode, G. Ayoola, A. S. Adesegun, J. Bamiro, I. E. Anyim and C. Anyakora (2012). Antiplasmodial and phytochemical investigation of leaf of *Anthocleista vogelii* (planch), *J. Nat. Prod*. **5**, 60-67.
[5] L. B. Gboeloh, O. E.Okon and S. E.Udoh (2014). Antiplasmodial effect of *Anthocleista vogelii* on albino mice experimentally infected with Plasmodium berghei berghei (NK 65). J. Parasitol. Res. Article ID 731906, 6 pages, 2014. doi:10.1155/2014/731906.
[6] B. O. Othuke, A. Uwakwe and C. C. A. Monago (2012). Antiplasmodial Activity of Methanolic Stem Bark Extract of *Anthocleista grandiflora* in Mice, *Int. J. Appl. Sci. Technol.* **2**, 142-148.
[7] R. N. Mbouangouere, P. Tane, D. Ngamga, S. N. Khan, M. I. Choudhary and B. T. Ngadjui (2007). A new steroid and *α*-glucosidase Inhibitors from *Anthocleista schweinfurthii*, *Res. J. Med. Plant*. **3**, 106-111.
[8] M. Tene, P. Tane, J-R. Kuiate, J. D Tamokou and J. D Connolly (2008). Anthocleistenolide, a new rearranged Nor-secoiridoid derivative from the stem bark of *Anthocleista vogelii*, *Planta Med*. **74**, 80-83.
[9] D. A. Okorie (1976). A new phthalide and xanthones from *Anthocleista djalonensis* and *Anthocleista vogelii*, *Phytochemistry* **15**, 1799-1800.
[10] P. A. Onocha, D. A. Okorie, J. D. Connolly and D. S. Roycroft (1994). Monoterpene diol, iridoid glucoside and dibenzo-α-pyrone from *Anthocleista djalonensis*, *Phytochemistry* **40**, 1183-1189.
[11] D. Zofou, T. K. Kowa, H. K. Wabo, P. Tane and V.P. K. Titandji (2011). *Hypericum lanceolatum* (Hypericaceae) as a potential source of new anti-malarial agents: a bioassay-guided fractionation of the stem bark, *Malar. J*. **10**, 1-7.
[12] T. K. Kowa, A. T. Tchinda, M. F. Tala, D. Zofou, R. Jumbam, H. K. Wabo, V. P. K. Titandji, M. Frédérich, N-H. Tan and P. Tane (2014). Antiplasmodial anthrqauinone and hemisynthetic derivatives from the leaves of *Tectona grandis* (Verbenaceae), *Phytochem lett*. **8**, 41-45.
[13] H. M. Malebo, W.Tanja, M. Cal, S. A. M. Swaleh, M. O. Omolo, A. Hassanali (2009). Anti-plasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants. Tanzan, *J. Health. Res.* **11**, 226-234.
[14] T. Mosmann (1983). Rapid Colorimetric Assay for Cellular Growth and Survival: Application to proliferation and phytotoxicity assays, *J. Immunol. Methods*. **65**, 55-63.
[15] D. Tazoo, K. Krohn, H. Hussain, S. F. Kouam and E. Dongoa (2007). Laportoside A and laportomide A: A New Cerebroside and a new ceramide from Leaves of *Laportea ovalifolia*, *Z. Naturforsch.* **62B**, 1208-1212.
[16] K. O. Eyong, K. Krohn, H. Hussain, G. N. Folefoc, A. E. Nkengfack, B. Schulz and Q. Hu (2005). Newbouldiaquinone and Newbouldiamide: A new naphthoquinone-anthraquinone coupled pigment and a new ceramide from *Newbouldia laevis, Chem. Pharm. Bull.* **53**, 616-619**.**
[17] N. Fusetani, K. Yasumuro and S. Matsunaga (1989). Haliclamines A et B, cytotoxic macrocyclic alkaloids from sponge of the genus *haliclona*, *Tetrahedron Lett*. **30**, 6891-6894.
[18] G. R. Pettit, Y. Tang and J. C. Knight (2005). Antineoplastic agents. 545. Isolation and structure of turbostatins 1-4 from the Asian marine mollusk Turbo stenogyrus, *J. Nat. Prod*. **68**, 974-978.
[19] M. Tomoki, S. Takeshi, I. Masanori, S. Osamu and H. Ryuichi (2005). Structure determination of glucocerobrosides from the Starfish *Linckia laevigata*, *Chem. Pharm. Bull*. **53**, 1255-1258.
[20] S. Muhammad, R. Naheed, I. Muhammad, N. Haq, M. Abdul and J. Abdul (2009). Phytochemical studies on *Asphodelus tenuifolius*, *J. Chem. Society of Pakistan*. **31**, 122-125.