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Antiplasmodial compounds from the stem bark of *Neoboutonia macrocalyx* Pax.

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Abstract

Ethnopharmacological relevance: The plant *Neoboutonia macrocalyx* has been reported in traditional medicine to be used in the treatment of malaria.

Aim of the study: To study the in vitro antiplasmodial activity of compounds from the stem bark of *N. macrocalyx*.

Materials and methods: Compounds were extracted and purified from stem bark of *N. macrocalyx* and their structure identified and confirmed by spectroscopic methods.

The crude ethylacetate extract, aqueous extract and the isolated compounds were evaluated for antiplasmodial activity against the chloroquine sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) strains of *Plasmodium falciparum.*

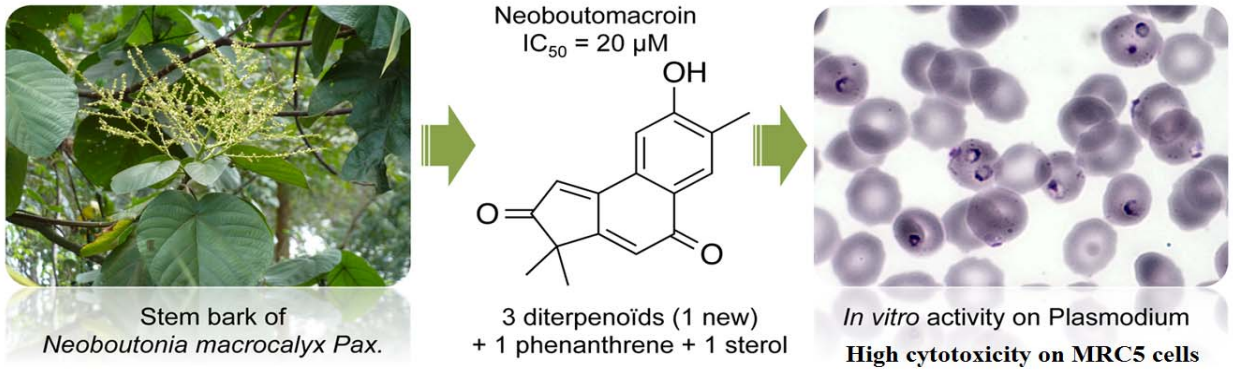
Results: Chemical investigation of the ethyl acetate extract of *N. macrocalyx* bark resulted in the identification of one new diterpenoid; neoboutomacroin (**1**) in addition to the four known compounds which included, a phenanthrene; 3,6-dihyroxy-1,7-dimethyl-9-methoxyphenanthrene (**2**), a sterol; 3-*O*-Acetyloleuritolic acid (**3**) and two diterpenoids; simplexin (**4**) and montanin (**5**). Compounds **1** and **5** displayed good antiplasmodial activity of IC_{50} values less than 10 µg/mL against both strains. However, all the compounds tested displayed high cytotoxic activity against MRC5 cell line with IC_{50} less than 10µM.

Conclusions: Despite an indirect *in vitro* antiplasmodial activity of some compounds isolated from the stem bark of *N. macrocalyx,* the identification of these bioactive compounds indicates that they may play a role in the pharmacological properties of this plant.

Key words**:** *Neoboutonia macrocalyx;* Euphorbaceae; antiplasmodial activity; diterpenoids; phenanthrene; sterol.

Graphical abstract

Chemical investigation of the extract from the stem bark of *Neoboutonia macrocalyx* led to the isolation of one new diterpenoid with two known diterpenoids, a phenanthrene and a sterol. The compounds displayed moderate to good antiplasmodial activity but with high cytotoxicity.



1. Introduction:

The genus *Neoboutonia* which belongs to the family Euphorbiaceae consists of three species endemic to tropical Africa i.e. *N. macrocalyx, N. Melleri,* and *N. glabrescens* (*N. Mannni*) (Radcliffe-Smith, 1974). *Neoboutonia* plants have been reported in Uganda, Kenya and Cameroon for their use in ethnomedicine for treatment of malaria, fevers, headache, stomach pains, diabetes and worms (Kirira et al., 2006; Long et al., 2011; Muthaura et al., 2007; Thomas et al., 1989). Although these plants are used in medicine, their pharmacology and phytochemistry has not been extensively studied. However, in 1998, Zhao et al. (1998) isolated mellerin A and B and known sterols including β-sitosterol from the leaves of *N. melleri*. Tchinda et al. (2003) reported the isolation of neoglabrescens A and B, baliosperm and montanin from the stem bark of *N. glabrescens*. In 2008, Neoboutomannin and manniorthoquinone among others were isolated from the stem bark of *N. mannii* by Tene and co-workers (Tene et al., 2008). Long et al. (2011) also reported the isolation of 30 new cycloartane derivatives from the leaves of *N. melleri*.

Neoboutonia macrocalyx Pax. (Euphorbiaceae) is a tree which grows in medium altitude tropical rain forests. The stem bark of this plant is reported to be used in Meru and Kilifi Districts of Kenya to treat headaches and fevers and, in Uganda, it has been reported in malaria treatment (Kirira et al., 2006; Namukobe et al., 2011).

The antiplasmodial activity of the stem extract has been reported by Kirira et al. (2006). Kirira et al. (2007) also reported tigliane diterpenoids from the stem bark of the same plant. Recently, cycloartane triterpenes have been isolated from the leaves of this plant and their antiplasmodial potential was investigated (Namukobe et al., 2014).

In our continued search for biologically active compounds from the Ugandan flora, we report the isolation of one new diterpenoid in addition to four known compounds from the ethylacetate extract of the stem bark of *N. macrocalyx*. With an urgent need for new antimalarial compounds coupled with the antimalarial use for the plant itself, we further investigated the in vitro antiplasmodial properties against *Plasmodium falciparum.*

2. Material and Methods

2.1. General experimental procedures

The NMR spectra of compounds **1, 3, 4** and **5** were recorded on a Bruker Avance 300 MHz and those of compounds **2,** were recorded on 500 MHz (Bruker, Avance; Switzerland) NMR instruments. High-resolution MS data were obtained on a LCT Premier XE (Waters) ESI-ToF mass spectrometer using a Waters Acquity UPLC. Column chromatography was performed on a medium pressure column with silica gel (6-32 μm). Analytical and preparative HPLC separation was performed with a C18 column Sunfire (5 μm; 4.6×150 mm) and (5 μm; 19×150 mm) (Waters) respectively. Analytical TLC was carried out on precoated silica gel 60F_{254} (Merck) and the spots were visualized by heating after spraying with Ammonium Molybdate. Preparative TLC was done using TLC glass plates prepared with Silica gel 60 F_{254}.

2.2 Plant material

The plant material was collected from Kibale National Park and identified at the Botany department, Makerere University, Uganda. The plant material was air dried and ground into a powder**.** A voucher specimen under reference UFC-380 has been deposited in Makerere University herbarium.

2.3. Extraction and isolation

The stem bark powder (387 g) was extracted three times with EtOAc at room temperature to obtain 1.4 g of the extract. The extract was subjected to medium pressure column chromatography packed with silica gel (6-32 μm) using a gradient system of hexane-EtOAc and EtOAc-MeOH to yield 12 fractions (1—12). Fractions that consisted of major compounds in the extract were subjected to further purification. Purification of fraction 4 was done using preparative HPLC with a gradient elution 70:30 to 0:100 (solvent A: H_2O+0.1% HCOOH, B: AcN+0.1% HCOOH) to obtain 22.7 mg of compound **1** and 4.0 mg of compound **2**. Fraction 2 was purified by PTLC using n-heptane/Acetone 70/30 v/v solvent system to obtain 20 mg of compound **3**. Preparative HPLC was done on fraction 5 with a gradient elution 20:80 to 0:100 (solvent A: H_2O+0.1% HCOOH, B: AcN+0.1% HCOOH) and this led to the isolation of 7.9 mg of compound **4** and 34.6 mg of compound **5**.

In order to check for the presence of the isolated compounds in the aqueous extract, the stem bark powder (85 g) was added to hot water and the contents were left to boil for 30 min. The extraction was done in a way that mimics the traditional preparation where a decoction of the stem bark is prepared. The aqueous extract (30 mg) was then dissolved in ethylacetate and the aqueous and organic phases obtained were separated and concentrated. A concentration of 2 mg/mL of the organic phase was prepared and analyzed for the presence of the isolated compounds using UPLC-MS.

2.4. Chemical elucidation of compounds

Neoboutomacroin (**1**); Yellow solid; ^{1}H NMR (300 MHz, DMF-d7): δ_H 7.12 (H-1), 6.50 (H-6), 7.59 (H-11), 7.92 (H-14), 2.33 (H-15), 1.30 (H-18), 1.30 (H-19); ^{13}C NMR (175 MHz, DMF-d7): δ_C 128.8 (C-1), 208.8 (C-3), 46.5 (C-4), 162.6 (C-5), 121.6 (C-6), 184.4 (C-7), 124.8 (C-8), 131.2 (C-9), 155.8 (C-10), 112.5 (C-11), 161.5 (C-12), 130.9 (C-13) 130.3 (C-14), 17.0 (C-15), 23.8 (C-8) 23.8 (C-19). HR-ESI-MS (positive ion mode) *m/z* 255.1015 $[M+H]^+$ (calcd for $C_{16}H_{14}O_3$, 255.1021).

3,6-dihydroxy-1,7-dimethyl-9-methoxyphenanthrene **(2**); White powder, ^{1}H NMR (500 MHz, MeOD): δ_H 2.60 (H-1a), 6.92 (H-2), 7.64 (H-4), 7.80 (H-5), 2.38 (H-7a), 7.97 (H-8), 4.02 (H-9a), 6.86 (H-10); ^{13}C NMR (125 MHz, MeOD): δ_C 136.c0 (C-1), 20.2 (C-1a), 118.8 (C-2), 154.4 (C-3), 105.5 (C-4), 128.5 (C-4a) 132.3 (C-4b) 107.0 (C-5), 156.4 (C-6), 127.0 (C-7), 16.7 (C-7a) 125.0 (C-8), 121.5 (C-8a) 152.9 (C-9), 55.5 (C-9a), 96.5 (C-10), 126.7 (C-10a). HR-ESI-MS (positive ion mode) *m/z* 269.1166 $[M+H]^+$ (calcd for $C_{17}H_{16}O_3$, 269.1178).

3-*O*-Acetyloleuritolic acid (**3**); white powder; ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 4.45 (H-3), 2.01 (H-3b), 5.5 (H-15), 2.33, 1.93 (H-16); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 37.5 (C-1), 23.6 (C-2), 81.1 (C-3), 171.2 (C-3a), 21.5 (C-3b), 38.1 (C-4), 55.8 (C-5), 18.9 (C-6), 40.9 (C-7), 30.2 (C-8), 49.2 (C-9), 37.8 (C-10), 17.5 (C-11), 33.6 (C-12), 37.6 (C-13) 160.7 (C-14), 117,0 (C-15), 31.5 (C-16), 51.7 (C-17), 41.6 (C-18), 35.5 (C-19), 29.5 (C-20),33.5 (C-21), 30.9 (C-22), 28.1 (C-23), 16.8 (C-24), 15.8 (C-25), 26.3 (C-26), 22.6 (C-27), 184.4 (C-28), 32.0 (C-29), 28.8(C-30). HR-ESI-MS (positive ion mode) *m/z* 499.3785 $[M+H]^+$ (calcd for $C_{32}H_{50}O4$, 499.3787).

Simplexin (**4**); colourless gum; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.59 (H-1), 4.23 (H-5), 3.41 (H-7), 2.88 (H-8), 3.72 (H-10), 2.43 (H-11), 1.64, 2.19 (H-12), 4.35 (H-14), 4.87, 5.00 (H-16), 1.76 (H-17), 1.15 (H-18), 1.78 (H-19), 3.76, 3.85 (H-20), 1.92 (H-2'), 0.87 (H-10'), 1.20-1.30 (CH_2)n; ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 161.6 (C-1), 136.8 (C-2), 210.1 (C-3), 72.4 (C-4), 72.3 (C-5), 60.6 (C-6), 64.6 (C-7), 36.7 (C-8), 78.9 (C-9), 48.4 (C-10), 35.0 (C-11), 36.9 (C-12), 84.3 (C-13) 82.0 (C-14), 146.5 (C-15), 111.4 (C-16), 19.3 (C-17), 20.5 (C-18), 10.1 (C-19), 65.5 (C-20), 119.6 (C-1'), 35.1 (C-2'), 14.3 (C-10'). 22.9-32.1 $(CH_2)_n$. HR-ESI-MS (positive ion mode) *m/z* 533.3124 $[M+H]^+$ (calcd for $C_{30}H_{44}O_8$, 533.3114).

Montanin (**5**); colourless gum; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.57 (H-1), 4.22 (H-5), 3.40 (H-7), 2.87 (H-8), 3.72 (H-10), 2.41 (H-11), 1.63, 2.18 (H-12), 4.34 (H-14), 4.86, 4.99 (H-16), 1.75 (H-17), 1.14 (H-18), 1.77 (H-19), 3.75, 3.84 (H-20), 1.92 (H-2'), 0.85 (H-12'), 1.20-1.30 (CH_2)n. ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 161.6 (C-1), 136.8 (C-2), 210.1 (C-3), 72.5 (C-4), 72.2 (C-5), 60.6 (C-6), 64.5 (C-7), 36.9 (C-8), 78.9 (C-9), 48.4 (C-10), 35.1 (C-11), 36.7 (C-12), 84.3 (C-13) 82.0 (C-14), 146.5 (C-15), 111.4 (C-16), 19.3 (C-17), 20.5 (C-18), 10.1 (C-19), 65.4 (C-20), 119.6 (C-1'), 35.0 (C-2'), 14.3 (C-12'), 22.9-32.1 $(CH_2)_n$. HR-ESI-MS (positive ion mode) *m/z* 561.3408 $[M+H]^+$ (calcd for $C_{32}H_{48}O_8$, 561.3427).

2.5. Antiplasmodial activity

The extracts and compounds were assayed using a non-radioactive Malaria SYBR Green I assay technique (Smilkstein et al., 2004) with modifications (Yenesew et al., 2012) to determine a concentration that inhibits growth of 50% of parasites in culture (IC_{50}). In this method, two different *Plasmodium falciparum* strains, chloroquine sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2), were grown as described by Trager and Jensen, (1976); with minor modifications (Johnson et al. (2007). Drugs, extracts and compounds were dissolved in 99.5% dimethylsulfoxide (DMSO) (Sigma–Aldrich) and diluted in complete Roswell Park Memorial Institute 1640 series of Cell Culture Media (RPMI 1640) enriched with human serum. The RPMI 1640 medium was prepared accordingly as described by Akala and co-workers (Akala et al., 2011). Briefly, the basic culture medium was prepared from RPMI 1640 powder (10.4 g; Invitrogen, Inc. augmented with 2 g glucose (Sigma Inc.) and 5.95 g of HEPES (Sigma Inc.), dissolved to homogeneity in 1 liter of de-ionized water and sterilized with a 0.2 µM filter. Complete RPMI 1640 media, used for all parasite culture and drug dilutions, consisted of basic RPMI 1640 media with 10% (v/v) human ABO pooled plasma, 3.2% (v/v) sodium bicarbonate (Thermo Fisher Scientific Inc.) and 4 µg/mL hypoxanthine (Sigma Inc.). Drug preparation entailed two-fold serial dilutions of chloroquine (1.953–1000 ng/mL), mefloquine (0.488–250 ng/mL) and test sample (97.7–50,000 ng/mL) were prepared on a 96-well plate, such that the

final proportion of DMSO was equal to or less than 0.0875 %. The culture-adapted *P. falciparum* at 2% hematocrit and 1% parasitemia, were then added on to the plate containing dose range of drugs and incubated in a gas mixture (5% CO2, 5 % O2, and 90 % N2) at 37 ◦C. The assay was terminated 72 h later by freezing at −80 ◦C and parasite growth inhibition was quantified as described by Johnson et al. (2007) and the results presented as Mean IC_{50} ± SD.

2.6. Cytotoxicity studies of the compounds

Cytotoxicity was done against MRC-5 (human diploid embryonic lung cell). Samples were dissolved in DMSO to make a final concentration of 100 µM. Samples were then added to a plate containing cells in a fixed volume of DMSO and incubated for 72 h. DMSO was used in the control experiments. The percentage inhibition was then calculated from the number of viable cells measured at 490 nm with the MTS reagent with reference to the control. The cytotoxicity assays were performed according to published procedures (Tempete et al., 1995). Taxotere was used as a control compound and experiments were performed in triplicate.

3. Results and discussion

3.1. Characterisation of compounds from the stem bark extract

Phytochemical investigation on the stem bark of *Neoboutonia macrocalyx* led to the isolation and identification of one new diterpenoid (**1**) in addition to the four known compounds **(2-5)** which include a phenanthrene (**2**), an acetyl oleuritolic acid (**3**), simplexin (**4**), and montanin (**5**). Spectroscopic methods which included MS, ^{1}H and ^{13}C NMR spectra along with 2-dimensional COSY, HMQC, HMBC, and NOESY experiments were used to elucidate the structures of the new diterpene. In addition to the above experiments, the known compounds were identified by comparison of their NMR and MS spectral data with that published in the literature.

Compound **1** was obtained as a yellow solid and its molecular formula was deduced from its HR-ESI-MS as C_{16} H_{14} O_3 from the positive ion at m/z 255.1015 $[M+H]^+$. This information showed that compound **1** has 10 sites of unsaturation. The ^{1}H NMR spectrum of compound **1**, exhibited four aromatic singlets at δ_H 7.92, 7.59 7.12 and 6.50. The ^{1}H NMR also indicated three methyl groups, one of which was an aromatic methyl at δ_H 2.33 and the remaining two at δ_H 1.30. Detailed analysis of ^{1}H NMR, ^{13}C-NMR and HSQC spectra revealed that compound **1** was a diterpenoid with four aromatic protons, three methyls, two carbonyl carbons at δ_C 208.8 and 184.8 and one hydroxyl group. The location of the H-1 was established through ^{1}H---^{13}C long-range HMBC correlations. H-1 had a cross peak with C-3, C-4, C-5, C-9 and C-10 at δ 208.8, 46.5, 162.6, 131.2 and 155.8 respectively. H-14 had cross peaks with Me-15 and C-7. $^{1}H/^{13}C$ HMBC correlations between the methyl at C-13 enabled the location of OH at C-10. The location of the rest of the protons and carbons was achieved through comparison with data in literature of a related compound, neoboutomannin (Tchinda et al 2003).

Compound **1** was numbered according to the numbering system used for neoboutomannin (Tchinda et al., 2003), which reflects its putative diterpenoid biogenetic origin. Thus compound **1** is a new degraded diterpenoid closely related to the dimer; “neoboutomannin” isolated from *N.*

manni (Tene et al., 2008) and *Trigonostemon flavidus* (Euphorbiaceae) (Tang et al., 2012). The difference between compound **1** and neoboutomannin is the presence of a proton H-1 at δ_H 7.12 in **1** and its absence in neoboutomannin. Compound **1** also has the same skeleton as Trigoxyphin N, recently isolated from *Trigonostemon xyphophylloides* (Yang et al., 2013). Trigoxyphin N is similar to **1** but with a methoxy group at C-11. Compound **1** is here assigned a trivial name 'neoboutomacroin' (Figure 1).

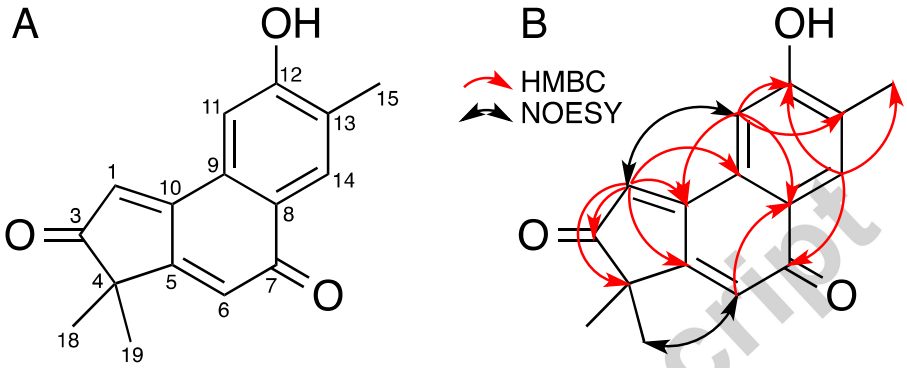


Figure 1**:** Structure of neoboutomacroin **(1)** (A); Key HMBC and NOSY correlations of neoboutomacroin (**1**) (B)

Compound **2** was obtained as a yellow solid. The molecular formula $C_{17}H_{16}O_3$ was deduced from its HR-ESI-MS positive ion at m/z 269.1166 $[M+H]^+$, ^1H ,^{13}C NMR and HSQC spectra. The ^{13}C NMR showed the presence of 17 carbons, including four oxygenated carbons at δ_C 55.5 for the methoxy group, δ_C 156.4, 154.4 and 152.9 for the aromatic oxygenated carbons. Compound **2** was found to be 3,6-dihyroxy-1,7-dimethyl-9-methoxyphenanthrene (Figure 2) by detailed NMR analysis and comparison of its ^1H NMR and ^{13}C NMR data and with those reported in literature (Long et al., 1997). This compound has been isolated before from *N. glabrescens* (Tchinda et al., 2003).

Compound **3** was isolated as a white amorphous solid and its molecular formula was deduced from its HR-ESI-MS as C_{32} H_{50} O_4 from the positive ion at m/z at 499.3785 $[M+H]^+$.
The ^1H NMR, ^{13}C NMR and DEPT spectra of **3** showed characteristic features of a 3-*O*-Acetyloleuritolic acid. The structure of compound **3** was identified by detailed ^1H NMR and ^{13}C NMR and also by comparison with data reported in the literature as 3-*O*-Acetyloleuritolic acid, a known compound that has been isolated previously from *Neoboutonia glabrescens* and other plant species (Addae-Mensah et al., 1992; Maciel et al., 1998; Prachayasittikul et al., 2009; Tchinda et al., 2003; Woo & Kang, 1985).

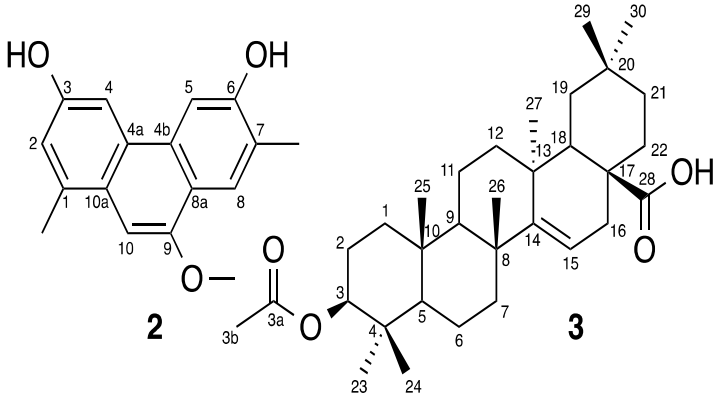


Figure 2: Structure of 3, 6-dihydroxy-1, 7-dimethyl-9-methoxyphenanthrene (**2**) and 3-*O*-Acetyloleuritolic acid (**3**)

Compounds **4** and **5** were isolated as colourless gums. Compound **4** exhibited an ion at $[M+H]^+$ m/z 533.3124 from its HR-ESI-MS data and a molecular formula of C_{30} H_{44} O_8 was deduced. The molecular formula for compound **5** was determined from its positive ion mode HR-ESI-MS data of 561.3408, $[M+H]^+$ as C_{32} H_{48} O_8. The ^1H and ^{13}C NMR data of these two compounds presented resonances close to those of mellerin B previously isolated from the leaves of *N. macrocalyx* and *N. melleri* (Namukobe et al., 2014; Zhao et al., 1998). The almost identical ^1H NMR and ^{13}C NMR chemical shifts of compounds **4** and **5** to mellerin B suggested the same daphnane type of skeleton with a difference in the aliphatic chain. As confirmed from the molecular formula, the ^1H and ^{13}C NMR revealed that the aliphatic chain of compounds **4** and **5** contained two more and four more methylene groups than mellerin B respectively**.** By detailed MS and NMR data analysis, compound **4** was identified as simplexin, a known compound previously isolated from the genus *Pimelea* (Thymelaeaceae) including *P.simplex* and *P. elongata* and other plant species (Asada et al., 2011; Hayes et al., 2010). Compound **5** was identified as montanin, from its MS and NMR data analysis and also in comparison with data from literature. This known compound has been isolated from *N. glabrescens*, *Baliospermum montanum* (Euphorbiaceae) and *Cunuria spruceona* (Euphorbiaceae) and its anticancer properties have been demonstrated (Tchinda et al., 2003; Gunasekera et al., 1979).

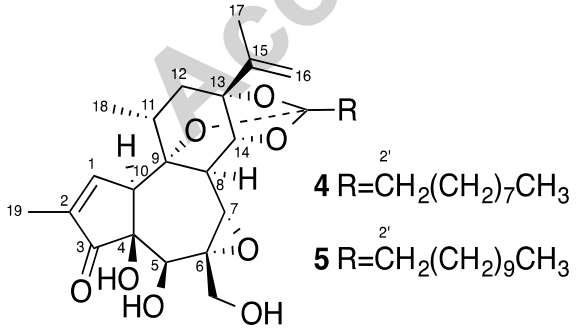


Figure 3: Structures of simplexin (**4**) and montanin (**5**)

3.2. Antiplasmodial and cytotoxic activity of the stem extracts and compounds

The extracts and the compounds were active against the D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *P. falciparum* (Table 1). In describing *in vitro* antiplasmodial activities of natural products, pure compounds are considered to be inactive when they show IC_{50} > 200 µM, whereas those with an IC_{50} of 100-200 µM have low activity; IC_{50} of 20-100 µM, moderate activity; IC_{50} of 1-20 µM good activity; and IC_{50} < 1 µM excellent/potent antiplasmodial activity [Batista et al., 2009]. Similarly, crude extract activities are categorized as IC_{50} < 10 µg/mL, good activity; IC_{50} of 10-50 µg/mL, moderate activity; IC_{50} of 50-100 µg/mL, low activity; and IC_{50} > 100 µg/mL, inactive (Basco et al., 1994). Based on these classifications, compound **1, 3** and **5** had good activities on W2 strain and moderate on D6 strain while compound **4** had moderate activity (Table 1). Compounds that were tested displayed significant cytotoxic activity (Table 1). These results indicated that the antiplasmodial activity is not specific and could be related to the cytotoxic nature of these compounds. The cytotoxic nature of compounds **4** and **5** had been reported before by Freeman et al (1979) and Gunasekera et al (1978). Due to insufficient quantities of compound **2**, the antiplasmodial testing of this compound was not carried out. The EtOAc extract had good activity while aqueous extract was curiously inactive. The traditional preparation and use of this plant and indeed for many other herbs, entails boiling in water to attain a decoction that is taken in large quantity. Compound **5,** which was the major compound in the EtOAc extract and the most active, was found in trace amounts in the aqueous extract using UPLC-MS experiments (Figure 4). This could be the reason for the lack of activity of the aqueous extract, but its efficiency as a traditional remedy.

Table 1: Antiplasmodial and cytotoxicity activities of the stem extract and compounds

Compound/ Reference	**Antiplasmodial activity IC_{50} (µg/mL)**		**Cytotoxicity activity IC_{50} (µM)**
	D6 strain (CQ sensitive)	W2 strain(CQ resistant)	MRC5
1	4.9±1.9 (19.3)*	3.6±0.3 (14.2)*	1.3 ± 0.5
3	15.4±1.6 (30.9)*	4.5±1.1 (9.0)*	6.7 ± 0.9
4	34.7±7.7 (64.2)*	30.8±9.6 (57.9)*	5.1 ± 0.8
5	3.9±0.3 (22.7)*	2.3±0.3 (10.2)*	5.7 ± 0.5
EtOAc extract	12.7±2.8	5.7±0.5	NT
Aqueous extract	Inactive	Inactive	NT
Chloroquine (CQ)	0.001±0.001	0.06±0.02	NT
Mefloquine (MQ)	0.006±0.0005	0.0015±0.0008	NT
Taxotere	NT	NT	31.5 ± 4.5

*IC_{50} values in µM.
NT: Not Tested

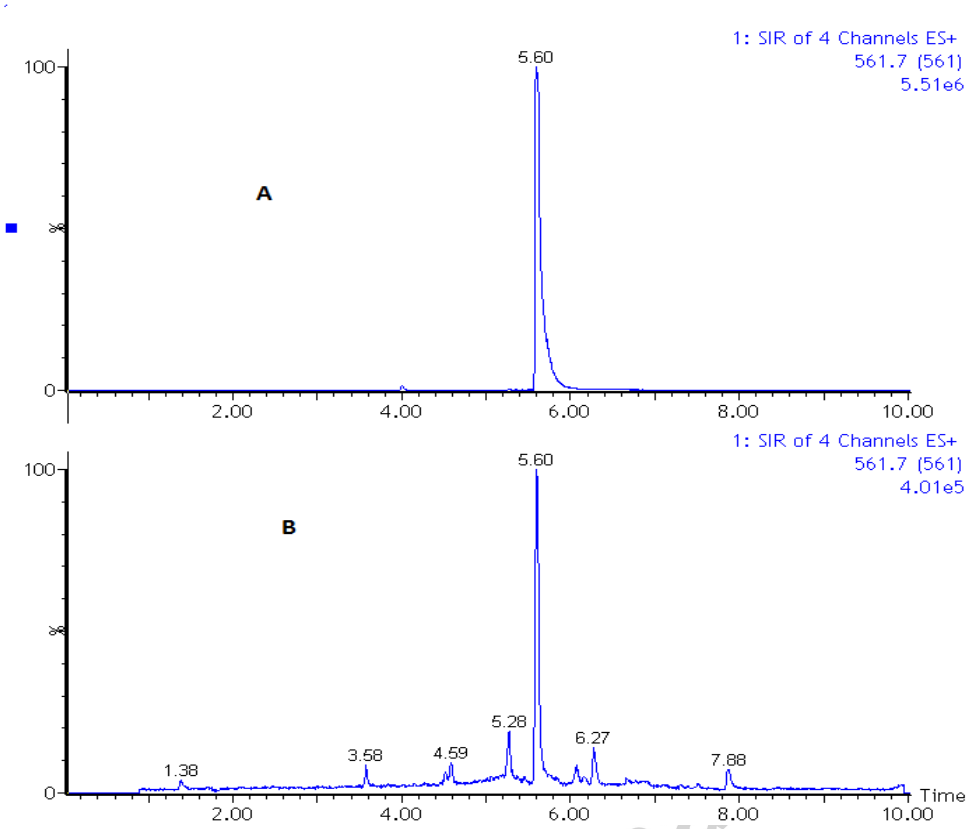


Figure 4: Chromatograms showing the presence of compound **5** (figure 4A) in the aqueous extract (Figure 4B**)**

4. Conclusion

In conclusion, this is the first report on antiplasmodial activity of the compounds isolated from the stem bark of *N. macrocalyx*. Unfortunately, this *in vitro* activity could be related to their cytotoxic nature and their potential as anti malarial compounds is thus limited. Despite an indirect *in vitro* antiplasmodial activity of some compounds isolated from the stem bark of *N. macrocalyx*, the identification of these bioactive compounds indicates that they may play a role in the pharmacological properties of this plant. The presence, in the trace amounts of the active compounds in the aqueous extract, mimicking the traditional use of the drug, does not provide direct supportive *in vitro* antiplasmodial activity evidence for the indicated use of *N. macrocalyx* in traditional medicine for the treatment of malaria. An alternative "reverse pharmacology" approach where a clinical evaluation of the phytomedicine is done, could be used to explain better the use of the plant.

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Table 1: Antiplasmodial and cytotoxicity activities of the stem extract and compounds

Compound/ Reference	Antiplasmodial activity IC_{50} (µg/mL)		Cytotoxicity activity IC_{50} (µM)
	D6 strain (CQ sensitive)	W2 strain(CQ resistant)	MRC5
1	4.9±1.9 (19.3)*	3.6±0.3 (14.2)*	1.3 ± 0.5
3	15.4±1.6 (30.9)*	4.5±1.1 (9.0)*	6.7 ± 0.9
4	34.7±7.7 (64.2)*	30.8±9.6 (57.9)*	5.1 ± 0.8
5	3.9±0.3 (22.7)*	2.3±0.3 (10.2)*	5.7 ± 0.5
EtOAc extract	12.7±2.8	5.7±0.5	NT
Aqueous extract	Inactive	Inactive	NT
Chloroquine (CQ)	0.001±0.001	0.06±0.02	NT
Mefloquine (MQ)	0.006±0.0005	0.0015±0.0008	NT
Taxotere	NT	NT	31.5 ± 4.5

*IC_{50} values in µM.
NT: Not Tested

Graphical abstract

Chemical investigation of the extract from the stem bark of *Neoboutonia macrocalyx* led to the isolation of one new diterpenoid with two known diterpenoids, a phenanthrene and a sterol.
The compounds displayed moderate to good antiplasmodial activity but with high cytotoxicity.