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Bioactive secondary metabolites from the leaves of *Secamone africana* (Olive.) Bullock

Peter SEKANDI[1], Jane NAMUKOBE[1*], Robert BYAMUKAMA[1], Hoseah M. AKALA[2], Redemptah A. YEDA[2] and Matthias HEYDENREICH[3]

[1]*Department of Chemistry, College of Natural Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda.*
[2]*Department of Emerging and Infectious Diseases (DEID), United States Army Medical Research Directorate-Africa (USAMRD-A), Kenya Medical Research Institute (KEMRI) Kisumu, Kenya.*
[3]*Institute of Chemistry, University of Potsdam, Germany.*
[*]*Corresponding author; E-mail: jnamukobe@cns.mak.ac.ug, Tel: +256700830095*

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ABSTRACT

Secamone africana leaves are used in the treatment of malaria and other ailments in Uganda. The aim of the study was to characterize the antiplasmodial compounds from the leaves of *Secamone africana*. The leaves were extracted sequentially using dichloromethane (DCM) and methanol (MeOH). The crude extracts and isolated compounds were evaluated for their antiplasmodial activity against the chloroquine sensitive Sierraleone I (D6) and chloroquine-resistant Indochina I (W2) strains of *Plasmodium falciparum*. Isolation and purification were done using chromatographic techniques including column chromatography and high performance liquid chromatography. The isolated compounds were characterized using spectroscopic methods. The MeOH extract (IC_{50} = 5.45 µg/mL) was found to be more active than the DCM extract (IC_{50}= 15.93 µg/mL) against the D6 malaria parasite. Chemical investigation of the MeOH extract yielded one new compound; 2-(2,4-dimethyloxetan-2-yl) acetic acid (**3**) in addition to the six known compounds; α-linolenic acid (**1**), conduritol B (**4**), β-sitosterol (**5**), 3,4-dihydroxybenzoic acid (**6**), 4-hydroxybenzoic acid (**7**) and coumaric acid (**8**). The DCM extract yielded one known compound: 1-methyl cyclobutene (**2**). The presence of these compounds with good anti-plasmodial activities and other bioactivities reported in literature, appears to argue for the therapeutic potential of *Secamone africana*.

Keywords: *Secamone africana*,, anti-plasmodial activity, chromatography, secondary metabolites.

INTRODUCTION

Secamone are small herbs that are well known for their medicinal properties and therefore used in traditional medicine in the management of many ailments (Malan et al., 2015; Vaiyapuri et al., 2015). *Secamone africana* is locally known in Luganda dialect as “akatakura” and “akateganende” in Rutooro

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dialect. The plant is a liana that climbs on trees, has smooth leaves and produces milky latex on damage. In Uganda, *Secamone africana* has been reported for treatment of malaria, syphilis, constipation, menstrual pains, swelling in children as well as antenatal diseases (Hamill 2003; Zabri et al., 2008; Namukobe et al., 2011). The ethanolic and water crude extracts of *Secamone africana* has been reported to have a high worm motility inhibition, anti-plasmodium and purgative activity as well as anthelmintic activity against *Ascaris suum* (Katuura et al., 2007; Nalule et al., 2013). Previous phytochemical analysis of the extracts of *Secamone africana* yielded alkaloids, flavonoids, phenols Terpenoids and glycosides as the major metabolites (Nalule et al., 2013; Vaiyapuri et al., 2015). Furthermore, reducing sugars, coumarines, proteins, tannins, sterols, polyterpenes, quinones, Anthocyanins, glycosides and aglycones have been extracted from *Secamone afzelii* (Zabri et al., 2008; 2009) in addition to antioxidants such as quercetin and rutin (Magid et al., 2016). The presence of these phytochemicals have aided the plant to possess medicinal values such as anti-inflammatory, anticancer, cytotoxic, antioxidant and antimalarial activities (Zabri et al., 2008; Wong et al., 2013; Mensah et al., 2014). Malaria is one of the leading causes of infections and deaths recording 455,000 deaths in 2017 worldwide with most deaths in sub-Saharan Africa (WHO, 2018). The increasing prevalence of resistant strains of *plasmodium* and difficulties to access and buy effective antimalarial are the major factors responsible for the increasing mortality rates that occur mainly in Africa (Atang et al 2019). Hence the need to use medicinal plants as complementary and alternative medicine for malaria treatment. Moreover, most of the current antimalarial like artemisinin and quinine are based on compounds isolated from plant extracts. It is therefore important to analyze plants for their bioactive constituents as this is very important in increasing their full exploitation and utilization in modern medicine (Chichir et al 2018; Mayaka et al 2019). The aim of the study therefore was to assess the antiplasmodial activity of the compounds isolated from the leaves of *Secamone africana*.

MATERIALS AND METHODS

Plant material collection and preparation of samples

After identification and authentication by a taxonomist, the leaves of *S. africana* were collected from Buikwe District in Uganda (00 08. 102 N, 33 00. 380 E), in a forested area that exists along the shores of Lake Victoria. A voucher specimen number SP 001 has been deposited at Makerere University Herbarium, Department of Plant Science, Microbiology and Biotechnology. The leaves were air dried at room temperature for 10 days. Dried samples were then pounded to a fine powder using a grinder. The powder sample (1.0 kg) was extracted sequentially using DCM and MeOH at room temperature. The extraction was carried out three times using 2 L of the solvent for each time of extraction. Filtration of the resultant extracts was done using cotton wool and Whatman No. 1 filter paper. The extracts were concentrated using a rotary evaporator at 40 ^{0}C and the dried extracts were transferred into sample bottles. In order to remove traces of water, the sample bottles were placed in a desiccator containing anhydrous sodium sulphate and later stored in a refrigerator awaiting further analysis. Dichloromethane extraction yielded 37.0 g while methanol extraction yielded 43.0 g. Reference drugs were provided by World Wide Antimalarial Resistance Network (WWARN), Malaria Drug Reference Material Programme. The reagents for reference clones were obtained from Biodefense and Emerging Infections Research Resources Repository (BEI Resources), National Institute of Allergy and Infectious Diseases, National Institutes of Health: *Plasmodium falciparum*, Strains D6, MRA-285; Strain W2, MRA-157 were contributed by Dennis E. Kyle.

Anti-plasmodial activity of the extracts and isolated compounds

The extracts and isolated compounds were assayed using a non-radioisotopic assay technique described by Smilkstein and co-

workers (2004) with modifications (Juma et al., 2011; Cheruiyot et al., 2016). Briefly, chloroquine sensitive SierraLeone I (D6) and chloroquine-resistant Indochina I (W2) strains of *Plasmodium falciparum* were cultured as described by Cheruiyot et al. (2016). Standard reference drugs and compounds were dissolved in 99.5% dimethylsulfoxide (DMSO) (Sigma-Aldrich) and diluted in complete Roswell Park Memorial Institute 1640 series of Cell Culture Medium (RPMI 1640) prepared as described by Akala et al. (2011). Briefly, the basic culture medium was prepared from 10.4 g RPMI 1640 powder (Invitrogen, Inc.) augmented with 2 g glucose (Sigma Inc.) and 5.95 g of HEPES (Sigma Inc.) dissolved to homogeneity in one liter of de-ionized water and sterilized with a 0.2 µm filter.

Complete RPMI 1640 media, used for all parasite cultures and drug dilutions, consisted of basic RPMI 1640 media with 10% (vol/vol), human ABO pooled plasma, 3.2% (vol/vol) sodium bicarbonate (Thermo Fisher Scientific Inc.) and four µg/ml hypoxanthine (Sigma Inc.). Complete RPMI 1640 media was stored at 4 °C and used within two weeks. Concurrently, two-fold serial dilutions of chloroquine (0.977 to 2,000 ng/ml), mefloquine (0.244 to 500 ng/ml) and test compounds or extracts (24.414 to 50,000 ng/ml) were prepared on a 96-well plate, such that the amount of DMSO was equal to or less than 0.0875%. The 10 doses for each drug or compound were added to specified wells on a row of the 96-well drug plate.

In vitro drug testing was initiated when the culture-adapted *P. falciparum* at 5% hematocrit with greater than 3% parasitemia were adjusted to 2% hematocrit and 0.5% parasitemia, then added on to the plate containing a dose range of drugs and incubated in gas mixture comprising 5% CO_2, 5% O_2, and 90% N_2 at 37 °C. Each drug was tested in three biological replicates i.e. in three 96-well plates. The assay was terminated after 72 hours, SYBR Green dye in the lysis buffer was added and then kept in dark for 24 hours (Cheruiyot et al., 2016). The fluorescence intensity was measured from the bottom of the plate with a GENios Plus plate reader with excitation wavelengths of 485 nm, emission wavelengths of 535 nm, gain set at 60 and number of flashes set at 10 (Akala et al., 2011). Parasite growth inhibition was quantified using GraphPad Prism software version 5.02 from GraphPad Software Inc. CA, USA (Johnson et al., 2007) and presented as mean ± standard deviation (mean IC_{50} ± SD).

Isolation and purification of compounds

The MeOH extract (21.0 g) was loaded on a column filled with silica gel and eluted with a gradient solvent system of Petroleum ether (PE) - Methyl *tert*-butyl ether (MTBE) and then PE: MTBE: MeOH to obtain purified fractions. Compound **1** (11.1 mg) was obtained from fraction 3 having eluted from the column with a solvent system of PE: MTBE (90:10; v/v). Compound **4** was obtained from fraction 21 at solvent system of PE: MTBE: MeOH (50:40:10 v/v)**.** The rest of the fractions that consisted of major compounds in the extract were subjected to further purification. Purification using Sephadex LH-20 column with MeOH and DCM (1:1; v/v) of fractions 17, 2, and 19 yielded compound **3** (10.3 mg), compound **5** (24.7 mg), and compound **6 (**5.9 mg**)** respectively. Fraction 15 was purified using a preparative HPLC with a gradient elution of ACN: H_2O + 0.05% TFA starting from 50: 50 to 0: 100 and finally 50:50 (ACN:H_2O) to obtain compounds **7** (1.5 mg) and **8** (2.5 mg). The DCM extract was fractionated on a silica gel column using a gradient system of PE - MTBE to yield 12 fractions (1-12). Fractions 5-9 which consisted of the major compounds in the extract were combined and purified on a Sephadex column using MeOH: DCM (1:1 v/v) to yield compound **2** (10.0 mg).

Optical rotation and Force-field calculations of compound 3

The optical rotation of compound **3** was determined using a polarimeter. The polarimeter was set to optical rotation mode and left to warm for 10 minutes. The optical rotation of $CHCl_3$ and that of the sample was measured. For calculating the energy minimum conformations of **3** the FFF

minimization option within the PERCH Suite software was used on an Intel® Core™ i7 CPU (Marcelo et al., 2012).

Identification of the isolated compounds

Identification of the isolated compounds was achieved using Nuclear Magnetic Resonance (NMR) and Mass spectroscopic (MS) techniques according to the method described by Byamukama et al (2015). Generally, the sample was dissolved in $CDCl_3$ and transferred into an NMR tube. The sample was then loaded onto the NMR auto-sampler spectrometer (Bruker Avance 500) where 1-dimensional ^1H and ^{13}C- NMR spectra along with 2- dimensional COSY, HMQC, HMBC, and NOESY experiments were used to elucidate the structures. For MS, each sample solution was introduced into the mass spectrometer (GC-TOF Micromass or Micromass Q-TOF micro, Waters Inc.) for analysis. All the spectra were analysed and the results were compared with published information in literature in order to elucidate and confirm structures of the known isolated compounds.

Linolenic acid (**1**); yellow oily liquid; ^1H-NMR (500 MHz, $CDCl_3$): δ_H1.25 (H-2), 2.35 (H-3), 1.63 (H-4), 1.25 (H-5), 1.25 (H-6), 1.25 (H-7), 2.80 (H-8), 5.36 (H-9), 5.36 (H-10), 2.06 (H-11), 5.36 (H-12), 5.36 (H-13), 2.80 (H-14), 5.36 (H-15), 5.36 (H-16), 2.06 (H-17), 0.97 (H-18). ^{13}C-NMR (125MHz, $CDCl_3$): δ_C179.3(C-1), 29.0 (C-2), 33.8 (C-3), 24.6 (C-4), 29.6 (C-5), 29.5 (C-6), 29.1 (C-7), 25.5 (C-8), 127.0 (C-9), 127.7 (C-10), 20.5 (C-11), 128.2 (C-12), 128.2 (C-13), 25.5 (C-14), 130.2 (C-15), 131.9 (C-16), 27.1 (C-17), 14.2 (C-18). EI-MS (positive ion mode) m/z 278 $[M]^+$, $C_{18}H_{30}O_2$. IR (cm^{-1}); 3050, 2854, 2925, 1709, 1453, 910, 732.

1-Methylcyclobutene (**2**); yellow oily liquid; ^1H-NMR (500 MHz, $CDCl_3$): δ_H 5.12 (H-2), 2.04 (H-3), 2.03 (H-4), 1.67 (H-5). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 135.2 (C-1), 125.0 (C-2), 26.3 (C-3), 32.1 (C-4), 23.4: (C-5). EI-MS (negative ion mode) m/z 67 $[M-H]^+$, C_5H_8.

Conduritol B (**4**); white crystals; ^1H-NMR (500 MHz, D_2O): δ_H 3.35 (H-1), 4.07 (H-2), 5.51 (H-3). ^{13}C-NMR (125 MHz, D_2O): δ_C 77.6 (C-1), 73.8 (C-2), 130.9 (C-3). ESI-MS (positive ion mode) m/z 169.04 $[M+Na]^+$, $C_6H_{10}O_4$.

β-Sitosterol (**5**); White needle-like crystals; ^1H-NMR (500 MHz, $CDCl_3$): δ_H 1.76 (H-1), 1.43 (H-2), 3.46 (H-3), 2.19 (H-4), 5.28 (H-6), 1.92 (H-7), 1.76 (H-8); 0.85 (H-9),1.40 (H-11), 1.93 (H-12), 0.93 (H-14), 1.52 (H-15), 1.76 (H-16), 1.03 (H-17), 0.60 (H-18), 0.93 (H-19), 1.28 (H-20), 0.85 (H-21), 2.27 (H-22), 1.08 (H-23), 0.85 (H-24), 1.25 (H-25), 0.74 (H-26), 0.76 (H-27), 1.18 (H-28), 0.77 (H-29); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.8 (C-7) 31.8 (C-8), 50.1 (C-9), 36.4 (C-10), 21.0 (C-11), 39.7 (C-12), 42.3 (C-13), 56.7 (C-14), 24.2 (C-15), 28.2 (C-16),56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 36.1 (C-20), 18.7 (C-21), 33.9 (C-22), 26.0 (C-23), 45.8 (C-24), 29.0 (C-25), 19.0 (C-26), 19.8 (C-27), 23.0 (C-28), 11.9 (C-29). EI-MS (positive ion mode) m/z 414 $[M+H]^+$, $C_{29}H_{50}O$.

3,4-Dihydroxybenzoic acid (**6**); yellow powder; ^1H-NMR (500 MHz, CD_3OD): δ_H 7.25 (H-2), 6.77 (H-5), 6.95 (H-6). ^{13}C-NMR (125 MHz, CD_3OD):δ_C 113.8 (C-1), 116.0 (C-2), 150.4 (C-3) 156.3 (C-4), 118.6 (C-5), 124.6 (C-6), 173.6 (C-7). ESI-MS (positive ion mode) m/z 154.02 $[M + Na]^+$, $C_7H_6O_4$. IR (cm^{-1}); 3215, 1671, 1019, 1484, 1458.

4-Hydroxybenzoic acid (**7**); white solid; ^1H-NMR (500 MHz, CD_3OD): δ_H 7.45 (H-2), 6.91 (H-3), 6.87 (H-5), 7.84 (H-6). ^{13}C-NMR (125 MHz, CD_3OD): δ_C 129.0 (C-1), 136.5 (C-2), 118.1 (C-3) 163.1 (C-4), 120.0 (C-5), 131.5 (C-6), 173.6 (C-7). EI-MS (positive ion mode) m/z 138.03 $[M]^+$, $C_7H_6O_3$. IR (cm^{-1}); 3345, 2502, 1655, 1019, 1484, 1458.

Coumaric acid (**8**); white-yellowish powder; ^1H-NMR (500 MHz, CD_3OD): δ_H 7.45 (H-2), 6.81 (H-3), 7.61 (H-7), 6.29 (H-8). ^{13}C-NMR (125MHz, CD_3OD):δ_C 127.2 (C-1), 131.0 (C-2), 116.7 (C-3) 161.1 (C-4), 146.6 (C-7), 115.5 (C-8), 171.0 (C=O). EI-MS (positive ion mode) m/z 164 $[M]^+$, $C_9H_8O_3$. IR (cm^{-1}); 3317, 2506, 1170, 1680, 1024, 1603, 1514, 832.

RESULTS

Characterization of compounds

Analysis of the NMR data of the isolated compounds led to the identification of one new compound (**3**) in addition to seven known compounds which included linolenic acid (**1**), 1-methylcyclobutene (**2),** conduritol B (**4**), β-sitosterol (**5**), 3,4-hydroxybenzoicacid (**6**), 4-hydroxybenzoicacid (**7**) and coumaric acid (**8**). The chemical structures of the known compounds (Figure **1**) were confirmed by comparison of the experimental results with those published in literature.

Compound 3 was isolated as a dark green powder and its molecular formula was established as $C_7H_{12}O_3$ on the basis of the basis of its NMR and MS data (m/s 85 [M-CH_2 COOH]$^+$, 58 [CH2 COO]$^+$, 143 [M-H]$^+$). This formula indicated that the compound has two double bond equivalents. The IR spectrum showed abroad absorption peak at cm^{-1}; 3415 typical for a carboxylic O-H stretch, 1721 for C=O, 2975 for C-H aliphatic stretch, 1247 and 1084 for C-O group. Analysis of the ^{1}H-NMR spectrum (Table 1) showed one oxy methine proton at δ_H 4.36, two methylene groups at δ_H 2.55/2.61 (typical of protons near the carbonyl group) and at δ_H 1.77/2.01 ppm. The ^{1}H-NMR also showed two methyl groups at δ_H 1.37 and 1.38 ppm. The ^{13}C-NMR showed a carboxylic signal at δ_C 172.0, oxymethine carbon at δ_C 73.9, two methyls at δ_C 21.4 and 29.2 ppm, and the δ_C 68.8 ppm was assigned to a quaternary carbon. In order to account for the two double bond equivalents, these results indicated that the compound contains a ring. The position of the C=O was determined from correlation from the HMBC spectrum at δ_H 2.55 (C*H*$_2$-COOH) with δ_C 172.0 (C=O) and 68.8 (C-2) ppm. The COSY spectrum revealed correlations between protons at δ_H 1.77 with 4.36 ppm which also correlated with protons at δ_H 1.38. The NOESY spectrum indicated that C*H*$_2$-COOH correlated with H-4 (δ_H4.36 ppm) and H-3 (δ_H 1.77). Thus, compound **3** is 2-(2,4-dimethyloxetan-2-yl) acetic acid (Figure 2). This is the first report of this compound. The optical rotation value of **3** was found to be-29.9^0 (MeOH). To get more information about the relative configuration of **3** the energy minimum conformations of **RR-3** and **SR-3** were force-field calculated by the PERCH Suite (Marcelo et al., 2012) and are shown in Figure 3.

Selected distances from the calculation of both stereoisomers are compared with experimental NOE's (Table 2). In this way the relative stereo configuration can be determined. For this, it is necessary to distinguish the two protons at position 3. Fortunately, one of these protons show a long-range coupling of 0.8 Hz ($^4J_{H,H}$, 'W'-coupling) to the C*H*$_2$COO protons. In case of **RR-3,** it must be the pro-R and for **SR-3,** it is the pro-S proton. It can be clearly seen that only the RR configuration fits the experimental NOE data. Thus, the relative stereochemistry is 2R*4R*.

Antiplasmodial activity of the crude extract and isolated compounds

The crude MeOH extract and isolated compounds were tested for their antiplasmodial activity and inhibited the growth of both the chloroquine-sensitive (D6) and chloroquine resistant (W2) malaria parasite strains. The crude DCM extract was tested for its antiplasmodial activity against D6 malaria parasite strains (Table 3). Antiplasmodial activity of the crude extracts and isolated compounds was classified as follows; extracts with IC_{50} < 5 µg/mL were highly active, IC_{50} between 5-15 µg/mL were moderately active and promising, low activity at IC_{50} between 15–50 µg/mL and inactive at IC_{50}>50 µg/mL (Batista et al., 2009; Namukobe et al., 2015). Basing on the above classification, the MeOH crude extract was moderately active against D6 (IC_{50}= 5.45 µg/mL) and W2 (IC_{50} = 12.34 µg/mL) malaria strains. The activity of the DCM crude extract (IC_{50}= 15.93 µg/mL) was very low against D6 malaria strain. Compound **2** was the most active (0.44 µg/mL) followed by compound **1** (2.54 µg/mL) and compound **3** with IC_{50} of 24.63 µg/mL against the chloroquine-sensitive D6 strains.

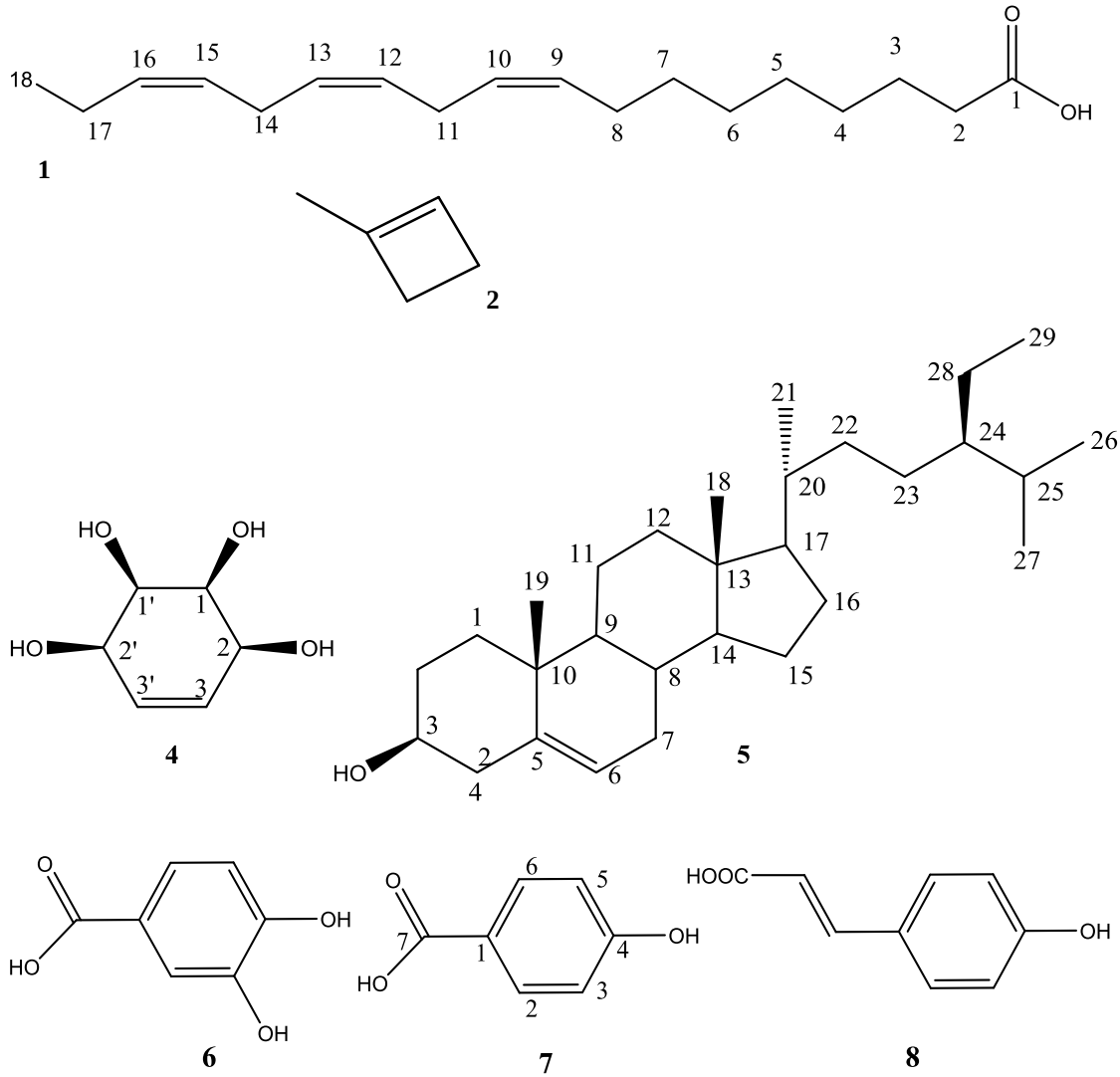

Figure 1: Chemical structures of compounds **1-2** and **4-8** isolated from the leaves of *Secamone africana.*

Table 1: ^{1}H-NMR and ^{13}C-NMR data of compound **3** isolated from the leaves of *Secamone Africa*

^{1}H/^{13}C No.	**^{1}H- NMR (ppm), J**	**^{13}C- NMR (ppm)**
C=O		172.0
CH$_2$-COOH	2.61 (*dd*, 16.2, 0.7 Hz)	
	2.55 (*d*, 16.2 Hz)	44.9
2		68.8
3	1.77: (*dd*, 14.1, 11.6 Hz)	45.0
	2.01 (*ddd*, 14.2, 3.7, 0.8 Hz)	
4	4.36 (*m*)	73.9
4-Me	1.38: (*d*, 6.4 Hz)	21.4
2-Me	1.37 (*s*)	29.2

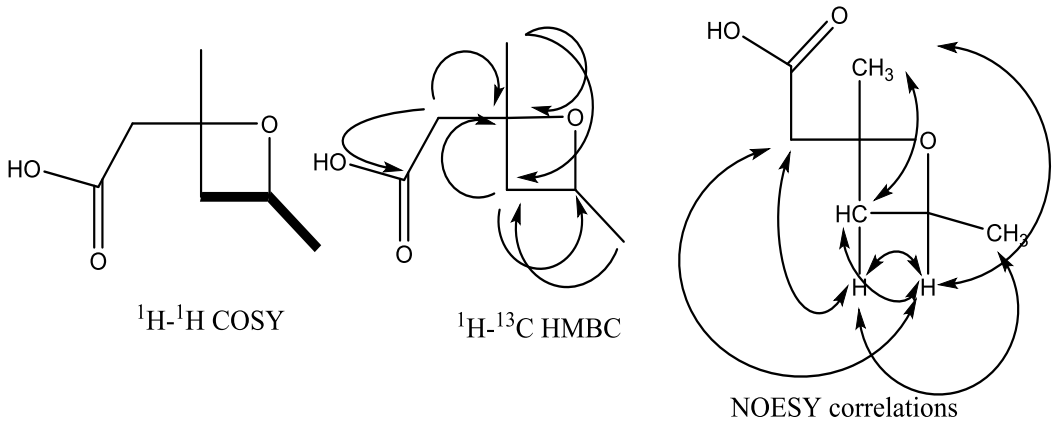

Figure 2: Structure of compound **3** showing ^{1}H-^{1}H COSY, ^{1}H-^{13}C HMBC and NOESY correlations.

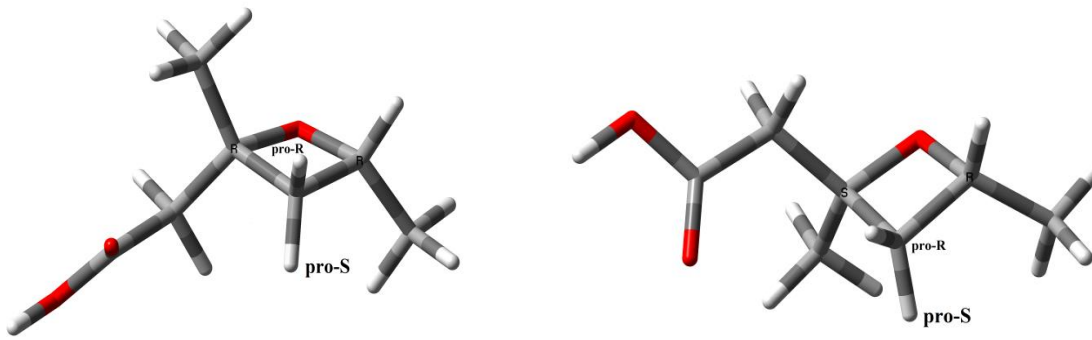

Figure 3. Energy-minimized conformations of **RR-3** (left) and **SR-3** (right) of the new compound **3** isolated from *Secamone africana.*

Table 2: Comparison of selected distances with experimental NOE's for **RR-3** and **SR-3.**

RR-3		**SR-3**		**Experiment**
δ ^{1}H (ppm)	distance to H-4 (Å)	δ ^{1}H (ppm)	distance to H-4 (Å)	NOE
pro-R: 2.01	2.42	pro-S: 2.01	3.05	strong
pro-S: 1.77	3.06	pro-R: 1.77	2.46	medium

Table 3: Antiplasmodial activity of the extracts and compounds against Chloroquine sensitive Sierraleone I (D6) and chloroquine-resistant Indochina I (W2) strains of *Plasmodium falciparum*.

Drug/Compound	Antiplasmodial activity IC_{50} (µg/mL)	
	W2 (CQ Resistant Clone ± SD	D6 (CQ Sensitive Clone) ± SD
Chloroquine	0.0621 ± 0.0141	0.0043 ± 0.0003
Mefloquine	0.0029 ± 0.0010	0.0118 ± 0.0004
DCM extract	NT	15.93 ± 3.46
MeOH extract	12.34 ± 7.10	5.45 ± 3.98
Compound 1	inactive	2.54 ± 0.63
Compound 2	Inactive	0.44 ± 0.18
Compound 3	Inactive	24.63 ± 10.06
Compound 4	Inactive	Inactive
Compound 5	inactive	Inactive
Compound 6	Inactive	Inactive
Compound 7	Inactive	Inactive
Compound 8	Inactive	Inactive

SD = Standard Deviation; Activities with IC_{50} above 50 µg/mL were regarded as inactive (Batista et al., 2009).

DISCUSSION

The activity of the crude MeOH extract could be due to the presence of Linolenic (**1)** and the new compound; 2-(2,4-dimethyloxetan-2-yl) acetic acid (**3)** while that of the DCM could be due to 1-methylcyclobutene (**2)**. The presence of these anti-plasmodial compounds in presence of other compounds that have shown other bioactivities justifies the use of the plant in malaria treatment and other ailments. There exist many pharmacological reports on the compounds that have been isolated from this plant. For instance, Linolenic acid (**1**) has been reported to have medicinal properties such as anti-inflammatory (Gdula-Argasińska et al., 2017). Though there are no reported pharmacological activities reported for 1-methyl cyclobutene (**2**), related compounds with cyclobutane ring such as tripartilactum from *Streptomyces* species have been reported to act as N^+/K^+ ATPase inhibitor (Park et al., 2012). This study however, is the first to report the antiplasmodial activity of 1-methyl cyclobutene (**2**).

2-(2,4-dimethyloxetan-2-yl) acetic acid (**3**) belongs to oxetanes group, and oxitanes forinstance the well-known paclitaxel, or Taxol used in cancer chemotherapy is one of the oxetanes that has been isolated from plants (Bull et al., 2016). Similar compounds such as penicillin scaffolds containing β-lactam are well known for their antibacterial activity (Bbosa et al., 2014). Heterocyclic oxygen containing compounds such as iridoids have been reported to exhibit a wide range of biological properties such as antimalarial, antibacterial and antioxidant property (Schripsema et al., 2007). Protocatechuic (**6**) acid and other phenolic acids have shown biological properties such as anti-inflammatory and anti-antimicrobial activity (Sahil et al., 2014; Takuji et al., 2011) 4-hydroxylbenzoic (**7**) acid and its derivatives have been reported to have antimicrobial and antibacterial properties (Batista et al., 2009; Heleno et al., 2015). Coumaric acid (**8**) has

been reported to have antioxidant and antibacterial properties (Torres et al., 2001; Lou et al., 2012). The obtained antiplasmodial activity of the plant and other reported activities in literature could explain the use of the plant in the treatment of malaria and other diseases like swellings in children and syphillis.

Conclusion

The research study has yielded one new compound (2,4-dimethyl-oxetan-2-yl) acetic acid and seven known compounds; α-linolenic acid (**1**), 5-cyclohexene-1,2,3,4-tetrol (**4**), β-sitosterol (**5**), protocatechuic acid (**6**), 4-hydroxylbenzoic acid (**7**), coumaric acid (**8**), and 1-methyl cyclobutene (**2**) from the leaves of *Secamone africana.* The isolated compounds have been reported for the first time in this plant and compound **2** has been found to have a high antiplasmodial activity. Though the rest of the isolated compounds did not show significant antiplasmodial activity, their presence with different bioactivities reported in literature indicate that they are important in the therapeutic potential of *Secamone africana*. Although the bioassays revealed substantial antiplasmodial activities, further cytotoxicity testing on the crude extract and isolated compounds should be carried out to establish their selectivity. Meanwhile these findings support the use of *Secamone africana* for the traditional treatment of malaria and other ailments.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

PS: data collection, data analysis and manuscript writing; JN: research design, data collection, data analysis and manuscript writing; RB: data analysis and manuscript writing; HMA: data collection, analysis and manuscript writing; RAY: data collection and analysis; MH: data analysis and manuscript writing.

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