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

Peter SEKANDI¹, Jane NAMUKOBE¹*, Robert BYAMUKAMA¹, Hoseah M. AKALA², Redemptah A. YEDA² and Matthias HEYDENREICH³

¹Department of Chemistry, College of Natural Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda.
²Department of Emerging and Infectious Diseases (DEID), United States Army Medical Research Directorate-Africa (USAMRD-A), Kenya Medical Research Institute (KEMRI) Kisumu, Kenya.
³Institute of Chemistry, University of Potsdam, Germany.

*Corresponding author: E-mail: jnamukobe@cns.mak.ac.ug, Tel: +256700830095

ACKNOWLEDGEMENTS
We acknowledge RISE competitive funds for graduates through the Natural Products Research Partnership for funding the Research. We further acknowledge the OPCW fellowship programme (L/ICA/ICB/210805/17) for the Internship support.

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 Sierra Leone 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 (IC₅₀ = 5.45 µg/mL) was found to be more active than the DCM extract (IC₅₀ = 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.

© 2020 International Formulae Group. All rights reserved.

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
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 °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-
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) 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: H₂O + 0.05% TFA starting from 50: 50 to 0: 100 and finally 50:50 (ACN:H₂O) 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 CHCl₃ and that of the sample was measured. For calculating the energy minimum conformations of 3 the FFF 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 with a 0.2 μl PE: MTBE) with a gradient elution system of PE: MTBE (90:10; v/v). 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 IC₅₀ ± SD).
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 CDCl₃ 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 ¹³C-NMR spectra along with 2-dimensional COSY, HMOC, 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, CDCl₃): δH 1.25 (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). ¹³C-NMR (125 MHz, CDCl₃): δC 179.3 (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]+, C₀₉H₁₈O₂. IR (cm⁻¹): 3050, 2854, 2925, 1709, 1453, 910, 732.

1-Methylocyclobutene (2); yellow oily liquid; ¹H-NMR (500 MHz, CDCl₃): δH 5.12 (H-2), 2.04 (H-3), 2.03 (H-4), 1.67 (H-5). ¹³C-NMR (125 MHz, CDCl₃): δC 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]+, C₅H₆.

Conduritol B (4); white crystals; ¹H-NMR (500 MHz, D₂O): δH 3.35 (H-1), 4.07 (H-2), 5.51 (H-3). ¹³C-NMR (125 MHz, D₂O): δC 77.6 (C-1), 73.8 (C-2), 130.9 (C-3). ESI-MS (positive ion mode) m/z 169.04 [M+Na]+, C₁₀H₁₀O₄.

β-Sitosterol (5); White needle-like crystals; ¹H-NMR (500 MHz, CDCl₃): δH 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); ¹³C-NMR (125 MHz, CDCl₃): δC 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]+, C₂₃H₃₈O₇.

3,4-Dihydroxybenzoic acid (6); yellow powder; ¹H-NMR (500 MHz, CD₃OD): δH 7.25 (H-2), 6.77 (H-5), 6.95 (H-6). ¹³C-NMR (125 MHz, CD₃OD): δC 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]+, C₆H₆O₃. IR (cm⁻¹): 3215, 1671, 1019, 1484, 1458.

4-Hydroxybenzoic acid (7); white solid; ¹H-NMR (500 MHz, CD₃OD): δH 7.45 (H-2), 6.91 (H-3), 6.87 (H-5), 7.84 (H-6). ¹³C-NMR (125 MHz, CD₃OD): δC 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]+, C₇H₆O₃. IR (cm⁻¹): 3345, 2502, 1655, 1019, 1484, 1458.

Coumaric acid (8); white-yellowish powder; ¹H-NMR (500 MHz, CD₃OD): δH 7.45 (H-2), 6.81 (H-3), 7.61 (H-7), 6.29 (H-8). ¹³C-NMR (125MHz, CD₃OD): δC 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]+, C₇H₆O₃. 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-methyleclobutene (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 C₇H₁₂O₃ on the basis of the basis of its NMR and MS data (m/z 85 [M-CH₂COOH]+, 58 [CH₂COO]+, 143 [M-H]+). This formula indicated that the compound has two double bond equivalents. The IR spectrum showed broad 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 ¹H-NMR spectrum (Table 1) showed one oxy methine proton at δH 4.36, two methylene groups at δH 2.55/2.61 (typical of protons near the carbonyl group) and at δH 1.77/2.01 ppm. The ¹H-NMR also showed two methyl groups at δH 1.37 and 1.38 ppm. The ¹³C-NMR showed a carboxylic signal at δC 172.0, oxymethine carbon at δC 73.9, two methyls at δC 21.4 and 29.2 ppm, and the δC 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 δH 2.55 (CH₂-COOH) with δC 172.0 (C=O) and 68.8 (C-2) ppm. The COSY spectrum revealed correlations between protons at δH 1.77 with 4.36 ppm which also correlated with protons at δH 1.38. The NOESY spectrum indicated that CH₂-COOH correlated with H-4 (δH4,3.46 ppm) and H-3 (δH 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 -29.9⁰ (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 (³JHH, W-coupling) to the CH₂-COOH 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 antimalarial 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 antimalarial activity against D6 malaria parasite strains (Table 3). Antiplasmodial activity of the crude extracts and isolated compounds was classified as follows; extracts with IC₅₀ < 5 µg/mL were highly active, IC₅₀ between 5-15 µg/mL were moderately active and promising, low activity at IC₅₀ between 15–50 µg/mL and inactive at IC₅₀>50 µg/mL (Batista et al., 2009; Namukobe et al., 2015). Basing on the above classification, the MeOH crude extract was the most active (0.44 µg/mL) followed by compound 1 (2.54 µg/mL) and compound 3 with IC₅₀ 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 ($dd$, 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-R: 2.01 | 3.05 | strong   |
| pro-S: 1.77 | 3.06       | pro-R: 1.77 | 2.46 | medium   |
DISCUSSION
The activity of the crude MeOH extract could be due to the presence of Linolenic (1) and the new compound; 2-(2,4-dimethyltetan-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-dimethyltetan-2-yl) acetic acid (3) belongs to oxetanes group, and oxetanes for instance 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

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

| Drug/Compound   | Antiplasmodial activity IC50 (µg/mL) |
|-----------------|--------------------------------------|
|                 | W2 (CQ Resistant Clone ± SD)         |
|                 | D6 (CQ Sensitive Clone) ± SD         |
| Chloroquine     | 0.0621 ± 0.0141                       |
| Mefloquine      | 0.0029 ± 0.0010                       |
| DCM extract     | NT                                   |
| MeOH extract    | 12.34 ± 7.10                         |
| Compound 1      | inactive                             |
| Compound 2      | Inactive                             |
| Compound 3      | Inactive                             |
| Compound 4      | Inactive                             |
| Compound 5      | inactive                             |
| Compound 6      | inactive                             |
| Compound 7      | inactive                             |
| Compound 8      | inactive                             |

SD = Standard Deviation; Activities with IC50 above 50 µg/mL were regarded as inactive (Batista et al., 2009).
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 syphilis.

**Conclusion**

The research study has yielded one new compound (2,4-dimethyl-oxytan-2-y1) 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-hydroxybenzoic 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.

**ACKNOWLEDGEMENTS**

We are indebted to the members in the groups of Prof. Dr. Heiko Möller and Prof. Dr. Bernd Schmidt both at Department of Chemistry, University of Potsdam (Germany), where part of this work was carried out.

**REFERENCES**

Akala HM, Eyase FL, Cheruiyot AR, Omondi AA, Ogutu BR, Waters NC, Johnson JD, Polhemus ME, Schnabel DC, Walsh DS. 2011 Anti-malarial drug sensitivity profile of western Kenya Plasmodium falciparum field isolates determined by a SYBR green I in vitro assay and molecular analysis. Am. J. Trop. Med. Hyg., 85(1): 34–41. DOI: http://dx.doi.org/10.4269/ajtmh.2011.10-0674.

Atang AD, Azi JY, Sani FO, Oyi RA, Ehinmidu JO. 2019. Malaria prevalence and In vitro susceptibility of Plasmodium falciparum isolates to selected antimalarial agents in Bauchi, Nigeria. Int. J. Biol. Chem. Sci., 13(6): 2714-2725. DOI:https://dx.doi.org/10.4314/ijbcs.v13i6.23.

Batista R, De Jesus Silva Junior A, de Oliveira A. 2009. Plant-derived antimalarial agents: New leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules., 14(8): 3037–3072. DOI: http://dx.doi.org/10.3390/molecules14083037.

Bbosa G, Mwebaza N, Odda J, Kyegombe B, Ntale M. 2014. Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance. Health., 6(5): 410-425. DOI: http://dx.doi.org/10.4236/health.2014.65059.

Bull JA, Croft RA, Davis OA, Doran R, Morgan KF. 2016. Oxetanes: Recent Advances in Synthesis, Reactivity, and Medicinal Chemistry. Chem. Rev., 116(19): 12150-12233. DOI: http://dx.doi.org/10.1021/acs.chemrev.6b00274.

Byamukama R, Ganza B, Namukobe J, Heydenreich M, Kiremire BT. 2015. Bioactive compounds in the stem bark of Albizia coriaria (Welw. ex Oliver). Int. J. Biol. Chem. Sci., 9(2): 1013-1024. 1013-1024. DOI: http://dx.doi.org/10.4314/ijbcs.v9i2.37.
Cheruiyot AC, Auschwitz JM, Lee PJ, Yeda RA, Okello CO, Leed SE, Talwar M, Murthy T, Gaona HW, Hickman MR, Akala HM, Kamau E, Johnson JD. 2016. Assessment of the Worldwide Antimalarial Resistance Network Standardized Procedure for In Vitro Malaria Drug Sensitivity Test using SYBR Green Assay for Field Samples with Varying Initial Parasitemia. Antimicrob. Agents Chemother., 60(4): 2417-2424. DOI:http://dx.doi.org/10.1128/AAC.00527-15.

Chichir KD, Cheploqoi KP, Omolo OJ, Langat KM. 2018. Chemical constituents of Solanum mauense (Solanaceae) and Dovyalis abyssinica (Salicaceae). Int. J. Biol. Chem. Sci., 12(2): 999-1007. DOI: http://dx.doi.org/10.4314/ijbcs.v12i2.29

Gdula-Argasińska J, Paśko P, Sülkowska-Ziaja K, Kała K, Muszyńska B. 2017. Anti-inflammatory activities of garlic sprouts, a source of α-linolenic acid and 5-hydroxy-L-tryptophan, in RAW 264.7 cells. Acta Biochim. Pol., 64(3): 551-559. http://dx.doi.org/10.18388/abp.2017_1534.

Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD. 2003. Traditional herbal drugs of southern Uganda, II: literature analysis and antimicrobial assays. J. Ethnopharmacol. 84(1): 57–78. DOI: http://dx.doi.org/10.1016/s0378-8741(02)00289-1.

Heleno SA, Martins A, Queiroz MJRP, Ferreira ICFR. 2015. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. Food Chem., 173: 501-513. DOI:http://dx.doi.org/10.1016/j.foodchem.2014.10.057.

Johnson JD, Dennull RA, Gerena L, Lopez-Sanchez M, Roncal NE, Waters NC. 2007. Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. Antimicrob. Agents Chemother., 51(6): 1926–1933. DOI: http://dx.doi.org/10.1128/AAC.01607-06.

Juma WP, Akala HM, Eyase FL, Muiva LM, Heydenreich M, Okalebo FA, Gitu PM, Peter MG, Walsh D, Imbuga M, Yenesew A. 2011. Terpurinflavone: An antiplasmodial flavone from the stem of Tephrosia Purpurea, Phytochem. Lett., 4(2): 176–178. DOI: http://dx.doi.org/10.1016/j.phytolet.2011.02.010.

Kakkar S, Baris S. 2014. A Review on Protocatechuic Acid and Its Pharmacological Potential. ISRN Pharmacology, 2014. DOI: http://dx.doi.org/10.1155/2014/952943.

Katuura E, Waako P, Ogwal-Okeng J, Bukenya-Ziraba R. 2007. Traditional treatment of malaria in Mbarara District, western Uganda. Afr. J. Ecol., 45(s1): 48-51. DOI: http://dx.doi.org/10.1111/j.1365-2028.2007.00737.

Lou Z, Wang H, Rao S, Sun J, Ma C, Li J. 2012. p-Coumaric acid kills bacteria through dual damage mechanisms. Food Control, 25(2): 550-554. DOI: http://dx.doi.org/10.1016/j.foodcont.2011.11.022.

Magid AA, Yao-Kouassi PA, Gossan DPA, Mairot C, Voutquenne-Nazabadioko L. 2016. New Antioxidant Flavonoids from the Aerial Parts of Secamone Afzelii. J. Antioxid. Act., 1(2): 8-16. DOI: http://dx.doi.org/10.14302/issn.2471-2140.jaa-15-887.

Maland DF, Neuba DFR, Kouakou KL. 2015. Medicinal plants and traditional healing practices in ehotile people, around the aby lagoon (eastern littoral of Côte d’Ivoire). J. Ethnobiol. Ethnomed., 11(1): 21. DOI: http://dx.doi.org/10.1186/s13002-015-0004-8.

Marcelo A, Nury PH, Mariano W, Guillermo SH, Pedro JN. 2012. Absolute Configuration and 1H NMR Characterization of Rosmaridiphenol Diacetate. J. Nat. Prod., 75: 779–778. DOI: http://dx.doi.org/10.1021/np200951y.

Mayaka RK, Langat MK, Njue AW, Cheploqoi PK, Omolo JO. 2019. Chemical compounds from the Kenyan polypore Trametes elegans (Spreng:Fr.) Fr (Polyporaceae) and their antimicrobial activity. Int. J. Biol. Chem. Sci., 13(4): 2352-2359. DOI:https://dx.doi.org/10.4314/ijbcs.v13i4.37

Mensah AY, Mireku EA, Okwuonu V. 2014. Anti-inflammatory and anti-oxidant activities of Secamone afzelii (Rhoem)
Asclepiadaceae. *J. Medical Biomed. Sci.* 3(1): 23-30. DOI: http://dx.doi.org/10.4314/jmbs.v3i1.4.

Nalule AS, Mbaria JM, Kimenju JW. 2013. In vitro anthelmintic potential of *Vernonia amygdalina* and *Secamone africana* on gastrointestinal nematodes. *Agric. Biol. J. N. Am.*, 4(1): 54-66. DOI: http://dx.doi.org/10.5251/abjna.2013.4.1.54.66.

Namukobe J, Kiremire BT, Byamukama R, Kasenene JM, Akala H, Kamau E, Dumontet V. (2015). Anti-plasmodial compounds from the stem bark of *Neoboutonia macrocalyx pax*, *J. Ethnopharmacol.*, 162: 317–322. DOI: http://dx.doi.org/10.1016/j.jep.2014.12.018.

Namukobe J, Kasenene JM, Kiremire BT, Byamukama R, Kamatenesi-Mugisha M, Krief S, Dumontet V, Kabasa JD. 2011. Traditional plants used for medicinal purposes by local communities around the Northern sector of Kibale National Park, Uganda. *J. Ethnopharmacol.*, 136(1): 236-245. DOI: http://dx.doi.org/10.1016/j.jep.2011.04.044.

Park S-H, Moon K, Bang H-S, Kim S-H, Kim D-G, Oh K-B, Shin J, Oh D-C. 2012. Tripartilactam, a cyclobutane bearing Tricyclic lactam from a *Streptomyces sp.* In a Dung Beetle’s Brood Ball. *Org. Lett.*, 14(5): 1258-1261. DOI: http://dx.doi.org/10.1021/ol300108z.

Schripsema J, Caprini GP, ver der Heijden R, Bino R, de Vos R, Dagnino D. 2007. Iridoids from *Pentas lanceolata*. *J. Nat. Prod.*, 70: 1495–1498. DOI: http://dx.doi.org/10.1021/np070116+.

Smilkstein M, Siswaiajaraon N, Kelly JX, Wilairat P, Risoeo M. 2004. Simple and inexpensive fluorescence-based technique for highthroughput antimalarial drug screening. *Antimicrob. Agents Chemother.*, 48(5): 1803–1806. DOI: http://dx.doi.org/10.1128/aac.48.5.1803-1806.2004.

Takuji T, Takahiro T, Mayu T. 2011. Potential Cancer Chemopreventive Activity of Protocatechuic Acid. *J. Exp. Clin. Med.*, 3(1): 27-33. DOI: http://doi:10.1016/j.jecm.2010.12.005.

Torres y Torres JL, Rosazza JPN. 2001. Microbial Transformations of p-Coumaric Acid by *Bacillus megaterium* and *Curvularia lunata*. *J. Nat. Prod.*, 64(11): 1408-1414. DOI: http://dx.doi.org/10.1021/np010238g.

Vaiyapuri M, Raju K, Karuppusamy S. 2015. Preliminary phytochemical investigation on *Secamone emetica* (Retz.) R.Br. (Apocynaceae) – An endemic medicinal plant species of southern India. *J. Pharmacogn. Phytochem.*, 3(6): 58-61. E-ISSN: 2278-4136.

Wong SK, Lim YY, Chan EWC. 2013. Botany, uses, phytochemistry and pharmacology of selected Apocynaceae species: A review. *Pharmacogn. Comm.*, 3(3): 2-11. DOI: http://dx.doi.org/10.5530/pc.2013.3.2.

World Health Organization. 2018. World Malaria Report. World Health Organization. WHO Press: Geneva, Switzerland

Zabri H, Doe A, Mambo V. 2009. Purification column on silica and chemical characterization of coumarin isolated from methanol excerpt of the stems of plant *Secamone afzelii* (Aclepiedaceae) from Abidjan - Ivory Coast. *Electron. J. Biomed.*, 1: 18-23. Corpus ID: 27136462.

Zabri H, Kodo C, Benie A, Bekro JM, Bekro YA. 2008. Phytochemical screening and determination of flavonoids in *Secamone afzelii* (Aclepiedaceae) extracts. *Afr. J. Pure Appl. Chem.*, 2(8): 080-082.