Chemical constituents of two Cameroonian medicinal plants: *Sida rhombifolia* L. and *Sida acuta* Burm. f. (Malvaceae) and their antiplasmodial activity

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**ABSTRACT**

An extensive phytochemical investigation of the EtOH/H$_2$O (7:3) extracts of *Sida rhombifolia* L. and *Sida acuta* Burm. f., yielded a previously undescribed ceramide named rhombifoliamide (1) and a xylitol dimer (2), naturally isolated here for the first time, as well as the thirteen known compounds viz, oleanolic acid (3), β-amyrin glucoside (4), ursolic acid (5), β-sitosterol glucoside (6), tiliroside (7), 1,6-dihydroxyxanthone (8), a mixture of stigmasterol (9) and β-sitosterol (10), cryptolepine (11), 20-Hydroxyecdyson (12), (E)-suberenol (13), thamnosmonin (14) and xanthyletin (15). Their structures were elucidated by the analyses of their spectroscopic and spectrometric data (1D and 2D NMR, and HRESI-MS) and by comparison with the previously reported data. The crude extracts, fractions, and some isolated compounds were tested against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *Plasmodium falciparum* strains. All the tested samples demonstrated moderate and/or significant activities against 3D7 (IC$_{50}$ values: 0.18-20.11 μg/mL) and Dd2 (IC$_{50}$ values: 0.74-63.09 μg/mL).
1. Introduction

Malaria is a parasitic disease caused by a protozoan of the genus *Plasmodium* asexually replicating inside the human body and transmitted from mosquitoes to humans during a blood meal. Despite the continuous evolution of drug search, malaria is the deadliest and most dangerous parasitic infection in Sub-Saharan Africa, making the search for new antimalarial drugs an imperative of the overall health goal and probably one of the greatest public health challenges facing humanity (Gontijo et al. 2019). Despite extensive control efforts, the incidence of the disease is not decreasing and constitutes a major public health issue, principally in developing countries. According to the latest *World malaria report*, released on 30 November 2020, around 229 million malaria cases were reported compared to 228 million cases in 2018 where children under 5 years of age are the most vulnerable group affected by malaria; in 2019 and accounting for 67% (274 000) of all malaria deaths worldwide (WHO. 2020). Responsible for 99.7% of malaria cases in 2017, *Plasmodium falciparum* is the most prevalent malaria parasite in Sub-Saharan Africa. Until the development of an effective vaccine, chemotherapy remains a major frontline strategy for the control and future elimination of malaria. The current chemotherapy against malaria relies on Artemisinin Combination Therapy (WHO 2020). Plants of the genus *Sida* (Malvaceae) are widely used in indigenous communities for their nutritive values and for treating various ailments such as gonorrhoea, piles, rheumatism, gastrointestinal infections, varicella, variola, and malaria (Gupta et al. 2009). *Sida* genus is one of the most diverse in the Malvaceae family, with about 200 species distributed worldwide (Brandao et al. 2017). Phytochemical and pharmacological studies performed on some species have led to the identification of antibacterial lipid compounds from *S. cordata* Burm. f. and *S. acuta* Burm. f. (Adindu and Oguzie 2017), alkaloids with anti-inflammatory and antiparasitic potential from *S. rhombifolia* L. and *S. cordifolia* L. (Chaves et al. 2017; da Rosa et al. 2018). Polyphenols, triterpenes, and steroids have also been identified (da Rosa et al. 2015; Mah et al. 2017; Kumar et al. 2019). Currently, there are various empiric formulations based on *Sida* species (e.g., *S. acuta* Burm. f., *S. cordifolia* L. and *S.
rhombifolia L.) for the treatment of neurological and rheumatic problems, and which also act as antimalarial drugs (Rodrigues and Oliveira 2020) but little is known about their chemical composition. In our continuous search for bioactive compounds from Cameroonian medicinal plants (Fotso et al. 2017, Mboungia et al. 2020), we have carried out the chemical and biological study of two Cameroonian medicinal plants: S. rhombifolia L. and S. acuta Burm. f. The choice of these plants was motivated by the fact that they are traditionally used to treat malaria, but no antiplasmodial constituent has been described from them to date. We herein report the isolation and structure elucidation of a new ceramide named rhombifoliamide (1) and a xylitol dimer (2), naturally isolated for the first time, together with thirteen known compounds as well as their antiplasmodial activity.

2. Results and discussion

2.1. Isolation and structure elucidation

S. rhombifolia whole plant was extracted using the mixture of EtOH/H2O (7:3, v/v). It is worth noting that this solvent system was chosen firstly because the hydroethanolic extract displayed the best antiplasmodial activity after micro-extraction compared to the DCM-MeOH extract and secondly because of the use of white wine in traditional medicine for plant maceration. The resulting crude extract was subjected to repeated silica gel and Sephadex LH-20 column chromatography (CC) to afford a previously undescribed ceramide named rhombifoliamide (1, 10.4 mg), together with eight known compounds viz, oleanolic acid (3, 6.2 mg) (Wouamba et al. 2020), β-amyrin glucoside (4, 7.8 mg) (Alam et al. 2012), ursolic acid (5, 4.3 mg) (Wouamba et al. 2020), β-sitosterol glucoside (6, 9.7 mg) (Wouamba et al. 2020), tiliroside (7, 8.1 mg) (Danielly et al. 2007), 1,6-dihydroxyxanthone (8, 6.4 mg) (Lien Do et al. 2020), mixture of stigmasterol (9) and β-sitosterol (10) (Wouamba et al. 2020) and 20-hydroxyecdysone (12, 2.2 mg) (da Rosa et al. 2018). Similarly, S. acuta whole plant was extracted using the same solvent mixture. The crude extract obtained was subsequently purified using the above-mentioned chromatographic techniques to yield a xylitol dimer (2, 9.3 mg), naturally isolated for the first time, together with five known secondary metabolites namely: cryptolepine (11, 3.9 mg) (Banzouzi et al. 2004), 20-hydroxyecdysone (12, 5.3 mg) (da Rosa et al. 2018), (E)-suberenol (13, 5.1 mg) (Bissim et al. 2019), thamnosmonin (14, 4.2 mg) (Tian-Shung et al. 1994) and xanthyletin (15, 5.4 mg) (Ngo et al. 2020) (Figure 1). The structures of the known compounds were identified by comparison of their spectroscopic and spectrometric data with those reported in the literature.

Compound 1 was obtained as a white powder. Its molecular formula C43H85NO5 was established from its HRESI-MS spectrum (Figure S1), showing the pseudo-molecular ion peak [M + H]+ at m/z 696.6506 (C43H86NO5; calcd. 696.6501), indicating two degrees of unsaturation. Its IR spectrum (Figure S2) showed characteristic absorption bands for free OH groups (3329-3215 cm⁻¹) and an amide group (1620 cm⁻¹) (Yue et al. 2001; Wonkam et al. 2020). The structure of 1 was fully assigned after careful analyses of its 1H, 13C, 1H-1H COSY, HMQC, HMBC, tandem MS spectra and methanolysis reaction (Figure S3–S12, Table 1). Indeed, the 1H NMR spectrum of 1 (Figure S4) in
conjunction with $^{13}$C-NMR DEPT 135 spectra and HSQC (Figure S5–S7) displayed a set of signals characteristic of a ceramide as described by Simo et al. 2008. This was confirmed by the signals of the carbonyl of an amide at $\delta_C$ 175.4 and the signal of a nitrogen-attached sp$^3$ carbon at $\delta_C$ 51.5. Specifically, the NC-H proton appeared at $\delta_{H/C}$ 4.03(1H, m)/51.5 while the broad signal centered at $\delta_H$ 1.18 was attributed to the methylene protons of the aliphatic long chain; a distorted triplet at $\delta_H$ 0.79 (6H, t, 6.9) characterized the two terminal methyl groups. In addition, the spectrum displayed two diastereotopic protons of an oxymethylene at $\delta_{H/C}$ 3.72 (1H, dd, 4.6, 11.5, H-1a)/60.9 and 3.66 (1H, dd, 4.6, 11.4, H-1b)/60.9 as well as three oxymethine protons at $\delta_{H/C}$ 3.46/75.3 (C-3), 3.45/72.1 (C-4) and 3.95/71.8 (C-2') respectively. Correlations between these protons were observed on the $^1$H–$^1$H COSY spectrum (Figure S8). In addition, the presence of a signal at $\delta_H$ 5.32(2H, m) showing cross peaks on the HSQC with two olefinic carbons at 129.8 and 130.5 ppm suggested the presence of a double bond in the structure of 1 (Wouamba et al. 2020). The length of this fatty acid moiety was deduced by the analysis of the ESI-MS/MS spectra of 1 (Figure S11) showing the fragment ion peak [(CH$_3$(CH$_2$)$_{22}$CH(OH)CO + 2H]$^+$ at m/z 383.4 and further confirmed...
by the methanolysis using 0.9 N, HCl/MeOH, at 70 °C for 20 H to yield the fatty acid methyl ester (1a) and the sphingosine (1b) (Simo et al. 2008 (Figure S3). Specifically, the peak at m/z 216.2 [M + H]^+ corresponding to molecular formula C_{18}H_{38}NO_{3}^+ was attributable to the long chain base (1b) and implying one degree of unsaturation. Furthermore, this molecular formula of sphingosine suggested that the olefinic moiety is located in the long chain base (LCB). In the HMBC spectrum of compound 1 (Figure S9), 2J correlations were observed between the olefinic proton at \( \delta_H 5.32 \) and carbon C-12 (\( \delta_C 32.5 \)); H-15 at \( \delta_H 1.21 \) with C-16 carbon (\( \delta_C 31.8 \)). Finally, H-17 at \( \delta_H 1.20 \) correlates in 3J with C-18 carbon corresponding to the terminal methyl. All of these correlations (Figure S11) made it possible to locate the double bond at \( \Delta^10 \) on the long basic chain. This information was confirmed by the ESI-MS/MS spectrum (Figure S12a and S12b) on which the ions peaks [M + H - C_{9}H_{17}]^+ at m/z 571.5 and [M + H - C_{7}H_{15}]^+ at m/z 619.5 corresponding to the allylic cleavages of the double bond, respectively for C_{9}-C_{10} and C_{11}-C_{12} were observed (Figure S10). The \textit{trans} configuration of the C=C bond was evident from the chemical shifts of the allylic C-atoms at \( \delta_C 32.5 \) and 32.0, which should have been less than 29.0 ppm if the configuration was cis (Simo et al. 2008, Wouamba et al. 2020). In addition, the absolute configurations at C(2), C(3), C(4), and C(2') were determined as (S), (S), (R), and (R) according to biogenetic consideration and previously reported data (Ishii et al. 2006, Wonkam et al. 2020). Therefore, the structure of 1 was unambiguously determined as \((2S,20R,3S,4R,10E)-N-[2'-hydroxypentacosanoyl]-2-amino-octadec-10-ene-1,3,4-triol\), to which the trivial name rhombifoliamide was given.

2.2. Antiplasmodial and cytotoxicity activities

Crude extracts, fractions and isolated compounds were screened for their antiplasmodial and cytotoxicity activities using SyBr Green-Based assay and resazurin-based assay respectively. Results showed that, extracts and fractions exhibited moderate to strong antiplasmodial activities against 3D7 (IC_{50} values: 0.18-20.11 \( \mu \)g/mL) and Dd2 (IC_{50} values: 0.74-63.09 \( \mu \)g/mL) \textit{Plasmodium falciparum} strains. Interestingly, two compounds, oleanolic acid (3) and cryptolepine (11) isolated from the EtOAc-soluble fraction of \textit{S. rhombifolia} and \textit{S. acuta} displayed strong antiplasmodial activity with IC_{50} of (3.56 ± 0.62 and 2.02 ± 0.27) for 3; (0.18 ± 0.01 and 0.74 ± 0.09) for 11 respectively against 3D7 and Dd2 \textit{P. falciparum} strains (Table S1). \( \beta \)-amyrin glucoside (4) and tiliroside (7) showed moderate activity only against the multidrug resistant (Dd2) and no activity against sensitive strain of \textit{P. falciparum}. Except for cryptolepine (11), all extracts, fractions and compounds with activity against asexual \textit{P. falciparum} parasites exhibited no cytotoxicity against Raw cells (selectivity indices (SI) > 10. To the best of our knowledge, this study provides the first report of antiplasmodial activity of isolated oleanolic acid against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) \textit{P. falciparum} strains. However, previous studies showed that the strong antiplasmodial activity of dichloromethane twig extract of \textit{keetia leucantha} is attributed to the presence of eight triterpenic esters and the major antiplasmodial triterpenic acids, ursolic and oleanolic acids identified by HPLC-UV methods (Beaufay et al. 2017). In addition, cryptolepine previously showed showed varied interaction with the 4-
aminoquinolines, amodiaquine, and chloroquine. The combination of cryptolepine with amodiaquine showed a synergistic effect in vitro (mean $\Sigma \text{FIC} = 0.235 \pm 0.15$), whereas an additive effect (mean $\Sigma \text{FIC} = 1.342 \pm 0.34$) have been seen with chloroquine (Forkuo et al. 2016). Additionally, cryptolepine has already been reported to show high inhibitory activity against the late-stage gametocytes ($\text{IC}_{50} = 1965 \text{nM}$) (Forkuo et al. 2016) of $P. falciparum$ (NF54). Summing-up, these studies report the good potential of cryptolepine as a promising antimalarial hit for both malaria treatment and transmission-blocking therapy (Forkuo et al. 2016). Based on the pronounced antiplasmodial activity of this compound, further chemical studies such as structural-activity relationship and/or medicinal chemistry are needed to obtain a lead compound that responds to pharmacokinetics and pharmacodynamics properties for antimalarial drugs. All experiments were performed in triplicate and the main results obtained are recorded in Table S2.

3. Experimental (supplementary data)

3.3.1. Rhombifoliamide (1)

White powder; IR (KBr): 3329 cm$^{-1}$, 3215 cm$^{-1}$ and 1620 cm$^{-1}$; HRESI-MS: [M + H]$^+$ at $m/z$ 696.6506 ($C_{43}H_{86}NO_5$)$^+$; calcd. 696.6501; $^1$H-NMR (600 MHz, CDCl$_3$/CD$_3$OD) & $^{13}$C-NMR (150 MHz, CDCl$_3$/CD$_3$OD) see Table S1.

4. Conclusion

The phytochemical investigation on the EtOH/H$_2$O (7:3) extracts of $S. rhombifolia$ and $S. acuta$ yielded a previously undescribed ceramide named rhombifoliamide (1) and a xylitol dimer (2), naturally isolated here for the first time, as well as the thirteen known compounds viz, oleanolic acid (3), $\beta$-amyrin glucoside (4), ursolic acid (5), $\beta$-sitosterol glucoside (6), tiliroside (7), 1,6-dihydroxyxanthone (8), a mixture of stigmasterol (9) and $\beta$-sitosterol (10), cryptolepine (11), 20-Hydroxyecdysone (12), (E)-suberenol (13), thamnosmonin (14) and xanthyletin (15). Crude extracts, fractions, and compounds 1-12 were evaluated for their antiplasmodial activity against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) $P. falciparum$ strains. Two tops ‘hits’ antiplasmodial compounds 3 and 11, with $\text{IC}_{50} < 3 \mu\text{g/mL}$ isolated from EtOAc-soluble fraction of $S. rhombifolia$ and $S. acuta$ respectively, were identified in this study as potential lead compounds for antimalarial drug discovery. The hydroethanolic extract of $S. rhombifolia$ and EtOAc-soluble fractions from $S. rhombifolia$ and $S. acuta$ had a good antiplasmodial activity with low preparation cost may be useful for further investigation in view to develop improved traditional medicines (ITM) to combat malaria disease.

Supplementary material

Experimental section, NMR, and MS data of compounds 1 are available alongside Figures S1–S12.
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Disclosure statement

No conflict of interest was reported by the authors.

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