



# Constituents from ripe figs of *Ficus vallis-choudae* Delile (Moraceae) with antiplasmodial activity

Hardo Suzy Donfack Chouna<sup>1,2</sup> · Darline Dize<sup>3</sup> · Donald Ulrich Kenou Kagho<sup>2</sup> · Jean Jules Kezetas Bankeu<sup>4</sup> · Yannick Stéphane Fotsing Fongang<sup>5</sup> · Mariscal Brice Tchatat Tali<sup>3</sup> · Beudelaire Kemvoufo Ponou<sup>1</sup> · Gabin Thierry Mbahbou Bitchagno<sup>1</sup> · Angelbert Fusi Awantu<sup>4</sup> · Léon Azefack Tapondjou<sup>1</sup> · Bruno Ndjakou Lenta<sup>6</sup> · Fabrice Boyom Fekam<sup>3</sup> · Norbert Sewald<sup>7</sup> · Silvère Augustin Ngouela<sup>1,2</sup>

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## Abstract

Ripe figs, barks, and wood of *Ficus vallis-choudae* are used in traditional medicine against several conditions including nausea and malaria. However, its use is still to be scientifically documented and validated. Hence, the aim of the present work was to evaluate the antiplasmodial activity of the dichloromethane-methanol (DCM-MeOH (1:1)) crude extract, their hexane, dichloromethane, ethyl acetate, and methanoli fractions, as well as the isolated chemical constituents. The chemical study of the DCM-MeOH (1:1) crude extract of *F. vallis-choudae* figs led to the isolation of fifteen (15) known compounds identified based on their spectroscopic data [one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR), mass spectrometry] and by comparison of these data with those reported in the literature. Some of the isolated compounds were assessed in vitro for their antiplasmodial activity against *Plasmodium falciparum* chloroquine-sensitive 3D7 (*Pf3D7*) and multidrug-resistant Dd2 strains. The dichloromethane fraction exhibited very good antiplasmodial activity against both strains with IC<sub>50</sub> values of 13.86 µg/mL and 8.18 µg/mL, respectively. Among the tested compounds, wighteone (**2**) was the most active against *P. falciparum* 3D7 (IC<sub>50</sub> = 24.6 ± 1.5 µM) and Dd2 (IC<sub>50</sub> = 11.9 ± 2.4 µM) strains. The obtained results could justify the traditional uses of *F. vallis-choudae* against malaria. Wighteone appears to be the most active ingredient. However, further consideration of this compound as starting point for antimalarial drug discovery will depend upon its selectivity of action towards *Plasmodium* parasites.

## Highlights

- 15 (fifteen) compounds were isolated from the dichloromethane-methanol extract of *Ficus vallis-choudae*.
- Their structures were determined on the basis of their spectroscopic data.
- The dichloromethane fraction showed promising activities on the *Pf3D7* and *PfDd2* strains with IC<sub>50</sub> values of 13.86 and 8.18 µg/mL, respectively.
- Wighteone was the most active compound against *PfDd2* (IC<sub>50</sub> = 11.9 ± 2.4 µM).

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✉ Beudelaire Kemvoufo Ponou  
beudelaireponou@yahoo.fr

✉ Bruno Ndjakou Lenta  
lentabruno@yahoo.fr

<sup>1</sup> Department of Chemistry, University of Dschang, P.O. Box 67, Dschang, Cameroon

<sup>2</sup> Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon

<sup>3</sup> Department of Biochemistry, Faculty of Science, University of Yaoundé I, P.O. Box: 812, Yaoundé, Cameroon

<sup>4</sup> Department of Chemistry, Faculty of Science, The University of Bamenda, P.O. Box 39, Bamili, Cameroon

<sup>5</sup> Department of Chemistry, Higher Teachers Training College, University of Maroua, P.O. Box 55, Maroua, Cameroon

<sup>6</sup> Department of Chemistry, Higher Teachers Training College, University of Yaoundé I, P.O. Box 47, Yaoundé, Cameroon

<sup>7</sup> Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University, P.O. Box 100131, 33501 Bielefeld, Germany

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## Introduction

Malaria is an endemic disease in most tropical regions of Africa, Asia, and Latin America and its incidence have been increasing these recent times due to drug resistance and the emergence of the Covid-19 pandemic. An estimated 228 million cases of malaria occurred worldwide in 2018 with the largest burden of morbidity (93%) in Africa (WHO 2019). Chemotherapy remains a key component of malaria control and elimination strategies. Artemisinin-based combination therapy was the most commonly used drug-based therapy between 2010 and 2018 in African public health sectors (WHO 2019), but the high cost and the low availability in rural areas is still an important health problem. The emergence of artemisinin-resistant *Plasmodium falciparum* strains in Africa turned the situation worse (Huayun et al. 2017). Hence, understanding and controlling the spread of antimalarial resistance, particularly to artemisinin and its partner drugs remains a top priority. Therefore, alternative antimalarial drugs need to be developed, and medicinal plants are known as promising sources of potential antimalarial compounds.

The genus *Ficus* (Moraceae) is one of the largest genera of angiosperms with more than 800 species of trees, shrubs, hemiepiphytes, climbers, and creepers in the tropics and subtropics worldwide. It is an important genetic resource due to its high economic and nutritional values and also an important part of the biodiversity in the rainforest ecosystem. *Ficus* species are also a good source of food for fruit-eating animals in tropical areas (Rønsted et al. 2007). *Ficus vallis-choudae* Dellile (synonyms *Ficus schweinfurthii* Miquel) is a shrub or small tree up to 8 m tall. It is distributed in tropical Africa from Senegal to Cameroon, from Sudan to Ethiopia and Malawi (Vivien and Faure 1996). Fruits, bark, and wood are the most used parts of *Ficus vallis-choudae* against several conditions including nausea and malaria (Oliver 1960). Several phytochemical and pharmacological surveys were already done worldwide on different *Ficus* species. However, only a few research studies were conducted on *Ficus vallis-choudae*. Our research group has previously reported the isolation of one ceramide, steroids, and triterpenoids from the methanolic extract of the figs of this plant as well as the  $\alpha$ -glucosidase inhibitory activity and DPPH radical scavenging potency (Bankeu et al. 2017). However, to provide scientific validation to the traditional use of this plant to treat malaria, in-depth investigation of stem bark and other parts of *Ficus vallis-choudae* for antiplasmodial was required. In the present article, we report the antiplasmodial activity of the dichloromethane-methanol extract *F. vallis-choudae* figs,

its hexane, dichloromethane, ethyl acetate, and methanolic fractions, as well as the isolated chemical constituents.

## Materials and methods

### General experimental procedures

The used equipments were those reported by Chouna et al (2021). Column chromatography (CC) was performed on silica gel (230–400 mesh). Fractions were monitored by thin-layer chromatography (TLC) using Merck precoated silica gel sheets (60 F<sub>254</sub>), and the identification of spots on the TLC plate was carried out by spraying with sulfuric acid reagent solution and heating the plate to about 80 °C.

### Plant material

The figs of *Ficus vallis-choudae* Dellile were collected in May 2019 at Egona II (Center region of Cameroon). Mr. NANA Victor, botanist at the National Herbarium, Yaoundé, by comparison with the existing voucher specimen No 5115/HNC4, identified the plant material.

### Extraction and isolation

The plant material (1.5 kg) was air-dried, pulverized, and extracted three times with 4 L of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1:1) mixture at room temperature (24°C) (each time for 24 h). The solvent was evaporated under reduced pressure to afford 119.1 g of crude extract. A part of the extract (118.1 g) was partitioned with *n*-hexane, dichloromethane (DCM), ethyl acetate, and methanol to afford fractions of 72.9, 6.5, 11.1, and 25.6 g, respectively.

The *n*-hexane fraction (FH) was subjected to column chromatography (CC) over silica gel (Merck, 230–400 mesh) eluting with the mixtures of *n*-hexane-EtOAc (1:0 → 0:1) and EtOAc/MeOH (1:0 → 0:1) with increasing polarities. Fractions of 300 mL each were collected and combined according to their TLC profiles to afford five major sub-fractions labeled FH1–FH5. Sub-fraction FH1 (10.5 g) was submitted to CC over silica gel using *n*-hexane-EtOAc (1:0 → 4:6) as solvent systems to yield **7** (50.3 mg) and **8** (55.2 mg). Compound **14** (40 mg) crystallized in sub-fraction FH2 (9.7 g) and was filtered off by simple filtration. Sub-fraction FH3 (13.9 g) was submitted to purified CC over silica gel eluted with *n*-hexane-EtOAc (1:0 → 2:8) to afford compounds **5** (9.2 mg), **4** (8.7 mg), and **11** (25.1 mg). The purification of sub-fraction FH4 (22.8 g) over silica

gel CC using *n*-hexane–EtOAc (1:0 → 2:8) as elution solvents yielded compounds **2** (20.5 mg), **3** (5.4 mg), and **1** (6.9 mg). The sub-fraction FH5 (15.4 g) was subjected to CC with *n*-hexane–EtOAc (6:4 → 0:1) and EtOAc/MeOH (1:0 → 85:15) over silica gel to afford compounds **6** (5 mg) and **15** (30 mg).

Likewise, the DCM fraction (6.5 g) was subjected to the CC over silica gel (Merck, 230–400 mesh) eluting with *n*-hexane–EtOAc (1:0 → 0:1) and EtOAc/MeOH (1:0 → 0:1) systems of increasing polarities. Fractions of 300 mL each were collected and combined according to their TLC profiles to afford four major sub-fractions labeled (FD1–FD4). The sub-fraction FD1 (1.43 g) was further subjected to CC over silica gel and eluted with *n*-hexane–EtOAc (1:0 → 0:1) to afford compound **9** (5.4 mg). Further CC of the sub-fraction FD2 (3.21 g) with *n*-hexane–EtOAc (8:2 → 0:1) afforded compounds **10** (20.8 mg) and **12** (6.2 mg). The sub-fraction FD3 (1.12 g) was subjected to CC over silica gel with *n*-hexane–EtOAc (6:4 → 0:1) to yield compound **13** (15.7 mg).

### Physical and spectroscopic data of the isolated compounds

**5,7,4'-trihydroxyisoflavone (Genistein) (1):** Yellow powder from *n*-hexane–EtOAc (1:1), mp 289–291 °C [Literature (Lit.) 290–292 °C (Mykhailenko et al. 2017)],  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 600 MHz):  $\delta_{\text{H}}$  (ppm) = 8.08 (1H, s, H-2), 7.40 (2H, d,  $J=8.9$  Hz, H-2'/6'), 6.87 (2H, d,  $J=8.9$  Hz, H-3'/5'), 6.37 (1H, d,  $J=2.2$  Hz, H-8), 6.25 (1H, d,  $J=2.2$  Hz, H-6);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 150 MHz):  $\delta_{\text{C}}$  (ppm) = 182.6 (C-4), 166.0 (C-7), 163.9 (C-5), 159.8 (C-9), 158.8 (C-4'), 154.8 (C-2), 131.4 (C-2'/6'), 123.8 (C-3), 123.3 (C-1'), 116.2 (C-3'/5'), 106.3 (C-10), 100.1 (C-6), 94.8 (C-8).

**Wightone (Erythrinin B) (2):** Yellow powder from *n*-hexane–EtOAc (4:6), mp 218–220 °C [Lit. 219–220 °C (Kinoshita et al. 1990)],  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 600 MHz):  $\delta_{\text{H}}$  (ppm) = 13.03 (1H, s, HO-5), 7.98 (1H, s, H-2), 7.28 (2H, d,  $J=8.5$  Hz, H-2'/6'), 6.74 (1H, d,  $J=8.5$  Hz, H-3'/5'), 6.31 (1H, s, H-8), 5.13 (1H, m, H-2''), 3.21 (2H, d,  $J=7.3$  Hz, H-1''), 1.67 (3H, d,  $J=1.5$  Hz, 5''-CH<sub>3</sub>), 1.56 (3H, d,  $J=1.5$  Hz, 4''-CH<sub>3</sub>);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 150 MHz):  $\delta_{\text{C}}$  182.0 (C-4), 163.4 (C-5), 160.4 (C-7), 158.8 (C-4'), 157.3 (C-9), 154.6 (C-2), 132.2 (C-3''), 131.5 (C-2'/6'), 124.3 (C-3), 123.5 (C-2''), 123.4 (C-1'), 116.3 (C-3'/5'), 112.7 (C-6), 106.1 (C-10), 94.0 (C-8), 26.2 (C-4''), 22.4 (C-1''), 18.2 (C-5'').

**Lupiwightone (3):** Yellow powder from *n*-hexane–EtOAc (4:6), mp 133–135 °C [Lit. 133–134 °C (Al-Maharik and Botting 2003)],  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 600 MHz):  $\delta_{\text{H}}$  (ppm) = 8.16 (1H, s, H-2), 7.40 (2H, d,  $J=8.9$  Hz, H-2'/6'), 6.87 (2H, d,  $J=0.8.9$ .Hz, H-3'/5'), 6.30 (1H, s, H-6), 5.23 (1H, m, H-2''), 3.44 (2H, d,  $J=7.1$  Hz, H-1''), 1.88 (3H, d, s, H-5''), 1.70 (3H, d, s, H-4'');  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 150 MHz):  $\delta_{\text{C}}$  (ppm) = 182.6 (C-4), 163.3 (C-7),

161.4 (C-5), 158.8 (C-4'), 156.9 (C-9), 154.8 (C-2), 132.4 (C-3''), 131.4 (C-2'/6'), 124.4 (C-1'), 123.4 (C-3), 123.4 (C-2''), 116.2 (C-3'/5''); 107.9 (C-8), 106.3 (C-10), 99.6 (C-6), 25.9 (C-4''), 22.3 (C-1''), 17.9 (C-5'').

**Derrone (4):** Yellow powder from *n*-hexane–EtOAc (3:7), mp 178–179 °C [Lit. 179–181 °C (Máximo et al. 2002)],  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 600 MHz):  $\delta_{\text{H}}$  (ppm) = 12.89 (1H, s, HO-5), 7.88 (1H, s, H-2), 7.38 (2H, d,  $J=8$  Hz, H-2'/6'), 6.88 (1H, d,  $J=8$  Hz, H-3'/5'), 6.68 (1H,  $J=10.0$  Hz, H-4''), 6.33 (1H, s, H-6), 5.60 (1H, d,  $J=10.0$  Hz, H-3''), 5.15 (1H, s, HO-4'), 1.48 (6H, s, CH<sub>3</sub>);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 150 MHz):  $\delta_{\text{C}}$  (ppm) = 181.2 (C-4), 162.4 (C-5), 159.7 (C-7), 156.1 (C-4'), 152.4 (C-9), 130.5 (C-2'/6'), 127.6 (C-3''), 123.8 (C-3), 123.1 (C-1'), 115.8 (C-3'/5'), 114.7 (C-4''), 106.2 (C-10), 100.5 (C-6), 101.3 (C-8), 78.3 (C-2''), 28.4 (C-5''/6'').

**Alpinumisoflavone (5):** Yellow powder from *n*-hexane–EtOAc (7:3), mp 212–214 °C [Lit. 213–214 °C (Ndemangou et al. 2013)],  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 600 MHz):  $\delta_{\text{H}}$  (ppm) = 13.18 (1H, s, HO-5), 7.82 (1H, s, H-2), 7.39 (2H, d,  $J=8$  Hz, H-2'/6'), 6.89 (1H, d,  $J=8$  Hz, H-3'/5'), 6.73 (1H,  $J=10.0$  Hz, H-4''), 6.33 (1H, s, H-8), 5.62 (1H, d,  $J=10.0$  Hz, H-3''), 5.15 (1H, s, HO-4'), 1.47 (6H, s, CH<sub>3</sub>);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 150 MHz):  $\delta_{\text{C}}$  (ppm) = 181.1 (C-4), 159.7 (C-7), 157.5 (C-9), 157.0 (C-5), 156.0 (C-4'), 130.5 (C-2'/6'), 128.4 (C-4''), 123.7 (C-3), 123.3 (C-1'), 115.7 (C-3'/5'), 115.6 (C-3''), 106.3 (C-10), 105.8 (C-6), 96.0 (C-8), 78.2 (C-2''), 28.5 (C-5''/6'').

**Oleanolic acid-28-O-β-D-glucopyranosyl ester (6):** white amorphous powder from *n*-hexane–EtOAc (3:7),  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 150 MHz): **Aglycone:**  $\delta_{\text{C}}$  (ppm) = 174.5 (C-28), 140.5 (C-13), 122.3 (C-12), 79.7 (C-3), 56.3 (C-5), 50.3 (C-9), 48.0 (C-9), 47.2 (C-19), 46.0 (C-18), 42.5 (C-17/14), 39.0 (C-8), 38.6 (C-4), 38.5 (C-1), 36.9 (C-10), 34.4 (C-21), 34.1 (C-22), 31.9 (C-7), 31.8 (C-29), 29.9 (C-20), 29.1 (C-23), 27.4 (C-2/15), 23.9 (C-27), 22.8 (C-11/16), 21.0 (C-6), 19.8 (C-30), 19.5 (C-26), 14.3 (C-25), 11.8 (C-24), **Glucose:**  $\delta_{\text{C}}$  (ppm) = 94.0 (C-1'), 76.5 (C-3'), 76.4 (C-5'), 72.2 (C-2'), 69.6 (C-4'), 61.3 (C-6').

**β-palmitate (7):** white amorphous powder from *n*-hexane–EtOAc (98:2),  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 150 MHz):  $\delta_{\text{C}}$  (ppm) = 145.5 (C-13), 121.8 (C-12), 80.7 (C-3), 55.4 (C-5), 47.7 (C-9), 47.4 (C-18), 46.9 (C-19), 41.9 (C-14), 40.0 (C-8), 38.4 (C-1), 37.9 (C-4), 37.8 (C-22), 37.3 (C-10), 34.8 (C-21), 33.5 (C-29), 32.7 (C-17 and C-20), 32.6 (C-7), 28.5 (C-28), 28.2 (C-23), 27.1 (C-2), 26.3 (C-15 and C-16), 26.1 (C-27), 23.8 (C-11 and C-30), 18.4 (C-6), 17.0 (C-26), 16.8 (C-24), 15.7 (C-25); **Palmitoyl:** 173.9 (C-1'), 31.2 (C-2'), 29.3–29.9 (C-4'-14'), 25.3 (C-3'), 22.8 (C-15'), 14.3 (C-16').

**β-Amiryn acetate (8):** white amorphous powder from *n*-hexane–EtOAc (97:3),  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 150 MHz):  $\delta_{\text{C}}$  (ppm) = 145.4 (C-13), 121.8 (C-12), 81.1 (C-3), 55.4 (C-5), 47.7 (C-9), 47.4 (C-18), 46.9 (C-19), 41.9 (C-14), 38.4

(C-1), 38.4 (C-4/8), 37.3 (C-22), 37.3 (C-10), 34.8 (C-21), 33.5 (C-29), 32.6 (C-7), 32.7 (C-20), 31.2 (C-17), 28.2 (C-24/28), 27.1 (C-2/16), 26.3 (C-15), 26.1 (C-27), 23.7 (C-30), 23.6 (C-11), 18.4 (C-6), 16.9 (C-26), 16.7 (C-25), 15.7 (C-23); **Acetyl**: 171.2 (C-1'), 21.5 (C-2').

**Capparaside (9)**: white amorphous powder from *n*-hexane–EtOAc (7:3), <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz): δ<sub>H</sub> (ppm) = 7.87 (1H, s, H-2), 2.23 (3H, s, CH<sub>3</sub>); <sup>13</sup>C RMN (CD<sub>3</sub>OD): δ<sub>C</sub> (ppm) = 170.3 (COOH), 151.8 (C-4), 145.8 (C-3), 142.8 (C-5), 140.4 (C-2), 14.8 (CH<sub>3</sub>).

**5-Hydroxymethylfuran-3-carboxylic acid (10)**: white powder from *n*-hexane–EtOAc (1:1), mp 144–146 °C [Lit. 145–147 °C (Ma et al. 2015)], <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz): δ<sub>H</sub> (ppm) = 7.96 (1H, s, H-2), 6.50 (1H, s, H-4), 4.42 (2H, s, CH<sub>2</sub>O); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz): δ<sub>C</sub> (ppm) = 176.9 (COOH), 170.4 (C-5), 147.4 (C-3), 141.0 (C-2), 110.7 (C-4), 61.2 (CH<sub>2</sub>OH).

**Oleanolic acid (11)**: White crystals from methanol, mp 304–306 °C [Lit. 303.306 °C (Cai and Wu 1996)].

**Maslinic acid (12)**: <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz): white amorphous powder from *n*-hexane–EtOAc (1:1), δ<sub>C</sub> (ppm) = 180.9 (C-28), 144.8 (C-13), 123.4 (C-12), 84.5 (C-3), 69.5 (C-2), 56.7 (C-5), 48.9 (C-9), 48.1 (C-1), 47.7 (C-17), 47.3 (C-19), 42.9 (C-14), 42.8 (C-18), 40.5 (C-4), 39.3 (C-8/10), 34.6 (C-21), 33.8 (C-22), 33.6 (C-29), 33.9 (C-7), 31.6 (C-20), 29.3 (C-23), 28.8 (C-15), 24.1 (C-11), 24.0 (C-16), 23.7 (C-27/30), 19.6 (C-6), 17.8 (C-26), 17.4 (C-25), 17.1 (C-24).

**Erythrinin C (13)**: Yellow crystals from *n*-hexane–EtOAc (7:3), <sup>1</sup>H NMR (CDCl<sub>3</sub>, 150 MHz): Yellow amorphous powder, <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ<sub>H</sub> (ppm) = 13.18 (1H, s, 5-OH), 9.64 (1H, s, 4'-OH), 8.10 (1H, s, H-2), 7.38 (2H, d, *J* = 8.5 Hz, H-2'/6'), 6.84 (2H, d, *J* = 8.5 Hz, H-3'/5'), 6.43 (1H, s, H-8), 4.81 (2H, m, H-2''), 3.10 (2H, d, *J* = 8.5 Hz, H-3''), 1.31 (3H, s, H-6''), 1.26 (3H, s, H-5''); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz): δ<sub>C</sub> (ppm) = 182.3 (C-4), 168.1 (C-7), 159.5 (C-5), 158.7 (C-9), 157.9 (C-4'), 93.1 (C-2''), 154.9 (C-2), 131.4 (C-2'/6'), 124.8 (C-3), 123.1 (C-1'), 116.1 (C-3'/5'), 110.8 (C-6), 107.1 (C-10), 89.9 (C-8), 72.2 (C-4''), 27.4 (C-3''), 25.3 (C-6''), 23.5 (C-5'').

**β-Sitosterol (14)**: White crystals from methanol, mp: 134–135 °C [Lit. 136–137 °C (Sen et al. 2012)].

**β-Sitosterol-3-O-β-D-glucopyranoside (15)**: White crystals from methanol, mp: 290–292 °C [Lit. 290–291 °C (Wang et al. 2009)].

## Antiplasmodial assay

Chloroquine-sensitive (*Pf3D7*) and multidrug-resistant (*PfDd2*) strains of *P. falciparum* were continuously cultured as reported by Chouna et al. (2021). The previously SYBR green I-based fluorescence method described by Smilkstein et al. (2004) was used to evaluate the antiplasmodial activity. The growth inhibition percentage for each test sample

was determined using Microsoft Excel and data analysis was performed with GraphPad Prism 5.0 fitting by non-linear regression and dose–response curves were drawn to determine the inhibitory concentration that reduces parasite viability by 50% (IC<sub>50</sub>).

The degree of resistance was determined by comparison of the antiplasmodial IC<sub>50</sub> of inhibitors on the chloroquine-sensitive and multi-resistant strains of *P. falciparum* using the following formula:

$$RI = IC_{50} \text{ multidrug-resistant strain} / IC_{50} \text{ chloroquine-sensitive strain.}$$

## Results and discussion

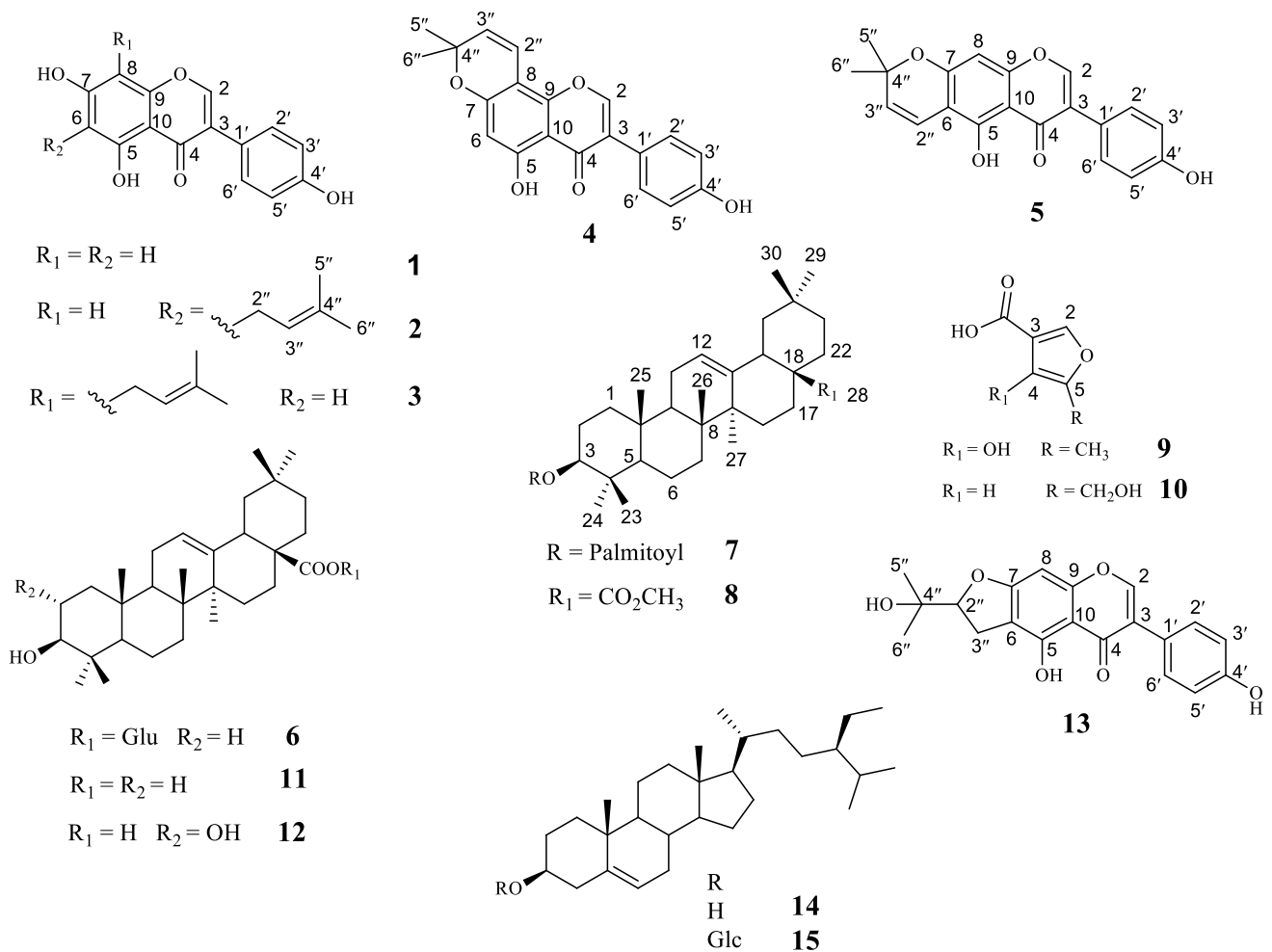
### Phytochemical analysis

The dichloromethane-methanol (1:1) crude extract of the figs of *Ficus vallis-choudae* was repeatedly subjected to silica gel column chromatography (CC) to yield fifteen compounds identified as 5,7,4'-trihydroxyisoflavone (**1**) (Sordon et al. 2017); wighteone (**2**) (Kinoshita et al. 1990); lupiwighteone (**3**) (Al-Maharik and Botting 2003); derrone (**4**) (Máximo et al. 2002); alpinumisoflavone (**5**) (Rahman et al. 2007; El-Masry et al. 2002); oleanolic acid-28-*O*-β-D-glucopyranosyl ester (**6**) (Young et al. 1997); β-amyrin palmitate (**7**) (Bankeu et al. 2017); β-amyrin acetate (**8**) (Du et al. 2003; Okoye et al. 2014); capparaside (**9**) (Yang et al. 2010); 5-hydroxymethylfuran-3-carboxylic acid (**10**) (Evidente et al. 2009); maslinic acid (**12**) (Tanaka et al. 2003); erythrinin C (**13**) (Chen et al. 2019). The remaining compounds were identified by co-TLC with authentic samples and melting points measurement as oleanolic acid (**11**) (Cai and Wu 1996), β-sitosterol (**14**) (Sen et al. 2012), and 3-*O*-β-D-glucopyranoside of β-sitosterol (**15**) (Wang et al. 2009) (Fig. 1). The isolation of isoflavonoids, triterpenes, and steroids is in agreement with the previous results obtained from *Ficus* species (Bankeu et al. 2011, 2017; Fongang et al. 2015).

### Antiplasmodial activity

The crude extract, fractions, and isolated compounds were assessed in vitro for their antiplasmodial activity against chloroquine-sensitive (*Pf3D7*) and multidrug-resistant (*PfDd2*) strains of *P. falciparum*. Out of the tested fractions, the dichloromethane fraction (FVFD) exhibited good antiplasmodial activities on the *Pf3D7* and *PfDd2* with IC<sub>50</sub> values of 13.86 and 8.18 μg/mL, respectively (Table 1). Four compounds (**9**, **10**, **12**, **13**) were isolated through its repeated purification. The multi-targeted activity of maslinic acid (**12**) as an antimalarial natural compound was previously demonstrated (Moneriz et al. 2011), and might justify the activity observed for FVFD. However, the low amount of





**Fig. 1** Structures of compounds 1–15

**12** isolated in this work did not allow the evaluation of its antiplasmodial activity against both *P. falciparum* strains. Compound **2** (wighteone) was the most active against *PfDd2* ( $\text{IC}_{50} = 11.9 \pm 2.4 \mu\text{M}$ ) and *Pf3D7* ( $\text{IC}_{50} = 24.6 \pm 1.5 \mu\text{M}$ ). However, a recent report indicated that wighteone inhibits cell proliferation, suppressed EGFR signalling pathway, caused cell cycle redistribution and induced cell apoptosis, and could provide a novel potential therapeutic strategy for NSCLC patients with T790M mutation (Sun et al. 2021). For further consideration of this compound (**2**) as starting point for antimalarial drug discovery, cytotoxicity assessment using normal mammalian cells is required. The main structural difference between **1** and **2** is the presence of a prenyl group at C-6 of **2**, thus suggesting that the prenyl moiety may contribute to the high activity of **2**. This result corroborates previous findings as prenyl moiety including isoflavonoids have been reported to considerably increase various biological activities such as antiplasmodial, antifungal, and anticancer potencies of secondary metabolites

(Zeleafack et al. 2012; Yang et al. 2015). These results could support the traditional use of *Ficus vallis-choudae* for the treatment of malaria. Wighteone, maslinic acid, and genistein may be eventually qualified as antiplasmodial active principles of this medicinal plant.

## Conclusion

Fifteen (15) known compounds including six isoflavonoids, five triterpenoids, two steroids, and two furan derivatives were isolated from the dichloromethane-methanol (1:1) crude extract of *Ficus vallis-choudae* figs. This is the first report of the isolation of these compounds (excepted **7**, **11**, and **14**) from *Ficus vallis-choudae*. The dichloromethane fraction showed promising activities on the *Pf3D7* and *PfDd2* strains with  $\text{IC}_{50}$  values of 13.86 and 8.18  $\mu\text{g/mL}$ , respectively. Wighteone was the most active against *PfDd2* ( $\text{IC}_{50} = 11.9 \pm 2.4 \mu\text{M}$ ) and *Pf3D7* ( $\text{IC}_{50} = 24.6 \pm 1.5 \mu\text{M}$ ).

**Table 1** Antiplasmodial activity of compounds and fractions from the dichloromethane-methanolic extract of *F. vallis-choudae*

	Codes	<i>Pf3D7</i> IC <sub>50</sub> ± SD* (µg/mL)	<i>PfDd2</i> IC <sub>50</sub> ± SD* (µg/mL)	Resistance index (RI)
Extract	FVDMe	74.3 ± 3.3	15.9 ± 0.02	0.21
Fractions	FVFH	74.8 ± 2.5	60.6 ± 0.1	0.80
	FVFD	13.9 ± 0.2	8.2 ± 2.1	0.59
	FVFEA	47.6 ± 3.6	28.6 ± 0.2	0.60
	FVFMe	>25	7.5 ± 1.3	-
	Compounds	N0	IC <sub>50</sub> ± SD* (µM)	IC <sub>50</sub> ± SD* (µM)
	1	>36	>36	0.99
	2	24.6 ± 1.5	11.9 ± 2.4	0.48
	4	>36	>36	-
	5	>36	>36	-
	6	>36	>36	-
	7	>36	19.5 ± 2.9	-
	8	>36	>36	-
	10	>36	>36	-
Positive control (µM)	Chloroquine	0.057 ± 0.006	0.43 ± 0.04	
	Artemisinin	0.023 ± 0.002	0.012 ± 0.001	

\*Samples were tested in triplicate against *P. falciparum* in culture; data points are means ± standard deviation (SD); *FV*, *Ficus vallis-choudae*; *FVDMe*, dichloromethane-methanol crude extract of *FV*; *FVFH*, hexane fraction of *FV*; *FVFD*, dichloromethane fraction of *FV*; *FVFEA*, ethyl acetate fraction of *FV*; *FVFMe*, methanolic fraction of *FV*

Wightone and maslinic acid might be suggested as the backbone of the antiplasmodial potency of *Ficus vallis-choudae*. However, their safety towards normal mammalian cells should be demonstrated.

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**Author contributions** Conceptualization: Norbert Sewald; methodology: Hardo Suzy Donfack Chouna, Darline Dize; formal analysis and investigation: Gabin Thierry Mbahbou Bitchagno, Donald Ulrich Kenou Kagho; writing—original draft preparation: Yannick Stéphane Fotsing Fongang, Mariscal Brice Tchatat Tali; writing—review and editing: Angelbert Fusi Awantu, Léon Azefack Tapondjou; funding acquisition: Bruno Ndjakou Lenta, resources: Jean Jules Kezetas Bankeu, Fabrice Boyom Fekam; supervision: Beaudelaire Kemvoufo Ponou, Silvère Augustin Ngouela.

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## Declarations

**Competing interest** The authors declare no competing interests.

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