In vitro activities of crude extracts and triterpenoid constituents of Dichapetalum crassifolium Chodat against clinical isolates of Schistosoma haematobium

Mary Anti Chama a,*, Henry Akwaffo Onyame a, Claudine Fleischera, Dorcas Osei-Safo a, Reiner Waibel b, Joseph Otchere c, Ivan Addae-Mensaha, Michael Wilsonc

a Department of Chemistry, School of Physical and Mathematical Sciences, P.O Box LG 56, University of Ghana, Legon, Ghana
b Department of Chemistry and Pharmacy, Friederich Alexander University of Erlangen-Nurnberg, Schuhstrasse 19, 91052 Erlangen, Germany
c Parasitology Department, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon, Ghana

ARTICLE INFO
Keywords:
Natural product chemistry
Organic chemistry
Pharmaceutical chemistry
Dichapetalum crassifolium
Schistosoma haematobium
Dichapetalin A
Dichapetalin M
Maslinic acid
Pomolic acid

ABSTRACT
Dichapetalum crassifolium Chodat (Dichapetalaceae) is widely distributed in Africa, Tropical Asia and Latin America. As part of our quest for potential bioactive lead compounds for various neglected tropical diseases, we report the anti-schistosomal potential of the crude extracts and chemical constituents of the stems and roots of Dichapetalum crassifolium. Column chromatography of extracts of the stems and roots led to the isolation and identification of three oleanane-type triterpenoids, friedelan-3β-ol (1), friedelan-3-one (2), and maslinic acid (3); the ursane-type triterpenoid, pomolic acid (4) and the dammarane-type tetracyclic triterpenoids, dichapetalin A (5) and dichapetalin M (6). Dichapetalin A was isolated from only the roots. Isolated compounds were identified by comparison of their physico-chemical and spectral data with published data. The highest in vitro anti-schistosomal activity (IC50) of the crude extracts against clinical isolates of Schistosoma haematobium (Bilharz 1852) was 248.6 μg/ml for the ethyl acetate extract of the root while dichapetalin A gave the highest activity at 151.1 μg/ml among the compounds compared with the 15.5 μg/ml for the standard drug, praziquantel. The rest of the compounds showed activities in the order 177.9, 191.0, and 378.1 μg/ml respectively for mixture of β-sitosterol/stigmasterol, dichapetalin M and friedelan-3-one. The least active extract was the methanol extract of the stem (893.7 μg/ml). The constituents of D. crassifolium showed activity against the S. haematobium that are below praziquantel. It is envisaged that the presence of multiple layers and the minute sizes of pores in the egg shells, may preclude penetration of eggs by the compounds.

1. Introduction

Naturally-occurring pentacyclic triterpenoids of the lupane, oleanane and ursane classes are known to be a wide range of biological activities. Quite a number of these triterpenoids have been isolated and identified from some plant species of the Dichapetalaceae family. One of the hitherto uninvestigated species is Dichapetalum crassifolium Chodat widely distributed in Africa, Tropical Asia and Latin America (Breteler, 1978). It is typically found in the rain or gallery forests, primitive woods, shady places, and among rocks (Breteler, 1978; Hiern et al., 1901). Even though there is no documented ethnobotanical use and phytochemical investigation for D. crassifolium, other species of the genus have indicated the presence of a wide range of secondary metabolites with diverse biological

* Corresponding author.
E-mail addresses: antichama@yahoo.com, machama@ug.edu.gh (M.A. Chama).

https://doi.org/10.1016/j.heliyon.2020.e04460
Received 1 April 2020; Received in revised form 8 June 2020; Accepted 10 July 2020
2405-8440/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Apart from the cytotoxic and antiproliferative activities exhibited by the dichapetalins (Achenbach et al., 1995; Addae-mensah et al., 1996; Jing et al., 2014; Osei-Safo et al., 2017), this unique class of triterpenoids has also shown anthelmintic (Chama et al., 2015; Jing et al., 2014), antifungal, feeding deterrent, inhibition of intracellular release of nitric oxide (NO) and acetylcholinesterase (AChE) activities (Jing et al., 2014). As part of our quest for potentially active constituents against parasitic and other causative agents of various neglected tropical diseases, we report the anti-schistosomal activity of the crude extracts and constituents of the stems and roots of the hitherto uninvestigated *D. crassifolium* against clinical isolates of *Schistosoma haematobium* Bilharz 1852, (Tan and Ahana, 2007).

2. Materials and methods

2.1. Materials

The roots and stems of *D. crassifolium* were obtained from the Bobiri Forest Reserve in the Bosomtwe district of the Ashanti Region in July 2013. Identification was done by John Ntim-Gyakare formerly of the Forestry Commission, Kumasi. Voucher specimen (DCR001) has been deposited in the Ghana Herbarium, Department of Plant and Environmental Biology, University of Ghana.

TLC was performed on aluminium foil slides pre-coated with silica gel (thickness 0.2 mm, type Kieselgel 6 F254, Merck, Rogers, AR); detection: *I*₂ vapour, vanillin stain and anisaldehyde spray reagent. Column chromatography was carried out on silica gel 60 (Fluka Analytical, Bellefonte, PA). Melting points (uncorrected) determined on a Stuart Scientific Melting Point Apparatus (Sigma Aldrich, St. Louis, MO). IR spectra were obtained on an FT IR spectrometer at the Food and Drugs Authority in Ghana. Visualisation of spots under UV light was done with UVGL-58 Handheld UV lamp at 254–365 nm. Organic solvents were concentrated using Buchi Rotary Vacuum Evaporator.

NMR were run on a 500 or 600 MHz Bruker Avance instrument at 90, 125 or 150 MHz for ¹³C NMR and 360, 500 or 600 MHz for ¹H NMR. Depending on the solubility of a particular compound, solvents used were CDCl₃/CD₃OD, DMSO-d₆, acetone-d₆ or CD₃OD with TMS as the internal standard. Schistosomal activity testing was carried out at the parasitology laboratory of the Noguchi Memorial Institute for Medical Research, University of Ghana. Ethical clearance for the anti-schistosomal work was obtained from the Noguchi Memorial Institute for Medical Research (NMIRM-IRB CPN 059/13–14). Schistosome egg recovery and concentration from infected urine samples was by the modified Kotze et al. method (Kotze et al., 2005). Urine reagent strips were obtained from URIT Medical Electronic Co, Ltd, China. Different pore sizes of sieves for filtration of suspensions were obtained from Nonaka Rikaki Co. Ltd, Japan.

2.2. Methods

2.2.1. Sample collection of clinical isolates

*S. haematobium* eggs were obtained from urine samples collected from 120 school children in Tomefa in the Ga South District of Accra and stored in air-tight plastic containers. More than 80% of participants were between ages 8 and 14 years. Samples were first tested with urine reagent strips, URIT 10V to identify cases of haematuria and then kept in a Styrofoam box and transported to the parasitology laboratory at the Noguchi Memorial Institute for Medical Research.

2.2.2. Recovery, purification and identification of *S. haematobium* eggs from urine samples

Portions (10 ml) of collected urine samples were centrifuged and observed using a low power microscope to determine the presence of *S. haematobium*, whose eggs were identified by the presence of terminal spines (Figure 7). Urine samples that were positive for schistosoma eggs were pooled into 50 ml falcon tubes and centrifuged at 500xg for 5 min. The supernatant was discarded and the deposits suspended in 50 ml of normal saline (0.9%, NaCl) and centrifugation repeated. The sediment was suspended in 40 ml of 0.015% Brij-35 and shaken vigorously. It was centrifuged again at 500xg for 5 min. The supernatant was discarded and the sediment re-suspended in normal saline into a 200 ml beaker. More saline was added to make up to the 150 ml mark. Due to the different particle sizes of faecal samples which do not usually produce clean egg cells of the parasite, sieves of different pore sizes were used.

For differential separation to obtain cleaner schistosoma eggs, the suspension was filtered through a stack of three sieves of pore size 180 μm, 150 μm and 80 μm respectively.

The stack of sieves was thoroughly flushed with a jet of normal saline to wash the eggs. To prevent air lock, the 150 μm and 80 μm sieves were occasionally separated. At the end of washing, the 80 μm sieves were removed and inclined at approximately 45° to the horizontal and a jet of saline applied to it to wash off the eggs into a beaker. The suspension was centrifuged at 200xg for 10 min and left for 30 min after which the supernatant was aspirated down to 5 ml. Three 30 μl suspensions of the concentrated schistosome eggs were pipetted and observed under light microscope, the number of eggs per 30 μl was noted and hence the average number of eggs per 50 μl determined. From this result, the approximate number of *S. haematobium* eggs in the 5 ml concentrated egg solution was calculated.

2.3. Extraction, isolation and structure elucidation of compounds

Soxhlet extraction of 5 kg each of pulverized roots and stems in batches of 500 g of *D. crassifolium* was carried out exhaustively using 5 L of petroleum ether for 24 h to give 54 g root and 34 g of stem extracts after concentration, Figure 1. Each plant residue was further extracted with EtOAc and MeOH respectively after drying. Concentration of the root extracts gave 77 g (EtOAc extract) and 108 g (MeOH extract) crude material. The stem yielded 65 g (EtOAc extract) and 23 g (MeOH extract) of crude material, Figure 1.

Column chromatography of the petroleum ether extract of the stem (20 g) on 230 g silica gel, eluting with petroleum ether/EtOAc (10:1 to 0:10, v/v) gave three main fractions, S1–S3, Figure 2. Fraction S2 (12.6 g) was re-chromatographed on silica gel (80 g) with petroleum ether/CHCl₃ (1:3, v/v) to give three fractions (S4–S6). Fraction S4 gave compound 1 (180 mg), while fraction S6 yielded compound 2 (80 mg), both as white crystals, as well as a mixture of the two compounds. Similar separations of the petroleum ether extract of the roots also gave compounds 1 (215 mg), and 2 (82 mg) (Figure 2). The EtOAc extract (40 g) of the stem was also column chromatographed on silica gel (450 g) with petroleum ether/EtOAc solvent gradient (10:1 to 0:10, v/v) to give five main fractions S7–S11. Fraction S7 yielded compounds 1 and 2 identified respectively as friedelan-3β,8α-ol and friedelan-3β-ol and friedelan-3-ol (Figures 3 and 4) while S8 gave a mixture of β-sitosterol/stigmasterol (7 and 8, 25 mg), Figures 4 and 5. Fraction S9 precipitated solids recrystallized from a mixture of Et₂O/CHCl₃ (10:3, v/v) to afford a white powdery compound 4 identified as pomolic acid (60 mg, Figure 3). Fraction S11 was re-chromatographed in similar manner to afford compounds 6 (dichapetalin M, 20 mg) as a white amorphous solid, recrystallized from a mixture of Et₂O/CHCl₃ (2.5, v/v), and compound 3, maslinic acid (Figure 3) as a creamy powder (32 mg) recrystallized from a mixture of Et₂O/CH₃Cl (2.5, v/v), Figure 4. Similar separation procedures by column chromatography were also carried out on the crude EtOAc extract of the roots to yield compounds 3, 4, 5 and 6. Column chromatography of the EtOAc extract (38 g) on 450 g silica gel, eluting with petroleum ether/EtOAc solvent gradient (10:0 to 0:10, v/v) gave nine main fractions R1–R9. Fractions R1–R2 gave compound 3, while compound 4 was obtained from fraction R3 and compound 5 from fraction R4. Compound 5, 40 mg, was isolated from fraction R7 and recrystallized from Et₂O/EtOAc (10:1, v/v) as yellowish flakes (Figure 4). All compounds were characterised by comparison of their physico-chemical properties (melting point, TLC...
profile in various solvent systems), as well as their spectroscopic data (IR, \(^{1}H\) and \(^{13}C\) NMR) with literature data as indicated below.

2.3.1. Physico-chemical properties and spectral data of isolated compounds

(3S,4R,4aS,6aS,6bR,8aR,12aR,14aS,14bS)-4,4a,6a,6b,8a,11,11,14a-octamethyl-2,4,5,6,6a,7,8,9,10,12,12a,13,14,14b-tetradecahydro-1H-picen-3-one (Friedelan-3-one, 1): 53 mg as white crystals; mp: 274–276 °C (Lit. 274–276 °C, (Utami et al., 2013); Anisaldehyde: yellow; IR \(v_{\text{max}}\) (KBr) cm\(^{-1}\): 3477 (O–H), 2932, 2871 (C–H), 1448, 1385 [C(CH3)2]; Co-TLC with authentic sample in 100% CHCl3, Rf 0.81; petroleum ether/EtOAc (14:0.5), Rf 0.61; and petroleum ether/MesCO (12:0.5), Rf 0.5.

4,4a,6a,6b,8a,11,11,14a-octamethyl-2,4,5,6,6a,7,8,9,10,12,12a,13,14,14b-tetradecahydro-1H-picen-3-one (Friedelan-3-one, 2): 215 mg as white crystals; mp: 249–251 °C (Lit. 249–251 °C, (Utami et al., 2013); Anisaldehyde: purple; IR \(v_{\text{max}}\) (KBr) cm\(^{-1}\): 2927, 2870 (C–H), 1716 (C=C), 1462, 1389 [C(CH3)2]; Co-TLC

Figure 1. Flow diagram of the work done on petroleum ether, ethyl acetate and methanol extracts of the stem and root of \(D.\) crassifolium. PE: Petroleum ether.

Figure 2. Chromatographic separation of the petroleum ether extracts of the stem and root of \(D.\) crassifolium.

PE: Petroleum ether
with authentic sample in 100% CHCl₃, R f 0.94; petroleum ether/EtOAc (14:0.5), R f 0.87; and petroleum ether/Me₂CO (12:0.5), R f 0.82.

(2α,3β)-2,3-Dihydroxyolean-12-en-28-oic acid (Maslinic acid, 3), 50 mg as creamy powder: mp: 249–251°C (Lit. 248–250°C, (Hossain and Ismail, 2013); Anisaldehyde: purple; IR ν max (KBr) cm⁻¹: 3443 (O–H), 2941 (C–H), 1692 (C=O), 1461, 1385 (C–H), 1270 (C–O–H) and 1051.37 (C–O). ¹H and ¹³C NMR: see Table 1 (Woo et al., 2014) and Supplementary material Figures S1 and S2. TLC: petroleum ether/Et₂O (3:7), R f 0.68; petroleum ether/EtOAc (10:3), R f 0.53; petroleum ether/Me₂CO (10:3), R f 0.40; petroleum ether/EtOAc (10:6), R f 0.88.

(1R,2R,4αS,6αR,6aβ,8αR,8bR,10αS,12αR,14βS)-1,10-dihydroxy-1,2,6α,6β,9,9,12α-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14β-

Figure 3. Structures of friedelan-3β-ol 1, friedelan-3-one 2, maslinic acid 3 and pomolic acid 4.

Figure 4. Chromatographic separation of the ethyl acetate extracts of the stem and root of D. crassifolium. PE: Petroleum ether.
tetradecahydropicene-4-carboxylic acid (Pomolic acid, 4): 71 mg as white powder; mp: 268–270 °C (Lit. 276–278 °C); Vanillin stain: purple; anisaldehyde: violet; IR νmax (KBr) cm⁻¹: 3567, 3419 (O–H), 2937, 2874 (C–H), 1686, 1618 (C=O); ¹H and ¹³C NMR: See Table 1 (Chama et al., 2015; Lee et al., 2005; Zhu et al., 2012) and Supplementary material Figures S3 and S4; TLC: petroleum ether/Et₂O (3:7), Rf 0.81; petroleum ether/EtOAc (10:3), Rf 0.45; petroleum ether/Mg₂CO (10:3), Rf 0.29; petroleum ether/EtOAc (10:6), Rf 0.77; petroleum ether/EtOAc (1:1), Rf 0.71.

(3S,5R)-5-[(E)-3-hydroxy-2-methylprop-1-enyl]−3−{(1S,2R,3R,5R,6R,9S,14S,15R,18S,19S)-3-hydroxy-2,6,14-trimethyl-9-phenyl-8-oxahexacyclo[16.3.1.0¹,18.0²,15.0⁵,14.0⁶,11]docosa-11,16-dien-19-yl]oxolan-2-one (Dichapetalin A, 5): 40 mg as yellow flakes; mp: 210–213 °C (from Et₂O/EtOAc (10:1, v/v)) (Lit.: 212–213 °C, Osei-Safo et al., 2008); Vanillin stain: blue; Anisaldehyde: purple; IR νmax (KBr) cm⁻¹: 3573, 3552, 3369 (O–H), 2957, 2932, 2873 (C–H), 1746 (C=O), 1655 (C=O), 1096, 1039 (C=O–O), 767 (aromatic C–H); ¹H and ¹³C NMR: see Table 2 (Chama et al., 2015; Osei-Safo et al., 2008) and

![Figure 5. Structures of β-sitosterol (7) and stigmasterol (8).](image-url)

| Position | Maslinic acid (3) (CD₃OD/CDCl₃) | Pomolic acid (Acetone-D₆) |
|----------|---------------------------------|--------------------------|
|          | δH (J, Hz) (Woo et al., 2014)   | δH (J, Hz) (Lee et al., 2005; Zhu et al., 2012) |
|          | δC/ppm                          | δC/ppm                   |
| 1        | 47.7                            | 39.5                     |
| 2        | 68.8                            | 36.6(1H, ddd, J 1 = 10.3, J2 = 8.5, J3 = 6.5 Hz) | 27.5 (2.63(2H, ddd, J1 = 8.8, J2 = 9.6, J3 = 6.6 Hz) |
| 3        | 83.7                            | 3.45(1H, d, 10.3 Hz)     | 3.78(1H, d, J = 4.5, J2 = 10 Hz) |
| 4        | 39.3                            | -                        | 39.5                     |
| 5        | 55.4                            | 53.0                     | -                        |
| 6        | 18.5                            | 18.0                     | -                        |
| 7        | 32.7                            | 32.4                     | -                        |
| 8        | 39.4                            | 38.3                     | -                        |
| 9        | 49.0                            | 1.90(1H, dd, J1 = 4.2, J2 = 7.8 Hz) | 48.2 (2.55(1H, s) |
| 10       | 38.3                            | -                        | 37.2                     |
| 11       | 23.6                            | 1.61(2H, dd, J1 = 11.3, J2 = 11.3 Hz) | 23.8 (11H, s) |
| 12       | 122.2                           | 5.27(1H, t, J = 3.5 Hz)  | 128.5 (5.28(1H, t) |
| 13       | 144.1                           | -                        | 137.8                    |
| 14       | 42.0                            | -                        | 40.9                     |
| 15       | 39.8                            | -                        | 29.2                     |
| 16       | 33.0                            | 1.95(2H, ddd, J1 = 11.6, J2 = 9.6, J3 = 9.6 Hz) | 26.3 (1.95(2H, d, J = 5 Hz) |
| 17       | 46.5                            | -                        | 47.8                     |
| 18       | 41.3                            | 2.83(1H, dd, J1 = 7.8, J2 = 8.3 Hz) | 54.8 (2.80(1H, s) |
| 19       | 46.3                            | -                        | 72.5                     |
| 20       | 30.8                            | -                        | 40.8                     |
| 21       | 34.0                            | -                        | 26.4                     |
| 22       | 33.2                            | -                        | 38.1 (1.94(2H, d, J = 4.6 Hz) |
| 23       | 28.7                            | 1.02(3H, s)              | 27.8 (2.55(3H, s) |
| 24       | 16.6                            | 0.78(3H, s)              | 16.0 (2.25(3H, s) |
| 25       | 16.8                            | 0.98(3H, s)              | 15.1 (1.35(3H, s) |
| 26       | 17.0                            | 0.81(3H, s)              | 18.1 (1.21(3H, s) |
| 27       | 25.7                            | 1.06(3H, s)              | 25.1 (0.99(3H, s) |
| 28       | 18.0                            | -                        | 18.0                     |
| 29       | 32.7                            | 0.90(3H, s)              | 25.6 (0.95(3H, d, J = 6.5 Hz) |
| 30       | 23.6                            | 0.93(3H, s)              | 15.5 (0.79(3H, d, J = 6.5 Hz) |

Table 1. ¹H and ¹³C NMR data of maslinic acid and pomolic acid isolated from D. crassifolium.
Supplementary material Figures S5 and S6; Co-TLC with authentic sample in petroleum ether/Me₂CO (10:3), Rf 0.40; petroleum ether/EtOAc (10:4), Rf 0.82; petroleum ether/EtOAc (10:6), Rf 0.20.

2.4. In vitro screening of test samples against schistosome eggs using the 96-well plate-egg hatch assay

For each of the test compounds; namely, friedelan-3-one (2), dichapetalins A (5) and M (6) and mixture of β-sitosterol/stigmasterol (6, 7), 2

Table 2. ¹H and ¹³C NMR data of dichapetalins A and M isolated from D. crassifolium.

| Position | Dichapetalin A (5) | Dichapetalin M (6) |
|----------|-------------------|-------------------|
| δc (ppm) | δH (Mult)        | δc (ppm)         | δH (Mult) |
| 1(CH₂)   | 41.3 2.15, s      | 39.9 2.90,1.65   |
| 2(CH)    | 119.3 5.45, m    | 116.6 5.40       |
| 3(C)     | 141.2 -           | 141.3 -          |
| 4(C)     | 39.5 -            | 39.8 -           |
| 5(CH)    | 42.5 2.00⁺        | 59.1 1.60        |
| 6(CH₂)   | 24.2 1.87,m       | 71.3 4.60,d,3.80 |
| 7(CH)    | 73.2 3.92 (t, J = 1.5) | 213.7 -         |
| 8(C)     | 37.4 -            | 47.0 -           |
| 9(CH)    | 45.1 1.75 (d, J = 2.5) | 52.1 2.00       |
| 10(C)    | 37.2 -            | 36.9 -           |
| 11(CH)   | 124.1 5.43 (dd, J = 10, 2.5Hz) | 120.1 5.40,dd    |
| 12(CH)   | 131.1 6.25 (dd, J = 10, 3Hz) | 131.6 6.35,dd    |
| 13(C)    | 30.6 -            | 31.6 -           |
| 14(C)    | 37.5 -            | 36.5 -           |
| 15(CH₂)  | 26.2 2.04⁺        | 26.7 2.00,2.20   |
| 16(CH)   | 22.9 1.13, s      | 23.6 1.30,1.80,m |
| 17(CH)   | 41.8 2.62, m      | 40.5 2.55        |
| 18(CH)   | 18.4 0.94, s      | 17.1 1.10        |
| 19(CH)   | 18.6 -            | 18.2 1.30,s      |
| 20(CH)   | 42.6 3.30, m      | 47.3 3.00,dd,2.45 |
| 21(C)    | 181.3 -           | 174.0 -          |
| 22(CH₂)  | 31.4 2.37,m       | 72.1 4.20,dd     |
| 23(CH)   | 77.1 5.26, m      | 111.3 -          |
| 24(CH)   | 123.1 5.50, s     | 45.7 2.90,2.50,d |
| 25(C)    | 143.2 -           | 85.1 -           |
| 26(CH₂)  | 67.3 3.98, s      | 78.5 4.35,d,4.10,d |
| 27(CH)   | 14.1 1.75, s      | 22.0 1.70,s      |
| 28(CH)   | 24.7 1.32, s      | 26.5 1.55,s      |
| 30(CH)   | 16.9 0.85 (d, J = 5Hz) | 14.4 1.30,d,1.05 |
| 2'(CH₂)  | 73.6 3.56 (d, J = 10.5Hz) | 71.3 4.35,3.95,d |
| 5'(CH₂)  | 42.6 2.20 (dd, J = 13.5, 4Hz) | 40.1 2.70, 2.25 |
| 6'(CH)   | 83.6 4.27 (dd, J = 11.5, 2.5Hz) | 81.3 4.35,dd    |
| 1'(C)    | 143.90 -          | 142.5 -          |
| 2''/6''/5'' (CH) | 126.97 7.37, m | 128.4 7.45,d    |
| 3''/5'' (CH) | 129.5 7.33, m | 125.7 7.40,t    |
| 4'(CH)   | 128.5 7.26, m     | 127.5 7.30,i     |
| AcCO(C)  | 170.4 -           | -                |
| AcCH₃(CH₂) | 21.8 2.00, s    | 2.00,s          |

* Overlapping signals.
mg was dissolved in 1 ml of 5% DMSO to give 2 mg/ml stock solution. For each of petroleum ether, EtOAc, and MeOH crude extracts of the roots and stems, 5 mg was dissolved in 1 ml of 5% DMSO to obtain a stock solution of 5 mg/ml.

For each test sample of the stock solution, 100 μl was pipetted in duplicate into the first wells of 96-well plate. Serial dilutions of the 2 mg/ml solutions produced 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/ml. Serial dilutions with concentrations 2.5, 1.25, 0.625, 0.3125, 0.15625, and 0.078125 mg/ml of the stock (5 mg/ml) solution of the crude extracts were also prepared. From the suspension of purified eggs, 50 μl was added to each well and the presence of eggs was ascertained under an inverted microscope. The number (#) of hatched eggs and unhatched eggs, 50 μl was added to each well and the presence of eggs was ascertained under an inverted microscope. The final concentration of DMSO was kept below 0.1%. The choice of DMSO as a solvent and selection of suitable concentration that had no effect on the morphology of the eggs followed the method of Treger et al. (2014). Water and praziquantel (2 mg/ml stock solution) were used as negative and positive controls respectively. Pra-ziquantel, because of its partial solubility in cold water, was dissolved in warm water. The assays were incubated for 24 h at ambient temperature.

Egg hatch inhibition (%EHI) was calculated as:

$$\text{%EHI} = \frac{\text{# of unhatched eggs}}{\text{# of unhatched eggs} + \text{# of hatched eggs}} \times 100$$

The %EHI was plotted against log of the concentration. Extrapolation of the 50% EHI on the curve gave the half maximal inhibitory concentration (IC50) of each test sample using GraphPad Prism v.7.

### Results and discussion

Compound 1 was identified as friedelan-3β-ol (Figure 3), mp 272–274 °C (Lit. 274–276 °C, Utami et al., 2013) and stained purple with anisaldehyde spray reagent. Compound 2 was also identified as friedelan-3-one (Figure 3), mp 249–251 °C, (Lit. 249–251 °C, Utami et al., 2013; Sousa et al., 2012) and stained yellow on TLC with anisaldehyde spray reagent. Other physico-chemical and spectroscopic properties were consistent with those reported in literature (Chama, 2007; Chama et al., 2015; Osei-Safo et al., 2008). Compound 3 was identified similarly as maslinic acid (Figure 3, Table 1), creamy powder which stained purple with anisaldehyde spray reagent, mp 249–251 °C (Lit. 248–250 °C, (Hossain and Ismail, 2013; Lozano-Mena et al., 2014; Tanaka et al., 2003; Woo et al., 2014). This is the first report of maslinic acid from the genus Dichapetalum aside its isolation from Crataegus oxy-acantha and Eriobotrya japonica. Compound 4 was characterised as pomolic acid (Figure 3, Table 1). Melting point and spectral data were consistent with literature values (Chama et al., 2015). Compounds 5 and 6 (Figure 6) were each identified as dichapetalin A and M, respectively upon comparison of their physico-chemical and spectral data with literature, Table 2. Melting point of dichapetalin A (210–213 °C) and spectral data (Table 2) were consistent with literature (Lit: 212–213 °C, Achenbach et al., 1995; Addae-mensah et al., 1996; Chama et al., 2015; Osei-Safo et al., 2008). Melting point of dichapetalin M, 282–284 °C and spectral data were also consistent with that of known values (Lit: 280–282 °C, Osei-Safo et al., 2008). This is the first report of the presence of these two dichapetalins in D. crassifolium and this could be of chemotaxonomic importance. With the exception of compounds 1 and 2 which were isolated from the petroleum ether extracts, the rest of the compounds including 1 and 2 were isolated from the EtOAc extracts. Phytochemical screening indicated the petroleum ether extracts contained only terpenoids in the stem and terpenoids, saponins and cardiac glycosides in the roots. Also, terpenoids and tannins were present in the EtOAc extracts of both the stem and root extracts in addition to saponins and cardiac glycosides which were present only in the roots. The methanol extracts contained terpenoids, tannins, and cardiac glycosides.

Dichapetalin A was first isolated from D. madagascariense (Achenbach et al., 1995) as the major constituent of the roots. Later the compound was isolated from D. gelonoides (Fang et al., 2016), D. ruhlandii, D. mombutense, D. zerkerti, D. eickii, D. filicula (Chama et al., 2015) and D. pullidum (Osei-Safo et al., 2017). Dichapetalin M has so far been isolated from only two other species, D. Madagascariense (Osei-Safo et al., 2008) and D. eickii (Long et al., 2013). Pomolic acid has also been isolated from D. filicula (Chama et al., 2015) and D. pullidum (Osei-Safo et al., 2017). Earlier isolation of friedelan-3β-ol and friedelan-3-one was reported in D. barteri, D. madagascariense (Osei-Safo et al., 2008), D. filicaule (Chama...
et al., 2015) and D. pallidum (Osei-Safo et al., 2017). β-Sitosterol and stigmasterol have previously been isolated from D. barteri and D. madagasca
crassifolium. β-Sitosterol and stigmasterol commonly occur in most plants and differ structurally only at the C22-C23 due to the presence of unsaturation in the latter and as a result both are often isolated together as a mixture.

The most abundant of the triterpenoids were the friedelan-3β-ol and friedelan-3-one. Even though phytochemical screening indicated the presence of terpenoids, tannins and cardiac glycosides in the roots and terpenoids, tannins in the stem of the ethyl acetate extracts, only triterpenoids were isolated suggesting that they constituted the abundant phytochemicals in the plant whiles tannins and cardiac glycosides occurred in least amounts.

3.1. Results of in vitro anti-schistosomal activity against Schistosoma haematobium

The picture of Schistosoma haematobium eggs identified under a low power microscope is presented in Figure 7. The half maximal inhibitory concentrations, IC₅₀ (µg/ml, ± SEM) of reference standard, compounds and extracts were determined for duplicate experiments (n = 2) as presented in Table 3.

Generally, the isolated compounds showed higher ovicidal activity against S. haematobium eggs than the extracts though activities for both samples were low compared to the standard praziquantel (Table 3). Among the compounds isolated from the stem, the mixture of β-sitosterol/stigmasterol showed the highest potency (IC₅₀ 177.90 µg/ml) but this was about 11 times less potent than the standard praziquantel drug (15.47 ± 0.06 µg/ml) (Table 3, Figure 8). The next highest was dichapetalin A (151.10 µg/ml) whiles friedelan-3-one showed the least potency with IC₅₀ 378.10 µg/ml (Table 3, Figures 8 and 9). Dichapetalin M from the root extract gave an IC₅₀ of 191.00 µg/ml, (Table 3, Figure 9).

The most abundant of the triterpenoids were the friedelan-3β-ol and friedelan-3-one. Even though phytochemical screening indicated the presence of terpenoids, tannins and cardiac glycosides in the roots and terpenoids, tannins in the stem of the ethyl acetate extracts, only triterpenoids were isolated suggesting that they constituted the abundant phytochemicals in the plant whiles tannins and cardiac glycosides occurred in least amounts.

3.1. Results of in vitro anti-schistosomal activity against Schistosoma haematobium

The picture of Schistosoma haematobium eggs identified under a low power microscope is presented in Figure 7. The half maximal inhibitory concentrations, IC₅₀ (µg/ml, ± SEM) of reference standard, compounds and extracts were determined for duplicate experiments (n = 2) as presented in Table 3.

Generally, the isolated compounds showed higher ovicidal activity against S. haematobium eggs than the extracts though activities for both samples were low compared to the standard praziquantel (Table 3). Among the compounds isolated from the stem, the mixture of β-sitosterol/stigmasterol showed the highest potency (IC₅₀ 177.90 µg/ml) but this was about 11 times less potent than the standard praziquantel drug (15.47 ± 0.06 µg/ml) (Table 3, Figure 8). The next highest was dichapetalin A (151.10 µg/ml) whiles friedelan-3-one showed the least potency with IC₅₀ 378.10 µg/ml (Table 3, Figures 8 and 9). Dichapetalin M from the root extract gave an IC₅₀ of 191.00 µg/ml, (Table 3, Figure 9).

All the triterpenoids were isolated from the polar EtOAc extracts. This might be the reason why the most active compound, dichapetalin A was isolated from the most active root EtOAc extract. The root crude extracts were generally more active than the stem crude extracts (Table 3) even though the root EtOAc extract contained additional saponins and cardiac glycosides aside the terpenoids and tannins which were also contained in the stem EtOAc extract. This could be because of the roots containing more of the terpenoids as observed in the greater yield of the root extracts (1.5%) compared with the stem extracts (1.3%).

The IC₅₀ values for the root extracts were 248.60 (EtOAc), 546.40 (petroleum ether) and 566.30 (MeOH) µg/ml while the stem crude extracts gave values of 443.70 (petroleum ether), 638.00 (EtOAc) and 893.70 (MeOH) µg/ml (Table 3, Figures 10 and 11).

Against the beet armyworm (Spodoptera exigua), dichapetalin A showed antifeedant activity with an EC₅₀ of 3.1 µg/cm² (Jing et al., 2014). The compound also indicated nematicidal activity of 15.3% rate at 100 µg/ml over 72 h against Panagrellus redivivus (Jing et al., 2014) and anti-hookworm activity against Necator americanus eggs with IC₅₀ of 162.4 µg/ml (Chama et al., 2015). Against parasitic fungi, dichapetalin A was almost as active as nystatin with growth inhibition of 12.8 ± 0.8 and 10.5 ± 0.02 at 50 µg/disk on Colletotrichum musae and Rhizoctonia solani respectively (Jing et al., 2014).

Except for the anti-schistosomal activity, dichapetalin M has not been tested for its antiparasitic activities. Antiplasmodial activity evaluated for friedelan-3-one (2) against W2 strain of Plasmodium falciparum gave an IC₅₀ of 7.7 Min (Ndjakou Lenta et al., 2007). Stigmasterol showed potency on both 3D7 and K1 strains of Plasmodium falciparum with EC₅₀ of 3
S. mansoni (Sass et al., 2014). The diterpene phytol, from chlorophyll has also cellular molecules of parasites to cause morphological changes (de Moraes, 2015). Monoterpenes with derivatives 4–62, 5-dihydrobudlein A and 4,5-11-tetrahydrobudlein A,13-tetrahydrobudlein A, and its derivatives 4α, 5-dihydrobudlein A and 4α,5-11β,13-tetrahydrobudlein A gave 100% mortality at the respective concentrations of 50 and 200 μM (Sass et al., 2014). The diterpene phyto, from chlorophyll has also indicated promising in vitro and in vivo activity with infected mice against adult S. mansoni (de Moraes et al., 2014). Aside these, triterpenes such as betulin isolated from Schefﬁeria vinosa plant was effective in vitro against S. mansoni adult worms at a concentration of 100–200 μM (Canha et al., 2012). The triphenylphosphonium derivatives of betulin and betulinic acid showed in vitro antischistosomal activity against newly transformed schistosomula and adult worms of S. mansoni at concentrations between 10 μM and 2 μM, respectively (Spivak et al., 2014). Moreover, balsaminol F and karavilagenin C are cucurbitane-type triterpenes from Momordica balsamina with effective in vitro antischistosomal activity against S. mansoni adult worms at LC50 values of 15 and 29 μM respectively (Ramalhete et al., 2012). Crude extracts and fractions of different families of plants have also indicated antischistosomal activity at varying degrees of potency. The stem bark and roots of Rauvolfia vomitoria killed all cercariae stage of S. mansoni within 2 h of exposure at a respective concentration range of 62.5–1000 μg/ml and 250–1000 μg/ml. The LC50 values after 1 and 2 h of exposure were 207.4 μg/ml (stem) and 61.18 μg/ml (root). In addition, both the stem and root of the plant showed 100 mortality of the adult worm within 120 h of incubation at a concentration range of 250–1000 μg/ml (Tekwu et al., 2017). The MeOH extracts of Curcuma longa L. (Zingiberaceae) and Nerium oleander L. (Apocynaceae) showed 100% S. mansoni worm mortality after a 24 h incubation period at concentrations up to 100 μg/ml (Abdel-Hameed et al., 2008). Also, MeOH extracts of five plants Dryopteris ﬁliax (Dryopteridaceae), Tanacetum vulgare (Asteraceae), Juglans nigra (Juglandaceae), Syzygium aromaticum (Myrtaceae) and Allium sativum (Liliaceae) exhibited strong potency at minimum effective concentrations of 50 μg/ml after 24 h against adult S. mansoni worms (Metwallie, 2015). In a study to evaluate the effect of MeOH extracts on S. mansoni infected Swiss Albino Mice, Malus domestica (Rosaceae) showed signiﬁcant (P < 0.05) antischistosomal activity at concentrations 300 mg/kg and 200 mg/kg with respective worm reduction of 85.93% and 72.22%. Allium cepa (Liliaceae), at 500 mg/kg and 300 mg/kg indicated 72.59% and 58.52% worm reductions respectively, whereas Citrus limon (Rutaceae) showed the least worm reduction of 42.96% at 200 mg/kg and 26.63% at 100 mg/kg (Moema et al., 2015). In a similar study, different solvent extracts of Ocimum americanum (Lamiaceae) and Bridelia micrantha (Phyllanthaceae) gave signiﬁcant antischistosomal activity against S. mansoni infected Swiss Albino Mice (Waiganjo et al., 2016).

4. Conclusion

This study has established the presence of tetracyclic and pentacyclic triterpenoids from the stems and roots of D. crassifolium. The identiﬁcation of dichapetalins from the plant is chemotaxonomically signiﬁcant since other species of the genus have been shown to contain this unique class of triterpenoids. So far, nine species of this genus have been shown to contain the dichapetalin class of compounds. However, only the roots of D. crassifolium contained both dichapetalins A and M, the stem yielded only dichapetalin M. For the ﬁrst time, maslinic acid and pomolic acid have been isolated from both the stems and roots of the plant. The activity of both extracts and isolated triterpenoids against S. haematobium was very low to merit consideration as potential lead compounds for development into any anti-schistosomal agent. The extracts and compounds might have an effect on other stages of the diseases such as the schistosomulae, miracidiae and the cercariae. Thus, a study of infections from experimental animals might reveal useful information on this. The general low activity of the tested compounds and extracts may be attributed to the facts that eggs of schistosomes have multiple layers between the shell and the larva. Hence, the presence of these layers, together with the minute size of pores in the egg shells, may preclude penetration of eggs by the extracts used.

Declarations

Author contribution statement

Mary Anti Chama: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data. Wrote the paper.

Dorcas Osei-Safo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ivan Addae-Mensah: Conceived and designed the experiments. Contributed reagents, materials, analysis tools or data.

Henry Akwaffo Onyame, Claudine Fleischer, Reiner Waibel, Joseph Otchere: Performed the experiments.

Funding statement

This work was supported by the University of Ghana Research Fund [Grant numbers URF/5/ILG-009/2011-2012].
Compelling interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04460.

Acknowledgements

The authors acknowledge the people of Toméfa in the Ga South District of Greater Accra for providing urine samples.

References

Abdel-Hameed, E., El-Nahas, H., Abo-Sedera, S., 2008. Antichistosomal and antimicrobial activities of some Egyptian plant species. Pharmacol. Biocat. 46 (9), 626–633.

Achcabach, H., Anunka, S., Waibel, R., Adad-Mansh, I., Oppong, I., 1995. Dichapetalin A, a novel plant constituent from Dichapetalum madagascariense with potential antineoplastic activity. Nat. Prod. Lett. 7 (2), 93–100.

Adad-Mansh, I., Adu-Kumia, S., Waibel, R., Oppong, I., 2007. A novel D:A-friedelanone triterpenoid and other constituents of the stem bark of Dichapetalum tortuor Engl. Alkaloid 2007 (9), 71–75.

Adad-mansh, I., Waibel, R., Anunka, S.A., Oppong, I.V., Achenbach, H., 1996. The dichapetalins–A new class of triterpenoids. Phytochemistry (3), 649–656.

Ado-Kumi, S., 1997. Phytochemical And Bioactivity Studies On the Chemical Constituents species and their isolated compounds. Eine base Compl. Alternative Med. 2012.

Ado-Kumi, S., 1997. Phytochemical And Bioactivity Studies On the Chemical Constituents species and their isolated compounds. Eine base Compl. Alternative Med. 2012.

Ado-Kumi, S., 1997. Phytochemical And Bioactivity Studies On the Chemical Constituents species and their isolated compounds. Eine base Compl. Alternative Med. 2012.

Afro-African American Association. The African Dichapetalaceae IV: a Taxonomical Revision: Wageningen.

Africar J. 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of A. C. Kotze, A.C., Coleman, G.T., Mai, A., McCarthy, J.S., 2005. Field evaluation of Hossain, M.A., Ismail, Z., 2013. Isolation and characteri...