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Chemical constituents and antibacterial activity of *Annickia chlorantha* (Oliv.) Setten & Maas (Annonaceae) and their chemophenetic significance

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Abstract

Background: *Annickia chlorantha* (Annonaceae) is an ornamental tree, which may grow up to 30 m high with dense foliage and spreading crown. It is used in traditional medicine for its anti-infective properties particularly in the treatment of fungal infections, malaria, hepatitis, jaundice and other bacterial infections. This study describes the chemical and antibacterial investigation of ethanol extract of the stem bark of *A. chlorantha*.

Methods: The compounds were obtained by separation using silica gel and Sephadex LH-20 CC. Their structures were elucidated through the analysis of their NMR and MS data. The antibacterial activities of the crude extract and isolated compounds were carried out using microdilution method against five sensitive bacterial strains

Results: The chemical investigation of the stem bark of *A. chlorantha* led to the isolation of ten known secondary metabolites classified into seven alkaloids (**1**-**7**), one triterpene (**9**), and two steroids (**8a-8b**, **10**) including 1,2,3-trimethoxy-4,5-dioxo-6a,-dehydroaporphine A (**1**), cepharadione B (**2**), *O*-methyl-moschatoline (**3**), palmatine (**4**), jatrorrhizine (**5**), columbamine (**6**), pseudojatrorrhizine (**7**), the mixture of *β*-sistosterol (**8a**) and stigmasterol (**8b**), cyclofoetigenine A (**9**) and *β*-sitosterol-3-*O*-*β*-*D*-glucopyranoside (**10**). Their structures were elucidated through the analysis of their NMR and MS data. The crude extract exhibited weak antibacterial activity against *Staphylococcus aureus* (MIC = 1000 *µ*g/mL). Compounds **2** and **4** exhibited moderate activity against *Staphylococcus aureus* (MIC = 125 µg/mL), while compound **5** displayed the same activity against *Shigella flexneri*. The chemophenetic significance of the isolated compounds was discussed.

Conclusion: The phytochemical and antibacterial investigation carried out on *A. chlorantha* showed that this plant contains some antibacterial compounds justifying its uses in traditional medicine for the treatment of bacterial infections.

Keywords: *Annickia chlorantha;* Annonaceae; chemophenetic significance; antibacterial activity; phytochemical.

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Background

The Annonaceae family is a pantropical flowering plant, which constitutes the most species-rich family of Magnoliales with approximately 108 genera and 2400 species worldwide [1]. The family is divided into four subfamilies including Ambavioideae, Anaxagoreoideae, Annonoideae, and Malmeoideae [1]. The genus *Annickia* is one of the five genera of the Piptostigmateae tribe, which is one of the seven tribes of the Malmeoideae subfamily [1]. Also called “Mpol” (“Moambé jaune” in French) by Bakola pygmies in Cameroon, *Annickia chlorantha* (Oliv.) Setten & Maas (Annonaceae) is one of the eight species that comprise the genus *Annickia* [2]. *A. chlorantha* is widely distributed along the coasts of West and Central Africa. It is an ornamental tree that may grow up to 30 m high with dense foliage and a spreading crown. The outer back (thin and dark brown) is fissured geometrically, while the inner bark is brown above and pale cream [3]. The stem is fluted and aromatic, while the leaves are elliptic with about 0.14-0.14 m long and 0.05-0.14 m broad [3]. *A. chlorantha* is used in traditional medicine for its anti-infective properties, particularly in the treatment of fungal infections, malaria, hepatitis, jaundice, and other bacterial infections [2, 4-6].

Previous phytochemical works undertaken on the bark of *A. chlorantha* led to the isolation of alkaloids [7-9] and sesquiterpenes [2]. Extracts and isolated compounds from A. chlorantha exhibited antimicrobial, antimalarial, antileishmanial, antiprotozoal, antiviral, antifungal, anti-ulcer, antitripanosomial activities [2, 10] and anticancer activity [11]. Other species of the genus *Annickia* exhibited antimicrobial, antiplasmodial, and antimalarial activities for *A. polycarpa* [12-13] and antiplasmodial, and antileishmanial activities for *A. kummeriae* [14].

Methods

General experimental procedure

For this study, ethanol and dichloromethane were used for the extraction of the plant material; *n*-hexane, dichloromethane, ethyl acetate, and methanol were used as pure or binary mixtures at different polarities for the purification of compounds. Column chromatographies were carried out on silica gel 230-400 mesh, Merck (Merck, Darmstadt, Germany), 63-200 mesh (Merck), or Sephadex LH-20 (Sigma-Aldrich, Munich, Germany). Thin-layer chromatography (TLC) was performed on Merck pre-coated silica gel (60 F_{254}) aluminum foil (Merck) and compound spots were detected by spraying plates with diluted sulfuric acid before heating at about 100°C. Mass Spectra were obtained with a QTOF Compact Spectrometer (Bruker, Germany). The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 500 MHz and 600 MHz NMR spectrometers (Bruker Corporation, Brussels, Belgium) in deuterated solvents.

Plant material

The stems of *A. chlorantha* were collected in Yaoundé-Cameroon in June 2018 and identified by Mr. Victor NANA at the National Herbarium of Cameroon by comparison with voucher specimen N° 45569/HNC.

Extraction and isolation

The air-dried and powdered vegetative stems of *A. chlorantha* (4 kg) were macerated twice using dichloromethane-ethanol-ammonia (1:1:0.25, v/v/v) for 72 hours at room temperature to yield 300.0 g of the crude extract. The crude extract (295 g) was acidified with 5% aqueous HCl (300 mL) [15] and extracted with methylene chloride to afford 85.0 g of the neutral fraction. The residual part was treated with NH_4OH (pH= 9) and extracted with dichloromethane and *n*-butanol to afford 150.0 g and 50.0 g of alkaloids fractions, respectively. Part of the neutral fraction (80.0 g) was subjected to silica gel column chromatography using a gradient of *n*-hexane and ethyl acetate from 100:0 to 0:10 (v/v). One hundred and eighty five (185) sub-fractions of 300 mL each were collected and combined according to their TLC profiles to afford four fractions F1[2.3 g, *n*-hexane-EtOAc (90:10, v/v)]; F2 [10.8 g, *n*-hexane-EtOAc (30:70, v/v)]; F3 [1.3 g, *n*-hexane-EtOAc (10:90, v/v)]; F4 [10.5 g, EtOAc-MeOH (90:10, v/v)].

Part of fraction F1 (2.0 g) was subjected to column chromatography (CC) over silica gel, eluting with the isocratic *n*-hexane-EtOAc (95:5, v/v) to yield 1.0 g of the mixture of compounds **8a** and **8b**. Fraction F2 (10.0 g) was subjected to CC over silica gel eluting with the mixture of *n*-hexane-EtOAc in increasing polarities followed by separation on a Sephadex LH-20 CC to give compounds **1** (40.7 mg; *n*-hexane-EtOAc, 80:20, v/v), **2** (30.4 mg; *n*-hexane-EtOAc, 80:20, v/v), **4** (100.5 mg; *n*-hexane-EtOAc, 85:15, v/v). Fraction **3** (1.0 g) was also chromatographed on a silica gel column with mixture of *n*-hexane-EtOAc and EtOAc-MeOH as eluent in increasing polarities to afford compounds **3** (10.6 mg; *n*-hexane-EtOAc, 20:80, v/v), **9** (8.7 mg; *n*-hexane-EtOAc, 40:60, v/v) and **10** (300.8 mg; EtOAc-MeOH, 98:2, v/v). Fraction F4 (10.5 g) was an insoluble gum that was not further investigated. 140.0 g of the alkaloid fraction were purified using a gradient of CH_2Cl_2-MeOH over a silica gel CC previously basified with NH_4OH 10% to afford compounds **4** (5.0 g; CH_2Cl_2-MeOH, 95:5, v/v), **5** (1.0 g; CH_2Cl_2-MeOH, 90:10, v/v), **6** (500 mg; CH_2Cl_2-MeOH, 90:10, v/v) and **7** (200 mg; CH_2Cl_2-MeOH, 80:20, v/v).

Bacteria strains and sample preparation

The microorganisms used in this study consisted of five sensitive bacterial strains namely *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC43300, *Shigella flexneri* 518, *Pseudomonas aeruginosa* NR and *Klebsiella pneumoniae* NR 41597. Stock solutions of extracts and compounds were prepared at 10 mg/mL by dissolving 10 mg of extract and compound in 1mL of 10% DMSO. Ciprofloxacin was prepared under the same conditions, at 1 mg/mL in acidified sterile distilled water and served as a positive control during the tests.

Determination of Minimal Inhibitory Concentration (MIC)

The antibacterial assay of extracts and some compounds was performed using microdilution method [16] to determine the minimal inhibitory concentration. The tests were carried out in triplicate in sterile 96-well microplates. Indeed, 160 µL and 180 µL of MHB culture medium were introduced, respectively into the first wells corresponding to the extracts and to the compounds and 100 µL into the rest of the wells of the plate. Subsequently, 40 µL of a sterile solution of extracts and 20 µL of a solution of compounds concentrated at 10 mg/mL were taken and introduced into the corresponding wells, followed by a series of 4 dilutions of geometric ratio of order 2. Finally, 100 µL of a bacterial suspension with a load of $1x10^6$ cells/mL were distributed in the test wells and those of the negative control. The concentrations of extracts,

compounds, and ciprofloxacin in the wells ranged from 1000 µg/mL to 62.5 µg/mL, from 500 µg/mL to 31.25 µg/mL, and from 0.24 µg/mL to 0. 0076 µg/mL respectively and the final inoculum load in each well was 5x105 cells/mL. The sterility control consisted solely of culture medium. The positive control consisted of the culture medium, inoculum, and ciprofloxacin. Microplates were covered and then incubated at 37°C for 24 hours. At the end of the incubation period, 20 µL of a freshly prepared resazurin solution (0.15 mg/mL) was added to all wells and the plates were again incubated under the same conditions for 30 minutes. The lowest concentration before the color change (from blue to pink) was considered as the minimal inhibitory concentration.

Results and discussion

The structures of the isolated compounds **1**-1**0** were determined on the basis of the spectroscopic and mass spectrometric data as 1,2,3-trimethoxy-4,5-dioxo-6a,7-dehydroaporphine A (**1**) [17], cepharadione B (**2**) [18], *O*-methyl-moschatoline (**3**) [19], palmatine (**4**) [14], jatrorrhizine (**5**) [14], columbamine (**6**) [14], pseudojatrorrhizine (**7**) [20], the mixture of *β*-sistosterol (**8a**) and stigmasterol (**8b**) [21], cyclofoetigenine A (**9**) [22] and *β*-sitosterol--3-*O*-*β*-*D*-glucopyranoside (**10**) [23].

1,2,3-Trimethoxy-4,5-dioxo-6a,7-dehydroaporphine (**1**) : yellow powder; ESI MS: *m/z* 374,094 [M+Na], ^{1}H NMR data (500 MHz, $CDCl_3$): δ_H (ppm) 7.65 (1H, *s*, H-7), 7.91 (1H, *m,* H-8), 7.65 (1H, *m*, H-9), 7.65 (1H, *m*, H-10), 9.48 (1H, *m,* H-11), 3.86 (3H, *s*, 6-NCH_3), 4.09 (3H, *s*, 3-OCH_3), 4.18 (3H, *s*, 2-OCH_3), 4.13 (3H, *s*, 1-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) 160.1 (C-1), 147.4 (C-2), 158.4 (C-3), 121.4 (C-3a), 174.5 (C-4), 157.0 (C-5), 131.7 (C-6a), 117.1 (C-6b), 115.1 (C-7), 131.8 (C-7a), 128.8 (C-8), 127.6 (C-9), 127.6 (C-10), 127.3 (C-11), 127.0 (C-11a), 121.3 (C-11b), 30.9 (6-NCH_3), 62.2 (3-OCH_3), 60.1 (2-OCH_3), 61.3 (1-OCH_3).

Cepharadione B (2) : yellow powder; ESI MS: *m/z* 344,123 [M+Na], ^{1}H NMR data (500 MHz, $CDCl_3$): δ_H (ppm) 8.30 (1H, *s*, H-3), 7.55 (1H, *s*, H-7), 7.90 (1H, *m,* H-8), 7.67 (1H, *m*, H-9), 7.67 (1H, *m*, H-10), 9.55 (1H, *m,* H-11), 3.90 (3H, *s*, 6-NCH_3), 4.17 (3H, *s*, 2-OCH_3), 4.14 (3H, *s*, 1-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) 155.3 (C-1), 152.6 (C-2), 112.9 (C-3), 127.0 (C-3a), 175.8 (C-4), 156.4 (C-5), 132.1 (C-6a), 119.9 (C-6b), 114.5 (C-7), 133.0 (C-7a), 129.2 (C-8), 127.8 (C-9), 128.2 (C-10), 127.3 (C-11), 129.1 (C-11a), 123.3 (C-11b), 30.6 (6-NCH_3), 56.7 (2-OCH_3), 60.5 (1-OCH_3).

***O*-Methylmoschatoline** (**3**) : orange needles ; ^{1}H NMR data (500 MHz, $CDCl_3$): δ_H (ppm) 8.26 (1H, *dd*, *J* = 8.3; 1.0 Hz, H-4), 9.02 (1H, *d*, *J* = 5.4, H-5), 8.61 (1H, *dd*, *J* = 7.9; 1.7 Hz, H-8), 7.58 (1H, *m*), 7.78 (1H, *ddd*, *J* = 8.7; 7.1; 1.0 Hz, H-10), 9.14 (1H, *dd*, *J* = 8.3; 1.0 Hz, H-11), 4.22 (3H, *s*, 3-OCH_3), 4.13 (3H, *s*, 2-OCH_3), 4.11 (3H, *s*, 1-OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) 156.5 (C-1), 147.4 (C-2), 148.9 (C-3), 145.5 (C-3a), 119.1 (C-4), 144.4 (C-5), 146.5 (C-6a), 122.8 (C-6b), 182.5 (C-7), 131.1 (C-7a), 128.9 (C-8), 128.1 (C-9), 134.3 (C-10), 127.6 (C-11), 134.4 (C-11a), 115.7 (C-11b), 61.8 (3-OCH_3), 61.5 (2-OCH_3), 61.0 (1-OCH_3).

Palmatine (**4**) : yellow needles ; ^{1}H NMR data (500 MHz, CD_3OD): δ_H (ppm) 7.64 (1H, *s*, H-1), 7.05 (1H, *s*, H-4), 3.31 (2H, *t*, *J* = 6.4 Hz, H-5), 4.96 (2H, t, *J* = 6.4 Hz, H-6), 9.75 (1H, *s*, H-8), 8.11 (1H, *d*, *J* = 9.1 Hz, H-11), 7.84 (1H, *d*, *J* = 9.1 Hz, H-12), 8.80 (1H, *s*, H-13), 4.00 (3H, *s*, 2-OCH_3), 4.95 (3H, *s*, 3-OCH_3), 4.22 (3H, *s*, 9-OCH_3), 4.00 (3H, *s*, 10-OCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C (ppm) 108.2 (C-1), 149.5 (C-2), 152.5 (C-3), 110.2 (C-4), 129.8 (C-4a), 27.0 (C-5), 57.1 (C-6), 145.3 (C-8), 124.5 (C-8a), 144.5 (C-9), 151.0 (C-10), 127.3 (C-11), 123.0 (C-12), 136.0 (C-12a), 120.0 (C-13), 139.6 (C-13a), 119.8 (C-13b), 57.8 (2-OCH_3), 57.0 (3-OCH_3), 62.0 (9-OCH_3), 58.0 (10-OCH_3).

Jatrozzhizine (5): orange crystals; ^{1}H NMR data (500 MHz, CD_3OD): δ_H (ppm) 7.73 (1H, *s*, H-1), 6.90 (1H, *s*, H-4), 3.28 (2H, *t*, *J* = 6.1 Hz, H-5), 4.79 (2H, t, *J* = 6.1 Hz, H-6), 9.82 (1H, *s*, H-8), 8.10 (1H, *d*, *J* = 9.1 Hz, H-11), 8.05 (1H, *d*, *J* = 9.1 Hz, H-12), 8.86 (1H, *s*, H-13), 4.05 (3H, *s*, 2-OCH_3), 4.22 (3H, *s*, 9-OCH_3), 4.15 (3H, *s*, 10-OCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C (ppm) 108.5 (C-1), 149.5 (C-2), 148.5 (C-3), 114.1 (C-4), 129.0 (C-4a), 26.2 (C-5), 55.9 (C-6), 144.1 (C-8), 121.8 (C-8a), 144.2 (C-9), 150.0 (C-10), 127.2 (C-11), 122.0 (C-12), 134.4 (C-12a), 119.1 (C-13), 138.4 (C-13a), 117.8 (C-13b), 55.9 (2-OCH_3), 61.5 (9-OCH_3), 56.5 (10-OCH_3).

Columbamine (6): red powder; ^{1}H NMR data (500 MHz, CD_3OD): δ_H (ppm) 7.35 (1H, *s*, H-1), 6.54 (1H, *s*, H-4), 3.09 (2H, *t*, *J* = 6.4 Hz, H-5), 4.79 (2H, t, *J* = 6.4 Hz, H-6), 9.50 (1H, *s*, H-8), 8.96 (1H, *d*, *J* = 9.1 Hz, H-11), 7.84 (1H, *d*, *J* = 9.1 Hz, H-12), 8.42 (1H, *s*, H-13), 4.91 (3H, *s*, 3-OCH_3), 4.17 (3H, *s*, 9-OCH_3), 4.06 (3H, *s*, 10-OCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C (ppm) 107.3 (C-1), 162.9 (C-2), 151.8 (C-3), 116.3 (C-4), 129.6 (C-4a), 26.5 (C-5), 56.0 (C-6), 143.4 (C-8), 120.8 (C-8a), 143.7 (C-9), 149.0 (C-10), 126.5 (C-11), 122.6 (C-12), 134.8 (C-12a), 116.7 (C-13), 141.0 (C-13a), 120.4 (C-13b), 54.8 (3-OCH_3), 61.0 (9-OCH_3), 56.1 (10-OCH_3).

Pseudojatrozzhizine (**7**) : yellow crystals ; ^{1}H NMR data (500 MHz, CD_3OD): δ_H (ppm) 7.50 (1H, *s*, H-1), 7.07 (1H, *s*, H-4), 3.08 (2H, *t*, *J* = 6.4 Hz, H-5), 4.77 (2H, t, *J* = 6.4 Hz, H-6), 9.51 (1H, *s*, H-8), 7.69 (1H, *s*, H-9), 7.69 (1H, *s*, H-12), 8.42 (1H, *s*, H-13), 3.89 (3H, *s*, 2-OCH_3), 4.00 (3H, *s*, 10-OCH_3), 4.07 (3H, *s*, 11-OCH_3); ^{13}C NMR (125 MHz, CD_3OD) δ_C (ppm) 112.4 (C-1), 146.9 (C-2), 150.6 (C-3), 112.0 (C-4), 127.4 (C-4a), 26.6 (C-5), 55.3 (C-6), 146.4 (C-8), 122.3 (C-8a), 107.6 (C-9), 152.8 (C-10), 158.2 (C-11), 106.0 (C-12), 138.8 (C-12a), 118.0 (C-13), 139.3 (C-13a), 119.8 (C-13b), 56.2 (2-OCH_3), 56.6 (10-OCH_3), 57.0 (11-OCH_3).

***β*-Sitosterol (8a) + stigmasterol** (**8b**): White crystals from methanol: mp: 135°C-137°C [lit. 134°C-136°C] [21].

Cyclofoetigenine A (**9**): white powder; ^{1}H NMR data (500 MHz, $CDCl_3$): δ_H (ppm) 0.39 (1H, *d*, *J* = 4.1Hz, H-19a), 0.62 (1H, *d*, *J* = 4.1 Hz, H-19b), 4.43 (1H, *m*, H-16), 4.59 (1H, *m*, H-24), 3.24 (1H*, m*, H-3), 0.94 (3H, *s*, H-21), 0.83 (3H, *s*, H-30), 0.98 (6H, *s*, H-28 and H-26), 1.16 (3H, *s*, H-29), 1.19 (6H, *s*, H-18 and H-27) ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 31.8 (C-1), 30.5 (C-2), 79.3 (C-3), 40.2 (C-4), 47.8 (C-5), 20.8 (C-6), 25.9 (C-7), 48.0 (C-8), 19.0 (C-9), 27.1 (C-10), 25.8 (C-11), 33.7 (C-12), 45.0 (C-13), 46.3 (C-14), 48.3 (C-15), 71.8 (C-16), 56.6 (C-17), 17.3 (C-18), 29.8 (C-19), 29.6 (C-20), 18.0 (C-21), 32.6 (C-22), 28.1 (C-23), 78.0 (C-24), 72.4 (C-25), 25.2 (C-26), 24.6 (C-27), 19.6 (C-28), 23.4 (C-29), 13.3 (C-30).

***β*-Sitosterol 3-*O*-*D*-glucopyranoside** (**10**): White crystals from methanol, mp: 290°C-292°C [Lit. 290°C-291°C] [22].

The antibacterial activity of the ethanol (EtOH) extract as well as compounds **1**-**7** against five pathogenic sensitive bacteria classified as one Gram-positive bacteria (*Staphylococcus aureus* ATCC43300) and four Gram-negative bacteria (*Shigella flexneri* 518*, Pseudomonas aeruginosa* NR*, Escherichia coli* ATCC25922, *Klebsiella pneunomiae* NR 41597) were carried out and the results are presented in table 1. According to Kuete and Efferth, the activity of the plant extract can be classified as highly active if MIC < 100 *µ*g/mL, moderate when 100 ≤ MIC ≤ 625 *µ*g/mL, and weakly active when MIC > 625 *µ*g/mL whereas for pure isolates this can be classified as highly MIC < 10 *µ*g/mL, moderate when 10 ≤ MIC ≤ 100 *µ*g/mL, weakly active when MIC > 100 *µ*g/mL [23]. The tested samples showed weak to, moderate activity (125 ≤ MIC ≤ 1000 *µ*g/mL). The EtOH crude extract was not active against

all the tested Gram-negative bacterial strains and was weakly active against *Staphylococcus aureus*. Concerning the isolated compounds, none of them were active against *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Compounds **1**, **2**, **4**, and **5** were weakly active against *Staphylococcus aureus*, while compounds **3**, **6**, and **7** were not active. Only compound **4** was active against *Shigella flexneri* and compounds **4** and **7** were weakly active against *Escherichia coli*.

Chemophenetic significance

The present work describes the isolation and structure elucidation of ten known compounds (Figure 1) including three aporphine alkaloids (**1**-**3**), four protoberberine alkaloids (**4**-**7**), one cycloartane triterpene (**9**), mixture of *β*-sistosterol (**8a**) and stigmasterol (**8b**), and the glucoside of *β*-sitosterol (**10**). To the best of our knowledge compounds **1**-**3**, **7** and **9** are being isolated in this genus for the first time. Compounds **4**-**6** have been isolated several times in different species of the genus *Annickia* [9-10, 13, 24-25]. Although compound **1**-**3** and **7** was isolated in this genus for the first time, they are already reported in the annonaceae family [24, 26, 27, 19] while compound **9** has been isolated for the first time in this family. The isolation of compounds **4**-**7** from *A. chloranta* strengthened the previous findings and the chemotaxonomic significance of aporphinic and protoberberine alkaloids at the genus and family level.

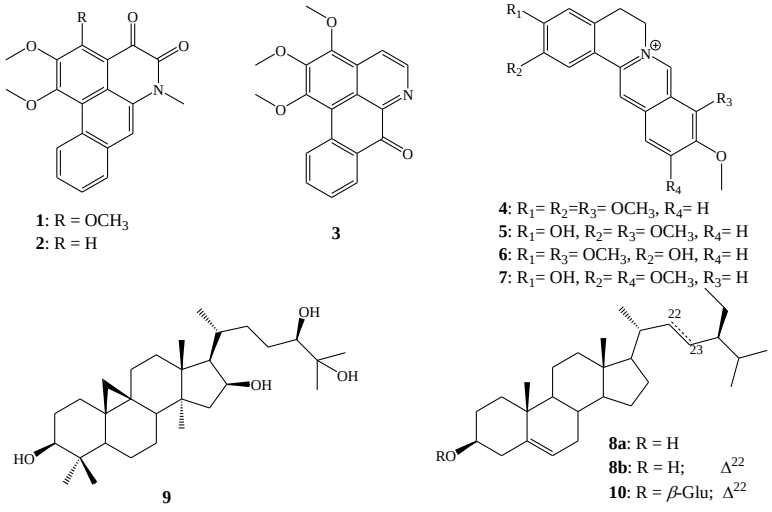

Figure 1. Structures of the isolated compounds (**1**-**10**)
[1,2,3-trimethoxy-4,5-dioxo-6a,-dehydroaporphine A (**1**), cepharadione B (**2**), *O*-methyl-moschatoline (**3**), palmatine (**4**), jatrorrhizine (**5**), columbamine (**6**), pseudojatrorrhizine (**7**), the mixture of *β*-sistosterol (**8a**) and stigmasterol (**8b**), cyclofoetigenine A (**9**) and *β*-sitosterol-3-*O*-*β*-*D*-glucopyranoside (**10**)]

Table 1. Antibacterial activities of extract and some isolated compounds from *A. chlorantha*.

Samples	Parameters (*μ*g/ mL)	Sf 518	Pa NR	Ec, ATCC25922	Kp NR 41597	Sa, ATCC43300
EtOH extract	MIC	Na	na	na	Na	1000
1	MIC	Na	na	na	Na	125
2	MIC	Na	na	na	Na	500
3	MIC	Na	na	na	Na	na
4	MIC	Na	na	250	Na	125
5	MIC	125	na	na	Na	250
6	MIC	Na	na	na	Na	na
7	MIC	Na	na	500	Na	na
Ciprofloxacin	MIC	0.062	0.031	0.031	0.015	0.015

Sf: *Shigella flexneri;* **Pa**: *Pseudomonas aeruginosa*; **Ec**: *Escherichia coli*; **Sa**: *Staphylococcus aureus*; **Kp**: *Klebsiella pneunomiae;* **MIC**: Minimum Inhibitory Concentration; **na**: non active; **Ciprofloxacin**: reference drug. 1,2,3-trimethoxy-4,5-dioxo-6a,-dehydroaporphine A (**1**), cepharadione B (**2**), *O*-methyl-moschatoline (**3**), palmatine (**4**), jatrorrhizine (**5**), columbamine (**6**), pseudojatrorrhizine (**7**).

Conclusion

The chemical investigation of the stem bark of *A. chlorantha* led to the isolation of ten known compounds including seven alkaloids (**1**-**7**), one triterpene (**9**), and two steroids (**8a**-**8b**, **10**). The crude extract and the isolated compounds were subjected to antibacterial activities. The crude extract exhibited weak antibacterial activity against *Staphylococcus aureus* (MIC = 1000 *μ*g/mL). Compounds **2** and **4** have shown moderate activity against *Staphylococcus aureus* (MIC = 125 *μ*g/mL), while compound **5** had similar activity against *Shigella flexneri*. These results enrich the natural product

database of the Annonaceae family in general and *Annickia* genus in particular.

Additional file

NMR spectra of the studied compounds. Available at: https://www.investchempharma.com/imcp69-supplementary-file-noume-et-al/ (PDF)

Abbreviations

CC: Column Chromatography
TLC: Thin-Layer Chromatography
MIC: Minimum Inhibitory Concentration
1H NMR: Proton Nuclear Magnetic Resonance
13C NMR: Carbon 13 Nuclear Magnetic Resonance
MS: Mass Spectrometry
MHB: Mueller Hinton Broth

Authors' Contribution

VLN isolated the compounds. VLN, JFZ, RNN, AKD, and JRC contributed to compounds identification, carried out the antibacterial activity of samples. VLN, RNN and JRC wrote the original draft. VLN, JFZ, RNN, AKD and JRC methodology. JRC and PNEA supervised the work. All authors have read and agree to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest

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