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Antiplasmodial and antimicrobial potential of *Canthium subcordatum* extracts and isolates

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ABSTRACT

Phytochemical studies on the stem bark of *Canthium subcordatum* afforded eight known compounds: β-sitosterol (1), ursolic acid (2), cerbinal (3), quinovic acid (4), Cerberinic acid (5), 3-*O*-β-D-glucopyranosylquinovic acid (6), 3-*O*-β-D-glucopyranosyloleanolic acid (7) and Clemahexapetoside B (8). Interestingly, 3, 5 and 8 were isolated and characterized for the first time from the genus *Canthium*. Their structures were elucidated using their physical and spectroscopic data. Furthermore, antiplasmodial potency of *Canthium subcordatum* and antimicrobial activities of 6, 5 and 8 has not yet been investigated. Fractions and isolates were tested for their antiplasmodial potency. Only the methanol fraction inhibited the growth of *P. falciparum* with an IC_{50} value of 3.044 µg/mL. The isolates were also tested against eight bacteria and fungi strains. 4 and 5 were the most active with inhibition diameter zones equal or above 12 mm and MIC equal or lower than 39 µg/mL.

Keywords: *Canthium subcordatum*; Rubiaceae; antiplasmodial; antimicrobial activity, macrocyclic glycoside.

INTRODUCTION

The genus *Canthium* is a genus of flowering plants in the Rubiaceae family. It is a poorly defined genus with about 50 species, including *Canthium subcordatum* in Africa and Asia. They are usually shrubs or small trees that are sometimes spiny. They can be recognized by their usually thorny stem, the leaves that are deciduous and usually placed opposite each other, the flowers that are hermaphroditic, small and usually green to white or pale yellow in colour and the fruits that are usually brown, yellow, or red; often drupaceous, subglobose, ellipsoid or diccocious when fully developed [1]. *C. subcordatum* is found in some countries in Africa. It is found in Southern Nigeria, Ivory Coast (Abidjan), Guinea (Mount Benna), Gambia, Ghana (Kumasi), Liberia (Kakatown), Sierra Leone, Angola, Sao Tomé and Cameroon (Mount Muanenguba, Nkongsamba-Loum, Idenau, Bomana and Mbalmayo) [2].

Plants of the Rubiaceae family have been shown to exhibit antimalarial, antimicrobial, antihypertensive, anti-diabetic, antioxidant and anti-inflammatory activities [3].

In folk medicine, the roots, leaves and stem bark of some species of *Canthium* are used for medicinal purposes including the treatment of malaria (Benin), fever, headache, conjunctivitis, diarrhea (Burkina Faso), diarrhea, fever, leucorrhoea, intestinal worms, and general debility (Ayurvedha), eczema (Ethiopia) sexually transmitted diseases (Guinea) and anti-venom in the treatment of snakebite and other wounds in some villages of Shimoga district in Karnataka, India. In Cameroon, the stem of *Canthium sp.* is used against ascariasis [4, 5].

The stem bark, leaves and roots of *C. subcordatum* have been exploited for their medicinal value: The stem bark Alcoholic extract has potential antidiabetic properties. Alcoholic extract of the roots are used for the treatment of fever, malaria, inflammation and cardiovascular diseases. Anti-inflammatory activity of the Petroleum ether and dichloromethane extracts of *C. subcordatum* has been reported [6]. Local traditional healers and some inhabitants of Mbalmayo, Centre Region, Cameroon reveal that the plant *C. subcordatum* is used by the local population to treat stomach disorders especially in the season when new crops are being consumed.

Malaria caused by the protozoan parasites of the genus *Plasmodium* remains one of the most important infectious diseases. *P. falciparum* is the species responsible for nearly all severe malaria cases and deaths [7]. History reveals that medicinal plants have always been considered as an important source of chemotherapeutic agents against malaria [8] such as quinine and artemisinin. Exploration of medicinal plants remains a promising strategy to identify new antimalarial agents.

To the best of our knowledge, very little phytochemical studies on *C. subcordatum* has so far been reported. Literature reports the isolation of iridoids and their derivatives [9, 10, 11]. Also, *C. subcordatum* antiplasmodial potency has not yet been investigated. This study is aimed at investigating the antiplasmodial and antimicrobial activities of *C. subcordatum* extract as well as the isolated compounds.

MATERIALS AND METHODS

General: The masses of the isolates were taken using an electronic balance, mark MELTER PC 2000. A Büchi-540 apparatus was used to determine Melting points. Optical rotations (measured at room temperature) in $CHCl_3$ were realized on a Perkin Elmer Polarimeter (Model 241). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker spectrometer containing 5 mm ^{1}H and ^{13}C probes, respectively operating at 500 MHz and 125 MHz, using as internal standard, TMS. Flash and column chromatography was carried out on Silica gel 230-400 mesh (Merck) and silica gels (Merck 230-400 and 70-230 mesh), and TLC (with different eluent mixtures of ether, hexane, ethyl acetate, methanol and acetone) was done with pre-coated aluminium silica gel 60 F_{254} sheets; spots viewed under UV lamps (254 and 365 nm) or sprayed with 50% H_2SO_4 reagent, heated and viewed.

Plant Material: The bark of *Canthium subcordatum* was harvested on the 28th of August 2008 from Mbalmayo, Centre Region of Cameroon, along the Nyong River. Mr. Nana Victor of the National Herbarium of Cameroon (Yaoundé), where a voucher specimen (N°1957/SRF/CAM) is deposited, identified the plant.

Extraction and isolation: C. subcordatum stem bark was chopped, air-dried and ground into fine powder (2.6 kg). The powdered plant material (2.6 kg) was extracted by soaking in 8 litres of methylene chloride/methanol (1:1) solvent mixture for 5 days. After filtration, the extract was concentrated with the use of a rotary evaporator under reduced pressure. A methylene chloride/methanol (1:1) crude extract was obtained. This crude extract was further concentrated to dryness under vacuum at low temperature (30°C) to afford 95 g CH_2Cl_2/MeOH extract. This crude extract was further fractionated into three fractions [Hexane fraction (3.3 g), Ethyl acetate fraction (76 g) and Methanol fraction (12.1 g). Based on the thin layer chromatography (TLC) profiles of the three fractions, 75 g of the ethyl acetate fraction was made to undergo flash chromatography over silica gel and eluted with solvents of increasing polarity (*n*-hexane, *n*-hexane-EtOAc, EtOAc and EtOAc-Methanol) and grouped using their thin layer chromatography profiles in 5 sub-fractions. Sub-fractions 1 to 4 were mixed and eluted with *n*-hexane-EtOAc solvent mixtures of increasing polarity, on silica gel to furnish 1 (4.3 mg), Hex/EtOAc (90:10); 2 (3.9 mg), Hex/EtOAc (82:18); 3 (5.8 mg), Hex/EtOAc (85:15); 4 (3.2 mg), Hex/EtOAc (80:20); 5 (4.9 mg), Hex/EtOAc (80:20); 6 (3.6 mg), Hex/EtOAc (30:70); 7 (4.2 mg), EtOAc/MeOH (90:10) and 8 (4.7 mg), EtOAc/MeOH (88:12).

In vitro antiplasmodial against Plasmodium falciparum W2: The experiment was conducted as previously described by Tchokouaha and collaborators (2015) using a Chloroquine and others antimalarial resistant strain, *Plasmodium falciparum* W2 maintained at 37°C in RPMI 1640, pH 7.4 containing 25 mM HEPES, 10% heat inactivated human serum and 2% hematocrit under 5% CO_2, 3% O_2 and 91% N_2 atmosphere [12]. Serial treatment with 5% Sorbitol (Sigma, Germany) allowed for the synchronization of malaria parasite in the ring stage (Lambros and Vanderberg, 1979). The compound stock solution was prepared at 1 mg/ml in DMSO, serially diluted in supplemented medium. Culture at 1% parasitemia and 4% hematocrit was treated with compounds in triplicate with the highest dilution being 10 µg/mL and DMSO concentration did not exceed 0.1% in the final reaction medium [8]. Negative control consisted in equal amount of DMSO while artemisinin (Sigma, Germany) served as positive control. After 48h incubation, parasites were fixed by 1% formaldehyde in PBS after removal of the medium. A 50 µL of each culture was added to a tube containing 0.5 mL of PBS supplemented with 1 nM YOYO nuclear dye (Molecular Probes) and 0.1% Triton X-100 then added to the 5 mL round-bottom polystyrene tubes. Becton-Dickinson FACSort flow cytometer that count nucleated (parasitized) erythrocytes was used to monitor the parasitemia of control and treated cultures by. CellQuest software was used for data acquisition. The concentration that inhibit 50% of the parasites growth (IC_{50}) was determined by non-linear regression using Prism 5.0 software (GraphPad, CA, USA) [12].

Antimicrobial assay using Paper disk diffusion and Microbroth dilution methods: A total of 8 microorganisms; 3 Gram-positive, 3 Gram-negative bacteria and 2 yeast species were tested. The Gram+ bacteria species were *Staphylococcus aureus*, *Staphylococcus saprophiticus*, *Streptococcus faecalis*; and the Gram- bacteria species *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and two yeast strains of Candida (*Candida albicans* and *Candida krusei)*. The strains were clinical isolates obtained from patients diagnosed with urinogenital tract infections consulting in Tiko Hospital (Cameroon). Appropriate cultural and biochemical procedures were used for their isolation and identification. Isolated colonies of new cultures of the different germs were diluted in 5 ml Nutrient broth (NB, Oxoid), in well-labeled sterile bottles, and incubated for 24 hours at 37 °C before antimicrobial susceptibility testing.

Reference antibiotics include Gentamycin (Sigma, USA) and Nystatin (Sigma, USA) for yeasts and bacteria species, respectively.

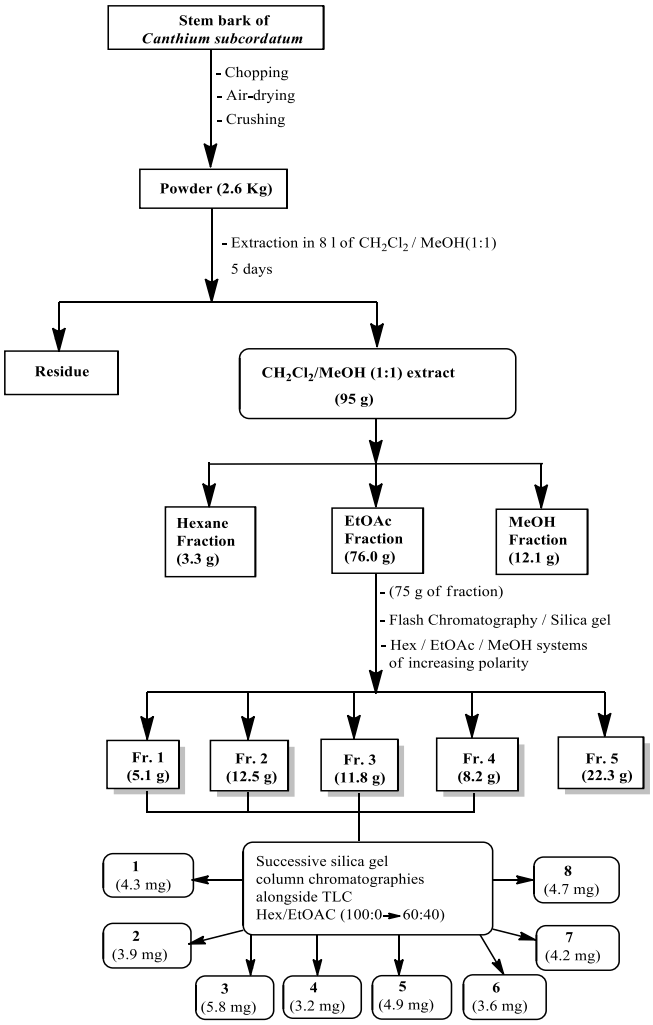

Figure 1: Isolation scheme of 1- 8

A stock solution of pure compounds (1, 2, 3, 4, 5, 6, 7, 8, Gentamicin, Nystatin), was obtained with sterile distilled water (1 mg/mL) a volume of 50 µl was then imbibed on filter paper (Filter sterile papers 6 mm in diameter) at 120°C for 1 hr) to yield a 50 µg/disk. The discs of reference antibiotics were placed on the culture plates already inoculated with bacteria (Mueller Hinton Agar Plates) and and yeast (Sabouraud

Deaxtrose Agar Plates) and left at 37ºC for incubation. The zones of inhibition were measured after 24 h [13, 14].

The minimum inhibitory concentration (MIC), which is the lowest concentration of the sample that inhibits visible growth of microorganisms, was determined by the micro broth dilution method [13, 14] in Mueller Hinton or Sabouraud broth containing 10% glucose and 0.5% phenol red supplements. A serial dilution (was distributed from the first to the twelfth row on a 96 wells microplate) of dry products (1, 2, 3, 4, 5, 6, 7, 8 and reference drugs (Gentamicin, Nystatin) initially in DMSO (20%) (100 µL) was made in microwells using supplemented Mueller Hinton or Sabouraud broth (50 µL). A total of 11 twofold dilution was made ranging from 2.4 to 2496 µg/mL

After adding 50 µL of each dissolved sample successive dilutions were then carried out by transferring the mixture/solution (50 µL) from the first to the eleventh well from which 50 µL was removed. The twelfth well served as growth control. The inoculum (50 µL, 10^5 colony forming units/mL (CFU/mL) properly obtained were then added in each microwell. Tests were incubated aerobically at 37 °C for 24 and 48 hours, for bacteria and fungi cultures, respectively. The Minimum Inhibitory Concentration was identified by monitoring any change in color from red to yellow due to the formation of acidic metabolites corresponding to microbial growth.

RESULTS AND DISCUSSION

Structures of the eight known isolates were established as ß-sitosterol (4.3 mg) (1), ursolic acid (3.9 mg) (2), cerbinal (an iridoid with $\Delta^{3,5,7,9(1)}$ –tetraene skeleton with 10-formyl and 11-carbomethoxy residues) (5.8 mg) (3) [15], quinovic acid (3.2 mg) (4), Cerberinic acid (an iridoid with $\Delta^{3,5,7,9(1)}$ –tetraene skeleton with 10-formyl and 11-carboxylic acid residues) (4.9 mg) (5) [15], 3-*O*-ß-D-glucopyranosylquinovic acid (3.6 mg) (6), 3-*O*-ß-D-glucopyranosyloleanolic acid (4.2 mg) (7) and Clemahexapetoside B (a symmetric macrocyclic glycoside) (4.7 mg) (8) [16] (Figure 2).

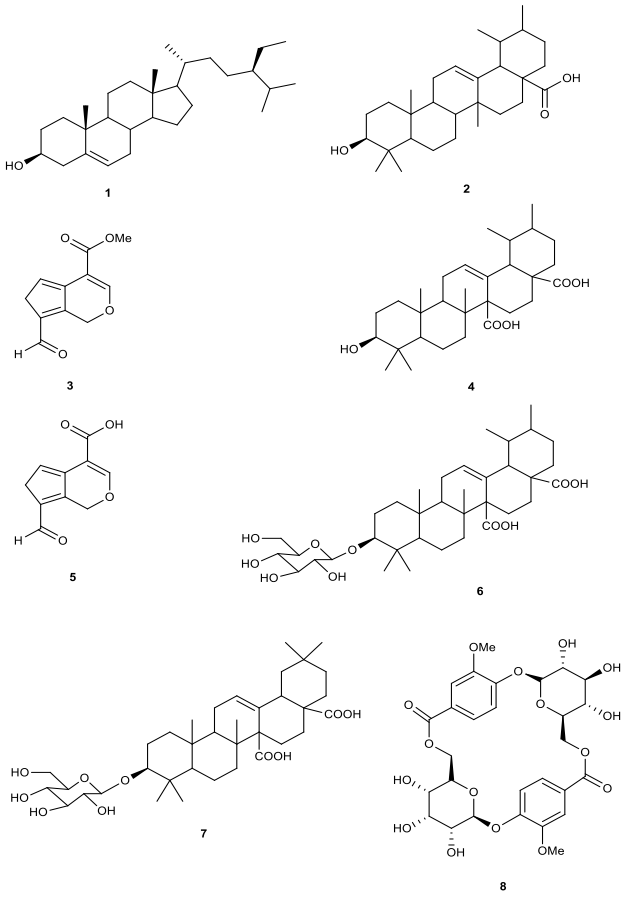

Figure 2: Strutures of 1- 8

β-sitosterol (compound 1): Colourless needles, mp 139-140.5 ºC. ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 38.3 (C-1), 30.6 (C-2), 73.5 (C-3), 39.6 (C-4), 141.8 (C-5), 122.9 (C-6), 33.1 (C-7), 33.4 (C-8), 51.7 (C-9), 37.8 (C-10), 22.3 (C-11), 41.0 (C-12), 43.5 (C-13), 58.3 (C-14), 25.3 (C-15), 29.4 (C-16), 57.4 (C-17), 12.2 (C-18), 19.8 (C-19), 37.5 (C-20), 19.3 (C-21), 35.0 (C-22), 27.1 (C-23), 47.2 (C-24), 30.3(C-25), 20.2 (C-26), 19.4 (C-27), 24.2 (C-28), 12.5 (C-29). EI-MS *m/z* 414 (64), 396 (40), 382 (14), 303 (23), 255 (20), 159 (23), 81 (37), 43 (100).

Ursolic acid (compound 2): White crystals, mp 290-292 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 3.15 (q, *J* = 10.0 Hz, H-3), 2.20 (d, *J* = 7.5 Hz, H-18). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 39.8(C-1), 27.8 (C-2), 79.7 (C-3), 39.9 (C-4), 56.6 (C-5), 17.8 (C-6), 34.3 (C-7), 40.2 (C-8), 40.7 (C-9), 37.9 (C-10), 21.7 (C-11), 126.7 (C-12), 139.5 (C-13), 40.3 (C-14), 29.2 (C-15), 25.3 (C-16), 43.2 (C-17), 54.2 (C-18), 38.0 (C-19), 35.9 (C-20), 31.7 (C-21), 36.8 (C-22), 28.8 (C-23), 16.4 (C-24), 16.1(C-25), 17.7 (C-26), 24.3 (C-27), 181.6 (C-28), 19.4 (C-29), 24.3 (C-30).

Cerbinal (compound 3): Orange-yellow powder, mp 188-190 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.94 (s, H-10), 9.14 (s, H-1), 8.51 (s, H-3), 7.94 (d, *J* = 4.0 Hz, H-7), 7.13 (d, *J* = 4.0 Hz, H-6), 4.00 (s, OMe). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 149.6(C-1), 147.9 (C-3), 114.9 (C-4), 130.2 (C-5), 113.4 (C-6), 148.0 (C-7), 124.9 (C-8), 124.3 (C-9), 184.9 (C-10), 164.5 (C-11), 52.4 (OMe). EI-MS *m/z* 204 (94), 203 (100), 173 (6.6), 145 (6.8).

Quinovic acid (compound 4): White crystals, mp 302-304 ºC. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 38.5(C-1), 26.9 (C-2), 78.5 (C-3), 39.7 (C-4), 55.0 (C-5), 18.2 (C-6), 36.5 (C-7), 39.9 (C-8), 47.8 (C-9), 38.2 (C-10), 22.5 (C-11), 129.1 (C-12), 134.7 (C-13), 57.3 (C-14), 29.7 (C-15), 25.4 (C-16), 47.1 (C-17), 53.5 (C-18), 37.9 (C-19), 37.1 (C-20), 31.5 (C-21), 36.5 (C-22), 28.3 (C-23), 15.6 (C-24), 16.8 (C-25), 18.4 (C-26), 176.5 (C-27), 179.9 (C-28), 19.6 (C-29), 20.5 (C-30).

Cerberinic acid (compound 5): Orange-yellow powder, mp 183-186 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 9.84 (s, H-10), 8.50 (s, H-1), 8.81 (s, H-3), 8.1 (d, *J* = 4.0 Hz, H-7), 7.3 (d, *J* = 4.0 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 150.2 (C-1), 147.6 (C-3), 115.0 (C-4), 130.1 (C-5), 113.5 (C-6), 148.2 (C-7), 125.0 (C-8), 124.4 (C-9), 184.9 (C-10), 171.5 (C-11).

3-O-β-D-glucopyranosylquinovic acid (compound 6): White crystals, mp 247-250 ºC. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 39.4(C-1), 27.7 (C-2), 87.8 (C-3), 39.6 (C-4), 55.2 (C-5), 18.1 (C-6), 36.2 (C-7), 39.4 (C-8), 47.2 (C-9), 36.4 (C-10), 22.3 (C-11), 127.9 (C-12), 132.5 (C-13), 55.2 (C-14), 25.5 (C-15), 25.5 (C-16), 46.9 (C-17), 55.2 (C-18), 39.2 (C-19), 39.1 (C-20), 29.7 (C-21), 36.2 (C-22), 18.1 (C-23), 27.7 (C-24), 16.0 (C-25), 17.7 (C-26), 176.1 (C-27), 178.3 (C-28), 16.5 (C-29), 21.1 (C-30), 105.3 (C-1′), 73.9 (C-2′), 76.8 (C-3′), 71.1 (C-4′), 76.8 (C-5′), 61.2 (C-6′).

3-O-β-D-glucopyranosyloleanolic acid (compound 7): White crystals, mp 249-252 ºC. ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 39.5(C-1), 27.9 (C-2), 86.7(C-3), 39.6 (C-4), 55.4 (C-5), 18.3 (C-6), 35.9 (C-7), 39.3 (C-8), 47.2 (C-9), 36.5 (C-10), 22.1 (C-11), 127.7 (C-12), 132.4 (C-13), 55.0 (C-14), 25.6 (C-15), 25.5 (C-16), 46.8 (C-17), 55.1 (C-18), 43.2 (C-19), 30.4 (C-20), 29.7 (C-21), 36.3 (C-22), 18.1 (C-23), 27.7 (C-24), 16.1 (C-25), 17.5 (C-26), 176.3 (C-27), 178.2 (C-28), 16.7 (C-29), 21.3 (C-30), 105.3 (C-1′), 73.8 (C-2′), 76.7 (C-3′), 71.1 (C-4′), 76.6 (C-5′), 61.1 (C-6′).

Clemahexapetoside B (compound 8): Colourless amorphous powder, mp 316-319 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 7.35 (d, *J* = 1.8 Hz, H-2a), 7.36 (d, *J* = 8.7 Hz, H-5a), 7.77 (dd, *J* = 1.8, 8.7 Hz, H-6a), 3.76 (s, H-3a-MeO), 5.33 (d, *J* = 7.5 Hz, H-1'a), 3.31-3.45 (m, H-2'a), 3.31-3.45 (m, H-3'a), 3.13-3.19 (m, H-4'a), 3.31-3.45 (m, H-5'a), 4.12 (dd, *J* = 5.0, 11.5 Hz), 3.30-3.94 (m, H-6'a), 7.38 (d, *J* = 1.8 Hz, H-2b), 7.31 (d, *J* = 8.7 Hz, H-5b), 7.75 (dd, *J* = 2.0, 8.7 Hz, H-6b), 3.76 (s, H-3b-MeO), 5.18 (d, *J* = 6.5 Hz, H-1'b), 3.30-3.46 (m, H-2'b), 3.53 (t, *J* = 2.5 Hz, H-3'b), 3.30-3.5 (m, H-4'b), 3.89-3.93 (m, H-5'b), 4.37 (dd, *J* = 5.5, 11.5 Hz), 4.10 (*br* d, *J* = 11.5 Hz, H-6'b). ^{13}C NMR (250 MHz,

DMSO-d_6) δ_C 122.6(C-1a), 110.4 (C-2a), 146.4 (C-3a), 148.1 (C-4a), 112.3 (C-5a), 119.8 (C-6a), 164.8 (C-7a), 53.9 (C-3a-MeO), 96.4 (C-1'a), 71.1 (C-2'a), 75.5 (C-3'a), 69.2 (C-4'a), 72.3 (C-5'a), 64.2 (C-6'a), 122.1 (C-1b), 11.2 (C-2b), 147.1 (C-3b), 148.5 (C-4b), 112.3 (C-5b), 122.1 (C-6b), 164.4 (C-7b), 53.6 (C-3b-MeO), 96.8 (C-1'b), 71.2 (C-2'b), 69.5(C-3'b), 67.9 (C-4'b), 70.8 (C-5'b), 64.4 (C-6'b).

Antiplasmodial assay: Except for the methanol extract (C3) that inhibited the growth of *P. falciparum* with an IC_{50} value of 3.044 mL, the hexane, ethyl acetate fractions and all afforded compounds were inactive at the tested concentration.

The results of the antiplasmodial assay suggest that constituents of *Canthium subcordatum* might act in synergy to provide the antiplasmodial activity and fractionation will result to a loss of activity. Nevertheless, the antiplasmodial activity of its methanol extract supports the use of the stem bark and root of *C. subcordatum* in the traditional treatment of malaria and fever [17].

Antimicrobial assay: Results of antimicrobial testing are presented in table 1. In general, it is observed that all the pure compounds tested presented an activity on at least three of the tested strains. Based on the inhibitory zones and MIC obtained, we noticed that 3, 4 and 5 presented comparable activities with the reference drug (Nystatin) on *Candida albicans*. *Candida krusei* was highly susceptible to 4, 5 and 6 with inhibitory zones of 12-14 mm and MIC ranging from 9.7 to 39 µg/mL. Sa was highly susceptible to 4 (ID=16 and MIC= 9.7 µg/mL) and 1 (ID= 12 mm and MIC 39 µg/mL) products. Ss on its part showed marked susceptibility to 4 products. These included 4 (ID=14; MIC=19.5), 5 (ID=12; MIC=39, NM15 ((ID=12; MIC=39) and 8 (ID=14; MIC=19.5). Sf appeared to be markedly susceptible only to 01 product; 5 (ID=14; MIC=19.5) Ec and Kp were susceptible to two products 3 (ID=14; MIC=19.5) and 5 then 4 and 5 respectively. Pa was sensitive to 1, 4 and 5. 1 was active on two strains (Sa and Kp). 4 and 5 appeared to be the most active products on being able to inhibit the growth of six strains including fungi, Gram + and Gram -; with inhibition diameter zones equal or above 12 and MIC equal or lower than 39 µg/mL.

Table 1: Results of antimicrobial assay

Product code	Mass/disk	Ca		Ck		Sa		Ss		Sf		Ec		Kp		Pa	
		ID	MIC	ID	MIC	ID	MIC	ID	MIC	ID	MIC	ID	MIC	ID	MIC	ID	MIC
1	50	14	19.5	10	39	12	39	0	NT	10	78	10	78	0	NT	12	39
2	50	10	39	10	39	10	78	10	78	10	78	10	78	14	19.5	10	39
3	50	16	9.7	10	39	8	78	10	78	10	78	12	78	10	78	10	78
4	50	18	4.8	14	9.7	16	9.7	14	19.5	0	NT	10	39	14	19.5	14	19.5
5	50	16	9.7	14	19.5	10	39	12	39	14	19.5	14	19.5	16	9.7	16	4.8
6	50	10	78	12	39	0	NT	10	78	0	NT	0	NT	10	ND	10	ND
7	50	10	78	12	39	10	78	12	39	0	NT	0	NT	10	ND	10	ND
8	50	10	78	10	19.5	10	78	14	19.5	10	78	10	78	10	78	10	ND
Gent.	50	NT	NT	NT	NT	20	2.4	30	2.4	30	2.4	30	2.4	25	2.4	30	2.4
Nyst.	50	16	4.8	18	4.8	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Key 1: ID in (mm) and MIC in (µg/ml). **Key 2:** NT: not tested; ND: Not determined; Sa: *Staphylococcus aureus*, Ss: *Staphylococcus saprophiticus, Ss: Streptococcus faecalis*; Ec: *Escherichia coli*, Kp: *Klebsiella pneumonia*, Pa: *Pseudomonas aeruginosa*; Ca: *Candida albicans*; Ck: *Candida krusei.* Genta. : Gentamicin; Nyst. : Nystatin

The following ranges defined cut-off points for antimicrobial activities. For pure compounds: (MIC <10 µg/mL) for significant activity, (10 < MIC ≤ 100 µg/mL) for moderate activity, and (MIC > 100 µg/mL) for low activity [18].

Apart from 3-*O*-β-D-glucopyranosylquinovic acid (**6**), 3-*O*-β-D-glucopyranosyloleanolic acid (**7**) and Clemahexapetoside B (**8**) for which little is known about their antimicrobial properties, all isolated compounds have been previously reported for either antibacterial activity or antifungal activity. β-sitosterol (**1**), isolated from *Vitex agnus*castus by bio-assay guided fractionation showed antibacterial activity by inhibiting the growth of *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. faecalis* and *E. coli* [19]. It was suggested that beta-sitosterol act by inhibition DNA polymerase beta lyase activity [20]. Ursolic acid (**2**), antibacterial properties have been previously assayed against different bacterial species, and the results suggested the important antibiotic properties. Indeed, **2** showed activity against *Streptococcus mutans* and *S. sobrinus* with a MIC_{50} of 2.0 µg/mL, indicating their usefulness in inhibiting caries in teeth [21, 22, 23]. Additionally, **2** has also been reported to possess antimicrobial activity [24]. Ursolic Acid (2) displayed some pleiotropic antibacterial mechanisms against methicillin-resistant *Staphylococcus aureus* (MRSA) [24]. Cerbinal (**3**) showed antifungal activity close to that of nystatin. In fact, Cerbinal is a well-known plant-derived antifungal compound previously isolated from *Gardenia jasminoides* leaves [25]. Quinovic acid (**4**), antimicrobial properties have been corroborated by many authors who indicated the inhibition of the growth of both Gram-positive and Gram – negative and *Aspergillus* species and *C. albicans* [26, 27]. Cerberinic acids (**5**), good antifungal activity is in line with previously reported antifungal and antibacterial activity of iridoids [28].

CONCLUSION

In this study, antiplasmodial and antimicrobial potential of *Canthium subcordatum* extracts and isolates were investigated. Antiplasmodial activity testing on fractions and isolates revealed that the methanol fraction was active inhibiting the growth of *P. falciparum* with an IC_{50} value of 3.044 µg/mL. Antimicrobial activity testing on isolates against eight bacteria and fungi strains showed two compounds as the most active with inhibition diameter zones equal or above 12 mm and MIC equal or lower than 39 µg/mL.

Three known isolates were obtained and characterized from the genus *Canthium* for the very first time. Furthermore, antiplasmodial activity of *Canthium subcordatum* and antimicrobial activities of three isolates are herein reported for the first time.

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REFERENCES

1. Bridson D, Thulin M. Flora of Somalia. Royal botanic gardens 3.
2. Hutchinson J, Dalziel MD. Flora of West Tropical Africa. Journal of Royal botanic gardens 1963; 2:180-185.
3. Sasmal D, Sanjeeb KP, Papiamitra M, Behera P, Lal UR, Dash SK, Padhy RK. Review on *Canthium*: special reference to *Canthium coromandelicum*. Am J Phytomed Clin Ther 2014; 6:796-813.
4. Simplice DK, Tchadjobo T, Denise PI, Jacques S. Sub-Saharan Rubiaceae: A review of their traditional uses, phytochemistry and biological activities. Pakistan J Biol Sci 2011; 14:149-169.
5. Pulate PV, Wagay NA, Deshmukh VR. Phytochemical, ethno-medicinal and anatomicalstudy of *Canthium parviflorum*. World J Pharm Pharm Sci 2015; 4(11):1464-1482.
6. Joubouhi C, Ngnokam D, Mabou FD, Tebou PF, Harakat D, Laurence VN. New iridoid dimmers from the fruits of *Canthium subcordatum* DC. Phytochem Lett. 2015; 13:348-354.
7. World Health Organization. World Health Statistics 2016: Monitoring Health for the SDGs Sustainable Development Goals. World Health Organization: Geneva.
8. Boyom FF, Fokou PV, Yamthe LR, Mfopa AN, Kemgne EM, Mbacham WF, *et al*. Potent antiplasmodial extracts from Cameroonian Annonaceae. J Ethnopharmacol 2011; 134:717-24.
9. Achenbach H, Waibel R, Addae-Mensah I. Shanzhisin methyl ester gentiobioside, a new iridoid-Isolation and synthesis. Tetahedron Lett. 1980; 21(38):3677-3678.
10. Murai Y, Kashimura S, Tamezawa S, Hashimoto T, Takaoka S, Asakwa Y, *et al*. Absolute configuration of (6S,9S)-reseoside from *Polygonum hydropiper*. Planta Med. 2010; 67(5):480-481.
11. Achenbach H, Waibel R, Raffelsberger B, Addae-Mensa I. Iridoid and other constituents of *Canthiun subcordatum*. Phytochemistry 1981; 20(7):1591-1595.
12. Singh A, Rosenthal PJ. Comparison of efficacies of Cysteine Protease Inhibitors against five strains of *Plasmodiun falciparum*. Antimicrob. Agents Chemother. 2001; 45(3):949-951.
13. Carbonnelle B, Denis F, Marmonier A, Pignon G, Vargue R. Bactériologie Médicale. In: Techniques Usuelles. SIMEP, Paris, 1987, 2006; 130-282.
14. Berghe VA, Vlietinck AJ. Screening methods for antibacterial and antiviral agents from higher plants. In: Hostettmann, K, (eds), Methods in Plant Biochemistry, vol. 6. Academic Press Limited, London, 1991; pp. 47-69.
15. Fumiko A, Hikaru O, Tatsuo Y. Cerbinal and its derivatives, yellow pigments in the bark of *Cerbera mamghas* L. Chem Pharm Bull 1977; 25(12):3422-3424.
16. Shi S, Dong C, Jiang D, Tu P. Macrocyclic Glycosides from *Clematis hexapetala*. Helv Chim Acta 2006; 89:3002-3006.
17. Chukwujekwu JC, Van Staden J, Smith P, Meyer JJM. Antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants. S Afr J Bot 2005; 71:316-325.
18. Kuete V, Efferth T. Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacol. 2010; 1:123. doi: 10.3389/fphar.2010.00123.
19. Arokiyaraj S, Anbuselvan Vimalarasan, Hemachandran M, Dhayalan, Priya. Antibacterial activity of beta-sitosterol of *Vitex agnus*castus. Int. J. of Appl. Biol. 2011; 2(3):12-15.
20. Prakash CVS, Gao Z, Hecht SM, Jones SH, Kingston DG. A new acylated oleanane triterpenoid from *Couepiapolyandra* that inhibits thelyase activity of DNA polymerase beta. J Nat Prod 2003; 66(11):1463-5.
21. Kim MJ, Kim CS, Park JY. Antimicrobial effects of ursolic acid against mutans Streptococci isolated from Koreans of. Int J Oral Sci 2011; 36(1):7-11.
22. Kozai K, Miyake Y, Kohda H. Inhibition of glucosyltransferase from *Streptococcus mutans* by oleanolic acid and ursolic acid, Caries Research, 1987; 21(2):104-108.
23. Jéssica AJ, João HGL, Márcia DL, Eduardo SY, Luiz FDP. Antimicrobial Activity of Oleanolic and Ursolic Acids: An Update Evidence-Based Complementary and Alternative Medicin. 2015; Article ID 620472, 14 pages
24. Wang CM, Jhan YL, Tsai SJ, Chou CH. The Pleiotropic Antibacterial Mechanisms of Ursolic Acid against Methicillin-Resistant *Staphylococcus aureus* (MRSA). Molecules (Basel, Switzerland) 2016**;** 21 (7).
25. Razzaghi-Abyaneh M, Rai M. Antifungal Metabolites from Plants. Springer Berlin Heidelberg, 2013.
26. Ryu YB, Park S, Kim YM, Lee J, Seo WD, Chang SJ, *et al*. SARS-COV3Clproinhibitory effects of quinone-methide triterpenes from *Triptergiumregelii*, Bioorganic Med. Chem. Lett, 2010; 20:1873-1876.
27. Ezem SN, Akpuaka MU, Ajiwe VIE. Pharmaceuticals from the whole root of *Nauclea latifolia* (Rubiaceae). International Journal of Pharmaceutical Chemistry 2015; 5(10):333-342.
28. Amaral AC, Ramos AD, Ferreira JL, Santos AR, Falcão DQ, Silva BO, *et al*. A General Description of Apocynaceae Iridoids Chromatography. Column Chromatography Dean F. Martin and Barbara B. Martin, Intech Open, DOI: 10.5772/55784. Available from: https://www.intechopen.com/books/column-chromatography/a-general-description-of-apocynaceae-iridoids-chromatography. 2013, Chapter 6.

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