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), 3-0-β-D-glucopyranosyloquinovacid (6), 3-0-β-D-glucopyranosyleanolic 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 IC₅₀ 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), Gambie, Ghana (Kumasi), Liberia (Kakatown), Sierra Leone, Angola, Sao Tome 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 artesiminin. 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 CHCl₃ were realized on a Perkin Elmer Polarimeter (Model 241). ¹H-NMR and ¹³C-NMR spectra were recorded using a Bruker spectrometer containing 5 mm H and ¹³C 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 F₂₅₄ sheets; spots viewed under UV lamps (254 and 365 nm) or sprayed with 50% H₂SO₄ reagent, heated and viewed.

Plant Material: The bark of Canthium subcordatum was harvested on the 28th of August 2008 from Mblomanyo, 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, 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 CHCl₃/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/Methanol (90:10) and 8 (4.7 mg), EtOAc/Methanol (88:12).

In vitro antiplasmodial against Plasmodium falciparum W2: The experiment was conducted as previously described by Tchokouaha and collaborators [52] using a Chloroquine and other 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% CO₂, 3% O₂ and 91% N₂ atmosphere [12]. Serial treatment with 5% Sorbitol (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 (IC50) 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 Grams-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 urogenital 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 37°C for incubation. The zones of inhibition were measured after 24 h.\textsuperscript{[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\textsuperscript{[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) 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 twofold well growth was used as growth control. The inoculum (50 μL, 10\textsuperscript{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 β-sitosterol (4.3 mg) (1), ursolic acid (3.9 mg) (2), cebernal (an iridoid with Δ\textsubscript{5,7(9)}-tetrane skeleton with 10-formyl and 11-carboxyl acid residues) (5.8 mg) (3)\textsuperscript{[13]}, quinovic acid (3.2 mg) (4), Cerberinic acid (an iridoid with Δ\textsubscript{5,7,9,11}-tetrane skeleton with 10-formyl and 11-carboxyl acid residues) (4.9 mg) (5)\textsuperscript{[13]}, 3-O-β-D-glucopyranosylquinovic acid (3.6 mg) (6), 3-O-β-D-glucopyranosylsorboanic acid (4.2 mg) (7) and Clemahexapetoside B (a symmetric macrocyclic glycoside (4.7 mg) (8)\textsuperscript{[16]} (Figure 2).

![Figure 2: Structures of 1-8](image-url)
DMSO-δ6 δC 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 IC50 value of 3.04 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 in 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 possessed 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). *Sa* 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 | Kc | Sa | Ss | Sf | Ec | Kp | Pa |
|--------------|-----------|----|----|----|----|----|----|----|----|
|              | ID | MIC | ID | MIC | ID | MIC | ID | MIC | ID |
| 1            | 50 | 14  | 19.5 | 10 | 39 | 12 | 39 | 0  | NT |
| 2            | 50 | 10  | 39 | 10 | 10 | 78 | 78 | 10 | 78 |
| 3            | 50 | 16  | 9.7 | 10 | 39 | 8  | 78 | 10 | 78 |
| 4            | 50 | 18  | 4.8 | 14 | 9.7 | 16 | 9.7 | 14 | 19.5 |
| 5            | 50 | 16  | 9.7 | 14 | 19.5 | 10 | 39 | 12 | 39 |
| 6            | 50 | 10  | 78 | 12 | 39 | 0  | NT | 10 | 78 |
| 7            | 50 | 10  | 78 | 12 | 39 | 10 | 78 | 12 | 78 |
| 8            | 50 | 10  | 78 | 10 | 19.5 | 10 | 78 | 14 | 19.5 |
| Gent.        | 50 | NT  | NT | NT | NT | NT | NT | NT | NT |
| Nyst.        | 50 | 16  | 4.8 | 18 | 4.8 | NT | NT | NT | NT |

**Key:** 1: ID in (mm) and MIC in (µg/mL). 2: ID = not tested; NT = Not determined; Sa: *Staphylococcus aureus*; Sc: *Staphylococcus saprophyticus*; 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-glucopyranosyleanolonic 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 (I), 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 MIC50 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 mecillinam-resistant *Staphylococcus aureus* (MRSA) [24]. Cervinal (3) showed antifungal activity close to that of nystatin. In fact, Cervinal 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 IC50 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.

**Acknowledgements**

Awantu A. F. thanks DAAD, Germany, for financial support and a research stay at Bielefeld University, Germany. Many thanks also to Mr. Nana Victor of the Cameroon National Herbarium for harvesting *C. subcordatum* in Mbalmayo, Centre Region of Cameroon.
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. Saamal D, Sanjeeb KP, Papainmita M, Behora P, LaI UR, Dash SK, Padhy RK. Review on Canthium coromandelicum. Am J PhytoMed Clin Ther 2014; 6:796-813.
4. Simplice DK, Tchadjobio T, Denise PI, Jacques S. Sub-Saharan Rubiaeaceae: A review of their traditional uses, phytochemistry and biological activities. Pakistan J Biol Sci 2011; 14:149-169.
5. Pulate PV, Wagy NA, Deshmukh VR. Phytochemical, ethno-medicinal and anatomical study of Canthium purvil Höhe. World J Pharm Sci 2015; 4(11):1464-1482.
6. Jouboubi C, Ngokon 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, Waiel R, Addae-Mensah I. Shanzhisin methyl ester gentiobioside, a new iridoid-Isolation and synthesis. Tetrahedron Lett. 1980; 21(38):3677-3678.
10. Murai Y, Kashimura S, Tamezawa S, Hashimoto T, Takaoka S, Asakawa Y, et al. Absolute configuration of (6S,9S)-resesidase from Polygonum hydropiper. Planta Med. 2010; 67(5):480-481.
11. Achenbach H, Waiel R, Raffelsberger B, Addae-Mensa I. Iridoid and other constituents of Canthium subcordatum. Phytochemistry 1981; 20(7):1591-1595.
12. Singh A, Rosenthal PJ. Comparison of efficacies of Cysteine Protease Inhibitors against five strains of Plasmodium 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, Vlie tinck AJ. Screening methods for antibacterial and antiviral agents from higher plants. In: Hostettmann, K, (eds), Methods in Inhibitors against five strains of Plasmodiun falciparum. In: Techniques Usuelles. SIMEP, Paris, 1987; 481.
15. Fumiko A, Hikaru O, Tatsuo Y. Cerbinal and its derivatives, yellow pigments in the bark of Cerbera manghas L. Chem Pharm Bull 1977; 25(12):3422-3424.
16. Shi S, Dong C, Jiang D, Tu P. Macroyclic Glycosides from Clematis hexapetala. Helv Chim Acta 2006; 89:3002-3006.
17. Chukwujekwu IC, Van Staden J, Smith P, Meyer JM. 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. Arokidyaraj S, Anbuselvan Vimalarasan, Hemachandran M, Dhayalan, Priya. Antibacterial activity of beta-sitosterol of Vitex agnuscastus. 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.
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 Oleandronic and Ursolic Acids: An Update Evidence-Based Complementary and Alternative Medicine. 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, Rair M. Antifungal Metabolites from Plants. Springer Berlin Heidelberg, 2013.
26. Ryu YB, Park S, Kim YM, Lee J, Soo WD, Chang SJ, et al. SARS-COV3Clpro inhibitory effects of quinone-methide triterpenes from Tripterygium regelii, Bioorganic Med. Chem. Lett, 2010; 20:1873-1876.
27. Ezem SN, Akpuaka MU, Ajije 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.

HOW TO CITE THIS ARTICLE

Awantu AF, Fotsing PYS, Bankeu KJJ, Lenta NB, Tsouh FPV, Boyom FF, et al. Antiplasmodial and antimicrobial potential of Canthium subcordatum extracts and isolates. J Phytopharmacol 2019; 8(2):52-56.