Antioxidant, antitumor and antimicrobial activities of the crude extract and compounds of the root bark of *Allanblackia floribunda*

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Running title: Bioactivity of Allanblackia floribunda

Keywords: *Allanblackia floribunda;* Guttiferae; antitumor; antioxidant; antimicrobial.

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Abstract

Context: *Allanblackia floribunda* Oliver (Guttiferae) is an African medicinal plant used traditionally to treat a variety of ailments.

Objective: We investigated the antitumor, radical scavenging, antimycobacterial, antibacterial and antifungal activities of the root bark extract of *A. floribunda* and three isolated phenolics, namely 1,7-dihydroxyxanthone (**1**), morelloflavone (**2**) and 7′-*O*-glucoside of morelloflavone (**3**).

Materials and methods: The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging assay was used for antioxidant test while crown gall tumor assay was used for assay of antitumor activity. The *p*-iodonitrotetrazolium chloride (INT) colorimetry and Microplate Alamar Blue Assay (MABA) were used for antimicrobial investigations.

Results: Moderate tumor reducing activity was observed with the extract while better activities were recorded with compounds **2** and **3**. The antimycobacterial and antitumor activities of the extract are being reported for the first time. The DPPH radical scavenging test showed that, all the studied samples were able to scavenge more than 50% of the free radical, with compound **3** showing the best inhibitory activity (IC_{50} of 49.08 µg/ml). Compounds **1** to **3** prevented the growth of *Mycobacterium smegmatis* and that both extract and compound **2** were active on *M. tuberculosis.* The lowest MIC value for the extract (9.76 µg/ml) was recorded against *Enterobacter aerogenes* while the corresponding value for the compounds (4.88 µg/ml) was obtained with compound **2** on *Trichophyton rubrum*.

Discussion and conclusion: The overall results of the present work provide baseline information for the potential use of the root bark extract of *A. floribunda* as an antimicrobial, antitumor and antioxidant phytomedicine.

Introduction

A large part of the world's population today relies on natural product remedies to treat a variety of ailments. The World Health Organization (WHO) estimates that 80% of the population in some Asian and African countries depend on traditional medicine for primary health care (WHO, 2002). Medicinal plants and their components are widely used in traditional medicine and have led to the development of new pharmaceutical drugs (Lewis & Elvin-Lewis, 1977). Approximately 25% of the active substances prescribed in the United States come from plant materials (Céspedes et al., 2006). It is estimated that nearly 20,000 species from several families are useful for this purpose (Penso, 1982). Our research on herbal medicine includes plants of the Guttiferae family. Most of these plants and their metabolites have been found to possess significant biological properties (Viven & Faure, 1979; Nkengfack et al., 2002a,b; Ouahouo et al., 2004; Mbaveng et al., 2008a). In this study, we targeted another plant of this family, *Allanblackia floribunda* Oliver. Different parts of *A. floribunda* are used traditionally to treat many ailments. In Cameroon, the decoction of the stem bark is used to treat dysentery or as gargle against toothache. The seeds are used in the manufacture of ointment against itching (Viven & Faure, 1979). The extracts from leaves, stem bark, and roots are used alone or combined with other plants in several African countries such as Gabon, Congo, and Cameroon to treat respiratory infections, dysentery, diarrhea, and toothache (Viven & Faure, 1979). The present work was therefore, undertaken to evaluate the antitumor, antioxidant, antimycobacterial, antibacterial and antifungal activities of the root bark extract of *A. floribunda* and three phenolic compounds purified from this extract.

Materials and methods

Plant material

The root bark of *Allanblackia floribunda* was collected at Mont Kala, Central Region of Cameroon, in October 2006. The plant was identified by Dr L. Zapfack of Botany Department, University of Yaoundé I, where a voucher specimen was deposited.

Extraction and purification

The air-dried and powdered root bark (5 Kg) of *A. floribunda* were successively macerated in CH_2Cl_2-MeOH (1:1) for 24 h and MeOH (20 l) for 4 h. Each filtrate was then concentrated in a vacuum under reduced pressure and the two concentrated filtrates were then combined after TLC analysis to give the crude extract (AFR; 182 g).

AFR (150 g) was subjected to vacuum flash chromatography using silica gel (70-230 mesh; 900 g), and eluted sequentially with hexane (2000 ml), hexane-ethyl acetate 50:50 v/v (1600 ml), pure ethyl acetate (1600 ml) and ethyl acetate-methanol 90:10 v/v (EtOAc-MeOH; 2800 ml). Twenty fractions of 400 ml each were collected and pooled on the basis of their TLC profiles in three fractions named A (fractions 1-5), B (6-13) and C (14-20).

Fraction A (15 g) was column chromatographed using silica gel 60 (100 g) and eluted with hexane and hexane-ethyl acetate gradient (97.5:2.5; 95:5; and 90:10 v/v). 144 fractions of 150 ml each were collected and pooled on the basis of their TLC profiles. Fractions 14 and 15 crystallized after 24 h to yield a yellow powder, 1,7-dihydroxyxanthone $C_{13}H_8O_4$ (**1**; 50 mg; MW: 228; m.p.: 240°C) (Monache et al., 1983). Fraction B (40 g) was column chromatographed using silica gel 60 (300 g), with CH_2Cl_2 (1250 ml) and CH_2Cl_2/MeOH gradients [97.5:2.5 v/v (2000 ml); 95:5 v/v (950 ml); 90:10 v/v (1250 ml); 85:15 v/v (1250 ml); and 75:25 v/v (550 ml)] as eluents. 145 fractions of 50 ml each were collected and fractions 50-62 eluted with CH_2Cl_2/MeOH 97.5: 2.5 v/v yielded a yellow powder, morelloflavone $C_{30}H_{20}O_{10}$ (**2**; 300 mg; MW: 556; m.p.: 244-245°C) (Locksley & Murray,

1971). Fractions 87-102 (4 g) eluted with CH_2Cl_2/MeOH (90:10 v/v) on silca gel 60 (35 g) column yielded another yellow powder, 7′-*O*-glucoside of morelloflavone $C_{36}H_{30}0_{16}$ (**3**; 150 mg; MW: 760; amorphous powder) (Monache et al., 1983). The chemical structures of the isolated compounds are shown in Figure 1.

General experimental procedure

IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer as KBr disc. ^{1}H-NMR, ^{13}C-NMR, two-dimensional COSY, ROESY, HSQC and HMBC analysis were performed on a Bruker Avance DPX instrument (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). The 2.50 and 40.0 ppm resonances of residual CD_3SOCD_3 were used as internal references for ^{1}H and ^{13}C-NMR spectra, respectively. Mass spectra were recorded on a micro TOF instrument. All melting points were determined on a micro-melting point apparatus and are uncorrected. The structures of the compounds were confirmed by comparing with reference data from available literature.

Antioxidant investigation: DPPH assay

The free radical scavenging activity of the extract and compounds was evaluated as described by Mensor et al. (2001). Briefly, the test samples were dissolved in pure dimethylsulfoxide (DMSO; Sigma-Aldrich, St Quentin Fallavier, France) and mixed with a 0.3 mM 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH; Sigma) solution in ethanol. After 30 min at room temperature, the absorbance was measured at 517 nm and converted into percentage of antioxidant activity. Ascorbic acid was used as a standard control. Each assay was repeated thrice and the results recorded as mean of the triplicate experiments (Figure 3). The inhibition ratio (%) was calculated as follows: % inhibition = [(Absorbance of control−Absorbance of test sample)/Absorbance of control] × 100. IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from a calibration curve by a linear regression (Joshi et al., 2010).

Preliminary antitumor test: Potato disc tumor induction (Crown gall) assay

The antitumor assay was carried out as described by Coker et al. (2003) and McLaughlin & Roger (1998). Briefly, *Agrobacterium tumefaciens* LMG 184 (from the Laboratory of Microbiology, University of Gent, Belgium) was grown on yeast extract medium (YEM) for 48 h at 28°C. Red potatoes (*Solanum tuberosum* L.) discs were impregnated with *A. tumefaciens* suspension [10^9 colony forming units (CFU) in phosphate-buffered saline (PBS)] and extract or compounds dissolved in pure DMSO at a final concentration of 100 µg/disc. Vinblastin (Sigma) at 5 µg/disc was used as positive control. Negative controls included pure DMSO with PBS; pure DMSO without the bacterium and pure DMSO with the bacterium. At day 12 of incubation at 28°C, the discs were stained with Lugol's reagent, and the tumors were counted under a dissecting microscope. Twelve replicates were analyzed for each sample, and the final results were graphically reported (Fig. 2).

Antimicrobial assays

Microbial strains

The test organisms included mycobacteria, fungi, Gram-negative and Gram-positive bacteria. Mycobacteria were obtained from American Type Culture Collection. Other microbial species were clinical isolates from Yaoundé General Hospital (Cameroon). Their identification was confirmed before use at the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) (Faculty of Science, University of Yaoundé I). This was followed by culturing on the specific media and biochemical test using API system (Mbaveng et al., 2008b).

Culture media

M. smegmatis was cultured on Middlebrook 7H11 agar (7H11;) and allowed to grow for 24 h. *M. tuberculosis* was plated onto Löwenstein-Jensen medium and allowed to grow for 3-4 weeks at 37°C. The Middlebrook 7H9 broth (Becton Dickinson, Sparks, MD) [supplemented

with 1% casamino acids (Fisher, Pittsburgh, PA), 0.2% glycerol (Fisher), 0.2% glucose, and 0.05% Tween-80 for *M. smegmatis* or supplemented with 0.2% glycerol (Sigma Chemical Co., St. Louis, MO) and 10% OADC (oleic acid-albumin-dextrose-catalase; Becton Dickinson), 0.5% glycerol, and 0.05% Tween 80 for *M. tuberculosis*] was used to determine the MIC and the minimal bactericidal concentration (MBC)of the test samples on *M. smegmatis* and *M. tuberculosis*. Nutrient Agar (NA) containing Bromocresol purple was used for the activation of *Bacillus cereus* while NA was used for other bacteria. Sabouraud Glucose Agar was used for the activation of the fungi. The Mueller Hinton Agar (MHA) was also used for the determination of the minimal microbicidal concentration (MMC) of the test samples.

Chemicals for antimicrobial assay

Ciprofloxacin and isoniazid (INH) (Sigma) were used as positive control for *M. smegmatis* and *M. tuberculosis* respectively. Nystatin (Maneesh Pharmaceutic PVT) and gentamycin (Jinling Pharmaceutic Group Corp.) were used as reference antibiotics (RA) respectively against fungi and bacteria other than *M. smegmatis* and *M. tuberculosis*.

Antimycobacterial assays

Microplate susceptibility testing against M. smegmatis.

All samples were tested against *M. smegmatis* using INT microplate dilution method. The MIC, MBC and bacterial preparation were performed in 96-well microplates according to Salie et al. (1996) and Newton et al. (2002). The extract and compounds (in 10% DMSO/7H9 both) were tested in the concentrations ranging from 1.22-625 µg/ml. Ciprofloxacin served as the positive drug control. DMSO at 2.5% served as solvent control Tests were done in triplicates. The cultured microplates were incubated at 37ºC for 24 h. The MIC of samples was detected following addition (40 µl) of 0.2 mg/ml of INT (Sigma-Aldrich, South Africa) and incubated at 37°C for 30 min (Eloff, 1998). MIC was defined as the lowest sample concentration that prevented the color shift (yellow to pink). The MBC was determined by

adding 50 µl aliquots of the preparations (without INT), which did not show any growth after incubation during MIC assays, to 150 µl of 7H9 broth. These preparations were incubated at 37°C for 48 h. The MBC was regarded as the lowest concentration of extract, which did not produce a color change after addition of INT as mentioned above.

Antituberculosis assay using M. tuberculosis: MABA susceptibility testing

The activities of all test samples against *M. tuberculosis* were evaluated using the MABA according to Collins & Franzblau (1997) as modified by Jimenez-Arellanes et al. (2003). *M. tuberculosis* was cultured at 37°C in Middlebrook 7H9 broth. The extract, compounds and INH were dissolved in 10% DMSO/7H9 broth to final concentrations ranging from 0.31 to 625 µg/ml. The final concentration of DMSO in all assays was 2.5% or less. The samples were assayed twice in duplicate. Test inoculum was 6×10^6 CFU/ml. Microplates were incubated for 5 days at 37°C in a 5% CO_2 atmosphere and growth was detected by observing color shift (blue to pink) following addition of Alamar Blue solution (Sigma) and 20% sterile Tween-80 (Sigma) 1:1 v/v. The MIC corresponded to the greatest dilution of sample in which the color shift from blue to pink was not observed.

Determination of mycobactericidal effect (MBC)

Samples with detected MIC values following MABA (Collins & Franzblau, 1997; Jimenez-Arellanes et al., 2003) were assayed for their mycobactericidal effect as follows. 5 µl of the mycobacterial suspensions (showing no growth) was transferred from the former to a new microplate that contained 195 µl of fresh culture medium. The microplates were incubated and developed with Alamar Blue solution as for MABA. The MMC corresponded to the minimum sample concentration that did not cause a color shift in cultures re-incubated in fresh medium.

Antimicrobial assay on Gram-positive, Gram-negative bacteria and fungi

Sensitivity test: Agar disc diffusion assay

Preparation of discs

Whatmann filter paper (No.1) discs of 6 mm diameter impregnated with extract at 200 µg/disc, isolated compounds at 80 µg/disc and RA at 40 µg/disc, were prepared using 100%DMSO as solvent. Three discs were prepared for each sample. Negative control discs were also prepared as above with 10 µl of the 100%DMSO solution.

Diffusion test

The antimicrobial disc diffusion test was carried out as described by Kuete et al. (2007a; 2008a,b) using a cell suspension of about 1.5×10^6 CFU/ml obtained from a McFarland turbidity standard N° 0.5. The suspension was standardized by adjusting the absorbance to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer). A disc prepared with 100%DMSO was used as negative control. The plates were incubated at 30°C for 48 h (*Microsporum audouinii*) or 37°C for 24 h (other organisms). Antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disc. The assay was repeated thrice and results were recorded as mean ± SD of the three experiments.

MIC and MMC determinations

The MICs of the extract and compounds were determined in a microdilution assay as previously described (Kuete et al., 2007a,b,c, 2008a,b). The test samples were dissolved in 10%DMSO/MHB (Mueller Hinton Broth) to a final concentration ranges of 1.22 to 625 µg/ml. Inoculum concentration was standardized at 1.5×10^6 CFU/ml. The final concentration of DMSO in each well was less than 1%. The microplates were incubated at 30°C for 48 h (*M. audouinii*) or 37°C for 24 h (other organisms). The assay was repeated thrice. The MICs of samples were detected following addition (40 µl) of 0.2 mg/ml *p*-iodonitrotetrazolium

chloride and incubated at 37°C for 30 min (Kuete et al., 2009a,b). The MIC corresponded to the greatest dilution of sample in which the color shift from yellow to pink was not observed

For the determination of MMC, a portion of liquid (5 µl) from each well that showed no change in color was plated on MHA and incubated at 30°C for 48 h (*M. audouinii*) or 37°C for 24 h (other organisms). The lowest concentration that yielded no growth after this sub-culturing was taken as the MMC (Kuete et al., 2007a,b,c, 2008a,b)

Results

The purification of *A. floribunda* extract led to the isolation of three major compounds, 1,7-dihydroxyxanthone (**1**) (MW: 228; m.p.: 240°C) (Monache et al., 1983), morelloflavone (**2**) (MW 556; m.p.: 244-245°C) (Locksley & Murray, 1971) and 7′-*O*-glucoside of morelloflavone (**3**) (MW: 760; amorphous powder) (Monache et al., 1983). The three phenolic compounds belong to the classes of xanthone (**1**) and biflavonoids (**2** and **3**) (Figure 1). In the present report, we evaluated the antitumor, antioxidant, antimycobacterial, antibacterial and antifungal activities of the extract and compounds from *A. floribunda*.

In the antitumor experiment, it appeared from the results of the bacterial viability test that the tested concentrations of the plant extract do not alter *A. tumefaciens* growth at 10, 20, 30 min and 1 h of treatment. The three controls used in this assay included DMSO with PBS, DMSO without the bacterium and DMSO with the bacterium. The two controls did not induce tumor, showing that neither DMSO nor PBS interfere with the activity of *A. tumefaciens* or induce tumor themselves. DMSO with *A. tumefaciens* induced an average of 33 tumors. The antitumor activity of the tested samples is summarized in Figure 2. Moderate tumor reducing activity was observed with the extract (42.46% at 100 µg/disc). Better activity was recorded with compounds **2** and **3**, their tumor inhibition percentages being 51.29% and 56.17% respectively. Compound **1** with 13.89% activity was less active compared to **2** and **3**.

However, the reference drug, vinblastin at 5 µg/disc was still more active (98.47%) than the compound **2** (51.29%) and **3** (56.17%) at 100 µg/disc.

Figure 3 summarizes the DPPH• scavenging activity of the extract and compounds isolated from the root bark of *A. floribunda*. It appeared that at the concentration of 500 µg/ml, all the studied samples were able to scavenge more than 50% of the free DPPH radical. Compound **3** showed the best activity, exhibiting 91.08% inhibition. This activity was not significantly ($P < 0.05$) different from that of ascorbic acid used as reference antioxidant compound. The IC_{50} as determined by graphic extrapolation were 45.7, 49.08, 62.8, 76.3 and 488.53 µg/ml, respectively for vitamin C, compounds **3**, **2**, the crude extract and compound 1.

The results of the antimycobacterial assays (Table 1) showed that the extract as well as compounds **1** to **3** were able to prevent the growth of *M. smegmatis* in the tested concentration range. Only the extract and compound **2** were active on *M. tuberculosis.* The MIC value of 39.06 µg/ml for the extract and 19.53 µg/ml for compound **2** were recorded on *M. smegmatis*. Results of the MMC determination (Table 1) showed detectable values for the samples on several organisms.

Tables 2-3 also summarize the results of the antimicrobial assays against fungi, Gram-positive and negative bacteria. Results of the diffusion test (Table 2) demonstrated that the extract and compound **2** prevented the growth of all the tested organisms. The IZ obtained ranged from 7 to 22 mm and 7-22.5 mm, respectively, for the extract and compound **2**. Compound **3** was active on 11 of the 18 (61.1%) studied organisms including Gram-positive and Gram-negative bacteria, and fungi. The results of MIC determinations (Table 3) indicated values ranging from 19.53 to 312.50 µg/ml for the extract on most of the tested microorganisms. As previously observed, compound **3** was selectively active. The MIC value of 9.76 µg/ml for the extract was recorded against *E. aerogenes*. The MIC of 4.88 µg/ml was noted with compound **2** on *Trichophyton rubrum*. The reference antibiotics exhibited MICs ranging from 2.44 to

19.53 µg/ml. The inhibition potentials of the extract and compound **2** can be considered important when regarding the antibacterial and antifungal activities of the RA. This was as active as nystatin on *T. rubrum*. The results of the MMC determinations (Table 3) showed microbicidal activity on 83.3% (15/18), 77.8% (14/18) and 27.7% (5/18) of the tested organisms for the extract, compounds **2** and **3,** respectively. The MMC values (ranging from 4.88-39.06 µg/ml) obtained with reference antibiotics were generally lower than those of the extract and compounds in the corresponding microbial species. Nevertheless, the value obtained once with compound **2** on *T. rubrum* was lower than that of nystatin, highlighting its good antimicrobial potency.

Discussion

The role of plant secondary metabolite as antitumor compounds is well known, with flavonoids shown to possess antimutagenic and anticarcinogenic activity (Brown, 1980; Hirano et al., 1989). The inhibition of *Agrobacterium tumefaciens*-induced tumors (or crown gall) in potato disc tissue is an assay based on antimitotic activity and have been used to detect a broad range of known and novel antitumor agents (Coker et al., 2003). Crown Gall is a neoplastic plant disease caused by *A. tumefaciens*. The validity of this bioassay is based on the observation that certain tumorigenic mechanisms are similar in plants and animals (Liu et al., 2007). It has been shown that the inhibition of crown gall tumor initiation on potato discs and subsequent growth showed good correlation with compounds and extracts active in the 3PS leukemic mouse assay (Galsky et al., 1980). A number of well-known antineoplastic agents such as podophyllin, taxol, camptothecin, vincristine and vinblastine have all shown significant tumor inhibition of crown gall (Coker et al., 2003). This experiment therefore indicates the possible use of this plant for anticancer treatment, and shows that some of its components could be more useful from the perspective of the development of antitumor

medicine. However, further studies on more specific tumor cell lines will be necessary to confirm this hypothesis.

In the DPPH radical scavenging assay, all the three studied phenolic compounds were active with IC_{50} closer to that of ascorbic acid. This result is in conformity with the role of phenolics as antioxidant compounds (Liu et al., 2007) and also consistent with the role of ascorbic acid as DPPH scavenging agent, as the IC_{50} (45.7 µg/ml)obtained is closer (40.2 µg/ml) to that obtained by Bhandari et al. (2010).

Observation of MBC values of samples against the mycobacteria indicated that they were not more than fourfold than their corresponding MICs. This suggests that bactericidal effect of studied samples could be expected (Mims et al., 1993). The data obtained when samples were tested against fungi, Gram-positive and negative bacteria also confirmed that they could have killing effect on most of the tested organisms (Kuete et al., 2007a, b).

The use of *M. smegmatis* in this assay was a preliminary step to select the concentration range to be tested on *M. tuberculosis* species. The results obtained validated the necessity of such experiments. However, it is well known that the sensitivity of *M. smegmatis* is closer to that of M. tuberculosis and that this non-pathogenic mycobacterial species can be used in selecting samples for *M. tuberculosis* studies (Newton et al., 2002).

When regarding the structure-activity relationship, it appeared that transformation of morelloflavone (**2**) to 7′-*O*-glucoside of morelloflavone (**3**) significantly reduced the antimicrobial activity of the latter compound. This could be due to the ability of the microorganisms to break the 7-O-glucoside bond of compound **3** to yield glucose and compound **2**. The release glucose can therefore be used as source of energy for their growth (Bacq-Calberget al., 1999). In the antitumor assay, such structure-related activity is not pronounced. This confirms the fact that the two compounds do not prevent the growth of *A. tumefaciens*, but act directly on the tumor-inducing mechanism. The glucose moiety also

increases the antioxidant potency of compound **3**, explaining why the resultant activity is better than that of compound **2**.

To the best of our knowledge, the antimycobacterial and antitumor activities of the extract of *A. floribunda* is being reported for the first time. However, the ability of this crude extract to prevent *in vitro* the growth of *Candida albicans,* and that of some Gram-positive and Gram-negative bacteria has been demonstrated (Ajibesin et al., 2008). The results obtained in the present work corroborate the earlier report and confirm also the activity of *A. floribunda* on filamentous fungi such as *Trichophyton rubrum* and *M. audouinii*. In the present study, compound **1** was not tested against fungi, Gram-positive and Gram-negative bacteria. However, this compound from the stem bark of *Vismia rubescens* has been found to exhibit both antibacterial and antifungal activities (Tamokou et al., 2009). The antitumor activity could essentially be due to the presence of anticancer compounds such as morelloflavone or 7′-*O*-glucoside of morelloflavone. Morelloflavone is known to inhibit tumor growth and tumor angiogenesis of prostate cancer in mouse tumor model *in vivo*, suggesting that the inhibition of tumorigenesis by targeting angiogenesis could be its mode of action (Xiufeng et al., 2009).

Conclusions

The overall results of this study provide baseline information for the use of the extract of *A. floribunda* as well as some of its components as sources of antimycobacterial, antibacterial, antifungal, antitumor and antioxidant drug. However, further toxicological studies need to be done to confirm this hypothesis.

Acknowledgments

Authors acknowledge the financial support of the International Foundation for Science (IFS) (Grant no. F /4579-1 to VK) for the antimycobacterial assays and both IFS and the Organization for the Prohibition of Chemical Weapons, The Hague, Netherlands (OPCW),

grant F/3969 to AAGB, and the technical support of Mr. P. Lunga of the University of Dschang.

Declaration of interest

The authors report no declaration of interest. The authors alone are responsible for the content and writing of the paper.

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Tables

Table 1.
Antimycobacterial activity of the crude extract, compounds isolated from *Allanblackia floribunda* and reference antibiotics

Tested samples[a]	Tested microorganisms[b]			
	M. smegmatis		*M. tuberculosis*	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC
Crude extract				
AFR	39.06	156.25	39.06	156.25
Compounds				
1	312.50	nd	>625	>625
2	19.53	78.12	39.06	78.12
3	312.50	625	>625	>625
Reference antibiotics				
Ciprofloxacin	0.61	1.22	nt	nt
Isoniazid	nt	nt	0.12	0.24

[a]Tested samples: AFR: crude extract from the root bark of *Allanblackia floribunda*, **1:** 1,7-dihydroxyxanthone, **2:** morelloflavone, **3:** 7′-*O*-glucoside of morelloflavone
[b] Tested mycobacteria were *M. smegmatis* ATCC 700084 and *M. tuberculosis* H37Rv ATCC 27264
MIC: minimal inhibition concentration, MBC: Minimal bactericidal concentration
(nd): Not determined because the sample was not active at the highest tested concentration
(nt): Not tested

Table 2.
Inhibition zone diameters (mm) of the extract and compounds isolated from *Allanblackia floribunda* and reference antibiotics as determined by diffusion test[a]

Microorganisms[b]	Tested samples[c]				
	Crude extract	Compounds		Reference antibiotics	
	AFR	**2**	**3**	Gentamicin	Nystatin
Gram-negative bacteria					
Citrobacter freundii	19.0 ± 1.0	7.0 ± 0.0	7.0 ± 0.0	23.7 ± 0.8	nt
Enterobacter aerogenes	22.0 ± 0.0	15.0 ± 0.5	12.0 ± 0.0	22.5 ± 0.5	nt
Enterobacter cloacae	18.2 ± 0.3	14.0 ± 0.0	-	19.0 ± 1.0	nt
Escherichia coli	18.0 ± 0.0	15.2 ± 0.3	14.3 ± 0.3	24.7 ± 0.3	nt
Klebsiella pneumoniae	7.0 ± 0.0	-	-	18.3 ± 0.3	nt
Morganella morganii	13.0 ± 0.0	14.7 ± 0.8	9.0 ± 0.0	25.0 ± 0.0	nt
Proteus mirabilis	14.5 ± 1.0	16.0 ± 0.0	-	23.7 ± 0.8	nt
Proteus vulgaris	18.5 ± 0.5	12.2 ± 0.3	9.0 ± 0.0	26.0 ± 1.0	nt
Pseudomonas aeruginosa	10.0 ± 0.0	10.0 ± 0.0	-	19.0 ± 0.0	nt
Shigella dysenteriae	20.7 ± 1.2	14.5 ± 0.5	7.0 ± 0.0	23.0 ± 0.0	nt
Salmonella typhi	19.0 ± 0.0	16.0 ± 0.5	-	23.3 ± 0.3	nt
Gram-positive bacteria					
Streptococcus faecalis	18.0 ± 0.0	14.0 ± 0.0	7.0 ± 0.0	20.3 ± 0.3	nt
Staphylococcus aureus	19.3 ± 0.3	7.0 ± 0.0	7.0 ± 0.0	21.2 ± 0.3	nt
Bacillus subtilis	14.2 ± 0.3	15.0 ± 0.5	-	22.0 ± 0.0	nt
Fungi					
Candida albicans	19.5 ± 0.5	17.2 ± 0.3	14.3 ± 0.3	nt	24.0 ± 1.0
Candida glabrata	16.0 ± 0.0	17.0 ± 1.0	14.2 ± 0.3	nt	23.3 ± 0.6
Microsporum audouinii	7.0 ± 0.0	7.0 ± 0.0	-	nt	25.3 ± 1.2
Trichophyton rubrum	17.0 ± 0.0	22.5 ± 0.5	13.0 ± 0.0	nt	23.0 ± 0.0

[a]Samples were tested at 80 µg/disc for compounds, 40 µg/disc for reference antibiotics, 200 µg/disc for the extract;
[b]Tested organisms were Methicillin-resistant *Staphylococcus aureus* LMP805, *Streptococcus faecalis* LMP 806, *Bacillus cereus* LMP 716 (Gram-positive bacteria), β-lactamase positive *Escherichia coli* LMP701, Ampicillin-resistant *Klebsiella pneumoniae* LMP803, Carbenicillin-resistant *Pseudomonas aeruginosa* LMP804, Chloramphenicol-resistant *Salmonella typhi* LMP706, Chloramphenicol-resistant *Citrobacter freundii* LMP802 (five Gram-negative bacteria), *Candida albicans* LMP709U and *Microsporum audouinii* LMP725D (fungi)
[c]Tested samples: AFR: crude extract from the roots of *Allanblackia floribunda*, **2:** morelloflavone, **3:** 7′-*O*-glucoside of morelloflavone
(-): not active
(nt) : not tested

Table 3.
Minimal inhibition concentration (MIC) and minimal microbicidal concentration (MMC) (in parethesis) of the extract and compounds isolated from *Allanblackia floribunda* and reference antibiotics as determined by microdilution assay

Microorganisms[a]	Tested samples[b], MIC (µg/ml) and MMC in parenthesis (µg/ml)				
	Crude extract	Compounds		Reference antibiotics	
	AFR	**2**	**3**	Gentamicin	Nystatin
Gram-negative bacteria					
Citrobacter freundii	39.06 (78.12)	>625 (nd)	>625 (nd)	4.88 (9.76)	nt (nt)
Enterobacter aerogenes	9.76 (19.53)	39.06 (78.12)	78.12 (312.50)	4.88 (9.76)	nt (nt)
Enterobacter cloacae	78.12 (156.25)	39.06 (156.25)	- (-)	9.76 (19.53)	nt (nt)
Escherichia coli	78.12 (156.25)	39.06 (78.12)	39.06 (156.25)	2.44 (4.88)	nt (nt)
Klebsiella pneumoniae	>625 (nd)	- (-)	- (-)	19.53 (39.06)	nt (nt)
Morganella morganii	312.50 (>625)	39.06 (156.25)	312.50 (>625)	2.44 (4.88)	nt (nt)
Proteus mirabilis	156.25 (625)	39.06 (156.25)	(-)	4.88 (9.76)	nt (nt)
Proteus vulgaris	39.06 (78.12)	78.12 (312.50)	312.50 (>625)	2.44 (4.88)	nt (nt)
Pseudomonas aeruginosa	625 (625)	156.25 (625)	- (-)	9.76 (19.53)	nt (nt)
Shigella dysenteriae	19.53 (39.06)	39.06 (156.25)	>625 (nd)	4.88 (9.76)	nt (nt)
Salmonella typhi	39.06 (78.12)	39.06 (78.12)	- (-)	4.88 (9.76)	nt (nt)
Gram-positive bacteria					
Streptococcus faecalis	78.12 (312.50)	39.06 (156.25)	>625 (nd)	4.88 (9.76)	nt (nt)
Staphylococcus aureus	39.06 (156.25)	>625 (nd)	>625 (nd)	4.88 (9.76)	nt (nt)
Bacillus subtilis	156.25 (312.50)	39.06 (78.12)	- (-)	4.88 (9.76)	nt (nt)
Fungi					
Candida albicans	39.06 (156.25)	19.53 (78.12)	39.06 (156.25)	nt (nt)	4.88 (9.76)
Candida glabrata	78.12 (156.25)	19.53 (78.12)	39.06 (156.25)	nt (nt)	4.88 (9.76)
Microsporum audouinii	>625 (nd)	>625 (nd)	- (-)	nt (nt)	4.88 (9.76)
Trichophyton rubrum	156.25 (625)	4.88 (9.76)	78.12 (156.25)	nt (nt)	4.88 (9.76)

[a]Tested organisms were Methicillin-resistant *Staphylococcus aureus* LMP805, *Streptococcus faecalis* LMP 806, *Bacillus cereus* LMP 716 (Gram-positive bacteria), β-lactamase positive *Escherichia coli* LMP701, Ampicillin-resistant *Klebsiella pneumoniae* LMP803, Carbenicillin-resistant *Pseudomonas aeruginosa* LMP804, Chloramphenicol-resistant *Salmonella typhi* LMP706, Chloramphenicol-resistant *Citrobacter freundii* LMP802 (Gram-negative bacteria), *Candida albicans* LMP709U and *Microsporum audouinii* LMP725D (fungi)
[B]Tested samples: AFR: crude extract from the root bark of *Allanblackia floribunda*, **2:** morelloflavone, **3:** 7′-*O*-glucoside of morelloflavone
(-): not determined as the compound was not active following diffusion test
(nd): not determined as the MIC>625 µg/ml
(nt): not tested

Figures

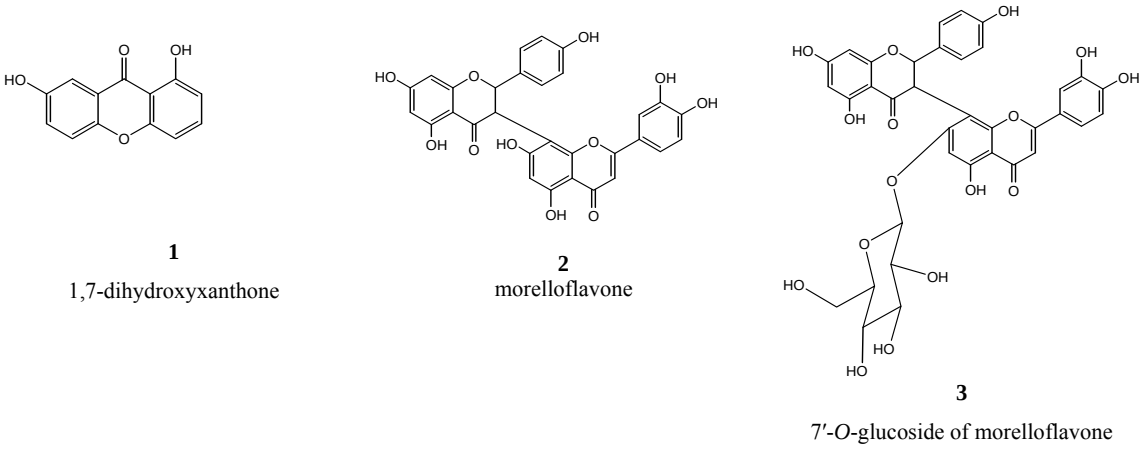

Figure 1. Chemical structure of compounds isolated from *A. floribunda*

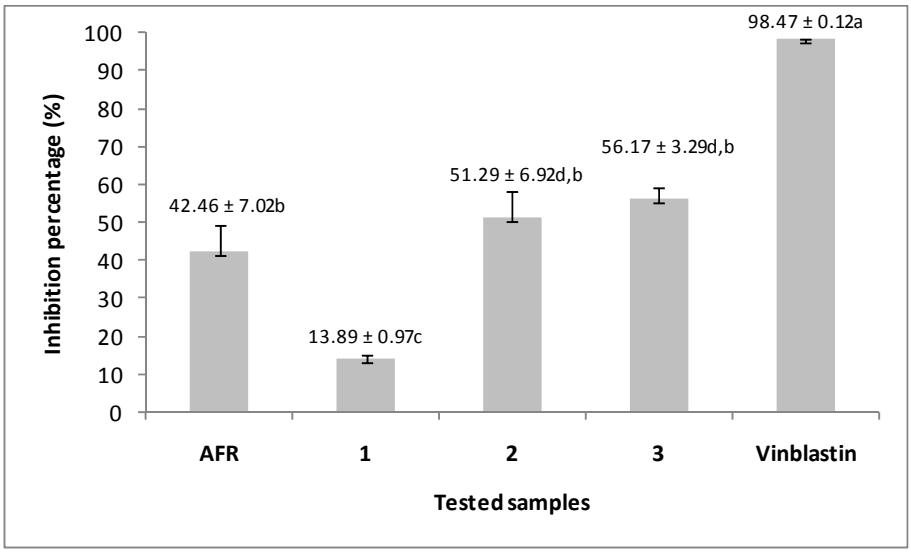

Figure 2. Antitumor activity of the crude extract and compounds isolated from *A. floribunda* (AFR: crude extract from the root bark of *Allanblackia floribunda*), **1:** 1,7-dihydroxyxanthone, **2:** morelloflavone, **3:** 7′-*O*-glucoside of morelloflavone; values with the same letter are not significantly different, P< 0.05; ANOVA)

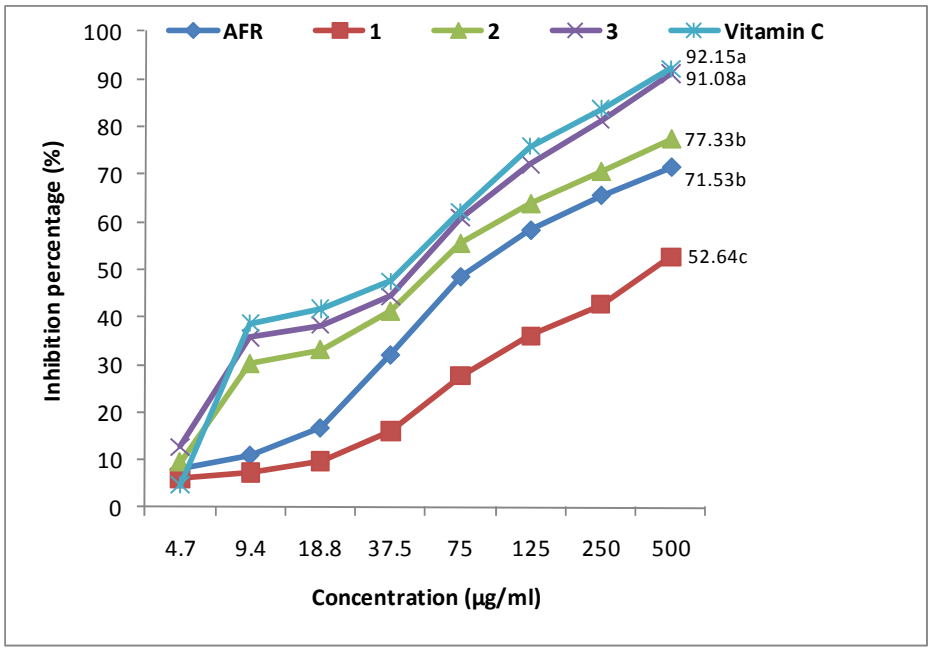

Figure 3. Antioxidant activity of the crude extract and compounds isolated from *A. floribunda* (AFR: crude extract from the root bark of *Allanblackia floribunda*), **1:** 1,7-dihydroxyxanthone, **2:** morelloflavone, **3:** 7′-*O*-glucoside of morelloflavone; values with the same letter are not significantly different, P< 0.05; ANOVA)