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Antibacterial and antifungal activities of the crude extract and compounds from *Dorstenia turbinata* (Moraceae)

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Abstract

The aim of this study was to evaluate the antimicrobial activity of the crude extract of the twigs of *Dorstenia turbinata* (DTT) as well as that of five of the nine compounds isolated from this extract, namely 5-methoxy-3-[3-(ß-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen (**1**), 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen (**2**), (2′S, 3′R)-3′-hydroxymarmesin (**3**), 4-hydroxy-3-methoxybenzaldehyde (**4**) and 4-methoxyphenol (**5**). Gram-positive, Gram-negative bacterial species as well as fungi were used. The agar disc diffusion test was used to determine the sensitivity of the tested samples while the well micro-dilution was used to determine the minimal inhibition concentrations (MIC) and the minimal microbicidal concentration (MMC) of the active samples. The results of the disc diffusion assay showed that the crude extract (DTT), compounds **1** to **3** were able to prevent the growth of all the tested pathogens at the tested concentrations. Compounds **4** and **5** showed moderate and selective activities. The results of MIC determinations indicated values ranging from 19.53 to 78.12 µg/ml for the DTT and from 9.76 to 78.12 µg/ml for compound **2**. The MIC values recorded on 91% of the tested organisms for compounds **1** and **3**. The lowest MIC value for the crude extract of *D. turbinata* (19.53 µg/ml) was noted on *Trichophyton rubrum* and *Escherichia coli*. The corresponding value for the tested compounds (9.76 µg/ml) was obtained with **2** and **3** on *T. rubrum*. The antimicrobial activity of this plant as well as that of compounds **1**-**2** is being reported for the first time. The overall results provide promising baseline information for the potential use of the crude extracts from DTT as well as some of the isolated compounds in the treatment of bacterial and fungal infections.

Keywords: Antimicrobial activity; Compounds; *Dorstenia turbinate*; Moraceae

1. Introduction

Evidence of the efficiency of herbal drugs used in African folk medicine in the treatment of several ailments is being provided continuously and intensively today (Lall and Meyer, 1999, 2000, 2001; Kuete et al., 2004; Masoko et al., 2005; Kuiate et al., 2006; Mbaveng et al., 2008). Many research institutes and centers throughout the continent focus their activities on the biological study of African medicinal plants. This is aimed to obtain baseline information for the development of efficient and more accessible herbal drugs, taking in account that about 80% of African population used traditional healing for first health care. Our research group directed its research toward the investigation of antimicrobial agents from African medicinal plants. This includes plants of the family Sapotaceae, Guttiferae, Solanaceae, Melianthaceae, Bignoniaceae and Moraceae. In our program, intensive investigations are continuing with plants of the Moraceae family. Within this family, the biological activities of

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plants-species belonging to the genus *Dorstenia* is well documented (Kuete et al., 2007a,b; Mbaveng et al., 2008). Numerous antimicrobial compounds were isolated from *Dorstenia* species. Compounds such as isobavachalcone, 4-hydroxylonchocarpin, kanzonol C and amentoflavone were identified as antimicrobial principles from *D. barteri* Bureau (Mbaveng et al., 2008). Gancaonin Q, stipulin, angusticornin B and bartericin A, isolated from *D. angusticornis*, showed significant inhibitory effects against pathogenic bacteria and fungi (Kuete et al., 2007b). Psoralen, O-[3-(2, 2-dimethyl-3-oxo-2H-furan-5-yl) butyl]bergaptol, bergapten, O-[3-(2, 2-dimethyl-3-oxo-2H-furan-5-yl)-3-hydroxybutyl]bergaptol, and 3-(3,3-dimethylallyl)-4,2′,4′-trihydroxychalcone isolated from *D. elliptica* were lately reported for their antimicrobial activity (Kuete et al., 2007a,c). In the present study, we targeted another species of the genus *Dorstenia*, namely, *D. turbinata*. *D. turbinata* is used traditionally in the treatment of infectious diseases including gastroenteritis and skin infections, and rheumatism (personal communication). Previously, compounds known for their antimicrobial activity such as 4-hydroxylonchocarpin and kanzonol C (Mbaveng et al., 2008) were isolated from the twigs of *D. turbinata* (Ngameni et al., 2006). In the present study, 5-methoxy-3-[3-(ß-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen (**1**), 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen (**2**), (2′S, 3′R)-3′-hydroxymarmesin (**3**), 4-hydroxy-3-methoxybenzaldehyde (**4**) and 4-methoxyphenol (**5**) were also isolated from the twigs of *D. turbinata* and subjected to antibacterial and antifungal investigations.

2. Materials and methods

2.1. Plant material

The twigs of *D. turbinata* were collected in Kumba, South West province of Cameroon, in June 2007, and identified at the Cameroon National Herbarium in Yaounde, where the voucher specimen (No. 28158/SRF/Cam) was deposited.

2.2. Isolation and structure elucidation

The air-dried powdered twigs (without leaves and flowers) of *D. turbinata* (2 kg) were extracted with methanol to give 71 g extract. The extract was chromatographed on silica gel 60 (0.063–0.200 mm) using Hex/AcOEt (F1: 50/50; F2: 30/70; F3: 0/100 each 2 × 500 ml) and AcOEt/MeOH (F4: 90/10; F5: 80/20) to yield 5 fractions. Fractions F1–3 were submitted to column chromatography over silica gel 60 (0.063–0.200 mm) using *n*-hexane–AcOEt as eluent with a continuous gradient (from 95:5 to 60:40, and finally with pure AcOEt), followed by gel filtration chromatography over Sephadex LH-20 using CH_2Cl_2:MeOH (9:1) as eluent and by preparative TLC to afford 4-methoxyphenol (**5**, 15 mg, M_W: 124, mp 54–56 °C) (Paterson and Tipman, 1962), 4-hydroxy-3-methoxybenzaldehyde (**4**, 24 mg, M_W: 152, mp 80–81 °C) (Klinck and Stothers, 1962), kanzonol C (**7**, 75 mg, M_W: 392, mp 190–194 °C) (Fukai et al., 1994), 4-hydroxylonchocarpin (**8**, 86 mg, M_W: 322, mp 203–204 °C) (Ngadjui et al., 2000), umbelliferone (**9**, 25 mg, M_W: 162, mp 225–228 °C) (Cussans and Huckerby, 1975), psoralen (**6**, 12 mg, M_W: 186, mp: 163–164 °C) (Elgamal et al., 1979), and (2′S, 3′R)-3′-hydroxymarmesin (**3**, 24 mg, M_W: 262, mp 173–175 °C) (Vilegas and Pozetti, 1993). Fractions F4 and F5 were chromatographed over silica gel using solvents of increasing polarity from CH_2Cl_2, CH_2Cl_2–MeOH mixtures to MeOH as eluent, followed by gel filtration chromatography over Sephadex LH-20 using CH_2Cl_2:MeOH (3:2) as eluent and by preparative TLC to afford (2′S, 3′R)-3′-hydroxymarmesin (**3**, 25 mg, M_W: 262, mp 173–175 °C) (Vilegas and Pozetti, 1993), 5-Methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen (**2**, 10 mg, M_W: 318, amorphous solid) (Franke et al., 2001) and 5-Methoxy-3-[3-(β-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen (**1**, 35 mg, M_W: 480, amorphous solid) (Ngameni et al., 2006).

2.3. General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker AMX-500 spectrometer or Bruker AV-300 spectrometers. ESI-MS spectra were recorded on a Kratos Concepts IIH mass spectrometer. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.5 mm) were used for PTLC. Thin layer chromatography (TLC) was performed on silica gel F_{254} (Merck) precoated aluminium sheets and spots were visualized under UV and by spraying with molybdenum solution and heating.

2.4. Microbial strains

The tested organisms included Methicillin-resistant *Staphylococcus aureus* LMP805 (Gram-positive bacterium), six Gram-negative bacteria namely β-lactamase positive (βL^+) *Escherichia coli* LMP701, βL^+-*Shigella dysenteriae* LMP606, Ampicillin-resistant *Klebsiella pneumoniae* LMP803, Carbenicillin-resistant *Pseudomonas aeruginosa* LMP804, Chloramphenicol-resistant *Salmonella typhi* LMP706, Chloramphenicol-resistant *Citrobacter freundii* LMP802 and four fungi namely *Candida albicans* LMP709U, *Candida gabrata* LMP0413U, *Microsporum audouinii* LMP725D, and *Trichophyton rubrum* LMP0723D. These microbial species were clinical isolates from Yaoundé General Hospital (Cameroon). Their identify was confirmed before use at the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) (Faculty of Science, University of Yaoundé I) by culturing on the specific media followed by biochemical test using the API system as previously reported (Mbaveng et al., 2008). They were maintained on agar slants at 4 °C at the LMP. These strains were sub-cultured on a fresh appropriate agar plate 24–48 h prior to any antimicrobial test.

2.5. Antimicrobial assays

2.5.1. Culture media

Nutrient Agar (NA) was used for bacteria. Sabouraud Glucose Agar was used for the activation of the fungi. The Mueller Hinton broth (MHB) was used to determine the minimal inhibition concentration (MIC) of all samples against the tested pathogens. The MHB and Mueller Hinton Agar

(MHA) were used to determine the minimal microbicidal concentration (MMC) of the active samples.

2.5.2. Chemicals

Nystatin (Maneesh Pharmaceutic PVT. Ltd., Govandi, Mumbai, 400 043 India) and gentamycin (Jinling Pharmaceutic Group corp., Zhejang Tieng Feng Pharmaceutic Factory, No. 11 Chezhan Road, Huzhou city, Zhejang, China) were used as reference antibiotics (RA) against fungi and bacteria, respectively.

2.5.3. Sensitivity test: agar disc diffusion assay

2.5.3.1. Preparation of discs. Whatmann filter paper (No 1) discs of 6 mm diameter were impregnated with 10 µl of the solution of crude extract at 10 mg/ml (100 (µg/disc) and isolated compounds at 4 mg/ml (40 (µg/disc) prepared using DMSO. The discs were evaporated at 37 °C for 24 h. The RA discs were prepared as described above using the appropriate concentrations to obtain discs containing 40 µg of drug. Two discs were prepared for each sample.

2.5.3.2. Diffusion test. The antimicrobial diffusion test was carried out as described by Kuete et al. (2006) using a cell suspension of about $1.5 \cdot 10^6$ CFU/ml obtained from a McFarland turbidity standard No 0.5. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer) (Kuete et al., 2007a,b,c, 2008a). This was used to inoculate by flooding the surface of MHA plates. Excess liquid was air-dried under a sterile hood and the impregnated discs were applied at equidistant points on top of the agar medium. A disc prepared with only the corresponding volume of DMSO was used as negative control. The plates were incubated at 30 °C for 48 h (*M. audouinii* and *T. rubrum*) 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 twice and results were recorded as mean ± SD of the duplicated experiment.

2.5.4. MIC and MMC determinations

The MICs of compounds **1** to **6**, and reference antibiotics (RA) (gentamycin for bacteria and nystatin for fungi) were determined as follows. The test sample was first of all dissolved in 4% dimethylsulfoxide (DMSO). The solution obtained was added to MHB to give a final concentration of 78.12 µg/ml. This was serially diluted two fold to obtain concentration ranges of 0.31 to 78.12 µg/ml. 100 µl of each concentration was added in a well (96-wells microplate) containing 95 µl of MHA and 5 µl of inoculum (standardized at $1.5 \cdot 10^6$ CFU/ml by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer) (Kuete et al., 2007a,b,d, 2008a). The final concentration of DMSO in the well was less than 1% (preliminary analyses with 1% (v/v) DMSO do not affect the growth of the test organisms). The negative control well consisted of 195 µl of MHB and 5 µl of the standard inoculum (Kuete et al., 2007a,b,d, 2008a). The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker and incubated at 30 °C for 48 h (*M. audouinii* and *T. rubrum*) or 37 °C for 24 h (other organisms). The assay was repeated trice. The MIC of samples was detected following addition (40 µl) of 0.2 mg/ml *p*-iodonitrotetrazolium chloride and incubated at 37 °C for 30 min (Kuete et al., 2008a,b). Viable bacteria reduced the yellow dye to a pink color. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth.

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* and *T. rubrum*) or 37 °C for 24 h (other organisms). The lowest

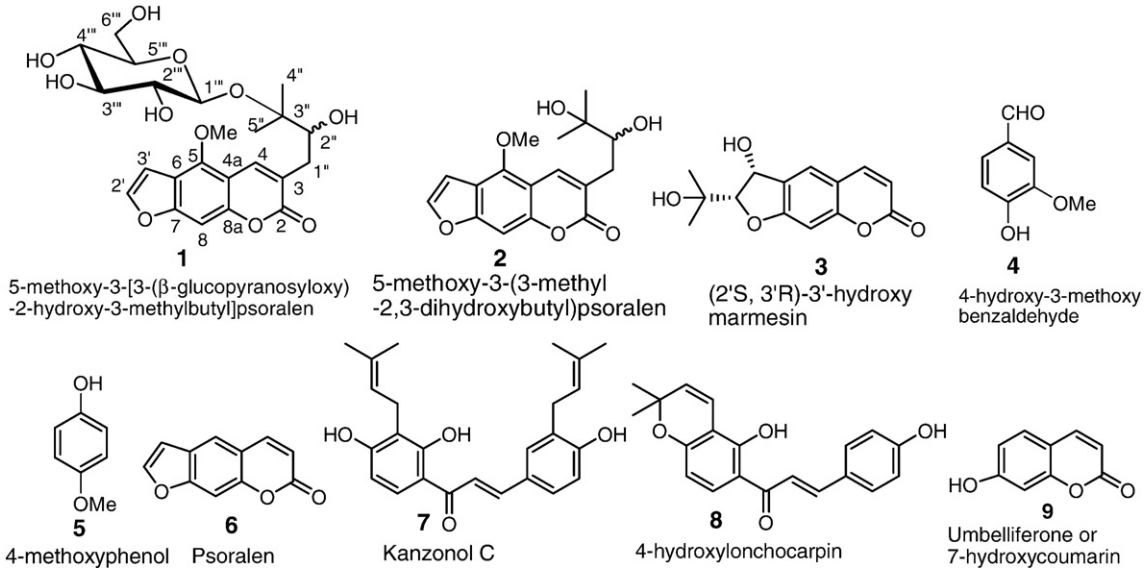

Fig. 1. Chemical structures of compounds isolated from the twigs of *Dorstenia turbinata*.

concentration that yielded no growth after this sub-culturing was taken as the MMC (Kuete et al., 2007a,b,d).

3. Results and discussions

The structures of the isolated compounds (Fig. 1) were found to be 5-methoxy-3-[3-(β-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen (**1**), 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen (**2**), (2′S, 3′R)-3′-hydroxymarmesin (**3**), 4-hydroxy-3-methoxybenzaldehyde (**4**), 4-methoxyphenol (**5**), psoralen (**6**), kanzonol C (**7**), 4-hydroxylonchocarpin (**8**) and umbelliferone (**9**). The presence of coumarins such as compounds **1**–**3**, **6** and **9** in other plants of the genus *Dorstenia* as well as that of prenylated chalcones (compounds **7** and **8**) is well documented (Paterson and Tipman, 1962; Parrish et al., 1974; Cussans and Huckerby, 1975; Huang et al., 1997; Ngadjui et al., 1999, 2000; Franke et al., 2001; Abegaz et al., 2004; Ngameni et al., 2004, 2006). In this study, we evaluated the antimicrobial potential of the crude extract from the twigs of *D. turbinata* and that of compounds **1**–**5**. The results are reported in Tables 1–3.

The results of the diffusion test (Table 1) showed that the crude extract (DTT), and compounds **1** to **3** were able to prevent the growth of all the pathogens at the tested concentrations. Compounds **4** and **5** showed moderated and selective activities. Their inhibition effects were observed on all the fungi. Meanwhile compound **4** and **5** were active on three (48.86%) and four (57.14%) of the seven tested bacterial species. The highest inhibition zone diameters were obtained against *T. rubrum* (23 mm and 21 mm respectively for the crude extract, and compounds **1** and **2**).

The results of MIC determinations recorded in Table 2 indicate values ranging from 19.53 to 78.12 μg/ml for the DTT and from 9.76 to 78.12 μg/ml for compound **2**. Within the tested interval (0.31–78.12 μg/ml), compounds **1** and **3** showed evident MIC values on 10 (91%) of the 11 tested organisms. No detectable MIC value was detected with compound 4 on all the tested microbial species while compound 5 showed a measurable MIC only against *E. coli*. The lowest MIC value for the crude extract of *D. turbinata* (19.53 μg/ml) was noted on *T. rubrum* and *E. coli*. The corresponding value for the tested compounds (9.76 μg/ml) was obtained with **1** and **2** on *T. rubrum*. This can be correlated with the diffusion test results (Table 1), as the highest inhibition zone diameters were also obtained from the corresponding compounds on *T. rubrum*. The reference antibiotics were generally more active than the tested compounds. Their MIC values ranged from 4.88 to 39.06 μg/ml. However, the antimicrobial activity of the crude extract and compounds **1** to **3** can be considered promising. The lowest MIC value obtained with compounds **1** and **2** (9.76 μg/ml) is equivalent to that of nystatin on *T. rubrum*. The lowest MIC value of 19.53 μg/ml recorded with the crude extract is only 2 and 4 fold greater than that of the RA on *E. coli* and *T. rubrum*, respectively, confirming the interesting inhibition potential of *D. turbinata*. The results of the MMC determinations (Table 3) presented noticeable values for DTT and compounds **1** to **3** on most of the tested organisms. Tables 2 and 3 indicate that some of the MMC values obtained are generally four fold less than the MICs on corresponding organisms indicating that cidal effects of the test samples could be expected (Kuete et al., 2007a,b,c, 2008a). The activity of DTT and compounds **1** to **3** could be considered as most important, taking in account that the tested organisms were resistant to the commonly used antibiotics.

Regarding the structure activity relationship of the tested compounds, it can be noted (Tables 1–3) that compounds **1**, **2** and **3** belonging to the class of coumarins showed closer inhibitory effects. The antimicrobial effect of coumarin-like compounds is well documented (Bruneton, 1999; Cowan, 1999;

Table 1
Antimicrobial activity[a] of the crude extracts, compounds isolated from *D. turbinata* and reference antibiotics determined by the disc diffusion test.

Microorganisms	Inhibition zone diameters of the test samples[b] (mm)						
	DTT	1	2	3	4	**5**	RA
Bacteria							
Staphylococcus aureus LMP805	16.5±0.5	10.0±0.0	14.5±0.5	8.0±0.0	–	–	22.5±1.5
Escherichia coli LMP701	22.5±1.0	12.0±0.0	12.0±0.0	12.0±0.0	10.0±0.0	11.5±0.5	24.0±2.0
Shigella dysenteriae LMP606	18.5±0.0	14.0±0.0	15.5±0.0	14.0±0.0	–	8.0±0.0	21.5±0.5
Klebsiella pneumoniae LMP803	16.0±0.5	10.5±0.4	11.0±0.0	11.0±0.0	–	–	13.0±0.0
Pseudomonas aeruginosa LMP804	16.0±0.0	17.0±0.0	16.0±0.0	15.5±1.00	–	–	20.5±1.5
Salmonella typhi LMP706	19.5±0.0	13.5±0.0	13.5±0.5	15.0±0.0	8.0±0.0	8.0±0.0	18.0±0.0
Citrobacter freundii LMP802	16.5±0.0	14.5±1.5	15.0±0.0	15.0±0.0	8.0±0.0	10.0±0.0	21.5±1.0
Fungi							
Candida albicans LMP709U	20.5±0.5	18.5±1.0	19.0±0.0	19.0±0.0	9.0±0.0	10.5±0.5	18.0±0.0
Candida gabrata LMP0413U	18.0±0.0	16.0±1.0	16.0±1.0	14.5±1.0	8.0±0.0	8.0±0.0	17.5±0.0
Microsporum audouinii LMP725D	19.0±1.0	19.0±0.0	18.5±0.5	17.0±0.0	10.0±0.0	10.0±0.0	19.0±0.0
Trichophyton rubrum LMP0723D	23.0±1.0	21.0±1.0	21.0±1.0	16.5±0.5	8.0±0.0	8.0±0.0	20.0±0.0

(–): Not active.

[a] Antimicrobial activity: crude extract was tested at 100 μg/disc and compounds and RA at 40 μg/disc.

[b] The test samples were crude extract from the twigs of *D. turbinata* (DTT), **1**: 5-methoxy-3-[3-(ß-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen; **2**: 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen; **3**: (2′S, 3′R)-3′-hydroxymarmesin; **4**: 4-hydroxy-3-methoxybenzaldehyde; **5**: 4-methoxyphenol; Reference antibiotics (Gentamycin for bacteria, Nystatin for fungi).

Table 2
Minimum inhibition concentration (μg/ml) of the crude extracts, compounds isolated from *D. turbinata* and reference antibiotics.

Microorganisms	MIC for test samples[a] (μg/ml)						
	DTT	1	2	3	4	**5**	RA
Bacteria							
Staphylococcus aureus LMP805	78.12	>78.12	39.06	>78.12	nd	nd	9.76
Escherichia coli LMP701	19.53	78.12	78.12	78.12	>78.12	78.12	4.88
Shigella dysenteriae LMP606	39.06	39.06	39.06	39.06	nd	>78.12	9.76
Klebsiella pneumoniae LMP803	78.12	78.12	78.12	78.12	nd	nd	39.06
Pseudomonas aeruginosa LMP804	78.12	39.06	39.06	39.06	nd	nd	9.76
Salmonella typhi LMP706	39.06	39.06	39.06	39.06	>78.12	>78.12	19.53
Citrobacter freundii LMP802	78.12	39.06	39.06	39.06	>78.12	>78.12	9.76
Fungi							
Candida albicans LMP709U	39.06	19.53	19.53	19.53	>78.12	>78.12	19.53
Candida gabrata LMP0413U	39.06	39.06	39.06	78.12	>78.12	>78.12	19.53
Microsporum audouinii LMP725D	39.06	19.53	19.53	19.53	>78.12	>78.12	19.53
Trichophyton rubrum LMP0723D	19.53	9.76	9.76	19.53	>78.12	>78.12	9.76

(nd): Not determined as the sample was not active following the diffusion test.
[a] The test samples were a crude extract from the twigs of *D. turbinata* (DTT), **1**: 5-methoxy-3-[3-(ß-glucopyranosyloxy)-2-hydroxy-3-methylbutyl]psoralen; **2**: 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen; **3**: (2′S, 3′R)-3′-hydroxymarmesin; **4**: 4-hydroxy-3-methoxybenzaldehyde; **5**: 4-methoxyphenol; Reference antibiotics (Gentamycin for bacteria, Nystatin for fungi).

Kuete et al., 2008a,b).These compounds are more active than the two simple phenol derivatives (compounds **4** and **5**). No significant differences (Tables 1–3) are recorded between compounds **1** and **2** (an aglycon of **1**). This predicts that the antimicrobial activity of compound **1** is due to the aglycon part.

When comparing the powerful antimicrobial activity of the crude extract of *D. turbinata* and that of the tested compounds on the studied microbial species, it could be suspected that other compounds more active than compounds **1** to **5** can also be implicated in the observed activity. In fact, compounds such as kanzonol C (**7**) and 4-hydroxylonchocarpin (**8**) also isolated from this plant were reported for their powerful antimicrobial activity against fungi, and Gram-positive and negative bacteria (Mbaveng et al., 2008).

Finally, the inhibition potency of *D. turbinata* might be due to the presence of compounds with both antibacterial and antifungal activities. This study highlighted the antimicrobial potential of this plant and thereby confirming the activity of the genus *Dorstenia*. *D. turbinata* as well as other species of the genus *Dorstenia* are rich in secondary metabolites such as prenylated chalcones, flavonoids, coumarins, simple phenolics, known for their antimicrobial activity (Paterson and Tipman, 1962; Parrish et al., 1974; Cussans and Huckerby, 1975; Huang et al., 1997; Ngadjui et al., 1999, 2000; Franke et al., 2001; Abegaz et al., 2004; Ngameni et al., 2004, 2006; Kuete et al., 2008a,b; Mbaveng et al., 2008).

The overall results provide promising baseline information for the potential use of the crude extracts from DTT as well as some of the isolated compounds in the treatment of bacterial and fungal infections.

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Table 3
Minimum microbicidal concentration (μg/ml) of the crude extracts, compounds isolated from *D. turbinata* and reference antibiotics.

Microorganisms	MMC for test samples[a] (μg/ml)						
	DTT	1	2	3	4	**5**	RA
Bacteria							
Staphylococcus aureus LMP805	>78.12	nd	78.12	nd	–	–	19.53
Escherichia coli LMP701	39.06	>78.12	>78.12	>78.12	nd	>78.12	9.76
Shigella dysenteriae LMP606	78.12	78.12	78.12	78.12	–	nd	19.53
Klebsiella pneumoniae LMP803	>78.12	>78.12	>78.12	>78.12	–	–	78.12
Pseudomonas aeruginosa LMP804	>78.12	78.12	78.12	>78.12	–	–	19.53
Salmonella typhi LMP706	>78.12	78.12	78.12	78.12	nd	nd	39.06
Citrobacter freundii LMP802	>78.12	78.12	78.12	78.12	nd	nd	19.53
Fungi							
Candida albicans LMP709U	78.12	78.12	39.06	78.12	nd	nd	39.06
Candida gabrata LMP0413U	78.12	>78.12	78.12	>78.12	–	–	39.06
Microsporum audouinii LMP725D	78.12	39.06	39.06	78.12	–	–	39.06
Trichophyton rubrum LMP0723D	39.06	19.53	19.53	78.12	–	–	19.53

(–): Not tested as the MIC was not determined; (nd): not determined because the MMC >78.12 μg/ml.
[a] The tested samples were a crude extract from the twigs of *D. turbinata* (DTT), **1**: 5-methoxy-3-[3-(ß-glucopyranosyloxy)-2-hydroxy-3-methylbutyl] psoralen; **2**: 5-methoxy-3-(3-methyl-2,3-dihydroxybutyl)psoralen; **3**: (2′S, 3′R)-3′-hydroxymarmesin; **4**: 4-hydroxy-3-methoxybenzaldehyde; **5**: 4-methoxyphenol; Reference antibiotics (Gentamycin for bacteria, Nystatin for fungi).

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