Antimicrobial activity of the methanolic extracts and compounds from *Treculia africana* and *Treculia acuminata* (Moraceae)

V. Kuete [a,*], R. Metuno [b], B. Ngameni [b], A.T. Mbaveng [a], F. Ngandeu [c], M. Bezabih [d], F.-X. Etoa [a], B.T. Ngadjui [b], B.M. Abegaz [d], V.P. Beng [a]

[a] *Department of Biochemistry, Faculty of Science, University of Yaoundé I. PO Box 812 Yaoundé, Cameroon*
[b] *Department of Organic Chemistry, Faculty of Science, University of Yaoundé I. PO Box 812 Yaoundé, Cameroon*
[c] *Department of Organic Chemistry, Faculty of Science, University of Dschang, PO Box 67, Dschang, Cameroon*
[d] *Department of Chemistry, Faculty of Science, University of Botswana, Private Bag 00704, Gaberone, Botswana*

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Abstract

The crude methanolic extracts from *Treculia africana* and *Treculia acuminata*, three compounds isolated from *T. africana* and identified as, Phyllocoumarin (**1**), Catechin (**2**) and 6, 9-dihydroxy-megastigmane-3-one (**3**), four compounds namely **2**, **3**, 2,3-dihydroxypropyl-heptadecanoate (**4**), and Ferulic acid (**5**) isolated from *T. acuminata* were tested for their antimicrobial activity against Gram-positive bacteria (six species), Gram-negative bacteria (12 species) and three *Candida* species. The micro-dilution method for the determination of the minimal inhibition concentration (MIC) and the Minimal microbicidal concentration (MMC) was used. The MIC values obtained with the crude extracts varied from 78 to 156 μg/ml against 12 (57.14%) and 20 (95.24%) of the 21 tested microorganisms respectively for *T. acuminata* and *T. africana*. Apart from compound **2** that prevented the growth of all the tested microorganisms, other bioactive compounds showed selective activity. The obtained results provide promising baseline information for the potential use of these crude extracts as well as some of the isolated compounds in the treatment of bacterial and fungal infections.
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Keywords: Antimicrobial activity; Compounds; Moraceae; *Treculia acuminata*; *Treculia africana*

1. Introduction

Traditional healing plays an integral role in black African culture as it provides for the primary heath care needs for a large majority (about 80%) of the population (WHO, 2002). In Cameroon, there is a rich tradition in the use of herbal medicinal for the treatment of various infectious diseases, inflammations, injuries and other diseases (Adjanohoun et al., 1996). Our herbal medicine researches include plants of the Moraceae family. Within this family, the genus *Treculia* contains three species, which are traditionally used to treat skin diseases, dental allergy, amoebic dysentery and AIDS (Berg et al., 1985; Bokesch et al., 2004) namely *Treculia obovoidea* N.E. Brown, *Treculia africana* Decaisne and *Treculia acuminata* Baillon (Berg et al., 1985). These species are distributed in the humid regions of Africa, from Nigeria to Congo, including Cameroon. To the best of our knowledge, the antimicrobial activity of these two *Treculia* species as well as that of compounds such as **1**, **3** and **4** has not yet been reported. The aim of this investigation was to evaluate the antibacterial and anticandidal activities of the crude extracts and compounds isolated from *T. africana* and *T. acuminata*, two of the three species that comprises the genus *Treculia*.

2. Methodology

2.1. Plant material

The twigs of *T. acuminata* Baillon and the leaves of *T. africana* Decaisne were collected in August 2004 in Kumba,

* Corresponding author. Tel.: +237 7735 59 27, +237 7533 84 55, +237 7592 71 81; fax: +237 222 60 18.
E-mail address: kuetevictor@yahoo.fr (V. Kuete).

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South-West Province of Cameroon. The botanical identification of the plants was done at the Cameroon National Herbarium, where the voucher specimens were conserved under the reference numbers 29053/SRF/Cam and 2921/SRF/Cam respectively for *T. africana* and *T. acuminata*.

2.2. Purification and general procedures

2.2.1. *T. africana*

The air-dried and powdered leaves of *T. africana* (1 kg) were macerated in Methanol (5 L) at room temperature for 24 h. Solvents were removed under reduced pressure to give a dark green residue TAL (60 g). Part (55 g) of this extract was submitted to Vacuum Liquid Chromatography (VLC) on silica gel 60 (170 g) using petroleum ether followed by petroleum ether 40–60/ethyl acetate gradient, and finally with EtOAc–MeOH mixtures. A total of 40 fractions, 250 ml each, were collected and similar fractions were combined on the TLC basis. Fractions 1–11 (2 g) eluted with petroleum ether (40–60), contained mainly mixtures of oils and were not investigated further. Combined fractions 12–30 (35 g) eluted with 20%, 30% and 60% petroleum ether–EtOAc mixtures, respectively, were passed through a Sephadex LH-20 column and eluted with $CHCl_3$–MeOH (2:1) mixtures. The post-chlorophyll fractions were also combined and subjected, successively to silica gel CC and repeated preparative TLC (petroleum ether–$CHCl_3$ 2:8) to afford Phyllocoumarin (**1**) (20 mg; M_W: 342) (Foo, 1989); Catechin (**2**) (25 mg; M_W: 290) (Ting Ting and Meei-Yueh, 1993; Gonzalez et al., 1994; Siddiqui et al., 2003) and 6, 9-dihydroxy-megastigmane-3-one (**3**) (15 mg; M_W: 224) (Ting Ting and Meei-Yueh, 1993; Gonzalez et al., 1994; Siddiqui et al., 2003). The following fractions 31–36 (7 g) and 37–40 (3 g) eluted with EtOAc and EtOAc–MeOH 15%, respectively, gave after repeated CC on silica gel two compounds, (**1**) (40 mg) and (**2**) (30 mg).

2.2.2. *T. acuminata*

The air-dried and powdered twigs of *T. acuminata* (800 g) were macerated in Methanol (10 L) at room temperature for 24 h. The filtrate was concentrated under vacuum to give a dark green crude extract (TAT) (48 g).

A part of TAT (40 g) was subjected to vacuum liquid chromatography (VLC) on silica gel (35–70 μm, 250 g) and eluted with petroleum ether, petroleum ether/EtOAc mixtures and EtOAc to give 46 fractions of 250 ml each. Fractions 1–25 (18 g) were diluted in acetone and after 24 h a precipitate was formed. After filtration, Glycerol-1-hexadecanoate (**4**) (12 mg; M_W: 330) (Sultana et al., 1976) was obtained. The resulting mother liquid was subjected to column chromatography over silica gel, followed by preparative TLC (petroleum ether/$CHCl_3$, 2:8) to afford 8 mg of compounds **2** and a long chain ester of Ferulic acid (**5**), (19 mg; M_W: 558) (Wandji et al., 1990; Achenbach et al., 1985). Fractions 26–46 (15 g), were passed through column chromatography over silica gel and followed by preparative TLC to give 6,9-dihydroxymegastigmate-3-one (**3**) (15 mg; M_W: 224) (Ting Ting and Meei-Yueh, 1993; Gonzalez et al., 1994; Siddiqui et al., 2003).

2.2.3. General procedure

Aluminium sheet pre-coated with silica gel 60 F254 nm (Mereck) was used for thin layer chromatography and the isolated spots were visualized using both ultra-violet light (254 and 366 nm) and 50% H2SO4 spray reagent. Petroleum ether–$CHCl_3$ 2:8 mixtures were used for the preparative TLC. The chemical structure of each of the isolated compound was determined on the basis of spectral data produced by one and two-dimensional nuclear magnetic resonance (NMR), recorded on Brüker DRX-400 instrument. This spectrometer was equipped with 5-mm, 1H- and 13C-NMR probes operating at 400 and 100 MHz, with tretramethylsilane as internal standard. Mass spectra were recorded on a API QSTAR pulsar mass spectrometer. The structures of the compounds were confirmed by comparing with reference data from available literature.

2.3. Microbial strains

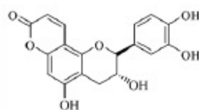
Twenty one species of microorganisms namely *Bacillus cereus* LMP0404G, *Bacillus megaterium* LMP0204G, *Bacillus stearothermophilus* LMP0104G, *Bacillus subtilis* LMP0304G, *Staphylococcus aureus* LMP0206U, *Streptococcus faecalis* LMP0207U (Gram positive bacteria), *Escherichia coli* LMP0101U, *Shigella dysenteriae* LMP0208U, *Proteus vulgaris* LMP0103U, *Proteus mirabilis* LMP0504G, *Shigella flexneri* LMP0313U, *Klebsiella pneumoniae* LMP0210U, *Pseudomonas aeruginosa* LMP0102U, *Salmonella typhi* LMP0209U, *Morganella morganii* LMP0904G, *Enterobacter aerogens* LMP 1004G, *Citrobacter freundii* LMP0904G, *Enterobacter cloacae* LMP1104G (Gram negative bacteria), *Candida albicans* LMP0204U, *Candida gabrata* LMP0413U and *Candida krusei* LMP0311U (yeasts) were used in this study. Three *Bacillus* species were provided by '*Institut Appert de Paris*', while *B. cereus* was provided by the A.F.R.C Reading Laboratory of Great Britain. Other strains were clinical isolates from '*Centre Pasteur du Cameroon*', Yaoundé. The microbial isolates were maintained on agar slant at 4 °C in the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP) (Faculty of Science, University of Yaoundé I) where the antimicrobial tests were performed. The strains were subcultured on a fresh appropriate agar plate 24 h prior to any antimicrobial test.

2.4. Antimicrobial assays

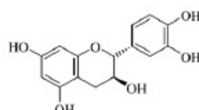
2.4.1. Culture media

Nutrient Agar (NA) containing Bromocresol purple was used for the activation of *Bacillus* species while NA was used for other bacteria. Sabouraud Glucose Agar was used for the activation of the fungi. Nutrient broth containing 0.05% phenol red and supplemented with 10% glucose (NBGP) was used for MIC and MMC (Minimal bactericidal concentration for bacteria and minimal candicidal concentration for *Candida* species) determinations. The Mueller Hinton Agar (MHA) was also used for the determination of the MMC.

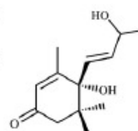
Flavonoids **Terpenoid**



1
Phyllocoumarin

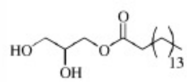


2
Cathechin

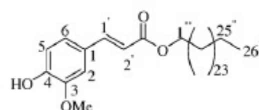


3
6,9-dihydroxy-megastigmane-3-one or Vimifoloil or blumerol

Esters



4
Glycerol-1-hexadecanoate or 2,3-hydroxypropylheptadecanoate



5
Ester of Ferulic acid

Fig. 1. Chemical structures of compounds isolated from the *T. africana* leaves (**1, 2, 3**) and *T acuminata* twigs (**2, 3, 4, 5**).

2.4.2. Chemicals

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

2.4.3. MIC and MMC determinations

MICs of test samples and RA were determined as follows: the test sample was first of all dissolved in Dimethylsulfoxide (DMSO). The solution obtained was added to NBGP to a final concentration of 156 µg/ml for each sample and RA. This was serially diluted two fold to obtain concentration ranges of 0.31 to 156 µg/ml. The RA solutions (in DMSO) were also prepared following the same concentration ranges as above. 100 µl of each concentration was added in a well (96-wells microplate) containing 95 µl of NBGP and 5 µl of inoculum (standardised at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm SHIMADZU UV-120-01 spectrophotometer) (Tereschuk et al., 1997). The final concentration of DMSO and Tween in the well was less than 1% (preliminary analyses with 1% (v/v) DMSO/NBGP and 10% v/v Tween 20/NBGP affected neither the growth of the test organisms nor the change of color due to this growth). The negative control well consisted of 195 µl of NBGP and 5 µl of the standard inoculum (Zgoda and Porter, 2001; Kuete et al., 2007). The plates were covered with a sterile plate sealer, then agitated to mix the content of the wells using a plate shaker and incubated at 37 °C for 24 h. The assay was repeated twice. Microbial growth was determined by observing the change of color in the wells (red when there is no growth and yellow when there is growth). The lowest concentration showing no color change was considered as the MIC (Delaras, 1998; Kuete et al., 2006, 2007). 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 37 °C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MMC (Delaras, 1998; Kuete et al., 2006, 2007).

3. Results and discussion

The structural identification of the isolated compounds **1**–**5** was done by comparing the spectroscopic and physical data with that of available literature and/ or authentic markers. The compounds isolated from *T. africana* (Fig. 1) were two flavonoids identified as Phyllocoumarin (**1**) and Catechin (**2**), and a unique ionone known as 6, 9-dihydroxy-megastigmane-3-one (**3**) while compounds **2**, **3** and two esters were isolated from *T. acuminata* namely 2,3-dihydroxypropylhexadecanoate (**4**), and aliphatic long chain ester of ferulic acid (**5**). Many compounds from secondary metabolites classes such as flavonoids and terpenoids have been reported from their antimicrobial activity (Cowan, 1999). The inhibition potential of compounds reported in this study is therefore in accordance with those studies. The antibacterial and anticandidal activities of the crude extracts and isolated compounds were evaluated and the results are reported in Tables 1 and 2. In general, there were differences in growth inhibition between compounds on various microbial cultures. The crude extracts from *T. africana*,

Table 1
Minimum inhibition concentrations (µg/ml) of the methanolic extracts, compounds isolated from *Treculia africana* leaves[a], *Treculia acuminata* twigs[b] and reference antibiotics[c]

Microorganisms	Tested samples							
	TAL	TAT	**1**	**2**	**3**	**4**	**5**	RA
Gram-negative bacteria								
Citrobacter freundii	78	–	39	10	–	–	–	5
Enterobacter aerogens	78	78	78	20	78	78	–	10
Enterobacter cloacae	156	78	78	39	39	78	–	5
Escherichia coli	78	156	78	2	78	–	–	1
Klebsiella pneumoniae	78	–	–	5	–	–	–	2
Morganella morganii	156	–	156	10	–	–	–	2
Proteus mirabilis	78	156	39	5	–	78	–	2
Proteus vulgaris	78	156	78	10	–	–	–	1
Pseudomonas aeruginosa	156	–	39	20	–	–	–	2
Shigella dysenteriae	78	–	78	5	–	–	–	2
Shigella flexneri	–	–	–	39	78	–	–	2
Salmonella typhi	156	78	156	78	39	–	–	1
Gram-positive bacteria								
Streptococcus faecalis	78	156	78	20	78	78	–	2
Staphylococcus aureus	156	–	–	10	–	–	–	5
Bacillus cereus	78	–	39	10	–	–	–	2
Bacillus megaterium	78	78	78	20	39	–	–	1
Bacillus stearothermophilus	156	–	78	20	–	–	–	5
Bacillus subtilis	78	156	78	20	78	–	–	2
Yeasts								
Candida albicans	78	156	78	5	39	–	–	2
Candida gabrata	156	156	156	20	39	–	–	2
Candida krusei	78	156	78	10	78	–	–	10

[a] Compounds isolated from the leaves of *Treculia africana* (TAL): Phyllocoumarin (**1**) and Cathechin (**2**) and 6, 9-dihydroxy-megastigmane-3-one (**3**).
[b] Compounds isolated from the twigs of *Treculia acuminata* (TAT): compound **2**; **3**; 2, 3-dihydroxypropyl heptadecanoate (**4**), and Ferulic acid (**5**).
[c] RA: Reference antibiotics (Gentamicin for bacteria, nystatin for yeast); (–): MIC>156 µg/ml.

and *T. acuminata*, compounds **1**, **2** and **3** showed both antibacterial and anticandidal activities at the tested MIC limit of 156 µg/ml (Table 1). The MIC values obtained with the crude extracts varied from 78 to 156 µg/ml on 12 (57.14%) and 20 (95.24%) of the 21 tested microorganisms respectively for TAT and TAL. Compound **2** isolated from TAL was active on all the tested microorganisms while compound **1** inhibited the growth of 18 (85.71%) of them. Compound **5** was not active at the tested MIC limit. Apart from compound **2**, no other compound isolated from the two crude extracts was able to prevent the growth of all Gram-negative cultures. Regarding the degree of activity of the isolated compounds, the lowest MIC value (2 µg/ml) was observed with compound **2** on *E. coli*. This compound appeared to be very active, with the MIC value of 5 µg/ml also observed on 4 of the tested microbial cultures namely *K. pneumoniae*, *P. mirabilis*, *S. dysenteriae* and *C. albicans*. The results of the MMC determinations (Table 2) indicated that the MMC values lower than 156 µg/ml were observed with crude extracts on 25% (3/12) and 65% (13/20) of the sensitive microbial species respectively for TAT and TAL. Within the tested interval (0.31–156 µg/ml), the MMC values were obtained with compounds on 0 to 100% of the tested microbial species.

When comparing the MIC interval of the antimicrobial activity of the tested samples to that of Gentamicin (1–10 µg/ml) and nystatin (2–10 µg/ml) used as reference antibiotics, the inhibition potency of tested compounds (except compound **5**) as well as that of the two crude extracts could mostly be considered as important. The results of the MMC determinations (Table 2) indicated that cidal effect of many of the tested sample could be expected. However, a keen look of the results of MIC (Table 1) and MMC (Table 2), showed that the MIC values obtained are 4 times lesser than the MMCs on corresponding (sensitive) microorganisms, confirming the microbicidal effects of the concerned samples (Carbonnelle et al., 1987).

The locally isolated microorganisms were mostly used in this study, in order to close up our experiment to the local condition of the use of *T. africana and T. acuminata*. To the best of our knowledge, the antibacterial and the anticandidal activities of the two extracts as well as that of compounds **1**, **3** and **4** is being reported for the first time. Nevertheless, this study supports the traditional use of plants of the genus *Treculia* in the treatment of

Table 2
Minimum microbicidal concentrations (µg/ml) of the methanolic extracts, compounds isolated from *Treculia africana* leaves[a], *Treculia acuminata* twigs[b] and reference antibiotics[c]

Microorganisms	Tested samples							
	TAL	TAT	**1**	**2**	**3**	**4**	**5**	RA
Gram-negative bacteria								
Citrobacter freundii	156	–	78	20	–	–	–	10
Enterobacter aerogens	156	156	156	39	156	156	–	20
Enterobacter cloacae	nd	nd	156	156	156	156	–	10
Escherichia coli	156	nd	nd	5	156	–	–	2
Klebsiella pneumoniae	156	–	–	10	–	–	–	5
Morganella morganii	nd	–	nd	20	–	–	–	5
Proteus mirabilis	156	nd	78	20	–	156	–	5
Proteus vulgaris	156	nd	156	20	–	–	–	2
Pseudomonas aeruginosa	nd	–	78	39		–	–	5
Shigella dysenteriae	156	–	156	10	–	–	–	5
Shigella flexneri	–	–	–	78	nd	–	–	5
Salmonella typhi	nd	156	nd	nd	156	–	–	2
Gram-positive bacteria								
Streptococcus faecalis	156	nd	156	39	156	nd	–	5
Staphylococcus aureus	nd	–	–	20	–	–	–	10
Bacillus cereus	156	–	156	39	–	–	–	5
Bacillus megaterium	156	156	156	39	78	–	–	2
Bacillus stearothermophilus	nd	–	156	78	–	–	–	10
Bacillus subtilis	156	nd	156	39	156	–	–	5
Yeasts								
Candida albicans	156	nd	156	20	78	–	–	5
Candida gabrata	nd	nd	nd	39	78	–	–	5
Candida krusei	156	nd	nd	20	156	–	–	20

[a] Compounds isolated from the leaves of *Treculia africana* (TAL): Phyllocoumarin (**1**) and Cathechin (**2**) and 6, 9-dihydroxy-megastigmane-3-one (**3**).
[b] Compounds isolated from the twigs of *Treculia acuminata* (TAT): compound **2**; **3**; 2, 3-dihydroxypropyl heptadecanoate (**4**), and Ferulic acid (**5**).
[c] RA: Reference antibiotics (Gentamicin for bacteria, nystatin for yeast); (-): Not tested because the MIC was not determined; (nd): not determined because MMC>156 µg/ml.

infectious illness such as skin diseases, dysentery and AIDS (Berg et al., 1985; Bokesch et al., 2004). The flavonoids isolated from the crude extracts exhibited a very good antibacterial and anticandidal activities. However, the antimicrobial activity of Catechin against bacteria species and fungi has previously been reported by Bruneton (1999), and this is confirmed by the present study. The activity of flavonoids tested in this study (**1** and **2**) seems to be related to their degree of hydroxylation. From the results of Table 1, it can be observed that Catechin with 5 hydroxy (–OH) groups prevents the growth of 100% of the tested microorganisms. However, several authors have previously confirmed this hypothesis (Sato et al., 1996; Cowan, 1999).

The known antimicrobial mechanisms associated to each class of chemical to which the isolated compounds belong, may explain the antimicrobial potency of the crude extracts and compounds from *T. africana* and *T. acuminata*. The activity of flavonoids such as compounds **1** and **2** might be due to their ability to complex with bacterial cell wall (Cowan, 1999) and therefore, inhibiting the microbial growth. Finally, the antimicrobial activity of the two extract from *Treculia* species may be due to the presence of both antifungal and antibacterial compounds. The present study provides an important basis for the use of extracts from these plants for the treatment of infections associated to the studied microorganisms. The crude extracts as well as the isolated compounds found active could be useful for the development of new antimicrobial drug. However, pharmacological and toxicity studies currently going on in our laboratory will be necessary to confirm this hypothesis.

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