Department of Biochemistry[1], Department of Organic Chemistry[2], Yaounde University I, Yaounde, Cameroon, Department of Chemistry[3], University of Paderborn, Germany

Antimicrobial activity of the methanolic extract and of the chemical constituents isolated from *Newbouldia laevis*

V. Kuete[1], K. O. Eyong[2], G. N. Folefoc[2], V. P. Beng[1], H. Hussain[3], K. Krohn[3], A. E. Nkengfack[2]

Received November 3, 2006, accepted November 30, 2006

Dr Victor Kuete, Department of Biochemistry, Yaounde University I, P.O. Box 812, Yaounde, Cameroon
kuetevictor@yahoo.fr

Pharmazie 62: 552–556 (2007) *doi: 10.1691/ph.2007.7.6757*

The methanolic extract (NLB) and ten compounds isolated from the root bark of *Newbouldia laevis* Seem, namely chrysoeriol (**1**), newbouldiaquinone (**2**), 2-acetylfuro-1,4-naphthoquinone (**3**), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (**4**), lapachol (**5**), β-sitosterol-3-*O*-β-D-glucopyranoside (**6**), oleanolic acid (**7**), canthic acid (**8**) newbouldiamide (**9**) and 2-(4-hydroxyphenyl)-ethyltrioctanoate (**10**), were tested for *in vitro* antimicrobial activity. Twenty one microorganisms belonging to six Gram-positive and twelve Gram-negative bacterial species as well as three yeasts from *Candida* species were tested for their susceptibility to NLB and the pure isolated compounds based on the Agar Hole Diffusion test and the Liquid Dilution method. The Hole Diffusion assay indicated that NLB and compound **7** were active against all tested pathogens while other compounds showed selective activity with the antimicrobial spectra varying from 76% (compound **10**) to 95 % (compound **6**). Minimal inhibitory concentrations (MIC) also illustrated the important antimicrobial activity of NLB and of the isolated compounds. MIC values obtained varied from 9.76 to 312.50 μg/ml for NLB, and 0.038 to 9.76 μg/ml for pure compounds against most of the tested microorganisms. The antimicrobial activities of compounds **2**, **4** and **9** are described here extensively for the first time. The results indicate a promising basis for the use of *Newbouldia laevis* and some of its active principles in the treatment of infectious diseases.

1. Introduction

Newbouldia laevis is widely used in the African folk medicine for the treatment of several illnesses and diseases as an astringent in diarrhoea, dysentery and in the treatment of various diseases such as worms, malaria, sexually transmitted diseases, and in the reduction of dental caries (Eyong et al. 2005). A number of authors have reported the antimicrobial activities of compounds from the roots of this plant such as phenyl propanoid glycosides, naphthoquinone and alkaloids (Ahmad et al. 1986; Adesanya et al. 1994; Houghton et al. 1994; Gafner et al. 1997; Aladesanmi et al. 1998; Okeke et al. 2002). In a preliminary study of biological activity we showed that newbouldiaquinone (**2**) was moderately antibacterial against Gram-positive *Bacillus megaterium* (Eyong et al. 2005).

Recently, we reported Newbouldiaquinone A, a new naphthoquinone-anthraquinone from the root bark of this plant, which was found to be active against Gram-positive, Gram-negative bacteria and yeasts (Eyong et al. 2006). In this study, we have evaluated the inhibitory potency of the methanolic extract (NLB) of the root bark and of ten pure compounds isolated from NLB. Among the pure compounds were one flavonoid (**1**), four quinones (**2** to **5**), one sterol (**6**), two triterpenes (**7** and **8**), one ceramide (**9**), and one phenolic derivative (**10**).

2. Investigations, results and discussion

The results of the Hole Diffusion test (Table 1) show that NLB prevents the growth of all tested pathogens including Gram-positive, Gram-negative bacteria and yeasts. This can justify the extensive use of the plant in African folk medicine to treat a spectrum of infectious diseases. This study also shows that active compounds from this extract belong to several groups of secondary metabolites such as flavonoids [chrysoeriol (**1**)], quinones [newbouldiaquinone (**2**), 2-acetylfuro-1,4-naphathoquinone (**3**), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (**4**) and lapachol (**5**)], sterols [β-sitosterol-3-*O*-β-D-glucopyranoside (**6**)], triterpenes [oleanolic acid (**7**) and canthic acid (**8**)], ceramide [newbouldiamide (**9**)] and one phenolic derivative [2-(4-hydroxyphenyl)-ethyltriacontanoate (**10**)].

The results in Table 1 also show an important and selective inhibition potency of most of the tested samples: NLB and compound **7** were active against all of the tested pathogens, while compound **8** was active against 95%, compounds **1** and **5** against 81%, and compounds **6**, **9** and **10** against 76% of the test organisms. Apart from compound **7**, no other compound was able to inhibit the growth of all Gram-negative bacteria. Otherwise, compounds **3**, **7**, **8** and **9** were active against 6 out of 6 tests against Gram-positive bacterial species. Seven out of ten test compounds, namely compounds **1**, **2**, **4**, **6**, **7**, **8** and **9**, inhibited the entire set of candidal cultures.

Table 1: Microbial susceptibility[a] to the methanolic extract (NLB) and pure compounds from *Newbouldia laevis* and reference antibiotics

Microbial strains	Isolated compounds											
	NLB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	RA[c]
Gram-negative bacteria												
Citrobacter freundii	++	+	++	−	++	+	−	++	++	−	−	++
Enterobacter aerogenes	++	−	+	−	−	+	++	+	−	−	−	++
Enterobacter cloacae	++	+	−	+	++	+	+	++	++	+	++	++
Escherichia coli	++	+	+	++	++	++	−	++	++	++	+	++
Klebsiella pneumoniae	++	++	−	++	−	++	+	++	++	−	−	++
Morganella morganii	++	+	++	++	+	+	++	++	++	+	++	++
Proteus mirabilis	++	+	++	+	++	++	++	++	++	++	++	++
Proteus vulgaris	++	++	−	++	+	++	+	+	++	+	+	++
Pseudomonas aeruginosa	++	+	−	+	++	+	+	++	++	+	++	++
Shigella dysenteriae	++	−	−	++	−	−	−	++	+	−	−	++
Shigella flexneri	++	++	−	+	+	++	++	+	+	++	+	++
Salmonella typhi	++	+	−	+	+	++	+	+	+	+	+	++
Gram-positive bacteria												
Streptococcus faecalis	++	++	++	+	+	++	++	+	+	++	+	++
Staphylococcus aureus	+	+	−	+	+	+	−	+	+	+	+	++
Bacillus cereus	++	−	+	++	++	−	+	++	++	++	++	++
Bacillus megaterium	++	−	+	++	−	+	−	+	++	+	+	++
Bacillus stearothermophilus	++	+	+	++	+	+	+	++	++	+	−	++
Bacillus subtilis	++	++	++	++	++	++	+	++	++	+	+	++
Yeasts												
Candida albicans	++	+	++	−	++	+	+	+	+	−	+	++
Candida krusei	++	+	+	+	++	−	+	++	++	+	++	++
Candida glabrata	++	++	+	++	++	−	+	++	++	++	++	++

[a] Results recorded as mean IZ of two experiments and expressed as signs: (−) for not active samples, (+) for samples with 6 mm < IZ < 10 mm, (++) for samples with IZ ≥10 mm; [b] Tested samples (Sample tested at 3 mg/ml for NLB (crude extract), pure compounds and RA, No effect of DMSO used as dilution solvent was observed on the tested microbial strains; [c] RA: Reference antibiotics (Gentamycin for bacteria, Nystatin for yeast)

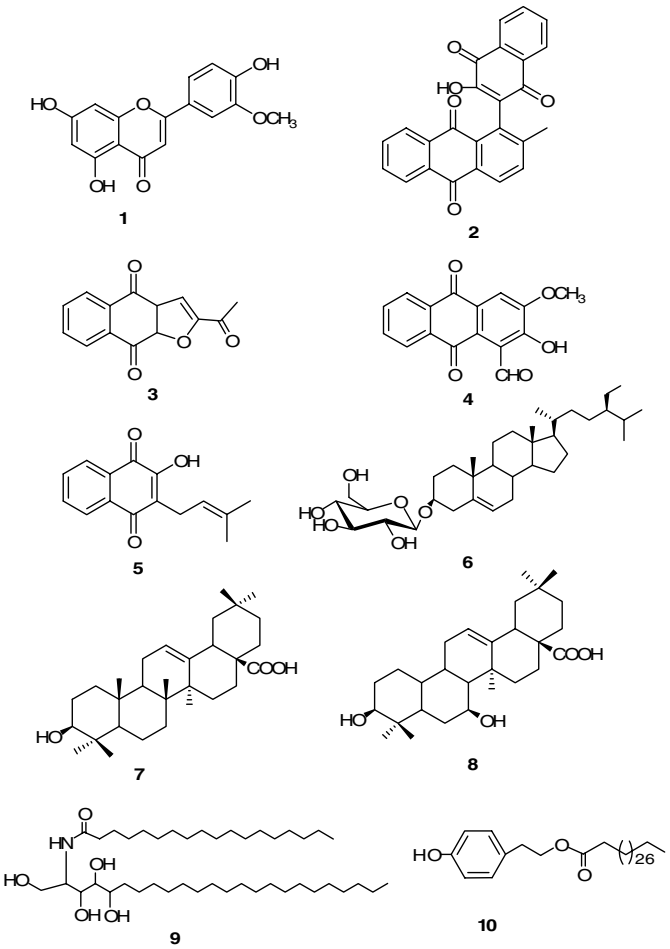

Table 2: Minimal inhibitory concentration (μg/ml) for the methanolic extract (NLB) and pure compounds from *Newbouldia laevis* Seem and reference antibiotics

Microbial strains	Isolated compounds											
	NLB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*RA
Gram-negative bacteria												
Citrobacter freundii	78.12	9.76	2.44	–	9.76	9.76	–	1.22	0.61	–	–	4.88
Enterobacter aerogens	39.06	–	4.88	–	–	9.76	0.61	9.76	–	–	–	9.76
Enterobacter cloacae	78.12	>9.76	–	9.76	1.22	9.76	9.76	1.22	2.44	9.76	0.61	4.88
Escherichia coli	78.12	4.88	4.88	1.22	2.44	2.44	–	1.22	0.15	1.22	9.76	1.22
Klebsiella pneumoniae	156.25	2.44	–	2.44	–	2.44	9.76	1.22	1.22	–	–	2.44
Morganella morganii	78.12	9.76	4.88	2.44	9.76	9.76	1.22	1.22	1.22	9.76	2.44	2.44
Proteus mirabilis	78.12	9.76	4.88	9.76	0.31	9.76	1.22	0.31	1.22	0.61	1.22	2.44
Proteus vulgaris	156.25	1.22	–	0.61	9.76	2.44	>9.76	>9.76	0.31	9.76	9.76	1.22
Pseudomonas aeruginosa	78.12	9.76	–	9.76	0.61	9.76	9.76	1.22	2.44	9.76	0.31	4.88
Shigella dysenteriae	78.12	–	–	2.44	–	–	–	4.88	>9.76	–	–	2.44
Shigella flexneri	78.12	1.22	–	9.76	4.88	2.44	4.88	4.88	>9.76	4.88	9.76	2.44
Salmonella typhi	78.12	>9.76	–	9.76	>9.76	4.88	9.76	9.76	9.76	9.76	9.76	2.44
Gram-positive bacteria												
Streptococcus faecalis	78.12	4.88	0.31	9.76	9.76	2.44	0.31	9.76	9.76	2.44	9.76	4.88
Staphylococcus aureus	312.50	9.76	–	9.76	>9.76	9.76	–	>9.76	>9.76	9.76	9.76	4.88
Bacillus cereus	9.76	–	9.76	0.15	1.22	–	9.76	1.22	0.038	1.22	2.44	2.44
Bacillus megaterium	9.76	–	4.88	0.15	–	9.76	–	4.88	0.076	9.76	9.76	4.88
Bacillus stearothermophilus	9.76	9.76	9.76	0.076	9.76	9.76	9.76	2.44	0.31	9.76	–	4.88
Bacillus subtilis	9.76	2.44	2.44	0.038	1.22	2.44	9.76	0.31	0.038	9.76	9.76	2.44
Yeasts												
Candida albicans	156.25	9.76	4.88	–	2.44	–	9.76	9.76	9.76	–	9.76	4.88
Candida krusei	156.25	9.76	4.88	9.76	2.44	–	9.76	1.22	1.22	9.76	4.88	4.88
Candida glabrata	156.25	2.44	9.76	0.31	0.31	–	9.76	0.076	1.22	1.22	0.31	9.76

* RA: Reference antibiotics (gentamycin for bacteria, nystatin for yeasts); (–): Not tested, because the sample was not active following diffusion assay

MIC values varied from 9.76 to 312.50 μg/ml for NLB and from 0.038 to a value up to 9.76 μg/ml for pure compounds. The MIC values of reference antibiotics (RA, gentamycin and nystatin) varied from 1.22 to 9.76 μg/ml (Table 2). The lowest MIC value (9.76 μg/ml) from the crude extract was obtained against *Bacillus* strains. The lowest MIC values of pure compounds (0.038 μg/ml) were observed with compound **8** on *Bacillus subtilis* and *Bacillus cereus*. MIC values lower than 9.76 μg/ml were observed on 19 out of 21 tested pathogens for compound **7**, 18 out of 21 (compounds **3** and **8**), 16 out of 21 (compounds **5**, **9** and **10**), 15 out of 21 (compounds **1**, **4** and **6**), and 13 out of 21 (compound **2**). However, MIC values lower than or equal to that of the RA were obtained with each of the purified compounds on at least one of the pathogens. Also, many of these compounds exhibited both antibacterial and anticandicidal activity. The antibacterial and antifungal activities of naphthoquinones from *Newbouldia laevis* such as lapachone derivatives have been reported against a limited number of strains (Gafner et al. 1996, 1997) and the antimicrobial potency of compound **5** is in accordance with the obtained results. In our previous study (Eyong et al. 2005), newbouldiaquinone (**2**) was found to be antibacterially active against Gram-positive *Bacillus megaterium*. Similarly, newbouldiaquinone A was found to be active against Gram-positive, Gram-negative bacteria and yeasts (Eyong et al. 2006).

As for the results in Table 3, it was observed that MMC values for NLB ranged from 19.53 to 625 μg/ml for 20 out of 21 pathogens, and from 0.076 to 9.76 μg/ml for compounds on most of the test microorganisms. In this interval, the MIC values are generally less than four fold smaller than MMCs. This suggests that the microbicidal effect of test compounds against most of the sensitive pathogens could be expected (Carbonnelle et al. 1987; Mims et al. 1993).

A number of mechanisms associated with compounds from each chemical group can explain the powerful potential of *N. laevis.* The microbicidal effect observed with these compounds suggests membrane disruption as one of the likely mechanism of action (Arvind et al. 2004). This action can be attributed to oleanolic (**7**) and canthic (**8**) acids (triterpenes) and β-sitosterol-3-*O*-β-D-glucopyranoside (**6**) (sterol) (Cowan 1999). Also, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to the inactivation of proteins and loss of function (Stern et al. 1996). This mechanism of action can, therefore, be attributed to newbouldiaquinone (**2**), 2-acetylfuro-1,4-naphathoquinone (**3**), 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde (**4**) and lapachol (**5**), and other antimicrobial quinones previously isolated from this plant (Eyong et al. 2006). Many studies have documented the effectiveness of flavonoids against a wide range of Gram-positive bacteria as well as fungi (Cowan 1999). The antimicrobial activity noted in this study corroborates those findings. Flavonoids are known to complex with cell wall components and adhesins to prevent the microbial growth (Rojas et al. 1992; Perrett et al. 1995) and this is possibly the mechanism by which chrysoeriol (**1**) exhibits its harmful effects.

The present results can be considered very promising, due to the fact that all test pathogens were selected as multiresistant strains and that they are medically very important. *Bacillus* species especially *B. cereus* are agents of food poisoning (Sleigh and Tiburury 1998; Avril 1997). *Salmonella typhimurium* is etiologically the most important agent of food toxi-infections (Avril et al. 2000). *Candida albicans* and other *Candida* species, the agents of candidiasis, are increasingly important diseases distributed worldwide, due to the fact that they are frequent opportunistic pathogens in AIDS patients (Avril et al. 2000). The

Table 3: Minimal microbicidal concentration (μg/ml) for the methanolic extract (NLB) and pure compounds from *Newbouldia leavis* Seem and reference antibiotics

Microbial strains	Isolated compounds											
	NLB	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	*RA
Gram-negative bacteria												
Citrobacter freundii	312.50	>9.76	4.88	–	>9.76	>9.76	–	2.44	1.22	–	–	9.76
Enterobacter aerogenes	156.25	–	9.76	–	–	>9.76	1.22	>9.76	–	–	–	>9.76
Enterobacter cloacae	156.25	ND	–	>9.76	2.44	>9.76	>9.76	2.44	4.88	>9.76	1.22	9.76
Escherichia coli	156.25	9.76	9.76	2.44	4.88	4.88	–	2.44	0.31	2.44	>9.76	2.44
Klebsiella pneumoniae	312.50	4.88	–	9.76	–	9.76	>9.76	2.44	4.88	–	–	4.88
Morganella morganii	156.25	9.76	9.76	4.88	>9.76	9.76	2.44	2.44	2.44	>9.76	4.88	4.88
Proteus mirabilis	156.25	>9.76	9.76	>9.76	0.61	>9.76	2.44	0.61	2.44	1.22	2.44	4.88
Proteus vulgaris	312.50	2.44	–	1.22	>9.76	4.88	ND	ND	0.61	>9.76	>9.76	2.44
Pseudomonas aeruginosa	156.25	>9.76	–	>9.76	1.22	>9.76	>9.76	2.44	4.88	>9.76	0.61	9.76
Shigella dysenteriae	156.25	–	–	4.88	–	–	–	9.76	ND	–	–	4.88
Shigella flexneri	156.25	2.44	–	>9.76	9.76	4.88	9.76	9.76	ND	9.76	9.76	4.88
Salmonella typhi	156.25	ND	–	>9.76	ND	9.76	>9.76	>9.76	>9.76	>9.76	>9.76	4.88
Gram-positive bacteria												
Streptococcus faecalis	156.25	9.76	0.61	>9.76	>9.76	4.88	0.61	>9.76	>9.76	4.88	>9.76	9.76
Staphylococcus aureus	>625	9.76	–	9.76	ND	>9.76	–	ND	ND	>9.76	9.76	9.76
Bacillus cereus	39.06	–	>9.76	0.31	2.44	–	>9.76	2.44	0.076	2.44	4.88	4.88
Bacillus megaterium	19.53	–	9.76	0.31	–	>9.76	–	9.76	0.15	>9.76	>9.76	9.76
Bacillus stearothermophilus	19.53	>9.76	>9.76	0.15	>9.76	>9.76	>9.76	4.88	0.61	9.76	–	9.76
Bacillus subtilis	78.12	4.88	4.88	0.076	2.44	4.88	>9.76	0.61	0.076	>9.76	>9.76	4.88
Yeasts												
Candida albicans	625	>9.76	9.76	–	4.88	–	9.76	>9.76	>9.76	–	>9.76	9.76
Candida krusei	312.50	>9.76	9.76	>9.76	4.88	–	9.76	2.44	2.44	>9.76	9.76	9.76
Candida glabrata	312.50	4.88	>9.76	0.61	0.61	–	>9.76	0.15	2.44	2.44	0.61	>9.76

* RA: Reference antibiotics (gentamycin for bacteria, nystatin for yeast); NT: Not tested (sample not active following hole diffusion assay); ND: Not determined (sample with MIC greater than 9.76 μg/ml).
(−): Not tested (compounds not active following hole diffusion assay)

incidence of the typhoid fever caused by *S. typhimurium* is increasing in developing countries nowadays.

The results of the present research work indicate a possible basis for the use of the methanolic extract from the root bark of *N. laevis* in the treatment of infections associated with the microorganisms used in this study. Isolated compounds, including canthic acid (**8**) could likely be used but this must be confirmed by further pharmacological studies currently going on in our laboratory.

3. Eperimental

3.1. Plant and extracts

The root bark of *N. laevis* Seem (Bignoniaceae) was collected at Mamfe, South West province of the Republic of Cameroon in December 2004, earlier identified by Mr. Ndive Elias (Plant taxonomist), Botanical Garden, Limbe Cameroon. A voucher specimen (No.1754/SRFK) has been deposited at the National Herbarium, Yaounde, Cameroon.

All compounds examined in this report were isolated from the methanolic extract of *Newbouldia laevis* (NLB) (Eyong et al. 2006) and compound **4** was previously isolated from *Tithonia diversifolia* (Bouberte et al. 2006). The purity and identity of samples were determined by chromatographic and spectrometric techniques. Gentamycin and nystatin were commercial samples available in the Laboratory of Applied Microbiology and Molecular Pharmacology (LMP), Faculty of Science, University of Yaoundé I, Cameroon.

3.2. Microbial strains

A total of 21 microbial cultures belonging to *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 krusei* LMP0311U and *Candida glabrata* LMP0413 (yeasts) were used in this study. Three *Bacillus* species were provided by 'l'Iinstitut Appert' in Paris (France), *Bacillus cereus* by the A.F.R.C. Reading Laboratory in Great Britain. Other strains were clinical isolates from patients in the 'Centre Pasteur du Cameroon'-Yaoundé. They were then maintained on agar slant at 4 °C in the LMP where the antimicrobial tests were performed.

3.3. Antimicrobial assays

3.3.1. Culture media

The strains were activated at 37 °C, 24 h prior to any antimicrobial test on the appropriate medium (Nutrient Agar (NA) containing bromocresol purple for *Bacillus* species, NA for other bacteria and Sabouraud glucose agar for fungi). The Nutrient broth supplemented with 10% glucose containing 0.01% phenol red as colour indicator (NBGP) was used for the determination of the Minimum Inhibition Concentration (MIC) and the Minimum Microbicidal Concentration (MMC). The Mueller Hinton Agar (MHA) was used in the diffusion test.

3.3.2. Diffusion test: determination of the inhibition zones

The inhibition zones were determined by hole diffusion as described by Berghe and Vlietinck (1991) using a cell suspension of about 1.5×10^6 CFU/ml obtained from a McFarland turbidity standard N° 0.5. The suspension was standardised by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer) (Tereschuck et al. 1997). A hole of 6 mm diameter was then made on the MHA plate (8 mm thick), inoculated by flooding and filled with 100 μl of NLB, compounds or RA prior diluted at 3 mg/ml in dimethylsulfoxide (DMSO). The inoculated plates were incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of growth inhibition (IZ) around the hole. The assay was repeated twice, and the results recorded as mean IZ were expressed as signs: (−) for not active samples, (+) for samples with 6 mm $<$ IZ $<$ 10 mm, (++) for samples with IZ $\geq$10 mm.

3.3.3. Microdilution method: MIC and MMC determinations

MICs of samples that were determined as active by diffusion test were evaluated against the pathogens. The inocula of microorganisms were prepared from 12 h broth culture and the suspensions were adjusted to

0.5 McFarland turbidity. The tested samples were first dissolved in DMSO, then in nutrient broth (NB) to the highest dilution of 625 µg/ml for NLB, 9.76 µg/ml for pure compounds and RA. Serial two-fold dilutions were then made in a concentration range from 1.22 to 625 µg/ml for NLB, 0.019 to 9.76 µg/ml for pure compounds and RA in the 96 wells microplate. The final concentration of DMSO was less than 1% v/v (preliminary analyses with 1% (v/v) DMSO/NBGP affected neither the growth of the test organisms nor the change of colour due to this growth). The negative control well was made of 195 µl of NBGP and 5 µl of standard inoculum (Zgoda and Porter 2001). The plates were covered with a sterile plate sealer, then shaken to mix the content of the wells using a plate shaker and incubated at appropriate temperature for 24 h. Microbial growth was determined by observing the change of colour in the wells (red to yellow when there is microbial growth). The lowest concentration that showed no change of colour was considered as the MIC.

For the determination of MMC, a portion of liquid (5 µl) from each well that showed no change of colour was plated on MHA and incubated accordingly for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MMC.

For MMC determination, the content of each tested tube showing no growth was used to inoculate by inoculating fresh antimicrobial free MHA. This was further incubated for 18 to 24 h at 37 °C, after which the lowest dilution that yielded no growth was considered as the MMC (Mims et al. 1993).

Acknowledgement: Authors acknowledge DAAD-Germany for financial support to K. O. Eyong the technical support of the Institut Appert (Paris, France), the A.F.R.C Reading Laboratory (Great Britain), the Centre Pasteur du Cameroun, the National Herbarium of Cameroon and Mr Oscar Betek for harvesting *N. laevis* in Mamfe.

References

Avril JL (1997) Nouveau Dictionnaire de Bactériologie Clinique. Ellipse, Paris, 154p.

Avril JL, Dabernat H, Denis F, Monteil H (2000) Bactérilogie Clinique. 3e ed. Ellipse, Paris. Pp 321–329.

Adesanya SA, Nia R, Fontaine C, Pais M (1994) Pyrazole alkaloids from *Newbouldia laevis*. Phytochemistry 35: 1053–1055.

Aladesanmi AJ, Nia R, Nahrstedt A (1998) New pyrazole alkaloids from the root bark of *Newbouldia laevis*. Planta Med 64: 90–91.

Ahmad A, Khan HA, Ahmad YU, Qasi S (1986) Antibacterial activity of juliflorine isolated from *Proscopis juliflora*. Planta Med 4: 285–288.

Arvind S, Reg FC, Enzo AP (2004) Identification of antimicrobial component of an ethanolic extract of the Australian medicinal plant, *Eremophila duttonii*. Phytother Res 18: 615–618.

Berghe VA, Vlietinck AJ (1991) Screening methods for antibacterial and antiviral agents from higher plants. Meth Plant Biochem 6: 47–68.

Bouberte MY, Krohn K, Hussain H, Dongo E, Schulz B, Hu Q (2006) Tithoniaquinone A and tithoniamide B: A new anthraquinone and a new ceramide from the leaves of *Tithonia diversifolia*. Z. Naturforsch 61b: 78–82.

Carbonnelle B, Denis F, Marmonier A, Pinon G, Vague R (1987) Bactériologie médicale: Techniques usuelles. Ed SIMEP, Paris, pp 228–282.

Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564–582.

Eyong OK, Krohn K, Hussain H, Folefoc NG, Nkengfack AE, Schulz B, Hu Q (2005) Newboudiaquinone and newboudiamide: A naphthoquinone-anthraquinone couple pigment and a new ceramide from *Newboudia laevis*. Chem Pharm Bull 53: 616–619.

Eyong OK, Folefoc NG, Kuete V, Beng, VP, Hussain H, Krohn K, Nkengfack AE, Ramadan SSS, Hoerauf A (2006) Newbouldiaquinone A: a naphthoquinone-anthraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *Newbouldia laevis*. Phytochemistry 67: 605–609.

Gafner S, Wolfender JL, Nianga M, Stoeckli-Evans H, Hostettmann K (1996) Antifungal and antibacterial naphthoquinones from *Newbouldia laevis* roots. Phytochemisty 45: 1315–1320.

Gafner S, Wolfender JL, Nianga M, Hostettmann K (1997) Phenyl propanoid glycosides from *Newbouldia laevis* roots. Phytochemistry 44: 687–690.

Houghton PJ, Pandey R, Hawkes JE, (1994) Naphthoquinones and an alkaloid from the roots of *Newbouldia laevis*. Phytochemistry 35: 1602–1603.

Mims CA, Playfair JHL, Roitt IM, Wakelin D, Williams R (1993) Antimicrobials and Chemotherapy. In: Mims et al. (Eds), Medical Microbiology Review 35: 1–34.

Okeke AS, Okeke MI, Iroegbu CU, Ebo PU (2002) Antibacterial activity of *Haranguna madagascariensis* leaf extracts. Phytother Res 16: 174–179.

Perrett S, Whitfield PJ, Sanderson L, Bartlrtt A (1995) The plant molluscide *Millettia rhortningii* (Leguminosae) as a topical antischistosomal agent. J Ethnopharmacol 47: 49–54.

Rojas A, Hernandez L, Perreda-Mirranda R, Matg R (1992) Screening for antimicrobial activity of crude drug extract and pure natural products from Mexican medicinal plants. J Ethnopharmacol 35: 275–283.

Sleigh DJ, Timbury MC (1998) Note on medical Bacteriology. 5th edition Churchill Livingstone, New York, p 482.

Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996) Phorotannin-protein interactions. J Chem Ecol 22: 1887–1899.

Tereschuck ML, Riera MVQ, Castro GR, Abdala LR (1997) Antimicrobial activity of flavonoid from leaves of *Tagetes minuta.* J Ethnopharm 56: 227–232.

Zgoda JR, Porter JR (2001) A convenient microdilution method screening natural products against bacteria and fungi. Pharm Biol 39: 221–225.