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South African Journal of Botany 74 (2008) 572 – 576

SOUTH AFRICAN JOURNAL OF BOTANY

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Antimicrobial activity of the methanolic extract and compounds from *Teclea afzelii* (Rutaceae)

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Received 30 July 2007; received in revised form 7 February 2008; accepted 7 February 2008

Abstract

The methanolic extract (TAE) together with three alkaloids identified as Kokusaginine (***1***), Maculine (***2***), Kolbisine (***4***) and a common terpenoid, Lupeol (***3***), isolated from the stem bark of *Teclea afzelii* were tested for their antimicrobial activity against Gram-positive and negative bacteria, fungi and *Mycobacterium smegmatis*. Agar diffusion assay was used for the determination of the sensitivity of test organisms to the samples. The micro-dilution method to determine the Minimal Inhibition Concentration (MIC) and the Minimal Microbicidal Concentration (MMC). The results of the diffusion test showed that only compound ***1*** was active on all the tested micro-organisms, whilst the inhibition effect of the crude extract and that of compounds ***2*** and ***4*** was observed on 87.5% of the tested microbial species. The lowest MIC value (19.53 μg/ml) for the crude extract was obtained on *Escherichia coli*, *Bacillus subtilis* and *Microsporum audorium*. The corresponding value for the tested compounds (2.44 μg/ml) was recorded with compound ***2*** on *B. subtilis*. The crude extract, compounds ***2*** and ***3*** showed moderate activity against *M. smegmatis*. The overall results provide promising basis for the use of the crude extract as well as the isolated alkaloids in the treatment of specific microbial infections.

Keywords: Alkaloids; Antimicrobial activity; Kokusaginine; Kolbisine; Maculine; *Teclea afzelii*

1. Introduction

Teclea afzelii Engl. (Rutaceae) is a shrub of about 5 m height that grows in the humid rain forest of tropical Africa, from Sierra Leone to Cameroon. It forms a part of the sub-family Toddaliodeae, which, as present in Africa, consist of 50 to 100 species distributed between a number of closely related and taxonomically difficult genera (Khalid and Waterman, 1981). This plant is used in the Cameroonian folk medicine in the treatment of wounds infections, abdominal pains, cough, fever and asthma (Adnan et al., 2003; Personal communication). From the available literature, no scientific report has yet been focussed on the antimicrobial activity of plant from the genus *Teclea*, though many phytochemical investigations on this genus resulted in the isolation of alkaloids and terpenoids, two known classes of bioactive metabolites. Some of the compounds isolated from this genus include skimmianine, flindersiamine, tecleaverdoornine, tecleaverdine, tecleine, montrifoline, nkolbisine, tecleamine, flindersiamine, tecleanatalensine A, tecleanatalensine B (alkaloids) and lupeol (terpenoid) (Tarus et al., 2005; Ayafor et al., 1982). Our continuing search of antimicrobial drugs from natural products includes plants of the family

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doi:10.1016/j.sajb.2008.02.004

Rutaceae and the genus *Teclea*. The present study was therefore undertaken to assess the antibacterial and antifungal activities of the methanolic extract from the stem bark of *T. afzelii*.

2. Materials and methods

2.1. Plant material and extraction

The stem bark of *T. afzelii* were collected in December 2005 at Elounden (Centre province of Cameroon). The botanical identification of the plant was done at the Cameroon National Herbarium, where the voucher specimen was conserved under the reference number 10674/SRF/Cam.

2.2. Purification and general procedures

The sun-dried and powdered stem bark (2.5 kg) of *T. afzelii* were macerated in Methanol (8 L) at room temperature for 48 h. The filtrate was then concentrated under vacuum to give a dark brown crude extract (TAE) (450 g).

One hundred and fifty grams (150 g) of TAE were subjected to column chromatography over silica gel 60 (180 g) and eluted with hexane-ethyl acetate (hexane-EtOAc) gradient. One hundred and twenty sub-fractions of 250 ml each were collected and pooled following analytic TLC. After 2 h, fraction 14 yielded Kokusaginine $C_{14}H_{13}O_4N$ (***1***) (20.5 mg; white powder, M_W: 259; m.p: 168–170 °C) (Briggs and Cambie, 1958; Mester and Inescu, 1971; Oliveira et al., 1996) as a precipitate. Fraction 20 after re-crystallization afforded Maculine $C_{13}H_9NO_4$ (***2***) (35.0 mg; white powder; M_W: 243; m.p: 196–197 °C) (Oliveira et al., 1996). Fraction 28 after re-crystallization within 1 h yielded white pallets identified as Lupeol $C_{30}H_{50}O$ (***3***) (15.8 mg; M_W: 426; m.p: 214–216 °C). Combined fractions 34–36, after 24 h at room temperature afforded brown crystals, Kolbisine $C_{18}H_{12}O_6N$ (***4***) (28.5 mg; M_W: 347; m.p: 190 °C) (Sener et al., 1987). The chemical structures of the isolated compounds are presented on Fig. 1.

Aluminium sheet pre-coated with silica gel 60 $F_{254\ nm}$ (Merck) was used for thin layer chromatography and the isolated spots were visualized using both ultra-violet light (254 and 366 nm) and 50% H_2SO_4 spray reagent. 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, ^{1}H- and ^{13}C-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.

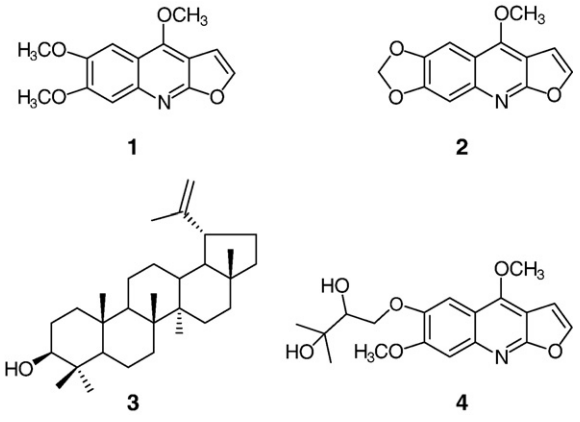

Fig. 1. Chemical structures of compounds isolated from the stem barks of *Teclea afzelii*.

2.3. Antimicrobial assays

2.3.1. Microbial strains

Nine species of microorganisms namely *Mycobacterium smegmatis* (ATCC 700084), *Bacillus subtilis* LMP0304G, *Staphylococcus aureus* LMP0206U, *Escherichia coli* LMP0101U, *Pseudomonas aeruginosa* LMP0102U, *Salmonella typhi* LMP0209U, *Candida albicans* LMP0204U, *Candida gabrata* LMP0413U and *Microsporum audorium* LMP 0724D were used in this study. *M. smegmatis* was obtained from American Type Culture Collection, MD, USA and the antimicrobial test on this strain was performed at the Department of Plant Science, University of Pretoria. Other strains were clinical isolates from '*Centre Pasteur du Cameroon*', Yaoundé. Their identity was confirmed before use at the LMP by culturing on the specific media followed by biochemical test using API system: *S. typhi* (SS agar; API 20E) (bioMérieux), *E. coli*, (C.L.E.D agar; API20E) (bioMérieux), *P. aeruginosa* (PSM agar, API20E) (bioMérieux), *S. aureus*. [Chapman agar; API 32Staph) (bioMérieux)], *B. subtilis* [NA (Oxoid) containing bromocresol purple; API 50 CH)], Candida sp [ChromagarTM *Candida* (Becton-Dickinson); API 20C AUX (bioMérieux)], *M. audorium* [SDA 1% chloramphenicol; API20C) (bioMérieux)] (Mbaveng et al., 2008).

They 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 conducted. These strains were sub-cultured on a fresh appropriate agar plate 24 h prior to any antimicrobial test.

2.3.2. Antimicrobial assays

2.3.2.1. Culture media. *M. smegmatis* was cultured onto Middlebrook 7H11 agar and allowed to grow for 24 h. 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 determination. The Mueller Hinton Agar (MHA) was also used for the determination of the MMC.

2.3.2.2. Chemicals for antimicrobial assay. Ciprofloxacin (Sigma-Aldrich Chemical Co., South Africa) was used as reference antibiotic (RA) for *M. smegmatis*. 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 RA against yeasts and other bacteria respectively.

2.3.2.3. Antimicrobial assays

2.3.2.3.1. Microplate susceptibility testing against M. smegmatis. All extracts were tested against *M. smegmatis* using microplate dilution method. The MIC, MBC and bacteria preparation were performed in 96-well microplates according to Salie et al. (1996) and Newton et al. (2002). The crude extracts were dissolved in 10% dimethylsulfoxide (DMSO) in sterile 7H9 broth to obtain a stock concentration of 1.250 mg/ml. Serial two-fold dilutions of each sample to be evaluated were made with 7H9 broth to yield volumes of 100 µl/well with final concentrations ranging from 9.76–312.50 µg/ml. Ciprofloxacin served as the positive drug control. 100 µl of *M. smegmatis* (0.2 log-phase, yielding 1.5×10^6 CFU/ml) was also added to each well containing the samples and mixed thoroughly to give final concentrations ranging from 0.31–19.53 µg/ml. The solvent control, DMSO at 2.5% or less in each well did not show inhibitory effects on the growth of the *M. smegmatis*. Tests were done in triplicates. The cultured microplates were sealed with parafilm and incubated at 37 °C for 24 h. The MIC of samples was detected following addition (40 µl) of 0.2 mg/ml *p*-iodonitrotetrazolium chloride (INT, Sigma-Aldrich, South Africa) and incubated at 37 °C for 30 min (Eloff, 1998; Mativandlela et al., 2006). Viable bacteria reduced the yellow dye to a pink colour. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth. 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 colour change after addition of INT as above mentioned.

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

2.3.2.3.2.1. Sensitivity test: agar disc diffusion assay

2.3.2.3.2.1.1. 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 20 mg/ml (200 µg/disc) and isolated compounds at 10 mg/ml (10 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 20 µg of drug. Two discs were prepared for each sample.

2.3.2.3.2.1.2. Diffusion test. The antimicrobial diffusion test was carried out 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) (Tereschuk et al., 1997). 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 37 °C for 24 h. 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.

2.3.2.3.2.2. MIC and MMC determinations. The MICs of test samples and RA were determined as follows: the test sample was first of all dissolved in DMSO. The solution obtained was added to NBGP to a final concentration of 78.12 µg/ml for each sample. This was serially diluted two fold to obtain concentration ranges of 0.61 to 78.12 µg/ml. 100 µl of each concentration was added in each 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 affected neither the growth of the test organisms nor the change of colour 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

Table 1
Inhibition zone diameters [a] (mm) of the crude extract, compounds isolated from *T. afzelii* and reference antibiotics determined by the disc diffusion test

Microorganisms	Tested samples [b]					
	Crude extract	Isolated compounds				
	TAE	*1*	*2*	*3*	*4*	RA
Mycobacteria						
M. smegmatis	nt	nt	nt	nt	nt	nt
Gram-negative bacteria						
Escherichia coli	17.5±0.5	21.0±1.0	–	10.0±0.0	–	22.0±0.0
Pseudomonas aeruginosa	–	–	12.5±0.5	–	–	20.0±1.0
Salmonella typhi	14.5±0.0	18.0±0.0	19.5±0.0	–	18.5±0.5	23.0±0.0
Gram-positive bacteria						
Staphylococcus aureus	–	13.0±0.0	–	–	–	22.0±0.0
Bacillus subtilis	17.5±0.5	22.5±0.0	24.0±0.0	–	21.0±0.5	22.0±0.5
Fungi						
Candida albicans	15.0±0.0	17.5±0.0	20.0±1.0	–	19.0±0.5	24.5±0.5
Candida gabrata	14.0±0.5	18.0±1.0	18.5±0.5	–	20.0±1.0	23.5±0.0
Microsporum audorium	16.0±1.0	22.0±0.0	21.0±0.0		22.0±0.0	26.0±0.0

(nt): Not tested; (–): Not active.

[a] Tested samples: crude extract was tested at 200 µg/disc, compounds at 100 µg/disc and RA at 20 µg/disc.

[b] The Tested samples were crude extract from *T. afzelii* (TAE), ***1***: Kokusaginine; ***2***: Maculine; ***3***: Lupeol; ***4***: Kolbisine; RA: Reference antibiotics (Gentamycin for bacteria, Nystatin for yeasts).

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 colour in the wells (red when there is no growth and yellow when there is growth). The lowest concentration showing no colour change was considered as the MIC.

For the determination of MMC, a portion of liquid (5 µl) from each well that showed no change in colour 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.

3. Results and discussions

The structures of the isolated compounds were established using spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments, COSY, HMQC and HMBC, and direct comparison with published information from literature data. The compounds isolated from the stem bark of *T. afzelii* (Fig. 1) were found to be three alkaloids and one terpenoid. The alkaloids were identified as Kokusaginine (***1***), Maculine (***2***) and Nkolbisine (***4***) while the isolated terpenoid was a known Lupeol (***3***). The isolation of Maculine and Kokusaginine from *Esenbeckia almawillia* and *E. grandifolia*, two plants of the family Rutaceae was previously reported (Oliveira et al., 1996) whilst Nkolbisine was also isolated from *T. nobilis* (Al-Rehaily et al., 2003), another plant of the genus *Teclea*.

Table 2
Minimal inhibition concentration (µg/ml) of the crude extracts, compounds isolated from *T. afzelii* and reference antibiotics

Microorganisms	Tested samples[a]					
	Crude extract	Isolated compounds				
	TAE	*1*	*2*	*3*	*4*	RA
Mycobacteria						
M. smegmatis	156.25	>312.50	156.25	312.50	>312.50	0.61
Gram-negative bacteria						
Escherichia coli	19.53	4.88	>78.12	78.12	–	4.88
Pseudomonas aeruginosa	>78.12	>78.12	39.06	–	>78.12	9.76
Salmonella typhi	39.06	9.76	9.76	–	9.76	2.44
Gram-positive bacteria						
Staphylococcus aureus	>78.12	39.06	>78.12	–	>78.12	4.88
Bacillus subtilis	19.53	4.88	2.44	–	4.88	4.88
Fungi						
Candida albicans	39.06	9.76	9.76	–	9.76	2.44
Candida gabrata	39.06	9.76	9.76	–	9.76	2.44
Microsporum audorium	19.53	4.88	4.88		4.88	1.22

(–): Not tested because samples were not active by disc diffusion.
[a] The Tested samples were crude extract from *T. afzelii* (TAE), ***1***: Kokusaginine; ***2***: Maculine; ***3***: Lupeol; ***4***: Kolbisine; RA: Reference antibiotics (Gentamycin for bacteria, Nystatin for yeasts; Ciprofloxacin for *M. smegmatis*).

Table 3
Minimal microbicidal concentration (µg/ml) of the crude extracts, compounds isolated from *T. afzelii* and reference antibiotics

Microorganisms	Tested samples[a]					
	Crude extract	Isolated compounds				
	TAE	*1*	*2*	*3*	*4*	RA
Mycobacteria						
M. smegmatis	312.50	>312.5	312.50	–	>312.5	1.22
Gram-negative bacteria						
Escherichia coli	78.12	9.76	–	>78.12	–	9.76
Pseudomonas aeruginosa	–	>78.12	>78.12	–	>78.12	19.53
Salmonella typhi	78.12	19.53	19.53	–	19.53	4.88
Gram-positive bacteria						
Staphylococcus aureus	>78.12	>78.12	>78.12	–	>78.12	9.76
Bacillus subtilis	39.06	9.76	4.88	–	9.76	9.76
Fungi						
Candida albicans	78.12	19.53	19.53	–	19.53	4.88
Candida gabrata	78.12	19.53	19.53	–	19.53	4.88
Microsporum audorium	39.06	9.76	9.76		9.76	2.44

(–): Not tested because samples were not active by disc diffusion.
[a] The Tested samples were crude extract from *T. afzelii* (TAE), *1*: Kokusaginine; *2*: Maculine; *3*: Lupeol; *4*: Kolbisine; RA: Reference antibiotics (Gentamycin for bacteria, Nystatin for yeasts; Ciprofloxacin for *M. smegmatis*).

The presence of alkaloids in many *Teclea* species is well known (Al-Rehaily et al., 2003; Tarus et al., 2005; Waffo et al., 2007). Therefore, the isolation of the three alkaloids from *T. afzelii* is in accordance with documented reports. The antimicrobial activities of the isolated compounds as well as that of the crude extract are reported in Tables 1–3.

The results of the diffusion test (Table 1) showed that only compound *1* was active on all the tested microbial species, while other compounds as well as the crude extract showed selective activity. The inhibition effect of the crude extract from *T. afzelii* and that of compounds ***2*** and ***4*** was observed on seven of the eight (87.5%) tested organisms. Compound ***3*** appeared to be the less active compound, its inhibition potency been observed only on *E. coli.* The difference observed in the activity of the tested compounds on *E. coli* and *S. typhi*, two bacteria of the family Enterobacteriaceae appeared to be strange. However, this might be related to the intrinsic specificity of the tested microbial species, or to the mode of action of the tested compounds.

The results of the MIC determination (Table 2) showed that the MIC values were ranged from 19.53 to 39.06 µg/ml were observed with the crude extract on 6 of the 9 tested microorganisms. This extract was moderately active on *M. smegmatis*, with greater MIC value (156.25 µg/ml). This experiment also confirmed to good activity of compounds ***1***, ***2*** and ***4*** on fungi, Gram-positive and negative bacteria, as observed with the diffusion assay. However, A part

on the tested *Mycobacterium* species, the MICs of these three compounds varied from 2.44 to 39.06 μg/ml on most of the tested microorganisms. The lowest MIC value (2.44 μg/ml) was observed with compound ***2*** on *B. subtilis*. This value was 2 fold lower than that of gentamycin used as reference antibiotic. The MIC values equal to that of the RA (4.88 μg/ml) were observed with compounds ***1*** and ***4*** on *B. subtilis* and compound ***2*** on *E. coli*. These data highlighted the selective activity activities of compounds ***1***, ***2*** and ***4*** as well as that of the crude extract on the tested microorganisms. This shows that the inhibition potency of these samples could be considered as very promising in the perspective of the development of antimicrobial drugs, with both antibacterial and antifungal activities. This can also be confirmed by the results of the MMC determination reported on Table 3. All the MMC values obtained were not more than four fold greater than the corresponding MICs, showing that cidal effect of the tested samples could be expected on most of the tested organisms (Carbonnelle et al., 1987; Mims et al., 1993). The MMC results also confirmed the inefficacy of the extract and compounds from *T. afzelii* on *M. smegmatis*. This indicates that an interesting inhibition effect on *M. tuberculosis* could not be expected as the sensitivity of this microbial species was found to be closer to *M. smegmatis*, a non pathogenic agent (Newton et al., 2002).

To the best of our knowledge, the antimicrobial activity of this plant as well as that of the tested alkaloids is being reported for the first time. Nevertheless, many alkaloids are known to possess antibacterial and antifungal activities (Bruneton, 1999; Cowan, 1999). Newton et al. (2002) have also documented the antimicrobial activity of two natural alkaloids, sanguinarine and chelerythrine, isolated from the roots of *Sanguinaria cadensis*.

The present study provide an important baseline information for the use of the crude extract from *T. afzelii as* well as that of the isolated alkaloids in the treatment of infections associated to the sensitive pathogens studied. Their pharmacological and cytotoxicity studies are being considered as our future objective, to confirm these samples as potential therapeutic agents.

Acknowledgements

The technical support of the '*Centre Pasteur du Cameroun*' and the National Herbarium of Cameroon. The Third World Academy of Science (TWAS) for its financial support through the 2007 training program, and the department of Plant Science, University of Pretoria (South Africa) for its financial and technical support.

References

Adnan, J.A., Mohammad, S.A., Ilias, M., Assad, A.A., Herman, P.P., 2003. Furoquinoline alkaloids from *Teclea nobilis*. Phytochemistry 66, 1405–1411.

Ayafor, F.J., Sondengam, B.L., Bilon, A.N., Tsamo, E., Kimbu, S.F., 1982. Furoquinoline alkaloids from *Teclea nobilis*. Journal of Natural Products 45, 714–717.

Al-Rehaily, A.J., Ahmad, M.S., Muhammad, I., Al-Thukair, A.A., Perzanowski, H.P., 2003. Furoquinoline alkaloids from *Teclea nobilis*. Phytochemistry 64, 1405–1411.

Berghe, V.A., Vlietinck, A.J., 1991. Screening methods for antibacterial and antiviral agents from higher plants. Methods for Plant Biochemistry 6, 47–68.

Briggs, L.H., Cambie, R.C., 1958. The constituents of *Phebalium nudum* hook-I: the bark. Tetrahedron 2, 256–270.

Bruneton, J., 1999. Pharmacognosie: Phytochimie, Plantes medicinales, 3e ed. Tec et Doc, Paris, pp. 263–309.

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

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Review 12, 564–582.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–713.

Khalid, A.S., Waterman, P., 1981. Alkaloids from stem barks of *Oricia renieri* and *Oricia gabonensis*. Phytochemistry 20, 2761–2763.

Kuete, V., Nguemeving, J.R., Penlap Beng, V., Azebaze, A.G.B., Etoa, F.-X., Meyer, M., Bodo, B., Nkengfack, A.E., 2007. Antimicrobial activity of the methanolic extracts and compounds from *Vismia laurentii* De Wild (Guttiferae). Journal of Ethnopharmacology 109, 372–379.

Mativandlela, S.P.N., Lall, N., Meyer, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. South African Journal of Botany 72, 232–237.

Mbaveng, A.T., Ngameni, B., Kuete, V., Konga Simo, I., Ambassa, T., Roy, R., Bezabih, M., Etoa, F.X., Ngadjui, B.T., Abegaz, B.M., Meyer, J.J.M., Lall, N., Penlap, B.V., 2008. Antimicrobial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae). Journal of Ethnopharmacology 116, 647–649.

Mester, I., Inescu, M., 1971. Synthesis of kokusaginine. Phytochemistry 10, 2205–2206.

Mims, C.A., Playfair, J.H.L., Roitt, I.M., Wakelin, D., Williams, R., 1993. In: Mims, C.A., et al. (Ed.), Antimicrobials and chemotherapy. Medical Microbiology Review, vol. 35, pp. 1–34.

Newton, S.M., Lau, C., Gurcha, S.S., Besra, G.S., Wright, C.W., 2002. The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. Journal of Ethnopharmacology 79, 57–67.

Oliveira, F.M., Sant'ana, A.E.G., Conserva, L.M., Maia, J.G.S., Guilhon, G.M.P., 1996. Alkaloids and coumarins from Esenbeckia species. Phytochemistry 41, 647–649.

Salie, F., Eagles, P.F.K., Leng, H.M.J., 1996. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of Ethnopharmacology 52, 27–33.

Sener, B., Nayonalpan, N., Tanker, N., Koyuncu, M., Lewis, J.R., Mutlugil, A., 1987. Pharmacognosic researches on Rutaceae plants growing in Turkies. International Journal of Crude Drug Research 25, 87–88.

Tarus, P.K., Philip, H., Coombes, N., Crouch, D.R., Mulholland, A., Moodley, B., 2005. Furoquinoline alkaloids from the southern African Rutaceae *Teclea natalensis*. Phytochemistry 66, 703–706.

Tereschuk, M.L., Riera, M.V.Q., Castro, G.R., Abdala, L.R., 1997. Antimicrobial activity of flavonoid from leaves of *Tagetes minuta*. Journal of Ethnopharmacology 56, 227–232.

Waffo, K.A.F., Coombes, P.H., Crouch, N.R., Mulholland, D.A., El Amin, S.M.M., Smith, J.P., 2007. Acridone and furoquinoline alkaloids from *Teclea gerrardii* (Rutaceae: Toddalioideae) of southern Africa. Phytochemistry 5, 663–667.

Zgoda, J.R., Porter, J.R., 2001. A convenient microdilution method screening natural products against bacteria and fungi. Pharmaceutical Biology 39, 221–225.

Edited by PJ Houghton