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Antimicrobial and antioxidant activities of extracts and ten compounds from three Cameroonian medicinal plants: *Dissotis perkinsiae* (Melastomaceae), *Adenocarpus mannii* (Fabaceae) and *Barteria fistulosa* (Passifloraceae)

F.S.T. Ndjateu [a,1], R.B.N. Tsafack [a,1], B.K. Nganou [a,1], M.D. Awouafack [a,b], H.K. Wabo [a], M. Tene [a], P. Tane [a], J.N. Eloff [b,*]

[a] Laboratory of Natural Products Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon
[b] Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa

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ABSTRACT

Background: We decided to investigate the antimicrobial and the antioxidant activities of extracts and compounds isolated from *Dissotis perkinsiae*, *Adenocarpus mannii* and *Barteria fistulosa*, three Cameroonian medicinal plants used for the treatment of skin diseases, wounds, fever, rheumatism, malaria and/or infectious diseases.
Methods: Standard chromatographic and spectroscopic methods were used to isolate and identify ten compounds from the three plant species [**1**–**5** (from *D. perkinsiae*), **2**, **6**–**8** (from *A. mannii*) and **2**, **4**, **9**, and **10** (from *B. fistulosa*)]. A two-fold serial microdilution method was used to determine the minimum inhibitory concentration (MIC) against a panel of fungal and bacterial species. The radical scavenging capacity using 2,2-diphenyl-1-picryhydrazyl (DPPH) was determined to evaluate the antioxidant activity of the samples.
Results: The compounds isolated were: ursolic acid (**1**), oleanolic acid (**2**), quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside (**3**), 3-*O*-*β*-D-glucopyranoside of sitosterol (**4**), ellagic acid (**5**), isoprunetin (**6**), chrysin 7-*O*-*β*-D-glucopyranoside (**7**), isovitexin (**8**), hederagenin (**9**) and shanzhiside methyl ester (**10**). The ethanol extract of *D. perkinsiae* had good antibacterial activity against *Enterococcus faecalis* (MICs 0.04 and 0.08 mg/ml), *Escherichia coli* (MIC 0.08 mg/ml) and *Staphylococcus aureus* (MIC 0.08 mg/ml). The extract of *B. fistulosa* had significant antifungal activity against *Cryptococcus neoformans* with an MIC of 0.08 mg/ml. Other extracts had moderate to poor antimicrobial activities with the MIC ranging from 0.16 to 2.50 mg/ml. The isolated compounds were generally more active against bacteria (MIC ranging from 16 to 250 μg/ml) than fungi (MIC between 31 and 250 μg/ml). Moderate antibacterial activity was obtained with compound **3** against *E. faecalis* and *E. coli* (MIC of 16 μg/ml in both cases), compounds **6** and **10** against *E. faecalis* (MIC of 16 μg/ml), and compound **9** against *E. faecalis* (MIC 31 μg/ml) and *S. aureus* (MIC 31 μg/ml). The *B. fistulosa* extract had the greatest radical scavenging activity (IC_{50} 100.16 μg/ml) followed by extracts of *D. perkinsiae* (IC_{50} 130.66 μg/ml), and *A. mannii* (IC_{50} 361.30 μg/ml). Compounds **3** and **5** had significant antioxidant activities with the IC_{50} of 9.84 and 9.99 μg/ml as compared to that of ascorbic acid (IC_{50} 2.41 μg/ml).
Conclusion: The results obtained support the traditional use of the three plant species (*D. perkinsiae*, *A. mannii* and *B. fistulosa*) in traditional medicine for the treatment of infections. Some extracts and isolated compounds could be useful in development of antimicrobial agents. We are currently investigating the toxicity and other pharmacological activities with the potential use as topical antimicrobial agents.

1. Introduction

The genus *Dissotis* is a member of the Melastomaceae family and comprises about 140 species in Africa (Loigier, 1994). They are climbing shrubs, shrubs or small trees of up to 2 m, and are found in some African countries such as Democratic Republic of Congo, Benin, Nigeria, Ivory Cost and Cameroon (Maluma, 2005). *Dissotis perkinsiae*, is a shrub with the height of 90–150 cm. The leaves are purple or pink with a diameter of about 10.5 cm. It is found in tropical areas of Nigeria, Togo and Cameroon (Hutchinson and Dalziel, 1954). This species is used in traditional medicine for the treatment of the skin diseases and malaria (Haxaire, 1979). To the best of our knowledge, no phytochemical work has yet been done on *D. perkinsiae*.

Adenocarpus mannii a member of the Fabaceae family is a tree of 5 to 6 m high with trifoliate leaves and yellow flowers. It is distributed in

* Corresponding author. Tel./fax: +27 12 529 8244.
E-mail address: kobus.eloff@up.ac.za (J.N. Eloff).
[1] These authors contributed equally to the experimental part of this work and each could have been considered as first author.

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north tropical Africa, south-west of Europe, central and southern zone of Italy (Cubas et al., 2010). Many of *Adenocarpus* species are used in traditional medicine. For instance the aerial part and fruits of *A. anisochilius* are used for the treatment of leprosy, scabies and acne (Cazarolli et al., 2008). According to the information from traditional healers, *A. mannii* is used in the Western Region of Cameroon to treat malaria and various infectious diseases. Previous phytochemical investigation on some species of the genus *Adenocarpus* led to the isolation of flavonoids (Essokne, 2011) and cyclobutane-containing alkaloids (Ribas and Talarid, 1950). Polyphenols and cyclobutane-containing alkaloids were also reported from *A. mannii* (Faugeras, 1970; Dembitsky, 2008).

Barteria fistulosa is a member of the Passifloraceae family with 18 genera and approximately 630 species. It has a height of 15 m, with alternate leaves, reddish stem bark and green fruit with a 3 cm diameter (Breteler, 1999). The plant is widely distributed in tropical regions around the world (Vanderplank, 1996) and is used locally in Cameroon for the treatment of wounds, fever and rheumatism (Gangoué-Piéboji et al., 2009). Plant species of Passifloraceae family contain some secondary metabolites including tannins (Dhawan et al., 2004), harmala alkaloids (Ingale and Hivrale, 2010), terpenoids (Yoshikawa et al., 2000), flavonoids (Qimin et al., 1991), iridoids and cyanoheterosides (Paris et al., 1969). Previous phytochemical investigation on *B. fistulosa* had reported the isolation of cyanogenic glycosides (Spencer and Seigler, 1984).

Traditional uses of these three plants motivated our effort to investigate the phytochemistry and antimicrobial and antioxidant activities. Ten compounds [ursolic acid (**1**), oleanolic acid (**2**), quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside (**3**), 3-*O*-*β*-D-glucopyranoside of sitosterol (**4**) and ellagic acid (**5**) (from *D. perkinsiae*), oleanolic acid (**2**), isoprunetin (**6**), chrysin 7-*O*-*β*-D-glucopyranoside (**7**) and isovitexin (**8**) (from *A. mannii*), and oleanolic acid (**2**), 3-*O*-*β*-D-glucopyranoside of sitosterol (**4**), hederagenin (**9**) and shanzhiside methyl ester (**10**) (from *B. fistulosa*)] were isolated and characterized. This is the first report on the isolation of compounds (**1**–**5**) from *D. perkinsiae* as well as compounds **2**, **6**–**8** from *A. mannii*, and compounds **2**, **4**, **9** and **10** from *B. fistulosa*. Antimicrobial and antioxidant activities of extracts of *D. perkinsiae*, *A. mannii* and *B. fistulosa* and some compounds that were isolated in sufficient quantities are reported here for the first time.

2. Material and methods

2.1. General experimental procedures

Mass spectral data [Electrospray ionization mass spectrometry (ESI-MS)] were measured on a Waters Synapt HDMS spectrometer. NMR spectra were recorded with a Varian spectrometer at 400 MHz. Chemical shifts (δ) were quoted in parts per million (ppm) from the internal standard tetramethylsilane (TMS). Deuterated solvents dimethyl sulfoxide (DMSO-d_6), and chloroform ($CDCl_3$) were used as solvents for the NMR experiments. Column chromatography was performed on silica gel 60 [(0.2–0.5 mm) and (0.2–0.063 mm)] mesh (Sigma-Aldrich, Germany). Pre-coated silica gel 60 F_{254} thin layer chromatography (TLC) plates (Merck, Germany) were used for monitoring fractions and spots were detected with UV light (254 and 365 nm) and then sprayed with 30% sulphuric acid (H_2SO_4) followed by heating to 110 °C.

2.2. Plant materials

Plant materials were collected in three locations of the Western Region of Cameroon: the stem barks of *Barteria fistulosa* in Bafou (November 2011), the leaves of *Dissotis perkinsiae* in Dschang (October 2010) and the leaves of *Adenocarpus mannii* in Mount Bamboutos (December 2011). They were all identified by Mr. Nana, a botanist at the Cameroon National Herbarium in Yaoundé where voucher specimens 24719/SRF/Cam (*D. perkinsiae*), 12495/SRF/Cam (*A. mannii*) and 26363/HNC (*B. fistulosa*) were deposited.

2.3. Extraction and isolation

2.3.1. Dissotis perkinsiae

The dried leaves (1.5 kg) of *D. perkinsiae* were extracted in ethanol (EtOH) (3.5 l) three times to yield 82 g of extract (DP) after filtration and removal of the solvent under vacuum. Part of this extract (80 g) was subjected to silica gel column chromatography (CC) eluting with a mixture of n-hexane/ethyl acetate (EtOAc) in increasing polarity to afford 66 fractions of 500 ml each. Eight combined fractions (F_1–F_8) were finally obtained after comparative TLC. Fraction F_2 obtained at a polarity of n-hexane/EtOAc (85:15) from the main CC, crystallized in acetone to give 1 (white powder, 8 mg, $C_{30}H_{49}O_3$ *m/z* 457 $[M + H]^+$) and 2 (white powder, 14 mg, $C_{30}H_{49}O_3$ *m/z* 457 $[M + H]^+$) after filtration. Fraction F_3 obtained with n-hexane/EtOAc (80:20 and 75:25) was subjected to Sephadex LH-20 using an isocratic mixture of dichloromethane/methanol (CH_2Cl_2/MeOH, 1:1, v/v) to afford compounds **3** (yellow powder, 15 mg, $C_{28}H_{25}O_{16}$ *m/z* 617 $[M + H]^+$) and **4** (white powder, 16 mg, $C_{35}H_{61}O_6$ *m/z* 577 $[M + H]^+$). Fraction F_7 was subjected to a similar Sephadex gel permeation procedure as fraction F_3 to afford compound **5** (grey powder, 21 mg, $C_{14}H_6O_8$ *m/z* 303 $[M + H]^+$).

2.3.2. Adenocarpus mannii

The leaves of *A. mannii* were dried and ground to yield 3 kg of powder that was macerated in EtOH (7 l × 3) for 24 h. After filtration and removal of the solvent in vacuo, 130 g of the crude extract (AD) was obtained. Part of this crude extract (110 g) was subjected to a silica gel column chromatography eluted with n-hexane/EtOAc and EtOAc/MeOH in gradient polarity to afford 51 fractions of 400 ml each that were combined using comparative TLC into four major fractions [A (15.2 g), B (12.5 g), C (22.2 g) and D (20.5 g)]. Fraction A contained mainly fatty acid derivatives and was not further investigated. Fractions B and C were similarly subjected to CC over silica gel eluting with a mixtures of n-hexane/EtOAc and EtOAc/MeOH in increasing polarity. Subfractions obtained were further purified over Sephadex LH-20 to afford four compounds: 2 (15 mg) as white powder in n-hexane/EtOAc (7:3), 6 (22 mg, $C_{16}H_{13}O_5$ *m/z* 285 $[M + H]^+$) as yellowish powder in CH_2Cl_2/MeOH (95:5), 7 (12 mg, $C_{21}H_{21}O_9$ *m/z* 417 $[M + H]^+$) as yellow powder in CH_2Cl_2/MeOH (9:1), and 8 (24 mg, $C_{21}H_{21}O_{10}$ *m/z* 433 $[M + H]^+$) as yellow powder in CH_2Cl_2/MeOH (85:15).

2.3.3. Barteria fistulosa

The dried and powdered stem barks of *B. fistulosa* (2.5 kg) were extracted in EtOH (4 l) for 3 days to yield a crude extract (BF) (100 g) after filtration and evaporation in vacuo. Part of this crude extract (80 g) was subjected to silica gel CC using n-hexane/EtOAc and in increasing polarity to afford a total number of 60 fractions of 500 ml each. A monitoring with Co-TLC of these fractions yielded four main fractions (I–IV). Fraction I was not investigated further while other fractions were subjected to similar fractionation by CC over silica gel eluting in gradient polarity with n-hexane–EtOAc and EtOAc/MeOH. Further purification of the resulting fractions over Sephadex LH-20 afforded compounds **4** (15 mg), **2** (8 mg), **9** (15 mg, $C_{30}H_{49}O_4$ *m/z* 473 $[M + H]^+$) as white powder in n-hexane–EtOAc (6:4) and **10** (10 mg, $C_{17}H_{27}O_{11}$ *m/z* 407 $[M + H]^+$) as white amorphous powder in EtOAc/MeOH (7:3).

2.4. Antimicrobial assay

The two-fold serial microdilution method was used to determine the minimum inhibitory concentration (MIC) values for extracts and isolated compounds against bacteria (Eloff, 1998) and fungi (Eloff, 1998) as modified by Masoko et al. (2005). The MIC of the samples were evaluated using two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212), two Gram-negative bacteria,

Pseudomonas aeruginosa (ATCC 27853) and *Escherichia coli* (ATCC 25922); and the four fungi *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans* (animal isolates) and *Candida albicans* (ATCC 10231). Some fungal strains used were cultured from clinical cases of fungal infectious diseases in animals, before treatment, in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science. *C. albicans* was isolated from a Gouldian finch, *C. neoformans* from a cheetah, while *A. fumigatus* was isolated from a chicken which suffered from a systemic mycosis.

A 100 μl of extract (10 mg/ml) or compound (1 mg/ml) was serially diluted two-fold in triplicate with sterile distilled water in 96-well microtitre plates and 100 μl of freshly prepared bacterial culture in Mueller Hinton broth (Fluka, Switzerland) was added to each well. DMSO (5%) and Gentamicin were used as negative and positive controls, respectively. The microtitre plates were sealed in plastic and were incubated overnight at 37 °C. Thereafter, 40 μl of 0.2 mg/ml of *p*-iodonitrotetrazolium violet (INT) was added to each well to indicate microbial growth. The microtitre plates were further incubated at 37 °C and minimal inhibitory concentration was determined 1 and 2 h after the addition of INT. MIC was determined as the lowest concentration inhibiting microbial growth, indicated by a decrease in the intensity of the red colour of the formazan (Shai et al., 2008).

Fractions and isolated compounds were dissolved in DMSO to a concentration of 10 mg/ml and 1 mg/ml, respectively. Fungal cultures were taken from agar culture plates and inoculated in fresh Sabouraud Dextrose broth and incubated at 37 °C for 4 h prior to conducting the assay. The extract or compounds (100 μl) in triplicate were serially diluted two-fold with sterile distilled water in 96-well microtitre plates. Thereafter, 100 μl of the fungal culture was added to each well. Amphotericin B and DMSO (5%) were used as positive and negative controls, respectively. To indicate the growth of microorganisms, 40 μl of 0.2 mg/ml of INT was added to each well. The microplates were sealed in air-tight plastic and then incubated at 37 °C in 100% relative humidity. MIC was taken as the lowest concentration of the extract that inhibited fungal growth after 16 and 36 h. Substantial differences would indicate that the effect was fungi static rather than fungicidal. The turbidity of the microbial suspension was adjusted to a McFarland standard 0.5 equivalent to concentrations of 1–5 × 10^8 and 1–5 × 10^7 cfu/ml for bacteria and fungi, respectively. The microbial suspensions were further diluted (1:100) in media to result in a final inoculum of approximately 1.5 × 10^6 cfu/ml for bacteria and 1.5 × 10^5 cfu/ml for fungi.

2.5. DPPH radical scavenging assay

The antioxidant activity was performed using the method described by Du Toit et al. (2001) with slight modifications. Samples were dissolved in HPLC-grade methanol (Sigma-Aldrich, Germany) and two-fold serially diluted to a concentration ranges of 2000 to 126 μg/ml for extracts and 200 to 12.6 μg/ml for isolated compounds or L-ascorbic acid (Sigma, Germany), a standard reference. Briefly, 200 μl of (3.7 mg/100 ml) methanolic solution of 2,2-diphenyl-1-picryhydrazyl (DPPH) was introduced in each well of a microtitre plate. Each sample and its dilutions (50 μl) were introduced into the wells and kept in the dark for 30 min before the measurement of the absorbance at 517 nm using a Multi-Mode Microplate Reader (BioTek, USA) with computer controlled software (BioTek Gen™). A 96 wells microplate (Bioster, Spain) was used for visible absorbance measurements. The free radical scavenging activity of each sample and the reference standard were determined as percent of the inhibition obtained from the following formula: Radical scavenging capacity (%) = $[(100 - (Ab_{sample} - Ab_{blank}) / Ab_{control}) \times 100]$. The concentration of samples reducing 50% of free radical DPPH (IC_{50}) was determined by plotting the percentage of inhibition against the sample concentrations. The assay was replicated three times and results are expressed as mean ± standard deviation.

3. Results and discussion

3.1. Phytochemical investigation

The extracts from *Dissotis perkinsiae*, *Adenocarpus mannii* and *Barteria fistulosa* were fractionated by silica gel column chromatography to afford ten compounds (**1**–**10**) (Fig. 1). Compounds obtained from *D. perkinsiae* were identified as ursolic acid (**1**) (Seebacher et al., 2003), oleanolic acid (**2**) (Seebacher et al., 2003), quercetin 3-*O*-(6″-*O*-galloyl)-β-galactopyranoside (**3**) (Söhretoglu et al., 2009), 3-*O*-β-D-glucopyranoside of sitosterol (**4**) (Al-Oqail et al., 2012) and ellagic acid (**5**) (Srivastava et al., 2007). From *A. mannii* compounds were identified as oleanolic acid (**2**) (Seebacher et al., 2003), isoprunetin (**6**) (Kulesh et al., 2008), chrysin 7-*O*-β-D-glucopyranoside (**7**) (Liu et al., 2010) and isovitexin (**8**) (Peng et al., 2005). Compounds isolated from *B. fistulosa* were identified as oleanolic acid (**2**) (Seebacher et al., 2003), 3-*O*-β-D-glucopyranoside of sitosterol (**4**) (Al-Oqail et al., 2012), hederagenin (**9**) (Joshi et al., 1999) and shanzhiside methyl ester (**10**) (Takeda et al., 1977; Da Silva et al., 2008). The structures of the compounds were determined by analysis of their NMR data and comparison with those reported in the literature. This is the first report on the isolation of any compounds (**1**–**5**) from *Dissotis perkinsiae* as well as compounds **2**, **6**, **7**, and **8** from *Adenocarpus mannii*, and compounds **2**, **4**, **9** and **10** from *Barteria fistulosa*.

3.2. Antimicrobial activity

Antimicrobial activity of extracts and isolated compounds from *D. perkinsiae*, *A. mannii* and *B. fistulosa* were determined against bacteria and fungi and the results are given as the minimal inhibitory concentration (MIC) (Table 1) and total activity (Table 2). Many authors consider the antimicrobial activities of extracts to be significant if the MIC value is 0.1 mg/ml or lower, moderate if 0.1 < MIC ≤ 0.625 mg/ml and weak if MIC > 0.625 mg/ml (Eloff, 2004; Kuete, 2010). When reporting the antimicrobial activities of the isolated compounds, it is significant if the MIC is 10 μg/ml or lower, moderate if 10 < MIC ≤ 100 μg/ml and low if MIC > 100 μg/ml (Kuete, 2010; Ríos and Recio, 2005). Based on these criteria, all the test samples had significant to weak antimicrobial activities with MICs between 0.04 and 2.50 mg/ml for extracts and 16 to 250 μg/ml for isolated compounds.

Among the crude extracts, *D. perkinsiae* had good antibacterial activity against *E. faecalis* (MICs of 0.04 and 0.08 mg/ml after 1 and 2 h incubations), *E. coli* (MIC 0.08 mg/ml) and *S. aureus* (MIC value of 0.08 mg/ml after 1 h incubation). The extract of *B. fistulosa* had significant antifungal activity against *C. neoformans* with an MIC of 0.08 mg/ml after 24 h incubation. The extract of *D. perkinsiae* had moderate activity against *S. aureus* (MIC 0.16 mg/ml after 2 h incubation), *P. aeruginosa* (MIC 0.16 mg/ml after 1 h incubation) and *A. fumigatus* (MIC 0.16 mg/ml). Moderate activity was also obtained for the extract of *A. mannii* against *E. faecalis* (MIC of 0.16 mg/ml after 1 h incubation), and *S. aureus* and *E. coli* with the MIC value of 0.16 mg/ml in both cases. All the fungi (MICs between 0.63 and 250 mg/ml) and one bacteria *P. aeruginosa* (MIC 0.63 mg/ml) were more resistant to the extract of *A. mannii*. Most of the microorganisms (fungi and bacteria) were also relatively resistant to the extract of *B. fistulosa* with the MIC values between 0.63 and 1.25 mg/ml.

Apart from compounds **4** and **7** that were not tested, all the isolated compounds had antimicrobial activity against the eight test microorganisms. The tested compounds were more active against bacteria (MIC ranging from 16 to 250 μg/ml) than fungi (MIC between 31 and 250 μg/ml). Some compounds had moderate antibacterial activity. This was the case with compound **3** against *E. faecalis* and *E. coli* (MIC of 16 μg/ml in both cases), compounds **6** and **10** against *E. faecalis* (MIC of 16 μg/ml after 1 h incubation), and compound **9** against *E. faecalis* (MIC 31 μg/ml) and *S. aureus* (MIC of 31 μg/ml after 1 h incubation). Compound **3** had weak antibacterial activity against *S. aureus* and

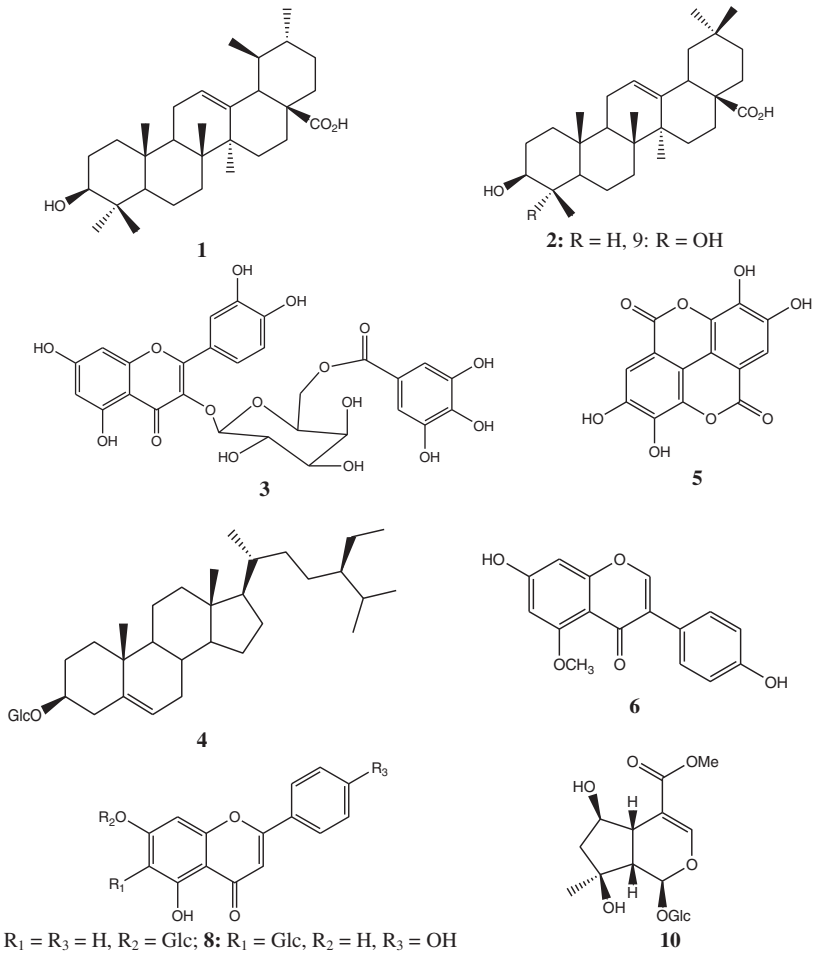

Fig. 1. Chemical structures of compounds[a] isolated from the leaves of *Dissotis perkinsiae* (**1**–**5**), *Adenocaropus mannii* (**2**, **6**–**8**), and the stem barks of *Barteria fistulosa* (**2**, **4**, **9**–**10**). [a]**1**: ursolic acid, **2**: oleanolic acid, **3**: quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside, **4**: 3-*O*-*β*-D-glucopyranoside of sitosterol, **5**: ellagic acid, **6**: isoprunetin, **7**: chrysin 7-*O*-*β*-D-glucopyranoside, **8**: isovitexin, **9**: hederagenin, **10**: shanzhiside methyl ester.

Table 1
Minimum inhibitory concentration (MIC) of the crude extracts in mg/ml and compounds in μg/ml from *D. perkinsiae*, *A. mannii* and *B. fistulosa* against fungi (C.a, C.A, A.f, C.n) and bacteria (S.a, P.a, E.f, E.c). The results are the means of three replicates and the standard deviation was zero.

Samples	Fungi[a]									Bacteria[b]								
	C. a		C.A		A.f		C.n [c]		Average	S.a		P.a		E.f		E.c		Average
	16 h	24 h	16 h	24 h	16 h	24 h	24 h	36 h		1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h	
Extracts[d]																		
DP	1.25	1.25	1.25	1.25	0.16	0.16	0.63	0.63	0.82	0.08	0.16	0.16	0.31	0.04	0.08	0.08	0.08	0.12
AM	0.63	0.63	0.63	0.63	1.25	1.25	1.25	2.50	1.1	0.16	0.16	0.63	0.63	0.16	0.31	0.16	0.16	0.30
BF	1.25	1.25	1.25	1.25	0.63	0.63	0.08	0.16	0.81	0.63	0.63	0.63	1.25	0.63	0.63	0.63	0.63	0.70
Compounds[e]																		
1	125	125	125	125	125	125	31	125	113.3	125	125	125	125	125	125	125	125	125
2	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125	125
3	125	125	125	125	125	125	250	250	156.3	63	63	63	63	16	16	16	16	39.5
5	125	125	125	125	125	125	31	63	105.5	250	250	125	125	250	250	125	125	187.5
6	125	125	125	125	125	125	250	250	156.3	125	125	125	125	16	125	125	125	111.4
8	–	–	–	–	–	–	–	–	–	250	250	–	–	125	125	125	125	–
9	125	125	125	125	63	63	63	63	94	31	63	125	125	31	31	125	125	82
10	125	125	125	125	63	125	250	250	148.5	125	125	125	125	16	125	125	125	111.4
Controls[f]																		
Amp B	16	16	8	8	16	16	>250	>250	72.5	–	–	–	–	–	–	–	–	
Gen	–	–	–	–			–	–	–	0.78	0.78	1.56	1.56	1.56	1.56	0.39	0.39	1.07

[a] C.a: *Candida albicans*(Isolate); C.A: *Candida albicans* (ATCC strain); C.n: *Cryptococcus neoformans*; A.f: *Aspergillus fumigatus*.
[b] E.c: *Escherichia coli*; E.f: *Enterococcus faecalis*; S.a: *Staphylococcus aureus*; P.a: *Pseudomonas aeruginosa*.
[c] With this microorganism little reaction was observed after 16 h and MIC were recorded after 24 h and 36 h.
[d] DP: *Dissotis perkinsiae*, BF: *Barteria fistulosa*, AM: *Adenocarpus mannii*.
[e] **1**: ursolic acid, **2**: oleanolic acid, **3**: quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside, **4**: 3-*O*-*β*-D-glucopyranoside of sitosterol, **5**: ellagic acid, **6**: isoprunetin, **7**: chrysin 7-*O*-*β*-D-glucopyranoside, **8**: isovitexin, **9**: hederagenin, **10**: shanzhiside methyl ester.
[f] Amp B: Amphotericin B (in μg/ml), Gen: *Gentamicin* (in μg/ml), –: not determined, compounds **4** and **7** were not tested.

Table 2
Total activity of the crude extracts in ml/g and compounds in ml/mg from *D. perkinsiae*, *A. mannii* and *B. fistulosa* against fungi (C.a, C.A, A.f, C.n) and bacteria (S.a, P.a, E.f, E.c).

Samples	Fungi[a]									Bacteria[b]								
	C. a		C.A		A.f		C.n[c]		Average	S.a		P.a		E.f		E.c		Average
	16 h	24 h	16 h	24 h	16 h	24 h	24 h	36 h		1 h	2 h	1 h	2 h	1 h	2 h	1 h	2 h	
Extracts[d]																		
DP	15.6	15.6	15.6	15.6	2	2	7.9	7.9	10.3	1	2	2	3.9	0.5	1	1	1	1.5
AM	5.7	5.7	5.7	5.7	11.4	11.4	11.4	22.7	9.9	1.5	1.5	5.7	5.7	1.5	2.8	1.5	1.5	2.7
BF	15.6	15.6	15.6	15.6	2	2	7.9	7.9	10.3	7.9	7.9	7.9	15.6	7.9	7.9	7.9	7.9	8.8
Compounds[e]																		
1	8	8	8	8	8	8	32.3	8	11	8	8	8	8	8	8	8	8	8
2	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
3	8	8	8	8	8	8	4	4	7	15.9	15.9	15.9	15.9	62.5	62.5	62.5	62.5	39.2
5	8	8	8	8	8	8	32.3	15.9	12	4	4	8	8	4	4	4	8	6
6	8	8	8	8	8	8	4	4	7	8	8	8	8	62.5	8	8	8	14.8
8	–	–	–	–	–	–	–	–	–	4	4	–	–	8	8	8	8	–
9	8	8	8	8	15.9	15.9	15.9	15.9	11.9	32.3	15.9	8	8	32.3	32.3	8	8	18.1
10	8	8	8	8	15.9	8	4	4	7.9	8	8	8	8	62.5	8	8	8	14.8

[a] C.a: *Candida albicans*(Isolate); C.A: *Candida albicans* (ATCC strain); C.n: *Cryptococcus neoformans*; A.f: *Aspergillus fumigatus*.
[b] E.c: *Escherichia coli*; E.f: *Enterococcus faecalis*; S.a: *Staphylococcus aureus*; P.a: *Pseudomonas aeruginosa*.
[c] With this microorganism little reaction was observed after 16 h and MIC were recorded after 24 h and 36 h.
[d] DP: *Dissotis perkinsiae*, BF: *Barteria fistulosa*, AM: *Adenocarpus mannii*.
[e] **1**: ursolic acid, **2**: oleanolic acid, **3**: quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside, **4**: 3-*O*-*β*-D-glucopyranoside of sitosterol, **5**: ellagic acid, **6**: isoprunetin, **7**: chrysin 7-*O*-*β*-D-glucopyranoside, **8**: isovitexin, **9**: hederagenin, **10**: shanzhiside methyl ester, –: not determined.

P. aeruginosa with an MIC of 63 μg/ml. Compound **9** was also weakly active after 2 h incubation against *S. aureus* (MIC 63 μg/ml). Bacteria such as *S. aureus*, *P. aeruginosa*, *E. coli* and/or *E. faecalis* were relatively more resistant to compounds **1**, **2**, **5**, **6** and **10** with MIC ranging from 125 to 250 μg/ml. The most active antifungal compounds were **2** and **5** with a moderate activity against *C. neoformans* with an MIC of 31 μg/ml (after 24 h incubation). Compound **9** had also moderate antifungal activity against *A. fumigatus* and *C. neoformans* (MIC 63 μg/ml) as well as compound **5** against *C. neoformans* (MIC 63 μg/ml after 36 h incubation) and compound **10** against *A. fumigatus* (MIC 63 μg/ml after 24 h incubation). The two strains of *C. albicans* were relatively resistant to all the compounds with an MIC of 125 μg/ml. *C. neoformans* was also resistant to compounds **3**, **6** and **10** with MICs of 250 μg/ml. The result of the antifungal activity of shanzhiside methyl ester (**10**) against *C. albicans* and *C. neoformans* obtained in this study is in agreement with previous investigation (Da Silva et al., 2008). Antifungal activity of isoprunetin (**6**) (isolated from *Spirotropis longifolia* Baill) against *C. albicans* ATCC strain in concentrations ranging from 64 to 0.125 μg/ml was previously reported (Basset et al., 2012).

Abere et al. (2010) reported significant antimicrobial activity of the aqueous EtOH leaf extract of *Dissotis rotundifolia* against *S. aureus* (MIC 0.05 mg/ml), *P. aeruginosa* (MIC 0.025 mg/ml), and *E. coli* (MIC 0.05 mg/ml) while Baba and Onanuga (2011) reported moderate antimicrobial activity of the MeOH extract from the same species. Our antimicrobial results on the EtOH leaf extract of *D. perkinsiea* are similar to those reported by Abere et al. (2010). The antimicrobial activity of the EtOH stem barks extract of *B. fistulosa* against *P. aeruginosa* (MICs 0.63 and 1.25 mg/ml) compared with previous work reported on the MeOH leaf extract (MIC ≥ 10 mg/ml) led to closed results but our sample had best activity (Gangoué-Piéboji et al., 2009). When comparing the average MICs of the extracts, *D. perkinsae* had higher antibacterial activity (0.12 mg/ml) with the average total activity of 1.5 ml/g, followed by *A. mannii* (average MIC 0.30 mg/ml), and *B. fistulosa* (average MIC 0.70 mg/ml). However, the average total activity (8.8 ml/g) of *B. fistulosa* indicated his overall superiority in terms of antibacterial activity among all the test extracts. The total activity, calculated by dividing mass in mg of the extract obtained from one gram of plant material with his MIC value, indicates to what volume the extract can be diluted and still inhibit the growth of the relevant microorganism (Eloff, 2004). This provides a useful measure to compare the antimicrobial activity and potential use of different plants (Eloff, 2000).

3.3. Antioxidant activity

The antioxidant activities of extracts (*D. perkinsiae*, *A. mannii* and *B. fistulosa*) and some isolated compounds (**3**, **5**–**10**) were determined using a DPPH method and the results (Table 3) are reported in terms of the concentration of the sample decreasing 50% of free radical scavenging (IC_{50}). Among the tested extracts *B. fistulosa* had the highest activity (IC_{50} of 100.16 μg/ml) followed by the extracts of *D. perkinsiae* (IC_{50} of 130.66 μg/ml), and *A. mannii* (IC_{50} of 361.30 μg/ml). Compounds **3** and **5** had significant antioxidant activities with the IC_{50} of 9.84 and 9.99 μg/ml compared to that of ascorbic acid (IC_{50} of 2.41 μg/ml). Apart from compound **8** that was moderately active (IC_{50} of 24.27 μg/ml), other test compounds had weak DPPH scavenging activities with IC_{50} ranging from 106.29 to 484.49 μg/ml. The antioxidant activity of compounds **3**, **5** and **8** could be assigned to their phenolic nature. The presence of *ortho*-hydroxyls and α,β-unsaturated ketone groups in the structures of **3** and **5** could be responsible of their interesting radical scavenging activities (Simić et al., 2007; Brand-Williams et al., 1995).

Table 3
Antioxidant activities of crude extracts and some compounds (**3**, **5**–**10**) isolated from *D. perkinsiae*, *B. fistulosa* and *A. mannii*.

Samples	IC_{50} (μg/ml)
Crude extracts[a]	
BF	100.16 ± 0.02
DP	130.66 ± 0.03
AM	361.30 ± 0.08
Compounds[b]	
3	9.99 ± 0.02
5	9.84 ± 0.06
6	185.46 ± 0.03
7	484.49 ± 0.04
8	24.27 ± 0.01
9	234.96 ± 0.02
10	106.29 ± 0.04
Reference	
L-ascorbic acid	2.41 ± 0.01

[a] DP: *Dissotis perkinsiae*, BF: *Barteria fistulosa*, AM: *Adenocarpus mannii*.
[b] **3**: quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside, **5**: ellagic acid, **6**: iso-prunetin, **7**: chrysin 7-*O*-*β*-D-glucopyranoside, **8**: isovitexin, **9**: hederagenin, **10**: shanzhiside methyl ester.

4. Conclusion

The results obtained support the traditional use of these three plant species (*D. perkinsiae*, *A. mannii* and *B. fistulosa*) in traditional medicine for the treatment of infections. Extract and some isolated compounds that were active in this study could be useful for development of new antimicrobial drugs. Nevertheless, further pharmacological and toxicity investigations are currently going on in our laboratory for their safely used as topical antimicrobial agents.

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