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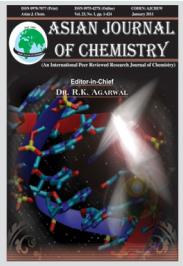

Chemical Constituents of *Dorstenia convexa* (Moraceae)

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Triterpenoids, sterols and coumarins were isolated from the leaves of *Dorstenia convexa*. Betulonic acid (**2**), platanic acid (**3**), betulinic acid (**4**), 3,20-dioxo-30-norlupan-28-oic acid (**5**), lupenone (**11**) and 29-norlupan-3,20-dione (**12**) are reported from *Dorstenia* genus for the first time. Some isolated compounds were evaluated for their antimicrobial activity. Compounds **5** and **12** as well as the crude $CHCl_3$/MeOH (1:1) extract exhibited significant antimicrobial activity at 40 µg/mL.

Key Words: *Dorstenia convexa*, Moraceae, Triterpenoid, Coumarin, Antimicrobial.

INTRODUCTION

There are about 170 species of the genus *Dorstenia* worldwide[1]. *Dorstenia convexa* De Wild. (Moraceae) is a prostrate herb of forest which undergrowth at roadsides and damp places. The leaves (2.5 cm × 6 cm) are alternate and have no petioles[2]. The leaf-infusion of *D. convexa* is used as an enema for a children's disease[2].

No phytochemical study on *D. convexa* De Wild. has been reported so far. However, the chemical constituents of the genus *Dorstenia* have been reported to be coumarins, furanocoumarins[3-7], flavonoids[3,8-16] and triterpenoids[17,18]. The widespread use of *Dorstenia* in indigenous medicine for different ailments, as well as the search for the chemical constituents of Cameroonian medicinal plants justified further attempts to isolate and identify active compounds[19].

EXPERIMENTAL

Melting point is uncorrected and was obtained with a micro melting point apparatus (Yanaco, Tokyo-Japan). Optical rotation values were measured with a Horiba SEPA-300 polarimeter and IR spectra were recorded with JASCO J-20A spectrophotometer. ^1H and ^{13}C NMR spectra were acquired with a Jeol EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Mass spectra were obtained with a Jeol JMS-700 instrument. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan), Sephadex LH-20 (Pharmacia, Sweden) and ODS (Fuji Silysia, Japan). TLC analysis was carried out by using precoated silica gel plates (Merck) and the spots were detected by spraying with H_2SO_4/10 % vanillin and then heating. Flash chromatography was carried out on silica gel (230-400 mesh). R_f values were measured on polygram SIL G/UV_{254} (Macherey-Nagel and Co.).

The leaves of *D. convexa* De Wild. (Moraceae) were collected from Kumba in the South West Province of Cameroon in January 2008 and identified by Mr. Victor Nana of the National Herbarium, Yaoundé where a voucher specimen (53450/H.N.C.) was deposited.

Extraction and isolation of organic compounds: Dried crushing leave (1.4 kg) of *D. convexa* were extracted with a mixture of $CHCl_3$/MeOH (1:1) during 18 h at room temperature. Evaporation of the solvents under reduced pressure yielded 47 g of extract. 45 g of this extract were fractionated by column chromatography over silica gel (70-230 mesh, 400 g) eluted with a mixture of *n*-hexane-EtOAc and EtOAc-MeOH of increasing polarity. 110 Fractions of 200 mL each were collected and monitoring on the basis of their TLC gave five major fractions (fractions I-V). Fraction I (3 g) was subjected to silica gel column chromatography (2 cm × 45 cm, 40 g), eluting with *n*-hexane/EtOAc gradient mixtures to afford lupeol (**1**, 57 mg)[20] and a mixture of sterols (112 mg, GC showed the presence of β-sitosterol, stigmasterol, campesterol and stigmastanol)[21,22]. Fraction II (10 g) was passed through Sephadex LH-20 column (4 cm × 150 cm, 300 g, Pharmacia) and eluted with $CHCl_3$/MeOH (2:1). Fractions collected were

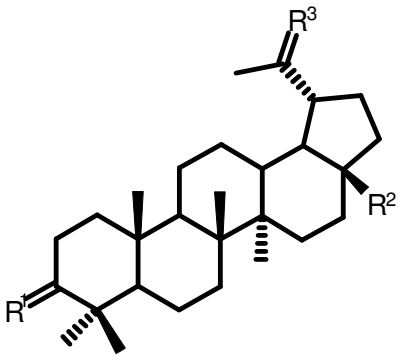
purified by preparative TLC and chromatography column using $CHCl_3$/MeOH (1:1) as eluent, to afford betulonic acid (**2**, 13.0 mg)[23], platanic acid (**3**, 8.3 mg)[24] and betulinic acid (**4**, 23.0 mg)[25,26]. Fraction III (1 g) was subjected to a small column chromatography over silica gel (1 cm × 50 cm, 20 g, 200-300 mesh) eluting with $CHCl_3$/MeOH gradient mixtures to yield 3,20-dioxo-30-norlupan-28-oic acid (**5**, 17.3 mg)[27] and psoralen (**6**, 9.3 mg)[28]. Fraction IV (4 g) was applied to a Sephadex LH-20 column and eluted with MeOH to yield bergapten (**7**, 10.0 mg)[29], 7-hydroxycoumarin (**8**, 5.8 mg)[30] and 7-methoxycoumarin (**9**, 17.0 mg)[33]. The last fraction (V, 5 g) was subjected to further separation and purification using preparative TLC and chromatography column to yield β-sitosterol glucoside (**10**, 135.7 mg)[31], lupenone (**11**, 11.6 mg)[32] and 29-norlupan-3,20-dione (**12**, 6.8 mg)[33].

Antimicrobial assay: Agar diffusion tests were performed in the usual manner[34] with *Bacillus subtilis* and *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient broth), *Streptomyces viridochromogenes* (M Test agar), the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar) and three microalgae (*Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*).

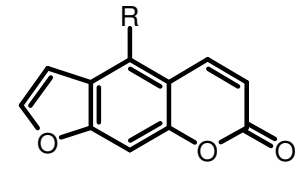
Compounds were dissolved in an azeotrope $CHCl_3$/MeOH (87:13) and 40 µg/mL pro paper disks (Ø 9 mm) were impregnated with each using a 100 µL syringe, dried for 1 h under sterile conditions and placed on the pre-made agar test plates. Bacteria and fungi plates were kept in an incubator at 37 ºC for 12 h, micro algae plates for 3 days at room temperature in a day light incubator. The diameter of inhibition zones was measured.

RESULTS AND DISCUSSION

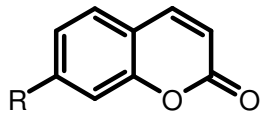
Identification of natural products: The chemical structures of the isolated compounds **1-12** (Fig. 1) were determined by spectral data 1D (^1H and ^{13}C NMR) and 2D (^1H-^1H COSY, HSQC and HMBC) and by comparison of their spectroscopic data with those reported in the literature. None of the compounds is new. However betulonic acid (**2**), platanic acid (**3**), betulinic acid (**4**), 3,20-dioxo-30-norlupan-28-oic acid (**5**), lupenone (**11**) and 29-norlupan-3,20-dione (**12**) are reported from this genus for the first time. On the other hand, 3,20-dioxo-30-norlupan-28-oic acid (**5**) and 29-norlupan-3,20-dione (**12**) were previously isolated from *Platanus hybrida* (Platanaceae), *Salacia chinensis* (Hippocrateaceae), respectively and they are reported in Moraceae family for the first time.



	R^1	R^2	R^3
1	H, OH (β)	CH_3	CH_2
2	O	COOH	CH_2
3	H, OH (β)	COOH	O
4	H, OH (β)	COOH	CH_2
5	O	COOH	O
11	O	CH_3	CH_2
12	O	CH_3	O



6: R = H
7: R = MeO



8: R = OH
9: R = MeO

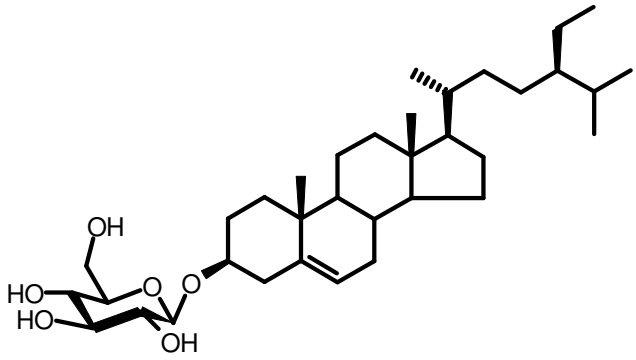


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Fig. 1. Compounds **1-12** from *D. convexa*

Biological assays: Compounds **1-5**, **11-12** as well as the crude $CHCl_3$/MeOH (1:1) extract were tested for their antimicrobial potential against two fungi (*Mucor miehi* and *Candida albicans*), four bacteria (*Bacillus subtilis*, *Streptomyces viridochromogenes*, *Staphylococcus aureus* and *Escherichia coli*) and three microalgae (*Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*) in the agar diffusion test, as show in Table-1.

The crude $CHCl_3$/MeOH (1:1) extract exhibited *in vitro* significant antimicrobial activity against *M. miehi* and *S. aureus*. 3,20-Dioxo-30-norlupan-28-oic acid (**5**) and 29-norlupan-3,20-dione (**12**) also exhibited *in vitro* significant antimicrobial activity against *M. miehi* and *C. albicans* compared to the nystatin as reference.

Chemotaxonomic: In this study, triterpenoids (lupeol, betulonic acid, platanic acid, betulinic acid, 3,20-dioxo-30-norlupan-28-oic acid, lupenone, 29-norlupan-3,20-dione), steroids (sterols, β-sitosterol glucoside) and coumarins/furanocoumarins (psoralen, bergapten, 7-hydroxycoumarin, 7-methoxycoumarin) were isolated. The coumarins/furanocoumarins as well as β-sitosterol glucoside, lupeol and sterols were previously isolated from some *Dorstenia* species (*D. angusticornis*, *D. barnimiana*, *D. barteri*, *D. brasiliensis*, *D. bryoniifolia*, *D. cayapiaa*, *D. contrajerva*, *D. dinklagei*, *D. drakena*, *D. elliptica*, *D. excentrica*, *D. foetida*, *D. gigas*, *D. kameruniana*, *D. lindeniana*, *D. mannii*, *D. multiradiata*, *D. picta*, *D. poinsettifolia*, *D. prorepens*, *D. psilurus*, *D. turbinata*, *D. zanzibrica* and *D. zenkeri*). Interestingly, lupeol (**1**), platanic acid (**3**) and betulinic acid (**4**) were previously isolated from

TABLE-1
ANTIMICROBIAL ACTIVITIES OF THE CRUDE $CHCl_3$/MeOH (1:1) EXTRACT AND COMPOUNDS **1-5**, **11-12**

Micro-organisms tested	Ext.	1	2	3	4	5	11	12	Nyst.
Bacillus subtilis	12	11	11	10	12	13	10	10	16
Streptomyces viridochromogenes	10	10	12	12	9	10	10	11	14
Staphylococcus aureus	15	11	10	10	10	10	9	9	12
Escherichia coli	11	13	12	10	10	10	12	12	14
Mucor miehi	17	13	11	11	10	19	14	16	15
Candida albicans	12	10	12	15	14	21	13	19	18
Chlorella vulgaris	10	12	11	13	10	10	11	11	–
Chlorella sorokiniana	11	10	10	11	13	13	10	10	–
Scenedesmus subspicatus	10	10	10	10	11	16	13	14	–

Ext. = Crude $CHCl_3$/MeOH (1:1) extract, Nyst. = Nystatin used as reference. Diameter of inhibition zones in mm.

Ficus benjamina[24]. This study can be used to establish relationship between *Ficus* and *Dorstenia*, two genera of the Moraceae family. The few differences between the secondary metabolites isolated from *D. convexa* and other *Dorstenia* species are may be related to the real specific differences or more probably to a geographic or environmental influence on biosynthesis[35].

The isolation of compounds **1** and **6-9** from *D. convexa* indicates that, these compounds could be chemotaxonomic markers for the *Dorstenia* genus. Betulinic acid is reported as having good activity against cancer cells of neuroectodermal origin and leukaemias[36] and it is highly selective inhibitors of HIV-1[37].

Flavonoids which are common on this genus were not isolated. Nevertheless, the isolation of seven triterpenoids, two steroids and four coumarins from *D. convexa* is a significant phytochemical report and can be used for further chemotaxonomic studies on the *Dorstenia* genus.

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