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Chemical constituents and antifungal effects of *Hyptis lanceolata* Poir.

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Abstract
This work focuses on the chemistry and antifungal effects of *Hyptis lanceolata* Poir. (Lamiaceae) a Cameroonian species. The phytochemical study led to the isolation and characterization of twelve known compounds. Seven triterpenes, namely betulin (1), betulinic acid (2), lupeol (3), *α*-amyrin (4), ursolic acid (5), oleanolic acid (6), and taraxerol (7), three phytosterols, stigmasterol (8), *β*-sitostrol (9) and *β*-sitosterol-3-*O*-*β*-*D*-glucopyranoside (10), two fatty acid namely hexadecanoid acid (11) and hexacosanol (12). The structures of these compounds were elucidated on the basis of their NMR and ESIMS experiments, in comparison with those reported in the literature. The crude extract and isolated compounds were evaluated for their antifungal activities on three fungal strains *Candida albicans*, *Candida krusei* and *Candida parasilosis*. Compound 5 and 6 showed significant antifungal activity on the strain *Candida krusei* with the same MIC value of 62.5 µg/mL while compounds 1, 2, 11 and 12 showed weakly activity with the same MIC value of 125 µg/mL. The crude extract, compounds 11, 1 and 2 exhibited weakly activity on the strain *Candida albicans* with the same MIC value of 125 µg/mL while compounds 6 and 11 showed weakly activity on *Candida parasilosis* with the same MIC value of 125 µg/mL.

Keywords: Lamiaceae, *Hyptis lanceolata* Poir., triterpene, antifungal activity

Introduction
The genus *Hyptis* Jacq. is one of the 240 genera of Lamiaceae family and it includes approximately 400 species, with five of them distributed in West tropical Africa including the different mountainous regions of Cameroon [1]. The plants from *Hyptis* genus are herbs and shrubs growing up to 2 m tall with opposite leaves. The plant of *Hyptis* genus are also known to be used in folk medicine for the treatment of various diseases, such as influenza and constipation (*Hyptis fruticosa* Salzm. ex Benth); respiratory diseases (*Hyptis macrostachys* Benth); stomach, intestinal disorders and bactericidal (*Hyptis martiusii* Benth.); colic and liver diseases (*Hyptis pectinata*); nasal and atrial disorders (*Hyptis umbrosa* Salzm. ex Benth.), and fever (*Hyptis suaveolens*) [2]. The chemical study of this genus revealed a wide variety of chemical constituents, including flavonoids [3], monoterpenes [3], diterpenes [4], triterpenes [5,6,7] and steroids [8, 9]. *Hyptis lanceolata* Poir. is an aromatic herbaceous broad leaf which growths up to 80 cm high. The leaves are simple and opposite, while the flowers are white and are grouped in globular clusters dense, located in the leaf axils [10]. The leaves of *Hyptis lanceolata* are used in the treatment of cutaneous and subcutaneous parasitic infections such as eczema, ringworm, rashes, athlete foot, etc [11]**.** In our best knowledge, it is the first time to carry out the phytochemical study of *Hyptis lanceolata,* but the GC-MS analysis of the methanolic extract from the leaves revealed the presence of α-Linolenic acid (45.86%), dihomo ɣ-linolenic acid (30.50%), squalene (8.27%) and Imidodicarbonimidic diamide, N,N-dimethyl- (6.61%) [12]. In this study we report herein the isolation, structural elucidation and antifungal effects of the isolated compounds from *Hyptis lanceolata*.

Materials and Methods
General procedures
IR spectra were determined on a JASCO Fourier transform IR-420 spectrometer (JASCO). Ultraviolet spectra were recorded in MeOH on a Hitachi UV 3200 spectrophotometer and infrared spectra on a JASCO 302-A spectrophotometer (Thermo Scientific, Waltham, MA, USA). ESI-HR mass spectra were measured on an Agilent 6220 TOF LCMS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and EI-MS on a Finnigan MAT 95 spectrometer (70 eV) (Thermo Fisher Scientific, Darmstadt, Germany) with

perfluorokerosene as reference substance for ESI-HR-MS. The ^{1}H- and ^{13}C-NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on Bruker DRX 500 NMR spectrometers (Bruker, Rheinstetten, Germany) in $CDCl_3$. Chemical shifts are reported in δ (ppm) using tetramethylsilane (TMS) (Sigma-Aldrich, Munich, Germany) as internal standard, while coupling constants (J) were measured in Hz. Column chromatography was carried out on silica gel 230–400 mesh (Merck, Darmstadt, Germany), silica gel 70–230 mesh (Merck, Darmstadt, Germany), and Sephadex LH-20 (Sigma-Aldrich, Munich, Germany). Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F254 aluminum foil (Merck, Darmstadt, Germany), and spots were detected using diluted sulfuric acid spray reagent after heating. The molecular composition of the isolated compounds was identified by exact mass determinations. All reagents used were of analytical grade.

Plant materials

The whole plants of *Hyptis lanceolata* were collected on 12th January 2019 at Loum, a locality in Littoral region of Cameroon. The plant was identified by the botanist Mr. Nana Victor of the National Herbarium of Yaounde - Cameroon, where a voucher specimen was deposited under reference number 24136/SRSCAM/HNC.

Extraction and isolation

The air-dried and powdered whole plants of *Hyptis lanceolata* (1.6 kg) were macerated twice in MeOH for 48h and 24h, respectively. The solvent was evaporated under reduced pressure to yield 25.5 g of crude extract. A portion of 25.0 g was chromatographed over silica gel 60 (70–230 mesh) using a gradient of EtOAc in hexane from 95.5: 0.5 to 0: 100 (v/v). 610 sub-fractions (200 mL each) were collected and combined on the basis of TLC analysis resulting in eight main fractions F1 – F8. Hexadecanoic acid (17.15 mg), the mixture of β-sitosterol and stigmasterol (15.20 mg), as well as β-sitosterol-3-*O*-β-D-glucopyranoside (50.25 mg) precipitated as white powders in the sub-fractions F1 [85.10 mg, Hexane – EtOAc (95.5 : 0.5, v/v)], F2 [60.55 mg, Hexane – EtOAc (90 : 10, v/v)], and F8 [150.30 mg, Hexane – EtOAc (30:70, v/v)], respectively. Sub-fraction F3 [75.10 mg, Hexane – EtOAc (85 : 15, v/v)] was further chromatographed on silica gel with an isocratic solvent system of Hexane – EtOAc (90:10, v/v) to obtain compounds **3** (7.25 mg), **4** (8.15 mg) and **7** (8.30 mg) while F4 [90.25 mg, Hexane – EtOAc (80 : 20, v/v)] was purified on silica gel column chromatography with an isocratic elution using the solvent system of Hexane – EtOAc (85 : 15, v/v) to afford compound **2** (5.2 mg) and **2** (7.25 mg). By the same means, using isocratic solvent system of Hexane – EtOAc (70 : 30, v/v), compound **5** (15.15 mg) and **6** (17.20 mg) was obtained from F5 [125.30 mg, Hexane – EtOAc (60 : 40, v/v)]. Finally, F6 [50.35 mg, Hexane – EtOAc (50 : 50, v/v)] was purified by silica gel column chromatography with Hexane - EtOAc (60 : 40 v/v), to afford compound **7** (10.25mg).

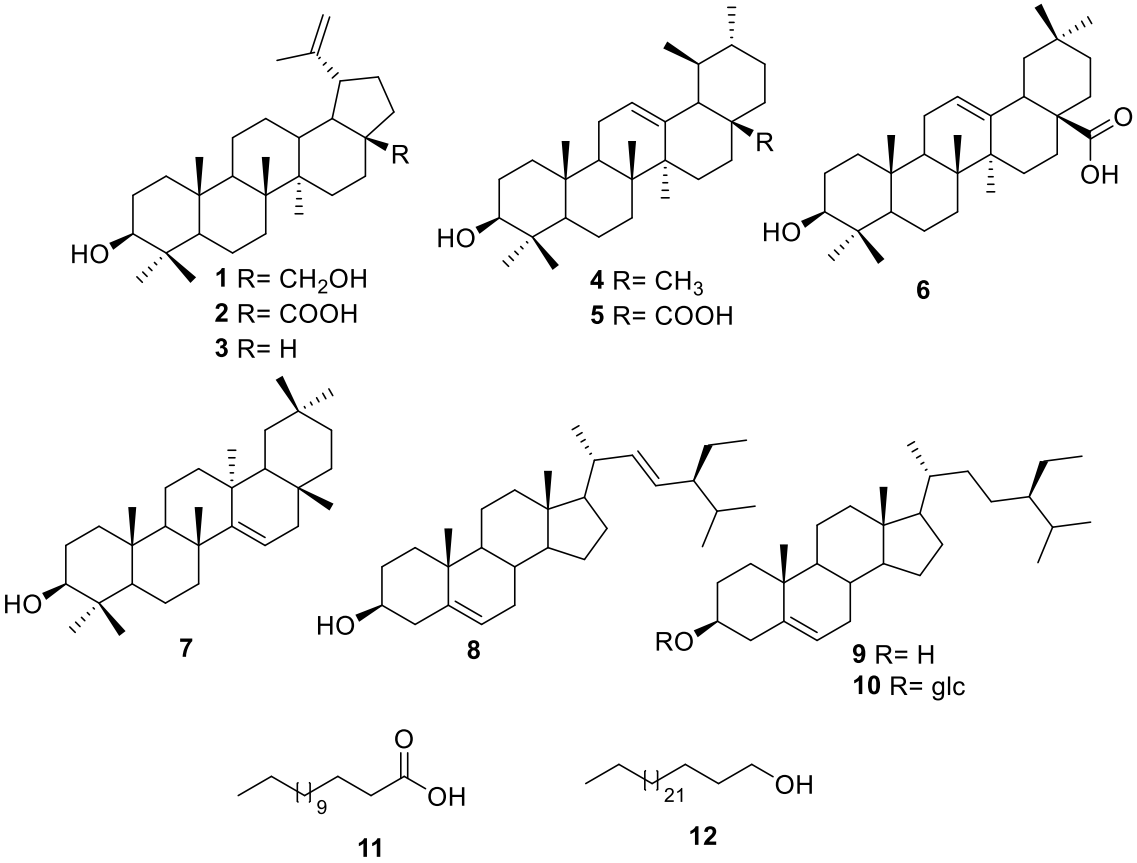

Fig 1: Chemical structures of compounds 1-12 from *Hyptis lanceolata.*

Betulin (1): White powder. ^{13}C NMR ($CDCl_3$, 125 MHz): 151.6 (C-20), 109.3 (C-29), 79.2 (C-3), 55.3 (C-5), 50.4 (C-9), 48.3 (C-19), 48.0 (C-18), 43.0 (C-17), 42.8 (C-14), 40.8 (C-8), 40.0 (C-22), 38.8 (C-4), 38.7 (C-1), 28.0 (C-13), 37.1 (C-10), 35.6 (C-16), 34.3 (C-7), 29.7 (C-21), 28.0 (C-23), 27.4 (C-2, C-15), 25.1 (C-12), 20.9 (C-11), 19.3 (C-30)18.3 (C-6), 68.9 (C-28), 16.1 (C-26), 15.9 (C-25), 15.3 (C-24), 14.5 (C-27).

Betulinic acid (2): White amorphous powder. ^{13}C NMR (CD_3OD, 125 MHz): 180.6 (C-28), 150.0 (C-20), 108.8 (C-29), 78.9 (C-3), 56.3 (C-17), 50.7 (C-9), 49.4 (C-19), 47.1 (C-18), 42.5 (C-14), 40.8 (C-8), 39.0 (C-1, C-4), 38.2 (C-13), 37.3 (C-10, C-22), 35.5 (C-5), 34.5 (C-7), 32.6 (C-16), 30.4 (C-15), 29.9 (C-21), 27.9 (C-23), 27.6 (C-2), 25.6 (C-12), 21.0 (C-11), 19.6 (C-30), 18.4 (C-6), 16.3 (C-26), 16.2 (C-25), 15.4 (C-24), 14.6 (C-27).

Lupeol (3): White needles. ^{13}C NMR ($CDCl_3$, 125 MHz): 151.6 (C-20), 109.3 (C-29), 79.0 (C-3), 55.3 (C-5), 50.4 (C-9), 48.3 (C-19), 48.0 (C-18), 43.0 (C-17), 42.8 (C-14), 40.8 (C-8), 40.0 (C-22), 38.8 (C-4), 38.7 (C-1), 28.0 (C-13), 37.1 (C-10), 35.6 (C-16), 34.3 (C-7), 29.7 (C-21), 28.0 (C-23), 27.4 (C-2, C-15), 25.1 (C-12), 20.9 (C-11), 19.3 (C-30)18.3 (C-6), 18.0 (C-28), 16.1 (C-26), 15.9 (C-25), 15.3 (C-24), 14.5 (C-27).

***α*-amyrin (4)**: white powder. ^{13}C NMR (CD_3OD, 125 MHz): 139.5 (C-13), 124.4 (C-12), 79.6 (C-3), 59.0 (C-18), 55.1 (C-5), 47.7 (C-9), 42.0 (C-14), 41.5 (C-22), 40.7 (C-8), 39.6 (C-19), 39.6 (C-20), 38.7 (C-1, C-4), 36.6 (C-10), 33.7 (C-17), 32.2 (C-7), 31.2 (C-21), 28.7 (C-2), 28.4 (C-28), 27.2 (C-15), 26.6 (C-16), 23.3 (C-11), 23.2 (C-25, C-27), 21.4 (C-30), 18.4 (C-6), 17.1 (C-29), 16.8 (C-24, C-26), 15.6 (C-23).

Ursolic acid (5): white powder. ^{13}C NMR (CD_3OD, 125 MHz): 138.2 (C-13), 125.2 (C-12), 79.1 (C-3), 47.5 (C-18), 55.4 (C-5), 47.8(C-9), 41.8 (C-14), 37.2 (C-22), 38.9 (C-8), 46.9(C-19), 31.2 (C-20), 38.8 (C-1), 38.9 (C-4), 37.6 (C-10), 32.6 (C-17), 32.8 (C-7), 34.8 (C-21), 27.3 (C-2), 28.5 (C-28), 26.3 (C-15), 27.0 (C-16), 23.6 (C-11), 15.6 (C-25), 26.1 (C-27), 23.7 (C-30), 18.5 (C-6), 33.4 (C-29), 15.6 (C-24), 16.9 (C-26), 28.3 (C-23).

Oleanolic acid (6): white powder. ^{13}C NMR (CD_3OD, 125 MHz): 143.2 (C-13), 121.9 (C-12), 79.1 (C-3), 47.5 (C-18), 55.4 (C-5), 47.8(C-9), 41.8 (C-14), 37.2 (C-22), 38.9 (C-8), 46.9(C-19), 31.2 (C-20), 38.8 (C-1), 38.9 (C-4), 37.6 (C-10), 32.6 (C-17), 32.8 (C-7), 34.8 (C-21), 27.3 (C-2), 28.5 (C-28), 26.3 (C-15), 27.0 (C-16), 23.6 (C-11), 15.6 (C-25), 26.1 (C-27), 23.7 (C-30), 18.5 (C-6), 33.4 (C-29), 15.6 (C-24), 16.9 (C-26), 28.3 (C-23).

Taraxerol (7): colourless crystal. ^{13}C NMR ($CDCl_3$, 125 MHz): 116.8 (C-15), 158.4 (C-14), 78.1 (C-3), 49.3 (C-18), 55.6 (C-5), 48.9 (C-9), 36.0 (C-12), 33.2 (C-22), 38.9 (C-8), 41.4 (C-19), 28.9 (C-20), 38.0 (C-1), 39.3 (C-4), 37.7 (C-10), 37.9 (C-17), 35.2 (C-7), 33.9 (C-21), 27.1 (C-2), 30.0 (C-28), 37.8 (C-13), 36.6 (C-16), 17.7 (C-11), 15.4 (C-25), 25.9 (C-27), 21.4 (C-30), 18.9 (C-6), 33.7 (C-29), 15.6 (C-24), 30.1 (C-26), 28.1 (C-23), 21.6 (CH_3CO-).

Hexadecanoic acid (11): white amorphous powder. ^{13}C NMR ($CDCl_3$, 125 MHz): 177.5 (C-1), 14.0 (C-16), 33.2 (C-2), 31.6 (C-3), 22.6-29.7 (C-4 – C-15).

Hexacosanol (12): white amorphous powder. ^{13}C NMR ($CDCl_3$, 125 MHz): 68.8 (C-1), 14.1 (C-26), 33.3 (C-2), 31.7 (C-3), 22.9-29.6 (C-4 – C-25).

Antifungal assay

The antifungal activities of the crude extract and compounds **1**–**12** were evaluated against three fungal strains *Candida albicans*, *Candida krusei* and *Candida parasilosis* using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical and Laboratory Standards, NCCLS) for yeasts (M27-A2) [13] with Nystatin as reference.

Result and Discussion

In the present work, twelve compounds were isolated and characterized from the whole plant of *Hyptis anceolata*. Their structures were elucidated based on the NMR and ESI-MS analysis, further supported by comparison with previous data reported in the literature but this chemical study is reported for the first time on this plant in the best of our knowledge. They are : betulin (1) [14], betulinic acid (2) [14], lupeol (3) [14], *α*-amyrin (4) [8], ursolic acid (5) [15], oleanolic acid (6) [15], taraxerol (7) [16], stigmasterol (8) [8,9], *β*-sitostrol (9) [8,9], *β*-sitostrol-3-*O*-*β*-*D*-glucopyranoside (10) [8,9], hexadecanoid acid (11) [17] and hexacosanol (12) [17]. Among them, compounds 7 is being reported for the first time from the genus *Hyptis*.

The isolated compounds and crude extract were tested for their antifungal properties against *Candida albicans*, *Candida krusei* and *Candida parasilosis*. According to the results (Table 1) compound 5 and 6 showed significant antifungal activity on the strain *Candida krusei* with the same MIC value of 62.5 µg/mL while compounds 1, 2, 11 and 12 showed weakly activity with the same MIC value of 125 µg/mL. The crude extract, compounds 11, 1 and 2 exhibit weak activities on the strain *Candida albicans* with the same MIC value of 125 µg/mL while compounds 6 and 11 showed weakly activities on *Candida parasilosis* with the same MIC value of 125 µg/mL.

Table 1: Results of the antifungal activities on the crude extract and isolated compounds

Samples **Parameters strains**				
		CA	**CK**	**CP**
Extract	MIC (µg/mL)	125	> 125	> 125
	MFC (µg/mL)	250	> 125	> 125
	MFC/MIC	2	ND	ND
Compound 5	MIC (µg/mL)	125	62.5	> 125
	MFC(µg/mL)	250	250	> 125
	MFC/MIC	2	4	ND
Compound 6	MIC (µg/mL)	> 125	62.5	125
	MFC (µg/mL)	ND	250	250
	MFC/MIC	ND	4	2
Compound 11	MIC (µg/mL)	> 125	125	> 125
	MFC (µg/mL)	ND	250	ND
	MFC/MIC	ND	2	ND
Compound 2	MIC (µg/mL)	125	125	> 125
	MFC (µg/mL)	250	250	ND

	MFC/MIC	2	2	ND
Compound 1	MIC (µg/mL)	125	125	125
	MFC (µg/mL)	250	250	250
	MFC/MIC	2	2	2
Compound 12	MIC (µg/mL)	> 125	125	> 125
	MFC (µg/mL)	ND	250	ND
	MFC/MIC	ND	2	ND
Compounds 3, 4, 7, 8, 9 and 10	MIC (µg/mL)	> 125	> 125	> 125
	MFC (µg/mL)	ND	ND	ND
	MFC/MIC	ND	ND	ND
Nystatin	MIC (µg/mL)	0.5	0.5	1
	MFC (µg/mL)	2	1	2
	MFC/MIC	4	2	2

[a]Abbreviation: Minimum inhibitory concentrations (MIC); Minimum fungicidal concentrations (MFC). CA: *Candida albicans*, CK: *Candida krusei*, CP: *Candida parasilosis*.

Conclusion

This study led to the isolation and antifungal activity of isolated compounds from the Cameroonian species *Hyptis anceolata*, thus, it increase the data on the chemical composition of this plant. By the significant antifungal activities of some compounds and crude extract, this work justify the use of this plant in folk medicine to treat cutaneous diseases. Further study will be necessary to evaluate the *in vivo* antifungal effects and toxicity of the crude extract.

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Declaration of interest

The authors report no conflict of interest

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