Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 745-749
Received: 13-09-2020
Accepted: 19-10-2020

Lidwine Ngah
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Jules Lobe Songue
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Jean Pierre Longue Ekon
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Willifred Dongmo Tékapi Tsopgni
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Moses K Langat
(1). Jodrell Laboratory, Natural Capital and Plant Health Department, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, United Kingdom
(2). Natural Products Research Group, Department of Chemistry, Faculty of Engineering and Physical Sciences, University of Surrey, Guildford, GU2 7XH, United Kingdom

Emmanuel Mpondo Mpondo
Department of Pharmaceutical Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, P.O. Box 2701 Douala, Cameroon

Jean Duplex Wansi
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Jean Claude Ndom
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Alain François Kamdem Waffo
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

Corresponding Author:
Alain François Kamdem Waffo
Department of Chemistry, University of Douala, Faculty of Science, 24157, Douala, Cameroon

The chemistry and biology activities of *Vitex phaseolifolius* Hildbr

Lidwine Ngah, Jules Lobe Songue, Jean Pierre Longue Ekon, Willifred Dongmo Tékapi Tsopgni, Moses K Langat, Emmanuel Mpondo Mpondo, Jean Duplex Wansi, Jean Claude Ndom and Alain François Kamdem Waffo

Abstract
We report the chemistry of *Vitex phaseolifolius* Hildbr. (Lamiaceae Martinov), a plant that is endemic to Cameroon, and the antibacterial, antioxidant and anti-inflammatory effects of the isolated compounds. Thirteen compounds, asperphenamate, the triterpenes, bauerenol, *β*-amyrin, oleanolic acid, lupeol, betulinic acid, taraxeryl acetate and friedelanol, the phytosterols, stigmasterol, sitostroland stigmasteryl-3-*O*-*β*-*D*-glucopyranoside and two fatty acids arachidic acid and palmitic acid were isolated and identified from this plant. The structures of these compounds were elucidated based on their NMR and ESIMS experiments, and compared with those reported in the literature. The compounds were found to be inactive against four bacterial strains *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas agarici.* Compounds 6 - 11 and crude extract were evaluated for their DPPH antioxidant properties and anti-inflammatory properties. The compounds were found to be inactive while the crude extract showed good antioxidant activity with an $IC_{50}$of 5.55 µg/mL and a weak percentage of protein denaturation with a percentage of 40%.

Keywords: Lamiaceae Martinov, *Vitex phaseolifolius* Hildbr., bauerenol, antioxydant effects, anti-inflammatory properties

Introduction
The genus *Vitex* L. (Lamiaceae Martinov) includes approximately 203 accepted species that occur as small trees and shrubs, and their native ranges being the tropics and subtropic.[1] There are 21 accepted species in Cameroon, including the endemic *V. phaseolifolius* Hildbr. *V. phaseolifolius* is a tree growing upto 10 m tall, with a bark that peels off in long small thin flakes. The wood is pale yellow and its leaves are papery, gloss and are medium to dark green above, and pale green beneath with red-brown mildrib. This plant was collected from the Centre region of Cameroon where it grows in a secondary forest [2]. It is locally called bepwaa and a water extract of the leaves is used as rectal medicine. The water extract is administered to six to seven month-old children to aid the children to develop walking skills quickly.[3] The chemistry of *V. phaseolifolius* is not documented but other species of the *Vitex* genus are reported to yield flavonoids, phenylpropanoids, diterpenoids, triterpenoids, iridoids glycosides, phytoecdysteroids, sterols, and fatty acids [4-7] These compounds are also reported to exhibit a wide range of bioactivities including as antibacterial, antimalarial, antiviral, antifeedant, antiproliferative, hepatoprotective, antioxidant agents [4-7] In this study we report the chemistry of *V. phaseolifolius* and the antibacterial effects of the isolated compounds. The isolated compounds includedone peptide derivative, asperphenamate (1) [8], the triterpenoids, bauerenol (2) [9], *β*-amyrin(3) [10], oleanolic acid (4) [11], lupeol (5) [10], betulinic acid (6) [12], taraxeryl acetate (7) [10], friedelanol (8) [13], the phytosterols stigmasterol (9), sitostrol, stigmasteryl-3-O-β-*D*-glucopyranoside [10] and the fatty acids arachidic acid [14] and palmitic acid [14]. the antibacterial, antioxidant and anti-inflammatory effects of the isolated compounds and crude extract are also reported.

Materials and Methods
General procedures
FTIR spectra were recorded using a Perkin-Elmer (2000) spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$and CD_3ODon a 500 MHz Bruker AVANCE NMR instrument at room temperature. Chemical shifts (δ) are expressed in ppm and were referenced against the solvent resonances at 7.26 and 77.23 ppm for ^1H and ^{13}C NMR respectively. ESI-HR mass spectra were measured on Agilent Techn. 6220 TOF LCMS mass spectrometer

(Agilent Technologies, Santa Clara, CA, USA) and EI-MS on a Finnigan MAT 95 spectrometer (70 ev) (Thermo Fischer Scientific, Darmstadt, Germany) with perfluorokerosene as reference substance for ESI-HR-MS. Column chromatography was per formed using silica gel of 70-230 mesh (Merck Darmstadt, Germany). Purity of compounds was monitored *via* thin layer chromatography (TLC) using pre-coated aluminium-backed plates (silica gel 60 F_{254}) and compounds were visualised by UV radiation at 254 nm and then using an anisaldehyde spray reagent (1% *p*-anisaldehyde:2% H_2SO_4: 97% cold MeOH) followed by heating. Final purifications used preparative thin layer chromatography (Merck 818133) and gravity column chromatography that was carried out using different column sizes (1-2 cm diameter), which were packed with silica gel (Merck Art. 9385) in selected solvent.

Plant materials

The roots of *V. phaseolifolius* were collected on 12thOctober 2019 at Nlong, a locality in Centre region of Cameroon. Authentication of the plant was performed by M. Nana Victor, taxonomist at the National Herbarium of Cameroon where a voucher specimen (ref. 10852HNC) has been deposited.

Extraction and isolation

The air-dried and powdered roots of *V. phaseolifolius* (1.50 kg) were macerated twice using methylene chloride-methanol (1:1) for 48h and 24h, respectively. The solvent was removed to afford crude extract (20.50 g), which was dissolved in water and partitioned with ethyl acetate (EtOAc) and n-butanol. The EtOAc-soluble fraction (10.35 g) was subjected to silica gel column chromatography using a gradient of EtOAc in hexane (Hex) from 95.5:4.5 to 0:100 (v/v) to afford eleven sub-fractions (V1–V11).Compound 12 (4.50 mg), compound 13 (5.85 mg), the mixture of compound 9 and compound11 (15.50 mg) precipitated after recrystallization as white powders in the sub-fractions V1 [115.01 mg, Hex–EtOAc (95.5:4.5, v/v)]. Sub-fraction V2 [150.20 mg, Hex–EtOAc (90:10, v/v)] was purified on silica gel column chromatography with an isocratic elution using the solvent system of Hex–EtOAc (95:5, v/v) to afford compound 8 (5.05 mg) and compound 7 (6.10 mg). Sub-fraction V3 [102.30 mg, Hex–EtOAc (85:15, v/v)] was further chromatographed on silica gel with an isocratic solvent system of Hex–EtOAc (92.5:7.5, v/v) to obtain compound 5 (5.25 mg), compound 3 and compound 2(8.65 mg), whereas V4 [65.20 mg, Hex–EtOAc (80:20, v/v)] was purified on silica gel column chromatography with an isocratic elution using the solvent system of Hex – EtOAc (90:10, v/v) to afford compound 6 (65.8 mg) and compound 4 (50.50 mg). By the same means, using isocratic solvent system of Hex–EtOAc (70:30, v/v), compound 1(10.20mg) was obtained from V7 [20.35 mg, Hex–EtOAc (60:40, v/v)]. Additionally, compound 10 (58.53 mg) precipitated directly from V9 [120.50 mg, Hex–EtOAc (40:60, v/v)].

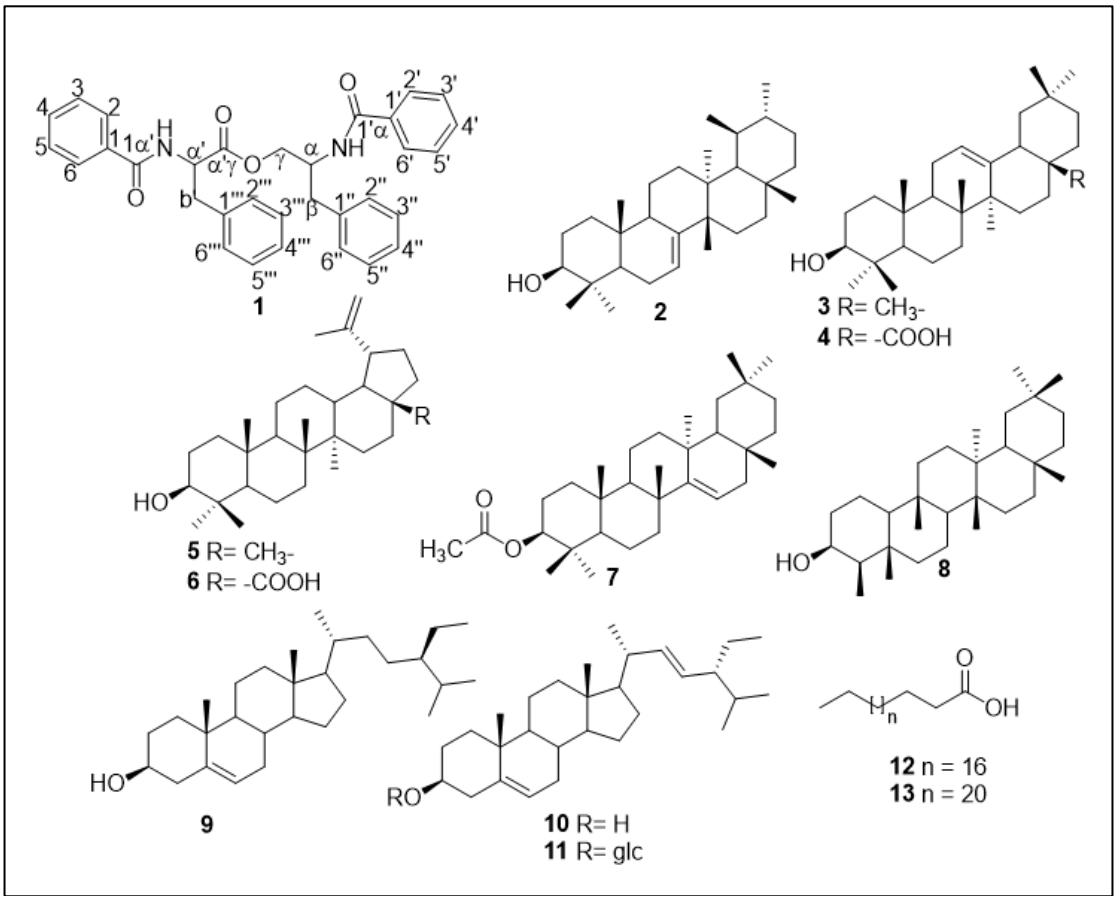

Fig 1: Chemical structures of compounds 1-13 from *V. phaseolifolius*

Asperphenamate (1):White powder;m/z 529.3 $[M+Na]^+$; ^1H NMR ($CDCl_3$, 500 MHz): 4.04 (1H, dd, *J* =11.3, 4.3Hz, H-1a), 4.54 (1H, dd, *J* =113, 3.3Hz, H-1b), 4.62 (1H, m, H-2), 3.00 (1H, dd, *J* =13.7, 6.5Hz, H-3a), 2.89 (1H, dd, *J* =13.7, 8.4Hz, H-3b), 7.40 (1H, m, H-5), 7.26 (1H, m, H-7), 7.70 (1H, m, H-12), 7.33 (1H, m, H-13), 7.53 (2H, m, H-14, H-15), 7.70 (1H, m, H-16), 4.92 (1H, q, *J* =6.7Hz, H-2'), 321 (1H, dd, 14.0, 6.3Hz, H-3'a), 3.30 (1H, dd, 14.0, 6.3Hz, H-3'b), 7.70 (1H, m, H-12'), 7.30 (1H, m, H-13', H-15'), 7.46 (1H, m, H-14'), 7.23 (1H, m, H-16'), 6.65 (1H, d, *J* =8.4Hz, N-

Ha), 6.54 (1H, d, *J* =65Hz, N-Hb). ^{13}C NMR ($CDCl_3$, 125MHz): 172.0 (C-1'), 167.5 (C-10), 167.3 (C-10'), 137.3 (C-4), 135.9 (C-4'), 133.5 (C-11), 134.3 (C-11'), 132.2 (C-14), 131.5 (C-14'), 129,0 (C-5, C-9, C-5', C-9'), 128.8 (C-13, C-15, C-13', C-15'), 128.6 (C-6, C-8, C-6', C-7', C-8'), 127.3 (C-12, C-16), 127.2 (C-12', C-16'), 126.9 (C-7), 65.1 (C-1), 54.6 (C-2'), 50.4 (C-2), 37.4 (C-3), 37.2 (C-3').

Bauerenol (2): white powder. ^{13}C NMR ($CDCl_3$, 125 MHz): 114.3 (C-7), 148.8 (C-8), 78.9 (C-3), 55.0 (C-18), 50.0 (C-5), 47.2 (C-9), 41.1 (C-14), 37.8 (C-22), 37.4 (C-13), 35.1 (C-19), 38.0 (C-20), 36.5 (C-1, C-4), 35.0 (C-10), 32.1 (C-17), 32.5 (C-12), 29.2 (C-21), 24.2 (C-2), 32.1 (C-28), 28.9 (C-15), 31.5 (C-16), 16.5 (C-11), 13.0 (C-25), 22.3(C-27), 22.1 (C-30), 24.0 (C-6), 25.6 (C-29), 15.3 (C-24), 23.6 (C-26), 27.5 (C-23).

***β*-amyrin (3)**: 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).

Oleanolic acid (4): 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).

Lupeol (5): 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).

Betulinic acid (6): 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).

Taraxeryl acetate (7): colourless crystal. ^{13}C NMR ($CDCl_3$, 125 MHz): 116.9 (C-15), 158.2 (C-14), 80.1 (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 ($\underline{C}H_3CO$-), 171.0 ($\underline{C}H_3\underline{C}O$-).

Friedelanol (8): colourless crystals. ^{13}C NMR ($CDCl_3$, 125 MHz): 39.5 (C-13), 30.2 (C-12), 72.7 (C-3), 42.8 (C-18), 37.7 (C-5), 37.8 (C-9), 38.8 (C-14), 39.2 (C-22), 52.5 (C-8), 35.2 (C-19), 28.3 (C-20), 20.1 (C-1), 49.0 (C-4), 61.3 (C-10), 30.6 (C-17), 17.8 (C-7), 32.6 (C-21), 34.9 (C-2), 31.9 (C-28), 32.6 (C-15), 36.2 (C-16), 35.5 (C-11), 17.9 (C-25), 18.5 (C-27), 31.8 (C-30), 41.6 (C-6), 34.9 (C-29), 14.5 (C-24), 20.2 (C-26), 11.8 (C-23).

Stigmasterol (9): white amorphous powder.^{1}H NMR ($CDCl_3$, 500 MHz): 1.15 (1H, m, H-1), 1.44 (11H, m, H-2, H-8, H-9, H-11, H-12, H-14), 3.52 (1H, m, H-3), 1.98 (2H, m, H-4), 5.35 (1H, m, H-6), 1.85 (2H, m, H-7), 1.54 (4H, m, H-15, H-16), 1.53 (1H, m, H-17), 0.68 (3H, s, H-18), 1.02 (3H, s, H-19), 2.27 (1H, m, H-20), 0.87 (3H, d, *J*=6.3Hz, H-21), 5.13 (1H, dd, *J*=12.0, 8.0Hz, H-22), 5.03 (1H, dd, *J* =12.0, 8.0Hz, H-23), 2.22 (1H, m, H-24), 1.85 (1H, s, H-25), 0.85 (3H, d, *J* =6.8Hz, H-26), 0.82 (3H, d, *J* =6.8Hz, H-27), 1.25 (3H, m, H-28), 0.82 (3H, d, *J* =6.9Hz, H-29).

Stigmasteryl 3-*O*-*β*-*D*-glucopyranoside (11): white amorphous powder. ^{13}C NMR (CD_3OD, 125 MHz): 141.0 (C-5), 138.8 (C-22), 129.6 (C-23), 121.9 (C-6), 102.6 (C-1'), 78.6 (C-3), 78.4 (C-3'), 78.2 (C-5'), 75.4 (C-2'), 71.8 (C-4'), 63.0 (C-6'), 57.0 (C-14), 56.2 (C-17), 51.5 (C-24), 50.4 (C-9), 42.4 (C-4, C-13), 40.8 (C-20), 39.4 (C-12), 37.5 (C-1), 37.0 (C-10), 32.2 (C-2, C-7), 32.1 (C-8, C-25), 29.3 (C-16), 25.7 (C-28), 24.6 (C-15), 21.3 (C-21), 19.5 (C-11, C-26), 19.3 (C-27), 19.2 (C-19), 12.5 (C-29), 12.2 (C-18).

Arachidic acid (12): white amorphous powder. ^{13}C NMR ($CDCl_3$, 125 MHz): 178.6 (C-1), 14.0 (C-20), 33.2 (C-2), 31.6 (C-3), 22.6-29.7 (C-4 – C-19).

Palmitic acid (13): white amorphous powder. ^{13}C NMR ($CDCl_3$, 125 MHz): 178.8 (C-1), 14.1 (C-16), 33.3 (C-2), 31.7 (C-3), 22.9-29.6 (C-4 – C-15).

Antibacterial assay

The antibacterial activities of the crude extractand compounds1–13were evaluated against four gram positive bacteria strains*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas agarici* using the micro-dilution broth susceptibility with ciprofloxacin as reference and they were found to be inactive.[15]

DPPH antioxidant activity

The DPPH antioxidant activity was estimated with the modified method of Govindarajan *et al*. [16] with few modifications. Accordingly, one milliliter of various concentrations (3.75-30 µg/mL) of the compounds and ascorbic acid (standard antioxidant) was added to 3 mL methanol solution of DPPH (20 mg/L) in a test tube. The reaction mixture was vigorously shaken and left to stand for 30 min in the dark at 25 °C. The absorbance of the residual DPPH was determined at 517 nm using a BIOBASE BK-UV-1600 PC single Beam. The concentration of each sample required for scavenging 50% of the free DPPH radicals (IC_{50}) was determined graphically by plotting the percentage of DPPH decrease as a function of the sample concentration (Eqn. 1):

$$\% \text{ DPPH radical scavenging} = 100\left(1 - \frac{OD_{sample}}{OD_{control}}\right) \quad \text{(Eqn. 1)}$$

Where OD means the optical density.

One milliliter (1.0 mL) methanol was added to 3 mL DPPH solution incubated at 25 °C for 30 minutes in the dark and used as control.

Anti-inflammatory bioassay *in vitro*

According to previously reported protocol [16], the reaction mixture consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of the test extract, by which the concentrations (µg/ml) became 400, 800, 2000, 4000, 8000, 16,000. This range was chosen because concentrations below 400 µg/ml gave very insignificant inhibition and above 16,000 µg/ml the value became too high. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37 °C ± 2 °C in a biological oxygen demand incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm (Systronix Spectrophotometer 150) by using vehicle as blank. Diclofenac sodium and rumalaya forte at the final concentrations (µg/ml) of 50, 100, 250, 500, 1000, 2000 were used as reference and traditional/herbal drugs respectively and treated similarly for determination of absorbance. Test extracts were chosen such that, they remained the nearest possible to the standard therapeutic mode. The percentage inhibition of protein denaturation was calculated byusing the following formula:

$$\% \text{ inhibition} = 100 \times ([Vt/Vc] - 1).$$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Result and Discussion

We isolated and characterized thirteen compounds from the roots of *V. phaseolifolius*. The structures of these compounds were elucidated based on NMR and ESI-MS analysis, further supported by comparison with previous reports in the literature. They are : asperphenamate (1) [8], bauerenol (2) [9], *β*-amyrin (3) [10], oleanolic acid (4) [11], lupeol (5) [10], betulinic acid (6) [12], taraxeryl acetate (7) [10], friedelanol (8) [13], mixture of stigmasterol (9) [10] and *β*-sitostrol (10) [10] and stigmasteryl-3-O-*β*-D-glucopyranoside (11) [10] arachidic acid (12) [14] and palmitic acid (13) [14]. Among them, compounds1and 2are being reported for the first timefrom the genus *Vitex*.

Compound 1 was obtained as a white powder. Its positive mode ESI mass spectrum showed a sodium adduct ion at *m / z* 529.3 $[M + Na]^+$ which corresponded to the molar mass of 506 g/mol and anempirical formula $C_{32}H_{30}O_4N_2$ which has 19 degrees of instaurations. The ^{1}H NMR spectrum (Table 1) showed a set of doublet of doublets and triplets of aromatic protons between δ_H 7.86 and 7.17 (20H, m) attributable to four mono-substituted benzene rings. In the strong fields, we noticed the presence of proton signals of *N*-methines at δ_H 4.80 (1H, t) and 4.58 (1H, m). In addition, we observedan oxymethylene signal at δ_H 4.20 (1H, dd) and 4.05 (1H, dd). We also noticed the presence of aliphatic methylenes at δ_H 2.92 (1H, dd), 2.87 (1H, dd), δ_H 3.28 (1H, dd); δ_H 3.12 (1H, dd). The analysis of the ^{13}C NMR spectrum confirmed the presence of aromatic rings with a set of aromatic carbon signals between δc 125.6 and 138.8. It also showed signals for *N*-methines and oxymethylenes at δc 55.9; 51.9 and 65.0 respectively. In addition, it showed signals for aliphatic methylenes at δc 38.8 and 37.9, signals for amide carbonyls at δc 166.6 and 170.1 and signalfor the carbonyl of an ester at δc 172.9. The complete molecule was obtained thanks to the HMBC spectrum (Fig. 2) which showed correlations between one of the aromatic protons at δ_H 7.72 and the carbonyl of theamide at δc 170,1; between the different protons of aliphatic methynes at 2.92, 2.87 and several aromatic carbons between δc 129.5 and 137.2. It also showed correlation between the protons of oxymethylene at δ_H 4.54 and 4.05 and the carbonyl of the ester δc 172.9. Furthermore, it showed correlation between the protons of the *N*-methyls at δ_H 4.80 and 4.58 and the carbonyls of the amides δc 170.1 and 166.6 respectively. All of these data compared to those obtained in the literature led to the identification of compound 1 as asperphenamate.

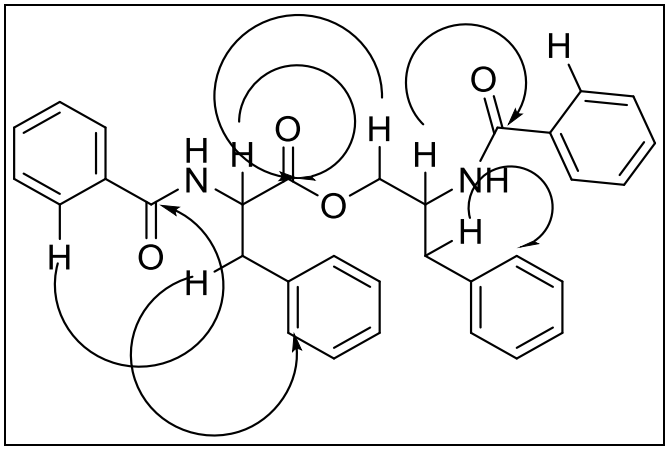

Fig 2: Key HMBC correlation for compound 1

Table 1: ^{1}H and ^{13}C NMR data of **1** in $CDCl_3$. (δ in ppm, 500 MHz for ^{1}H and 125 MHz for ^{13}C)[a]

	1	
Position	**δ_C**	**δ_H(Mult.; J)**
1-6 1'-6' 1''-6'' 1'''-6'''	125.6-138.8	7.23-8.03 (20H,m)
α'γ	172.9	-
1α'	170.1	-
1'α	166.6	-
α	51.9	4.58 (1H, m)
β	37.9	2.92(1H, dd); 2.87(1H, dd)
γ	65.0	4.20 (1H, dd); 4.05 (1H, dd).
α'	55.9	4.80 (1H, t)
β'	38.8	3.28 (1H, dd); 3.12(1H, dd)

[a]The chemical shifts are in *δ* values (ppm) from TMS. ^{13}C multiplicities were determined by HSQC experiment

Compound 2 was obtained as a white powder. It gave a purplish red coloration to the Liebermann-Burchard test, characteristic of the triterpenes. Its positive-mode ESI mass spectrum showed a sodium-adduct ion at m/z 449.3 ($[M + Na]^+$) which corresponded to the molar mass of 426 g/mol and the empirical formula $C_{30}H_{50}O$which has 6 degrees of insaturations. Its ^{1}H NMR spectrum showed 6 singlets forangular methyls at δ_H 0.69; δ_H 0.70; δ_H 0.74; δ_H 0.75; δ_H 0.91 and δ_H 0.96 and two doublet of tree protons each at δ_H 0.76 (3H, d, J= 6.6 Hz) and δ_H 0.82 (3H, d, J= 6.5 Hz) which are all characteristics of pentacyclic triterpenes of the ursane series. It also showed a signal at δ_H 5.16 (1H, dt, J = 6.3, 2.0 Hz) attributable to the proton H-7 of pentacyclic triterpene of the urs-7-ene series. In addition, it showed a signal at δ_H 3.15 (1H, dd, J = 11.6, 4.2 Hz) attributable to the proton of the oxymethine at position C-3 of ring A according to the biosynthesis of triterpenes. The ^{13}C NMR spectrum supported the above information since it exhibited thirty signals of carbons as indicated by the molecular formula. Among

them,the characteristic signals for the carbons C-7 and C-8 ofthe triterpenes of the urs-7-ene series at δ_C 114.3 and 148.8, respectively. In addition, we have a signal at δ_C 78.9 which correspond to the carbon of the oxymethine at C-3. The above data compared to those reportedinthe literature indicated that compound 2isbauerenol.

β-amyrin (3) [10], oleanolic acid (4) [11], lupeol (5) [10], betulinic acid (6) [12], taraxeryl acetate (7) [10], friedelanol (8) [13], mixture of stigmasterol (9) [10] and *β*-sitostrol (10) [10] and stigmasteryl-3-O-*β*-D-glucopyranoside (11) [10] arachidic acid (12) [14] and palmitic acid (13) [14].

The isolated compoundsand crude extract were evaluated for their antimicrobial properties against four bacterial strains *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas agarici*, and found to be inactive.

Compounds 6 - 11 and crude extract were evaluated for their DPPH antioxidant properties. Only the crude extract showed good activity with an $IC_{50}$of 5.55µg/mL, showing that the activity is due to the synergy of compounds. This activity justify the used of this plant as rectal medicine.

Compounds 6 - 11 and crude extract were also evaluated for their anti-inflammatory properties. Only the crude extract showed a weak percentage of protein denaturation with a percentage of 40%.

Conclusion

The different classes of secondary metabolites isolated in this work from the roots of *V. phaseolifolius* are triterpenoids, phytosterols and fatty acids. Some of these compounds were previously reported from other species of *Vitex* genus. Betulinic acid was isolated from *V. trifolia* [12] while oleanolic acid was isolated from *V. Negundo* [11]. Fatty acids were isolated from *V. agnus castus* Linn [13], friedelanol from *V. peduncularis* [16]; lupeol, *β*-amyrin, taraxeryl acetate, *β*-sitosterol, stigmasterol and stigmasteryl 3-*O*-*β*-*D*-glucopyranoside from *V. parvifolia* [10]. The oleanane-type triterpenes are the most reported pentacyclic triterpenes from species belonging to the genus *Vitex* [16]. The triterpenes and phytosterols isolated in this work were reported to be responsible of the antimutagenic activities of *V. trifolia* [12].

Acknowledgment

The authors wish to thank the Alexander von Humboldt Foundation, Germany for research group linkage funding 2015-2018, of Norbert Sewald/Jean Duplex Wansi research group cooperation, for the return fellowship 2019 and the generous support with Laboratory equipments.

Declaration of interest

The authors report no conflict of interest

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