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A New Stilbene Dimer and Other Chemical Constituents from *Monanthotaxis littoralis* with Their Antimicrobial Activities

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Abstract – A new dimer stilbene [Monalittorin (**1**)] and ten known compounds [engeletin (**2**), aurantiamide acetate (**3**), lupeol (**4**), friedelin (**5**), quercetin (**6**), tiliroside (**7**), rutoside (**8**), astragalin (**9**), isoquercitrin (**10**) and quercimeritroside (**11**)] have been isolated from the leaves of *Monanthotaxis littoralis* (Annonaceae). The structures of these compounds were established by interpretation of their data, mainly, HR-TOFESIMS, 1-D NMR (^{1}H and ^{13}C) and 2-D NMR (^{1}H-^{1}H COSY, HSQC, HMBC and NOESY) and by comparison with the literature. The evaluation of their antimicrobial activities against three bacteria (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* S2 (1) and *Pseudomonas aeruginosa* PA01) and three fungal strains (*Candida albicans* ATCC10231, *Candida tropicalis* PK233 and *Cryptococcus neoformans* H99) using broth micro dilution method, showed the largest antimicrobial activities of EtOAc fraction and compounds **1**, **5**, **6**, **8** and **11** (MIC = 8 - 64 μg/mL). In addition, EtOAc fraction presented synergistic effect with Vancomycin and fluconazole against the tested microorganisms.
Keywords – *Monanthotaxis littoralis*, Annonaceae, Monalittorin, Dimer stilbene, Antimicrobial activities, Synergistic effect

Introduction

Monanthotaxis littoralis is a persistent shrub with oblong elliptic leaves and solitary flowers belongs to the family Annonaceae and the genus *Monanthotaxis*. The Annonaceae family includes 130 genera and about 2300 species distributed in tropical and subtropical areas of America, Asia and Africa.[1] In Cameroon, *M. littoralis* is used in folk medicine in the treatment of headache, stomach ache, constipation, cough, hernia, febrile pains and hemorrhoids.[2] Previous studies on this plant reported the presence of flavonoids and essential oil with antifungal activities.[1,3] In the course of our continuing search for secondary metabolites with antimicrobial activities from Cameroonian medicinal plants,[4-6] we investigated the crude MeOH extract of the leaves of *M. littoralis* and isolated a new stilbene dimer, monalittorin (**1**) together with ten known compounds (**2-11**). The isolation and the structure elucidation as well as the antimicrobial activities of monalittorin (**1**) are reported in the present work.

Experimental

General and experimental procedures – The optical rotations were measured on a Bellingham & Stanley ADP 220 polarimeter (Bellingham + Stanley Ltd., United Kingdom). IR spectra were recorded with a Shimadzu FT-IR-8400S spectrometer. ^{1}H and ^{13}C NMR spectra were per-

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formed in deuterated solvent (CD_3OD) on a Bruker AVANCE III 600 spectrometer equipped with a cryoprobe (^1H at 600 MHz and ^{13}C at 150 MHz). 2D NMR experiments were recorded by means of standard Bruker microprograms (Xwin-NMR version 2.1 software TopSpin 3.2). All chemical shifts (δ) are given in ppm with reference to tetramethylsylane (TMS) as internal standard and the coupling constants (J) are in Hz. TOF-ESIMS and HR-TOFESIMS spectra were recorded using a Micromass Q-TOF micro instrument (Manchester, UK) equipped with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 μL min^{-1}. Column chromatography was run on Merck silica gel 60 (70 - 230 mesh) and gel permeation on Sephadex LH-20 while TLC was carried out on silica gel GF_{254} precoated plates with detection accomplished by spraying with 10% H_2SO_4 followed by heating at 90 °C, or by visual inspection under UV lamp at 254 and 365 nm.

Plant material – The leaves of *M. littoralis* were collected in Dschang (Menoua Division, Western Region of Cameroon), in January 2016. Authentication was done by Mr. Fulbert TADJOUTEU, a Botanist of the Cameroon National Herbarium, Yaounde, where the voucher specimen (N° 35048/HNC) has been deposited.

Extraction and isolation – The air-dried plant material (3.0 Kg) was powdered and extracted at room temperature with methanol (18 L, 72 h). The solvent was evaporated under reduced pressure to yield 620.9 g of crude extract. A part of this extract (610.9 g) was suspended in water and successively extracted with EtOAc and *n*-BuOH yielding respectively 206.9 g and 70.2 g of fractions after evaporation of solvent under reduced pressure. The EtOAc fraction was fractionated on silica gel column chromatography using *n*-hexane/EtOAc (85:15 → 0:100) gradient as eluent to afford eight fractions (MLE1 - MLE8). Fraction MLE3 (16.8 g) was subjected to sephadex LH-20 column chromatography using MeOH as eluent to provide three sub-fractions (MLE3.1 - MLE3.3). Sub-fraction MLE3.1 (6.23 g) was submitted to silica gel column chromatography, eluted with *n*-hexane/acetone (70:30) to yield compounds **1** (6.0 mg), **2** (3.5 mg) and **3** (8.1 mg) while fraction MLE2 (9.6 g) was purified on silica gel column chromatography with isocratic elution system, *n*-hexane/EtOAc (85:15) to give compounds **4** (30.0 mg) and **5** (6.0 mg). The *n*-BuOH fraction (MLB) was subjected to silica gel column chromatography using EtOAc/MeOH as eluent with gradient graduated elution (100:0 → 0:100) to give seven fractions (MLB1 - MLB7). Fraction MLB2 (8.55 g) was purified on sephadex LH-20 column chromatography with MeOH as eluent to provide five sub-fractions (MLB2.1 - MLB2.5). Sub-fraction MLB2.1 (102 mg) was submitted to silica gel column chromatography, eluted with hexane/EtOAc (95:5) to yield compound **6** (10.2 mg). The sub-fraction MLB2.4 (130 mg) and MLB2.5 (90.3 mg) were purified on silica gel column chromatography using the same elution system, *n*-hexane/acetone (1:1) to give compound **7** (18.5 mg) for the first sub-fraction and compounds **8** (12.3 mg), **9** (15.9 mg) and **10** (7.0 mg) for the second sub-fraction. Fraction MLB4 (3.57 g) was also subjected to a silica gel column chromatography and eluted with EtOAc/MeOH/H_2O (90:5:2) to yield three sub-fractions (MLB4.1 - MLB4.3). Compound **11** (6.5 mg) was obtained after purification of sub-fraction MLB4.3 (370 mg) on silica gel column chromatography using EtOAc/MeOH (98:2) as eluent.

Monalittorin (1) – Red oil. $[\alpha]_D^{25}$ -4.2° (C = 0.166, EtOH); UV λ_{max} (log ε): (MeOH): 226, (175), 317 (25) nm; IR (KBr): 3500 - 3400 cm^{-1} (hydroxyl groups), 1660 - 1600, 900 - 750 cm^{-1} (aromatic rings); ^1H and ^{13}C NMR see Table 1; HR-TOFESIMS: *m/z* 453.2065 $[M+H]^+$ (calcd. for $C_{30}H_{29}O_4$, 453.2066).

Engeletin (2) – Yellow powder. ^1H-NMR (CD_3OD, 600 MHz) δ_H: 7.36 (2H, *d*, J = 8.4 Hz, H-2′, 6′), 6.84 (2H, *d*, J = 8.4 Hz, H-3′, 5′), 5.92 (1H, *d*, J = 2.1 Hz, H-6), 5.90 (1H, *d*, J = 2.1 Hz, H-8), 5.14 (1H, *d*, J = 10.9 Hz, H-2), 4.62 (1H, *d*, J = 10.9 Hz, H-3) for aglycone; 4.26 (1H, *dq*, J = 6.2, 12.5 Hz, H-5″), 3.99 (1H, *s*, H-1″), 3.65 (1H, *dd*, J = 3.2, 9.7 Hz, H-3″), 3.49 (1H, *dd*, J = 1.7, 3.2 Hz, H-2″), 3.29 (1H, *brd*, J = 9.7, 12.5 Hz, H-4″), 1.18 (3H, *d*, J = 6.2 Hz, H-6″) representing the rhamnose moiety; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 196.2 (C-4), 168.6 (C-7), 165.5 (C-5), 164.2 (C-9) , 159.3 (C-4′), 130.1 (C-2′,6′), 128.6 (C-1′), 116.4 (C-3′,5′), 102.4 (C-10), 97.4 (C-6), 96.2 (C-8), 83.4 (C-2), 78.7 (C-3) for aglycone; 102.2 (C-1″), 71.8 (C-2″), 72.1 (C-3″), 73.8 (C-4″), 70.5 (C-5″), 17.9 (C-6″) for sugar moiety.

Auranthiamide acetate (3) – White powder. ^1H-NMR (DMSO-d_6, 600 MHz) δ_H: 4.17 (1H, *m*, H-2), 2.79 (1H, *dd*, J = 6.3, 13.8 Hz, H-3a), 2.77 (1H, *dd*, J = 7.1, 13.8 Hz, H-3b), 7.22 (2H, *m*, H-5, 9), 7.23 (2H, *m*, H-6, 8), 7.22 (1H, *m*, H-7), 3.95 (1H, *dd*, J = 4.3, 10.9 Hz, H-10b), 3.84 (1H, *dd*, J = 6.8, 10.9 Hz, H-10a), 1.96 (3H, *s*, H-12), 4.65 (1H, *q*, J = 4.7 Hz, H-13), 7.77 (2H, *dd*, J = 1.4, 6.9 Hz, H-16, 20), 7.44 (2H, *t*, J = 7.6 Hz, H-17,19), 7.51 (1H, *t*, J = 7.4 Hz, H-18), 2.98 (1H, *dd*, J = 5.9, 13.7 Hz, H-21b), 2.96 (1H, *dd*, J = 8.5, 13.7 Hz, H-21a), 7.29 (2H, *d*, J = 7.5 Hz, H-23, 27), 7.23 (2H, *d*, J = 7.5 Hz, H-24, 26), 7.15 (1H, *t*, J = 7.1 Hz, H-25), 8.16 (1H, *d*, J = 8.4 Hz, NH-b), 8.51 (1H, *d*, J = 8.3 Hz, NH-a); ^{13}C-NMR (DMSO-d_6, 150 MHz) δ_C: 170.6 (C-1), 49.3 (C-2), 36.7

(C-3), 138.2 (C-4), 129.3 (C-5, 9), 128.4 (C-6, 8), 128.8 (C-7), 64.8 (C-10), 171.4 (C-11), 20.8 (C-12), 55.1 (C-13), 166.4 (C-14), 134.1 (C-15), 127.6 (C-16, 20), 128.4 (C-17, 19), 131.6 (C-18), 37.4 (C-21), 138.4 (C-22), 129.3 (C-23, 27), 128.3 (C-24, 26), 126.5 (C-25).

Lupeol (4) – White powder. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ_H: 0.66 (1H, *d*, *J* = 9.1 Hz, H-5), 0.73 (3H, *s*, H-24), 0.76 (3H, *s*, H-28), 0.80 (3H, *s*, H-25), 0.92 (3H, *s*, H-27), 0.94 (3H, *s*, H-23), 1.00 (3H, *s*, H-26), 1.65 (3H, *s*, H-30), 1.82–1.96 (2H, *m*, H-21), 2.35 (1H, *dt*, *J* = 10.9, 5.5 Hz, H-19), 3.16 (1H, *dd*, *J* = 10.8, 5.1 Hz, H-3), 4.55 (1H, *brs*, H-29), 4.65 (1H, *brs*, H-29); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ_C 14.5 (C-27), 15.3 (C-24), 15.9 (C-25), 16.1 (C-26), 18.0 (C-28), 18.3 (C-6), 19.3 (C-30), 20.9 (C-11), 25.1 (C-12), 27.4 (C-2, C-15), 28.0 (C-23), 29.7 (C-21), 34.3 (C-7), 35.6 (C-16), 37.1 (C-10), 38.0 (C-13), 38.7 (C-1), 38.8 (C-4), 40.0 (C-22), 40.8 (C-8), 42.8 (C14), 43.0 (C-17), 48.0 (C-18), 48.3 (C-19), 50.4 (C-9), 55.3 (C-5), 79.0 (C-3), 109.3 (C29), 150.9 (C-20).

Friedelin (5) – White powder. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H: 0.72 (3H, *s*, H-24), 0.87 (3H, *s*, H-25), 0.88 (3H, *d*, *J* = 6.5 Hz, H-23), 0.95 (3H, *s*, H-29), 0.997 (3H, *s*, H-28), 1.00 (3H, *s*, H-26), 1.05 (3H, *s*, H-27), 1.18 (3H, *s*, H-30). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C: 22.3 (C-1), 41.5 (C-2), 213.2 (C-3), 58.2 (C-4), 42.1 (C-5), 41.3 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 59.4 (C-10), 35.6 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.4 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.3 (C-19), 28.2 (C-20), 32.8 (C-21), 39.2 (C-22), 6.8 (C-23), 14.6 (C-24), 17.9 (C-25), 20.2 (C-26), 18.7 (C- 27), 32.1 (C-28), 35.0 (C-29), 31.8 (C-30).

Quercetin (6) – Yellow powder. ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 7.75 (1H, d, *J* = 2.1 Hz, H-2'), 7.65 (1H, dd, *J* = 8.5, 2.1 Hz, H-6'), 6.90 (1H, d, H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, H-6). ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 176.2 (C-4), 164.1 (C-2), 161.1(C-4'), 157.1 (C-7), 147.5 (C-9), 146.5 (C-5), 144.7 (C-3'), 135.8 (C-3), 123.3 (C-1'), 120.3 (C-6'), 115.1 (C-5'), 114.5 (C-2'), 103.2 (C-10), 98.2 (C-6), 93.2 (C-8).

Tiliroside (7) – Yellow powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 7.98 (2H, *d*, *J* = 8.4 Hz, H-2′, 6′), 6.85 (2H, *d*, *J* = 8.4 Hz, H-3′, 5′), 6.37 (1H, *d*, *J* = 2.1 Hz, H-8), 6.14 (1H, *d*, *J* = 2.1 Hz, H-6) for aglycone; 5.44 (1H, *d*, *J* = 7.8 Hz, H-1″), 3.21 (1H, *m*, H-2″), 3.25 (1H, *m*, H-3″), 3.17 (1H, *m*, H-4″), 3.38 (1H, *m*, H-5″), 4.25 (1H, *d*, *J* = 10.3 Hz, H-6b″), 4.02 (1H, *dd*, *J* = 6.4, 11.9 Hz, H-6a″) for glucose; 7.36 (2H, *d*, *J* = 8.6 Hz, H-2‴, 6‴), 6.78 (2H, *d*, *J* = 8.6 Hz, H-3‴, 5‴), 7.33 (1H, *d*, *J* = 15.9 Hz, H-7‴), 6.09 (1H, *d*, *J* = 15.9 Hz, H-8‴) representing the *trans-p*-coumaroyl moiety; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 177.5 (C-4), 164.5 (C-7), 161.2 (C-5), 160.1 (C-4′), 156.5 (C-2), 156.4 (C-9), 133.1 (C-3), 130.9 (C-2′,6′), 120.9 (C-1′), 115.2 (C-3′,5′), 103.9 (C-10), 98.9 (C-6), 93.8 (C-8) for aglycone; 101.0 (C-1″), 74.2 (C-2″), 76.3 (C-3″), 70.0 (C-4″), 74.3 (C-5″), 63.0 (C-6‴) for sugar moiety; 125.0 (C-1‴), 130.3 (C-2‴, 6‴), 115.9 (C-3‴, 5‴), 159.9 (C-4‴), 144.7 (C-7‴), 113.7 (C-8‴), 166.3 (C-9‴) for *trans-p*-coumaroyl moiety.

Rutoside (8) – Yellow powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 8.06 (2H, *d*, *J* = 8.8 Hz, H-2′, 6′), 6.89 (2H, *d*, *J* = 8.9 Hz, H-3′, 5′), 6.40 (1H, *d*, *J* = 2.0 Hz, H-8), 6.20 (1H, *d*, *J* = 2.0 Hz, H-6) for aglycone; 5.13 (1H, *d*, *J* = 7.4 Hz, H-1″), 3.44 (1H, *m*, H-2″), 3.34 (1H, *m*, H-3″), 3.26 (1H, *m*, H-4″), 3.56 (1H, *m*, H-5″), 3.81 (1H, *d*, *J* = 10.1 Hz, H-6b″), 3.36 (1H, *m*, H-6a″) for glucose; 4.52 (1H, *sl*, H-1‴), 3.64 (1H, *d*, *J* = 1.6 Hz, H-2‴), 3.53 (1H, *dd*, *J* = 3.3, 9.5 Hz, H-3‴), 3.29 (1H, *m*, H-4‴), 3.45 (1H, *m*, H-5‴), 1.13 (3H, *d*, *J* = 6.2Hz, H-6‴) for rhamnose; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 179.4 (C-4), 165.9 (C-7), 162.9 (C-5), 161.5 (C-4′), 159.4 (C-2), 158.5 (C-9), 135.5 (C-3), 132.4 (C-2′,6′), 122.7 (C-1′), 116.1 (C-3′,5′), 105.6 (C-10), 99.9 (C-6), 94.9 (C-8) for aglycone; 104.5 (C-1″), 75.8 (C-2″), 77.2 (C-3″), 71.4 (C-4″), 78.1 (C-5″), 68.6 (C-6‴) for glucose moiety; 102.3 (C-1‴), 72.1 (C-2‴), 72.3 (C-3‴), 73.4 (C-4‴), 69.7 (C-5‴), 17.9 (C-6‴) for rhamnose moiety.

Astragalin (9) – Yellow powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 8.10 (2H, *d*, *J* = 8.3 Hz, H-2′, 6′), 6.90 (2H, *d*, *J* = 8.3 Hz, H-3′, 5′), 6.41 (1H, *d*, *J* = 2.0 Hz, H-8), 6.21 (1H, *d*, *J* = 2.0 Hz, H-6) for aglycone; 5.15 (1H, *d*, *J* = 7.8 Hz, H-1″), 3.78 (1H, *m*, H-2″), 3.53 (1H, *m*, H-3″), 3.82 (1H, *m*, H-4″), 3.44 (1H, *m*, H-5″), 3.61 (1H, *m*, H-6b″), 3.53 (1H, *m*, H-6a″) for glucose; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 179.8 (C-4), 166.1 (C-7), 163.1 (C-5), 161.6 (C-4′), 158.7 (C-2), 159.0 (C-9), 135.6 (C-3), 132.4 (C-2′,6′), 122.7 (C-1′), 116.1 (C-3′,5′), 105.7 (C-10), 99.9 (C-6), 94.1 (C-8) for aglycone; 104.9 (C-1″), 73.0 (C-2″), 75.0 (C-3″), 70.0 (C-4″), 77.1 (C-5″), 61.9 (C-6‴) for sugar moiety.

Isoquercetin (10) – Yellow powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 7.85 (1H, *d*, *J* = 2.2 Hz, H-2′), 7.58 (1H, *dd*, *J* = 8.5, 2.2 Hz, H-6′), 6.86 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.40 (1H, *d*, *J* = 2.0 Hz, H-8), 6.20 (1H, *d*, *J* = 2.0 Hz, H-6) for aglycone; 5.18 (1H, *d*, *J* = 7.8 Hz, H-1″), 3.79 (1H, *m*, H-2″), 3.54 (1H, *m*, H-3″), 3.84 (1H, *m*, H-4″), 3.46 (1H, *m*, H-5″), 3.60 (1H, *m*, H-6b″), 3.52 (1H, *m*, H-6a″) for glucose; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 179.7 (C-4), 166.2 (C-7), 163.0 (C-5), 158.7 (C-2), 158.5 (C-9), 149.9 (C-4′), 145.8 (C-3′), 135.7 (C-3), 122.9 (C-6′), 122.8 (C-1′), 117.8 (C-2′), 116.0 (C-5′), 105.6 (C-10), 99.8 (C-

6), 94.1 (C-8) for aglycone; 105.3 (C-1″), 73.2 (C-2″), 75.1 (C-3″), 70.0 (C-4″), 77.2 (C-5″), 61.8 (C-6‴) for sugar moiety.

Quercimeritroside (11) – Yellow powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ_H: 7.77 (1H, *d*, *J* = 1.8 Hz, H-2′), 7.68 (1H, *dd*, *J* = 1.8, 8.5 Hz, H-6′), 6.91 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.77 (1H, *d*, *J* = 2.0 Hz, H-8), 6.48 (1H, *d*, *J* = 2.0 Hz, H-6) for aglycone; 5.08 (1H, *d*, *J* = 7.3 Hz, H-1″), 3.51 (1H, *d*, *J* = 5.5 Hz, H-2″), 3.52 (1H, *d*, *J* = 6.9 Hz, H-3″), 3.44 (1H, *d*, *J* = 9.1 Hz, H-4″), 3.56 (1H, *m*, H-5″), 3.95 (1H, *dd*, *J* = 1.9, 10.3 Hz, H-6b″), 3.75 (1H, *dd*, *J* = 5.9, 12.2 Hz, H-6a″) for glucose; ^{13}C-NMR (CD_3OD, 150 MHz) δ_C: 176.1 (C-4), 163.0 (C-7), 160.7 (C-5), 147.6 (C-2), 156.3 (C-9), 147.3 (C-4′), 144.8 (C-3′), 136.2 (C-3), 120.5 (C-6′), 122.5 (C-1′), 114.7 (C-2′), 114.8 (C-5′), 104.8 (C-10), 98.8 (C-6), 94.1 (C-8) for aglycone; 100.2 (C-1″), 73.3 (C-2″), 76.4 (C-3″), 69.8 (C-4″), 76.9 (C-5″), 61.0 (C-6‴) for sugar moiety.

Microorganisms – The studied microorganisms were: one Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), two Gram-negative bacteria (*Escherichia coli* S2 (1) and *Pseudomonas aeruginosa* PA01) and three strains of yeasts (*Candida tropicalis* PK233, *Candida albicans* ATCC10231 and *Cryptococcus neoformans* H99) taken from our laboratory collection on the basis of their relevance as human pathogens. The bacterial and fungal species were grown at 37 ºC and maintained on nutrient agar (NA, Conda, Madrid, Spain) and Sabouraud Dextrose Agar (SDA, Conda) slants, respectively.

Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) – MIC values were determined by a broth microdilution method as described earlier,[7,8] with slight modifications. Each test sample was dissolved in dimethylsulfoxide (DMSO) and the solution was then added to Mueller Hinton Broth (MHB) for bacteria or Sabouraud Dextrose Broth (SDB) for yeasts to give a final concentration of 8192 μg/mL. This was serially diluted twofold to obtain a concentration range of 0.125 - 4096 μg/mL. Then, 100 μL of each concentration were added in each well (96-well microplate) containing 95 μL of MHB or SDB and 5 μL of inoculum for final concentrations varying from 0.0625 - 2048 μg/mL. The inoculum was standardized at 2.5×10^5 cells/mL for yeasts and 10^6 CFU/mL for bacteria using a JENWAY 6105 UV/Vis spectrophotometer. The final concentration of DMSO in each well was <1% [preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of the test organisms]. The negative control well consisted of 195 μL of MHB or SDB and 5 μL of the standard inoculum. The cultured micro plates were covered; then, the contents of each well were mixed thoroughly using a plate shaker (Flow Laboratory, Germany) and incubated at 35 ºC for 24 h (bacteria) and at 30 °C for 48 h (yeasts) under shaking. The assay was repeated three times. The MIC values of samples were determined by adding 50 μL of a 0.2 mg/mL *p*-iodonitrotetrazolium violet solution followed by incubation at 37 ºC for 30 min. Viable microorganisms reduced the yellow dye to a pink color. MIC values were defined as the lowest sample concentrations that prevented this change in color indicating a complete inhibition of microbial growth. For the determination of MMC values, a portion of liquid (5 μL) from each well that showed no growth of microorganism was plated on Mueller Hinton Agar or SDA and incubated at 37 ºC for 24 h (for bacteria) or 30 ºC for 48 h (for yeasts). The lowest concentrations that yielded no growth after this sub-culturing were taken as the MMC values. Vancomycin (Sigma-Aldrich, Steinheim, Germany) and fluconazole (Merck, Darmstadt, Germany) were used as positive controls for bacteria and yeasts, respectively.

Combined effect of ethyl acetate extract and antibiotics – The antimicrobial effects of a combination of the ethyl acetate fraction of *Monanthotaxis littoralis*, which exhibited the highest antimicrobial activity, and antibiotics (vancomycin and fluconazole) were assessed by the checkerboard test as previously described.[7,8] Serial dilutions of three different antimicrobial agents were mixed in Mueller-Hinton broth. After 24 - 48 h of incubation at 37 ºC / 30 ºC, the MICs were determined as described above. The fractional inhibitory concentration (FIC) index was calculated according to the following equation: FIC index = FICA + FICB = MIC of drug A in combination / MIC of drug A alone + MIC of drug B in combination / MIC of drug B alone. The FIC indices are the sum of the FICs of each of the drugs, which in turn is defined as the MIC of each drug when it is used in combination divided by the MIC of the drug when it is used alone. The interaction was defined as synergistic if the FIC index was less than or equal to 0.5, additive if the FIC index was greater than 0.5 and less than or equal to 1.0, indifferent if the FIC index was greater than 1.0 and less than or equal to 2.0, and antagonistic if the FIC index was greater than 2.0.[9] All the experiments were performed in triplicate.

Statistical analysis – Data were analyzed by one-way analysis of variance followed by Waller-Duncan Post Hoc test. The experimental results were expressed as the mean ± Standard Deviation (SD). Differences between groups were considered significant when $p < 0.05$. All analyses

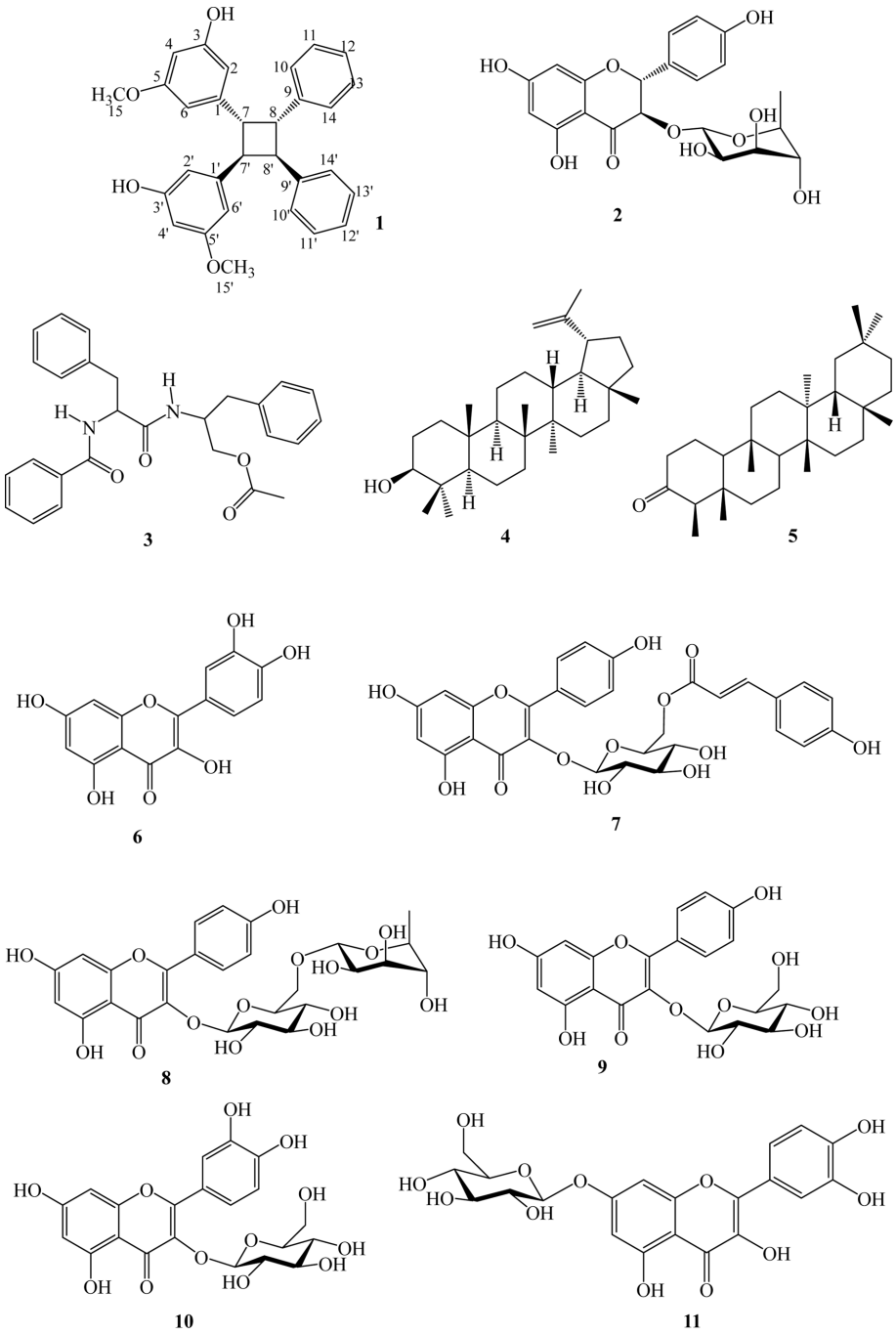

Fig. 1. Compounds isolated from the leaves of *Monanthotaxis littoralis*.

were performed using the Statistical Package for Social Sciences (SPSS, version 12.0) software.

Results and Discussion

The purification through silica gel and Sephadex LH-20 column chromatography of EtOAc and *n*-butanol extracts obtained from liquid-liquid partition of MeOH crude extract of *Monanthotaxis littoralis* led to the isolation of new stilbene dimer, monalittorin (**1**) together with ten known compounds (**2 - 11**) (Fig. 1).

Compound **1** was obtained as red oil. It molecular formula $C_{30}H_{28}O_4$, was deduced from the pseudo-molecular ion peak at *m/z* 453.2065 $[M+H]^+$ (calcd. for $C_{30}H_{29}O_4$ 453.2066) on the HR-TOFESIMS spectrum, indicating 17 degrees of unsaturation. The IR spectrum

Table 1. ^{13}C (150 MHz) NMR and ^{1}H (600 MHz) NMR data of compound **1**

Position	δ_c	δ_H (Int.; *mult.*; *J* in Hz)
1/1′	144.8	-
2/2′	108.9	6.31 (2H ; *brt* ; 1.5)
3/3′	159.1	-
4/4′	100.2	6.10 (2H ; *t* ; 2.2)
5/5′	161.9	-
6/6′	106.4	6.25 (2H ; *brt* ; 1.5)
7	48.8	4.37 (1H ; *dd,* 8.0, 6.1)
7′	48.9	4.38 (1H ; *dd,* 8.0, 6.1)
8	48.6	4.34 (1H ; *dd,* 8.0, 6.1)
8′	48.5	4.33 (1H ; *dd,* 8.0, 6.1)
9/9′	142.2	-
10/10′	129.3	7.08-7.13 (2H ; *m*)
11/11′	128.9	7.08-7.13 (2H ; *m*)
12/12′	126.8	7.03 (2H ; *m*)
13/13′	128.9	7.08-7.13 (2H ; *m*)
14/14′	129.3	7.08-7.13 (2H ; *m*)
15/15′	55.5	3.61 (6H ; *s*)

showed vibration bands for hydroxyl groups (3500 - 3400 cm^{-1}) and aromatic rings (1660 - 1600, 900 - 750 cm^{-1}). The ^{1}H NMR spectrum (Table 1) indicated the presence of characteristic signals in two distinct regions. The former, between δ_H (7.20 - 6.00) ppm, was constituted by two benzene rings with 1,3,5-trisubstituted at δ_H: 6.31 (2H, *brt, J* = 1.5 Hz, H-2/2'), 6.25 (2H, *brt, J* = 1.5 Hz, H-6/6'), 6.10 (2H, *t, J* = 2.2 Hz, H-4/4') and mono-substituted at δ_H: 7.13-7.08 (8H, *m*, H-10/10', 11/11', 13/13', 14/14') and 7.03 (2H, *m*, H-12/12'). The second region of the ^{1}H NMR spectrum, between δ_H (4.50-3.50) ppm, was characterized by methine signals at δ_H: 4.38 (1H, *dd, J* = 8.0 and 6.1 Hz, H-7'), 4.37 (1H, *dd, J* = 8.0 and 6.1 Hz, H-7), 4.34 (1H, *dd, J* = 8.0 and 6.1 Hz, H-8), 4.33 (1H, *dd, J* = 8.0 and 6.1 Hz, H-8') and methoxy signal at δ_H 3.61 (6H, *s,* H-15/15'). The integration values of different type proton signals suggested a symmetric in this compound. This suggestion was further confirmed by the ^{13}C NMR spectrum on which 17 carbon signals were observed at δ_c: 161.9 (C-5/5′), 159.1 (C-3/3′), 144.8 (C-1/1′), 142.2 (C-9/9′), 129.3 (C-10/10′, 14/14'), 128.9 (C-11/11′, 13/13'), 126.8 (C-12/12′), 108.9 (C-2/2′), 106.4 (C-6/6′), 100.2 (C-4/4′), 55.5 (15/15′), 48.9 (C-7′), 48.8 (C-7), 48.6 (C-8), 48.5 (C-8'). The comparison of ^{1}H and ^{13}C NMR data of compound **1** with those of the literature[10,11] indicated that, compound **1** was a stilbene dimer. However, the absence of methylene and olefinic proton signals suggested a [2+2] dimerization of two stilbene units to afford a cyclobutane ring with an aryl substituent and a

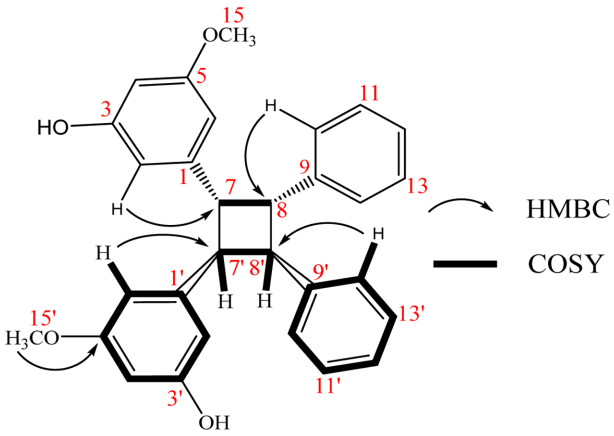

Fig. 2. Key COSY and HMBC correlations of compound **1**.

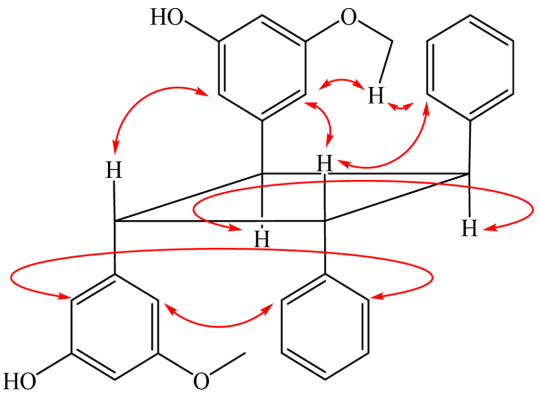

Fig. 3. Key NOESY correlations of compound **1**.

hydrogen at each ring carbon.[12] This suggestion was supported by the number of unsaturation and the presence of methinic protons at δ_H 4.38 (H-7′), 4.37 (H-7), 4.34 (H-8) and 4.33 (H-8′) which showed a cross correlation on the ^{1}H-^{1}H COSY spectrum (Fig. 2). The positions of the aromatic rings relative to cyclobutane ring were in agreement with the HMBC correlations observed between the protons at δ_H: 6.31 (H-2/2′), 6.25 (H-6/6′) and the carbon at δ_c 48.9 (C-7′) and 48.8 (C-7) on the one hand, and the proton at δ_H 7.13-7.08 (H-10/10′, H-14/14′) and the carbon at δc 48.6 (C-8) and 48.5 (C-8′) on the other (Fig. 2). Those information were supported by comparison of the differences in chemical shift between carbons C-7 and C-8 on the one hand, C-7 'and C-8' on the other with those of the literature.[13-17] The correlation observed on the same spectrum between the protons at δ_H 3.61 (H-15/15′) and the carbon at δc 161.9 (C-5/5′) allowed us to locate the methoxy groups at position C-5/5′. The coupling constant between H-7 and H-8 (*J* = 8.0 Hz), H-7 and H-8' (*J* = 6.1 Hz) indicated their *cis* and *trans* orientations respectively. The relative stereochemistry of cyclobutane

ring was further supported by the NOESY spectrum on which the correlation between the protons H-7 and H-8, H-7' and H-8' were observed (Fig. 3). Furthermore correlation observed on the same spectrum between H-2/6 and H-7'/8'/10 corroborated with this relative stereochemistry. Accordingly, the structure of compound **1** was established as 8,8'-diphenyl-7,7'-di[1-(3-hydroxy-5-methoxyphenyl)] cyclobutane and trivially named monalittorin. The ten

Table 2. Antimicrobial activity (MIC and MMC in μg/mL) of extracts, isolated compounds and reference antimicrobial drugs

Extracts/ Compounds	Inhibition parameters	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. tropicalis*	*C. albicans*	*C. neoformans*
MeOH extract	MIC	256	512	256	512	512	256
	MMC	512	512	512	1024	1024	512
	MMC/MIC	2	2	2	2	2	2
EtOAc fraction	MIC	64	64	64	256	256	256
	MMC	64	64	64	512	512	256
	MMC/MIC	1	1	1	2	2	1
n-BuOH fraction	MIC	128	128	128	512	512	512
	MMC	128	128	256	1024	512	512
	MMC/MIC	1	1	2	2	1	1
1	MIC	64	64	64	32	16	16
	MMC	128	64	64	64	16	16
	MMC/MIC	2	1	1	2	1	1
3	MIC	>256	256	128	>256	128	64
	MMC	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/
4	MIC	>256	>256	256	>256	256	256
	MMC	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/
5	MIC	64	32	32	64	32	16
	MMC	128	64	64	64	32	32
	MMC/MIC	2	2	2	1	1	2
6	MIC	16	16	16	16	16	8
	MMC	32	16	16	16	16	16
	MMC/MIC	2	1	1	1	1	2
7	MIC	256	>256	256	>256	>256	>256
	MMC	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/
8	MIC	16	16	8	8	8	8
	MMC	16	16	8	16	16	8
	MMC/MIC	1	1	1	2	2	1
9	MIC	>256	>256	>256	>256	>256	>256
	MMC	>256	>256	>256	>256	>256	>256
	MMC/MIC	/	/	/	/	/	/
11	MIC	64	16	16	32	32	16
	MMC	128	32	16	64	32	16
	MMC/MIC	2	2	1	2	1	1
Reference antibiotic*	MIC	32	16	0.5	0.5	1	2
	MMC	32	16	0.5	0.5	1	2
	MMC/MIC	1	1	1	1	1	1

MIC: minimum inhibitory concentration; MIC: minimum microbicidal concentration; /: not determined; EtOAc: ethyl acetate; *n*-BuOH : n butanol; * : vancomycin for bacteria and fluconazole for yeasts.

Table 3. Checkerboard assay of EtOAc fraction and reference antibiotics against pathogenic strains

Strains	Agent	MIC (μg/mL)		FIC	FICI	Outcome
		Alone	Combination			
E. coli	EtOAc fraction	64	16	0.25	0.375	Synergistic
	Vancomycin	32	4	0.125		
P. aeruginosa	EtOAc fraction	64	8	0.125	0.25	Synergistic
	Vancomycin	16	2	0.125		
S. aureus	EtOAc fraction	64	32	0.50	0.75	Additive
	Vancomycin	0.5	0.125	0.25		
C. tropicalis	EtOAc fraction	256	32	0.125	0.25	Synergistic
	Fluconazole	0.50	0.062	0.125		
C. albicans	EtOAc fraction	256	64	0.25	0.375	Synergistic
	Fluconazole	1	0.125	0.125		
C. neoformans	EtOAc fraction	256	8	0.0312	0.0937	Synergistic
	Fluconazole	2	0.125	0.0625		

FIC: fractional inhibitory concentration; FICI: fractional inhibitory concentration index; EtOAc: ethyl acetate

known compounds were identified by comparison of their spectroscopic data with literature values as engeletin (**2**),[18] acetate auranthiamide (**3**),[19] lupeol (**4**),[20] friedelin (**5**),[21] quercetin (**6**),[22] tiliroside (**7**),[23] rutoside (**8**),[24] astragalin (**9**),[25] isoquercitrin (**10**)[26] and quercimeritroside (**11**).[27]

After the elucidation of the different structures, the MeOH extract, EtOAc fraction and *n*-BuOH fraction as well as compounds were tested for their antimicrobial activities against three bacterial (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* S2 (1) and *Pseudomonas aeruginosa* PA01) and three fungal (*Candida albicans* ATCC10231, *Candida tropicalis* PK233 and *Cryptococcus neoformans* H99) strains and the result are presented in Table 2. In general, the test samples demonstrated varying degrees of inhibitory activities against bacterial and fungal strains ($64 \leq MIC \leq 512$ μg/mL). The MIC values obtained with the EtOAc fraction were smaller than those obtained with *n*-BuOH fraction and MeOH extract against the microbial strains. The observations suggest that, the fractionation of MeOH extract enhanced its antimicrobial activity. These results corroborated with those of Ngoufack et al. (2018).[5] On the basis of antimicrobial cutoff points defined in the literature for plant extract,[28] the antimicrobial activities of MeOH, EtOAc and *n*-BuOH extracts could be considered as significant ($MIC < 100$ μg/mL) or moderate ($100 \leq MIC \leq 512$ μg/mL) against the test microorganisms. The result obtained after combination of the EtOAc fraction, which exhibited the highest antimicrobial activity and antibiotics (vancomycin and fluconazole) are depicted in Table 3. These results showed that, the MIC values of EtOAc fraction in combination with antibiotics at MIC concentration are smaller than that of EtOAc fraction used alone (vice versa). According to the interpretation of FIC indices by Bone et al. (1994),[9] the combination of EtOAc with antibiotics demonstrated synergistic effect (FIC indices ≤ 0.5) against *E. coli*, *P. aeruginosa*, *C. albicans*, *C. tropicalis* and *C. neoformans* and additive effect ($0.5 <$ FIC indices < 1) against *S. aureus*. These results are in agreement with those of Kengne et al. 2018,[29] which demonstrated synergistic effect between MeOH extract of *Curcuma longa* and amoxicillin against *S. aureus*, *E. coli*, *S. flexneri*, *B. subtilis* and *P. auriginosa*. Concerning the isolated compounds, the lowest MIC value (8 - 16 μg/mL) were recorded with compound **8** while compounds **3**, **4**, **7** and **9** presented the high MIC values (> 256 μg/mL). The MIC values of compound **6** and **8** are lesser than those of vancomycin used as reference antibiotic. Considering the cutoff points of antimicrobial activities of pure compound defined in the literature,[28] the activities of isolated compounds could be considered as significant ($1 \leq MIC \leq 10$ μg/mL), moderate ($10 \leq MIC \leq 100$ μg/mL), weak ($100 \leq MIC \leq 1000$ μg/mL). Taking into account the importance of the test microbial species, the result can be consider as promising for the development of new antimicrobial drugs.

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References

(1) Clara, C.; Matasyoh, J. C.; Wagara, I. N.; Nakavuma, J. *Am. J. Chem. Appl*. **2014**, *1*, 54-60.

(2) Bele, M. Y.; Focho, D. A.; Egbe, E. A.; Chuyong, B. G. *Afr. J. Plant Sci.* **2011**, *5*, 237-247.

(3) Clara, C.; Matasyoh, J. C.; Wagara, I. N.; Nakavuma, J. *Int. J. Microbiol. Res. Rev.* **2013**, *2*, 103-109.

(4) Joubouhi, C.; Tamokou, J. d. D.; Ngnokam, D.; Voutquenne-Nazabadioko, L.; Kuiate, J. R. *BMC Complem. Altern. Med.* **2017**, *17*, 1-8.

(5) Tagousop, N. C.; Tamokou, J. d. D.; Kengne, I. C.; Ngnokam, D.; Voutquenne-Nazabadioko, L. *Chem. Cent. J*. **2018**, *12,* 97.

(6) Kenfack, J. N.; Ngoudjou, D. T.; Ponou, B. K.; Kühlborn, J.; Tsafack, B. T.; Teponno, R. B.; Opatz, T.; Barboni, L.; Gatsing, D.; Tapondjou, L. A. *Sci. J. Chem.* **2020**, *8*, 48-58.

(7) CLSI. M27 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th ed; Clinical and Laboratory Standards Institute; USA, 2017, pp 1-46.

(8) CLSI. M07-A8 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 8th ed; Clinical and Laboratory Standards Institute; USA, 2010, pp 1-65.

(9) Bone, R. C. *Arch. Intern. Med*. **1994**, *154*, 26-34.

(10) Pel, P.; Chae, H. S.; Nhoek, P.; Kim, Y. M.; Khiev, P.; Kim, G. J.; Nam, J. W.; Choi, H.; Choi, Y. H.; Chin, Y. W. *Bioorg. Chem.* **2020**, *99*, 103869.

(11) Niesen, D. B.; Craig, H.; Seeram, N. P. *J. Berry Res.* **2013**, *3*, 181-196.

(12) Garg, H. S.; Chaturvedi, R.; Bhakuni, D. S. *J. Nat. Prod.* **1991**, *54*, 104-109.

(13) Li, J.; Tan, L. H.; Zou, H.; Zou, Z. X.; Long, H. P.; Wang, W. X.; Xu, P. S.; Liu, L. F.; Xu, K. P.; Tan, G. S. *J. Nat. Prod.* **2020**, *83*, 216-222.

(14) Liu, Y.; Zhang, X.; Kelsang, N.; Tu, G.; Kong, D.; Lu, J.; Zhang, Y.; Liang, H.; Tu, P.; Zhang, Q. *J. Nat. Prod.* **2018***, 81,* 307-315.

(15) Zhang, X.; Chen, C.; Li, Y.; Chen, D.; Dong, L.; Na, W.; Wu, C.; Zhang, J.; Li, Y. *J. Nat. Prod.* **2016**, *79*, 1249-1258.

(16) Ma, G. L.; Xiong, J.; Yang, G. X.; Pan, L. L.; Hu, C. L.; Wang, W.; Fan, H.; Zhao, Q. H.; Zhang, H. Y.; Hu, J. F. *J. Nat. Prod.* **2016**, *79*, 1354-1364.

(17) Sagawa, T.; Takaishi, Y.; Fujimoto, Y.; Duque, C.; Osorio, C.; Ramos, F.; Garzon, C.; Sato, M.; Okamoto, M.; Oshikawa, T.; Ahmed, S. U. *J. Nat. Prod.* **2005**, *68,* 502-505.

(18) Ali, A. H.; Hassan, N. M.; Shukor, N. I.; Embi, N.; Latip, J.; Mohd Sidek, H. M. *Malays. Appl. Biol*. **2014**, *43*, 73-80.

(19) Zhou, B.; Yang, Z.; Feng, Q.; Liang, X.; Li, J.; Zanin, M.; Jiang, Z.; Zhong, N. *J. Ethnopharmacol.* **2017**, *199*, 60-67.

(20) Kaundal, M.; Akhtar, M.; Deshmukh, R. *J. Neurol. Neurosci.* **2017**, *8*, 1-8.

(21) Mann, A.; Ibrahim, K.; Oyewale, A. O.; Amupitan, J. O.; Fatope, M. O.; Okogum, J. I. *Am. J. Chem*. **2011**, *1*, 52-55.

(22) Sinha, R.; Gadhwal, M. K.; Joshi, U. J.; Srivastava, S.; Govil, G. *Int. J. Curr. Pharm. Res*. **2012**, *4*, 70-79.

(23) Larit, F.; Benyahia, S.; Benayache, S.; Benayache, F.; Léon, F.; Brouard, I.; Bermijo, J. *Int. J. Med. Arom. Plants* **2012**, *2,* 34-37.

(24) Dehaghani, Z. A.; Asghari, G.; Dinani, M. S. *J. Agric. Sci. Tech. A* **2017**, *7*, 45-51.

(25) Ghavan-Haghi, F.; Dinani, M. S. *J. Herbmed Pharmacol*. **2017**, *6*, 114-118.

(26) Li, H.; Liu, Y.; Yi, Y.; Miao, Q.; Lui, S.; Zhao, F.; Cong, W.; Wang, C.; Xia, C. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci*. **2017**, *1048*, 56-63.

(27) Legault, J.; Perron, T.; Mshvildadze, V.; Girard-Lalancette, K.; Perron, S.; Laprise, C.; Sirois, P.; Pichette, A. *J. Med. Food* **2011**, *14*, 1127-1134.

(28) Tamokou, J.-d.-D.; Mbaveng, T. A.; Kuete, V. In Medicinal Spices and Vegetables from Africa 1st Ed: Therapeutic Potential against Metabolic, Inflammatory, Infectious and Systemic Diseases; Academic Press; Amsterdam, 2017, pp 207-237.

(29) Kengne, I. C.; Tamokou, J. D. D. *Invest. Med. Chem. Pharmacol*. **2018**, *1*, 9.

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