Newsletter Tala *et al.*

ANTIOXIDANT XANTHONES, ANTHRAQUINONES AND SEMI-SYNTHETIC DERIVATIVES FROM VISMIA RUBESCENS AND VISMIA LAURENTII

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Summary

Chemical investigation of the methanol extract from V. rubescens yielded seven known compounds: friedelin, friedelanol, lupeol. 1,7-dihydroxyxanthone (1). 1.4.8trihydroxyxanthone (2), 1,2,8- trihydroxyxanthone (3) and physcion (4); that of the extract of V. laurentii also gave seven known compounds: laurenquinone A (5), laurenquinone B (6), xanthone V_1 (7), laurentixanthone C (8), bivismiaguinone (9), vismiaguinone (10) and vismiaquinone B (11). Laurenquinone B (6) and xanthone V_1 (7) were subjected to acetylation to afford two new acetylated derivatives: methyl 5,7-diacetoxy-6,11-dihydro-2,2,9-trimethyl-6,11-dioxo-2*H*-naphto[2,3-g]chromene-8-carboxylate (12) and 9-acetoxy-5,10-dihydroxy-2,2-dimethyl-12-(3,3-dimethylprop-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one (13) respectively. The antioxidant activities of compounds 2 and 3 were comparable to that of L-ascorbic acid used as the reference compound. The antioxidant activities of the crude extracts as well as those of compounds 4-13 are being reported here for the first time.

Keywords: *Vismia rubescens*, *Vismia laurentii*, Guttiferae, xanthones, anthraquinones, acetylated derivatives, antioxidant activity.

Newsletter

Introduction

In recent years, one of the areas which have attracted a great attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity (1-3). Plants have also been found to be useful in accelerating wound healing, a complex process involving the interplay of many biochemical and cellular mediators. Microbial infections, especially due to Staphylococcus, Streptococcus and Pseudomonas species, and the presence of oxygen free radicals, are known impediments to wound healing (4). Any agent capable of eliminating or reducing the number of microorganisms present in a wound, as well as reducing the level of reactive oxygen species (ROS), may facilitate the wound healing process. Vismia rubescens Oliv. and Vismia laurentii De Wild. are plants of Guttiferae family, found mostly in secondary forest in Tropical regions; they are used in West Tropical Africa in the treatment of infections and wounds (5-7). Previous studies have reported the presence of triterpenoids, anthraquinones, bianthraquinones, benzophenones, xanthones and lignans from V. rubescens and V. laurentii (6-9). Antimicrobial and antiplasmodial activities have also been reported for these plants (6-10). There are, however, no reports on the antioxidant properties of these plants. This study dealt with the isolation, identification and chemical transformations of compounds from the crude extracts of V. rubescens and V. laurentii. We also examined the in vitro antioxidant activities of the crude extracts, isolated compounds and some acetylated derivatives from these plants.

Material and Methods

General experimental procedures

Melting points were determined on a Büchi SMP-20 melting point apparatus and with a Reichert microscope and are uncorrected. UV spectra were measured with a UV-210 PC, UV.VIS scanning spectrophotometer (Analytikjena). IR spectra were recorded on a SHIMADZU FTIR-8400S spectrophotometer. EI-MS (ionization voltage 70 eV) and HREI-MS mass spectra were recorded on a Finnigan MAT double focusing spectrometer Model 8230. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were recorded in CDCl₃ or CDCl₃ + CD₃OD using a Bruker-Avance-500 MHz NMR spectrometer and TMS as internal standard. Silica gel 60 (70-230 mesh ASTM; Merck; Darmstadt, Germany) was used for column chromatography with step gradients of *n*-hexane-EtOAc and EtOAc-MeOH as eluents. Precoated silica gel plates (Merck, Kieselgel 60 F₂₅₄) were used for TLC. Spots were visualized at 254 nm and 365 nm, and by spraying with 50% H₂SO₄ followed by heating at 100 °C.

Plant material

The stem bark of *V. rubescens* Oliv. was collected in Bazou (West Region of Cameroon) and the stem bark and the seeds of *V. laurentii* De Wild. were collected in Mbalmayo (Centre Region of Cameroon) in March 2008. Botanical identification of the plants was done by comparison with voucher specimens (43288/HNC for *V. rubescens* and 1882/SRFK for *V. laurentii*) at the National Herbarium, Yaounde, Cameroon.

Extraction, fractionation and isolation

Vismia rubescens

The stem bark of V. rubescens was dried at room temperature $(25 \pm 2 \text{ °C})$ and ground. The resulting fine powder (1.0 kg) was extracted with MeOH (3 x 2 L, 72 h) to afford a crude extract (59 g; 5.9%, w/w) after evaporation under vacuum. A portion of this extract (55 g) was subjected to silica gel column chromatography eluted with step gradients of nhexane-EtOAc and EtOAc-MeOH. Twenty four fractions of 500 mL each were collected and combined on the basis of their TLC profiles into eight major fractions (A-H): A (6.9 g, nhexane-EtOAc 10:0, 95:5; v/v); B (7.5 g, n-hexane-EtOAc 9:1; v/v), C (3.9 g, n-hexane-EtOAc 8:2; v/v); D (5.4 g, n-hexane-EtOAc 7:3; v/v), E (7.0 g, n-hexane-EtOAc 1:1; v/v), F (4.6 g, *n*-hexane–EtOAc 0:1; v/v), G (5.3 g, EtOAc-MeOH 10:0, 95:5; v/v) and H (6.7 g, EtOAc-MeOH 9:1, 0:10; v/v). Fraction A contained mostly fatty material and was not further investigated. Fraction B was purified on a silica gel column with n-hexane-EtOAc (10:0, 95:5 and 9:1; v/v) to give friedelin (86 mg; 0.15%, w/w) and friedelanol (31 mg; 0.056%, w/w). Fraction C was subjected to silica gel column chromatography eluted with *n*-hexane-EtOAc (10:0, 98:2, 96:4, 94:6; v/v) to give eight subfractions (C1 to C8). Lupeol (33 mg; 0.06%, w/w) was crystallized from fraction C3 eluted with *n*-hexane-EtOAc (98:2; v/v) (1.8 g) while fraction C4 obtained with *n*-hexane-EtOAc (96:4; v/v) (900 mg) was further purified by preparative TLC using *n*-hexane-EtOAc (95:5; v/v) to yield 1,7-dihydroxyxanthone (1) (17 mg; 0.03%, w/w). Fraction D was subjected to another silica gel column chromatography and eluted with the mixture of *n*-hexane-EtOAc (10:0, 95:5, 9:1, 85:15, 8:2; v/v) to give physcion (4) (38 mg; 0.07%, w/w). Fraction E was rechromatographed on a silica gel column, eluting with *n*-hexane-EtOAc (10:0, 9:1, and 85:15, 8:2, 7:3, 1:1, 4:6; v/v) to give six subfractions (E1-E6). Subfraction E3 eluted with *n*-hexane-EtOAc (8:2; v/v) (1.2 g) was further purified by silica gel column chromatography with n-hexane-EtOAc (85:15; v/v) to afford 1,4,8trihydroxyxanthone (2) (162 mg; 0.29%, w/w) while subfraction E5 eluted with hexane-EtOAc (7:3) (750 mg) was purified by preparative TLC using *n*-hexane-EtOAc (8:2: v/v) to yield 1,2,8- trihydoxyxanthone (3) (32 mg, 0.058%; w/w).

Vismia laurentii

The seeds of *V. laurentii* were dried at room temperature $(25 \pm 2 \,^{\circ}\text{C})$ and ground. The resulting fine powder (158 g) was macerated with a mixture of CH₂Cl₂-MeOH (1:1; v/v) (3 x 1 L, 72 h) to afford a crude extract (12 g; 7.59%, w/w) after evaporation under vacuum. A portion of this extract (11.0 g) was subjected to silica gel column chromatography eluted with step gradients of *n*-hexane-EtOAc and EtOAc-MeOH. Twenty three fractions of 100 mL each were collected and combined on the basis of their TLC profiles yielding four main fractions (F1-F4): F1 (2.1 g, *n*-hexane-EtOAc 10:0, 9:1, 8:2; v/v), F2 (1.9 g, *n*-hexane-EtOAc 8:2; v/v), F3 (1.63 g, *n*-hexane-EtOAc 7:3; v/v) and F4 (3.7 g, *n*-hexane-EtOAc 6:4, 0:10; v/v; EtOAc-MeOH 9:1, 8:2, 0:10; v/v). Fraction F1 contained mostly fatty material and was not further investigated. Fraction F2 was subjected to repeated column chromatography over silica gel with a gradient of *n*-hexane-EtOAc to give laurenquinone B (6) (15 mg; 0.13%, w/w). Fraction F3 was rechromatographed over silica gel using a gradient of CH₂Cl₂-EtOAc. Subfractions eluted with CH₂Cl₂-EtOAc (9:1; v/v) were further purified through Sephadex LH-20 column using CH₂Cl₂-MeOH (1:1; v/v) to give laurenquinone A (5) (26 mg; 0.23%, w/w) and xanthone V₁ (7) (20 mg; 0.18%, w/w).

The stem bark of *V. laurentii* was dried at room temperature $(25 \pm 2 \text{ °C})$ and ground. The resulting fine powder (2.0 Kg) was extracted successively with acetone (3 x 4 L, 72 h) and MeOH(3 x 4 L, 72 h). Removal of the solvents under reduced pressure yielded 43 g (2.15%, w/w) and 56 g of respective extracts. Part of the acetone extract (40 g) was subjected to column chromatography over silica gel, eluting with *n*-hexane-EtOAc of increasing polarity. Twenty four fractions of 500 mL each were collected and combined on the basis of TLC to afford six major fractions (A-F): A (6.9 g, *n*-hexane-EtOAc 10:0, 95:5; v/v); B (5.4 g, *n*-hexane-EtOAc 9:1; v/v), C (5.0 g, *n*-hexane-EtOAc 8:2; v/v); D (3.3 g, *n*-hexane-EtOAc 7:3; v/v), E (8.0 g, *n*-hexane-EtOAc 1:1; v/v) and F (8.4 g, *n*-hexane-EtOAc 10:0; v/v). Fraction A contained mostly fatty material and was not further investigated. Fraction B was purified on a silica gel column eluting with *n*-hexane-EtOAc (10:0, 98:2, 96:4, 94:6, 9:1; v/v) to give laurentixanthone C (8) (500 mg; 1.25%, w/w). Fraction C was subjected to repeated column chromatography over silica gel with *n*-hexane-EtOAc (10:0, 98:2, 96:4, 94:6, 9:1, 85:15; v/v) to yield vismiaquinone (10) (15 mg; 0.037%, w/w). Fraction D was purified over silica gel using a gradient of *n*-hexane-EtOAc to afford bivismiaquinone (9) (88 mg; 0.22%, w/w). Further column chromatography of fraction F over silica gel, eluting with a step gradient of *n*-hexane-EtOAc gave five main subfractions (F1-F5). Vismiaquinone B (11) (6 mg; 0.015%, w/w) was crystallized from fraction F2 eluted with *n*-hexane-EtOAc (8:2; v/v).

Acetylation of laurenquinone B (9) and xanthone V_1 (11)

Laurenquinone B (9) (7 mg) was dissolved in 1.5 mL of AC₂O and 1 mL of pyridine, and was stirred at room temperature for 24 h. 10 mL of water was added to the mixture and stirred for 30 min. Extraction with CH_2Cl_2 and purification over a silica gel column with CH_2Cl_2 as solvent gave a new diacetyl derivative, identified as methyl 5,7-diacetoxy-6,11-dihydro-2,2,9-trimethyl-6,11-dioxo-2*H*-naphto[2,3-*g*]chromene-8-carboxylate (12) (5.0 mg, 58.57%).

Xanthone V₁ (**11**) (10 mg) was dissolved in 2 mL of AC₂O and 2 mL of pyridine, and was stirred at room temperature for 24 h. 10 mL of water was added to the mixture and stirred for 30 min. Extraction with CH_2Cl_2 and purification by prep. TLC (*n*-hexane-EtOAc 9:1) gave a new acetyleted derivative, namely 9-acetoxy-5,10-dihydroxy-2,2-dimethyl-12-(3,3-dimethylprop-2-enyl)pyrano[3,2-*b*]xanthen-6(2*H*)-one (**13**) (6.2 mg, 56.05%).

Methyl 5,7-diacetoxy-6,11-dihydro-2,2,9-trimethyl-6,11-dioxo-2H-naphto[2,3-g]chromene-8-carboxylate (12)

Red powder; m.p. = 193-194 °C; ¹H NMR (500 MHz, CDCl₃+ CD₃OD): δ (ppm) 8.00 (1H, s, H-10), 7.57 (1H, s, H-12), 6.45 (1H, d, J = 10.2 Hz, H-4), 5.83 (1H, d, J = 10.2 Hz, H-3), 3.95 (3H, s), 2.46 (3H, s), 2.44 (3H, s), 2.40 (3H, s, 11-CH₃), 1.49 (6H, s, C-13/C-14); ¹³C NMR (125 MHz, CDCl₃ + CD₃OD): δ (ppm) 181.3 (C-11), 179.2 (C-6), 168.9 (CH₃O-<u>C</u>O-), 168.5 and 166.0 (2CH₃<u>C</u>OO-), 158.1 (C-1a), 144.8 (C-5), 143.1 (C-7), 134.6 (C-9), 134.5 (C-10a), 134.4 (C-8), 134.3 (C-11a), 130.3 (C-3), 126.6 (C-6a), 123.6 (C-5a), 120.9 (C-4a), 120.5 (C-12), 115.6 (C-10), 115.2 (C-4), 112.7 (C-12), 78.2 (C-2), 52.6 (CH₃O), 28.5 (C-14), 28.4 (C-13), 21.0 (C-15), 20.9 and 20.1 (2<u>C</u>H₃-CO-); EIMS *m/z* (rel. int): 478 [M]⁺ (4); 435 [M - COMe]⁺ (15), 392 [M - 2COMe]⁺, (8), 379 (100) and 347 (93); HREIMS: *m/z* 478.1270 (calcd. for C₂₆H₂₂0₉, 478.1264).

9-acetoxy-5,10-dihydroxy-2,2-dimethyl-12-(3,3-dimethylprop-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one (13)

Yellow powder; m.p. = 175-176 °C; ¹H NMR (500 MHz, CDCl₃ + CD3OD): δ (ppm) 13.28 (1H, s, 5-OH), 7.57 (1H, d, J = 8.7 Hz, H-7), 6.77 (1H, d, J = 8.7 Hz, H-8), 6.44 (1H, d, J = 10.0 Hz, H-4), 5.71 (1H, d, J = 10.1 Hz, H-3), 5.17 (1H, t, J = 6.9 Hz, H-2'), 3.42 (2H, d, J = 6.6 Hz, H-1'), 2.51 (6H, s), 1.80 (3H, s, H-4'), 1.67 (3H, s, H-5'), 1.45 (3H, s, H-13), 1.44

(3H, s, H-14); ¹³C NMR (125 MHz, CDCl₃ + CD3OD): δ (ppm) 180.3 (C-6), 167.2 (Me<u>C</u>OO-), 156.7 (C-5), 153.9 (C-11a), 152.2 (C-1a), 143.1 (C-10a), 138.5 (C-9), 131.8 (C-10), 131.4 (C-5'), 126.8 (C-3), 122.0 (C-2'), 116.7 (C-8), 115.3 (C-4), 112.8 (C-6a), 112.5 (C-7), 108.6 (C-12), 104.4 (C-4a), 102.7 (C-5a), 77.6 (C-2), 28.2 (C-13 and C-14), 25.7 (C-3'), 21.2 (C-1'), 20.6 (<u>C</u>H3-CO-), 18.0 (C-4'); EIMS *m/z* (%): 436 [M]⁺ (6) and 393 [M - COMe]⁺ (20). HREIMS: *m/z* 436.1517 (calcd. for C₂₅H₂₄07, 436.1522).

Antioxidant assay

The test samples, were prior dissolved in DMSO (SIGMA) beforehand, then mixed with a 20 mg/L 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) methanol solution, to give final concentrations of 10, 50, 100, 500 and 1000 μ g/mL. Antioxidant activities of the crude extracts and compounds from *V. rubescens* and *V. laurentii* as well as the new acetylated derivates determined on the basis of their scavenging potential of the stable DPPH free radical .The inhibition ratio (%) and the amount of sample necessary to decrease by 50% the absorbance of DPPH (IC₅₀) were calculated.

Statistical analysis

The inhibition ratios and the IC_{50} of the test substances were expressed as the Mean \pm Standard Deviation and compared using Waller-Duncan's test. A value of p < 0.05 was considered statistically significant.

Results and Discussion

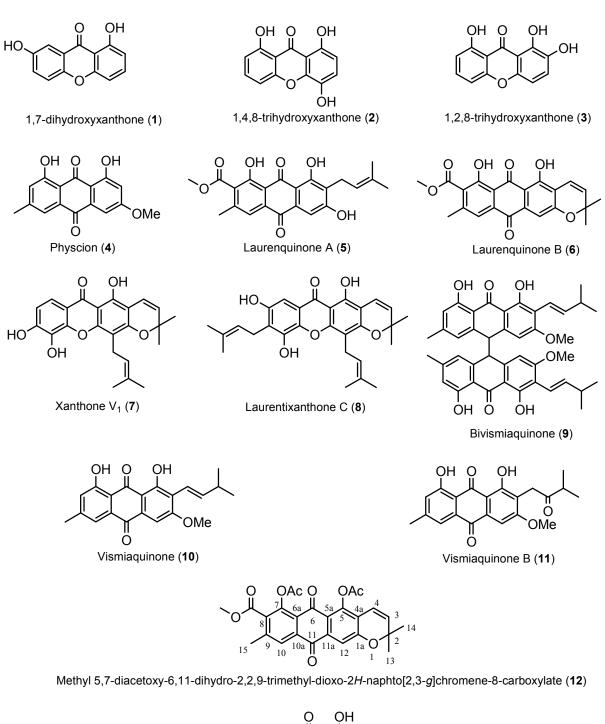
The structures of the isolated compounds (Figure 1) were elucidated on the basis of spectroscopic data (IR, ¹H NMR, ¹³C NMR and 2D NMR). Comparison of the data with those reported in the literature led to the identification of the compounds as friedelin (6), friedelanol (6), lupeol (11), 1,7-dihydroxyxanthone (1) (6), 1,4,8-trihydroxyxanthone (2) (6), 1,2,8trihydroxyxanthone (3) (12), physcion (4) (7), laurenquinone A (5) (7), laurenquinone B (6) (7), xanthone V_1 (7) (7). laurentixanthone C (8) (9), bivismiaquinone (9) (9), vismiaquinone (10) (9), vismiaquinone B (11) (10). The structures of the semi-synthetic derivatives, methyl 5,7-diacetoxy-6,11-dihydro-2,2,9-trimethyl-6,11-dioxo-2H-naphto[2,3-g]chromene-8-9-acetoxy-5,10-dihydroxy-2,2-dimethyl-12-(3,3-dimethylprop-2and carboxylate (12)envl)pyrano[3,2-b]xanthen-6(2H)-one (13) (Figure 1) were determined on the basis of ${}^{1}H$ NMR, ¹³C NMR and EIMS data and comparison with those of laurenquinone B (6) and xanthone V_1 (7) respectively. This is the first report concerning the isolation of lupeol and 1,2,8-trihydroxyxanthone from V. rubescens as well as the semi-synthesis of acetylated derivatives from laurenquinone B and xanthone V₁.

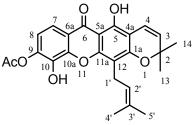
DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In this study, we have evaluated the antioxidant activities of the crude extracts and the isolated compounds from *V. rubescens* and *V. laurentii*. The results summarized in table 1 showed that the crude extracts of the two plants and compounds **1-13** exhibited different degrees of antioxidant activities on the DPPH radical. Comparable results have been reported from other species of the Guttiferae family (13-15). The inhibition percentage of free radical (DPPH) increased with the concentration of the tested substances. Friedelin, friedelanol and lupeol were found to be inactive (not shown). The MeOH extract of the stem bark of *V. rubescens* (IC₅₀ = 2.18 µg/mL) exhibited strong antioxidant activity. Its antioxidant activity was greater than those of the acetone-soluble fraction of the stem bark of *V. laurentii* (IC₅₀ = 2.57 µg/mL) and compounds **1, 4-6, 8-13**.

However, the antioxidant activity of the MeOH extract of V. rubescens was less than those of compounds 3 (IC₅₀ = 1.73 μ g/mL), 2 (IC₅₀ = 1.92 μ g/mL), 7 (IC₅₀ = 2.09 μ g/mL) and Lascorbic acid ($IC_{50} = 1.86 \mu g/mL$). The results of the antioxidant activities of crude extracts of V. rubescens and V. laurentii partially support the traditional use of these plants in the treatment of infectious diseases and wounds. Compound **3** (IC₅₀ = $1.73 \mu g/mL$) was the most active substance among the test samples. Moreover, its antioxidant activity and that of compound 2 (IC₅₀ = 1.96 μ g/mL) were found to be comparable (p \ge 0.05) to that of Lascorbic acid (IC₅₀ = $1.86 \mu g/mL$) used as the reference compound. This is interesting in line with the perspective of developing new antioxidant drugs from natural products. Although the antimicrobial activities of compounds 1-2 and 4-10 had been previously reported (6,7,9), this study reports the antioxidant activities of the crude extracts and compounds from V. rubescens and V. laurentii as well as some of their acetylated derivatives for the first time. The antioxidant properties of some individual xanthones and anthraquinones of plant origin are documented (14,16,17). As for the structure-activity relationship, compound 3 with the same basic skeleton as compounds 1 and 2 was more active (Table 1). This could be due to the number and position of hydroxyl groups in these compounds (Figure 1). In comparison with compound 8, compound 7 with the same basic skeleton was more active (Table 1). The presence of a hydroxyl group at position 9 in compound 7 and the presence of hydroxyl and isoprenyl groups at positions 8 and 9 respectively in compound 8 (Figure 1) could be responsible for the difference in the observed antioxidant activities. In comparison with compound 10, compound 4 with the same basic skeleton was more active (Table 1). The presence of the (E)-3-methylbutene group at position 2 in compound 10 (Figure 1) could be responsible for the difference in the observed antioxidant activity. Moreover, the presence of the methoxyl group at position 3 in compound 4 and the presence of isoprenyl, hydroxyl and methoxycarbonyl groups at positions 2, 3 and 7 respectively in compound 5 (Figure 1) could be responsible for their different activities (Table 1). In comparison with compound 10, compound 11 with the same basic skeleton was more active (Table 1). The presence of (E)-3methylbutene and 3-methylbut-2-one groups at position 2 in compounds 10 and 11 respectively (Figure 1) could be responsible for the difference in the observed antioxidant activities. The acetylation of compound 6 enhanced its antioxidant activity while that of compounds 7 decreased its activity (Table 1). The overall results suggest that the position and the degree of hydroxylation on the aromatic ring are the most important features for the antioxidant activities as previously reported (18). Flavonoids, hydroxycinnamates and other related phenolic compounds have been reported to function as potent antioxidants by virtue of their hydrogen-donating and metal-chelating properties (19-21). Therefore, the presence of these compounds could be responsible for the antioxidant activity found in the crude extracts. These results suggest that an investigation into the structural requirements for the scavenging activities of the different classes of compounds on free radicals and reactive oxygen species is needed.

Conclusion

The results of this study indicate that the crude extracts of *V. rubescens* and *V. laurentii* as well as some of their isolated compounds: 1,2,8-trihydroxyxanthone, 1,4,8-trihydroxyxanthone, laurenquinone A, xanthone V_1 , vismiaquinone B and bivismiaquinone possess potential antioxidant properties and therefore may be useful for treating oxidative damage. Further investigation into the antioxidant activity of these natural compounds in rats on hepatic glutathione, lipid peroxidation and catalase levels will be necessary to confirm this hypothesis.





9-acetoxy-5,10-dihydroxy-2,2-dimethyl-12-(3,3-dimethylprop-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one (13)

Fig. 1. Chemical structures of compounds isolated from *V. rubescens* (1-4) and *V. laurentii* (5-11) and of acetylated derivatives (12-13)

Extracts/compounds	IC ₅₀
V. rubescens	
MeOH extract	2.18 ± 0.11^{a}
1,7-dihydroxyxanthone (1)	134.87 ± 0.88^{d}
1,4,8-trihydroxyxanthone (2)	1.96 ± 0.07^{en}
1,2,8- trihydroxyxanthone (3)	1.73 ± 0.06^{b}
physcion (4)	$95.47 \pm 0.59^{\circ}$
V. laurentii	
Acetone extract	$2.57 \pm 0.14^{\rm f}$
laurenquinone A (5)	$4.78 \pm 0.08^{ m g}$
laurenquinone B (6)	$112.27 \pm 0.70^{\rm h}$
xanthone V1 (7)	2.09 ± 0.06^{ae}
laurentixanthone C (8)	$128.82 \pm 0.80^{\rm i}$
bivismiaquinone (9)	36.30 ± 0.54^{k}
vismiaquinone (10)	69.18 ± 0.65^{j}
vismiaquinone B (11)	3.80 ± 0.07^{1}
methyl 5,7-diacetoxy-6,11-dihydro-2,2,9-trimethyl-6,11-	
dioxo-2 <i>H</i> -naphto[2,3-g]chromene-8-carboxylate (12)	36.42 ± 0.38^{k}
9-acetoxy-5,10-dihydroxy-2,2-dimethyl-12-(3,3-	
dimethylprop-2-enyl)pyrano[3,2- <i>b</i>]xanthen-6(2H)-one (13)	44.66 ± 0.43^{m}
L-ascorbic acid (reference compound)	$1.86 \pm 0.09^{\text{bn}}$

Table 1- Inhibition concentrations of crude extracts and compounds from V. rubescens
and <i>V. laurentii</i> scavenging 50 % of DPPH radical (IC ₅₀) in µg/mL.

Values are expressed as mean \pm SD. In the same column, values affected by the different superscript letters (a-n) are significantly different (p < 0.05).

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