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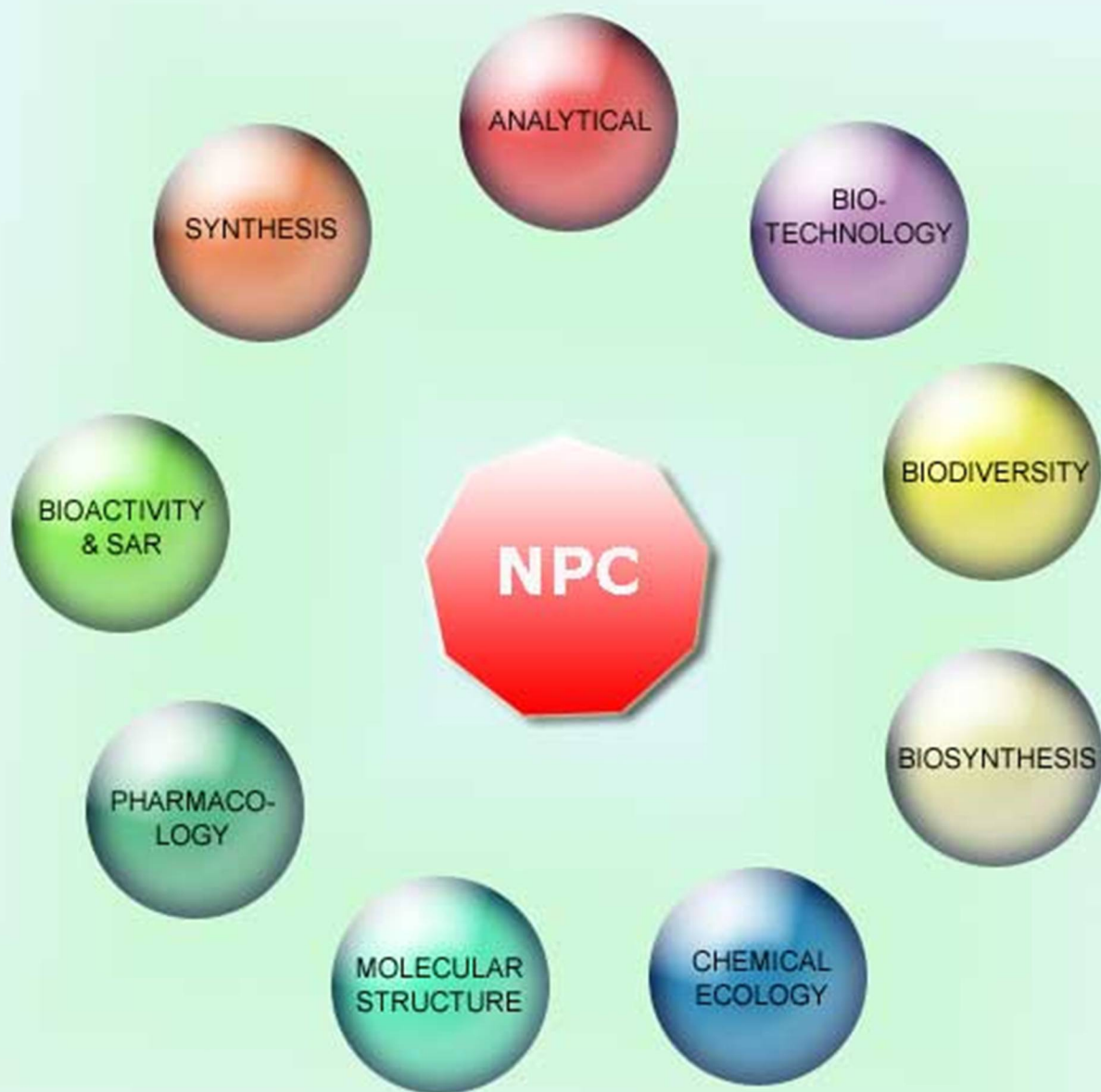
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NATURAL PRODUCT COMMUNICATIONS

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Volume 6. Issue 12. Pages 1799-1968. 2011
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
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A Novel Flavonoid and Furoquinoline Alkaloids from *Vepris glomerata* and their Antioxidant Activity

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Received: June 23rd, 2011; Accepted: October 4th, 2011

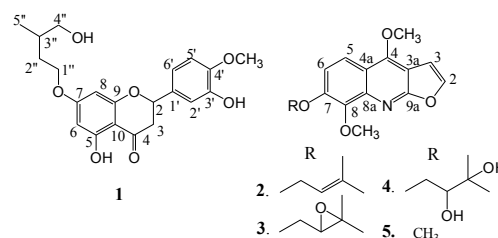
The dichloromethane extract of the aerial part of the plant *Vepris glomerata* (Rutaceae) yielded a new flavonoid, which was accorded the trivial name veprisinol (**1**), together with four known furoquinoline alkaloids: haplopine-3,3'-dimethylallyl ether (**2**), anhydroevoxine (**3**), evoxine (**4**) and skimmianine (**5**). The structures of the compounds were established by 1D and 2D NMR spectroscopy, as well as HREIMS. Compounds **1** and **2** have strong antioxidant potential, similar to and in some instances better than ascorbic acid and can be used as beneficial additives to antioxidant supplements.

Keywords: *Vepris glomerata*, veprisinol, furoquinoline alkaloids, antioxidant activity.

The African *Vepris* species have proved to be a good source of furoquinoline and acridone alkaloids that typify the genus as a whole. *V. bilocularis* has been found to have both furoquinoline as well as acridone alkaloids [1,2], while furoquinoline alkaloids alone have been found in *V. ampody* [3], *V. heterophylla* [4], *V. punctata* [5] and *V. stolzii* [6], and acridone alkaloids alone in *V. fitoravina* and *V. macrophylla* [7]. The alkaloids are reported to possess broad spectrum antimicrobial [8], antiradical [9], antioxidant [10], antiplasmodial [11], anticancer [12] and antimutagenic [13] activities. *V. glomerata* is used in African traditional medicine, where its aqueous root extract is used to treat malaria, epilepsy, psychosis and stroke, when mixed with tea [14]. Earlier pharmacological studies on this plant reported antiplasmodial activities of the ethanol extract [15].

Since the species of Rutaceae are often cited as antimalarials or febrifuges in African traditional medicine [14], and the antioxidant activity of alkaloids [10] and flavonoids [16] has previously been demonstrated, all the five compounds isolated were assessed for antioxidant activity using three methods.

Here we report on the isolation and structure elucidation of a new flavonoid, in addition to four known furoquinoline alkaloids: haplopine-3,3'-dimethylallyl ether (**2**), anhydroevoxine (**3**), evoxine (**4**) and skimmianine (**5**) from the dichloromethane extract of *V. glomerata*, together with their antioxidant activities *in vitro*. The structures of the known compounds **2-5** were determined by comparison



of their physical and spectroscopic data with those reported in literature; **2** and **3** [17], **4** [18] and **5** [19]. Only skimmianine was previously reported from the leaves of *V. glomerata* endemic to Ethiopia, in addition to kokusaginine [20]. It is not apparent if the different compounds found in this study are as a result of either geographical or seasonal differences.

Compound **1** was obtained as a yellow solid. Its mass was established to be 388.1573 amu, based on HREIMS data, corresponding to a molecular formula of $C_{21}H_{24}O_7$, which indicates a double bond equivalence of 10, eight being due to the aromatic rings, one being due to the carbonyl group and one to ring C of the flavanone skeleton. The IR spectrum showed a carbonyl stretching band at 1705 cm^{-1} and a hydroxyl absorption band at 3364 cm^{-1} . This compound was identified as a flavanone based on its characteristic ^1H NMR spectral pattern. The characteristic ABX coupling system of H-2 β , H-3 α and H-3 β appeared at δ_{H} 5.29 (1H, dd, $J = 12.84, 2.84\text{ Hz}$, H-2 β), δ_{H} 3.04 (1H, dd, $J = 17.12, 2.84\text{ Hz}$, H-3 α) and δ_{H} 2.75 (1H, dd, $J = 17.12, 12.84\text{ Hz}$, H-3 β). These signals also showed COSY and NOESY correlations with each other.

Another characteristic pattern was that of the trisubstituted aromatic B ring. The proton resonances of this ring occurred as a singlet at δ_H 7.00 (s, H-2'') and doublets at δ_H 6.89 and 6.84 (1H each, d, $J = 8.48$ Hz, H-5' and H-6'). The small coupling constant of about 2 Hz for $J_{H2'',H6'}$ could not be detected for the H-2'' resonance. The 1H NMR spectrum also showed the presence of a methoxy group at δ_H 3.88 (s), its position at C-4' being confirmed by both a 1D NOE and a NOESY correlation with the resonances at δ_H 6.89 and 6.84 (H-5' and H-6'). Five aromatic C-O resonances were seen at δ_C 164.0, 167.2, 162.8, 145.0 and 147.0 attributed to oxygenation at C-5, 7, 9, 3' and 4'.

A pair of doublets at δ_H 6.02 (1H, d, $J = 1.76$ Hz, H-6) and δ_H 6.00 (1H, d, $J = 1.76$ Hz, H-8) were attributed to the *meta* coupled, H-6 and H-8 protons on ring A. These two proton resonances showed NOESY correlations to 2H-1'' at δ_H 4.02, confirming the position of the side chain at C-7. Its corresponding carbon resonance showed HMBC correlations to two multiplets at δ_H 1.87 (overlapping resonances of H-2''a and H-3'') and δ_H 1.61 (H-2''b). The H-2'' resonances were diastereotopic and appeared as two separate resonances. COSY correlations were also observed between H-1'' and H-2''a and H-2''b and between H-2''b and H-3''. The H-3'' methine proton was coupled to the methyl proton resonance at δ_H 0.95 (d, $J = 6.52$ Hz) attributed to 3H-5'' and the methylene proton at δ_H 3.50 (2H-4'') in the COSY spectrum. These correlations formed a side chain which was attached to ring A by an ether linkage at C-7. Compound **1** was thus identified as 4H-1-benzopyran-4-one, 2, 3-dihydro-5-hydroxy-2-(4'-methoxy-3'-hydroxybenzyl)-7-O-(2-methyl butanol) ether, and given the trivial name veprisinol.

The results of the reducing potential (transformation of Fe^{3+} - Fe^{2+}) of the standard (ascorbic acid) and compounds **1-5** are shown in Figure 1. The activity of haplopine-3,3'-dimethylallyl ether, **2** and veprisinol (**1**) was significantly higher than the activity of the other three alkaloids at all concentrations. However, the reducing power of compound **1** was significantly lower than that of compound **2**. The reducing power of the compounds and standard followed the order: ascorbic acid > **2** > **1** > **3** > **4** > **5**.

The DPPH radical scavenging assay results are shown in Fig. 2. The results revealed that the scavenging activity of the standard ascorbic acid was significantly higher than all other compounds tested. At concentrations of $62.5 \mu g mL^{-1}$ and above, the activity decreased in the order ascorbic acid > **1** > **2** > **4** > **3**, whereas at the lower concentrations, 31.25 and $15.625 \mu g mL^{-1}$, evoxine (**4**) had the highest percentage antioxidant activity of 41%. The activity of compounds **1** and **2** was increased with their concentration and significantly higher than other compounds, particularly at higher concentrations (Figure 2).

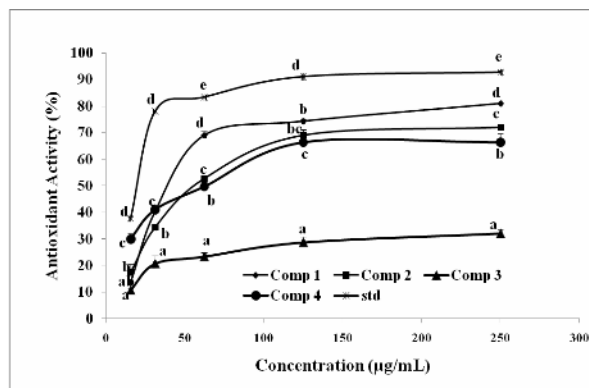


Figure 1: Free radical reducing potential of compounds **1-5** and standard ascorbic acid as evaluated by the spectrophotometric detection of the Fe^{3+} - Fe^{2+} transformation (FRAP method).

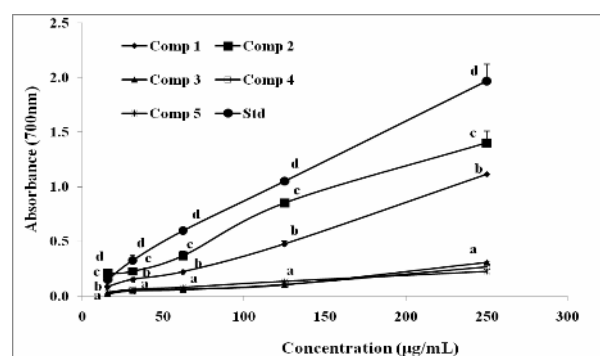


Figure 2: Antioxidant activity of compounds **1-4** and ascorbic acid standard, as measured by the DPPH method.

The hydroxyl radical scavenging activities in the deoxyribose assay are shown in Figure 3. The results revealed that compound **1** possessed significantly higher activity than all the other compounds tested, including the standard, ascorbic acid, at most concentrations. Compounds **1**, **2** and **4** had hydroxyl radical scavenging activity comparable with and in the case of **1** and **2**, better than that of ascorbic acid. Skimmianine (**5**) was not tested in either the DPPH or deoxyribose assays due to insufficient amount.

The three assays revealed that compounds **1** and **2** are good antioxidant compounds, while compound **4** shows high activity at a lower concentration in the DPPH assay. Flavonoids are known to be potent antioxidants and their activity is dependent on their molecular structure. The activity of **1** could be attributed to the hydroxyl (OH) groups in the molecule, which donate hydrogen to reduce the DPPH radical to DPPH-H. The alkaloids **2-5** have the same basic skeleton, the only difference being in their side chain. The reductive ability of **2** may be attributed to the double bond of the isoprenyl unit, rich in delocalized pi-electrons, which are easily donated during reduction of Fe^{3+} to Fe^{2+} . Sang *et al.* also reported that the double bond of the isoprenyl group was responsible for the antioxidant activity of garcinol [21]. The antioxidant activity of **2** in the DPPH assay, like **1**, could also be attributed to the hydroxyl groups in the molecule.

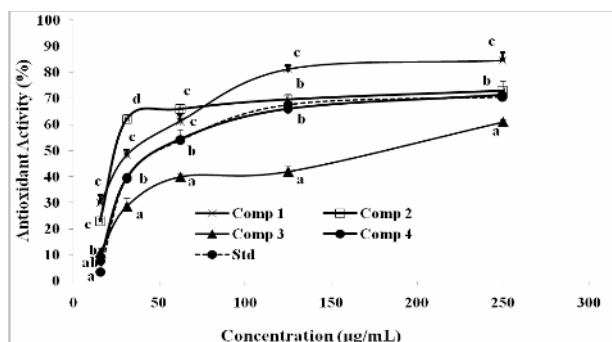


Figure 3: Hydroxyl radical scavenging activity of compounds 1-4 and standard ascorbic acid as measured by the deoxyribose method.

In conclusion, five compounds were isolated (a flavonoid and four alkaloids) from the aerial parts of *V. glomerata*. Verification of their antioxidant activities, as well as comparison with known antioxidants, will provide herbalists and traditional healers with scientific evidence for the use of the aerial parts of this plant as natural antioxidants.

Experimental

General experiment procedures: The melting points were recorded on an Ernst Leitz Wetzler micro-hot stage melting point apparatus and are uncorrected. UV spectra were obtained on a Varian Cary UV-VIS Spectrophotometer in chloroform. IR spectra were recorded on a Perkin-Elmer Universal ATR Spectrometer. The 1D and 2D NMR spectra were recorded using a Bruker Avance^{III} 400 MHz NMR spectrometer. All the spectra were recorded at room temperature using deuterated chloroform (CDCl₃) as solvent. The HREIMS was measured on a Bruker Micro TOF-QII instrument. Specific rotations were measured at room temperature in chloroform on a PerkinElmerTM, Model 341 Polarimeter with a 10 mm flow tube. The separation, isolation and purification of compounds were carried out by gravity CC and monitored by TLC. Merck silica gel 60 (0.040-0.063 mm) was used for CC. Merck 20 × 20 cm silica gel 60 F₂₅₄ aluminum sheets were used for TLC. TLC plates were analyzed under UV light (254 and 366 nm) before being sprayed with anisaldehyde: concentrated sulfuric acid: methanol [1:2:97] spray reagent and then heated.

Plant material: *Vepris glomerata* was collected from the Rift Valley province of Kenya and identified by Dr S. T. Kariuki from the Department of Botany, Egerton University, Kenya. A voucher specimen (Kiplimo 01) was deposited at the University of KwaZulu-Natal Ward Herbarium, Westville Campus, Durban, South Africa.

Extraction and isolation: The air-dried aerial parts (980 g) of *V. glomerata* were sequentially extracted with *n*-hexane, followed by dichloromethane in a Soxhlet apparatus for 48 h, yielding crude extracts of 46 and 32 g, respectively. The oily residue of the dichloromethane extract obtained after evaporation under vacuum, was separated by CC on silica

gel with *n*-hexane and then increasing the concentration of ethyl acetate from 10 to 80% in *n*-hexane, to give 10 fractions (fr.); fr. 8-16 (1.27 g), fr. 17-19 (0.5 g), fr. 20-26 (2.36 g), fr. 27-32 (2.35 g), fr. 33-39 (1 g), fr. 40-43 (2.1 g), fr. 44-49 (0.5 g), fr. 52-56 (3.9 g), fr. 57-62 (1.75 g) and fr. 63-67 (5.1 g).

Fraction 52-56 was separated by CC with *n*-hexane/EtOAc (7:3) as the solvent to afford sub-fractions A-C. Sub-fraction A was further purified using 100% dichloromethane to afford compound **2**, a green solid (51 mg). Sub-fraction B yielded compound **3**, a brownish solid (43 mg), which needed no further purification. Sub-fraction C was crystallized in methanol to afford **4** (62 mg). Fraction 44-49 was purified using 100% dichloromethane to afford **5** (60 mg). Fraction 63-67 was separated with *n*-hexane/EtOAc (4:1) to yield 4 sub-fractions A-D. Sub-fraction B was crystallized in methanol to afford yellow crystals of compound **1** (18 mg).

Veprisinol (1)

4H-1-Benzopyran-4-one, 2, 3-dihydro-5-hydroxy-2-(4'-methoxy-3'-hydroxybenzyl)-7-O-(2-methyl butanol) ether

Yellow solid.

M.p: 78-80°C.

[α]_D²⁰: +55.30 (c 0.056, CHCl₃).

IR: 3364 (O-H), 2928, 1705 (C=O), 1636, 1512, 1162 cm⁻¹.
UV λ_{max} (CHCl₃) nm (log ε): 337 (4.45), 285 (5.13), 239 (5.44).

¹H NMR (400 MHz, CDCl₃): 11.97 (H, s, OH), 7.00, (H, s, H-2''), 6.89 (H, d, *J* = 8.28 Hz, H-5'), 6.84 (H, d, *J* = 8.28 Hz, H-6'), 6.02 (H, d, *J* = 1.76 Hz, H-6), 6.00 (H, d, *J* = 1.76 Hz, H-8), 5.29 (H, dd, *J* = 12.84, 2.84 Hz, H-2β), 4.02 (2H, dd, *J* = 12.88, 6.24 Hz, 2H-1''), 3.88 (3H, s, OCH₃), 3.50 (2H, d, *J* = 5.68 Hz, 2H-4''), 3.04 (H, dd, *J* = 17.12, 12.84 Hz, H-3α), 2.75 (H, dd, *J* = 17.12, 2.84, Hz, H-3β), 1.87 (2H, m, H-2''a and H-3''), 1.61 (H, m, H-2''b), 0.95 (3H, d, *J* = 6.52 Hz, H-5'').

¹³C NMR: 195.97 (C, C-4), 167.29 (C, C-7), 164.05 (C, C-5), 162.85 (C, C-9), 147.02 (C, C-4'), 145.93 (C, C-3'), 131.52 (C, C-1'), 118.15 (CH, C-5'), 112.71 (CH, C-2'), 110.71 (CH, C-6'), 103.11 (C, C-10), 95.54 (CH, C-6), 94.60 (CH, C-8), 78.92 (CH, C-2), 67.86 (CH₂, C-4''), 66.70 (CH₂, C-1''), 56.06 (OCH₃), 43.15 (CH₂, C-3), 32.94 (CH, C-3''), 32.37 (CH₂, C-2''), 16.60 (CH₃, C-5'').

HREIMS *m/z* 388.1573 [M]⁺ (calcd. for C₂₁H₂₄O₇, 388.1522)

Antioxidant activity: The total reducing power was determined according to the method described previously [22]. The free radical scavenging activity (antioxidant capacity) of the plant phytochemicals on the stable radical 2, 2-diphenyl-β-picrylhydrazyl (DPPH) was evaluated by the method established by Shirwaikar *et al.* [23], and the deoxyribose assay for hydroxyl radical scavenging activity was performed as described previously by Chung *et al.* [24].

Statistical analysis: The data in Figures 1-3 are presented as mean \pm SD of triplicates. ^{a-d}Values with different superscript letters for a given concentration are significantly different from each of the other compounds. The data were statistically analyzed using a statistical software program SPSS (SPSS for Windows, version 18, SPSS Science, Chicago, IL, USA). One-way analysis of

variance (ANOVA) followed by Tukey's multiple range post-hoc test was employed to find the differences. The data were considered significantly different at $p < 0.05$.

Acknowledgments - The authors wish to acknowledge the financial support received from the Organization for Women in Science for the Developing World (OWSDW).

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