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## Short Communication

# Seven flavonoids with antibacterial activity isolated from *Combretum erythrophyllum*

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Leaf extracts of *C. erythrophyllum* yielded seven flavonoids by bioassay-guided fractionation. Four of these compounds were identified as flavonols and three were identified as flavones using Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS). Six of

these flavonoids are reported for the first time for the Combretaceae (kaempferol, rhamnocitrin, rhamnazin, quercetin-5,3'-dimethylether, genkwanin and 5-hydroxy-4',7-dimethoxyflavone).

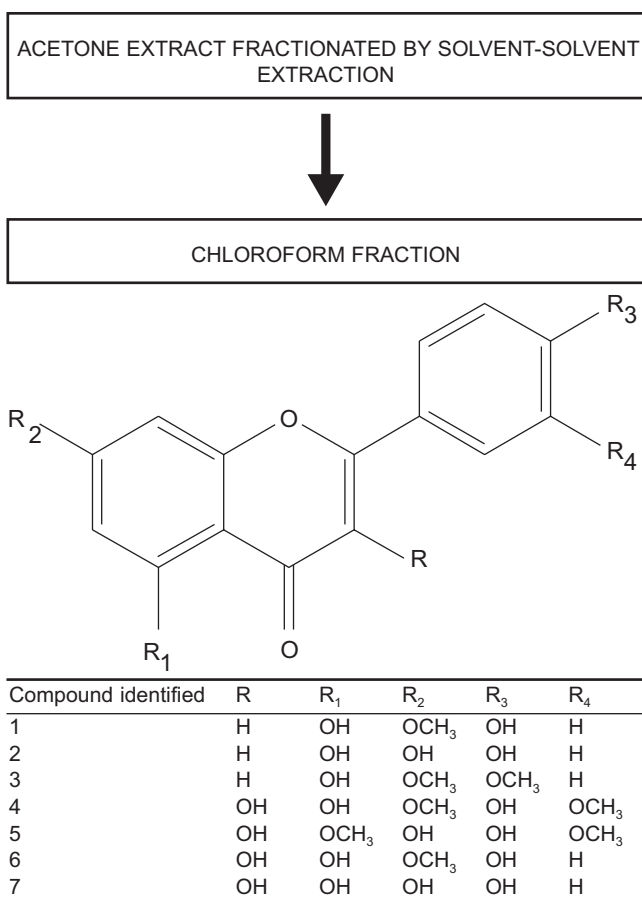
Considerable phytochemical work has been done on the genus *Combretum* (Letcher and Nhamo 1973, Osborne and Pegel 1985, Pegel and Rogers 1985, Rogers and Thevan 1986, Katerere 2001). Several phenanthrenes and stilbenes have been reported from *C. hereroense* (Letcher and Nhamo 1973), *C. apiculatum* (Rogers and Verotta 1996), *C. caffrum* (Pettit *et al.* 1987), *C. kraussii* and *C. erythrophyllum* (Schwikkard *et al.* 2000). These compounds commonly known as combretastatins are of interest due to their activity as anti-mitotic and anti-angiogenesis agents (Pettit *et al.* 1987). Pentacyclic triterpenes and their glycosides were isolated from *C. nigricans* (Jossang *et al.* 1996). Two triterpenes, arjunolic acid and mollic acid, as well as two flavonoids were reported from *C. leprosum* (Facundo *et al.* 1993). Triterpenoid acids and lactones from *C. erythrophyllum* have been isolated in the search for compounds responsible for the reported toxicity of this plant (Lawton and Rogers 1991). The representative aglycone isolated was named erythrophylllic acid. *C. erythrophyllum* contains many antibacterial compounds (Martini and Eloff 1998) and we attempted to identify these compounds.

*Combretum erythrophyllum* (Burch.) Sond. leaves were collected from a tree in the Pretoria National Botanic Gardens. The plant was identified by the label and a voucher specimen (CL Bredenkamp 1542) was deposited in the Pretoria National Herbarium.

The procedures for isolating the compounds briefly involved an acetone extraction of powdered leaves partitioned into six fractions by solvent-solvent fractionation (Eloff 1998). The chloroform fraction was chosen for silica gel column chromatography due to substantial yield and high antibacterial activity (Martini 2002). Bioautography showed that this fraction contained at least seven antibacterial com-

pounds. Subsequent fractionation was on Sephadex LH-20. Compounds were purified by recrystallisation from hexane, methanol or chloroform. TLC was used to determine the purity of the fractions and  $R_f$  values of the isolated compounds. Thin layer chromatography (5  $\mu$ l of a 20m ml<sup>-1</sup> solution) was on Merck TLC F<sub>254</sub> plates with chloroform:ethyl acetate:formic acid (CEF) (5:4:1), acetone:methylene dichloride (A:MDC) (2:3) and benzene: ethanol: ammonia (BEA) (90:10:1). Separated components were detected under visible and ultraviolet light (254nm and 360nm, Camac Universal UV lamp TL-600) or sprayed with 0.36% vanillin, 3.6% sulphuric acid in methanol and heated at 100°C to allow for development of colour. NMR spectra were obtained on either a 400 or 300 MHz AMX Bruker High Resolution Electron Impact Mass Spectroscopy (HREIMS) using a MASPEC II system (I132/A002).

Three flavones were isolated from the leaf extracts (Figure 1). Compound **1** was isolated as a yellow powder.  $R_f$  values with the three solvent systems were: 0.34 (CEF), 0.70 (BEA) and 0.93 (2A:3MDC). HREIMS showed the base peak and molecular ion  $M^+$  to be at  $m/z$  284 corresponding to  $C_{16}H_{12}O_5$ . Other prominent peaks were seen at  $m/z$  255 ( $(M-CO)^+$  (26%),  $m/z$  241 ( $(M-C_2H_3O)^+$  (10%),  $m/z$  166 ( $(M-C_8H_6O)^+$  (8%) and  $m/z$  118 ( $(M-C_8H_6O_4)^+$  (5.5%). A Long Range Correlation Spectroscopy (COSY-LR) experiment was performed to assist with the positioning of the methoxyl group on the A-ring of the flavone. Cross-peaks were visible between the methoxyl group and H-6 and H-8 confirming the positioning of the methoxyl group at C-7. Based on the NMR data and comparison with the literature, **1** was identified as 4',5-dihydroxy-7-methoxyflavone, commonly known as genkwanin (Agrawal 1989). It had been isolated from *Artemisia*, *Eupatorium*, *Alnus*, *Populus*, *Daphne* and



**Figure 1:** Flavonoids isolated from *C. erythrophyllum* leaf extracts

*Notholaena* species (Wollenweber and Dietz 1980). This is the first report in the Combretaceae.

Compound **2** was identified as 4',5,7-trihydroxyflavone (apigenin) (Agrawal 1989), an almost ubiquitous flavone only recently reported in *Combretum* spp. first isolated from the leaves of *C. apiculatum* (Katerere 2001).

Compound **3** was isolated from the chloroform fraction as a yellow crystalline solid and identified as 5-hydroxy-4',7-dimethoxyflavone. It has the following R<sub>f</sub> values in the three solvent systems: 0.70 (CEF), 0.78 (BEA) and 0.95 (2A:3MDC). It has previously been found in *Biota orientalis* (Yang *et al.* 1995) and in the leaves of *Rosmarinus officinalis* (Brieskorn and Domling 1967). This is the first report of its presence in Combretaceae.

Four flavonols that were isolated from the *C. erythrophyllum* leaf extracts are reported for the first time in the Combretaceae (Figure 1). Compound **4** was isolated as a yellow amorphous solid and identified as 4',5-dihydroxy-3',7-dimethoxyflavonol, commonly known as rhamnazin (Agrawal 1989). It has been found previously in *Artemisia pygmaea*, *Alnus* spp., *Betula* spp., *Aesculus* spp., *Polygonum hydropiper*, *Rhamnus* spp., *Populus* spp., *Larrea* spp., *Cheilanthes* spp. and *Notholaena* spp. (Wollenweber and Dietz 1980).

Compound **5** was isolated as a yellow amorphous powder

and had R<sub>f</sub> values of 0.38 with CEF, 0.74 with BEA and 0.93 with 2A:3MDC. Its <sup>1</sup>H-NMR was almost identical to that of **4** except that the methoxyl group was placed at C-5 because of the strong correlation with H-6 seen when COSY-LR was performed. It was concluded that **5** is 4',7-dihydroxy-3',5-dimethoxyflavonol.

Compound **6** was isolated as a yellow amorphous powder with the following R<sub>f</sub> values in the three solvent systems: 0.28 (CEF), 0.75 (BEA) and 0.94 (2A:3MDC). It was identified by NMR as 4',5-dihydroxy-7-methoxyflavonol, commonly known as rhamnocitrin (Agrawal 1989) previously reported in *Alnus* spp., *Betula* spp., *Ostrya* spp., *Aesculus* spp., *Rhamnus* spp. and *Populus* spp. (Wollenweber and Dietz 1980).

Compound **7** was isolated as a yellow powder. HREIMS gave a molecular ion peak at m/z 286 corresponding to C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>. The NMR spectra were similar to that of **6** except that there were no methoxyl functions. It was characterised as 4',5,7-trihydroxyflavonol (kaempferol) (Agrawal 1989).

All seven antibacterial compounds isolated are flavonoids and although they are not novel structures, six are reported for the first time in this family. They were hitherto not known to possess antibacterial activity. Apigenin has previously been isolated in *C. apiculatum* (Katerere 2001). Because we did not follow the methods employed by Rogers (1998) and focussed on bioassay-guided fractionation, it may explain why no terpenoids were isolated in the present study.

Flavonoids are secondary metabolites produced by plants fulfilling many different roles and are classified chemically under the phenolics. Most plants store phenolics attached to a hydrophilic moiety such as a sugar. This renders them more soluble and more easily handled by the plant but biologically less active. Certain phenolics are particularly bioactive and have pronounced effects on mammalian cells including antioxidant activity, modulation of gene expression, enzyme inhibition and receptor binding (Williamson *et al.* 2000).

The R<sub>f</sub> values of the flavonoids isolated may aid in avoiding the isolation of compounds of known structure from other members of the Combretaceae. If the compound investigated is present at a low concentration it may not be possible to visualise it on a TLC plate with regular spray reagents, but bioautography should indicate the R<sub>f</sub> value of the compound in three solvent systems and aid in deciding if it is worthwhile to isolate the compound. In those cases where R<sub>f</sub> values were determined, the eluent CEF was by far the most selective of the three solvent systems for separating the flavonoids isolated here.

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