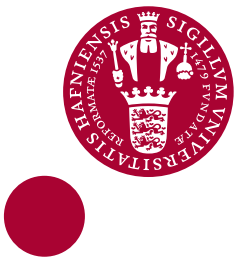

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Itoside A and 4-hydroxytremulacin from *Dovyalis caffra* and *Dovyalis zeyheri*

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1. Subject and source

Dovyalis caffra (Hook. f. & Harv.) Sim [syn. *Aberia caffra* Hook. f. & Harv.] (Salicaceae) is commonly known as kei apple (Palgrave, 1991). It is found in the eastern parts of southern Africa, and is a shrub or a small tree with edible fruits. The roots and thorns are used in African traditional medicine to treat amenorrhea and chest pain (Cumes et al., 2008), and *D. caffra* and other *Dovyalis* species are used by the Zulu to treat pain in rheumatic fever and rheumatism (Bryant, 1966). Twigs and leaves were collected in September 2008 at the University of KwaZulu-Natal Botanical Garden (S29°37E30°24), South Africa. A voucher specimen (accession number: Stafford 362 NU) has been deposited in the Herbarium at University of KwaZulu-Natal, Pietermaritzburg (NU Herbarium). *D. zeyheri* Warb. [syn. *Aberia zeyheri* Sond.] (Salicaceae) is commonly known as wild apricot due to its edible fruits, and is a small to medium-sized tree found in the same regions as *D. caffra*. Leaves as well as stem bark were collected in August 2008 at the University of KwaZulu-Natal Botanical Garden (S29°37E30°24), South Africa, and a voucher specimen (accession number: Stafford 361 NU) has been deposited in the above-mentioned herbarium.

2. Previous work

There is no literature report of phytochemical investigations of *D. zeyheri*. Fruits of *D. caffra* have been investigated for their composition of pectin and amino acids (Abdel-Fattah et al., 1975), and for the antioxidant activity of the polyphenols present

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in the fruit juice (Loots et al., 2006). Leaves of *D. caffra* have been investigated for their content of tannins (Saleh et al., 1969), and the extracts of fruits, leaves, stems, and roots have shown antibacterial activity (Basile et al., 1997; Zaki, 1975). Although alkaloids are generally uncommon in Salicaceae, two alkaloids have been identified in *D. caffra* (i.e, *A. caffra*) by Sayed et al. (2000), and a series of novel spermidine-alkaloids were recently isolated from *Dovyalis macrocalyx*, *Dovyalis hebecarpa*, and *Dovyalis abyssinica* (Staerk et al., 2003; Rasmussen et al., 2006).

3. Present study

Dried and ground plant material of *D. caffra* (twigs and leaves, 477 g) and *D. zeyheri* (stem bark, 213 g and leaves, 120 g) were successively extracted with a 1:1 mixture of dichloromethane and methanol (4 × 650 ml *D. caffra*, 5 × 540 ml *D. zeyheri* stem bark, and 4 × 400 ml *D. zeyheri* leaves) and pure methanol (2 × 900 ml *D. caffra* and 3 × 750 ml *D. zeyheri* stem bark). The combined extracts were dried *in vacuo* and the residues (57 g *D. caffra*, 16 g *D. zeyheri* stem bark, and 17 g *D. zeyheri* leaves) were dissolved in a 9:1 mixture of methanol and water and defatted with light petroleum to afford 35 g, 14 g, and 15 g defatted extract, respectively. The defatted extract from *D. caffra* was dissolved in 300 ml of water and extracted with 3 × 100 ml dichloromethane and followed by extraction with 3 × 100 ml ethyl acetate. During this procedure 390 mg of precipitate was obtained, which was identified as 4-hydroxytremulacin (**1**) (Fig. 1) by comparison of ^{1}H and ^{13}C NMR data with data from the literature (Rasmussen et al., 2006). Half (2.25 g) of the ethyl acetate fraction (4.5 g) was subjected to vacuum liquid chromatography (16 × 4 cm i.d. column, Merck silica gel 60 (15–40 μm), eluted with step-gradients of 5, 10, and 20% methanol in dichloromethane) to afford four fractions (VLC_1A-VLC_1D), of which fraction VLC_1A (eluted with 500 ml 5% methanol and 200 ml 10% methanol in dichloromethane) was combined with the other half of the ethyl acetate extract. The combined extract was chromatographed using the same VLC column, but with a less steep step-gradient (5, 7.5, 10, 15, and 20% methanol in dichloromethane) to afford six fractions (VLC_2A-VLC_2F). Fraction VLC_2E (700 mg) was identified as itoside A (**2**), by comparison of ^{1}H and ^{13}C NMR data with data from the literature (Chai et al., 2007). The isolated material corresponds to 0.6 mg/g of dry plant material (0.06% w/w) for **1** and 3 mg/g (0.3% w/w) for **2**.

The defatted extract of *D. zeyheri* stem bark was partitioned between water, dichloromethane, and ethyl acetate as described above, and the ethyl acetate fraction (1.68 g) was subjected to VLC (8 × 3 cm i.d. column, Merck silica gel 60 (15–40 μm) eluted with step-gradients of 5, 10, and 20% methanol in dichloromethane) to afford six fractions (VLC_3A-VLC_3F). Fraction VLC_3C (580 mg) was identified as 4-hydroxytremulacin (**1**). A portion (6.1 mg) of fraction VLC_3D (450 mg) was separated at 40 °C by RP-HPLC (150 × 4.6 mm i.d. Phenomenex C_{18}(2) Luna column (3 μm, 100 Å)), using isocratic elution (water-methanol 11:9 + 0.1% formic acid) with a flow rate of 0.8 ml/min. Collection of one peak (t_R 3.9 min) afforded 1.2 mg of material that was identified as itoside A (**2**). The isolated material corresponds to 2.7 mg/g of dry plant material (0.06% w/w) for **1** and 0.4 mg/g (0.04% w/w) for **2**.

A portion (1.5 mg) of the defatted extract of *D. zeyheri* leaves was separated at 40 °C by RP-HPLC (150 × 10 mm i.d. Phenomenex C_{18}(2) Luna column (3 μm, 100 Å)) using the same solvent composition as above with a flow rate of 3.78 ml/min. Peak 1 (t_R 3.7 min, 0.7 mg) was identified as itoside A (**2**), and peak 2 (t_R 6.6 min, 0.4 mg) was identified as 4-hydroxytremulacin (**1**).

4. Chemotaxonomic importance

The genus *Dovyalis* has traditionally been placed in the family Flacourtiaceae, which has long been recognized as a polyphyletic taxon, having a highly diverse and controversial circumscription (Chase et al., 2002). However, the cyanogenic tribes of Flacourtiaceae were recently included in the family Achariaceae, and the noncyanogenic tribes, including *Dovyalis*, were united with Salicaceae (APG II, 2003). Phenolic glycosides such as tremulacin, salicortin (a debenzoyl derivative of

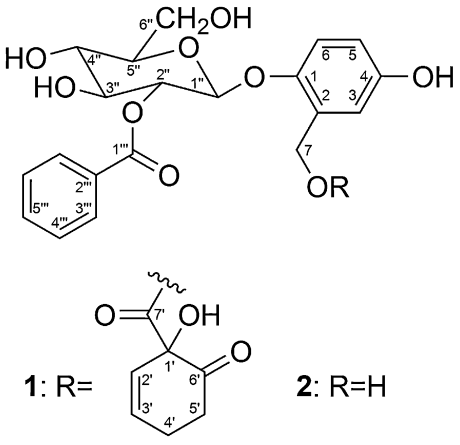

Fig. 1. Chemical structures of compounds **1-2**.

tremulacin), salicin, and their derivatives are known to be characteristic markers of many Salicaceous species (Nyman and Julkunen-Tiitto, 2005), and may be of common occurrence in the extended Salicaceae (Leskinen and Alström-Rapaport, 1999). An interesting common feature of the redefined Salicaceae is that many of the species included are host plants for the same genera of specialist butterflies (Nandi et al., 1998). The clue for this preference may very likely be the common occurrence of salicin derivatives. In this work, 4-hydroxytremulacin (**1**) was identified as a major constituent of both *D. caffra* and *D. zeyheri*. Until now **1** has only been identified in two genera within Salicaceae, i.e., *Dovyalis* [*D. abyssinica* and *D. hebecarpa* (Rasmussen et al., 2006)] and *Itoa* [*Itoa orientalis* (Chai et al., 2007, 2008)]. Compounds with 4-hydroxysalicin (= salirepin) as the core skeleton are frequently found in Salicaceae, but there have also been a few reports of these compounds in Achariaceae, Symplocaceae, Liliaceae, and Hypoxidaceae (see Supplementary data). Interestingly, however, is the fact that salirepin analogues with the 1-hydroxy-6-oxocyclohex-2-enecaboxylate moiety at C-7 are restricted to Salicaceae, and they should therefore be considered important Salicaceous chemical markers (see Supplementary data). A salirepin analogue with a related 1,2,6-trihydroxy-5-oxocyclohex-3-enecarboxylate moiety has been reported from *Homalium longifolium* (Shaari and Waterman, 1995) and has more recently been identified in stems of *Scolopia braunii* by Mosaddik et al. (2007). Both species belong to Salicaceae. Itoside A (**2**) is a 2-benzoyl analogue of salirepin, but due to the appearance of salirepin analogues outside Salicaceae, this compound is not a unique Salicaceous chemical marker. However, as a possible precursor of **1** its presence is an important finding, and the occurrence of both phenolic glycosides **1** and **2** in *D. caffra* and *D. zeyheri* supports the inclusion of these species in the extended Salicaceae. The spermidine-alkaloids found in *D. macrocalyx*, *D. hebecarpa*, and *D. abyssinica* (Staerk et al., 2003; Rasmussen et al., 2006) were neither identified in *D. caffra* or *D. zeyheri*, and the unusual occurrence of these rare alkaloids in Salicaceae continues therefore to be an interesting chemotaxonomic aspect of *Dovyalis* systematics worth further investigations.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bse.2010.02.006.

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