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Chemical Constituents from *Fadogia homblei* De Wild (Rubiaceae)

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ABSTRACT

The total of fourteen known compounds was isolated from the fruits, leaf and stem of *Fadogia homblei* De Wild. (Rubiaceae) and identified as: a coumarin, 7-hydroxy-6-methoxy-*2H*-1-benzopyran-2-one (scopoletin) **1**, two flavones; 3′,4′,5,7-tetrahydroxyflavone (luteolin) **2** and quercetin-3-*O*-β-*D*-galactoside **3**, four lupane triterpenoids; lupeol **4**, betulinic acid **5**, 3β-dodecanoyllup-20(29)-en-28-al **6**, lup-20(29)-en-3β-ylhexadecanoate **7**, and two steroids; sitosterol **8**, stigmasterol **9**, a lignan 4,4′-dihydroxy-3,3′-dimethoxy-7,9′:7′,9-diepoxylignan ((-)-pinoresinol) **10**, a phaeophytin A **11**, an uracil **12**, an oleanolic acid **13**, and an ursolic acid **14**. To our best knowledge, this is the first report for isolation of these compounds from this species. This finding is nevertheless significant as it is the first report of uracil from a plant source other than from various species of ferns.

***Keywords*:** *Fadogia homblei*; *F. monticola*; *F. fragrans*; Rubiaceae; Pavettamine; *gousiekte*

1. INTRODUCTION

Fadogia homblei De Wild (syn. *F. monticola* Robyns, *F. fragrans* Robyns) is a poisonous plant belonging to the family Rubiaceae [1]. It is common in parts of the former province Transvaal occurring from Pretoria eastwards to Swaziland and northwards to the Limpopo province and central Africa [2]. It is a perennial shrublet with subterranean branches from which the aerial stems grow.

The erect aerial stems are unbranched, squarish in cross-section, and 300 - 500 mm height. Three to five leaves are arranged in groups opposite to each other and borne in whorls at regular intervals along the stem. The flowers are small, fragrant and produced in clusters at

the nodes. The fruits are round, edible and green in colour but turn black when ripened [2]. Due to the resemblance of the fruit to a small date, it is commonly known as the "wild date" [3].

F. homblei has long been known as a cause of the economically-important fatal poisoning syndrome in ruminants, known locally as *gousiekte* („quick disease") [3]. Although a number of other Rubiaceae species from southern Africa including, *inter alia*, *Pavetta harborii* S. Moore, *Pavetta schumanniana* F. Hoffm. ex K. Schum., *Pachystigma pygmaeum* (Schltr.) Robyns and *Pachystigma thamnus* Robyns have also been implicated in *gousiekte* poisoning studies [4,5], the structure of pavettamine, the active principle involved, has remained elusive, finally appearing in print in 2010 [6], more than 15 years after it was first reported isolated in pure form [7].

The structure of pavettamine has very recently been reported, by Bode *et al*. (2010), to be (*2S*,*4R*,*8R*,*10S*)-1,11-diamino-6-aza-undecane-2,4,8,10-tetraol. To our best knowledge, no previous phytochemical work has been done on the southern African species of the genus *Fadogia* except a report on isolation of a polyamine known as pavettamine, from the aqueous extract of leaf of *Fadogia homblei* [7].

Other phytochemical analyses were done on non-southern African species of the genus *Fadogia* such as *Fadogia agrestis* (Schweinf. ex Hiern). The phytochemical investigation of *Fadogia agrestis* afforded six new monoterpene glycosides [8].

The aqueous extract of *Fadogia agrestis* leaves has been shown to exhibit *in vitro* antiplasmodial activity against *Plasmodium falciparum* at an IC_{50} value of 182 ng/mL compared to 185 ng/mL for standard chloroquine [9].

The aqueous extract of *Fadogia agrestis* stem is widely used in folk medicine as an aphrodisiac [10]. The current investigation sought to profile the plant chemistry and determine whether the constituents could reasonably be related to the documented livestock poisoning known as *gousiekte* in South Africa.

2. EXPERIMENTAL

2. 1. Materials and Methods

2. 1. 1. Plant material

Fadogia homblei was collected from Cullinan District, Gauteng Province in February 2006. This plant was collected by Professor Neil Crouch and the voucher specimen (*N Crouch & J. Meyer, 1072 NH*) was deposited at the KwaZulu-Natal Herbarium for verification purposes.

The air-dried, milled leaves (1.5 kg) and stem (0.8 kg) of *Fadogia homblei* were extracted separately and successively for 24 h each, in a Soxhlet apparatus with *n*-hexane, CH_2Cl_2, EtOAc and MeOH yielding 48.6, 46.5, 11.3 and 124.0 g, and 11.8, 6.2, 8.7 and 60.2 g, respectively, while the fruits (0.10 kg) were extracted with CH_2Cl_2 and MeOH only, yielding 5.0 and 17.0 g, respectively.

Exhaustive gravity column chromatography on Merck 9385 silica gel, and PTLC on aluminium backed analytical TLC (Merck 5554) plates, using *n*-hexane: CH_2Cl_2:EtOAc:MeOH mixtures. The structures of all isolated compounds were elucidated by analysis of their spectral data (IR, MS, 1D and 2D NMR spectra) and by comparison with the literature.

3. RESULTS AND DISCUSSION

The total of fourteen known compounds was isolated from the fruits, leaves and stem of *Fadogia homblei* De Wild. (Rubiaceae) and identified as: a coumarin, 7-hydroxy-6-methoxy-*2H*-1-benzopyran-2-one (scopoletin) **1**, two flavones; 3′,4′,5,7-tetrahydroxyflavone (luteolin) **2** and quercetin-3-*O*-β-*D*-galactoside **3**, four lupane triterpenoids; lupeol **4**, betulinic acid **5**, 3β-dodecanoyllup-20(29)-en-28-al **6**, lup-20(29)-en-3β-ylhexadecanoate **7**, and two steroids; sitosterol **8**, stigmasterol **9**, a lignan 4,4′-dihydroxy-3,3′-dimethoxy-7,9′:7′,9-diepoxylignan ((-)-pinoresinol) **10**, a phaeophytin A **11**, an uracil **12**, an oleanolic acid **13**, and an ursolic acid **14**. To our best knowledge, this is the first report for isolation of these compounds from this species.

Compound **1**, scopoletin, was isolated as a pale-yellow powder from the dichloromethane extract of the leaf and stem of *Fadogia homblei*. Scopoletin has been shown to exhibit antifungal [11], antispasmodic [12], antioxidant [13], anti-inflammatory [14] and anticancer [15] activities.

Compound **2**, luteolin, was isolated from the methanol extract of the fruits of *Fadogia homblei* as a yellow amorphous powder. Luteolin has been reported to possess antimutagenic, antitumor, antioxidant and anti-inflammatory activities [16].

Compound **3**, quercetin 3-*O*-β-*D*-galactoside, was isolated from the methanol extract of the leaf of *Fadogia homblei* as a yellow amorphous powder [17]. Compound **3** occurs widely in plants and it has been shown to exhibit biological activities such as antihypertensive, vasodilatory and is an active ingredient of herbal remedies.

Compound **4**, lupeol, was isolated as a white solid from the *n*-hexane extract of the fruits, leaves and stem of *Fadogia homblei*. Lupeol has been isolated previously from many plant species but it has shown a wide spectrum of biological activities such as antioxidant [18], antiangiogenic [18], antineoplastic, anti-inflammatory [19] and antimalarial [20].

Compound **5**, betulinic acid, was isolated as a white solid from the dichloromethane extract of the fruits, leaves and stem of *Fadogia homblei*. A variety of biological activities have been ascribed to betulinic acid including anti-inflammatory, *in vitro* antimalarial, anti-HIV and anticancer effects [21].

Compound **6**, 3β-dodecanoyllup-20(29)-en-28-al, was isolated as a white fatty material from the dichloromethane extract of the fruits of *Fadogia homblei*.

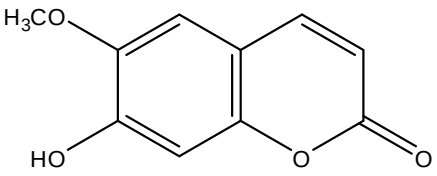
Compound **7**, lup-20(29)-en-3β-yl hexadecanoate, was isolated as a white fatty material from the dichloromethane extract of the fruits of *Fadogia homblei*. Lupeol fatty acid esters isolated from *Holarrhena floribunda* (Apocyanceae) have been shown to exhibit antimalarial activity [20].

Compound **8**, sitosterol, and compound **9**, stigmasterol, were isolated from this species and they are common compounds found in almost all plant species.

Compound **10**, pinoresinol, was isolated from the ethyl acetate extract of the stem of *Fadogia homblei* as a greenish brown solid. Pinoresinol was shown to inhibit 15-lipoxygenase with an IC_{50} value of 3.5 μM compared to 1.12 μM for the standard and was considered as active [22].

Compound **11**, phaeophytin A, was isolated as a fine very dark green powder from the dichloromethane extract of the leaf of *Fadogia homblei* [23].

Compound **12**, uracil, was isolated as a white powder from the ethyl acetate extract of the leaf of *Fadogia homblei*. Uracil has been previously isolated from ferns such as *Eudodia daniellii* Hemsley (Rutaceae) [24] and *Salicornia herbacea* L. (Chenopodiaceae) [25]. This finding is nevertheless significant as it is the first report of uracil from a plant source other than from various species of ferns.



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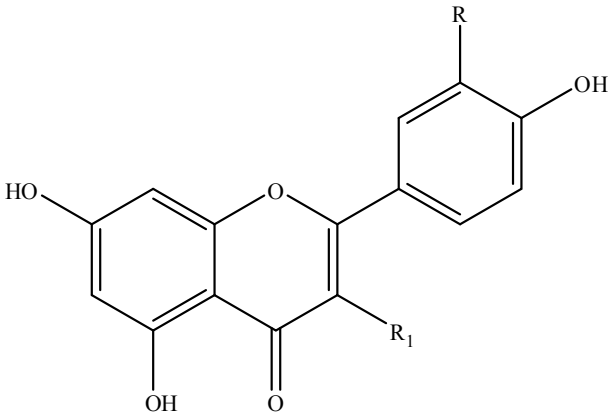
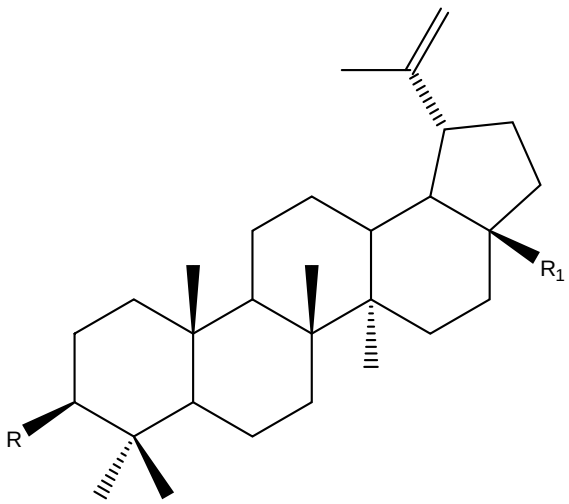


R = OH R_1 = H **2**

R = OH R_1 = OGal **3**

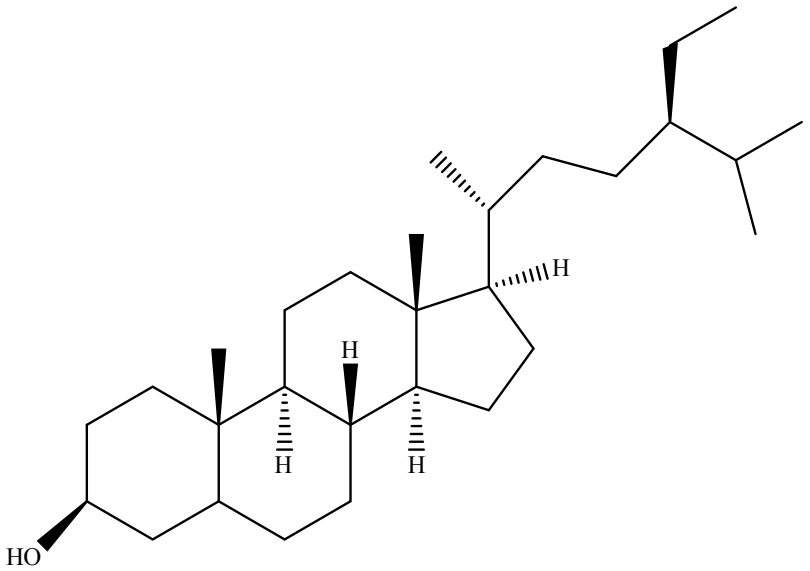


4 R = OH $R_1 = CH_3$

5 R = OH R_1 = COOH

6 R = $CH_3(CH_2)_9H_2COO$ R_1 = CHO

7 R = $CH_3(CH_2)_{13}H_2COO$ $R_1 = CH_3$



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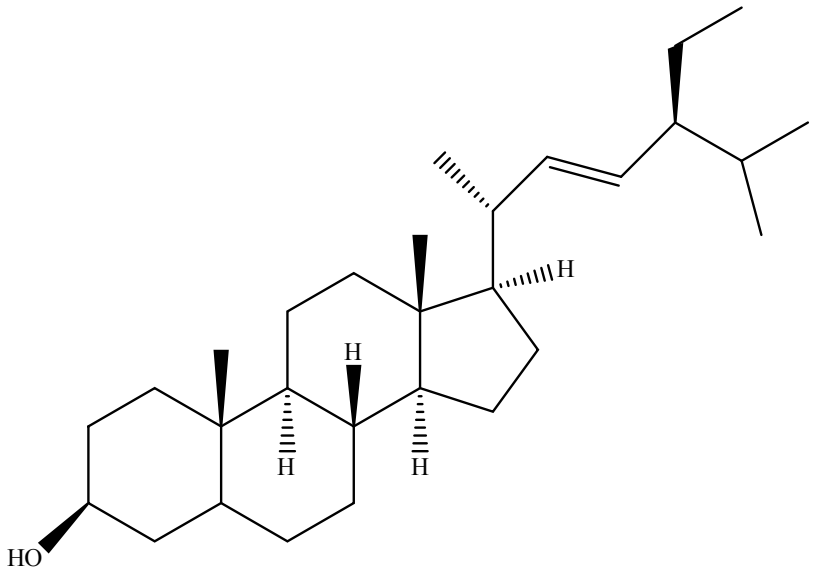

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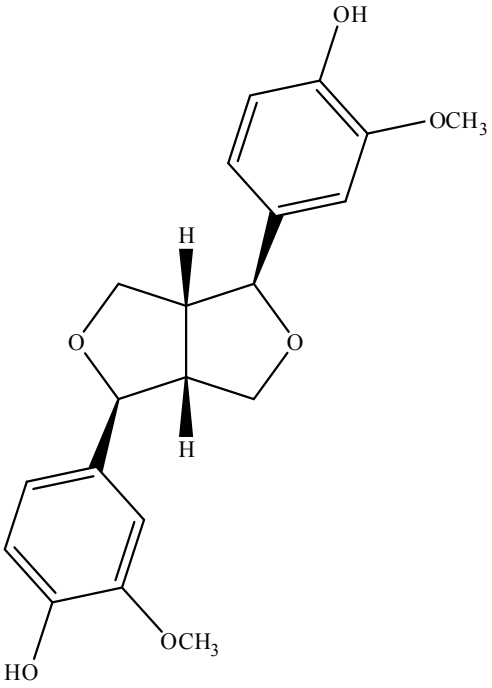

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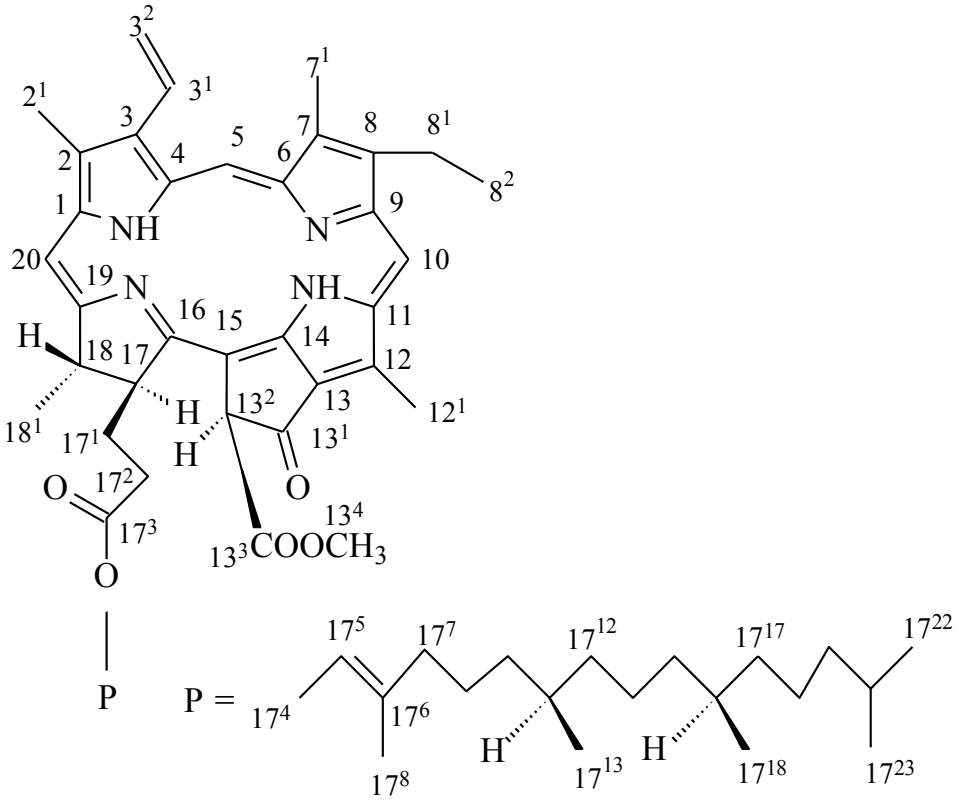

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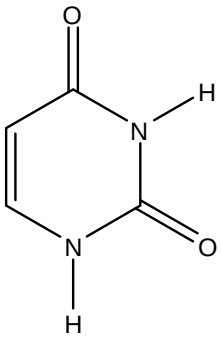

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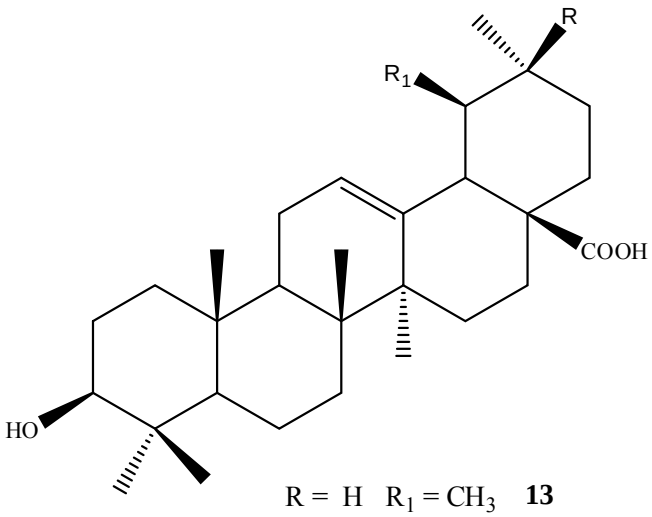

R = H $R_1 = CH_3$ **13**

R = CH_3 R_1 = H **14**

Fig. 1. Chemical structures of compounds **1-14.**

Compound **13**, oleanolic acid, was isolated as a white powder from the *n*-hexane extract of the leaf of *Fadogia homblei*. Oleanolic acid has been shown to exhibit anti-HIV activity by inhibiting HIV-1 replication in acutely infected H9 cells with an EC_{50} value of 1.7 µg/mL compared to the 0.012 µg/mL for the standard and it is considered as an active agent [26]. It also showed a moderate anti-tubercular activity against *Mycobacterium tuberculosis* with MIC value of 50 µg/ mL compared to the 0.16 µg/ mL for rifampin as a standard [27] and a moderate cytotoxicity against leukemia cells (L-1210) with IC_{50} value of 40 µg/ mL [28].

Compound **14**, ursolic acid, was isolated as a white powder from the *n*-hexane extract of the leaf of *Fadogia homblei*. Ursolic acid has been shown to be an antitrypanosomal agent against *Trypanosoma brucei* and *T. cruzi* with an IC_{50} value of 4.0 µM for both the parasites compared to the IC_{50} values of 0.08 µM and 0.39 µM for the standards, sumarin and nifurtimox, respectively. The activity was considered to be due to the presence of a free carboxyl group at position C-28 [29].

4. CONCLUSION

The total of fourteen known compounds was isolated from leaves, fruits and stem of *Fadogia homblei* De Wild. (Rubiaceae). To our best knowledge, this is a first report on isolation of these compounds from *Fadogia homblei*. This finding is nevertheless significant as it is the first report of uracil from a plant source other than from various species of ferns.

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