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

Abdelhafeez M. A. Mohammed¹,²,*, Philip H. Coombes¹, Neil R. Crouch¹,³, Dulcie A. Mulholland¹,⁴

¹School of Chemistry, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban 4000, South Africa
²Department of Chemistry, Alzaiem Alazhari University, PO Box 1432, Khartoum North, Sudan Tel: +249 9288 09281, Fax: +249 185 3480078
³Ethnobotany Unit, South African National Biodiversity Institute, PO Box 52099, Berea Road 4007, South Africa
⁴Division of Chemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, United Kingdom

*E-mail address: ahafeez61@yahoo.com

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-hexadecanoate 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-yI 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* (Apocynaceae) 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 IC50 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.
R = OH, \( R_1 = \text{H} \)  

R = OH, \( R_1 = \text{OGal} \)  

R = OH, \( R_1 = \text{CH}_3 \)  

R = \( \text{CH}_3(\text{CH}_2)_9\text{H}_2\text{COO} \), \( R_1 = \text{CHO} \)  

R = \( \text{CH}_3(\text{CH}_2)_{13}\text{H}_2\text{COO} \), \( R_1 = \text{CH}_3 \)
International Letters of Chemistry, Physics and Astronomy Vol. 14

10

11
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 \( \mu \)g/mL compared to the 0.012 \( \mu \)g/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 \( \mu \)g/mL compared to the 0.16 \( \mu \)g/mL for rifampin as a standard [27] and a moderate cytotoxicity against leukemia cells (L-1210) with IC\(_{50}\) value of 40 \( \mu \)g/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 \( \mu \)M for both the parasites compared to the IC\(_{50}\) values of 0.08 \( \mu \)M and 0.39 \( \mu \)M 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].

![Chemical structures of compounds 1-14.](image)
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.

ACKNOWLEDGMENTS

Thanks to Mr Dilip Jagjivan for NMR analysis, Mr Bret Parel for GC-MS analysis, and Dr Philip Boshoff at the Cape Technikon and Dr Colin Sparrow at Oxford University for HRMS analysis.

References


(Received 03 May 2013; accepted 07 May 2013)