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Flavonoids from *Cyclopia genistoides* and Their Xanthine Oxidase Inhibitory Activity

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Key words

- xanthine oxidase
- *Cyclopia genistoides*
- gout
- flavonoid
- benzophenone
- honeybush
- Fabaceae

Abstract

The present paper reports the chemical analysis of the methanolic extracts of fermented and non-fermented *Cyclopia genistoides* herbs and an investigation of the xanthine oxidase inhibitory activity of the isolated constituents. Chemical analysis of the leaves and stems of *C. genistoides* yielded the isolation and identification of two benzophenone glucosides, iriflophenone 2-*O*- β -glucopyranoside (1) and iriflophenone 3-*C*- β -glucopyranoside (2), two pterocarpan, (6aR,11aR)-(-)-2-methoxymaackiain (5) and (6aR,11aR)-(-)-maackiain (6), along with the flavanones liquiritigenin (9) and hesperetin (10), the flavone

diosmetin (11), the isoflavones afrormosin (7) and formononetin (8), piceol (3), and 4-hydroxybenzaldehyd (4). Among the eleven compounds, nine are reported for the first time from this species, and six from the genus *Cyclopia*. These compounds, together with previously isolated secondary metabolites of this species, were tested for xanthine oxidase inhibitory activity. The 5,7-dihydroxyflavones luteolin and diosmetin significantly inhibited the enzyme *in vitro*, while hesperetin (10) and 5,7,3',5'-tetrahydroxyflavone exerted weak activity.

Supporting information available online at <http://www.thieme-connect.de/products>

Introduction

Honeybush tea, a caffeine-free South African herbal beverage, is produced from *Cyclopia* species (Fabaceae) [1]. Due to its natural sweetness, honey-like aroma, and the absence of caffeine, the tea prepared from the fermented herbs of different *Cyclopia* species is becoming increasingly popular worldwide [2,3]. Four species of the genus, namely *Cyclopia intermedia* E. Mey., *Cyclopia genistoides* (L.) Vent., *Cyclopia subternata* Vogel, and *Cyclopia sessiliflora* Eckl. & Zeyh., are marketed and consumed worldwide as honeybush tea [1,4]. The export of honeybush from South Africa is growing rapidly, and has quadrupled between 1999 and 2010 [3].

The 23 species of the genus *Cyclopia* are distributed in a limited area in South Africa. *Cyclopia* bushes, depending on the species, are 1.5–3 m tall. Their herbs are traditionally used as a restorative or expectorant, but anecdotal evidence also exists about their consumption in order to stimulate milk production in breast-feeding women and to alleviate menopausal symptoms [1,5].

The polyphenolic composition of *C. intermedia* (fermented) and *C. subternata* (non-fermented) is well studied, and some polyphenols were also identified in *C. genistoides* and *C. sessiliflora* [6–8]. Recently, the phenolic profile of the hot water extracts of *C. genistoides* using HPLC-DAD and electrospray ionization mass spectrometry (ESI-MS, MS/MS) has also been elucidated [9]. *Cyclopia* species are valuable sources of bioactive compounds, as they contain a wide range of phenolic constituents, such as xanthenes, benzophenones, flavanones, flavanols, isoflavones. Although fermentation decreases the xanthone and flavonoid content of *Cyclopia*, it was shown that the total phenolic content of *C. genistoides* was the least affected by fermentation when compared to the other three commercially important *Cyclopia* species [8]. *C. genistoides* (methanolic extract) demonstrated the strongest estrogen receptor binding with the highest consistency [5]. The high polyphenolic content is likely to be responsible for the studied estrogen-like, antimutagenic, chemopreventive, pancreatic β -cell protective, and antioxidant activities [2,8,10–12]. Yet,

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Bibliography

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there are no data on the xanthine oxidase (XO) inhibitory activity of *Cyclopia* species.

Gout is the most prevalent form of inflammatory arthropathies, with the precondition of elevated serum urate levels, thus, urate-lowering XO inhibitors are the cornerstone of successful long-term gout management [13]. The first-line therapy of gout is based on the application of allopurinol, which needs to be gradually increased to achieve the therapeutic target. One of its adverse reactions is the rare but potentially lethal allopurinol hypersensitivity syndrome. Febuxostat is more expensive, which may, in part, limit its use. It is rarely associated with hypersensitivity vasculitis. Hence, new XO inhibitors are needed in gout therapy, but since hyperuricemia may also be an independent risk factor in cardiovascular and renal disease, inhibitors of this enzyme are the focus of scientific studies [14].

The aim of our study was to evaluate the chemical composition of the less hydrophilic, not yet studied fraction of the methanolic extract of *C. genistoides*. In our previous study, bioactivity-guided fractionation (estrogen-like activity) led to the isolation of genistein, naringenin, isoliquiritigenin, luteolin, helichrysin B, and 5,7,3',5'-tetrahydroxyflavone [under publication]. Here, we also report the assessment of the XO inhibitory activities of the compounds isolated by us from this plant.

Results and Discussion

Multistep chromatographic separation and purification procedures, including CC, preparative TLC, MPLC, VLC, RPC and HPLC, resulted in the isolation of pure compounds 1–11 (Fig. 1). Compounds 1 and 2 were identified as benzophenone derivatives based on their spectral characteristics. Compound 1 was identified as iriflophenone 2-*O*- β -glucopyranoside by comparing its spectral data with those reported in the literature [15]. Compound 2 was proved to be identical with iriflophenone 3-*C*- β -glucopyranoside, isolated earlier from *C. genistoides* [9] and *C. subternata* [16].

Compounds 5 and 6 were found to have a pterocarpans nucleus, substituted with methylenedioxy, hydroxyl, and methoxy groups. After detailed MS and NMR studies, 6 could be identified as (6aR,11aR)-(-)-maackiain [17] and 5 as (6aR,11aR)-(-)-2-methoxymaackiain [18,19]. Two-dimensional NMR investigations, including ^1H - ^1H COSY, NOESY, HSQC, and HMBC experiments, permitted unpublished ^1H and ^{13}C assignments for both compounds. This is the first isolation of maackiain (6) and 2-methoxymaackiain (5) from the *Cyclopia* genus. Previously, these compounds were published only from *Ulex* and other *Fabaceae* species [20–22].

Nine compounds [(iriflophenone 2-*O*- β -glucopyranoside (1), piceol (3), 4-hydroxybenzaldehyd (4), (-)-2-methoxymaackiain (5), (-)-maackiain (6), afrormozin (7), formononetin (8), liquiritigenin (9), and diosmetin (11)] were first isolated from the species and six [iriflophenone 2-*O*- β -glucopyranoside (1), piceol (3), 4-hydroxybenzaldehyd (4), (-)-2-methoxymaackiain (5), (-)-maackiain (6), and liquiritigenin (9)] from the genus *Cyclopia*. Both dichloromethane layers derived from the methanolic extract of the fermented and non-fermented plant material exerted XO inhibitor activity, and thus were subjected to further chromatography. The CH_2Cl_2 layer of the fermented and non-fermented plant material was separated into 14 and 12 fractions, respectively, by a polyamide column with mixtures of MeOH and H_2O as the eluents. Fractions PP8 from the non-fermented and P10 from the

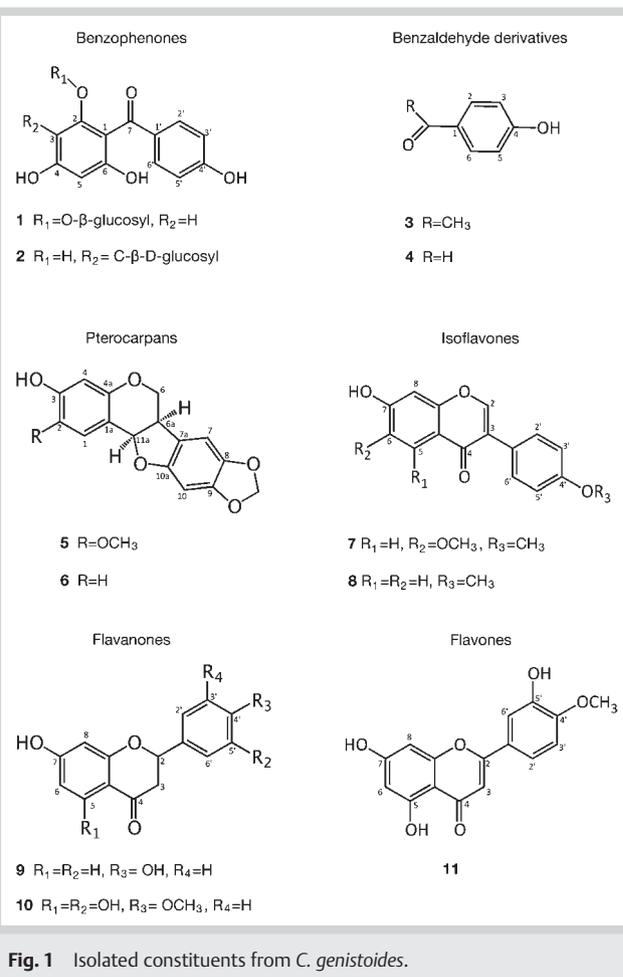


Fig. 1 Isolated constituents from *C. genistoides*.

fermented herbal substance were amongst the fractions that exhibited the strongest inhibition of xanthine oxidase. Further purification of these fractions led to the isolation of luteolin (10) and diosmetin (11), exerting a remarkable XO inhibitory effect with IC_{50} values of $0.84 \mu\text{M}$ (95% confidence interval 0.80 to $0.91 \mu\text{M}$) and $0.53 \mu\text{M}$ (95% confidence interval 0.40 to $0.80 \mu\text{M}$), respectively. The inhibitory activity of both compounds significantly exceeded that of allopurinol, which was used as a positive control. The IC_{50} of allopurinol (the concentration that inhibits 50% of enzyme activity) was $11.50 \mu\text{M}$ (95% confidence interval 11.40– $11.60 \mu\text{M}$).

Alongside with the bioactivity-guided isolation, all other isolated compounds were tested. From the other 15 isolated constituents, only 2 structurally close flavanones, hesperetin (10) and 5,7,3',5'-tetrahydroxyflavone (9), exhibited a weak inhibition [$\text{IC}_{50} = 55.20 \mu\text{M}$ (95% confidence interval 41.40 to $73.51 \mu\text{M}$) and $120.55 \mu\text{M}$ (95% confidence interval 101.71 to $142.86 \mu\text{M}$), respectively]. The rest of the isolated compounds showed no XO inhibition ($\text{IC}_{50} > 150 \mu\text{M}$; Table 1).

Materials and Methods

General experimental procedures

Vacuum liquid chromatography (VLC) was carried out on silica gel 60 GF₂₅₄ ($15 \mu\text{m}$, Merck); column chromatography (CC) on polyamide (ICN), silica gel (160–200 mesh, Qingdao Marine

Table 1 IC₅₀ values of the active compounds. CI: confidence intervals (95%). Fifty percent inhibitory concentrations (IC₅₀) were calculated using nonlinear regression curve fitting of log(inhibitor) vs. normalized response of GraphPad Prism 5.04 software (GraphPad Software, Inc.). Six to ten sample points were used in each graph. All XO activity measurements were made in triplicate.

Compound	Mw	IC ₅₀ µg/mL	CI (95%)	IC ₅₀ µM	CI (95%)
Diosmetin	300.26	0.16	0.12–0.24	0.53	0.40–0.80
Luteolin	286.24	0.24	0.23–0.26	0.84	0.80–0.91
5,7,3',5'-Tetrahydroxyflavanone	288.26	34.75	29.32–41.18	120.55	101.71–142.86
Hesperetin	302.38	16.69	12.52–22.24	55.20	41.40–73.55
Allopurinol	136.11	1.50	1.40–1.60	11.02	10.29–11.76

Chemical Co.), and Sephadex LH-20 (Sigma); preparative thin-layer chromatography (preparative TLC) on silica gel 60 F₂₅₄ and 60 RP-18 F_{254s} plates (Merck); and rotation planar chromatography (RPC) on silica gel 60 F₂₅₄ (Merck) using a Chromatotron instrument (Model 8924, Harrison Research). Medium-performance liquid chromatography (MPLC) was performed by a Büchi apparatus (Büchi Labortechnik AG) using a 40 × 150 mm RP18ec column (40–63 µm, Büchi).

HPLC was performed on a Waters Alliance 2695 separation module (Empower software) connected to a Waters 2478 dual absorbance detector and to a Waters 600 controller and pump (Waters Associates) using method 1 [reverse-phase HPLC, AcNi-H₂O 3.5:10, LiChroCART 250–4 RP-18e (5 µm, 250 × 4 mm), 0.75 mL/min] or the instrumentation for HPLC composed of dual Shimadzu LC-10AT pumps and a Shimadzu SPD-10 A UV-Vis detector using method 2 [normal-phase HPLC, *n*-hexane-CH₂Cl₂-MeOH 4:8:0.015, Phenomenex Luna CN (5 µm, 250 × 10.0 mm), 2 mL/min].

¹H-NMR (500 MHz), ¹³C-NMR (125 MHz), and 2D NMR were recorded in CD₃OD, CDCl₃, or DMSO using a Bruker Avance DRX 500 spectrometer or a JEOL ECS 400 MHz FT-NMR spectrometer, and chemical shifts are given in δ (ppm) relative to tetramethylsilane (TMS) as the internal standard. The signals of the deuterated solvents were taken as a reference. Two-dimensional experiments were performed with standard Bruker software. In the COSY, HSQC, and HMBC experiments, gradient-enhanced versions were used. MS spectra were recorded on an API 2000 Triple Quad mass spectrometer with an APCI or ESI ion source using both positive and negative modes.

Plant material

The herbs of the fermented (F) and non-fermented (nF) *C. genistoides* were a gift from Val Zyl and Mona Joubert, owners of Agulhas Honeybush Tea, from their farm near Bredasdorp in South Africa. Botanical identification was performed by Dr. Hannes de Lange. Fermentation was carried out according to the traditional method for this material [23]. Voucher specimens (no. 825-F and 826-nF) for both the fermented and the non-fermented plants have been deposited at the herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Extraction and isolation

The dried fermented and non-fermented plant materials (1.7 and 1.3 kg, respectively) were extracted via ultrasonication with methanol (12 L and 10 L) at room temperature for 30 min. The solvent was evaporated under reduced pressure to yield 228.2 g and 237.6 g of crude MeOH extracts, respectively. These extracts were subjected to solvent-solvent partition, affording *n*-hexane (F = 15.7 g, nF = 13.2 g), dichloromethane (F = 14.8 g, nF = 6.4 g),

ethyl acetate (F = 29.7 g, nF = 23.35 g), and the remnant aqueous layers (F = 128.7 g, nF = 121.4 g) and insoluble parts. For the schematic detailing of the fractionation process, see Fig. S1, Supporting Information.

The TLC profiles and ¹H NMR spectra of the EtOAc layers from the non-fermented and fermented *C. genistoides* were similar, thus only the EtOAc layer from the non-fermented plant material was further examined. It was separated into twelve fractions by VLC eluting with EtOAc – MeOH (1:0 to 0:1).

Fraction V7 was separated by MPLC with EtOAc-MeOH-H₂O (20:1:1 to 0:1:0) to yield 21 subfractions, M1 to M21. Among these subfractions, M5 and M6 were subjected to further chromatography. Fractions M6 (777.5 mg) and M5 (65.5 mg) were separated into twelve (M6/1–12) and six subfractions (M5/1–6) by MPLC using silica gel and MeOH-H₂O (2:8 to 1:0) as the eluent. Subfraction M5/2 (11.2 mg) and subfraction M6/11 (21.5 mg) were purified by reverse-phase preparative TLC eluting with MeOH-H₂O (4:6) to provide compounds **1** (1.8 mg) and **2** (4 mg), respectively.

The concentrated CH₂Cl₂ phases (F = 14.8 g, nF = 6.4 g) were chromatographed on a polyamide column eluting with MeOH-H₂O (2:3 to 1:0). The fractions were combined into 14 (F: P1–P14) and 12 fractions (nF: PP1–PP12) according to the TLC monitoring. Fraction P3 (570 mg) was chromatographed by RPC on silica gel and was eluted with cyclohexane-acetone (1:0 to 0:1) to give 15 subfractions. Subfraction N4 (38.5 mg) was further purified by normal-phase HPLC (method 2) to yield compounds **3** (2.3 mg) and **4** (2.8 mg).

Fraction P7 (300 mg) was also subjected to silica gel RPC, eluted with cyclohexane-acetone (1:0 to 0:1) to yield 17 subfractions (O1–O17), from which O6 was further separated by normal-phase HPLC (method 2) to provide compounds **5** (1.7 mg) and **6** (1.8 mg), whereas the recrystallization of O9 with CHCl₃-MeOH provided compound **7** (7.6 mg).

Fraction P8 (750 mg) was subjected to silica gel CC, eluted with *n*-hexane-acetone (5:1 to 0:1) to yield 22 subfractions (Q1–Q22). The combined subfractions Q8 +9 and Q14 were chromatographed by reverse-phase HPLC (method 1) to provide compounds **8** (1.45 mg) and compound **9** (1.7 mg), respectively. Recrystallization of subfraction 13 with CHCl₃-MeOH provided compound **10** (16.2 mg).

Fraction P10 (475.5 mg) was subjected to silica gel CC, eluted with *n*-hexane-acetone (3:1 to 0:1) to yield 13 (CE1–CE13) subfractions. CE10 was purified by RP-HPLC (method 1) to provide compound **11** (1.6 mg).

Xanthine oxidase assay

Inhibition of XO activity was measured using the protocol recommended by Sigma-Aldrich, readapted to an assay volume of 300 µL, and published in detail before [24,25]. Briefly, the en-

zyme activity at pH 7.5 was determined by the production of uric acid from xanthine. Uric acid was measured at 290 nm for 3 min in a 96-well plate using the plate reader FluoSTAR OPTIMA (BMG LABTECH). XO, isolated from bovine milk (lyophilized powder), and xanthine powder were purchased from Sigma-Aldrich. Allopurinol (Sigma-Aldrich, $\geq 99\%$), a well-known inhibitor of XO, was used as a positive control. Each compound or fraction was dissolved in DMSO. The final concentration of DMSO in the assay did not exceed 3.3% of the total volume. After the addition of all other reagents, the reaction was initiated by the automatic addition of XO solution. All XO activity measurements were made in triplicate.

Statistical analysis

Fifty percent inhibitory concentrations (IC_{50}) were calculated using nonlinear regression curve fitting of $\log(\text{inhibitor})$ vs. normalized response in GraphPad Prism 5.04 software (GraphPad Software, Inc.).

Spectral data

Iriflophenone 2-O- β -glucopyranoside (1): amorphous solid; $[\alpha]_D^{25} -28$ (c 0.1, MeOH); APCI-MS positive m/z 409 $[M + H]^+$, 247 $[(M + H) - C_6H_{10}O_5]^+$, 153 $[C_7H_5O_2 + MeOH]^+$, 121 $[C_7H_5O_2]^+$; HRESIMS: m/z 431.0940 $[M + Na]^+$ (calcd. for $C_{19}H_{20}O_{10}Na$ 431.0954); 1H - and ^{13}C -NMR data are identical with published data [15].

Iriflophenone 3-C- β -glucopyranoside (2): amorphous powder; APCI-MS m/z 409 $[M + H]^+$, 231, 219, 195; 1H NMR data were in agreement with those published earlier for DMSO- d_6 solution [26]. 1H -NMR in CD_3OD is published here for the first time (500 MHz), δ (ppm): 7.62 (2H, d, $J=8.7$ Hz, H-2', 6'), 6.79 (2H, d, $J=8.7$ Hz, H-3', 5'), 5.98 (1H, s, H-5), 4.87 (1H, d, $J=9.6$ Hz, H-1''), 3.88 (2H, m, H-2'', 6a''), 3.75 (1H, dd, $J=12.0$, 5.1 Hz, H-6b''), 3.48 (2H, m, H-3'', H-5''), 3.42 (1H, m, H-4'').

(6aR,11aR)-(-)-2-Methoxymaackiain (5): white powder; $[\alpha]_D^{25} -331$ (c 0.1, $CHCl_3$); APCI-MS positive m/z 315 $[M + H]^+$; 1H - and ^{13}C -NMR data in $CDCl_3$ were in good agreement with literature data [18, 19]. NMR data in DMSO- d_6 are published here for the first time: 1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 9.30 (1H, s, OH), 6.96 (1H, s, H-1), 6.93 (1H, s, H-7), 6.53 (1H, s, H-4), 6.32 (1H, s, H-10), 5.49 (1H, d, $J=6.7$ Hz, H-11a), 5.94 and 5.91 (2x1H, 2xs, -OCH₂O-), 4.19 (1H, m, H-6), 3.53 (2H, m, H-6, H-6a), 3.74 (3H, s, OCH₃); ^{13}C -NMR (125 MHz, DMSO- d_6) δ (ppm): 153.7 (C-10a), 149.6 (C-4a), 148.2 (C-9), 147.4 (C-3), 143.0 (C-2), 141.0 (C-8), 118.5 (C-7a), 113.8 (C-1), 110.1 (C-1a), 105.4 (C-7), 103.8 (C-4), 101.0 (-OCH₂O-), 93.2 (C-10), 78.2 (C-11a), 65.9 (C-6), 56.2 (OCH₃), 40.0 (C-6a).

(6aR,11aR)-(-)-Maackiain (6): white powder; $[\alpha]_D^{25} -177$ (c 0.1, $CHCl_3$); 1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 9.61 (1H, s, OH), 7.23 (1H, d, $J=8.4$ Hz, H-1), 6.96 (1H, s, H-7), 6.53 (1H, d, $J=1.8$ Hz, H-4), 6.52 (1H, s, H-10), 6.46 (1H, dd, $J=8.4$, 1.9 Hz, H-2), 5.94 and 5.91 (2x1H, 2xs, -OCH₂O-), 5.50 (1H, d, $J=6.9$ Hz, H-11a), 4.22 (1H, dd, $J=10.1$, 3.8, H-6), 3.58 (1H, m, H-6), 3.54 (1H, m, H-6a); ^{13}C -NMR (125 MHz, DMSO- d_6) δ (ppm): 158.7 (C-3), 156.3 (C-4a), 153.7 (C-10a), 14.4 (C-9), 141.0 (C-8), 132.0 (C-1), 118.4 (C-7a), 111.3 (C-1a), 109.7 (C-2), 105.3 (C-7), 101.0 (-OCH₂O-), 102.8 (C-4), 93.2 (C-10), 77.9 (C-11a), 65.8 (C-6), 39.0 (C-6a).

Hesperetin (10): APCI-MS positive m/z 303 $[M + H]^+$, 176, 153; 1H - and ^{13}C -NMR data were in good agreement with literature data [27], but in DMSO- d_6 are published here for the first time: 1H -NMR (500 MHz, DMSO- d_6) δ (ppm): 12.10 (1H, brs, OH), 6.93

(1H, d, $J=8.5$ Hz, H-5'), 6.92 (1H, d, $J=1.7$ Hz, H-2'), 6.86 (1H, dd, $J=8.4$, 1.7 Hz, H-6'), 5.88, and 5.86 (2x1H, 2xd, $J=1.9$ Hz, H-6, H-8), 5.42 (1H, dd, $J=12.3$, 2.9 Hz, H-2), 3.77 (3H, s, OCH₃), 3.18 (1H, dd, $J=17.1$, 12.5, H-3a), 2.69 (1H, dd, $J=17.1$, 3.0, H-3b); ^{13}C -NMR (125 MHz, DMSO- d_6) δ (ppm): 196.0 (C-4), 167.2 (C-7), 163.5 (C-5), 162.8 (C-9), 147.9 (C-4'), 146.5 (C-3'), 131.2 (C-1'), 117.7 (C-6'), 114.1 (C-5'), 112.0 (C-2'), 101.6 (C-10), 95.9 (C-6), 95.1 (C-8), 78.2 (C-2), 55.7 (OCH₃), 42.1 (C-3). NMR data for this solvent were not published previously.

The further compounds, identified by comparing their physical and spectroscopic data with reported data, were afromozin (7) [28], formononetin (8) [29], liquiritigenin (9) [26, 27], and diosmetin (11) [27]. Compound 3 was identified as piceol (=4-hydroxyacetophenone) and compound 4 as 4-hydroxybenzaldehyde based on their 1H , ^{13}C -NMR, and MS data.

Supporting information

A figure describing the isolation of compounds is available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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References

- Joubert E, Gelderblom WC, Louw A, de Beer D. South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides* – a review. *J Ethnopharmacol* 2008; 119: 376–412
- Louw A, Joubert E, Visser K. Phytoestrogenic potential of *Cyclopia* extracts and polyphenols. *Planta Med* 2013; 79: 580–590
- Joubert E, Joubert ME, Bester C, de Beer D, de Lange JH. Honeybush (*Cyclopia* spp.): From local cottage industry to global markets – The catalytic and supporting role of research. *S Afr J Bot* 2011; 77: 887–907
- Van Wyk BE. A broad review of commercially important southern African medicinal plants. *J Ethnopharmacol* 2008; 119: 342–355
- Verhoog NJ, Joubert E, Louw A. Screening of four *Cyclopia* (honeybush) species for putative phyto-oestrogenic activity by oestrogen receptor binding assays. *S Afr J Sci* 2007; 103: 13–21
- Kamara BI, Brandt EV, Ferreira D, Joubert E. Polyphenols from Honeybush tea (*Cyclopia intermedia*). *J Agric Food Chem* 2003; 51: 3874–3879
- Kamara BI, Brand DJ, Brandt EV, Joubert E. Phenolic metabolites from honeybush tea (*Cyclopia subternata*). *J Agric Food Chem* 2004; 52: 5391–5395
- Joubert E, Richards ES, Merwe JD, De Beer D, Manley M, Gelderblom WC. Effect of species variation and processing on phenolic composition and *in vitro* antioxidant activity of aqueous extracts of *Cyclopia* spp. (Honeybush Tea). *J Agric Food Chem* 2008; 56: 954–963

- 9 Beelders T, de Beer D, Stander MA, Joubert E. Comprehensive phenolic profiling of *Cyclopia genistoides* (L.) Vent. by LC-DAD-MS and -MS/MS reveals novel xanthone and benzophenone constituents. *Molecules* 2014; 19: 11760–11790
- 10 Marnewick JL, Batenburg W, Swart P, Joubert E, Swanevelde S, Gelderblom WC. Ex vivo modulation of chemical-induced mutagenesis by subcellular liver fractions of rats treated with rooibos (*Aspalathus linearis*) tea, honeybush (*Cyclopia intermedia*) tea, as well as green and black (*Camellia sinensis*) teas. *Mutat Res* 2004; 558: 145–154
- 11 Marnewick JL, van der Westhuizen FH, Joubert E, Swanevelde S, Swart P, Gelderblom WC. Chemoprotective properties of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) herbal and green and black (*Camellia sinensis*) teas against cancer promotion induced by fumonisin B1 in rat liver. *Food Chem Toxicol* 2009; 47: 220–229
- 12 Chellan N, Joubert E, Strijdom H, Roux C, Louw J, Muller CJ. Aqueous extract of unfermented honeybush (*Cyclopia maculata*) attenuates STZ-induced diabetes and beta-cell cytotoxicity. *Planta Med* 2014; 80: 622–629
- 13 Roddy E, Doherty M. Epidemiology of gout. *Arthritis Res Ther* 2010; 12: 223
- 14 Stamp LK, Chapman PT. Urate-lowering therapy: current options and future prospects for elderly patients with gout. *Drugs Aging* 2014; 31: 777–786
- 15 Lee SS, Tseng CC, Chen CK. Three new benzophenone glucosides from the leaves of *Planchonella obovata*. *Helv Chim Acta* 2010; 93: 522–529
- 16 Kokotkiewicz A, Luczkiewicz M, Sowinski P, Glod D, Gorynski K, Bucinski A. Isolation and structure elucidation of phenolic compounds from *Cyclopia subternata* Vogel (honeybush) intact plant and *in vitro* cultures. *Food Chem* 2012; 133: 1373–1382
- 17 Sato S, Takeo J, Aoyama C, Kawahara H. Na⁺-Glucose cotransporter (SGLT) inhibitory flavonoids from the roots of *Sophora flavescens*. *Bioorg Med Chem* 2007; 15: 3445–3449
- 18 Máximo P, Lourenço A. A pterocarpan from *Ulex parviflorus*. *Phytochemistry* 1998; 48: 359–362
- 19 Mizuno M, Tanaka T, Tamura KI, Matsuura N, Iinuma M, Phengkklai C. Flavonoids in the roots of *Euchresta horsfieldii* in Thailand. *Phytochemistry* 1990; 29: 2663–2665
- 20 Máximo P, Lourenço A, Feio SS, Roseiro JC. Flavonoids from *Ulex airensis* and *Ulex europaeus* ssp. *europaeus*. *J Nat Prod* 2002; 65: 175–178
- 21 Máximo P, Lourenço A, Feio SS, Roseiro JC. Flavonoids from *Ulex* Species. *Z Naturforsch C* 2000; 55: 506–510
- 22 Máximo P, Lourenço A, Feio SS, Roseiro JC. A new prenylisoflavone from *Ulex jussiaei*. *Z Naturforsch C* 2002; 57: 609–613
- 23 Alguhas honeybush tea. Fermentation process. Available at <http://www.agulhashoneybushtea.co.za/art-tea/>. Accessed 10 March, 2016
- 24 Hunyadi A, Martins A, Danko B, Chuang DW, Trouillas P, Chang FR, Wu YC, Falkay G. Discovery of the first non-planar flavonoid that can strongly inhibit xanthine oxidase: protoapigenone 1'-O-propargyl ether. *Tetrahedron Lett* 2013; 54: 6529–6532
- 25 Sigma-Aldrich. Protocol of inhibition of xanthine oxidase by Sigma-Aldrich. Available at https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/General_Information/xanthine_oxidase.pdf. Accessed 10 March, 2016
- 26 Severi JA, Lima ZP, Kushima H, Brito AR, Santos LC, Vilegas W, Hiruma-Lima CA. Polyphenols with antiulcerogenic action from aqueous decoction of mango leaves (*Mangifera indica* L.). *Molecules* 2009; 14: 1098–1110
- 27 Correia-da-Silva M, Sousa E, Duarte B, Marques F, Carvalho F, Cunha-Ribeiro LM, Pinto MMM. Flavonoids with an oligopolysulfated moiety: a new class of anticoagulant agents. *J Med Chem* 2011; 54: 95–106
- 28 Puebla P, Oshima-Franco Y, Franco LM, Santos MG, Silva RV, Rubem-Mauro L, Feliciano AS. Chemical constituents of the bark of *Dipteryx alata* Vogel, an active species against *Bothrops jararacussu* venom. *Molecules* 2010; 15: 8193–8204
- 29 Nessa F, Ismail Z, Mohamed N, Haris MRHM. Free radical-scavenging activity of organic extracts and of pure flavonoids of *Blumea balsamifera* DC leaves. *Food Chem* 2004; 88: 243–252

