JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2019, 67, 4967−4975

pubs.acs.org/JAFC

Safety Assessment of Phytochemicals Derived from the Globalized South African Rooibos Tea (*Aspalathus linearis*) through Interaction with CYP, PXR, and P-gp

Omer I. Fantoukh,[†,‡,§] Olivia R. Dale,[†] Abidah Parveen,[†,‡] Mohammed F. Hawwal,[†,‡,§] Zulfiqar Ali,[†] Vamshi K. Manda,[†] Shabana I. Khan,[*,†,‡] Amar G. Chittiboyina,[†] Alvaro Viljoen,[∥] and Ikhlas A. Khan[†,‡]

[†]National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States

[‡]Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States

[§]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 4545, Saudi Arabia

[∥]Department of Pharmaceutical Sciences and SAMRC Herbal Drugs Research Unit, Tshwane University of Technology, Pretoria 0183, South Africa

Supporting Information

ABSTRACT: Rooibos tea (*Aspalathus linearis*) is a well-known South African herbal tea enjoyed worldwide. Limited reports indicate the potential of rooibos tea to alter the activity of certain cytochrome P450 (CYP450) isozymes. In this study, the phytochemical investigation of MeOH extract of *A. linearis* (leaves and stems) resulted in the isolation and characterization of 11 phenolic compounds. The MeOH extract exhibited significant inhibition of the major human CYP450 isozymes (CYP3A4, CYP1A2, CYP2D6, CYP2C9, and CYP2C19). The strongest inhibition was observed by the extract for CYP3A4 (IC_{50} 1.7 ± 0.1 μg/mL) followed by CYP2C19 (IC_{50} 4.0 ± 0.3 μg/mL). Among the tested phytochemicals, the most potent inhibitors were isovitexin on CYP3A4 (IC_{50} 3.4 ± 0.2 μM), vitexin on CYP2C9 (IC_{50} 8.0 ± 0.2 μM), and thermopsoside on CYP2C19 (IC_{50} 9.5 ± 0.2 μM). The two major, structurally related compounds aspalathin and nothofagin exhibited a moderate pregnane-X receptor (PXR) activation, which was associated with increased mRNA expression of CYP3A4 and CYP1A2, respectively. These results indicate that a high intake of nutraceuticals containing rooibos extracts may pose a risk of herb−drug interactions when consumed concomitantly with clinical drugs that are substrates of CYP enzymes.

KEYWORDS: *Aspalathus linearis, rooibos, CYP450, flavonoids, aspalathin*

INTRODUCTION

Aspalathus genus is classified as a member of the pea family, *Leguminosae*, and it comprises more than 270 plant species. Interestingly, *Aspalathus linearis* (Burm.f.) R. Dahlgren, commonly known as rooibos tea, is recognized to be the only edible species so far. It is a perennial shrub endemic to the western parts of the Cape region in South Africa.[1,2] It grows naturally in the Cederberg Mountains encompassing the Citrusdal, Clanwilliam, and Nieuwoudtville regions, which are considered as the commercial epicenters of the rooibos industry. The leaves and stems are used for the production of the refreshing rooibos drink, which contains no caffeine and only minimal levels of tannins. In addition to antioxidant properties, it displays hypoglycemic, antiallergic, antispasmodic, photoprotective, anti-inflammatory, and estrogenic activities.[3−11]

Rooibos is a popular herbal tea in more than 37 countries, which has recently attracted greater global attention as a health beverage. The export price spiked dramatically between 2010 and 2014 because of the increased demand and reputation. Additionally, the Swiss Business Hub South Africa report anticipated that ordinary tea (*Camellia sinensis*) followed by rooibos tea would become the two most abundant and widely distributed teas in the world.[1] Oxidation and browning of green rooibos, which takes place during "fermentation", alters the phenolic profile and biological properties of the tea. Consequently, two types of rooibos teas are available, unfermented (green) and fermented (red).[1,12]

Although two clinical reports indicated the hepatotoxic effect of rooibos tea extract in humans, it is generally considered safe when ingested as a tisane.[13,14] Myriad herbal supplements may cause unexpected toxicity by altering drug-metabolizing enzymes such as cytochrome P450 (CYP450) and drug transporters when administered in conjunction with conventional pharmaceutical drugs.[15] Despite little evidence available to describe the adverse effects, herbal supplements containing rooibos ingredients were found to inhibit CYP2C11 isozyme expression.[16] When comparing the unfermented and fermented rooibos extracts, researchers observed the inhibitory

Received: February 2, 2019
Revised: April 4, 2019
Accepted: April 7, 2019
Published: April 8, 2019

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

activity of both extracts on CYP2C8, CYP2C9, and CYP3A4 isozymes.[17] Conversely, in an animal study, a rooibos extract was shown to induce the activity of intestinal and hepatic CYP3A isoforms and upregulate the CYP3A contents.[18] Moreover, recently published studies found that rooibos tea has modulated steroid biosynthesis and influenced rat liver enzymes.[19−21] Nevertheless, all studies on the biological properties, except one, lack key information on the chemical compositions of the infusions or extracts used in the experiments. The study highlighted the ability of aspalathin, a *C*-glucosyl dihydrochalcone derivative, to inhibit the activity of CYP3A4 in a time-dependent assay compared with erythromycin.[17]

In our continued quest to explore the quality and safety of botanical ingredients, we aim to investigate the herb−drug interaction (HDI) profile of the MeOH extract of unfermented rooibos tea and the isolated secondary metabolite constituents on drug-metabolizing enzymes and efflux transporter (CYPs and P-gp). The rationale for selecting MeOH was due to its superiority as an extraction solvent of the majority of secondary metabolites. To differentiate the integrity of secondary metabolism associated with *A. linearis*, unfermented material (dried leaves and stems) was selected for isolation, characterization, and drug-interaction potential studies.

MATERIALS AND METHODS

Biochemicals and Reagents. Inhibitor screening kits for recombinant human CYPs including CYP3A4/BQ, CYP2D6/AMMC, CYP1A2/CEC, CYP2C9/MFC, and CYP2C19/CEC along with substrates and buffer systems were purchased from BD Gentest (Woburn, MA, U.S.A.). HepG2 cells were from ATCC (Manassas, VA, U.S.A.). MDCK-II (parental) and hMDR1-MDCK-II (transfected) cells were obtained from Dr. Gottesman (NIH, Bethesda, MD, U.S.A.). All positive controls (ketoconazole, quinidine, furafylline, sulfaphenazole, tranylcypromine, rifampicin, and omeprazole) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Their purity was more than 95%. Troleandomycin, with purity higher than 98%, was obtained from Santa Cruz Biotechnology, Inc. Fetal bovine serum (FBS) was obtained from Hyclone Lab, Inc. (Logan, UT, U.S.A.). Hanks balanced salt solution (HBSS), minimal essential medium (MEM), Dulbecco's modified Eagle medium (DMEM), DMEM/F12, trypsin EDTA, sodium pyruvate, HEPES, streptomycin, and penicillin-G were all from GIBCO BRL (Invitrogen Corp., Grand Island, NY, U.S.A.).

The isolation and fractionation procedures were performed by column chromatography carried out on silica gel (32−63 μm, Dynamic Adsorbents, Inc., Atlanta, GA, U.S.A.), Sephadex LH20 (Sigma-Aldrich, St. Louis, MO, U.S.A.), or reversed-phase C-18 (Polar bond, J. T. Baker, Suwanee, GA, U.S.A.). The analytical thin-layer chromatography (TLC) was carried out on silica gel F254 aluminum sheet (20 × 20 cm, Sorbtech, Norcross, GA, U.S.A.) or Silica 60 RP-18 F254S aluminum sheet (20 × 20 cm, Sorbtech, Norcross, GA, U.S.A.). Savant Speed Vac Plus SC210A Concentrator was employed to concentrate and dry the samples. Visualization of the spots on TLC was observed under UV light at 254 or 365 nm and derivatized by spraying with 1% vanillin dissolved in 10% H_2SO_4/ethanol reagent and heating via heat gun for the detection of phenolic compounds. All solvents used in the isolation, fractionation, and purification steps were of analytical grade purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). The NMR spectra were obtained on either Varian Dual Broadband Probe or Bruker Avance DRX spectrometer at 400 MHz for ^{1}H experiments and 100 MHz for ^{13}C experiments. Using standard pulse instrument software, other 2D experiments (DEPTQ135, COSY, HSQC, HMBC, and NOESY) were recorded. Dimethyl sulfoxide (DMSO) or CD_3OD solvents were used as the internal standards and residual solvents to obtain and calibrate the spectrum, respectively. Chiroptical techniques were employed to assign the absolute configuration using an Olis DSM 20 circular dichroism spectrophotometer (Olis, Inc., Bogart, GA, U.S.A.) and confirmed by AUTOPOL IV Automatic Polarimeter (Rudolph, Hackettstown, NJ, U.S.A.) to measure specific rotations.

High-resolution electrospray ionization mass spectra (HR-ESI-MS) were acquired by injecting the samples directly to an Agilent Technologies 6230 series mass spectrometer in the negative ion mode equipped with an electrospray ionization interface that was controlled by Agilent software (Agilent MassHunter Workstation, A.02.01). All acquisitions were performed with a capillary voltage of 3000 V. Nitrogen was used as nebulizer gas (25 psig) as well as drying gas at 10 L/min at a drying gas temperature of 300 °C. The voltages of photomultiplier tube (PMT), fragmentor, and skimmer were set at 750, 175, and 65 V, respectively. Full scan mass spectra were acquired from m/z 100−1100. Data acquisition and processing were done using the MassHunter Workstation software (Qualitative Analysis Version B.07.00). Qualitative analysis of MeOH extract of *A. linearis* was performed on Waters UPLC system, and detailed conditions are outlined in the Supporting Information.

Culture of HepG2, MDCK-II, and hMDR1-MDCK-II Cells. HepG2 cells were cultured in DMEM/F12 supplemented with 10% FBS, 2.4 g/L sodium bicarbonate, 100 μg/mL streptomycin, and 100 U/mL penicillin-G at 37 °C, 5% CO_2, and 95% relative humidity. MDCK-II and hMDR1-MDCK-II cell lines were cultured in DMEM supplemented with 10% FBS, 1% nonessential amino acids, 1% L-glutamine, 100 μg/mL streptomycin, and 100 U/mL penicillin-G at 37 °C, 5% CO_2, and 95% relative humidity.

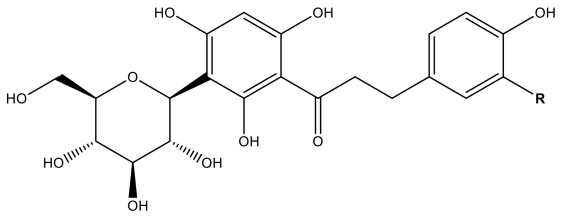
Plant Material. The dried leaves and stems of unfermented (green) *A. linearis* were received from South Africa as a gift from Rooibos Limited in Clanwilliam (Ms. Colette Cronje, sample no. 793; NCNPR no. 16850) in October 2014. A specimen (NCNPR no. 16850) was prepared and deposited by Dr. Vijayasankar Raman in the herbarium of NCNPR, School of Pharmacy, University of Mississippi. The morphological and chemical properties were compared with the reference sample (NCNPR no. 5488) at the NCNPR to establish the authenticity of the sample from Rooibos Limited.

Extraction and Isolation of Compounds. The dried leaves and stems of *A. linearis* (1.5 kg) were ground to a fine powder and extracted exhaustively by percolation process with MeOH (4 L, 5 times) at room temperature. The MeOH solution was then evaporated under reduced pressure at 40 °C to yield a dried crude extract (202.8 g). Around 100 g of crude extract was subjected to column chromatography on silica gel (8 cm × 73 cm) and eluted with ethyl acetate/chloroform/MeOH/water gradient systems (15:8:4:1, 10:6:4:1, and 6:4:4:1) to provide 20 parent fractions (1−20). Fraction 6 (0.95 g) was fractionated by silica gel with chloroform/MeOH/water (8:2:0.25) to afford vitexin (**5**, 73.4 mg). Fraction 8 (0.27 g) was separated on Sephadex LH-20 (92 cm × 4 cm) with MeOH/water mixtures (1:1, 3:1, and 1:0) followed by silica gel (102 cm × 2.7 cm) with chloroform/MeOH/water (8:2:0.25) to yield isoquercitrin (CAS: 482-35-9) (**7**, 76.6 mg), thermopsoside (**3**, 23.6 mg), and nothofagin (**2**, 306.6 mg). Aspalathin (**1**, 521.0 mg), isovitexin (**6**, 8.4 mg), and (*R*)/(*S*)-eriodictyol-6-*C*-β-D-glucopyranoside (**10**, 68.1 mg) were purified from fraction 12 (4.33 g) by repeated column chromatography [Sephadex LH-20 (92 cm × 4 cm), MeOH/water (1:1, 3:1, and 1:0); silica gel (104.5 cm × 2.7 cm), chloroform/MeOH/water (8:2:0.25); and silica gel (76 cm × 2 cm), chloroform/MeOH/water (8:2:0.25)]. Fraction 14 (1.86 g) was chromatographed on Sephadex LH-20 (98 cm × 4.5 cm) with MeOH to obtain isoorientin (**4**, 90.9 mg) and syringin (**11**, 68.8 mg). Fraction 17 (2.37 g) was fractionated by reversed-phase C-18 silica gel (47 cm × 3 cm) with water/MeOH (7:3 and 3:2) followed by Sephadex LH-20 column (101 cm × 3.2 cm) with MeOH to purify rutin (**8**, 59.6 mg) and bioquercetin (CAS: 52525-35-6) (**9**, 136.9 mg). Structure elucidation of the isolated compounds was performed by analysis of their spectroscopic data including 1D and 2D NMR, circular dichroism, IR, and mass analysis by HR-ESI-MS.

Inhibitory Activity with Recombinant CYP Assay. The assay to determine the inhibitory activity of test samples on the catalytic activity of CYPs was performed under similar conditions as earlier

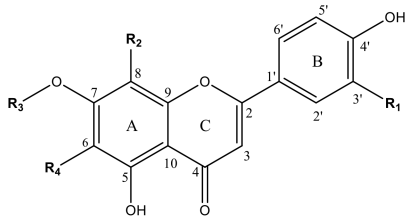
DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

I. Dihydrochalcone glycosides:



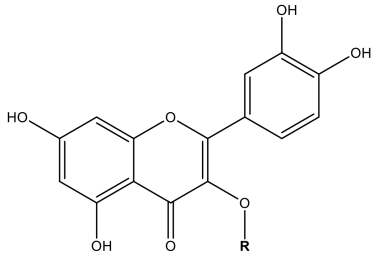
(1) Aspalathin: R = OH
(2) Nothofagin: R = H

II. Flavone glycosides:



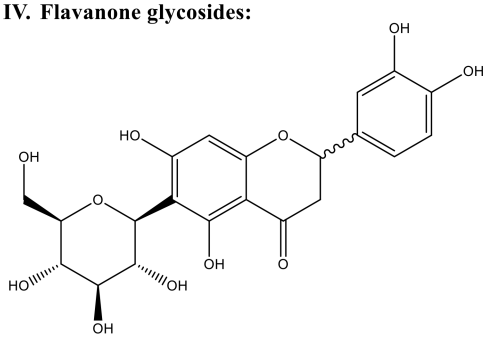
	R_1	R_2	R_3	R_4
(3) Thermopsoside:	O-CH_3	H	Glc	H
(4) Isoorientin:	OH	H	H	Glc
(5) Vitexin:	H	Glc	H	H
(6) Isovitexin:	H	H	H	Glc

III. Flavonol glycosides:



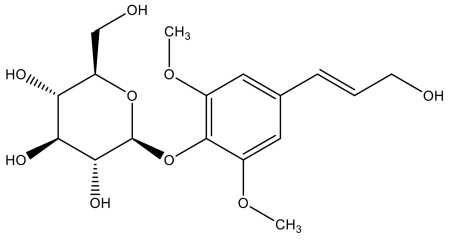
(7) Isoquercitrin: R= β-D-Glc
(8) Rutin: R= α-L-Rha-1➔6-β-D-Glc
(9) Bioquercetin: R= α-L-Rha-1➔6-β-D-Gal

IV. Flavanone glycosides:



(10) ***(R)/(S)*****-eriodictyol-6-C-β-D-glucopyranoside**

V. Phenyl propanoid glycoside:



(11) Syringin

Figure 1. Chemical structures of isolated phytoconstituents **1**−**11** from *A. linearis*.

reported.[22] The MeOH extract of *A. linearis* and its constituents along with positive controls were serially diluted in a solution (100 μL) of cofactors mix, CYP proteins (0.05 mg of protein/mL), and glucose phosphate dehydrogenase (G-6-PDH) to achieve six concentrations ranging from 0.1 to 100 μg/mL for the extract and 0.1 to 100 μM for the pure compounds. DMSO (0.25%) was used as vehicle control. Initial readings were recorded to report the inherent fluorescence, and then the plates were incubated at 37 °C for 10 min. Initiation of the enzymatic reaction was achieved by the addition of the enzyme substrate mixture (100 μL) followed by incubation for a specified time according to instructions provided for each enzyme. Termination of the enzymatic reaction was performed by the addition of 75 μL of ice cold acetonitrile/0.5 M Tris base (80:20). Fluorescence was measured on a Spectramax M5 plate reader (Molecular Devices, Sunnyvale, CA, U.S.A.) at specified excitation and emission wavelengths for each substrate. IC_{50} values were calculated from dose curves prepared by the tested concentrations against observed enzyme inhibition (%).

Assay for P-Glycoprotein Inhibition by Calcein-AM Uptake in Parental and Transfected MDCK-II Cells. The inhibition of the efflux activity of P-gp by the test samples was determined as mentioned earlier.[23] Cells were seeded in 96-well plates at a density of 70 000 cells/well in 200 μL of culture medium. After 24 h, the media were changed and cells were further incubated for 48 h. The test samples (0.4−100 μg/mL or μM) and the positive control (cyclosporine A 0.4−100 μM) were added to the cells at varying concentrations in 50 μL of transport buffer and incubated at 37 °C for 10 min. DMSO (0.3%) was used as the negative control. Calcein acetoxymethyl ester (Calcein-AM) (1 μM) was added to the plates as a probe substrate of P-gp. Immediately, the plates were placed on Spectramax to read the fluorescence up to 1 h at 15 min intervals at excitation and emission wavelengths of 485 and 530 nm, respectively. EC_{50} was determined by plotting the increase (%) in calcein-AM uptake against the log concentration of test samples to obtain the concentrations that caused 50% increase using GraphPad Prism Software V.7.0 (GraphPad Software, Inc., San Diego, CA, U.S.A.).

Pregnane-X Receptor Modulation by Luciferase Reporter Gene Assay. The pregnane-X receptor (PXR) modulation assay was conducted in HepG2 cells transiently transfected with pSG5-PXR (25 μg) and PCR5 plasmid DNA (25 μg) via electroporation at 180 V, 1 pulse for 70 ms, as described earlier.[24] In brief, the aforementioned cells were seeded in 96-well plates at a density of 50 000 cells per well.

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

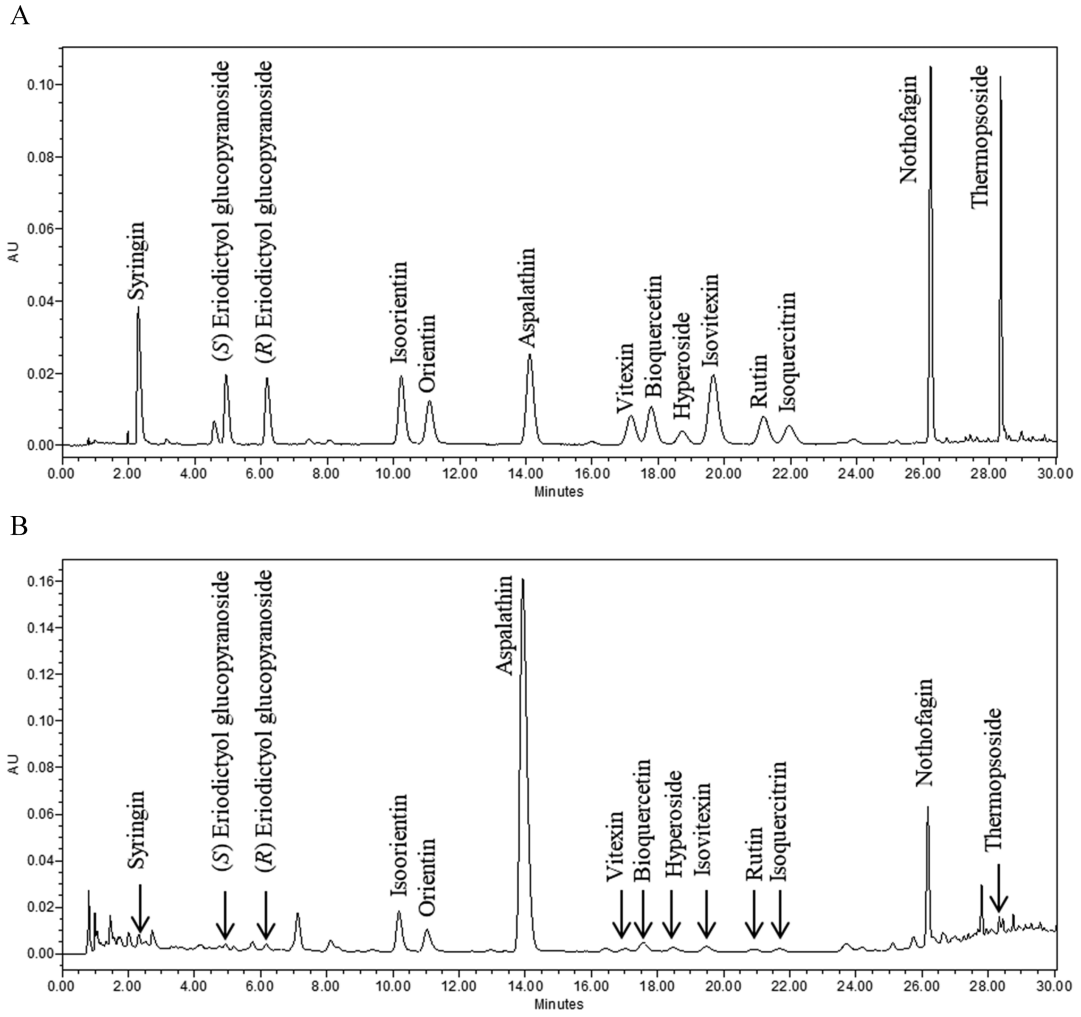


Figure 2. UHPLC-UV chromatograms of standard mixture (A) and MeOH extract (B) at 288 nm.

After the cells reached 90% confluency (24 h), the samples were added at several concentrations (0.3−30 μM for controls and isolated compounds; 1−30 μg/mL for the MeOH extract). After 24 h incubation, the media were aspirated and 40 μL of luciferase reagent (Promega Corporation, Madison, WI, U.S.A.) was added to each well, followed by measuring the luminescence on Spectramax M5 plate reader (Molecular Devices, Sunnyvale, CA, U.S.A.). Compared with vehicle-treated cells (0.3% DMSO), the fold induction in luciferase activity was calculated for the sample-treated cells.

RT-PCR Analysis of CYP3A4, CYP1A2, CYP2C9, and P-gp mRNA. The validated forward and reverse primers for CYP3A4 (qHsaCID0012316), CYP1A2 (qHsaCID0015160), CYP2C9 (qHsaCED0044817), and P-gp (qHsaCED0002291) were purchased from Bio-Rad Laboratories (Hercules, CA, U.S.A.). Transfected HepG2 cells were seeded into a 6-well plate at a density of 2×10^6 cells per well and cultivated for 24 h (>90% confluency). After that, cells were treated with several concentrations of test samples (12.5−50 μg/mL for the MeOH extract; 0.3−30 μM for pure compounds; 30 μM for positive controls). After 48 h, the cells were washed with phosphate-buffered saline (PBS), and the total RNA was extracted using the Quick-start protocol (Qiagen). Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out in a 96-well plate by a CFX connect real-time PCR detector system (Bio-Rad). The gene expression of CYP3A4, CYP1A2, CYP2C9, and P-gp was quantified relatively and normalized to the housekeeping gene GAPDH (qHsaCED0038674) as the control gene. Compared with vehicle-treated cells (0.3% DMSO), the fold induction in mRNA expression for treated cells was calculated.

Statistical Analysis. All values are represented as mean ± SD (n = 3). Nonparametric data were analyzed by one-way ANOVA, followed by Bonferroni's multiple comparison tests using GraphPad Prism Software V.7.0 (GraphPad Software, Inc., San Diego, CA, U.S.A.). The value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Isolation and Structure Elucidation. Phytochemical investigation of the MeOH extract of *A. linearis* mixed parts comprising leaves and stems resulted in the isolation and characterization of 11 flavonoid glycosides including two dihydrochalcone derivatives, aspalathin[25] (**1**) and nothofagin[26] (**2**); four flavone derivatives, thermopsoside[27] (**3**), isoorientin[28] (**4**), vitexin[29] (**5**) and isovitexin[29] (**6**); three flavonol derivatives, isoquercitrin[30] (**7**), rutin[31] (**8**), and bioquercetin[32] (**9**); one flavanone derivative, (*R*/*S*)-eriodictyol-6-*C*-β-D-glucopyranoside[12] (**10**); and one phenylpropanoid derivative, syringin[33] (**11**) (Figure 1). Structure elucidation was mainly achieved by spectroscopic data analysis including 1D and 2D NMR (^{1}H, ^{13}C, DEPTQ135, COSY, HSQC, and HMBC), and the compounds were confirmed by HR-ESI-MS and identified by comparison with the literature. The purity of phytoconstituents **1**−**11** was established as >90−95% (Supporting Information). Ultrahigh-performance liquid chromatography coupled with ultraviolet detector (UHPLC-UV) chromato-

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

Table 1. Inhibitory Activity of the MeOH Extract and Isolated Compounds from *A. linearis* on CYP Isozymes[a]

test sample	CYP3A4	CYP1A2	CYP2D6	CYP2C9	CYP2C19
IC_{50} μg/mL					
A. linearis extract	1.7 ± 0.1	19.0 ± 1.1	20.0 ± 1.8	10.0 ± 0.3	4.0 ± 0.3
IC_{50} μM					
aspalathin (**1**)	90.0 ± 3.8	–	–	>100	>100
nothofagin (**2**)	88.0 ± 3.4	–	–	>100	>100
thermopsoside (**3**)	6.0 ± 0.5	>100	12.0 ± 0.3	32.0 ± 1.4	9.5 ± 0.2
isoorientin (**4**)	10.0 ± 0.2	>100	40.0 ± 1.6	>100	24.0 ± 0.2
vitexin (**5**)	4.0 ± 0.3	–	>100	8.0 ± 0.2	13.0 ± 0.3
isovitexin (**6**)	3.4 ± 0.2	–	56.0 ± 1.2	57.0 ± 1.2	55.0 ± 1.6
isoquercitrin (**7**)	61.0 ± 1.2	–	–	>100	21.0 ± 0.3
rutin (**8**)	45.0 ± 2.4	85.0 ± 2.5	–	–	57.0 ± 1.3
bioquercetin (**9**)	56.0 ± 1.8	–	–	–	>100
eriodictyol-6-C-β-D-glucopyranoside (**10**)	44.0 ± 1.7	–	>100	>100	>100
syringin (**11**)	10.0 ± 1.2	–	–	>100	>100
ketoconazole[Ω]	0.040 ± 0.001				
furafylline[Ω]		1.5 ± 0.1			
quinidine[Ω]			0.050 ± 0.001		
sulfaphenazole[Ω]				3.0 ± 0.3	
tranylcypromine[Ω]					1.2 ± 0.1

[a](−) indicates the lack of inhibition, and ([Ω]) indicates the positive control for respective CYP450 isozyme. The data are represented as mean ± SD (n = 3).

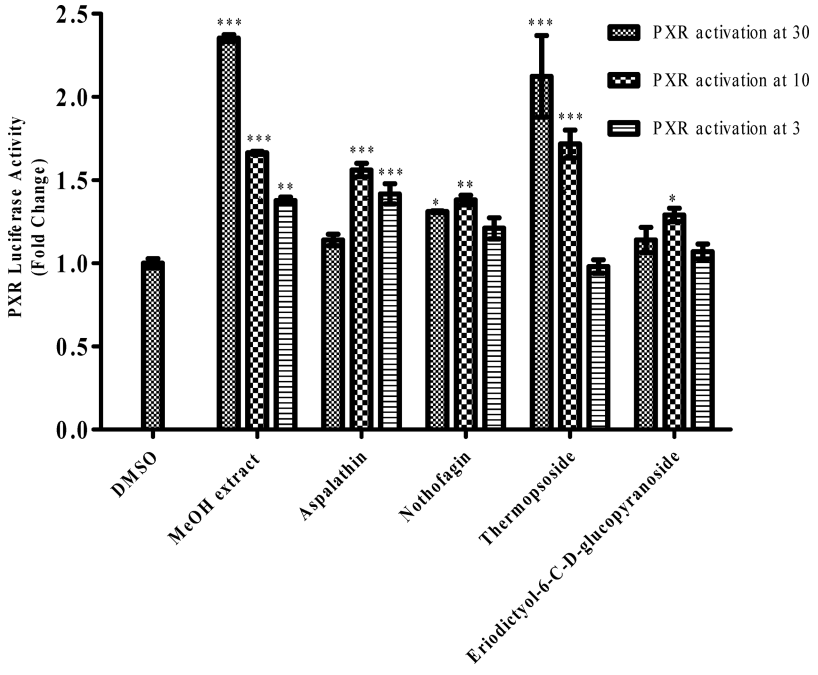


Figure 3. Effect of *A. linearis* MeOH extract and its constituents on PXR determined by reporter gene assay. Values are fold increase in PXR activity compared to DMSO control. An increase of 1.5-fold or more means there is an increase of 50% or more. The extract showed a concentration-dependent effect on PXR. The MeOH extract was tested at concentrations of 30, 10, and 3 μg/mL, whereas the pure compounds were tested at 30, 10, and 3 μM. The data are represented as mean ± SD of three experiments. *P < 0.05, **P < 0.01, and ***P < 0.001, determined by one-way ANOVA, followed by Bonferroni's post-tests.

grams of standards mixture and the authenticated plant sample (NCNPR no. 16850) at 288 nm are illustrated in Figure 2.

Inhibition of CYP Isoforms by the MeOH Extract of *A. linearis* and the Isolated Phytochemicals. The inhibitory effects of test samples on the catalytic activity of CYP isoforms were measured. A decrease in the formation of the respective metabolites was calculated, and the IC_{50} value (concentration responsible for a 50% decrease in activity) was determined from concentration−response curves. The crude MeOH extract of *A. linearis* demonstrated significant inhibition (IC_{50} < 20 μg/mL, Table 1) of the major human CYP450 isoforms (CYP3A4, CYP1A2, CYP2D6, CYP2C9, and CYP2C19), which are responsible for the metabolism of 80% of clinical drugs. For the extract, the strongest inhibition was observed for CYP3A4 (IC_{50} 1.7 ± 0.1 μg/mL) followed by CYP2C19 (IC_{50} 4.0 ± 0.3 μg/mL) isozymes. It was interesting to note that the activity of CYP3A4 was inhibited by almost all isolated secondary metabolites in varying degrees with the strong

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

inhibition exerted by isovitexin (IC_{50} 3.4 ± 0.2 μM), vitexin (IC_{50} 4.0 ± 0.3 μM), and thermopsoside (IC_{50} 6.0 ± 0.5 μM). In addition, thermopsoside (9.5 ± 0.2 μM) exhibited the strongest inhibition for CYP2C19 isozyme. Rutin, a common flavonoid also present in several other plant species, was found to be responsible for the CYP1A2 inhibition, while vitexin, isovitexin, isoorientin, and thermopsoside seem to be the major contributors for CYP2D6 and CYP2C9 inhibition as shown in Table 1.

P-Glycoprotein Inhibition by the MeOH Extract of *A. linearis* and its Isolates. P-glycoprotein (P-gp) is recognized as one of the efflux transporters in many tissues, and its inhibition has been found to be responsible for multiple herb−drug interactions. For assessing the efflux activity of P-gp in the presence of tested samples, Calcein-AM as a fluorescent substrate of P-gp in MDR-MDCK-II cells was utilized. Calcein-AM crosses the cell membrane and is rapidly hydrolyzed by cellular esterases to a fluorescent form (calcein free acid). Increased uptake of calcein-AM is an indicator of P-gp inhibition.[34] In this study, the MeOH extract and the isolated phytochemicals did not show inhibition of P-gp at 100 μg/mL and 100 μM, respectively.

PXR Modulation by the MeOH Extract of *A. linearis* and its Isolated Components. PXR reporter gene assay is a reliable method to assess the CYP induction potential of xenobiotics through the PXR mechanism. It was established to screen CYP450 inducers based on the activation of PXR determined in HepG2 cells. The MeOH extract was found to increase the activity of PXR in comparison to the vehicle. The effect was concentration-dependent, as shown in Figure 3. Of the 11 isolated secondary metabolites tested, thermopsoside showed about a 2-fold activation of PXR at 30 μM, while the two major dihydrochalcone derivatives, aspalathin and nothofagin, displayed a moderate activation at 10 μM (1.56 and 1.38, respectively) (Figure. 3). Rifampicin (10 μM) showed an activation of 4.09 under similar assay conditions.

mRNA Expressions of CYP3A4, CYP1A2, CYP2C9, and P-gp. The results from PXR reporter gene assay demonstrate that *A. linearis* extract and its constituents (aspalathin, nothofagin, and thermopsoside) caused an induction in PXR transcriptional activity. To further confirm if this increase in PXR activity resulted in the increased expression of four major downstream target genes—CYP3A4, CYP1A2, CYP2C9, and P-gp—we carried out real-time reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for the gene expression. The extract showed a marginal effect on the expression of CYP genes compared to the vehicle. However, aspalathin, a dihydrochalcone derivative, showed a significant induction (1.81-fold) in CYP3A4 expression at a concentration of 30 μM. Similarly, increases of 1.64- and 1.42-fold were observed at a concentration of 30 μM of nothofagin for CYP1A2 and CYP3A4 expression, respectively. On the one hand, thermopsoside activated PXR to a higher extent but have marginal effects on the expression of CYP450 target genes (Table 2). On the other hand, the extract as well as pure compounds did not show any increase in the expression of P-gp mRNA levels.

Table 2. Effect of Isolates on the Expression of CYPs: Values are Fold Increase in mRNA Expression Compared to DMSO Control[a]

test sample	fold increase in mRNA expression[b]		
	CYP1A2	CYP3A4	CYP2C9
A. linearis extract	1.14 ± 0.14	NA	NA
aspalathin (**1**)	NA	1.81 ± 0.18[c]	NA
nothofagin (**2**)	1.64 ± 0.33[c]	1.42 ± 0.57	NA
thermopsoside (**3**)	NA	1.16 ± 0.13	NA
eriodictyol-6-*C*-β-D-glucopyranoside (**10**)	NA	1.32 ± 0.17	NA
omeprazole[Ω]	5.78 ± 0.70		
rifampicin[Ω]		2.1 ± 0.8	7.6 ± 1.1

[a]An increase of 1.5-fold or more means there is an increase of 50% or more. [b]Fold increase in mRNA expression at 30 μg/mL of extract and 30 μM of pure compounds or drug controls. The data are represented as mean ± SD (n = 3). [c]$P < 0.05$, determined by one-way ANOVA, followed by Bonferroni's post-tests. NA = no activation. ([Ω]) indicates the positive control for respective mRNA expression.

DISCUSSION

Overview. Phytochemicals derived from natural origins are pivotal agents for treating a range of illnesses when administered orally or applied topically.[35] Simultaneously, they could be exploited for further optimization as a promising natural lead nucleus to synthesize potent therapeutic analogues. The quest to find a bioactive botanical, as either a standardized extract or a single natural compound, is a core research domain for a number of research laboratories around the world. However, investigating the safety aspects for these pharmacological candidates is as substantial as seeking for biological activity.[36] Limited information is available in the literature related to the safety profile of *A. linearis*. With the rapid increase in its global use, the possibility of herb−drug interaction needs to be explored.

Chemotaxonomical Significance of *A. linearis*. Because of the ability to biosynthesize unique and rare secondary metabolites, rooibos tea is known for its hypoglycemic activity.[37,38] To the best of our knowledge, aspalathin (acyclic dihydrochalcone) was isolated from *A. linearis* as the sole natural source. Yet, aspalathin recently was detected in a closely related and endangered species, *Aspalathus pendula* (the golden tea).[7,39] In addition to aspalathin, phytochemical investigation of *A. linearis* in this study afforded 10 additional phenolic secondary metabolites, and we also report for the first time the isolation of thermopsoside from this species.

Abundant Phytochemicals Detected in Unfermented *A. linearis* by UHPLC-MS. The UHPLC-MS and the fragmentation patterns indicated that the most abundant components were *C*-glycosides. The neutral mass loss of hexose equals 120 Da, usually observed compared with the 162 Da with the *O*-counterparts.[40] Also, the chemical shift value for the anomeric carbon, which appeared at the upfield region of the NMR spectra further supported identification of the compounds (Supporting Information). Unlike classical flavonoids (glycopyranosyloxy flavonoids), glycopyranosyl flavonoid derivatives have superior characteristic features. For instance, their glycone moiety is attached via the C-1″ directly to the flavonoid backbone, usually at the C-6 (**6**) or C-8 position (**5**) of the A ring, as shown in Figure 1. Because of these characteristic chemical features, glycopyranosyl derivatives are believed to possess unique biological activities, including superior stability and altered metabolism, compared to those of the classical *O*-glycosides. In fact, some of the *C*-glycosides are reported to resist the hydrolysis, absorb intact in the colon, and respond to liver enzymes differently than their counterparts.[41] Quantitative analysis by HPLC-UV previously indicated that the abundant phytochemicals in aqueous

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

unfermented rooibos extract were aspalathin followed in order by isoorientin, orientin, rutin, isovitexin, vitexin, and isoquercitrin.[42] As shown in Figure 2B, UHPLC-UV chromatogram at 288 nm reveals that aspalathin remains the main phytochemical present in MeOH unfermented rooibos extract in agreement with the previous study. Additionally, a pool of minor compounds was detected alongside aspalathin, and successful isolation of some components was achieved. Nevertheless, the absence of orientin in this study might be justified by the effects of fractionation conditions employed during the phytochemical investigation or accidental loss in other fractions.

Effects of Unfermented MeOH Extract and Dihydrochalcones on CYP Enzymes, PXR, and mRNA Expressions. In the present study, the effects of MeOH extract, as well as isolated phytochemicals from *A. linearis*, were evaluated on the major human CYP450 isozymes. Accordingly, CYP3A4 and CYP2C19 enzyme activities were inhibited potently by the MeOH extract. However, minimal inhibition of CYP3A4 was observed for aspalathin (IC_{50} = 90.0 ± 3.8 μM) and nothofagin (IC_{50} = 88.0 ± 3.4 μM), which are two major compounds of *A. linearis*. These results are in agreement with a recent study performed on aspalathin.[17] Moreover, these findings highlight the significance of the other minor compounds present in the unfermented rooibos extract and could synergistically account for the inhibitory activity of the MeOH extract toward CYP3A4. On the contrary, these two major components increased PXR activity to 1.56- and 1.38-fold, respectively. Moreover, further mRNA expression studies in HepG2 cells displayed a significant increase in CYP3A4 gene expression after treatment with aspalathin and nothofagin (30 μM). Our findings suggest that the inhibitory effect of these compounds on the activity of CYP3A4 isozyme is a negative compensatory mechanism for the increase of mRNA at lower concentrations. Therefore, further studies should be carried out to translate the studies in animal models to observe the overall effect.

Effects of Flavone Glycosides on Major CYP Enzymes. Flavone derivatives including vitexin, isovitexin, isoorientin, and thermopsoside exhibited inhibitory activities on CYP3A4 and CYP2C19. Except for isoorientin, they also displayed inhibitory action on CYP2C9 enzyme. Isoorientin, *C*-glucosyl flavone, has an additional hydroxy group attached to ring B, which potentially plays a role in reducing the inhibitory activity of this component on CYP2C9 compared to vitexin, isovitexin, and thermopsoside. The most potent inhibitory activities in this study were observed with isovitexin on CYP3A4, vitexin on CYP2C9, and thermopsoside on CYP2C19. Parallel pharmacological effects including antihypertensive and anti-inflammatory activities for vitexin and isovitexin were previously reported because of the structural resemblance. The distributions of vitexin and isovitexin are evident in a wide range of plants such as *Aspalathus pendula*, *Mimosa pudica*, *Passiflora* species, wheat leaves, and bamboo. The primary source and highest concentration of vitexin were determined in the leaves of *Crataegus pinnatifida* (9.53 mg/g).[43] Thermopsoside, on the other hand, was isolated for the first time from *Thermopsis alterniflora* and reported in other species including the fruits of *Rhus parviflora* and the aerial part of *Lycopus lucidus*.[27,44,45]

Bioactive *C*-glycoside flavones such as isovitexin and vitexin are structurally similar except for the position of sugar attachment. Both regioisomers inhibited CYP3A4 enzyme activity to a similar extent (IC_{50} of 4.0 ± 0.3 and 3.4 ± 0.2 μM, respectively). On the other hand, their abilities to inhibit CYP2C19 and CYP2C9 activities were dissimilar. While the former exhibited the inhibitory activity with an IC_{50} of 55.0 ± 1.6 μM and 57.0 ± 1.2 μM, respectively, the latter inhibited CYP2C19 isozyme with an IC_{50} of 13.0 ± 0.3 μM and CYP2C9 with an IC_{50} of 8.0 ± 0.2 μM. According to this result, it is possible that the CYP2C family has a distinguished binding site that plays a substantial role in this effect, providing higher selectivity toward vitexin compared with isovitexin. Preceding in vivo studies indicated that vitexin could inhibit both CYP3A1 and CYP2C11 but has no effect on CYP1A2 in rat.[46] Our in vitro study also confirms that CYP1A2 enzyme activity was not affected. We report for the first time the potent and selective inhibition of CYP3A4 (4.0 ± 0.3 μM), CYP2C9 (8.0 ± 0.2 μM), and CYP2C19 (13.0 ± 0.3 μM) isoforms by vitexin.

Effects of Flavonol Glycosides on Major CYP Enzymes. For the tested flavonols (isoquercitrin, bioquercetin, and rutin), CYP3A4 isozyme was found to be inhibited with an IC_{50} ranging from 45 to 61 μM. Among these three tested flavonols, bioquercetin showed a weak inhibitory activity (>100 μM) on CYP2C19. It is possible that the type of glycone moiety, galactopyranoside, contributed to reducing the inhibitory activity of bioquercetin compared with the other two compounds. As mentioned earlier, *O*-glycoside flavonoids are prone to hydrolysis, readily compared to *C*-glycoside counterparts. Even though the flavonoid disaccharide epimers such as rutin and bioquercetin show different in vitro IC_{50} values, they are expected to exert similar inhibitory effects in the animal model. This effect could be a consequence for the conversion of the aforementioned flavonols to the flavonol aglycone derivative, quercetin. The latter was extensively evaluated and found to inhibit several CYP isozymes including CYP3A4, CYP2D6, and CYP2C9 based on prior in vivo studies.[47,48]

Prospective Trends for the Safety Assessment of Rooibos Tea. Multiple endogenous substrates and exogenous xenobiotics are predominantly metabolized and biosynthesized by cytochrome P450. Moreover, these heme-containing monooxygenases are exclusively responsible for the conversion of the inactive prodrug to the pharmacologically active metabolites in the human body. Both CYP inducers and inhibitors interact with CYP isozymes and interfere with CYP substrates in the binding site.[49] While CYP inhibitors can intensify the toxicity profile of other medicines being accumulated in the body, CYP inducers have the ability to eliminate the drugs from the biological system, rapidly resulting in diminished activity. Clinically, two case reports indicated the potential hepatotoxicity associated with rooibos tea in patients who experienced elevated liver enzymes after ingestion, and they recovered liver functions after ceasing.[13,14] The possibility of rooibos constituents reacting with CYP enzymes leading to hepatotoxicity cannot be disregarded. A previous study indicated that concurrent consumption of fermented rooibos extract exhibited antagonistic effects with antibacterial agents such as ciprofloxacin and gentamicin against Gram-negative pathogen (*E. coli*) and antifungal therapy, amphotericin B, against yeasts (*C. albicans* and *C. neoformans*).[50] Another study determined a significant serum concertation reduction of midazolam in rats when consumed in conjunction with the rooibos tea.[18] Therefore, determination of components potentially responsible for rooibos interaction with clinical drugs via CYP enzymes is pivotal.

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

In conclusion, phytochemical investigation of unfermented *A. linearis* in this study afforded 11 secondary metabolites that were examined for potential herb−drug interactions. The current results demonstrated that a high intake of nutraceuticals containing rooibos extracts might lead to possible interactions when concomitantly ingested with medicines that are metabolized by CYP enzymes. On the basis of the former and current studies, which indicated the mechanistic potential for rooibos tea extract and phytochemicals to affect drug metabolism, further in vivo interaction studies with clinical treatments are warranted to support the findings and highlight the clinical significance.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b00846.

UV, HR-ESI-MS, and NMR spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: skhan@olemiss.edu.

ORCID

Zulfiqar Ali: 0000-0003-3902-5152

Shabana I. Khan: 0000-0002-0262-7101

Amar G. Chittiboyina: 0000-0002-7047-5373

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research is supported in part by "Science Based Authentication of Dietary Supplements" funded by the Food and Drug Administration (Grant no. 5U01FD004246-07). Rooibos Limited in South Africa is thanked for supplying the plant material used in this study. The authors thank Dr. Jon F. Parcher for his assistance in proofreading the manuscript.

■ ABBREVIATIONS USED

A. linearis, *Aspalathus linearis*; CYP450, cytochrome P450; PXR, pregnane-X receptor; P-gp, P-glycoprotein; MeOH, methanol; HDI, herb−drug interaction; mRNA, messenger ribonucleic acid; G-6-PDH, glucose-6-phosphate dehydrogenase

■ REFERENCES

(1) Joubert, E.; de Beer, D. Rooibos (*Aspalathus linearis*) beyond the farm gate: From herbal tea to potential phytopharmaceutical. *S. Afr. J. Bot.* **2011**, *77*, 869−886.

(2) Krafczyk, N.; Glomb, M. A. Characterization of phenolic compounds in rooibos tea. *J. Agric. Food Chem.* **2008**, *56*, 3368−76.

(3) Shimamura, N.; Miyase, T.; Umehara, K.; Warashina, T.; Fujii, S. Phytoestrogens from *Aspalathus linearis*. *Biol. Pharm. Bull.* **2006**, *29*, 1271−4.

(4) Son, M. J.; Minakawa, M.; Miura, Y.; Yagasaki, K. Aspalathin improves hyperglycemia and glucose intolerance in obese diabetic ob/ob mice. *Eur. J. Nutr.* **2013**, *52*, 1607−19.

(5) Gilani, A. H.; Khan, A. U.; Ghayur, M. N.; Ali, S. F.; Herzig, J. W. Antispasmodic effects of Rooibos tea (*Aspalathus linearis*) is mediated predominantly through K+ -channel activation. *Basic Clin. Pharmacol. Toxicol.* **2006**, *99*, 365−73.

(6) Kawano, A.; Nakamura, H.; Hata, S.; Minakawa, M.; Miura, Y.; Yagasaki, K. Hypoglycemic effect of aspalathin, a rooibos tea component from *Aspalathus linearis*, in type 2 diabetic model db/db mice. *Phytomedicine* **2009**, *16*, 437−43.

(7) Johnson, R.; Beer, D.; Dludla, P. V.; Ferreira, D.; Muller, C. J. F.; Joubert, E. Aspalathin from rooibos (*Aspalathus linearis*): a bioactive *C*-glucosyl dihydrochalcone with potential to target the metabolic syndrome. *Planta Med.* **2018**, *84*, 568−583.

(8) Baba, H.; Ohtsuka, Y.; Haruna, H.; Lee, T.; Nagata, S.; Maeda, M.; Yamashiro, Y.; Shimizu, T. Studies of anti-inflammatory effects of rooibos tea in rats. *Pediatr. Int.* **2009**, *51*, 700−4.

(9) Magcwebeba, T.; Swart, P.; Swanevelder, S.; Joubert, E.; Gelderblom, W. Anti-Inflammatory effects of *Aspalathus linearis* and *Cyclopia* spp. Extracts in a UVB/Keratinocyte (HaCaT) model utilising interleukin-1α accumulation as biomarker. *Molecules* **2016**, *21*, 1323.

(10) Smith, C.; Swart, A. C. Rooibos (*Aspalathus linearis*) facilitates an anti-inflammatory state, modulating IL-6 and IL-10 while not inhibiting the acute glucocorticoid response to a mild novel stressor *in vivo*. *J. Funct. Foods* **2016**, *27*, 42−54.

(11) Lee, W.; Bae, J. S. Anti-inflammatory effects of aspalathin and nothofagin from rooibos (*Aspalathus linearis*) *in vitro* and *in vivo*. *Inflammation* **2015**, *38*, 1502−16.

(12) Marais, C.; van Rensburg, W. J.; Ferreira, D.; Steenkamp, J. A. (*S*)- and (*R*)-eriodictyol-6-C-β-D-glucopyranoside, novel keys to the fermentation of rooibos (*Aspalathus linearis*). *Phytochemistry* **2000**, *55*, 43−9.

(13) Sinisalo, M.; Enkovaara, A. L.; Kivisto, K. T. Possible hepatotoxic effect of rooibos tea: a case report. *Eur. J. Clin. Pharmacol.* **2010**, *66*, 427−8.

(14) Engels, M.; Wang, C.; Matoso, A.; Maidan, E.; Wands, J. Tea not Tincture: Hepatotoxicity associated with rooibos herbal tea. *ACG Case Rep. J.* **2013**, *1*, 58−60.

(15) Fasinu, P. S.; Manda, V. K.; Dale, O. R.; Egiebor, N. O.; Walker, L. A.; Khan, S. I. Modulation of Cytochrome P450, P-glycoprotein and pregnane X receptor by selected antimalarial herbs-implication for herb-drug interaction. *Molecules* **2017**, *22*, 2049.

(16) Jang, E. H.; Park, Y. C.; Chung, W. G. Effects of dietary supplements on induction and inhibition of cytochrome P450s protein expression in rats. *Food Chem. Toxicol.* **2004**, *42*, 1749−56.

(17) Patel, O.; Muller, C.; Joubert, E.; Louw, J.; Rosenkranz, B.; Awortwe, C. Inhibitory interactions of *Aspalathus linearis* (rooibos) extracts and compounds, aspalathin and Z-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid, on cytochromes metabolizing hypoglycemic and hypolipidemic drugs. *Molecules* **2016**, *21*, 1515.

(18) Matsuda, K.; Nishimura, Y.; Kurata, N.; Iwase, M.; Yasuhara, H. Effects of Continuous Ingestion of Herbal Teas on Intestinal CYP3A in the Rat. *J. Pharmacol. Sci.* **2007**, *103*, 214−221.

(19) van der Merwe, J. D.; de Beer, D.; Joubert, E.; Gelderblom, W. C. Short-term and sub-chronic dietary exposure to aspalathin-enriched green rooibos (*Aspalathus linearis*) extract affects rat liver function and antioxidant status. *Molecules* **2015**, *20*, 22674−90.

(20) Schloms, L.; Swart, A. C. Rooibos flavonoids inhibit the activity of key adrenal steroidogenic enzymes, modulating steroid hormone levels in H295R cells. *Molecules* **2014**, *19*, 3681−95.

(21) Schloms, L.; Storbeck, K. H.; Swart, P.; Gelderblom, W. C.; Swart, A. C. The influence of *Aspalathus linearis* (Rooibos) and dihydrochalcones on adrenal steroidogenesis: quantification of steroid intermediates and end products in H295R cells. *J. Steroid Biochem. Mol. Biol.* **2012**, *128*, 128−38.

(22) Manda, V. K.; Dale, O. R.; Awortwe, C.; Ali, Z.; Khan, I. A.; Walker, L. A.; Khan, S. I. Evaluation of drug interaction potential of *Labisia pumila* (Kacip Fatimah) and its constituents. *Front. Pharmacol.* **2014**, *5*, 178.

(23) Manda, V. K.; Ibrahim, M. A.; Dale, O. R.; Kumarihamy, M.; Cutler, S. J.; Khan, I. A.; Walker, L. A.; Muhammad, I.; Khan, S. I. Modulation of CYPs, P-gp, and PXR by *Eschscholzia californica* (California poppy) and its alkaloids. *Planta Med.* **2016**, *82*, 551−8.

(24) Manda, V. K.; Avula, B.; Ali, Z.; Khan, I. A.; Walker, L. A.; Khan, S. I. Evaluation of *in vitro* absorption, distribution, metabolism,

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975

and excretion (ADME) properties of mitragynine, 7-hydroxymitragynine, and mitraphylline. *Planta Med.* **2014**, *80*, 568−76.
(25) Koeppen, B. H.; Roux, D. G. Aspalathin: a novel *C*-glycosylflavonoid from *Aspalathus linearis*. *Tetrahedron Lett.* **1965**, *6*, 3497−3503.
(26) Hillis, W. E.; Inoue, T. The polyphenols of *Nothofagus* species—II.: The heartwood of *Nothofagus fusca*. *Phytochemistry* **1967**, *6*, 59−67.
(27) Kattaev, N. S.; Nikonov, G. K. The structure of thermopsoside — A new flavonoid from *Thermopsis alterniflora*. *Chem. Nat. Compd.* **1973**, *9*, 108−109.
(28) Koeppen, B. H.; Roux, D. G. *C*-Glycosylflavonoids. The chemistry of orientin and iso-orientin. *Biochem. J.* **1965**, *97*, 444−448.
(29) Hillis, W.; Horn, D. Nuclear magnetic resonance spectra and structures of some *C*-glycosyl flavonoids. *Aust. J. Chem.* **1965**, *18*, 531−542.
(30) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. Carbon-13 NMR studies of flavonoids—III: Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* **1978**, *34*, 1389−1397.
(31) Koeppen, B.; Smit, C.; Roux, D. The flavone *C*-glycosides and flavonol *O*-glycosides of Aspalathus acuminatus (rooibos tea). *Biochem. J.* **1962**, *83*, 507−511.
(32) Rastrelli, L.; Saturnino, P.; Schettino, O.; Dini, A. Studies on the constituents of *Chenopodium pallidicaule* (Canihua) seeds. isolation and characterization of two new flavonol glycosides. *J. Agric. Food Chem.* **1995**, *43*, 2020−2024.
(33) Sutarjadi; Malingre, T. M.; Van Os, F. H. L. Iridoid and phenolic glycosides of *Fraxinus griffithii*. *Phytochemistry* **1978**, *17*, 564.
(34) Sarkadi, B.; Homolya, L.; Szakacs, G.; Varadi, A. Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system. *Physiol. Rev.* **2006**, *86*, 1179−236.
(35) Newman, D. J.; Cragg, G. M. Natural Products as Sources of New Drugs from 1981 to 2014. *J. Nat. Prod.* **2016**, *79*, 629−61.
(36) Ekor, M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol.* **2014**, *4*, 177.
(37) Muller, C. J.; Joubert, E.; de Beer, D.; Sanderson, M.; Malherbe, C. J.; Fey, S. J.; Louw, J. Acute assessment of an aspalathin-enriched green rooibos (*Aspalathus linearis*) extract with hypoglycemic potential. *Phytomedicine* **2012**, *20*, 32−9.
(38) Kamakura, R.; Son, M. J.; de Beer, D.; Joubert, E.; Miura, Y.; Yagasaki, K. Antidiabetic effect of green rooibos (*Aspalathus linearis*) extract in cultured cells and type 2 diabetic model KK-A(y) mice. *Cytotechnology* **2015**, *67*, 699−710.
(39) Stander, M. A.; Van Wyk, B. E.; Taylor, M. J. C.; Long, H. S. Analysis of phenolic compounds in rooibos tea (*Aspalathus linearis*) with a comparison of flavonoid-based compounds in natural populations of plants from different regions. *J. Agric. Food Chem.* **2017**, *65*, 10270−10281.
(40) Avula, B.; Bae, J. Y.; Raman, V.; Fantoukh, O. I.; Wang, Y. H.; Osman, A. G.; Wang, M.; Ali, Z.; Khan, I. A. Quantification of phenolic compounds from *Fadogia agrestis* and dietary supplements using UHPLC-PDA-MS. *Planta Med.* **2019**, *85*, 145−153.
(41) Zeng, P.; Zhang, Y.; Pan, C.; Jia, Q.; Guo, F.; Li, Y.; Zhu, W.; Chen, K. Advances in studying of the pharmacological activities and structure−activity relationships of natural *C*-glycosylflavonoids. *Acta Pharm. Sin. B* **2013**, *3*, 154−162.
(42) Bramati, L.; Aquilano, F.; Pietta, P. Unfermented rooibos tea: quantitative characterization of flavonoids by HPLC−UV and determination of the total antioxidant activity. *J. Agric. Food Chem.* **2003**, *51*, 7472−7474.
(43) He, M.; Min, J. W.; Kong, W. L.; He, X. H.; Li, J. X.; Peng, B. W. A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia* **2016**, *115*, 74−85.
(44) Shrestha, S.; Lee, D.-Y.; Park, J.-H.; Cho, J.-G.; Seo, W.-D.; Kang, H. C.; Jeon, Y.-J.; Yeon, S.-W.; Bang, M.-H.; Baek, N.-I. Flavonoid glycosides from the fruit of *Rhus parviflora* and inhibition of cyclin dependent kinases by hyperin. *Han'guk Eungyong Sangmyong Hwahakhoeji* **2012**, *55*, 689−693.
(45) Malik, A.; Yuldashev, M. P.; Obid, A.; Ismoil, T.; Ping, L. Y. Flavonoids of the aerial part of *Lycopus lucidus*. *Chem. Nat. Compd.* **2002**, *38*, 612−613.
(46) Wang, X. S.; Hu, X. C.; Chen, G. L.; Yuan, X.; Yang, R. N.; Liang, S.; Ren, J.; Sun, J. C.; Kong, G. Q.; Gao, S. G.; Feng, X. S. Effects of vitexin on the pharmacokinetics and mRNA expression of CYP isozymes in rats. *Phytother. Res.* **2015**, *29*, 366−72.
(47) Valentova, K.; Vrba, J.; Bancirova, M.; Ulrichova, J.; Kren, V. Isoquercitrin: pharmacology, toxicology, and metabolism. *Food Chem. Toxicol.* **2014**, *68*, 267−82.
(48) Song, M.; Hong, M.; Lee, M. Y.; Jee, J. G.; Lee, Y. M.; Bae, J. S.; Jeong, T. C.; Lee, S. Selective inhibition of the cytochrome P450 isoform by hyperoside and its potent inhibition of CYP2D6. *Food Chem. Toxicol.* **2013**, *59*, 549−53.
(49) Denisov, I. G.; Makris, T. M.; Sligar, S. G.; Schlichting, I. Structure and chemistry of cytochrome P450. *Chem. Rev.* **2005**, *105*, 2253−2278.
(50) Hübsch, Z.; Van Vuuren, S. F.; Van Zyl, R. L. Can rooibos (*Aspalathus linearis*) tea have an effect on conventional antimicrobial therapies? *S. Afr. J. Bot.* **2014**, *93*, 148−156.

DOI: 10.1021/acs.jafc.9b00846
J. Agric. Food Chem. 2019, 67, 4967−4975