Heliyon 9 (2023) e22897

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

New lignan glycosides from *Justicia secunda* Vahl (Acanthaceae) with antimicrobial and antiparasitic properties

Viviane Flore Kamlo Kamso [a], Yanick Kevin Dongmo Melogmo [b], Billy Toussie Tchegnitegni [c], Mariscal Brice Tchatat Tali [b], Darline Dize [b], Cyrille Njampa Ngansop [b], Pantaléon Ambassa [a], Judith Laure Ouete Nantchouang [a], Ingrid Simo Konga [c], Fabrice Fekam Boyom [b], Bonaventure Tchaleu Ngadjui [a,d], Ghislain Wabo Fotso [a,*]

[a] *Department of Organic Chemistry, Faculty of Science, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon*
[b] *Department of Biochemistry, Faculty of Science, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon*
[c] *Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon*
[d] *University Institute of Sciences, Technologies and Ethics, P.O. Box 30201, Yaoundé, Cameroon*

A R T I C L E I N F O

Keywords:
Lignan glycosides
Justicia secunda vahl
Justisecundosides a-c
Bacterial
Fungal
P. falciparum
L. donovani
T. brucei

A B S T R A C T

Three new lignan glucosides, namely, justisecundosides A (**1**), B (**2a**), and C (**2b**), were isolated from the whole plant of *Justicia secunda* together with seven known compounds (**3**—**9**). Their structures were established based on a comprehensive analysis of HR-ESI-MS, IR, UV, and CD, in conjunction with their 1D and 2D-NMR data. A putative biogenetic pathway of compounds **1**—**2a, b** from coniferyl alcohol was proposed. In addition, the antimicrobialactivities of the extract, fractions, and some isolated compounds were assessed against multiresistant bacterial and fungal strains. Furthermore, the antiplasmodial, antileishmanial, and antitrypanosomal activities were assessed against the sensitive (3D7) and multidrug-resistant (Dd2) strains of *P. falciparum*, promastigote and bloodstream forms of *L. donovani*, and *Trypanosoma brucei*, respectively. Compound **4** exhibited moderate antibacterial activity against *Staphylococcus aureus* SA RN 46003 with a MIC value of 62.5 μg/mL. Besides, compound **6** demonstrated a very good activity against sensitive (IC_{50} *Pf*3D7: 0.81 μg/mL) and multidrug-resistant (IC_{50} *Pf*Dd2: 14.61 μg/mL) strains of *P. falciparum* while compound **4** displayed good antitrypanosomal activity (IC_{50}: 1.19 μg/mL). Also, compound **1** was the most active on the promastigote form of *L. donovani* with an IC_{50} of 13.02 μg/mL.

1. Introduction

The development and spread of resistance to currently available antibiotics and antiparasitics is a global concern [1]. Many investigations have reported multidrug-resistant pathogens, including *Escherichia, Pseudomonas, Shigella, Staphylococcus, Candida,*

* Corresponding author.
E-mail addresses: kamsokamlo@yahoo.com (V.F. Kamlo Kamso), melogmoyanne@gmail.com (Y.K. Dongmo Melogmo), darline.dize@yahoo.fr (D. Dize), ambassa1993panataleon@gmail.com (P. Ambassa), ouetejudit@yahoo.fr (J.L. Ouete Nantchouang), simoingrid@yahoo.fr (I.S. Konga), fabrice.boyom@fulbrightmail.org (F.F. Boyom), ngadjuibt@yahoo.fr (B.T. Ngadjui), ghis152001@gmail.com (G.W. Fotso).

https://doi.org/10.1016/j.heliyon.2023.e22897
Received 6 September 2023; Received in revised form 21 November 2023; Accepted 22 November 2023
Available online 27 November 2023

Cryptococcus, Aspergillus, Fusarium, Plasmodium, and Leishmania sp [2–4]. The progress of bacterial resistance to available antibiotics is alarming and makes the treatment of even simple bacterial infections difficult [5]. This concern requires a continuous search for new and efficient lead antibacterial and antiparasitic agents. In this context, plants can play a key role in the discovery of new lead drugs, since in most African countries, people have been relying mainly on medicinal plants to treat themselves from bacterial, parasite diseases, and other infections. Plants have been reported as an important source of bioactive molecules [6]. It is the case of *Justicia secunda* Vahl belonging to the Acanthaceae family, which includes more than 600 species distributed mainly in southern China, Taiwan, Ethiopia, Eritrea, Kenya, Tanzania, Uganda, South Sudan, and Cameroon [7]. It is a perennial herb that grows up to 90 cm with a purplish green stem, evergreen leaves, and pink flowers. It is an upright shrub that grows well in humid soils and colonies around riverbanks and creeks [8]. This species originates from South America but also grows in tropical or subtropical African countries [9]. This plant is used in traditional medicine to treat many illnesses such as anemia, hypertension, and sickle cell disease [10,11]. Also called "Sanguinaria" in Venezuelan folk medicine [12], the leaves of this plant are commonly used in the South West region of Cameroon as a decoction for the treatment of anemia in humans [13,14]. Previous chemical studies on *J. secunda* led to the isolation of pyrrolidone alkaloids such as secundarellones A−C [15] and glycosylated flavonoids [9]. Furthermore, phytochemical screenings of the leaves of this plant revealed the presence of alkaloids, flavonoids, quinones, tannins, and anthocyanins [16]. It is worth mentioning that lignans including arylnaphthalides such as depauperatin A−E, depauperatosides A and B, procumbenosides I, K, L and M, represent the main class of secondary metabolites present in the *Justicia* genus [17–19]. Many of them have shown a wide range of biological activities, including cytotoxic, antibacterial, insecticidal, antiplatelet, antiangiogenic, anti-inflammatory, and antiviral activities [17]. Based on the traditional uses of the plant, and as part of our continuing effort in the search for bioactive natural products from Cameroonian medicinal plants [20–22], we herein report the isolation and characterization of three new lignans from the whole plant of *J. secunda* together with their antimicrobial and antiparasitic activities. This work reports the first chemical investigation of the Cameroonian specimen to the best of our knowledge.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were recorded on a JASCO DIP-3600 digital polarimeter (JASCO, Tokyo, Japan) using a 10 mm cell. UV–vis spectra were recorded on an Evolution 201 UV–visible spectrophotometer (Thermo Scientific, Waltham, MA, USA). A JASCO GA 10 spectrophotometer (JASCO Corporation, Tokyo, Japan) was used for circular dichroism (CD) measurements. IR spectra were recorded on a Tensor 27 FTIR spectrometer (Bruker, Billerica, MA, USA) equipped with a diamond ATR. NMR spectra were obtained on a Bruker Avance III 500 HD or Avance 600 NMR spectrometer. (Bruker, Bremen, Germany), TMS was used as an internal standard, and chemical shifts are expressed in δ values. HR-ESI-MS were recorded on a Micromass-Q-TOF Ultima-3-mass spectrometer (Waters, Milford, MA, USA) with a lock spray interface and a suitable external calibrant. Column chromatography was carried out on silica gel 230–400 mesh or silica gel 70–230 mesh (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} aluminum foil using a UV lamp (254–365 nm) and 10 % H_2SO_4 reagent followed by heating.

2.2. Plant material

The whole plant of *Justicia secunda* was collected at Bafoussam, West Region of Cameroon, in January 2017. The plant material was identified by Mr. NANA Victor, botanist at the Cameroon National Herbarium in comparison with Voucher specimen n° 67466/HNC.

2.3. Extraction and isolation

The air-dried and powdered whole plant of *J. secunda* (2.7 kg) was extracted with CH_2Cl_2/MeOH (20 L, 3:7, v/v) at room temperature to yield a crude CH_2Cl_2/MeOH extract (152.3 g). Part of the extract (150 g) was suspended in water (300 mL) and partitioned successively between *n*-hexane (4 × 500 mL), EtOAc (4 × 500 mL), and *n*-BuOH (4 × 500 mL) to realize 20.8 g, 39.5 g, and 78.3 g of each fraction, respectively. The EtOAc fraction (38.1 g) was chromatographed on silica gel using an *n*-hexane/EtOAc gradient system (0−100 %) to yield three main subfractions (Fr.1−3) after TLC interpretation. Purification of subfraction Fr.2 (6.5 g) using an *n*-hexane/EtOAc system (19:1–1:1) on normal-phase CC yielded three subfractions (Fr.2-1−Fr.2-3). Compound **7** (35 mg) was obtained after filtration of Fr.2-1 (703 mg).

The *n*-BuOH fraction (77.2 g) was subjected to silica gel CC using an acetone/MeOH gradient mixture (from 1:19 to 1:1) to afford four major subfractions (Fr.4−7). Fr. 4 (6.7 g) was subjected to CC over silica gel and eluted with a further solvent mixture EtOAc/MeOH/H_2O (95:5:2) to give three subfractions, Fr. 4-1−Fr. 4-3. Subfraction Fr. 4-2 (1.1 g) was subjected to reversed-phase C_{18} CC eluted with MeOH/H_2O (9:1) to afford compound **1** (15 mg) and a mixture of **2a,b** (12 mg). From Fr. 4-1 (1.7 g), compound **8** (300 mg) was obtained after filtration and washing of the solid residue with MeOH. Repeated CC over silica gel with an EtOAc/MeOH/H_2O system from 9:1:0.5 to 8:1.5:0.5 and Sephadex LH-20 (eluted with MeOH) of Fr.6 (3.2 g) led to isolation of compounds **3** (11.7 mg) and **4** (25.3 mg). Isocratic CC over normal-phase silica gel of Fr.5 (25.2 g) using EtOAc/MeOH/H_2O (8:2:1) solvent system afforded three subfractions (Fr.5-1−Fr.5-3). Purification of Fr.5-1 (2.3 g) over reversed-phase C_{18} eluted with MeOH/H_2O (3:2) led to compounds **5** (15 mg) and **6** (13.2 mg). Sephadex LH-20 CC (eluted with MeOH) of Fr.5-2 (6.2 g) afforded **9** (12 mg).

2.3.1. Justisecundoside A (1)

White finely divided solid; $[\alpha]_D^{20}$ −56.4 (*c* 0.5, CH_3CN); UV (CH_3CN) λ_{max} (log ε) 280 (5.20) and 340 (5.50) nm; IR ν_{max} 3376, 1744, 1646, 934 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS: *m/z* $[M+Na]^+$ 553.1327 (calcd for $C_{26}H_{26}O_{12}Na^+$: 553.1365).

2.3.2. Justisecundoside B/C (2a,b)

Brown finely divided solid; $[\alpha]_D^{20}$ −74.8 (*c* 0.5, CH_3CN); UV (CH_3CN) λ_{max} (log ε) 350 (5.12); CD (MeOH) (nm, ($\Delta\varepsilon$)) 200 (−6.644); IR ν_{max} 3383, 1738, 1639, 928 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS: *m/z* $[M+Na]^+$ 553.1329 (calcd for $C_{26}H_{26}O_{12}Na^+$: 553.1365).

2.4. Acid hydrolysis of 1 and 2a,b

Each compound (2 mg) was refluxed individually in 2 M HCl (5 mL) at 100 °C for 4 h. The reaction mixture was poured into water and extracted with EtOAc (5 mL, three times). The aqueous layer was concentrated and used for sugar identification by TLC and comparison of the optical rotation with that of D-glucose (+18.6°). (Sigma–Aldrich, Munich, Germany).

2.5. Determination of the biological activities

2.5.1. Antimicrobial assay

Some isolated compounds as well as crude extract and fractions were screened *in vitro* for their antibacterial and antifungal potencies against some selected bacterial and fungal strains using Ciprofloxacin and flucoxazole as reference drugs respectively. Both bioassays were carried out following the same protocols described in our recently reported works [22].

2.5.2. Antibacterial activity

The screenings were performed in duplicate three times in sterile 96-well microplates. Indeed, 2 μL of a sterile solution of extract/fractions (100 mg/mL, DMSO 100 %) and 5 μL of a solution of compounds (20 mg/mL, DMSO 100 %) were introduced into 98 μL and 95 μL of Muller Hinton Broth (MHB) culture medium respectively. Twofold serial dilutions were achieved to yield final concentrations ranging from 1000 μg/mL to 62.5 μg/mL, 500 μg/mL to 31.25 μg/mL, and from 0.25 μg/mL to 0.0153 μg/mL for extract/fractions, compounds, and ciprofloxacin used as positive control. Thereafter, bacterial suspension calibrated at 10^6 cells/mL was distributed in the test wells including those of the negative control. The final charge of the inoculum in each well was 5×10^5 cells/mL with 100 μL as

Table 1
^1H and ^{13}C -NMR data for **1**−**2a,b** (600, 150 MHz, TMS, δ ppm, DMSO-d_6).

No	1		2a		2b	
	δc	δ_H	δc	δ_H	δc	δ_H
1	128.6	–	115.5	–	117.0	–
2	110.6	7.86, d (1.7)	153.0	–	152.5	–
3	147.5	–	98.9	6.95 (s)	108.6	6.79 (s)
4	148.9	–	150.1	–	149.7	–
5	108.5	6.97, d (8.2)	142.7	–	142.0	–
6	127.7	7.34, dd (1.7, 8.2)	106.9	7.26 (s)	109.8	7.71 (s)
7	140.9	7.18, d (2.1)	131.7	7.82, d (2.2)	134.6	7.48 d (2.2)
8	124.5	–	124.5	–	126.4	–
9	169.1	–	172.3	–	169.7	–
10	101.9	6.09 (s)	102.4	6.08, d (1.0)	102.1	6.04, dd (1.0)
1′	130.2	–	132.1	–	132.6	–
2′	107.1	6.88, d (1.7)	109.6	6.80, d (1.7)	109.9	6.91, d (1.7)
3′	147.8	–	147.7	–	147.7	–
4′	147.6	–	146.3	–	146.1	–
5′	108.7	6.92, d (8.1)	108.6	6.78, d (8.0)	108.5	6.83, d (8:0)
6′	120.2	6.79, dd (1.7, 8.1)	122.5	6.64, dd (1.7, 8.0)	122.6	6.75, dd (1.7, 8:0)
7′	79.5	5.73, d (7.6)	36.9	2.50*	40.2	3.00, dd (4.9, 13.7)
				2.79, dd (4.4, 14.0)		2.67, dd (9.9, 13.7)
8′	46.2	3.76*	38.4	4.08, dtt (2.2, 4.5, 7.1)	43.1	3.45, dt (6.0, 11.8)
9′	67.9	3.65, dd (8.5, 10.5),	69.8	4.12, dd (2.4, 8.9)	69.6	4.02, dd (4.5, 8.2)
		3.12*		4.25, dd (7.6, 8.9)		4.23 dd (7.3, 8.2)
10′	101.6	6.04, d (1.2)	101.2	5.96 (s)	101.1	5.97 (s)
1"	102.9	4.02, d (7.7)	101.5	4.89, d (7.3)	103.2	4.67, d (7.3)
2"	73.9	2.95, dd (7.7, 9.0)	73.8	3.31 (m)	73.7	3.26 (m)
3"	76.9	3.11*	77.3	3.29-3.27 (m)	76.8	3.29-3.27 (m)
4"	70.4	3.02*	70.2	3.13-3.18 (m)	70.3	3.13-3.18 (m)
5"	77.9	3.03*	77.6	3.37-3.35 (m)	77.5	3.37-3.35 (m)
6"	61.5	3.62, dd (1.8, 11.8) 3.39, dd (5.5, 11.8)	61.2	3.45, dt (6.0, 11.8)	61.3	3.73 (m)
				3.71, dd (5.3, 11.8)		

*Overlapped with other signals.

the final volume. The sterility control was made up of a culture medium while the positive control consisted of the culture medium, inoculum, and ciprofloxacin. The negative control was constituted of the culture medium and bacteria suspension. The microplates were covered and incubated at 37 °C for 24 h. At the end of the incubation period, 10 μL of a freshly prepared resazurin solution (0.15 mg/mL) was added to each well, and the plates were once again incubated under the same conditions for 30 min. The smallest concentration at which there was no change in coloration from blue to pink corresponding to a lack of visible bacterial growth was considered as the MIC.

2.5.3. Antifungal activity

The extract, fractions, and compounds were also screened for their antifungal activity. The assay was conducted in duplicate two times in sterile 96-well plates. Note, 5 μL solution of each compound (20 mg/mL) and 4 μL solution of extract/fractions (100 mg/mL) were introduced into 96 μL and 95 μL of Sabouraud Dextrose Broth (SDB) respectively. Afterwards, twofold serial dilutions were performed to obtain the final concentrations respectively ranging from 2000 μg/mL to 62.5 μg/mL, 500 μg/mL to 3.890 μg/mL, and from 1.25 μg/mL to 0.0383 μg/mL, respectively for extract/fractions, compounds, and fluconazole used as a positive control. The final fungal concentration in each well was 10^4 cells/mL with the final volume of 100 μL. The sterility control was made up of the culture medium and the positive control consisted of the culture medium, inoculum, and fluconazole. The negative control was constituted of culture medium and fungal suspension. The microplates were covered and then incubated at 37 °C for 48 h. At the end of the incubation period, 10 μL of a freshly prepared resazurin solution (0.15 mg/mL) was added to all wells and the plates were once again incubated under the same conditions for 30 min. The smallest concentration at which there was no change in coloration from blue to pink corresponding to a lack of visible fungal growth was considered as the MIC.

2.5.4. SyBr green fluorescence-based antiplasmodial assay

The susceptibility of extract, fractions and some isolates from *J. secunda* were assayed against asexual-blood stages of sensitive (3D7) and multidrug resistant (Dd2) strains of *Plasmodium falciparum* as previously described by Smilkstein et al. (2004) using artemisinin as the positive control [23]. The IC_{50} obtained were used to calculate the resistance index using the following formula:

Resistance index = CC_{50} PfD2/CC_{50} Pf3D7.

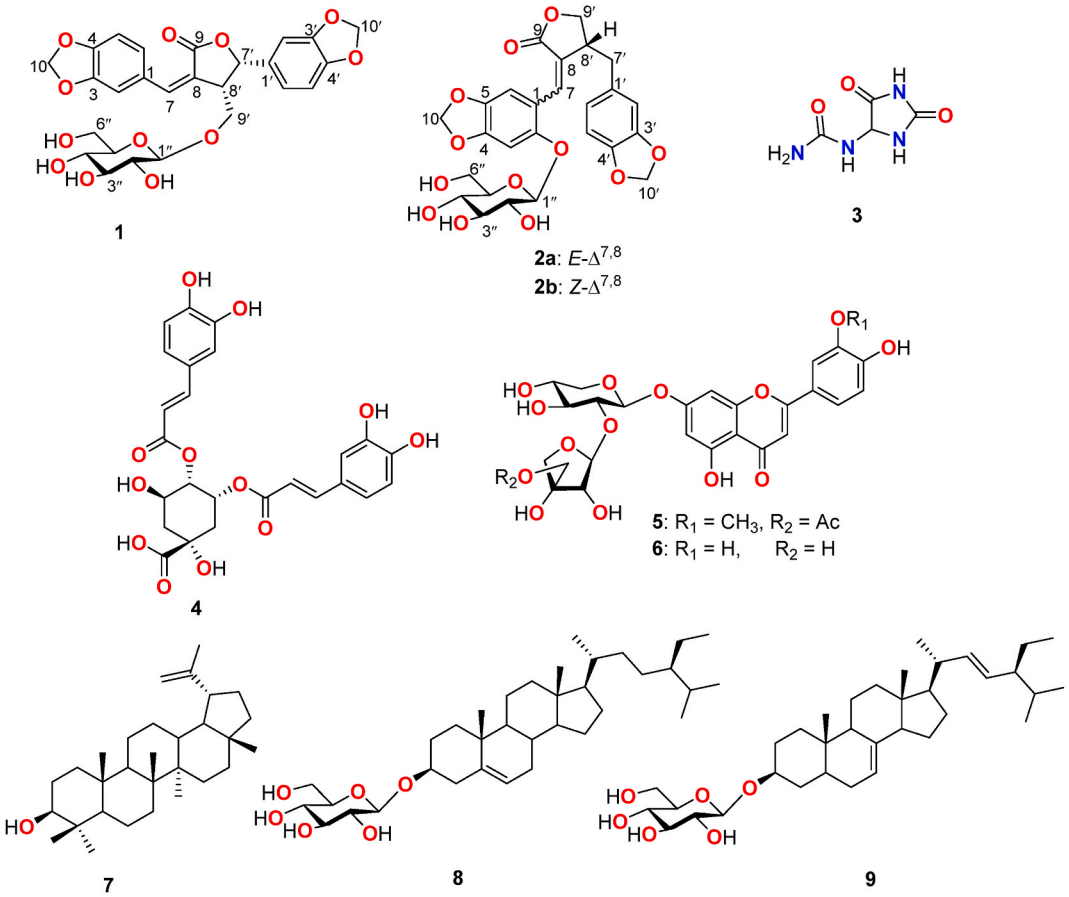

Fig. 1. Structures of compounds **1**–**9** from *Justicia secunda*.

2.5.5. Resazurin-based antileishmanial and antitrypanosomal assays

The growth of promastigote form of *L. donovani* and bloodstream form of *Trypanosoma brucei brucei* was determined *in vitro* using the resazurin-based viability assay as described by Dize et al. (2022) and Njanpa et al. (2021) respectively. Amphotericin B and Pentamidine isethionate were used as positive controls for antileishmanial and antitrypanosomal tests, respectively [24,25].

3. Results and discussion

The CH_2Cl_2/MeOH (3:7) extract of the whole plant of *J. secunda* was subjected to silica gel and Sephadex LH-20 column chromatography to afford three new lignans; justisecundosides A (**1**), B (**2a**), and C (**2b**). Additionally, allantoin (**3**) [9], 4,5-di-*O*-caffeoylquinic acid (**4**) [26], granduloside B (**5**) [27], luteolin-7-*O*-*β*-D-apiofuranosyl-(1 → 2)-*O*-*β*-D-xylopyranoside (**6**) [27], lupeol (**7**) [28], daucosterol (**8**) [28], and spinasterol-3-*O*-*β*-D-glucopyranoside (**9**) [29] were also obtained (Fig. 1).

Compound **1** was isolated as a white finely divided solid, $[\alpha]_D^{20}$ −56.4 (*c* 0.5, CH_3CN). Its molecular formula, $C_{26}H_{26}O_{12}$, implying 14° of unsaturation, was established from its NMR data and its HR-ESI-MS in positive mode (Fig. S1, Supplementary data), which showed the sodium adduct peak $[M+Na]^+$ at *m/z* 553.1327 (calcd for $C_{26}H_{26}O_{12}Na^+$, 553.1365). The IR spectrum of **1** (Fig. S2, Supplementary data) displayed a broad absorption band at 3376, and other weak bands at 1744, 1646, and 934 $cm^{-1,}$ indicating the presence of hydroxyl, *α,β*-unsaturated *γ*-lactone carbonyl, double bonds, and methylenedioxy groups, respectively [19,30]. Its UV spectrum (Fig. S3, Supplementary data) showed absorption bands at λ_{max} 280 and 340 nm, indicating the presence of benzene moieties [30]. The ^{1}H NMR spectrum of **1** (Fig. S4, Supplementary data) revealed the existence of two pairs of ABX aromatic protons: the first at $\delta_{H/C}$ 7.86 (d, $J = 1.7$ Hz, H-2)/110.6, 6.97 (d, $J = 8.2$ Hz, H-5)/108.5, and 7.34 (dd, $J = 1.7$, 8.2 Hz, H-6)/127.7, and the second at $\delta_{H/C}$ 6.88 (d, $J = 1.7$ Hz, H-2')/107.1, 6.92 (d, $J = 8.1$ Hz, H-5')/108.8, and 6.79 (dd, $J = 1.7$, 8.1 Hz, H-6')/120.2 (Table 1). These signals are characteristic for two 1,3,4-trisubstituted phenyl patterns [31]. The cross-peak signals integrating for two protons each at δ_H 6.09 (−OCH_2O−10) and 6.04 (−OCH_2O−10') (Fig. S4, Supporting information) suggested the presence of two methylenedioxy units [32,33]. The spectrum also showed signals of three methines, among which two aliphatic at δ_H 3.76 (m, H-8') and 5.73 (d, $J = 7.6$ Hz, H-7'), and one olefinic at δ_H 7.18 (d, $J = 2.1$ Hz, H-7). Two diastereotopic protons of oxymethylene were observed at δ_H 3.12 and 3.65 dd (8.5, 10.5) (H-9'). All these data were closely related to those of *trans*-(3) *E*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-4-(hydroxymethyl)dihydrofuran-2(3H)-one [20] and phellinsin A [31]. However, the ^{1}H and ^{13}C NMR spectra of **1** showed the presence of a sugar moiety with an anomeric proton at δ_H 4.02 (d, $J = 7.7$ Hz, H-1") and its corresponding carbon at δ_C 102.9 (C-1"). The above NMR data indicated that **1** is a dihydrofuran-type lignan glycoside. Based on the ^{13}C chemical shifts of the sugar moiety (Fig. S5, Supplementary data, Table 1) and the high coupling constant of its anomeric proton, it was unambiguously identified as a *β*-glucopyranose [34,35]. The acid hydrolysis of **1** confirmed the identification of the sugar as D-glucose after comparison of the optical rotation (+18.1°) with an authentic sample (+18.6°). The ^{1}H–^{1}H COSY spectrum of **1** (Fig. S7, Supplementary data) showed two main spin systems between protons H-2/H-6/H-5 on one hand and H-2′/H-6′/H-5′ on the other hand, confirmed the presence of two ABX systems in the molecule. The correlation between H-7′/H-8′/H-9′ permitted us to justify the aliphatic chain [31]. The HMBC correlations between H-7′ (δ_H 5.73), C-1′ (δ_C 130.2), C-2′ (δ_C 107.1), and C-6′ (δ_C 120.2) (Fig. 2) supported the linkage between C-1′ to C-7′. In addition, the correlations depicted from the olefinic proton at δ_H 7.18 (H-7) and C-2 (δ_C 110.6) and C-6 (δ_C 127.7) established the linkage between C-7 to C-1 (Fig. 2). Moreover, the correlation observed between H-7 (δ_H 7.18) and the olefinic carbon at δ_C 124.5 (C-8) plus the carbonyl of ester at δ_C 169.1 (C-9) strongly confirmed the presence of an *α,β*-unsaturated lactone moiety connected to C-1. Further HMBC analysis demonstrated important correlations between H-7′ (δ_H 5.73), C-8 (δ_C 124.5) and C-9 (δ_C 169.1); H-8′ (δ_H 3.76), C-9 (δ_C 169.1) and C-7′ (δ_C 79.5) (Fig. 2), suggesting a five-membered 7′,9-lactone ring in compound **1**. The locations of the two methylenedioxy groups were deduced by the HMBC correlations observed between the protons at δ_H 6.09 (−OCH_2O− 10) and 6.04 (−OCH_2O− 10′) and carbon atoms at δ_C 147.5/148.9 ppm (C-3/C-4) and δ_C 147.8/147.6 (C-3′/C-4′), respectively. The glucopyranosyl unit was linked to the aglycone based on the HMBC correlation observed between H-1" (δ_H 4.02) and C-9′ (δ_C 67.9). The lack of NOESY correlation between H-8′ and H-6 (Fig. S10, Supplementary data) suggested the *Z*-orientation $\Delta^{7,8}$ of the double bond, which was also confirmed by the low value of the chemical shift of H-7 (δ_H 7.18) in comparison to that of *E*-orientation $\Delta^{7,8}$ H-7 (δ_H 7.47–7.52) due to their proximity to the carbonyl group [30,36]. Additionally, the NOESY correlation between H-7 and H-8′ on one hand and between H-7 and H-9′ on the other hand strongly confirmed the *Z*-orientation $\Delta^{7,8}$ double bond. Furthermore, the relative configuration of H-7′ and H-8′ was deduced to be *cis* based on the NOESY correlation between H-7′ and H-8′ and the coupling constant of H-7′/H-8′ ($J_{H\text{-}7',H\text{-}8'}$

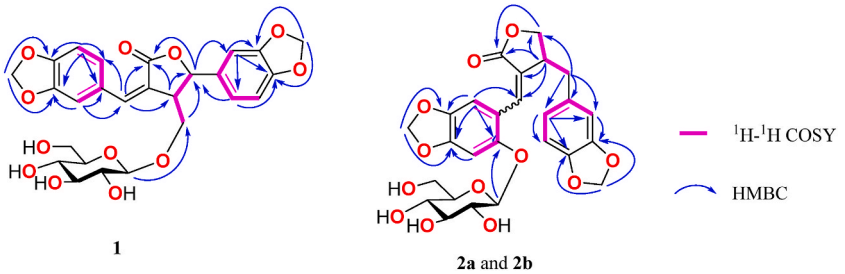

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations for **1**−**2a,b**.

= 7.6 Hz) compared to *trans* ($J_{H\text{-}7',H\text{-}8'}$ = 2.6 Hz) [30,36]. Consequently, compound **1** was characterized as (7′*S*, 8′*S*) *cis*-(3) *Z*-3-(3, 4-methylenedioxybenzylidene)-5-(3′,4′-methylenedioxyphenyl)-4-(1-*O*-*β*-D-glucopyranosyloxymethyl) dihydrofuran-2(3H)-one, a new lignan glucoside, to which the trivial name justisecundoside A is given.

Compounds **2a** and **2b** were obtained as an optically active mixture, $[\alpha]_D^{20}$ −74.8 (*c* 0.5, CH_3CN) of a brown finely divided solid with the same R_f on RP-18 thin layer chromatography in different solvent systems. They were present as a 3:1 mixture based on their NMR peak intensities. The HR-ESI-MS (Fig. S11, Supplementary data) displaying the sodium adduct peak $[M+Na]^+$ at *m/z* 553.1329 (calcd for $C_{26}H_{26}O_{12}Na^+$: 553.1365) led to the assignment of the molecular formula $C_{26}H_{26}O_{12}$ with 14 double-bond equivalents, suggesting that **2a** and **2b** are isomers. The IR spectrum (Fig. S12, Supplementary data) showed broad absorption bands at 3383, a stronger ones at 1738, 1639, and 928 $cm^{-1,}$ suggesting the presence of hydroxyl groups, *α,β*-unsaturated *γ*-lactone carbonyl groups, double bonds and methylenedioxy groups, respectively [30,37,38]. The ^1H NMR spectrum (Fig. S15, Supplementary data) of mixture **2a,b** (Table 1) exhibited two pairs of methylenedioxy units at δ_H 6.08/6.04 (−OCH_2O−10) and 5.96/5.97 (−OCH_2O−10′), attached to phenyl functionalities. It also exhibited signals of five pairs of aromatic protons, including one ABX-type 1,3,4-trisubstituted phenyl pattern at δ_H 6.80/6.91 (d, *J* = 1.7 Hz, H-2′), 6.78/6.83 (d, *J* = 8.0 Hz, H-5′), and 6.64/6.75 (dd, *J* = 1.7, 8.0 Hz, H-6′), and two para-hydrogenated atoms at δ_H 6.95/6.79 (s, H-3) and 7.26/7.71 (s, H-6), clearly indicating that the other aromatic ring possesses a 1, 2,4,5-substitution pattern. The doublet signal at δ_H 7.82/7.48 (d, *J* = 2.2, H-7) was attributable to the olefinic proton of a benzylidene unit [37,38]. The downfield chemical shifts of the geminal aliphatic protons at δ_H 4.12/4.25 (**2a**) and 4.02/4.23 (**2b**) (H-9′$_{a,b}$) allowed their assignment as the *γ*-hydrogens of a *γ*-butyrolactone ring [37,38]. These geminal protons are related to benzylic hydrogen pairs at δ_H 2.50/3.00 (**2a**) and 2.78/2.67 (**2b**) (H-7′$_{a,b}$) via a methine proton at δ_H 4.08/3.45 (H-8′). All this information was confirmed by the ^1H–^1H COSY correlations between H-9′$_{a,b}$/H-8′/H-7′$_{a,b}$. At this juncture, the data of **2a,b** were closely related to those of kaerophyllin and cappadocin [37,38]. Extensive analysis of the ^1H and ^{13}C spectra (Fig. S16, Supplementary data) of **2a, b** showed the presence of a sugar moiety with an anomeric signal at δ_H 4.89/4.67 (d, *J* = 7.3 Hz, H-1″) and the corresponding carbon at δ_C 101.5/103.2 (C-1″). The sugar moiety was ascertained to be a *β*-glucopyranose by comparison of ^{13}C chemical shifts (see Table 1) with those in the literature [34,35]. The absolute configuration of the glucose unit was determined to be D-glucose by comparison of its optical rotation with that of an authentic sample after acid hydrolysis of **2a,b**. The HMBC correlations between benzylic protons H-7′$_{a,b}$ (δ_H 2.50/2.79 for **2a**; δ_H 3.00/2.67 for **2b**) and C-8 (δ_C 124.5/126.4), C-9′ (δ_C 69.8/69.6), and C-8′ (δ_C 38.4/43.1) allowed the fixation or linkage of C-7′ to C-8′. Further correlations between H-7 (δ_H 7.82/7.48) and C-9 (δ_C 172.3/169.7), C-8′ (δ_C 38.4/43.1) and C-8 (δ_C 124.5/126.4) allowed the linkage of the benzylidene unit to C-8. The locations of the two methylenedioxy groups were confirmed thanks to the correlations between their protons at δ_H 6.08/6.04 (H-10) and 5.96/5.97 (H-10′) and carbons at [δ_C 150.1/142.7 (C-4/C-5) and 147.7/146.3 (C-3′/C-4′) for **2a**, and δ_C 149.7/142.0 (C-4/C-5) and 147.7/146.1 (C-3′/C-4′) for **2b**], respectively. Additionally, the HMBC correlations between H-8′ (δ_H 4.08/3.45) and C-8 (δ_C 124.5/126.4), C-9 (δ_C 172.3/169.7), and C-9′ (δ_C 69.8/69.6); H-9′ (4.12/4.25 (**2a**), 4.02/4.23 (**2b**) (H-9′$_{a,b}$)) and C-8 (δ_C 124.5/126.4),C-9 (δ_C 172.3/169.7) strongly confirmed the presence of a *γ*-butyrolactone ring [27, 28]. Furthermore, the cross-peak correlation between the anomeric proton H-1" (δ_H 4.89/4.67) and the aromatic carbon C-2 (δ_C 153.0/152.5), which is also correlated with H-3 (δ_H 6.95/6.79) and H-6 (δ_H 7.26/7.71), justified the linkage of the sugar at this position. The stereochemistry of the olefinic double bond C_7–C_8 was established as *E* in **2a** and *Z* in **2b** based on the comparison of their spectroscopic data with those of analogous compounds. Indeed, it is firmly established that the olefinic proton in the *E*-isomers of analogous compounds is deshielded by the proximal carbonyl group compared to the *Z* one [38–40]. Additionally, the NOESY correlations (Fig. 3) between H-7 (δ_H 7.48), H-8′ (δ_H 3.45), and H-7′$_{a,b}$ (3.00/2.67) in **2b** strongly confirms the *Z*-stereochemistry of the C_7–C_8 olefinic double bond, while the lack of NOESY correlation between the same protons in **2** confirms the *E*-stereochemistry of the C_7–C_8 olefinic double bond. All this information suggested that **2a,b** is a mixture of *E* and *Z* diastereoisomers with *E*-isomer (**2a**) as the major compound and *Z*-isomer (**2b**) as the minor compound. The absolute configuration at the only chiral center was unequivocally assigned as 8′*R* by comparison of the CD curve of **2a,b** (Fig. S14, Supplementary data) with those of analogous lignans of established structures [37,38]. Additionally, the negative sign of optical rotation of **2a,b** (−74.8°) in comparison to those of kaerophyllin (−72.6°) and (−)-cappadoside (−91.2°) also confirmed this stereochemistry [38]. Therefore, **2a,b** was characterized as a mixture of (8′*R*) (−)-*α*-(*E*-4,5-methylenedioxy-2-*O*-*β*-D-glucopyranosyloxybenzylidene)-*β*-(3′,4′-methylenedioxybenzyl)-*γ*-butyrolactone (**2a**) and (8′*R*) (−)-*α*-(*Z*-4,5-methylenedioxy-2-*O*-*β*-D-glucopyranosyloxybenzylidene)-*β*-(3′,4′-methylenedioxybenzyl)-*γ*-butyrolactone (**2b**), two previously undescribed lignan glucosides to which the trivial names justisecundoside B and justisecundoside C were given.

The antibacterial and antifungal activities of the crude extract, EtOAc and *n*-BuOH fractions, and some isolates (**1**−**6** and **9**) were

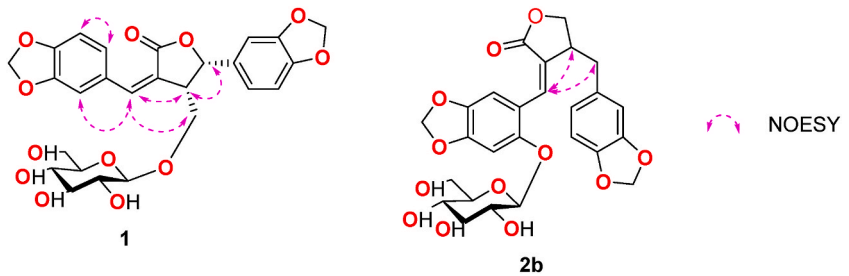

Fig. 3. Key NOESY correlations in **1** and **2b**.

evaluated against selected strains (Table 2). Compound **5** displayed moderate activity against *S. aureus* (MIC = 62.5 μg/mL), while compounds **5** and **6** displayed weak activity against *E. coli* (MIC = 250 μg/mL). Regarding the fungal strains (Table 3), only compounds **2a,b** (MIC = 125 μg/mL) and **5** (MIC = 250 μg/mL) exhibited activities against *C. tropicalis* [41]. The crude extract,EtOAc and *n*-BuOH fractions displayed weak activity against almost all the tested strains.

Besides, extract, fractions and some isolates were also screened *in vitro* for the ability to inhibit the proliferation of asexual-blood stages of *P. falciparum*; promastigote form of *L. donovani*, and bloodstream form of *T. brucei brucei* in culture. As results, the SyBr Green-based antiplasmodial assay led to the identification of eight (08) inhibitors with IC_{50} ranging from 0.81 to 96.43 μg/mL against both sensitive (*Pf*3D7) and multidrug resistant (*Pf*Dd2) strains of *P. falciparum* (Table 4). It is worth noting that the purification process from extract to pure compounds steadily increases antiplasmodial potency., from IC_{50} of 92.15 μg/mL for the crude extract to fractions (IC_{50} 27.07–75.76 μg/mL) and isolated compounds with IC_{50} ranging from 0.81 to 96.43 μg/mL (Table 4). Out of the screened chemical derivatives, compound **6** was the most active with moderate activity (IC_{50}: 14.61 μg/mL) on multidrug-resistant (Dd2) and very good activity (IC_{50}: 0.81 μg/mL) on sensitive (3D7) strains of *P. falciparum*. In fact, compound **6** belongs to the flavonoid chemical class of compounds which are reputed to possess strong activity against malaria parasites. It is the case of flavanone and chalcone derivatives such as the 6-methyl-3′,4′-dimethoxyflavanone and (2,4-dihydroxy phenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone, which displayed good activities against different strains of *P. falciparum* [42,43]. Tali et al. (2023) recently attributed the high antiplasmodial activity to the presence of peroxide bridge (like in the sesquiterpene lactone artemisinin) and phenyl group in the chemical structure [44]. Furthermore, these authors asserted that the presence of methyl groups in chemical structures decreases antimalarial activity. [44], which corroborates the shift in activity obtained with compounds **5** and **6**.

On the other hand, extract, fractions and some chemical derivatives were also screened for their ability to arrest the growth of *Leishmania* and *Trypanosoma* parasites in culture. As summarized in Table 5, results showed that, except for compound **6**, **3** and **5**, all the investigated samples demonstrated activity against the promastigote form of *Leishmania donovani* with IC_{50} values ranging from 13.02 to 78.19 μg/mL while all tested samples displayed promising activity on the bloodstream form of *Trypanosoma brucei brucei* with IC_{50} values ranging from 1.19 to 53.37 μg/mL. More interestingly, the antileishmanial and antitrypanosomal potency also displayed activity-guided profile. The antileishmanial and antitrypanosomal activities of the chemical pharmacophores might be due to the presence of lactone and phenyl moieties which are reputed to generate free radical or inducing oxidative stress leading to the parasite death.

A plausible biogenetic pathway to the formation of compounds **1**−**2a,b** (Fig. 4) from coniferyl alcohol (**10**) was proposed because biosynthetically, these compounds were originally derived from **10**, which has shikimic acid as a precursor [45]. Two coniferyl alcohols are connected via the aid of a dirigent protein (DIR) to give rise to pinoresinol (**11**), which is reduced sequentially to lariciresinol (**12**) and secoisolariciresinol (**13**) by the pinoresinol/lariciresinol reductase enzyme (PLR) [46]. The oxidation of the two methyl groups of **12** with cytochrome P450 (CYP) followed by lactonization by the secoisolariciresinol dehydrogenase (SDH) enzyme allows the formation of two methylenedioxy bridges [32,45], and leads to the intermediate **17**. Dehydrogenation with 7-dehydrogenase (7-DH) followed by enzymatic glucosylation catalyzed by glucosyl transferase (GT) gives justisecundoside A (**1**). The conversion of secoisolariciresinol (**13**) to retro-matairesinol (**14**) is evidenced by the secoisolariciresinol dehydrogenase (SDH) enzyme, which is subsequently oxidized by cytochrome P450 to give rise to hinokinin (**15**), which then undergoes dehydrogenation to hibalactone (**16**). Subsequent hydroxylation with hydroxylase enzyme (2-HL) and glucosylation by glucosyl transferase (GT) leads to justisecundoside B/C (**2a,b**).

4. Conclusion

In this study, three previously undescribed lignan glucosides were reported from the whole plant of *J. secunda,* confirming lignans as the main class of secondary metabolites of *Justicia*. The antimicrobial screening performed on extract, fractions and some isolates

Table 2
Antibacterial activity of extract, fractions and some isolated compounds.

Compounds/Extract /fractions	Bacteria strains					
	EC ATCC 25922	KP NR 41897	PA NR 48982	SF NR 518	SA NR 46003	SA ATCC 43300
1	>500	>500	>500	>500	>500	>500
2a,b	>500	>500	>500	>500	250	>500
3	>500	>500	>500	>500	>500	>500
4	>500	250	>500	>500	250	>500
5	250	>500	>500	>500	62.5	125
6	250	>500	>500	>500	125	125
9	>250	>500	>500	>500	>500	125
ECH_2Cl_2/MeOH	500	1000	500	>1000	1000	1000
FEtOAc	500	1000	500	>1000	1000	1000
Fn-BuOH	500	1000	500	>1000	1000	1000
Ciprofloxacin	0.031	0.015	0.031	0.0625	0.015	0.015

PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; KP: *Klebsiella pneumoniae*; SF: *Shigella flexneri*; EC: *Escherichia coli*; >1000- >250: nonactive; ECH_2Cl_2/MeOH: CH_2Cl_2/MeOH extract; FEtOAc: EtOAc fraction; Fn-BuOH: *n*-BuOH fraction.

Table 3
Antifungal activity of extract, fractions and some isolated compounds.

	Clinical isolates Fungal				
Compounds/Extract /fractions	CK 370S	CT 390S	CG 109B	CA 348S	CP0 31U
1	>500	>500	>500	>500	>500
2a,b	>500	125	>500	>500	>500
3	>500	>500	>500	>500	>500
4	>500	>500	>500	>500	>500
5	>500	>500	>500	>500	>500
6	>500	>500	>500	>500	>500
9	>500	>500	>500	>500	>500
ECH_2Cl_2/MeOH	1000	500	1000	1000	1000
FEtOAc	1000	500	1000	1000	1000
Fn-BuOH	1000	1000	1000	1000	1000
Fluconazole	0.153	0.306	0.306	0.0765	0.153

CA: *Candida albicans*, CK: *Candida krusei,* CG: *Candida glabrata*; CT: *Candida tropicalis*; CP: *Candida parapsilosis*; >1000- >250: nonactive; ECH_2Cl_2/MeOH: CH_2Cl_2/MeOH extract; FEtOAc: EtOAc fraction; Fn-BuOH: *n*-BuOH fraction.

Table 4
Antiplasmodial potency of extract, fractions, and some isolated compounds.

	IC_{50} ± SD (μg/mL)		Resistance index
Inhibitors	*Pf*Dd2	*Pf*3D7	
1	>100	>100	nd
2a,b	96.43 ± 0.91	>100	nd
3	>100	>100	nd
4	15 ± 0.82	26.11 ± 1.32	0.57
5	37.54 ± 1.38	28.62 ± 2.59	1.31
6	14.61 ± 1.49	0.81 ± 0.17	18.03
9	>100	>100	nd
ECH_2Cl_2/MeOH	92.15 ± 5.49	41.96 ± 0.62	2.19
FEtOAc	61.7 ± 0.42	27.07 ± 1.11	2.27
Fn-BuOH	75.76 ± 5.43	>100	nd
Artemisinin (μM)	0.02 ± 0.00	0.03 ± 0.00	0.66

ECH_2Cl_2/MeOH: CH_2Cl_2/MeOH extract; FEtOAC: EtOAc fraction; Fn-BuOH: *n*-BuOH fraction; IC_{50}: Inhibitory Concentration 50; SD: Standard Deviation; *Pf*Dd2: *Plasmodium falciparum* Dd2; *Pf*3D7: *Plasmodium falciparum* 3D7.

Table 5
Inhibitory potency of extract, fractions, and some isolated compounds against *Leishmania donovani* Promastigote and *Trypanosoma brucei brucei* Bloodstream forms.

IC_{50} ± SD (μg/mL)		
Inhibitors	Promastigote form of *L. donovani*	Bloodstream forms of *T. b. brucei*
1	13.02 ± 0.07	43.83 ± 1.64
2a,b	26.43 ± 0.11	53.37 ± 0.83
3	>100	52.06 ± 6.03
4	23.56 ± 0.06	1.19 ± 0.10
5	>100	44.70 ± 6.37
6	>100	31.67 ± 0.70
9	53.55 ± 0.11	53.02 ± 2.33
ECH_2Cl_2/MeOH	78.9 ± 0.03	25.13 ± 0.72
FEtOAc	36.77 ± 0.11	22.77 ± 0.96
Fn-BuOH	53.52 ± 0.098	22.72 ± 0.14
Amphotericin B (μM)	0.18 ± 0.00	–
Pentamidine (μM)	–	0.005 ± 0.00

ECH_2Cl_2/MeOH: CH_2Cl_2/MeOH extract; FEtOAC: EtOAc fraction; Fn-BuOH: *n*-BuOH fraction; IC_{50}: Inhibitory Concentration 50; SD: Standard Deviation.

revealed that compound **5** was the most active. In addition, compound **6** displayed the best activities against *P. falciparum* strains while, compounds **4** displayed good antitrypanosomal activity and compound **1** was the most active on the promastigote form of *L. donovani*. The isolation of flavonoids and lignans as chemical markers confirms the botanical identification of the plant. Furthermore, the results of preliminary screenings could justify the use of this plant in traditional medicine for the treatment of bacterial and parasitic infections.

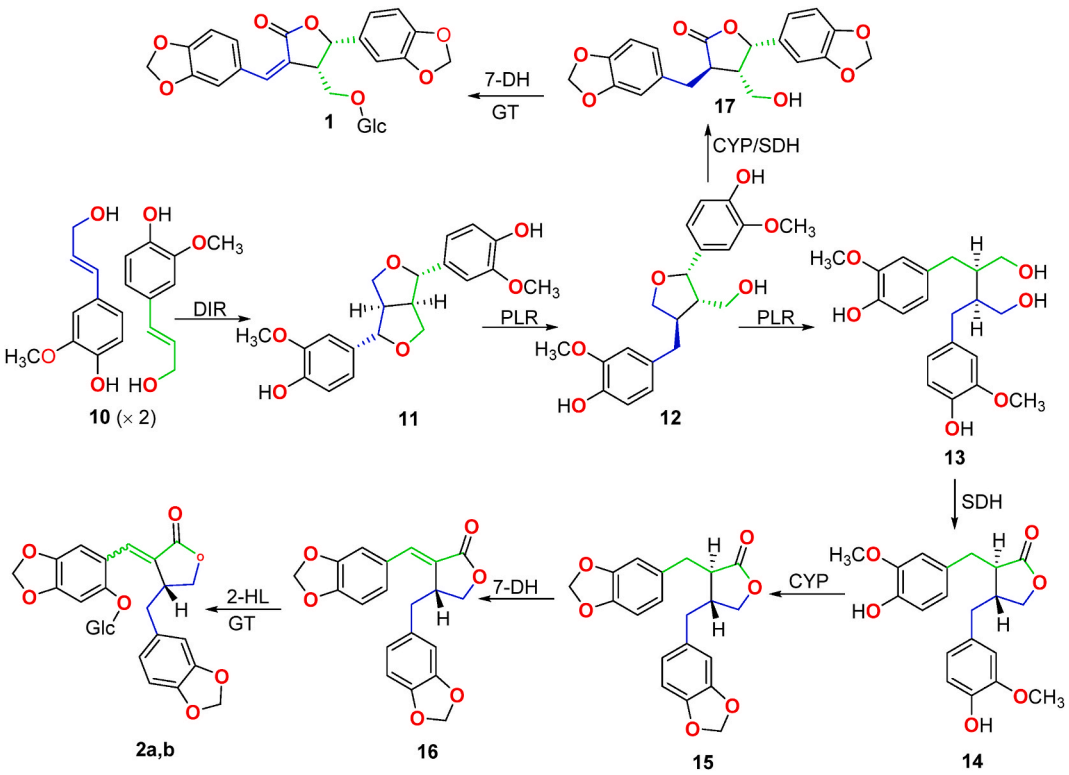

Fig. 4. Putative biogenetic pathway for the formation of compounds **1**−**2a,b** from coniferyl alcohol.

Data availability statement

Data included in article/supp. material/referenced in the article.

CRediT authorship contribution statement

Viviane Flore Kamlo Kamso: Writing – original draft, Investigation, Formal analysis. **Yanick Kevin Dongmo Melogmo:** Writing – original draft, Investigation. **Billy Toussie Tchegnitegni:** Software, Formal analysis. **Mariscal Brice Tchatat Tali:** Writing – original draft, Investigation. **Darline Dize:** Writing – original draft, Investigation. **Cyrille Njampa Ngansop:** Writing – original draft, Investigation. **Pantaléon Ambassa:** Software, Formal analysis. **Judith Laure Ouete Nantchouang:** Software, Formal analysis. **Ingrid Simo Konga:** Software, Formal analysis, Data curation. **Fabrice Fekam Boyom:** Writing – review & editing, Visualization, Validation, Supervision, Data curation. **Bonaventure Tchaleu Ngadjui:** Visualization, Validation, Supervision, Resources, Project administration, Data curation. **Ghislain Wabo Fotso:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Ghislain FOTSO WABO reports financial support was provided by Alexander von Humboldt Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

VFKK, GWF and BTN are particularly grateful to the Yaoundé–Bielefeld Bilateral Graduate School Natural Products with Antiparasitic and Antibacterial Activity (YaBiNaPA) [DAAD, project ID 57316173] for providing research facilities in Cameroon. GWF is thankful to the Alexander von Humboldt, Germany Foundation for a 6-month fellowship at the Leibniz Institute for Plant Biochemistry in Halle, Germany, through the Georg Foster Research Fellowship for Experienced Researchers program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22897.

References

[1] S.K. Chanda, K. Rakholiya, Combination therapy: synergism between natural plant extracts and antibiotics against infectious diseases, in: A. Mendez-Vilas (Ed.), Science against Microbial Pathogens: Communicating Current Research and Technological Advances, Formatex Research Center, Badajoz, 2011, pp. 520–529.
[2] H. Grundmann, M. de Kraker, P. Davey, Clinical impact of antimicrobial resistance: design matters, Lancet Infect. Dis. 11 (2011) 344–345.
[3] C.C. Lai, C.Y. Wang, C.C. Chu, C.K. Tan, C.L. Lu, Y.C. Lee, Y.T. Huang, P.I. Lee, P.R. Hsueh, Correlation between antibiotic consumption and resistance of gram-negative bacteria causing healthcare-associated infections at a university hospital in Taiwan from 2000 to 2009, J. Antimicrob. Chemother. 66 (2011) 1374–1382.
[4] G.A. Amang à Ngnoung, Y.O.N. Ditchou, P.B. Leutcha, D. Dize, S.J.N. Tatsimo, L.R.Y. Tchokouaha, T.K. Kowa, B. Tembeni, H. Mamoudou, M. Poka, P. H. Demana, X.S. Noundou, F.F. Boyom, A.M. Lannang, Antiplasmodial and antileishmanial activities of a new limonoid and other constituents from the stem bark of *Khaya senegalensis*, Molecules 28 (2023) 7227.
[5] B. Aslam, W. Wang, M.I. Arshad, M. Khurshid, S. Muzammil, M.H. Rasool, M.A. Nisar, R.F. Alvi, M.A. Aslam, M.U. Qamar, M.K.F. Salamat, Z. Baloch, Antibiotic resistance: a rundown of a global crisis, Infect. Drug Resist. 11 (2018) 1645–1658.
[6] A.M. Stafford, Plant cell cultures as a source of bioactive small molecules, Curr. Opin. Drug Discov. Dev 5 (2002) 296–303.
[7] S. Catarino, M.M. Romeiras, R. Figueira, V. Aubard, J.M. Silva, J.M. Pereira, Spatial and temporal trends of burnt area in Angola: implications for natural vegetation and protected area management, Diversity 12 (2020) 307.
[8] P.O. Ogunbamowo, M.B. Olaniyi, O.L. Awotedu, I.L. Oduola, Assessment of the foliar micromorphology, phytochemical and mineral composition of Justicia secunda Valh leaves, An. Biol. 42 (2020) 183–192.
[9] E.N. Koffi, C. Le Guernevé, P.R. Lozano, E. Meudec, F.A. Adjé, Y.A. Bekro, Y.F. Lozano, Polyphenol extraction and characterization of *Justicia secunda* Vahl leaves for traditional medicinal uses, Ind. Crops Prod. 49 (2013) 682–689.
[10] P. Manda, D.P. Abrogoua, C. Bahi, D.S. Dano, G. Gnahoui, B.J. Kablan, Evaluation of the antihypertensive activity of total aqueous extract of *Justicia secunda* Valh (Acanthaceae), Afr. J. Pharm. Pharmacol. 5 (2011) 1838–1845.
[11] W.M. Kone, A.G. Koffi, E.L. Bomisso, F.H.T. Bi, Ethnomedical study and iron content of some medicinal herbs used in traditional medicine in Cote d'Ivoire for the treatment of anemia, Afr. J. Tradit. Complement. Altern. Med. 9 (2012) 81–87.
[12] H.M. Hernando, R.R. Alfredo, V.O. Crescente, Biological activity of "Sanguinaria"(*Justicia secunda*) extracts, Pharm. Biol. 40 (2002) 206–212.
[13] A.G.N. Fongod, N.B. Modjenpa, M.C. Veranso, Ethnobotany of Acanthaceae in the mount Cameroon region, J. Med. Plants Res. 7 (2013) 2707–2713.
[14] N. Neba, M. Ngwa, C. Anuanwen, D.N. Bup, Simultaneous extraction and pasteurization (SEPA) of a drink from "blood root"(*Justicia secunda*) leaves part 1: kinetic studies, Heliyon 6 (2020), e03297.
[15] B.A. Theiler, S. Revoltella, M. Zehl, C. Dangl, L.O.E. Caisa, J. König, J. Winkler, E. Urban, S. Glasl, Secundarellone A, B, and C from the leaves of *Justicia secunda* Vahl, Phytochem. Lett. 10 (2014), https://doi.org/10.1016/j.phytol.2014.05.007.
[16] P.T. Mpiana, K.N. Ngbolua, M.T. Bokota, T.K. Kasonga, E.K. Atibu, D.S. Tshibangu, V. Mudogo, *In vitro* effects of anthocyanin extracts from *Justicia secunda* Vahl on the solubility of hemoglobin S and membrane stability of sickle erythrocytes, Blood Transfus 8 (2010) 248–254.
[17] G.M. Corrêa, A.F.D.C. Alcântara, Chemical constituents and biological activities of species of *Justicia*: a review, Rev. Bras. Farmacogn. 22 (2012) 220–238.
[18] H. Jin, S. Yang, J.X. Dong, New lignan glycosides from *Justicia procumbens*, J. Asian Nat. Prod. Res. 19 (2017) 1–8.
[19] T.B. Tchegnitegni, R.T. Nguengang, I.K. Mawabo, R.B. Teponno, J.J.K. Bankeu, J.R. Chouna, C.N. Nkenfou, L.A. Tapondjou, N. Sewald, B.N. Lenta, Bioactive arylnaphthalide lignans from *Justicia depauperata*, J. Nat. Prod. 85 (2022) 2731–2739.
[20] T.K. Tabopda, G.W. Fotso, J. Ngoupayo, A.C. Mitaine-Offer, B.T. Ngadjui, M.A. Lacaille-Dubois, Antimicrobial dihydroisocoumarins from *Crassocephalum biafrae*, Planta Med. 75 (2009) 1258–1261.
[21] G.W. Fotso, L.M. Kamdem, M. Dube, S.A. Fobofou, A.N. Ebene, N. Arnold, B.T. Ngadjui, Antimicrobial secondary metabolitesfrom the stem barks and leaves of *Monotes kerstingii* Gilg (Dipterocarpaceae), Fitoterapia 137 (2019), 104239.
[22] V.F.K. Kamso, F.C.C. Simo, I.M.K. Mbekou, B.T. Tchegnitegni, B.N. Lenta, F.B. Fekam, N. Sewald, M. Frese, B.T. Ngadjui, W.G. Fotso, Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies, Molecules 27 (2022) 8820.
[23] M. Smilkstein, N. Sriwilaijaroen, J.X. Kelly, P. Wilairat, M. Riscoe, Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening, Antimicrob. Agents Chemother. 48 (2004) 1803–1806.
[24] D. Dize, R.B. Tata, R. Keumoe, R.M.K. Toghueo, M.B. Tchatat, C.N. Njanpa, V.C. Tchuenguia, L.T. Yamthe, P.V.T. Fokou, B. Laleu, J. Duffy, O.T. Bishop, F. F. Boyom, Preliminary structure-activity relationship study of the MMV pathogen box compound MMV675968 (2,4-diaminoquinazoline) unveils novel inhibitors of *Trypanosoma brucei brucei*, Molecules 27 (2022) 6574.
[25] C.A.N. Njanpa, S.C.N. Wouamba, L.R.T. Yamthe, D. Dize, B.M.T. Tchatat, P.V.F. Tsouh, M.N. Pouofo, J.B. Jouda, B.L. Ndjakou, N. Sewald, S.F. Kouam, F. F Boyom, Bio-guided isolation of anti-leishmanial natural products from *Diospyros gracilescens* L. (Ebenaceae), BMC Complement. Med. Ther. 21 (2021) 106.
[26] J. Xiong, S. Li, W. Wang, Y. Hong, K. Tang, Q. Luo, Screening and identification of the antibacterial bioactive compounds from *Lonicera japonica* Thunb. leaves, Food Chem. 138 (2013) 327–333.
[27] C.N. Tagousop, D. Ngnokam, D. Harakat, L. Voutquenne-Nazabadioko, Three new flavonoid glycosides from the aerial parts of *Graptophyllum grandulosum* Turril (Acanthaceae), Phytochem. Lett. 19 (2017) 172–175.
[28] A.K.N. Wonkam, C.A.N. Ngansop, M.A.T. Tchuenmogne, B.T. Tchegnitegni, G.T.M. Bitchagno, A.F. Awantu, J.J.K. Bankeu, F.B. Fekam, N. Sewald, B. N Lenta, Chemical constituents from *Baphia leptobotrys* Harms (Fabaceae) and their chemophenetic significance, Biochem. Syst. Ecol. 96 (2021), 104260.
[29] T.H. Lee, S.M. Lee, D.Y. Lee, Y. Son, D.K. Chung, N.I. Baek, J. Kim, A glycosidic spinasterol from *Koreana stewartia* promotes procollagen production and inhibits matrix metalloproteinase-1 expression in UVB-irradiated human dermal fibroblasts, Biol. Pharm. Bull. 34 (2011) 768–773.
[30] P.H. Nguyen, J.L. Yang, M.N. Uddin, S.L. Park, S.I. Lim, D.W. Jung, W.K. Oh, Protein tyrosine phosphatase 1B (PTP1B) inhibitors from *Morinda citrifolia* (Noni) and their insulin mimetic activity, J. Nat. Prod. 76 (2013) 2080–2087.
[31] E.I. Hwang, B.S. Yun, Y.K. Kim, B.M. Kwon, H.G. Kim, H.B. Lee, S.U. Kim, A. Phellinsin, A novel chitin synthases inhibitor produced by *Phellinus sp*, PL3. J. Antibiot. 53 (2000) 903–911.
[32] B.T. Tchegnitegni, R.B. Teponno, C. Tanaka, A.F. Gabriel, L.A. Tapondjou, T. Miyamoto, Sappanin-type homoisoflavonoids from *Sansevieria trifasciata* prain, Phytochem. Lett. 12 (2015) 262–266.
[33] B.T. Tchegnitegni, R.B. Teponno, K. Jenett-Siems, M.F. Melzig, T. Miyamoto, L.A. Tapondjou, A dihydrochalcone derivative and further steroidal saponins from *Sansevieria trifasciata* Prain, Z. Naturforsch. C. 72 (2017) 477–482.
[34] T.H. Lee, Y.H. Chiang, C.H. Chen, P.Y. Chen, C.K. Lee, A new flavonol galloylrhamnoside and a new lignan glucoside from the leaves of *Koelreuteria henryi* Dummer, J. Nat. Med. 63 (2009) 209–214.
[35] P.K. Agrawal, NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides, Phytochemistry 1 (1992) 3307–3330.
[36] X. Wan, H. Liu, X. Huang, J. Luo, L. Kong, Biotransformation of caffeic acid by *Momordica charantia* peroxidase, Can. J. Chem. 86 (2008) 821–830.
[37] K.S. Khetwal, S. Harbola, P. Cai, J.K. Snyder, M.G. Newton, S.W. Pelletier, B.S. Joshi, X-ray crystal structure and absolute configuration of kaerophyllin (chaerophyllin), J. Nat. Prod. 56 (1993) 318–328.

[38] B. Gözler, M.A. Önür, T. Gözler, G. Kadan, M. Hesse, Lignans and lignan glycosides from Haplophyllum cappadocicum, Phytochemistry 37 (1994) 1693–1698.
[39] R. Estévez-Reyes, A. Estévez-Braun, A.G. Gonzalez, Lignanolides from *Bupleurum salicifolium*, Phytochemistry 31 (1992) 2841–2845.
[40] F. Jim-Min, L. Ching-Kuo, C. Yu-Shia, Lignans from leaves of *Juniperus chinensis*, Phytochemistry 31 (1992) 3659–3661.
[41] V. Kuete, Potential of Cameroonian plants and derived products against microbial infections: a review, Planta Med. 76 (2010) 1479–1491.
[42] S.S. Lim, H. Kim, D. Lee, *In vitro* antimalarial activity of flavonoids and chalcones, Bull. Korean. Chem. Soc. 28 (2007) 2495–2497.
[43] A.R. Hidayati, A. Widyawaruyanti, H. Ilmi, M. Tanjung, T. Widiandani, D. Syafruddin, A.F. Hafid, Antimalarial activity of flavonoid compound isolated from leaves of *Artocarpus altilis*, Pharmacogn. J. 12 (2020) 835–842.
[44] T.B. Tali, B.P. Kamdem, J.C. Tchouankeu, F.F. Boyom, Current developments on the antimalarial, antileishmanial, and antitrypanosomal potential and mechanisms of action of *Terminalia spp*, S. Afr. J. Bot. 156 (2023) 309–333.
[45] R.B. Teponno, S. Kusari, M. Spiteller, Recent advances in research on lignans and neolignans, Nat. Prod. Rep. 33 (2016) 1044–1092.
[46] S. Hemmati, T.J. Schmidt, E. Fuss, (+)-pinoresinol/(−)-lariciresinol reductase from *Linum perenne* Himmelszelt involved in the biosynthesis of justicidin B, FEBS Lett. 581 (2007) 603–610.