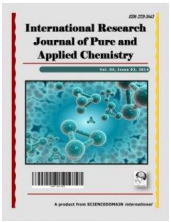

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Antimalarial Biflavonoids from the Roots of *Ochna serrulata* (Hochst.) Walp

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Authors' contributions

This work was carried out in collaboration between both authors. Author FRVH designed the study and mobilized the resources. Author MMN engaged in laboratory activities and wrote the first draft of the manuscript. Data analyses, reading and approving the manuscript were done by both authors.

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ABSTRACT

Aims: To isolate and evaluate the biological activities of the compounds present in the roots of *Ochna serrulata*.
Study Design: Laboratory isolation of compounds by using various chromatographic methods, and cytotoxicity and antimalarial evaluations of the isolated compounds.
Place and Duration of Study: School of Chemistry and Physics, College of Agriculture, Engineering and Science, University of KwaZulu-Natal (UKZN), from September 2009 to June 2012.
Methodology: Methanol extract of the pulverised roots of *O. serrulata* were subjected to different chromatographic techniques. Structures were established by using 1D and 2D NMR techniques and MS. The compounds were subjected to cytotoxicity tests to evaluate their growth inhibitory effects on renal (TK 10), melanoma (UACC62) and breast (MCF7) cancer cell lines using a sulforhodamine B (SRB) assay. Antimalarial evaluations were done against chloroquine-resistant strains of *Plasmodium falciparum* (FCR-3).

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Results: Three new biflavonoids; 4,4′,7-tri-O-methylisocampylospermone A (**1**), 4‴-de-O-methylafzelone A (**2**) and serrulone A (**3**) along with irisolone 4′-methyl ether (**4**), 3',4'-dimethoxy-6,7-methylenedioxyisoflavone (**5**), iriskumaonin 3′-methyl ether (**6**), lophirone L (**7**), a mixture of biflavanone 1 and campylospermone A, syringaresinol (**8**), and 16α,17-dihydroxy-ent-kauran-19-oic acid (**9**). Compound **1** showed antimalarial activity with IC_{50} 11.46 µM, followed by compound **3** (26.52 µM) and lastly compound **2** (38.43 µM). In the same test, **4** demonstrated antimalarial activity at IC_{50} 40.72 µM, **8** (42.66 µM), **6** (93.69 µM), **9** (106.48 µM) and the rest did not show any significant activity. Upon cytotoxicity evaluation against three different cancer cell lines, the compounds displayed no significant activity.
Conclusion: The genus is comprised of biflavonoids of varying nature, some of which expressed interesting biological activities with no/low toxicity.

Keywords: Biflavonoid; cytotoxicity; antimalarial; Ochna serrulata.

1. INTRODUCTION

The emergence of drug-resistant pathogens has devastated efforts of scientists in overcoming the burden of the diseases. The widespread and use of substandard medicines have fuelled the emergence of drug-resistant pathogens. Simply, drug resistance can be viewed as a persistence of a parasite even after proper treatment doses have been taken [1,2]. So far, drug resistance has become an obstacle towards disease eradication, thus creating an increasing gap for new medicines. Nature has been known to have made a significant contribution in drug discovery and pharmacology, in the fight against diseases, thus, provide a wealth of compounds as drug leads or novel structures. The use of plants for the medicinal purpose can be traced back since man begun existing, thus a need to tap into this rich source of novel structures and new drugs.

Locally, *O. serrulata* is called *Umbomvane*, *fynblaarrooihout* and *iliTye* in Zulu, Afrikaan and Xhosa respectively. The plant belongs to genus Ochna that has been deployed in various traditional medicine systems for treatment of various ailments. In Zulu traditional medicine, the root decoction of the plant is commonly used for bone disease treatment and *gangrenus proctitis* [3,4].

Although there are many reports on the structures of the secondary metabolites from the leaves and stems of the genus, not many reports on structures of compounds from the roots of Ochna species. Nonetheless, to the best of our knowledge, phytochemical studies on the roots of *O. serrulata* has never been done before, thus, this paper intends to report for the first time the results of the phytochemical studies and biological activities of the compounds from the roots of *O. serrulata*.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

Chromatotron (model 7924, Harrison Research), centrifugal chromatography was used for purification of the fractions, on a circular plate that were coated with 2 or 4 mm thickness preparative silica gel. Silica gel 60F254, (40-63 µm, Merck) was used for column chromatography. Acquisition of the ^{1}H, COSY, HSQC and HMBC NMR spectra recording at 500 MHz/400 MHz and 125 MHz/100 MHz for ^{13}C was achieved by using Bruker Avance III 500 or Bruker Avance III 400 spectrometers. Referencing of chemical shifts was achieved by using residual solvent peaks. that is; chloroform: ^{1}H 7.26 ppm, ^{13}C, 77.0 ppm, methanol-d4: ^{1}H 3.31 ppm, ^{13}C, 49.1 ppm, acetone-d6 ^{1}H 2.05 ppm, ^{13}C, 29.9 ppm. The LCT Premier mass spectrometer was used in Mass Spectrometry. Data recording was taken on the time-of-flight (TOF) Waters using electrospray ionisation. ADP 440+ model polarimeter (Bellingham and Stanley product) was used for optical rotations determinations. For separations monitoring, Thin-Layer Chromatographic plates (TLC; Kieselgel 60 F254, 0.25mm) sprayed with anisaldehyde reagent followed by heating. Anisaldehyde stain solution was prepared following: ice cooling of 465 mL methanol in a 1 L volumetric flask, on stirring, an addition of 5 mL acetic acid followed by 17 mL concentrated sulfuric acid and 13 mL of *p*-anisaldehyde.

2.2 Plant Materials

The plant was collected from the botanical garden of the University of KwaZulu-Natal (UKZN) and identified by Allison Young from the botanical garden in September 2009. At the

herbarium of UKZN, voucher specimen (reference number NU, M. Ndoile 01) was deposited.

2.2.1 Extraction and isolation

Roots of *O. serrulata* (air-dried and ground; 1.2 kg) were soaked in MeOH at room temperature for 48 h to yield 15 g of the extract. Initial fractionation in a short silica gel column with mixtures of EtOAc-hexanes eluents (increasing polarity) to give five fractions (R.1-R.5), with the fifth being MeOH wash. Upon dissolving fraction R.2 (850 mg) in MeOH, compound **9** precipitated out as an off-white powder, thus washed further with MeOH to afford 15 mg. The remaining filtrate was subjected to repeated chromatotron with a DCM-hexanes mixture (1:1) to obtain a pale yellow amorphous solid of 4,4′,7-tri-O-methylisocampylospermone A (**1**) (14.2 mg), isoflavonoids **4** (9.1 mg), **5** (10.0 mg) and **6** (11.2 mg).

Fraction R.3 was subjected to repeated purification on a chromatotron with DCM:EtOAc (1:1) eluent to afford lophirone A (8.1 mg), lophirone C (7.7 mg), afzelone B (10.1 mg) and 5-deoxyurundeuvine C (18.0 mg).

Fraction R.4 (2.5 g) was fractionated in a silica gel column (DCM-EtOAc (1:1) eluent) to obtain four fractions (R4.1-R4.4). Fraction R4.1 (400 mg) and R4.2 (500 mg) were combined and the resulting mixture was purified repeatedly on a chromatotron with DCM-EtOAc (1:1) as a mobile phase to yield a yellow powder of compound **2** (19.0 mg), yellow solids of compound **3** (27.5 mg) and compound **8** (15 mg). R4.3 was combined with R4.4 and repeatedly purified on a chromatotron with EtOAc:DCM (6:4) eluent to afford **3** (5.1 mg) and yellow solids of **7** (7.2 mg).

2.3 Biological Assays

2.3.1 Cytotoxicity

Growth inhibitory activity of all the isolated compounds was evaluated using renal (TK10), melanoma (UACC62) and breast (MCF7), following Wellington et al. 2013 protocol [5].

2.3.2 Antimalarial activities

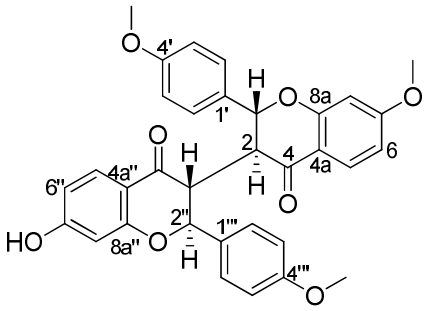
In vitro antimalarial tests as described by Chemaly et al. [6] with chloroquine-resistant strains of *P. falciparum* (FCR-3).

3. RESULTS AND DISCUSSION

3.1 Characterisation of the Compounds Isolated

Three new biflavonoids, namely, 4,4′,7-tri-O-methylisocampylospermone A (**1**), 4‴-de-O-methylafzelone A (**2**) and serrulone A (**3**) were isolated, along with 4′-methoxyirisolone (**4**), 3′,4′-dimethoxy-6,7-methylenedioxyisoflavone (**5**), 3′-methoxyiriskumaonin (**6**), lophirone L (**7**), syringaresinol (**8**), 16α,17-dihydroxy-ent-kauran-19-oic acid (**9**), lophirone A, lophirone C, afzelone B, 5-deoxyurundeuvine C and a mixture of biflavanone 1 and campylospermone A. The isolation of a furofuran lignan **8** (a major constituent of genus syringa) [7] from the family ochnaceae is hereby reported for the first time. A diterpene of ent-kauranoic acid type [16α,17-dihydroxy-ent-kauran-19-oic acid] was reported from Helianthus and Annona species [8,9], however, its isolation from the family ochnaceae is reported herein for the first time.

The structures of all secondary metabolites were established by NMR spectroscopic and mass spectrometric analyses, however, the observed and literature reported spectroscopic data were compared for further confirmation of the structures of the known compounds.



1

Compound **1** is a yellow non-crystalline solid with a molecular formula $C_{33}H_{28}O_8$ confirmed by molecular ion peak [M-H]- at m/z = 551 in mass spectrometry. The NMR spectra revealed the compound to be the 3,3″-biflavanone. The spectra also showed three *O*-methyl groups [δ_H 3.84 (3H) and 3.82 (6H)], two *p*-substituted aromatic rings [δ_H 7.21 and 6.98 (4H each)] and two *o,p*-disubstituted aromatic rings (δ_H 6.59, 6.28, 7.77 and 7.80, 6.65, 6.38). The signals for H-2 and H-3 for the two flavanone units behaved similarly with the heterocyclic proton signals of

biflavanone 1. These were observed as unresolved broad signals which almost disappear into the base line, probably due to restricted rotation at 30°C [10].

The cross peaks (HMBC) between δ_H 7.21 and 6.98 for the two *p*-substituted aromatic rings, and C-2 and C-2″ of ring C and C″ (δ_C 82.8 and 82.6, respectively) were expected for flavanone dimers. Two of the three methoxy groups displayed signals in the same chemical shift, a clear indication of originating from the same chemical environment and thus either linked to the two disubstituted aromatic rings or the two *p*-substituted rings. The cross peaks (HMBC) between the two identical methoxy groups protons signals and C-4′/C-4‴ (δ_C 161.6) indicated their connectivity to the two p-substituted rings. Therefore, the remaining was confined to either of the two A- rings of flavonoid skeleton. It was confirmed that this methoxy was linked to the ring A (C-7 at δ_C 167.2) and not ring A′ due to the cross peaks between H-6 and H-8 (δ_H 6.65 and 6.38 respectively) and the carbon bearing this methoxy (δ_C 167.2). The described cross peaks are summarized in Fig. 1 and ^{13}C and ^{1}H NMR data are given in Table 1.

According to the analysis above, the structure of **1** was assigned as 4,4′,7-tri-O-methylisocampylospermone A.

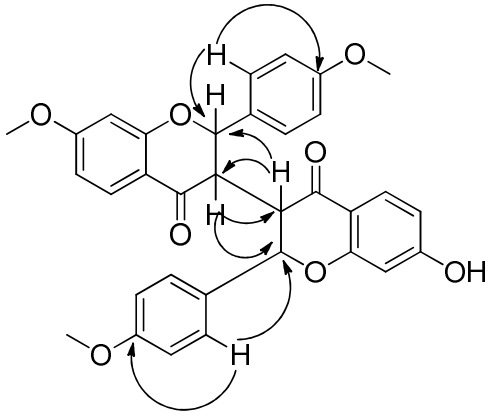


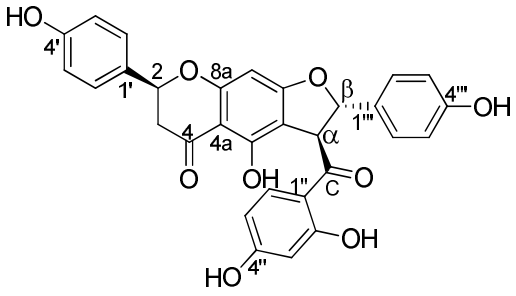
Fig. 1. Important HMBC correlations in structure 1

Table 1. ^{13}C and ^{1}H – NMR Spectral Data for 1 ((CD3)2CO)

Position	δ_H	*m* (*J* in Hz)	1 δ_C
4	-	-	190.7
4″	-	-	190.5
7	-	-	167.2
7″	-	-	165.4
8a, 8a″	-	-	164.3
4′,4‴	-	-	161.6
2′,6′,2‴,6‴	7.21	d (8.5)	130.7
1′	-	-	130.4
1‴	-	-	130.3
5″	7.77	d (8.6)	130.2
5	7.80	d (8.8)	129.7
3′,5′	6.98	d (8.5)	115.2
3‴,5‴	6.97	d (8.5)	115.0
4a,4a″	-	-	114.7
6″	6.59	dd (2.1, 8.6)	111.6
6	6.65	dd (2.3, 8.8)	111.1
8″	6.28	d (2.2)	103.5
8	6.38	d (2.3)	101.6
2	4.99	brs	82.8
2″	4.99	brs	82.6
3	3.72	brs	50.0
3″	3.72	brs	49.9
7-OMe	3.84	s	56.3
4‴,4′-OMe	3.82	s	55.8

Data summary:

Isolated as yellow amorphous solid substance, $[\alpha]^{21}_D$= -101 (*c* 0.1, MeOH). ^{1}H NMR (500 MHz, $(CD_3)_2CO$) and ^{13}C NMR (125 MHz, $(CD_3)_2CO$) see Table 1.



2

Compound **2**, a light yellow noncrystalline solid was determined to be a biflavonoid, specifically, a flavanone basing on the observation of the spectroscopic data. The unit was indicated by the ABX spin system (δ_H 3.15 (m) and 2.72 (m) and δ_H 5.39 (m) for H-3 and H-2 respectively), and confirmed by DEPT-135 experiment that indicated one CH_2 carbon at δ_C 44.0.

Moreover, the presence of two coupling protons typical for the aliphatic AB system (δ_H 5.75, J=5.7 Hz) and (5.22, J=5.7 Hz) was observed, along with two *p*-substituted, *o,p*-disubstituted and a *penta*-substituted benzoyl systems were observed.

The pronounced down field resonance (δ_C 202.7) of a carbonyl that expressed cross peaks with the AB aliphatic protons favoured the presence of a furan ring. The position of furan ring was confirmed by the correlation between AB protons and C-1‴, C-5, C-6, C-7 and C-c (δ_C 131.9, 159.5, 104.5, 167.5 and 202.7 respectively).

The linkage of the first *p*-substituted aromatic ring to C-2 of ring C (of flavonoid skeleton) was given by cross peaks observed between the H-2 and C-1′ (δ_C 131.0), and H-2′, 6′ (δ_H 7.34) and C-2 (δ_C 81.0). The second *p*-substituted ring protons (H-2‴,6‴ at δ_H 7.17) were correlated with C- β (δ_C 92.3), moreover, H-β was correlated with C-1‴ (δ_C 131.9). The *o,p*-disubstituted aromatic and α-protons were both correlated to carbonyl (δ_C 202.7) indicating their respective position. The described correlations are summarized in Fig. 2 and ^{13}C and ^{1}H NMR data are given in Table 2.

The NMR spectra of compound **2** gave peaks identical to afzelone A, the only difference being the absence of methoxy group. Thus, molecular formula of $C_{30}H_{22}O_9$ in agreement with [M-H]- peak at m/z 525, and hence named 4‴-de-O-methylafzelone A, a derivative of afzelone A [11]. A 5-deoxy analogue of compound **2** has been isolated from the stem of *Caesalpinia ferrea* (Pau-Ferro) in Middle and South America. It is reported to be the active ingredient in the stem of the plant having an inhibitory effect on type II DNA topoisomerase and as a result, the compound inhibits cell growth and induces apoptosis [12].

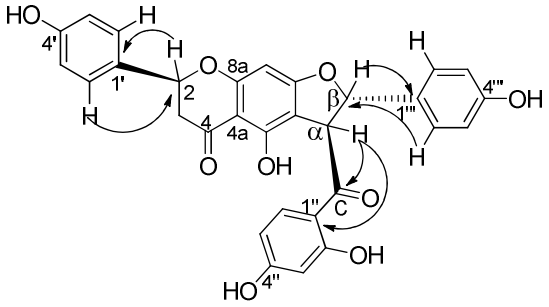
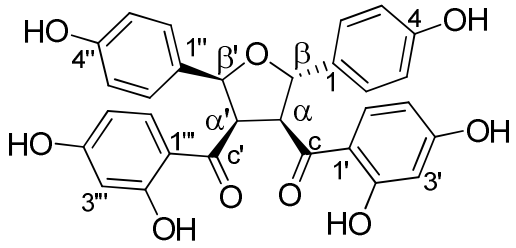


Fig. 2. Important HMBC correlations in structure 2



3

Analysis of the NMR spectra of **3** indicated the presence of two *p*-substituted aromatic rings, two *o,p*-disubstituted rings (protons with *o*-, *o/m*-, and *m*-coupling) and an array of four methine protons constituting a tetrahydrofuran ring having a substituent on each carbon.

The $^{1}H/^{13}C$ cross peaks in the HMBC spectrum between the two *p*-substituted aromatic ring H-2/H-6 and H-2″, 6″ (δ_H 7.39 and 7.20 respectively) and C-β/C- β′ (δ_C 85.7) confirmed the position of the two *p*-oxygenated aromatic rings. H-α (δ_H 5.47) expressed a cross peak with the carbonyl and C-1′ and C-1‴ of the two *o,p*-dioxygenated aromatic rings, thus confirming their positions. The above discussed correlations led to the confirmation of structure **3** in Fig. 3.

Table 2. ^{13}C and ^{1}H – NMR Spectral Data for 2 (CD3OD)

Position	δ_H	*m* (*J* in Hz)	2 δ_C
C	-	-	202.7
4	-	-	198.5
8a	-	-	170.3
7	-	-	167.5
4″	-	-	167.4
2″	-	-	166.5
4‴	-	-	160.2
5	-	-	159.5
4′	-	-	159.2
6″	7.54	d (8.8)	134.6
1‴	-	-	131.9
1′	-	-	131.0
2′,6′	7.34	d (8.4)	129.2
2‴,6‴	7.17	d (8.3)	128.9
3‴,5‴	6.83	d (8.3)	116.8
3′,5′	6.79	d (8.4)	116.5
1″	-	-	113.9
5″	6.26	dd (8.8, 2.2)	109.6
4a	-	-	106.9
6	-	-	104.5
3″	6.29	d (2.2)	103.9
β	5.75	d (6.0)	92.3
8	6.06	s	91.5
2	5.39	dd (13.0, 3.0)	81.0
α	5.22	d (6.0)	55.2
3	3.15	dd (13.0, 17.0)	44.0
	2.72	dd (3.0, 17.0)	

Data summary:

Isolated as a yellow powder, $[\alpha]^{22}_{D}$= +89.3 (*c* 0.4, MeOH). ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) see Table 2.

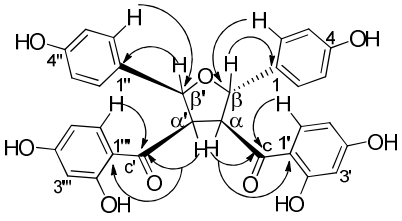
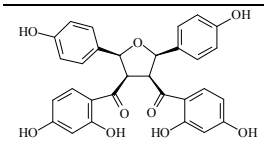
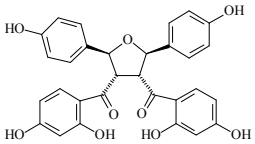
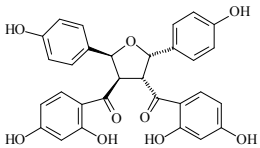
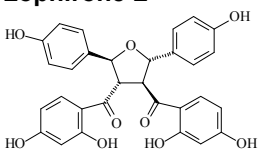
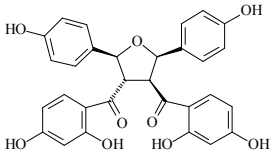
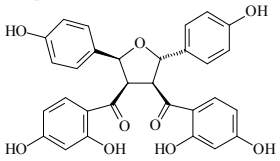


Fig. 3. Important HMBC Correlation in Structure 3

Compound **3** exhibited a pseudo-molecular ion [M-H]- peak at m/z 527 in the mass spectrum consistent with a molecular formula of $C_{30}H_{24}O_9$. Based on the spectroscopic data discussed, six possible structures (Table 3) are possible for this compound.

The first four structures in Table 3 are symmetrical (expected to show 13 carbon signals), thus, could not be the correct structures of **3** which is unsymmetrical (it showed 26 carbon signals). Therefore, the possibilities are left to the last two structures, but the observed NMR data for **3** are not in agreement with those of lophirone F, isolated from the *Lophira lanceolata* stem bark [13]. Thus, structure of compound **3** is the one with a *cis-cis-trans* configuration around the furan ring. Compound **3** is assigned as *cis-cis-trans*-3,4-bis(2,4-dihydroxybenzoyl)-2,5-bis(4-hydroxyphenyl)furan and is given the common name serrulone A. The absolute configuration of **3** has not yet been established. Table 4 summarizes the ^{13}C and ^{1}H – NMR spectral data for 3.

Table 3. Possible structures for 3 from the observed NMR data

Structure	Configuration	$[\alpha]_D$	^{13}C NMR signals
	*Cis*-*cis*-*cis*-isomer plane of symmetry *meso*-compound	0	13
	*Trans*-*cis*-*trans*-isomer plane of symmetry *meso*-compound	0	13
	*Cis*-*trans*-*cis*-isomer c_2-symmetry chiral	≠0	13
Lophirone L			
	*Trans*-*trans*-*trans*-isomer c_2-symmetry chiral	≠0	13
Lophirone G			
	*Cis*-*trans*-*trans*-isomer no symmetry chiral	≠0	26
Lophirone F			
	*Cis*-*cis*-*trans*-isomer no symmetry chiral	≠0	26

3.2 Biological Activities Evaluation

3.2.1 Cytotoxic activities

The isolated compounds were assayed for their cytotoxicity using sulforhodamine B (SRB) method, against melanoma (UACC62), renal (TK10), and breast (MCF7). None of the compounds exhibited any lethal activity (> 100 μM) on the mentioned three cancer cell lines.

3.2.2 Antimalarial activities

The compounds were evaluated against the chloroquine-resistant strains of *Plasmodium falciparum* (FCR-3) for their growth inhibitory effects. Table 5 summarizes antimalarial activities of compounds that showed moderate antimalarial activity.

With regard to the Structural Activity Relationship (SAR), the difference in the antimalarial activity of the structurally-related compound **1** (IC_{50} value of 11.46 μM) and isocampylospermone A (IC_{50} value of 0.16 μM) Ichino *et al* 2006 is noted [10]. The observation might have been attributed by methoxy substituents in former, thus altering its binding capacity.

A completely different level of antimalarial activity has been observed in the isomeric compounds, **3** and **7** at 26.52 μM and no significant activity respectively, this observation indicates the influence of relative and/or absolute configuration in biological activities.

Table 4. ^{13}C and ^{1}H – NMR Spectral Data for 3 ((CD3)2CO)

Position	δ_H	*m* (*J* in Hz)	3 δ_C
C	-	-	202.5
C′	-	-	201.1
2′	-	-	166.4
2‴	-	-	166.2
4′	-	-	165.70
4‴	-	-	165.68
4″	-	-	157.80
4	-	-	157.78
6‴	7.46	d (8.8)	133.9
6′	7.99	d (8.9)	133.4
1	-	-	129.8
1″	-	-	128.8
2,6	7.39	d (8.5)	128.4
2″,6″	7.20	d (8.5)	128.3
3,5	6.83	d (8.5)	116.1
3″,5″	6.59	d (8.5)	115.4
1‴	-	-	114.8
1′	-	-	113.8
5′	6.30	dd (8.9, 2.3)	108.9
5‴	6.08	dd (8.8, 2.2)	108.4
3′	6.18	d (2.3)	103.7
3‴	6.00	d (2.2)	103.2
β	5.94	d (5.7)	84.9
β'	6.30	d (8.8)	83.9
α'	4.51	dd (7.2, 15.7)	61.4
α	5.47	dd (6.1, 13.1)	56.4

Data summary:

Isolated as a yellow solid, $[\alpha]^{21}_{D}$= +55.9 (*c* 0.3, MeOH). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) and ^{13}C NMR (100 MHz, $(CD_3)_2CO$) see Table 4.

Table 5. Antimalarial activity of the isolated compounds

Compound	1	3	2	4	8	6	9
IC_{50} (μM)	11.46	26.52	38.43	40.72	42.66	93.69	106.48

*Key: 4,4',7-tri-O-methylisocampylospermone A (**1**), 4'''-de-O-methylafzelone A (**2**) serrulone A (**3**), irisolone 4'-methyl ether (**4**), iriskumaonin 3'-methyl ether (**6**), syringaresinol (**8**), and 16α,17-dihydroxy-ent-kauran-19-oic acid (**9**)*

If compound **2** is compared with its 5-deoxy analogy, moderate antimalarial activity at 38.43 μM, and no significant cytotoxicity activity for the former while the latter has been patented for its anticancer activities by Japanese scientists [12], stressing the influence of substituents in biological activities.

It is interesting to note that all new compounds demonstrated a higher antimalarial activity with no cytotoxicity indicating their suitability for antimalarial drug development.

4. CONCLUSION

All the new compounds isolated and characterised herein are of biflavonoid nature, indicating the plant species to be a rich source of these compounds. While all compounds exhibited no significant cytotoxic activity, higher

antimalarial activity is demonstrated by the new compounds, indicating their suitability for antimalarial development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lobel HO, Campbell CC. Malaria Prophylaxis and distribution of drug resistance. Clinics in Tropical Medicine and Communicable Diseases. 1986;1:225–242.
2. Bloland PB. Drug resistance in Malaria, World Health Organisation, Department of Communicable Disease Surveillance and Response; 2001.
3. Hutchings A., Scott AH, Cunnignham AB. Zulu Medicinal Plants. University of Natal Press, Scottsville, South Africa; 1996.
4. Hutchings A, Van Staden J. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. J. Ethnopharmacol. 1994;43:89-124.
5. Wellington KW, Qwebani-Ogunleye T, Kolesnikova NI, Brady D, de Koning CB. One-pot laccase-catalysed synthesis of 5,6-dihydroxylated benzo[b]furans and catechol derivatives, and their anticancer activity. Arch Pharm (Weinheim). 2013; 346:266-77.
6. Chemaly SM, Chen CT, Van Zyl RL. Naturally occurring cobalamins have antimalarial activity. J. Inorg. Biochem. 2007;101:764-773.
7. Deyama T. The Constituents of *Eucommia ulmoides*: Isolation of (+)-medioresinol di-O-β-D-glucopyranoside. Chem. Pharmaceut. Bull. 1983;31:2993-2997.
8. Hertz W, Kulanthaivel P. Ent-pimaranes, ent-kauranes, heliangolides and other constituents of three Helianthus species. Phytochem. 1984;23:1453-1459.
9. Wu YC, Hung YC, Chang FR, Cosentino M, Wang HK, Lee KH. Identification of ent-16β,17-dihydroxykauran-19-oic acid as an anti-HIV principle and isolation of the new diterpenoids annosquamosins A and B from *Annona squamosa*. J. Nat. Prod. 1996;59:635-637.
10. Ichino C, Kiyohara H, Soothornchareonnon N, Chuakul W, Ishiyama A, Seguchi H, et al. Antimalarial activity of biflavonoids from *Ochna integerrima*. Planta Med. 2006;72: 611-614.
11. Pegnyemb DE, Tih RG, Sondengam BL, Blond A, Bodo B. Flavonoids of *Ochna afzelii*. Phytochem. 2003;64:661-665.
12. Hiroshi N, Masashi Y, Hirohito S, Yukie S, Manabu I. Novel antitumoric compound isolated from plant "pau-ferro", *Caesalpinia ferrea*. 2009, Japan, PATENT, Japan Kokai Tokyyo Koho, JP2009107928 A 2020090521.
13. Tih RG, Sondengam BL, Martin MT, Bodo, B. Structure of the chalcone dimers lophirone F, and H from *Lophira lanceolata* stem bark. Phytochem. 1990;29:2289-2293.

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