Phytoconstituents from *Turraea obtusifolia* and their antiplasmodial activity

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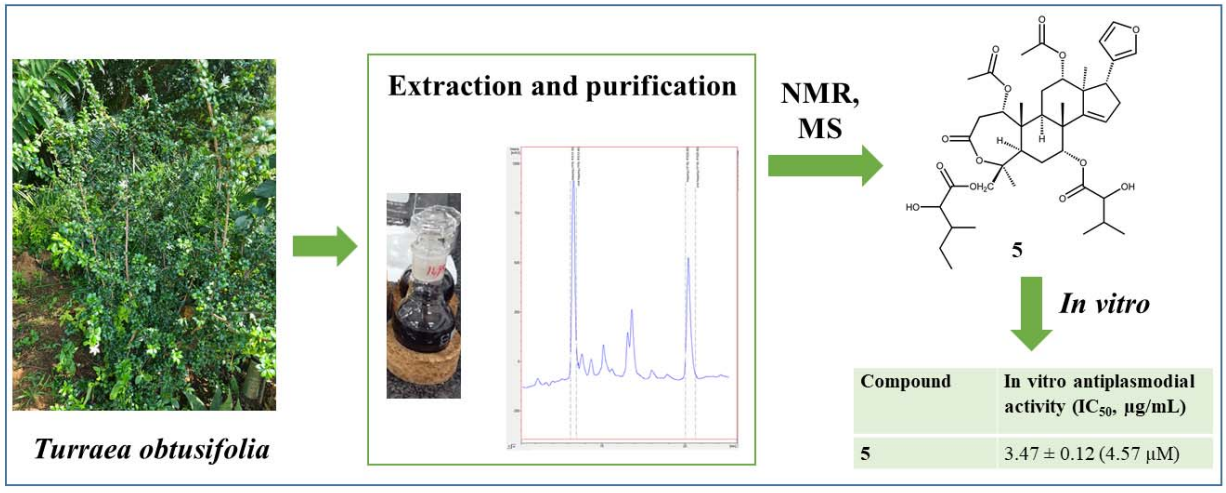
Abstract

Three new steroids, turranin M, N and O (**1-3**), together with four known limonoids, nymania 1 (**4**), rubralin B (**5**), aphapolynin C (**6**) and Trichillia substance Tr B (**7**), were isolated from the leaves of *Turraea obtusifolia*. Their chemical structures were elucidated using NMR and MS. Rubralin B (**5**) displayed good activity against the asexual parasites from the drug sensitive *Plasmodium falciparum* NF54 strain with an IC_{50} value of 3.47 µg/mL (4.57 µM), nymania 1 (**4**) showed a weak activity (IC_{50} 13.36 µg/mL (19.40 µM)) and the rest of compounds had IC_{50} > 20 µg/mL.



Key words: *Turraea obtusifolia*, steroids, limonoids, *Plasmodium falciparum*.

1. Introduction

Turraea obtusifolia Hochst. (Meliaceae) is a deciduous shrub, known as the small honeysuckle tree, native to South Africa, Mozambique, Swaziland, Botswana and Zimbabwe (Notten 2007). It can reach up to 3 m in height, bearing dark glossy green leaves, which varies from narrowly

oblanceolate to obovate. The plant produces large, showy and pure white flowers which are distributed in small clutters amongst the leaves (Notten 2007). *T. obtusifolia* used traditionally to treat stomach and intestinal ailments (Madikizela et al. 2012), as an enema, in topical applications to treat wounds in livestock (Luseba and Tshisikhawe 2013; Oyedeji-Amusa Mariam Oyefunke et al. 2021) and as insect repellent (Magwede et al. 2019). *T. obtusifolia* leaf and bark extracts have been reported to have antimicrobial activity (Oyedeji-Amusa M. O. et al. 2020). *T. obtusifolia* is known for producing limonoids like most members of the Meliaceae family (Akinniyi et al. 1986; Mulholland and Monkhe 1993; Fraser et al. 1995; Sarker et al. 1997). This class of natural products has been reported for having antiplasmodial activity (Tundis et al. 2014).

Malaria is a life-threatening which remains a global health threat, particularly in sub-Saharan Africa (WHO 2020). The current WHO recommended first-line treatment regimen for uncomplicated malaria, artemisinin-based combination therapies (ACT), have significantly reduced morbidity and mortality associated with malaria. However, strains of *Plasmodium falciparum* resistant to this regimen have emerged, a phenomenon which will undermine their continued efficacy and the control of malaria (Dondorp et al. 2010; Saunders et al. 2014; Woodrow and White 2017; Balikagala et al. 2021; Rosenthal 2021; White 2021). Novel, safe and effective antimalarial agents are urgently needed to prevent a global malaria emergency.

The value of plants in antimalarial drug discovery has been proven with the successful story of *Cinchona* sp. and *Artemisia annua* (Cox 2010). Additionally, the variety of chemical matter present in nature suggest that natural products continue to be a non-negligible source of unique molecular scaffolds in the search for new antimalarial drugs. In the frame of setting up a natural product library at the University of Pretoria, a subset of extracts and fractions were generated and tested for their antiplasmodial activity, among which was *T. obtusifolia* leaves. In this study, *T.*

obtusifolia leaf acetone extract which displayed good activity was further explored. We herein report on the chromatographic isolation and purification of compounds from *T. obtusifolia* leaf acetone extract, their structure elucidation and antiplasmodial activity.

2. Results and discussion

2.1 Isolation and structure elucidation

The leaf extract of *T. obtusifolia* was fractionated using positive pressure solid phase extraction (SPE) workstation to produce seven fractions as part of the University of Pretoria Natural Product Library. These were screened for *in vitro* inhibitory activity against the NF54 strain of *P. falciparum* parasites in a full dose-response assay. The crude extract and three fractions (Fractions 5, 6 and 7) exhibited IC_{50} values of less than 10 µg/mL (Table 1), which was considered as good as per the previously outlined criterion (Moyo et al., 2016). The antiplasmodial activity of *T. obtusifolia* has not been previously reported, however, there are records of good antiplasmodial activity of other *Turraea*, such as *T. robusta*, *T. nilotica* (Irungu et al., 2015; Irungu et al., 2007) and *T. floribunda* (Clarkson et al., 2004; Mokoka et al., 2013). Hence, the plant was selected for further investigation. The fractions and crude extract were analysed by UPLC-QTOF-MS for tentative identification of the active compounds, which was achieved through accurate mass determination and fragmentation patterns. Analysis of compounds using several databases (MetFrag, PubChem, Dictionary of Natural Products) after generating the molecular formulas through MassLynx revealed two main classes of compounds as limonoids and pregnan steroids. However, no positive identification was deduced due to the possibility of several isomers. These classes had previously been isolated from *Turraea* species. Therefore, peaks with masses belonging from these two classes were targeted for isolation. The purification of the crude acetone

extract using flash silica chromatography and mass-directed preparative HPLC led to the isolation of three novel steroids (compounds **1**-**3**) and four known limonoids (compounds **4**-**7**).

Table 1: *In vitro* full dose-response activity of *T. obtusifolia* leaf acetone extract and subsequent SPE fractions. Concentration range: 100 µg/mL to 0.39 µg/mL (9-point concentration, technical duplicates, n=1). Chloroquine used as control with IC_{50} of 10.4 nM.

Sample name	**IC_{50} value (µg/mL)**
Crude acetone extract	4.8
Fraction 1	139.2
Fraction 2	112.0
Fraction 3	35.3
Fraction 4	11.7
Fraction 5	5.9
Fraction 6	4.4
Fraction 7	8.0

Compound **1** was isolated as a white powder and had the molecular formula $C_{21}H_{34}O_4$, which was deduced from its QTOF mass spectrum at *m*/*z* 351.2528 $[M+H]^+$ (calcd for $C_{21}H_{35}O_4^+$, 351.2530). The ^{13}C NMR spectrum exhibited 21 carbon signals, which were classified by DEPT as eight methines, eight methylenes, two methyls and three quaternary carbons, suggesting a tetracyclic ring system. The DEPT revealed the presence of one olefinic methylene group resonating at δ_C 115.1. This was further confirmed by the ^1H NMR spectrum displaying two protons signal at δ_H 4.88 – 4.93 (2H, m, H-21), which were connected to the same carbon (δ_C 115.1) as deduced from HSQC spectrum. A downfield proton appeared at δ_H 5.75 (1H, ddd, J = 7.8, 10.6, 17.0 Hz, H-20) in the ^1H NMR spectrum showing COSY interactions with the olefinic protons and a proton resonating at δ_H 1.95 (1H, q, J = 8.7 Hz, H-17). The data indicated that compound **1** was likely a C21 steroid with a vinyl side chain at C-17 (Wang et al. 2006). The position of the vinyl group at

C-17 was further confirmed by HMBC correlations of H-20 with C-17 and 18-CH_3 and H-17 with C-20, C-21 and 18-CH_3. Further, in the ^{1}H NMR spectrum, three oxygenated methine protons were resonating at δ_H 4.02 (1H, q, *J* = 3.2 Hz, H-2), 3.70 (1H, dd, *J* = 3.2, 4.8 Hz, H-1), and 3.67 (1H, t, *J* = 3.2 Hz, H-6), with two of them showing COSY interactions, while the third had COSY interactions with two methylenic protons, which appeared at δ_H 1.67 (1H, m, H-7a) and 1.22 (1H, m, H-7b). Additionally, two tertiary methyl singlets appeared at δ_H 0.60 (3H, s, 18-CH_3) and 1.11 (3H, s, 19-CH_3). 18-CH_3 displayed HMBC correlations with C-17, C-13 and C-12, while 19-CH_3 correlated with C-1, C-5, C-10, C-8 and C-11, in the HMBC spectrum. Further, HMBC correlations between H-7b (δ_H 1.22, 1H, m) and C-8 and C-9; H-2 (δ_H 4.02, 1H, q, *J* = 3.2 Hz) and C-5, C-8 and C-10; H-4a (δ_H 1.69, 1H, m) and C-1 and C-10; H-3b (δ_H 1.80, 1H, m) and C-1 and C-5 and H-2 (δ_H 4.02) and C-5 enabled the construction of rings A and B.

The relative stereochemistry of compound **1** was established by NOESY spectrum, ^{1}H and ^{13}C NMR. The NMR data for 18-CH_3 (δ_H 0.60, δ_C 13.3) were consistent with it being β-orientated and the rings C/D trans-fused, as reported for pregnane steroids (Dorta et al. 2004; Díaz-Marrero et al. 2011), which is in agreement with the biosynthesis of pregnanes (Nes 2011; Lindemann 2015). The proton assigned at H-20 showed NOESY interactions with 18-CH_3 suggesting that they were on the same side. Further, 18-CH_3 also displayed NOESY interactions with H-8, which in turn showed NOESY correlations with 19-CH_3, indicating that they were both on the same side. NOESY interactions were also seen between H-9 and H-17, implying they were both α-oriented. Additionally, NOESY correlations between 19-CH_3 and H-2, 19-CH_3 and H-6, H-2 and H-3 indicated that they were all β-oriented. Therefore, the OHs were α-oriented. The NOE interaction between H-4a and 19-CH_3 indicated a trans-fused pattern between rings A and B (Wang et al. 2006), which implied that 5-OH was α-oriented (Veleiro et al. 1999). Therefore, the structure of

compound **1** (Figure 1) was deduced as 2α,3α,5α,6α-tetrahydroxy- pregn-20-en and given the name turranin M, based on similar steroids that have been isolated from *T. pubescens*.

Compound **2** was isolated as a yellow powder, which had a molecular formula $C_{21}H_{34}O_4$ deduced from its QTOF mass spectrum at *m*/*z* 351.2527 $[M + H]^+$ (calculated for $C_{21}H_{35}O_4^+$, 351.2530). The ^1H and ^{13}C NMR spectra showed similarities with compound **1**, suggesting that compound **2** was also a C21 steroid with a vinyl side chain at C-17. The ^1H NMR spectrum revealed that the 19-CH_3 was oxygenated with signals appearing at δ_H 4.12 (1H, d, *J* = 11.6 Hz, H-19a) and 3.73 (1H, d, *J* = 11.6 Hz, H-19b) as deduced from their HMBC correlations with C-1 and C-5. Further, a broad singlet resonating at δ_H 3.97 (1H, br s, H-6) displayed HMBC correlation with C-10. Another broad singlet appearing at δ_H 3.91 was assigned at position C-7 as deduced from its HMBC correlations with C-10 and C-8. Additionally, in the HMBC spectrum, the proton at δ_H 2.30 (1H, dd, *J* = 1.3, 14.5 Hz, H-1a) correlated with C-3. The methylene protons at δ_H 2.10 (1H, q, *J* = 12.6 Hz, H-4a) and 1.57 (1H, m, H-4b) showed COSY interactions with H-3 (δ_H 3.63, m) and H-5 (δ_H 1.34, br s) and HMBC correlations with C-3. Further, the proton at δ_H 0.74 (1H, m, H-14) displayed COSY interactions with H-8 (δ_H 1.89, m) and HMBC correlation with 18-CH_3 (δ_H 0.61, s). The 18-CH_3 correlated with C-13, C-17 and C-12, in the HMBC spectrum, while H-12a (δ_H 1.70, 1H, m) correlated with C-13, C-14 and C-16.

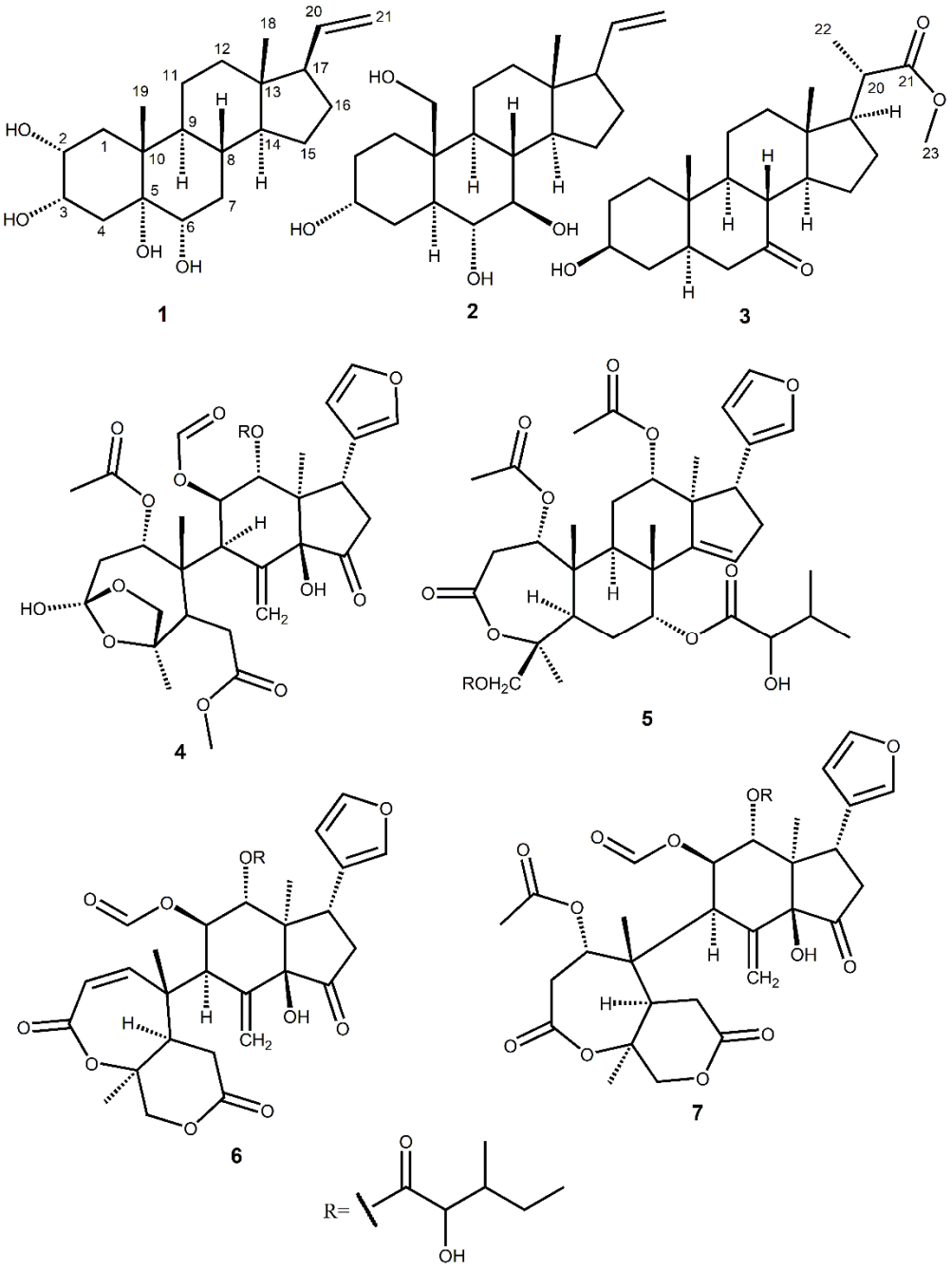


Figure 1: Structures of compounds **1**-**7** from the leaves of *Turraea obtusifolia*

The relative stereochemistry was deduced from NOESY spectrum, ^{1}H and ^{13}C NMR. Similar to compound **1**, the NMR data of compound **2** for 18-CH_3 (δ_H 0.61, δ_C 13.4) were consistent with the methyl being β-orientated and the rings C/D trans-fused (Dorta et al. 2004; Díaz-Marrero et al.

2011). The NOESY interactions between 19-CH_2 and H-8 indicated that they were on the same face. There were no correlations between 19-CH_2 and H-5, while H-19b displayed NOESY interactions with H-4b, which implied that rings A and B were trans-fused (Wang et al. 2006). Furthermore, the NOESY correlations of both H-3, H-6 and H-19b with H-4b indicated that they were on the same side (β-oriented). Therefore, OH-3 and OH-6 were α-oriented. H-5 showed NOESY interactions with H-7, which indicated that OH-7 was β-oriented. The structure of compound **2** (Figure 1) was deduced as 3α,6α,7β,19- tetrahydroxy- pregn-20-en and given the name turranin N.

Compound **3** was isolated as a white solid with the molecular formula $C_{23}H_{36}O_4$ deduced from its QTOF mass spectrum at *m*/*z* 377.2831 $[M+H]^+$ (calcd. for $C_{23}H_{37}O_4^+$, 377.2692). The ^{13}C NMR spectrum displayed 23 signals, which were classified as four methyls, eight methylenes, seven methines, and four quaternary carbons based on the DEPT spectrum, suggesting that compound **3** possessed a tetracyclic ring system. The ^1H NMR spectrum showed two tertiary methyl groups at δ_H 0.67 (3H, s, 18-CH_3) and δ_H 1.08 (3H, s, 19-CH_3), one secondary methyl group at δ_H 1.18 (3H, d, J = 7.0 Hz, 21-CH_3), and one oxygenated methyl group at δ_H 3.64 (3H, s, O-CH_3). The HMBC correlations between methyl protons 19-CH_3 with C-9 and C-5; methylene protons H-1a (δ_H 1.02, 1H, m) and H-1b (δ_H 1.77, 1H, m) with C-3, C-5, C-10, C-2 and 19-CH_3; H-4b (δ_H 1.43, 1H, m) with C-3 and C-5; H-6b (δ_H 2.03, 1H, m) with C-8, C-5, C-1 and C-7, enabled the assignments of rings A and B. Furthermore, the HMBC correlations observed between 18-CH_3 and C-17, C-14, C-13, C-12; H-16a (δ_H 1.75, 1H, m) and C-13, C-15, as well as between H-13a (δ_H 1.92, m) and C-9, C-14, C-13, C-11 and 18-CH_3, assisted the construction of rings C and D. Additionally, COSY interactions of H-20 (δ_H 2.41, m) with both H-17 (1.60, m) and 21-CH_3 (δ_H 17.30, d) and HMBC

correlations between 21-CH_3 and C-17, C-20, C-22 and between the methoxy (δ_H 3.64, 3H, s) and C-22, constructed the side chain assigned at C-17.

The relative configuration was determined from NOESY spectrum and ^{13}C NMR. The chemical shifts of 19-CH_3 being in the upfield region (δ_C 11.9) indicated an A/B trans junction as in the A/B cis junction the chemical shift of the methyl group would be in a range of 22 – 24 ppm (Zhang F et al. 2016). This assumption was supported by the absence of NOESY interactions between H-5 and 19-CH_3. Likewise, the NMR data for 18-CH_3 (δ_H 0.67, δ_C 12.4) were consistent with it being β-orientated and the rings C/D trans-fused (Dorta et al. 2004; Díaz-Marrero et al. 2011). Further, cross-peaks of H-8 and 19-CH_3 and 18-CH_3 suggested that they were both β-oriented, while H-3, H-5, H-9, and H-14 where α-oriented as deduced from cross-peaks H-3/H-5, H-5/H-9, H-9/H-14 observed in the NOESY spectrum. The structure of compound **3** (Figure 1) was established as 3α-hydroxy-23-bisnorchol-7-one-22-oate and was given the name turranin O. Similar compounds had previously been isolated from the twigs of *T. pubescens*, such as turranin E, which differed from compound **3** by the presence of a β-OH at C-6 and absence of ketone at C-7 (Yuan et al. 2013). Turranin C (which has the Δ6(7) and Δ8(14) conjugated double bond) and turranin D (with Δ7(8) unsaturation) are also similar steroids isolated from *T. pubescens* (Yuan et al. 2013).

The known limonoid compounds **4-7** (Figure 1) were identified as nymania 1 (MacLachlan and Taylor 1982; L. Musza et al. 1994), rubralin B (Musza et al. 1995), aphapolynin C (Zhang Y et al. 2013) and Trichillia substance Tr B (Gunatilaka et al. 1998) by comparing their observed spectroscopic data with that which was reported (The NMR data are provided in the supplementary data).

2.2 Antiplasmodial results of compounds 1-7

Compounds **1**-**7** were tested for their antiplasmodial activity against asexual parasites from the drug sensitive *P. falciparum* NF54 strain using a SYBR Green I-based fluorescence assay. Compounds **6** and **7** were initially tested as a mixture. Following separation using LC-MS-SPE-NMR, they were re-evaluated individually against the parasites. Compound **5** (rubralin B) displayed good activity with an IC_{50} value of 3.47 µg/mL (4.57 µM) (Table 2), based on the classification criteria used by Ntie-Kang et al. (2014). The mixture of compounds **6** and **7** exhibited an IC_{50} value of 6.59 µg/mL. However, individually, they exhibited IC_{50} values > 20 µg/mL. This indicated that they may have had an additive or synergistic effect when used as a mixture.

Table 2: *In vitro* activity of compounds isolated from *T. obtusifolia* leaves against asexual *P. falciparum* NF54 parasites.

Compounds	**In vitro antiplasmodial activity (IC_{50}, µg/mL)**	
1	> 40	
2	> 40	
3	20.34 ± 0.43 (54.02 µM)*	
4	13.36 ± 0.2 (19.40 µM)*	
5	3.47 ± 0.12 (4.57 µM)*	
6	6.59 ± 0.28[#]	> 20
7		> 20
Chloroquine	0.009 ± 0.005 µM	

* IC_{50} in µM

Data are from two independent biological repeats, each performed in technical triplicates. Means ± S.E. indicated.

[#]The mixture of compound **6** and **7** had an IC_{50} of 6.59 ± 0.28 µg/mL, while the individual IC_{50}s were >20 µg/mL.

The most active compound, **5**, had an IC_{50} value slightly lower than the extract indicating that it contributed strongly to the antiplasmodial activity of the crude extract. Among limonoids isolated from *Turraea* species, the most active compound has thus far been azadironolide, with IC_{50} values

of 2.4 (5.12 μM) and 1.1 (2.34 μM) μg/mL against the D6 and W2 strains of *P. falciparum*, respectively (Irungu et al. 2015). This makes compound **5** one of the most active antiplasmodial compounds isolated from species of the genus *Turraea*.

3. Conclusion

In conclusion, our study, using mass-directed purification, led to the isolation of seven compounds including three novel steroids, namely turranin M (**1**), turranin N (**2**) and turranin O (**3**) from the acetone extract of *T. obtusifolia* leaves. Their structures were elucidated based on MS and NMR data analyses. Compound **5** displayed a good activity against asexual *P. falciparum* parasites which merits consideration for advancement in the malaria drug discovery pipeline. *T. obtusifolia* is not extensively studied and so further studies seeking to more previously undescribed antiplasmodial compounds from it are merited.

4. Experimental

4.1 General

Extraction was carried out at room temperature under constant agitation at 200 rpm using the FMH 200 shaker (FMH Electronics, RSA). Column chromatography fractionation was carried out on a BUCHI Pure C-815-Flash system using BUCHI prepacked flash silica columns procured from Labotech (Gauteng, South Africa). Preparative high-performance liquid chromatography (HPLC) was carried out on a Waters chromatographic system equipped with a photodiode array (PDA) detector (Model 2998) and interfaced with an Acquity QDa detector (Waters, Milford, MA, USA) with a flow rate of 20 mL/min. Data were collected using MassLynx 4.1 software (Waters, USA). Preparative HPLC separation was achieved on an XBridge Prep C18 column (19 x 250 mm, 5 μM, Waters). Targeted peaks were collected in test tubes (about 8 mL capacity) using the Waters 2767

fraction collector. Hyphenated LC-MS-SPE-NMR was also used for purification, which was carried out on Agilent 1260 Infinity HPLC with PDA detector coupled to a Bruker AmaZon SL ion trap MS, Bruker Prospect II SPE Interface, and Bruker Sample Pro sample handler. The MS data were acquired on a UPLC coupled to QTOF Synapt G2 HDMS (Waters Corp., MA, USA) operating in both positive and negative electrospray ionisation (ESI) modes. Analytical and technical grade solvents used in extraction, fractionation and preparative HPLC were procured from Merck South Africa (Merck, Darmstadt, Germany) and Romil-SpS™, Microsep, South Africa (Waterbeach, Cambridge, United Kingdom). All solvents were used without further treatment. 1D and 2D NMR spectra were recorded at room temperature on Bruker AVANCE III 500 MHz spectrometer. Deuterated chloroform ($CDCl_3$) (Sigma-Aldrich) was used to dissolve the compounds. The chemical shifts are reported in ppm (δ-scale) and the calibrations of the spectra were done by using the trace protons from the deuterated solvents, δ_H – 7.26 and δ_C – 77.16. The coupling constants "J" are given in Hertz (Hz). ECD and UV data were acquired on an Applied Photophysics Chirascan spectrometer.

4.2 Plant material

Leaves of *Turraea obtusifolia* Hochst. (Meliaceae) were collected at the University of Pretoria Hatfield campus in April 2021 with the help of the University's curator, Jason Sampson. A voucher specimen was identified and deposited at the H.G.W.J. Schweickerdt Plant Herbarium at University of Pretoria (Voucher specimen code: PRU 129518). The collected leaves were oven dried at 30˚C and subsequently ground to a fine coarse powder using a grinder (Polymix, PX-MFC 90 D, Lasec, Gauteng, South Africa).

4.3 Extraction, SPE fractionation and isolation

Dry powdered leaves of *T. obtusifolia* (787 g) were extracted repeatedly (3 times) for 12 hours with 3.5 L of acetone at room temperature. The extract was filtered and concentrated under vacuum using a rotary evaporator to yield 74.7 g of a gum type crude extract. The dry extract was stored in the cold room. A small quantity of the crude extract (230 mg) was fractionated using a ppSPE workstation on a reverse phase C8 cartridge with the eluent of water (H_2O), MeOH and acetonitrile (MeCN) in the following ratios: for fractions 1 to 6: H_2O:MeOH 95:5, 80:20, 60:40, 40:60, 20:80, 0:100, respectively, and for fraction 7: MeOH:MeCN 50:50. The solvents were evaporated to dryness using a Genevac HT Series (Genevac Ltd., Ipswich, United Kingdom). The extract and fractions were then tested for the antiplasmodial activity.

The dried acetone crude extract (59.5 g) was re-suspended in MeOH/H_2O (85:15) and sequentially partitioned with n-hexane and DCM. The partitioned DCM fraction (8.5 g) was subjected to fractionation on a BUCHI Pure C-815 Flash system using a gradient of DCM-EtOAc (95:5 → 90:10 → 85:15 → 80:20 → 70:30 → 50:50 → 0:100, v/v), then the column was gradually washed with EtOAc-MeOH (95:5 → 70:30, v/v). The separation provided 47 sub-fractions (Sf 1-47). Sf 5 (110 mg) was further purified by mass-directed preparative HPLC using gradient of H_2O + 0.1% FA and MeOH + 0.1% FA (50:50 → 20:80 → 0:100 → 50:50) to yield compound **1** (2.58 mg). Sf 11 (331 mg) was further purified by mass-directed preparative HPLC using gradient of H_2O + 0.1% FA and MeOH + 0.1% FA (50-50 → 0:100 → 50:50) to yield compound **2** (2.57 mg). Sf 3 (3.3 g) was further fractionated on the BUCHI Pure C-815 Flash system using a prepacked silica FP EcoFlex Si 80 g column (50 µm irregular, maximum pressure rating 250 psi) using gradient of DCM- EtOAc (100:0 → 90:10 → 80:20 → 70:30 → 50:50 → 0:100) and then the column was gradually washed with EtOAc-MeOH (95:5 → 70:30). The separation provided 19 sub-sub-

fractions (SbF1-SbF19). SbF7 (540 mg) was subjected to mass-directed HPLC purification using gradient of H_2O + 0.1% FA and MeOH + 0.1 % FA (50:50 → 20:80 → 0:100 → 50:50) to yield compounds **3** (3.62 mg), **4** (3.01 mg) and **5** (2.15 mg) and a mixture of two compounds (15.64 mg), which was further resolved using hyphenated LC-MS-SPE-NMR with a gradient of H_2O + 0.1% TFA and MeCN + 0.1% TFA (60:40 → 20:80 → 0:100 → 60:40) to provide compound **6** (1 mg) and **7** (2.06 mg).

The isolated compounds were analysed using the UPLC-QTOF-MS to resolve their exact masses. They were run on a Waters Acquity UPLC System equipped with a binary solvent delivery system and an autosampler. The Waters BEH C18 (2.1 mm × 100 mm, 1.7 μm) column was used with a mobile phase of solvents A = H_2O + 0.1% formic acid and B = MeOH +0.1% formic acid, applied in gradient mode (0 min 3% B, 0.10 min 3% B, 14 min 100% B, 16 min 100% B, 16.5 min 3% B, 20 min 3% B). The flow rate was 0.3 mL/min and the injection volume was 5 μL. The separated compounds were analysed by a Waters Synapt G2 high definition QTOF mass spectrometer, which was run in electrospray ionization positive and negative modes. The following MS source parameters were set for both positive and negative mode: source temperature 120 ºC, sampling cone 20 V, extraction cone 4.0 V, desolvation temperature 300 ºC, cone gas flow 10.0 L/h, desolvation gas flow 600 L/h, capillary 2.6 kV. It was constantly infused at a rate of 3 μL/min through a separate orthogonal ESI probe to compensate for experimental drift in mass accuracy. The trap collision energy was 28 V. Respective compound's molecular formulas were generated from MassLynx V 4.

Turranin M (**1**): white powder, UV (MeOH) λ_{max} (log ε): 282 nm (0.1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.75 (1H, ddd, 7.8, 10.6, 17.0 Hz, H-20), 4.98 (1H, m, H-21a), 4.95 (1H, ddd, 1.2, 2.2, 10.0 Hz, H-21b), 4.06 (1H, s, 5-OH), 4.02 (1H, q, 3.2 Hz, H-3), 3.70 (1H, dd, 3.2, 4.8 Hz, H-2),

3.67 (1H, t, 3.2 Hz, H-6), 3.26 (1H, s, 3-OH), 2.45 (1H, s, 2-OH), 2.03 (1H, dd, 2.9, 15.9 Hz, H-4a), 1.95 (1H, q, 8.7 Hz, H-17), 1.84 (1H, dd, 2.8, 4.1 Hz, H-11a), 1.80 (1H, m, H-4b), 1.79 (1H, m, H-16a), 1.77 (1H, m, H-8), 1.73 (1H, m, H-12a), 1.69 (1H, m, H-1a), 1.672 (1H, m, H-7a), 1.59 (1H, m, H-15a), 1.57 (2H, m, H-1b, H-16b), 1.42 (1H, dq, 4.2, 13.0 Hz, H-15b), 1.25 (1H, m, H-11b), 1.22 (1H, m, H-7b), 1.13 (1H, d, 4.1 Hz, H-14), 1.11 (3H, s, H-19), 1.07 (1H, dd, 4.1, 15,6, H-12b), 0.999 (1H, m, H-9), 0.60 (3H, s, H-18); and ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.0 (C-20), 115.1 (C-21), 75.3 (C-6), 74.7 (C-5), 70.6 (C-3), 67.3 (C-2), 55.8 (C-9), 55.6 (C-17), 45.2 (C-14), 44.0 (C-13), 43.0 (C-10), 37.7 (C-12), 36.3 (C-4), 35.3 (C-1), 35.2 (C-11), 30.4 (C-8), 27.5 (C-16), 25.0 (C-7), 21.5 (C-15), 19.0 (C-19), 13.3 (C-18); HRESIMS: *m*/*z* 351.2528 $[M + H]^+$ (calcd for $C_{21}H_{35}O_4^+$, 351.2530).

Turranin N (**2**): yellow powder, UV (MeOH) λ_{max} (log ε): 280 nm (0.3); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (1H, ddd, 7.6, 6.9, 10.15 Hz, H-20), 4.92-4.99 (2H, m, H-21), 4.12 (1H, d, 11.6 Hz, H-19a), 3.97 (1H, brs, H-6), 3.91 (1H, brs, H-7), 3.73 (1H, d, 11.6 Hz, H-19b), 3.63 (1H, m, H-3), 2.30 (1H, dd, 1.3, 14.5 Hz, H-1a), 2.10 (1H, m, H-4a), 1.93 (1H, m, H-17), 1.89 (1H, m, H-8), 1.83 (1H, m, H-2a), 1.77 (1H, m, H-16a), 1.70 (1H, dt, 3.0, 12.4 Hz, H-12a), 1.66 (1H, m, H-15a), 1.63 (1H, m, H-11a), 1.57 (1H, m, H-4b), 1.56 (1H, m, H-16b), 1.34 (1H, brs, H-5), 1.27 (1H, m, H-11b), 1.22 (1H, m, H-1b), 1.21 (1H, m, H-15b), 1.19 (1H, m, H-2b), 0.99 (1H, m, H-9), 0.95 (1H, m, H-12b) 0.74 (1H, dt, 3.0, 13.2 Hz, H-14), 0.61 (3H, s, H-18) and ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.8 (C-20), 114.9 (C-21), 72.1 (C-3), 71.9 (C-7), 69.4 (C-6), 67.7 (C-19), 55.9 (C-14), 55.7 (C-9), 55.5 (C-17), 48.0 (C-5), 44.0 (C-13), 43.1 (C-1), 40.0 (C-2), 38.8 (C-10), 37.8 (C-12), 32.3 (C-4), 31.5 (C-8), 27.3 (C-16), 24.8 (C-15), 21.1 (C-11), 13.4 (C-18); HRESIMS: *m*/*z* 351.2527 $[M + H]^+$ (calcd for $C_{21}H_{35}O_4^+$, 351.2530).

Turranin O (**3**): white powder, UV (MeOH) λ_{max} (log ε): 235 (0.9), 285 (0.1) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.64 (3H, s, O-CH_3), 3.61 (1H, tt, 4.9, 10.9 Hz, H-3), 2.41 (1H, m, H-20), 2.36 (1H, d, 2.9 Hz, H-6a), 2.34 (1H, brs, H-8), 2.23 (1H, m, H-15a), 2.03 (1H, dd, 3.1, 12.5 Hz, H-6b), 1.92 (1H, m, H-12a), 1.86 (1H, m, H-2a), 1.77 (1H, m, H-1a), 1.75 (1H, m, H-16a), 1.61 (1H, m, H-4a), 1.60 (1H, m, H-17), 1.59 (1H, m, H-11a), 1.53 (1H, m, H-5), 1.51 (1H, m, H-11b), 1.48 (1H, m, H-2b), 1.47 (1H, m, H-14), 1.43 (1H, br d, 4.7 Hz, H-4b), 1.32 (1H, m, H-16b), 1.20 (1H, m, H-12b), 1.18 (1H, d, 7.0 Hz, H-21), 1.12 (1H, m, H-9), 1.08 (3H, s, H-19), 1.02 (1H, d, 5.0 Hz, H-1b), 1.00 (1H, m, H-15b), 0.67 (3H, s, H-18) and ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.7 (C-7), 177.3 (C-OO), 70.8 (C-3), 55.2 (C-9), 52.1 (C-17), 51.5 (O-CH_3), 50.0 (C-8), 48.7 (C-14), 46.8 (C-5), 46.1 (C-6), 42.8 (C-13), 42.4 (C-20), 38.6 (C-12), 38.0 (C-4), 36.2 (C-10), 36.1 (C-1), 31.2 (C-2), 27.4 (C-16), 25.1 (C-15), 21.9 (C-11), 17.3 (C-21), 12.4 (C-18), 11.9 (C-19); HRESIMS: *m*/*z* 377.2831 $[M + H]^+$ (calcd for $C_{23}H_{37}O_4^+$, 377.2692).

4.4 Determination of antiplasmodial activity

Antiplasmodial activity of extracts, fractions and isolated compounds was carried out using the malaria SYBR Green I-based fluorescence assay (Smilkstein et al. 2004; Johnson et al. 2007; Verlinden et al. 2011). Parasitology work and volunteer human blood donation at the University of Pretoria was covered under ethical approval from the Health Sciences Ethics Committee (506/2018) and Natural and Agricultural Sciences Ethics Committee (180000094) to LB. The *P. falciparum* parasites were maintained at 37 ˚C in human erythrocytes (O^+) suspended in complete culture medium [RPMI 1640 medium (Sigma-Aldrich) supplemented with 25 mM HEPES (Sigma-Aldrich), 20 mM D-glucose (Sigma-Aldrich), 200 µM hypoxanthine (Sigma-Aldrich), 0.2% (w/v) sodium bicarbonate, 24 µg/mL gentamycin (Sigma-Aldrich) and 0.5% (w/v) AlbuMAX II] in a hypoxic atmosphere of 90% N_2, 5% O_2, and 5% CO_2. The culture was

synchronised using D-sorbitol (5% w/v) to achieve an >95% homogeneous ring-stage asexual parasite culture. Full dose-response was determined from a 2-fold serial dilution over 9 concentrations (range of 0.156 to 40 µg/mL). Two biological repeats were performed in s in technical triplicates. Chloroquine served as a positive control for inhibition of parasite proliferation. Sigmoidal dose-response analyses were performed using GraphPad Prism (v5) to determine the concentration required to inhibit proliferation of 50% of the parasites (IC_{50}). Results are reported as mean ± S.E..

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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