Antiplasmodial and cytotoxic activities of the constituents of *Turraea robusta* and *Turraea nilotica*

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**A R T I C L E   I N F O**

Article history:
Received 2 July 2015
Received in revised form 15 August 2015
Accepted 26 August 2015
Available online 28 August 2015

Keywords:
*Turraea robusta*
*Turraea nilotica*
Antiplasmodial activity
Cytotoxicity
Limonoid
Toonapubesins F
Toonacinil
Azadironolide

**A B S T R A C T**

**Ethnopharmacological relevance:** *Turraea robusta* and *Turraea nilotica* are African medicinal plants used for the treatment of a wide variety of diseases, including malaria. The genus *Turraea* is rich in limonoids and other triterpenoids known to possess various biological activities.

**Materials and methods:** From the stem bark of *T. robusta* six compounds, and from various parts of *T. nilotica* eleven compounds were isolated by the use of a combination of chromatographic techniques. The structures of the isolated compounds were elucidated using NMR and MS, whilst the relative configuration of one of the isolated compounds, toonapubesin F, was established by X-ray crystallography. The antiplasmodial activities of the crude extracts and the isolated constituents against the D6 and W2 strains of *Plasmodium falciparum* were determined using the semiautomated micro dilution technique that measures the ability of the extracts to inhibit the incorporation of (G-3H, where G is guanine) hypoxanthine into the malaria parasite. The cytotoxicity of the crude extracts and their isolated constituents was evaluated against the mammalian cell lines African monkey kidney (vero), mouse breast cancer (4T1) and human larynx carcinoma (HEP2).

**Results:** The extracts showed good to moderate antimalarial activities, where the extract of the stem bark of *T. robusta* was also cytotoxic against the 4T1 and the HEP2 cells (IC_{50} < 10 μg/ml). The compounds isolated from these extracts were characterized as limonoids, protolimonoids and phytosterol glucosides. These compounds showed good to moderate activities with the most active one being azadironolide, IC_{50} 2.4 ± 0.03 μM and 1.1 ± 0.01 μM against the D6 and W2 strains of *Plasmodium falciparum*, respectively; all other compounds possessed IC_{50} 14.4–40.5 μM. None of the compounds showed significant cytoxicity against vero cells, yet four of them were toxic against the 4T1 and HEP2 cancer cell lines with piscidinol A having IC_{50} 8.0 ± 0.03 and 8.4 ± 0.01 μM against the 4T1 and HEP2 cells, respectively. Diacetylation of piscidinol A resulted in reduced cytotoxicity.

**Conclusion:** From the medicinal plants *T. robusta* and *T. nilotica*, twelve compounds were isolated and characterized; two of the isolated compounds, namely 11-epi-toonacinil and azadironolide showed good antimalarial activity with the highest selectivity indices.

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1. Introduction

The genus *Turraea* (family Meliaceae) consists of circa 70 species, mainly shrubs and small trees, and is widely distributed in Eastern Africa. Several of its species are used in indigenous medicine for the treatment of gastrointestinal disorders (Kokwaro, 2009). For example *T. floribunda* is used as an emetic and purgative. *T. holstii* is used to treat diarrhea and constipation, and *T. mombassana* against excess bile, malaria and other fevers. *Turraea robusta* is utilized to treat malaria, stomach pain, diarrhea and gastrointestinal discomfort (Gathirwa et al., 2008; Kokwaro, 2009). Its leaves are used as an antidote for poisoning. The roots of...
Turracea nilotica are also used for the treatment of stomach disorders in traditional medicine (Kokwaro, 2009).

The methanolic root bark extract of T. robusta was previously reported to possess antiplasmodial and antimalarial activities, with negligible toxicity (mice oral LD₅₀ > 5000 mg/kg body weight) (Irungu et al., 2007; Gathiriwa et al., 2008). Previous phytochemical investigations of the root bark of T. robusta led to the isolation of triterpenoids which included five limonoids (Rajab et al., 1988; Bentley et al., 1992). There are no phytochemical and antiplasmodial reports on the stem bark of T. nilotica Kotschy & Peyr, and of T. robusta Gürke (BN/2011/1) were collected from the University of Nairobi in July 2011. The leaves, stem and root bark of T. nilotica Kotschy & Peyr. (BN/2012/1) were collected from the Mombasa District (03°38'17.06"E, 04°10'04.72"S) in February 2012. The plants were authenticated by Mr. Patrick Mutiso of the Herbarium, School of Biological Science, University of Nairobi where voucher specimens were deposited.

2. Materials and Methods

The stem bark of T. robusta Gürke (BN/2011/1) was collected from Chiromo Campus (01°16’31.34’S; 39°36’38.64’E) of the University of Nairobi in July 2011. The leaves, stem and root bark of T. nilotica Kotschy & Peyr. (BN/2012/1) were collected from the Mombasa District area (03°38’17.06”E, 04°10’04.72”S) in February 2012. The plants were authenticated by Mr. Patrick Mutiso of the Herbarium, School of Biological Science, University of Nairobi where voucher specimens were deposited.

2.1. Antiplasmodial assay

Continuous in vitro cultures of asexual erythrocytic stages of P. falciparum strains (W2 and D6) were maintained following previously described procedures (Kigondo et al., 2009). A drug assay was carried out following a modification of the semiautomated micro dilution technique that measures the ability of the extracts to inhibit the incorporation of (G-3H) hypoxanthine into the malaria parasite (Gathiriwa et al., 2008). Plates were harvested onto glass fiber filters and hypoxanthine (G-3H) uptake determined using a micro-beta trilux liquid scintillation and luminescence counter (Wallac, MicroBeta TriLux) and results recorded as counts per minute (cpm) per well at each drug concentration. Data was transferred into Microsoft Excel 2007, and expressed as percentage of the untreated controls. Results were expressed as the drug concentration required for 50% inhibition of (G-3H) hypoxanthine incorporation into parasite nucleic acid, using a non-linear regression analysis of the dose–response curve. The criterion for scoring activity of compounds described by Batista et al. (2009) was adopted: IC₅₀ < 1 μM, highly active; IC₅₀ ≥ 1 and < 20 μM, active; IC₅₀ ≥ 20-100 μM, moderate activity; IC₅₀ > 100 inactive.

2.2. Cytotoxicity assay

Rapid colorimetric assay was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Mosmann, 1983). This assay is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tetrazolium rings of the pale yellow MTT and thereby form dark blue formazan crystals, which are largely impermeable to cell membranes, resulting in their accumulation within healthy cells. The amount of generated formazan is directly proportional to the number of cells (Mosmann, 1983). In this assay, the mammalian cell lines African monkey kidney (vero), mouse breast cancer (4T1) and human larynx carcinoma (HEp2) were used. Cells were maintained in Eagle’s Minimum Essential Medium (MEM) containing 10% fetal bovine serum (FBS). A cell density of 20,000 cells per well in 100 μL serum were seeded on 96-well plates and incubated for 12 h at 37 °C and 5% CO₂ to attach to the surface. Following 12 h, the medium was replaced with maintenance medium containing the appropriate drug concentration, 0.14–100 μg/mL, or vehicle control (≤ 1% v/v DMSO). After an incubation of 48 h, cell viability was measured by addition of 10 μL of MTT reagent (5 mg MTT in 1 mL of PBS). The plates were incubated for an additional 4 hours at the same conditions. Next, all media was removed from the plates and 100 μL DMSO was added to dissolve the formazan crystals. The plates were read on a Multiskan EX Labsystems scanning multi-well spectrophotometer at 562 nm, and 620 nm as reference. The results were recorded as optical density (OD) per well at each drug concentration, the data was transferred into the software Microsoft Excel 2007 and expressed as percentage of the untreated controls. Percentage cytotoxicity (PC) as compared to the untreated controls was calculated using the following equation:

\[
P_C = \frac{A - B}{A} \times 100
\]

where A is the mean OD of the untreated cells and B is the mean OD at each drug concentration. The drug concentration required for 50% inhibition of cell growth, using nonlinear regression analysis of the dose–response curve, was calculated. Cytotoxicity was defined as toxic if IC₅₀ < 100 μg/mL.

2.3. Extraction and Isolation

2.3.1. Turracea robusta

The air dried and ground stem bark of T. robusta (1.4 kg) was extracted with MeOH/CH₂Cl₂ (1:1) at room temperature (2 × 3 L, 48 h each). The filtrate was dried in vacuo using a rotary evaporator to yield dark red oil (144 g). A 79 g portion of the extract was fractionated using silica gel column chromatography (600 g, 5 cm × 30 cm) with gradient elution of petroleum ether (40–60 °C) and increasing proportions of ethyl acetate. A total of 41 eluents, ca. 250 mL each, were collected and combined into 11 fractions, labeled TR1-TR11 on the basis of their TLC profile. Fraction TR4 (1.2 g) was re-chromatographed over silica gel column chromatography (500 g, 2 cm × 30 cm) using petroleum ether/acetone (95:5) to yield azadirone (1, 32.4 mg). Fraction TR7 (4.0 g) was fractionated on Sephadex LH-20 (40 g, 2 cm × 30 cm) eluting with methanol and was further purified by PTLC eluting with petroleum ether/chloroform/methanol (16:2:1) to yield 12α-acetoxy-7-deacylatedazadirone (2, 7.0 mg) and mizinone (3, 6.3 mg). Fraction TR8 (3.0 g) was re-chromatographed on silica gel column (30 g, 2 cm × 30 cm) eluting with petroleum ether/ethyl acetate (9:1) and a subfraction containing one major compound was purified on PTLC, eluting with petroleum ether/acetone (7:4) to yield azadironeol (5, 16.5 mg). Fractions TR9-T10 were combined (145.2 mg) and were separated on PTLC eluting with petroleum ether/chloroform/methanol (12:2:1) eluent gave turranolide (6, 17.7 mg).
2.3.2. Turraea nilotica

The air dried and grounded stem bark of *T. nilotica* (1.2 kg) was extracted and dried following the procedure described in Section 2.3.1, to yield a dark gum (59 g). A 58 g portion of the extract was fractionated by silica gel column chromatography (400 g, 5 cm × 30 cm) using petroleum ether (40-60 °C) and increasing proportions of ethyl acetate as eluent. A total of 50 fractions, ca. 250 mL each, were collected and combined into 20 major fractions, labeled TN1-20, upon TLC analyses. Repeated silica gel column chromatography (10 g, 2 cm × 30 cm) of fraction TN12 (1.35 g) eluting with 9:1 petroleum ether/acetone yielded niloticin (8, 9.3 mg). Fractions TN13-15 (7 g) were combined and purified by silica gel column chromatography (100 g, 2 × 30 cm) using petroleum ether and increasing portions of acetone; crystallization of the residue from an acetone/dichloromethane mixture yielded toonapubesin F (11, 2.01 mg). A repeated column chromatographic separation of fraction TN16 (3 g) in silica gel (30 g, 2 cm × 30 cm) yielded piscidinol A (10, 775.6 mg). Fraction TN18 was crystallized from methanol/dichloromethane (1:9) to yield hispidol B (9, 84.2 mg).

The root bark of *T. nilotica* (837 g) was extracted and dried as described in Section 2.3.1 above yielding 13 g of a yellowish gum. A portion of the extract (11 g) was fractionated by silica gel column chromatography (88 g, 2 cm × 30 cm) using a petroleum ether: acetone gradient, with increasing polarity. Forty six fractions, ca. 100 mL each, were collected and combined into 20 major fractions, labeled TN21-40, based on their TLC profile. Fraction TN27 (140.7 mg) was purified on PTLC eluting with petroleum ether/acetone (9:1) to yield azadirone (1, 8.3 mg). Fraction TN32 (366 mg) was subjected to RP-HPLC (CH3OH/water) yielding 12α-acetoxy-7-deacetylazadirone (2, 18.5 mg) and mizikonone (3, 4.4 mg). Repeated column chromatographic purification of fraction TN36 (358 mg) followed by PTLC with a petroleum ether/chloroform/methanol (10:2:1) mixture as eluent yielded 1α,3α-diacetyltigloylvinilisin (7, 11.6 mg).

The dried and grounded leaves of *T. nilotica* (500 g) were extracted as described in Section 2.3.1, yielding 30 g of a dark green gum. A 20 g portion of the crude extract was fractionated using silica gel column chromatography (200 g, 5 cm × 30 cm) with petroleum ether/acetone gradient with increasing polarity. A total of fifty eight fractions, ca 100 mL each, were collected and combined into the 12 major fractions and labeled TN41-52, based on TLC profiling. Fraction TN43 was crystallized from acetone giving β-sitosterol (15) and stigmasterol (16) as a mixture (4.7 mg). Fraction TN50 was crystallized from acetone yielding sitosterol-3-O-β-D-glucopyranoside acetate (12) and stigmasterol-3-O-β-D-glucopyranoside acetate (13) as a mixture (9.8 mg). Fraction TN52 was crystallized in acetone to yield sitosterol-3-O-β-D-glucopyranoside (14, 4.7 mg).

2.3.3. Structure elucidation

For structure elucidation 1H, 13C, gCOSY, gNOESY, gHSQC and gHMBC NMR spectra were acquired on a Bruker Avance III HD 800 MHz, on a Varian VNMRS-500 or a Varian 400 MR spectrometer. The spectra were processed using the MestReNova (v9.0.0) software. Chemical shifts were referred indirectly to tetramethysilane via the residual solvent signal (CHCl3, 1H at 7.26 ppm and 13C at 77.16 ppm). LC/ESI/MS spectra was acquired on a PE SCIEX API 150EX instrument (Perkin Elmer, Waltham, MA, USA) equipped with a Turbolon spray ion source (30 eV ionization energy) and a Gemini 5 mm-C18 110 Å HPLC column, using water: acetonitrile gradient (80:20 to 20:80).

2.4. X-ray diffraction

Crystals of compound 11 was selected and mounted under a Rigaku X-AXIS llc image plate system. Diffraction intensities were measured using graphite-monochromated Mo Kα (λ = 0.71073 Å) radiation from a RU-H3R rotating anode operated at 50 kV and 40 mA. Using the X-AXIS llc detector, 90 oscillation photos with a rotation angle of 2° were collected and processed using the CrystalClear software package. An empirical absorption correction was applied using the REQAB program under CrystalClear. All structures were solved by direct methods (SIR 97) (Altomare et al., 1999) and refined using full-matrix least-squares calculations on F2 (SHELXL-97) (Sheldrick, 2007) operating in the WinGX program package. Anisotropic thermal displacement parameters were refined for all the non-hydrogen atoms. Hydrogen atoms were included in calculated positions and refined using a riding model. Displacement ellipsoids are drawn with ORTEP-3 for Windows under WinGX.

2.5. Acetylation

A 20 mg portion of compound 8 or 10 was dissolved in 1 mL pyridine and then 1 mL acetic anhydride was added. The mixture was stirred overnight, and subsequently methanol was added and the solvent was removed under vacuum. The acetate precipitated by addition of water while stirring briskly, and it was filtered and dried. The procedure yielded niloticin acetate (17, 5 mg) and piscidinol A diacetate (18, 7 mg), respectively.

3. Results and discussion

3.1. Antiplasmodial and cytotoxic activities

The crude extracts of *T. nilotica* and *T. robusta* were evaluated for antiplasmodial activity against the chloroquine-resistant (W2) and chloroquine-sensitive (D6) *Plasmodium falciparum* strains (Table 1). The stem bark of *T. robusta* showed high antiplasmodial activity with IC50 2.8 ± 0.02 μg/mL and 2.3 ± 0.05 μg/mL against the W2 and D6 strains, respectively. The stem and root barks of *T. nilotica* also displayed considerable antiplasmodial activities (IC50 < 10 μg/mL) while its leaves showed only moderate activity.

Cytotoxicity of these extracts was evaluated against three mammalian cell lines, namely African green monkey kidney (vero), mouse breast cancer (4T1) and human larynx carcinoma (HEP2) (Table 1). The stem bark extract of *T. robusta* was cytotoxic against the 4T1 and the HEP2 cells (IC50 < 10 μg/mL), whereas the extract of *T. nilotica* possessed moderate cytotoxicity against the three cell lines studied. Notably, all the extracts had low selectivity index ( < 10), defined as the ratio of IC50 vero cells to IC50 *P. falciparum* (D6). This may indicate that the good to moderate antiplasmodial activity observed for the above extracts may be due to their general cytotoxicity. The fractions obtained from the crude extracts were evaluated for their cytotoxicity against vero cells. Generally, they had lower cytotoxicity than the crude extracts. Except for fraction TN12 (IC50 = 4.34 ± 0.04 μg/mL), most fractions showed moderate cytotoxicity.

From the stem bark of *T. robusta* Garke six known compounds were isolated: three ring A-D-intact limonoids, namely azadirone (1), 12α-acetoxy-7-deacetylazadirone (2) and mizikonone (3), and the ring B seco limonoid 11-epi-toonacinil (4) (Fig. 1). In addition, it gave two other triterpenoids azadironeolide (5) and turranolide (6). Compound 5 was isolated as an epimeric mixture, indicated by doubling of some of its NMR signals. Epimeric mixtures of limonoids having a hemiacetal functionality in place of a furan ring.
were previously reported (Cheplogoi and Mulholland, 2003; McFarland et al., 2004). The secondary metabolite content of *T. robusta* stem bark was similar to those reported for other *Turraea* species, which have limonoids as the main constituents. Compounds 2 and 4 were reported from this species for the first time. This is the first report on the occurrence of compound 5 in the genus *Turraea*.

From the root bark of *T. nilotica*, four limonoids azadirone (1), 12α-acetoxy-7-deacety lazadirone (2), mizikonone (3), (Fig. 1) and 1α,3α-diacety-7α-tigloyvlasin (7) (Fig. 2) were isolated. From its stem bark, four protolimonoids nilotin (8), hispidol B (9), piscidinol A (10) and toonapubesin F (11) were isolated. A mixture of sitosterol-3-O-β-D-glucopyranoside acetate (12) and stigmasterol-3-O-β-D-glucopyranoside acetate (13), sitosterol-3-O-β-D-glucopyranoside (14) as well as a mixture of 5-sitosterol (15) and stigmasterol (16) were isolated from the leaves. Compounds 1, 2, 3, 7 and 9 are reported here from this species for the first time. There is no previous report on compounds 11-14 from the genus *Turraea*.

The structure of toonapubesin F (11), first isolated from *Toona ciliate* var. *pubesins* (Wang et al., 2011), was further confirmed by an X-ray crystallographic analysis (Fig. 3) from a single crystal obtained by slow crystallization from a mixture of dichloromethane and acetone. The atomic coordinates are given in the Supplementary Information.

As the stereochemistry of the toonapubesin backbone is known (Wang et al., 2011), the chirogenic center at C4 is (S). Hence, this data allows identification of the oxidized methyl functionality on C4, i.e. C25 and not C24 (Fig. 3).

The roots, stem and leaves of *T. nilotica*, were found to contain different secondary metabolites. Limonoids were isolated from the root bark, and protolimonoids from the stem bark while phytosterols were isolated from the leaves. Limonoids, protolimonoids and steroids share a tetracyclic triterpenoid skeleton; however, they differ in the oxidation state of their side chain. Based on the previously proposed biosynthetic pathway of limonoids, (Champagne et al., 1992; Tan and Luo, 2011) *T. nilotica* is likely to follow the phytosterol (leaves)→protolimonoid (stem bark)→limonoid (root bark) biosynthetic pathway.

The antiplasmodial potency of some limonoids and other triterpenoids has been previously reported (Meneerat et al., 2008; Mohamad et al., 2009). Therefore, some of the compounds isolated in this work were tested for antiplasmodial activities against the D6 and W2 *Plasmodium falciparum* strains (Table 2). Their activity was scored according to the classification of Batista et al. (2009), where IC50 < 1 μM is highly active; 1 ≤ IC50 ≤ 20 μM is active, 20 μM ≤ IC50 ≤ 100 μM is moderately active, and IC50 > 100 μM is inactive. Of the eight compounds tested, two showed good activity with the most active substance being the epimeric mixture azadironolide (5) (IC50 = 2.4 μM), and six showed moderate activities against the W2 and D6 strains (Table 2). The antiplasmodial

![Fig. 1. The structures of the limonoids azadirone (1), 12α-acetoxy-7-deacety lazadirone (2), and mizikonone (3), the ring B seco limonoid 11-epi-toonacilin (4), the tri-terpenoids azadironolide (5), and turranolide (6), isolated from the stem bark of *Turraea robusta*.]
activity of compound 1 is in agreement with the previous literature data (Chianese et al., 2010).

The isolated compounds were also tested for cytotoxicity (Table 2) against the mammalian cell line African green monkey kidney (vero cells). Most of them showed moderate cytotoxicity (IC\textsubscript{50} > 20 \mu M) and a low selectivity index [SI=IC\textsubscript{50}(vero)/IC\textsubscript{50}(D6) < 10]. Thus, the observed moderate antimalarial activity of these compounds is likely due to general cytotoxicity, rather than due to a specific activity against the Plasmodium parasite. It should, however, be noted that compounds 4 and 5 that are classified as active, were observed to have comparably high selectivity index SI > 10.5 and 11.5, respectively, though significantly lower than chloroquine (SI=5702).

Although the investigated plants are in traditional medicinal use against malaria, and their crude extracts showed promising antimalarial activities, their isolated metabolites did not display considerable activity against P. falciparum. This may be explained by synergetic effects or by the loss of one or some minor yet highly active metabolites during the purification process. For an improved understanding of the traditional medicinal applicability of these plants further studies are necessary.

Some limonoids and protolimonoids isolated from other plants were previously reported to possess substantial cytotoxicity (Maneerat et al., 2008), which, along with the high to moderate cytotoxicity of the crude extracts and isolated compounds against ‘normal’ vero cells motivated the evaluation of cytotoxicity of the isolated constituents against the cancerous cell lines 4T1 and HEp2 (Table 2).

Compounds 1, 5, 8–10 showed high cytotoxicities against the

Table 2

| Compound     | D6   | Vero | 4T1 | HEp2 | Vero | SI<sup>+</sup> |
|--------------|------|------|-----|------|------|---------------|
| Azadirone (1) | 23.4 ± 0.2 | 29.6 ± 1.0 | 14.0 ± 0.0 | 12.8 ± 0.0 | > 229.4b | 9.8 |
| 12a-Acetoxy-7-deacetylazadirone (2) | 31.0 ± 0.2 | 30.2 ± 0.5 | 104.6 ± 7.1 | 40.3 ± 2.2 | 134.1 ± 2.9 | 4.3 |
| Mzikonone (3) | 36.6 ± 0.8 | 40.5 ± 3.7 | 38.8 ± 0.4 | 50.3 ± 0.0 | 139.6 ± 4.7 | 3.8 |
| 11-epi-Toonacin (4) | 17.1 ± 0.2 | 14.4 ± 0.5 | 88.6 ± 3.2 | 68.1 ± 1.3 | > 180.5b | > 10.5 |
| Azadironolide (5) | 2.4 ± 0.0 | 11.0 ± 0.0 | 14.7 ± 0.2 | 8.5 ± 0.5 | 276.0 ± 6.6 | 11.5 |
| Niloticin (8) | 48.2 ± 2.3 | 77.0 ± 5.7 | 14.5 ± 0.5 | 6.9 ± 0.6 | 145.4 ± 0.4 | 0.3 |
| Hispidol B (9) | 36.8 ± 2.0 | 37.2 ± 3.2 | 21.7 ± 3.2 | 7.4 ± 0.7 | 130.0 ± 3.1 | 3.5 |
| Piscidinol A (10) | 37.6 ± 1.4 | 36.3 ± 4.4 | 8.0 ± 0.0 | 8.4 ± 0.0 | 411.5 ± 5.8 | 1.1 |
| Niloticin acetate (15) | 68.3 ± 5.3 | 172.9 ± 4.5 | ND | 121.9 ± 0.8 | > 200.8b | ND |
| Piscidinol A diacetate (16) | ND | ND | ND | 15.2 ± 0.8 | > 179.2<sup>a</sup> | ND |
| Chloroquine | 7.7 ± 0.02<sup>b</sup> | 108.0 ± 0.1<sup>c</sup> | ND | ND | 43.9 ± 0.5 | 5701 |

The mean values of at least three independent experiments are reported. ND: not determined, due to the small amount of sample available.

<sup>a</sup> SI=IC\textsubscript{50} (vero)/IC\textsubscript{50}(D6).

<sup>b</sup> Values > 100 ug/ml not cytotoxic. Positive control: podophyllum resin, IC\textsubscript{50} (4T1)=0.47 ± 0.05 \mu g/ml, melarsoprol IC\textsubscript{50} (Vero)=0.76 ± 0.01 \mu g/ml.

<sup>c</sup> IC\textsubscript{50} half maximal inhibitory concentration given in nM for chloroquine.
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Table 3 Occurrence of some limonoids in Turraea species.

| Limonoid | Turraea species |
|----------|-----------------|
| Azadirone (1) | T. nilotica T. robusta |
| 12α-acetoxy-7-deacetylagadirone (2) | T. cornucopia T. parvifolia T. nilotica T. pubescens |
| Mzikonone (3) | T. robusta T. cornucopia T. pubescens T. robusta |
| 11-epi-toonacin (4) | T. robusta T. holstii T. pubescens T. cornucopia |
| 1x3,6-diacety-7α-tigloylvasin (7) | T. nilotica T. parvifola |

4T1 and HeP2 cell lines with IC_{50} < 20 µM. It should be stressed that these compounds showed moderate cytotoxicity against ‘normal’ vero cells, yet high cytotoxicity against cancerous cell lines. Hence these compounds may be promising leads for development of anticancer agents (Diantini et al., 2012).

For assessment of the importance of the free hydroxyl groups of compound 8 and 10 on their bioactivities, they were acetylated to give niloticin acetate (Fig. 4). The acetate derivatives 17 and 18 showed lower cytotoxicity as compared to the parent, non-acetylated compounds 8 and 10, indicating the importance of free hydroxyl group(s) in the side chain. A reduction in antiplasmodial activity of compound 17 in comparison to the parent compound 8 was also observed.

3.2. The chemotaxonomic significance of Turraea limonoids

Various classes of limonoids were reported from the genus Turraea with each species synthesizing more than one class of limonoids. Ring intact limonoids were reported from eight Turraea species, namely T. robusta, T. nilotica, T. cornucopia, T. parvifolia, T. floribunda, T. holstii, T. wakefieldii and T. pubescens (Bentley et al., 1992; Ndung’u et al., 2004; Owino et al., 2008; Yuan et al., 2013).

So far, ring A seco limonoids have only been isolated from T. wakefieldii (Ndung’u et al., 2003), whereas ring B seco limonoids were reported in T. floribunda, T. holstii and T. pubescens (Mulholland et al., 1998; McFarland et al., 2004; Yuan et al., 2013). Ring A-B seco limonoids were reported from T.ombambosa and T. obtusifolia, while ring C seco limonoids from T. holstii and T. pubescens (Adul et al., 1993; Sarker et al., 1997; Mulholland et al., 1998; Yuan et al., 2013).

Interestingly, so far no ring D seco limonoid was reported from the genus Turraea although several examples were isolated from other Meliaceae genera. The presence of compounds 1-4 and 7 in T. robusta and T. nilotica is in line with the fact that limonoids are common constituents of the genus Turraea. Except for compound 1 that was reported from another genera of the Meliaceae family (Zhou et al., 1997), the limonoids disclosed here have not been reported from any other genus. The occurrence of five limonoids in Turraea species is shown in Table 3, among which 3 was isolated from five species.

4. Conclusion

Six compounds were isolated from the stem bark of Turraea robusta. Four of them were limonoids and two of them triterpenoids. Of these six compounds, azadironolide (5) is new to the genus. The secondary metabolites present in the stem bark were also found in the root bark. Compound 3 is a common limonoid in the genus Turraea, and was previously reported from five Turraea species (Cheplogoi and Mulholland, 2003; Owino et al., 2008; Yuan et al., 2013). From the leaves, root and stem bark of Turraea nilotica twelve compounds were isolated. Toonapubesin F (11), sitosterol 3-O-β-D-glucopyranoside acetate (12), stigmasterol-3-O-β-D-glucopyranoside acetate (13) and sitosterol-3-O-β-D-glucopyranoside (14) are new to the genus. Different secondary metabolites were observed in the leaves, and thus the roots and the stem were observed to contain limonoids and protolimonoids, whereas the leaves contained phytosterols.

Out of the isolated compounds, 11-epi-toonaconin (4) and azadironolide (5) showed good antiplasmodial activity with the highest selectivity indices among the isolated compounds. Azadirone (5), niloticon (8), hispidol B (9) and piscidol A (10) were cytotoxic to HeP2 and 4T1 cells with IC_{50} < 20 µM. The cytotoxicity of the latter constituents against the ‘normal’ vero cell line was comparably low, indicating some degree of selectivity.

Acknowledgments

This work was supported by IFS (grant no. F/4575-2), UNICEF/UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (SPH-Q10-RTG-6 ID: A90208) and by the Swedish Research Council (grant no. 2012–6124).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2015.08.039.

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