Bioassay-guided isolation of antiplasmodial and antimicrobial constituents from the roots of Terminalia albida

Bioassay-guided isolation of antiplasmodial and antimicrobial constituents from the roots of *Terminalia albida*

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Abstract

Ethnopharmacological relevance: Terminalia albida (Combretaceae), widely used in Guinean traditional medicine, showed promising activity against *Plasmodium falciparum* and *Candida albicans* in previous studies. Bioassay-guided fractionation was carried out in order to isolate the compounds responsible for these activities.

Materials and methods: Fractionation and isolation were performed by flash chromatography, followed by semi-preparative HPLC-DAD-MS. The structural elucidation of the isolated compounds was carried out by 1D and 2D NMR as well as HR-ESI-MS. Isolated compounds were evaluated against *Plasmodium falciparum*, *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*, and their cytotoxicity against MRC-5 cells was determined.

Results: Bioassay-guided fractionation of *Terminalia albida* root resulted in the isolation of 14 compounds (**1**–**14**), and their antimicrobial properties were evaluated. Pantolactone (**1**) (IC_{50} 0.60 ± 0.03 µM) demonstrated significant activity against *P. falciparum.* Other compounds, including 3,4,3'-tri-O-methyl-ellagic acid **(3)**, the triterpenes arjunolic acid **(5),** arjungenin **(6)**, arjunic acid **(7)** and arjunglucoside II **(10),** and the phenol glycoside calophymembranside-B (**14**), were less active and showed IC_{50} values in the range 5 – 15 µM. None of the tested compound showed antibacterial or antifungal activity.

Conclusion: These results may explain at least in part the activity of the root extract of *T. albida* against *P. falciparum*.

Key words: *Terminalia albida*; Combretaceae; antiplasmodial activity; triterpenes; pantolactone

1.Introduction

Medicinal plants have been serving for many years as sources of therapeutic agents and have shown beneficial uses in multiple areas of application (Yuan et al., 2016). Indeed, many bioactive products discovered from natural sources so far have played a pivotal role in improving human health, in spite of facing a tough competition with synthetic compounds (Veeresham, 2012). Of the 1,562 drugs approved between 1981 and 2014 in USA by the Food and Drug Administration, 64 (4%) were unaltered natural products, 141 (9%) were botanical drugs (mixtures), 320 (21%) were natural derivatives and 61 (4%) were synthetic drugs but containing natural pharmacophores (Newman and Cragg, 2016). The increased interest in herbal medicine by the general public has stimulated a greater scientific awareness in exploring and understanding the pharmacologically active constituents of medicinal plants (Che and Zhang, 2019; Sharma and Gupta, 2015). Nowadays medicinal plants still represent an important pool for the identification of novel drug leads (Atanasov et al., 2015). However, the potential of many of them as new antimicrobial scaffolds has not sufficiently been explored (Bapela et al., 2019). Furthermore, the isolation and purification of plant compounds in an adequate yield remains a major concern (Savi et al., 2019).

In Guinea, medicinal plants play an important role in the management of the most common diseases, especially in rural areas. *Terminalia albida* Sc. Elliot (Combretaceae), distributed throughout the Guinean regions, is widely used in traditional medicine to treat a variety of ailments such as malaria, skin diseases, oral diseases and urinary disorders (Baldé et al., 2020; Balde et al., 2015; Traore et al., 2013; Magassouba et al., 2007). It has been demonstrated that the methanolic extract of the stem bark of *T. albida* considerably increased the survival rate in mice infected with *Plasmodium berghei* (Camara et al., 2019). A preliminary biological screening carried out as part of our research program confirmed the previously reported antiplasmodial activity and gave a possible explanation for the use of extracts in case of oral and skin diseases. Promising *in vitro* activities against *Candida albicans*, *Staphylococcus aureus* and *Plasmodium falciparum* were obtained for both methanolic and dichloromethane extracts of the root of *T. albida* (Baldé et al., 2020)**.** However, hitherto most of the compounds responsible for such activities have not been isolated or identified yet. Therefore, the bioassay-guided isolation of *Terminalia albida* root extracts has been carried out.

2.Materials and methods

2.1. Solvent and reagents

Dichloromethane, ethyl acetate, *n*-butanol, *n*-hexane, acetone, acetonitrile, acetonitrile far UV and methanol (all HPLC grade) were purchased from Fisher Scientific (Leicestershire, UK). All reagents, such as sulphuric acid and formic acid (eluent additive for HPLC) were purchased from Acros Organics (Geel, Belgium) or Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and formic acid (both for UPLC-MS) were obtained from Biosolve Chimie (Dieuze, France). Ultrapure water was obtained using a Milli-Q system from Millipore (Bedford, MA, USA). NMR deuterated solvents ($CDCl_3$, acetonitrile-d_3, methanol-d_4, DMSO-d_6, pyridine-d_5) were obtained from Sigma-Aldrich.

2.2. General experimental methods

Purification of extracts and fractions was carried out using a Grace Reveleris X2 flash chromatographic system (Lokeren, Belgium) equipped with an evaporative light scattering detector (ELSD), a diode array detector (DAD), and a fraction collector. The chromatographic profiles of fractions collected during different isolation steps were obtained on an Agilent HPLC system (1200 series) and/or by thin layer chromatography (TLC) on NP F254 plates (20 cm × 20 cm) from Merck (Darmstadt, Germany). The TLC plates were observed under UV light (254 and 366 nm) and under visible light after spraying with a vanillin-sulphuric acid reagent (prepared by mixing 5g vanillin with 475 mL ethanol and 25mL sulphuric acid).

A semipreparative HPLC system equipped with DAD and ESI-MS detectors was used for the isolation of the pure compounds. The system was composed of a sample manager, injector, and collector (2767), a quaternary gradient module (2545), a System Fluidics Organizer, an HPLC pump (515), a diode array detector (2998), and a Micromass Quattro TQD mass spectrometer, all supplied by Waters (Milford, MA, USA). For data processing MassLynx version 4.1 was used. Optical rotations were mesured on a JASCO P-2000 spectropolarimeter (Easton, MD,USA).

1D and 2D Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker DRX-400 NMR spectrometer (Rheinstetten, Germany) equipped with either a 3 mm inverse broadband (BBI) probe or a 5 mm dual $^{1}H/^{13}C$ probe using standard Bruker pulse sequences and operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C NMR spectra. The spectra were processed with Topspin (version 4.0.6).

Accurate mass measurements were carried out on a Xevo-G2XS-QTof mass spectrometer (Waters) coupled with an Acquity LC system equipped with MassLynx version 4.1 software. A Waters Acquity UHPLC BEH Shield RP18 column (3.0 mm x 150 mm, 1.7µm) was used with a mobile phase consisting of water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), which were pumped at a rate of 0.4 ml/min. The gradient system was set as follows: 2% B (0-1 min), 2-100% B (1-5 min), 100% B (5-7 min), 100-2% B (7-8 min), 2% B (8-10 min). For all analyses, full scan data were recorded in ESI (−) and ESI (+) mode from *m/z* 50 to 1500 in sensitivity mode (approximate resolution: 22000 FWHM) using a spray voltage at either −1.0 kV and +1.5 kV, respectively. Cone gas flow and desolvation gas flow were set at 50.0 L/h and 1000.0 L/h, respectively; and source temperature and desolvation temperature at 120 °C and 550 °C, respectively. Leucine encephalin was used as lock mass during the analysis.

2.3.Plant Material

Roots of *Terminalia albida* were harvested in Dubréka, Republic of Guinea in June 2016. The plant was identified by the botanists from the Research and Valorization Center on Medicinal Plants, Dubréka, where a voucher specimen (D36HK13) is kept. The collected root samples were dried at room temperature and milled.

2.4.Extraction and isolation

The dried and milled root of *Terminalia albida* (470.9 g), defatted four times wih *n*-hexane (2.0 L each 24 h) was extracted five times (2.5 L each 24 h) with 80% MeOH. The filtrate was concentrated under reduced pressure and freeze-dried to obtain the crude methanolic extract (110 g), which was redissolved in water and successively partitioned with CH_2Cl_2, EtOAc, and *n*-BuOH to yield a dichloromethane soluble fraction (A_D) (6.0 g), an ethyl actetate soluble fraction (A_E) (15.1 g), an *n*-butanol soluble fraction (A_N) (24.2 g) and a residual water fraction. The dichloromethane fraction was further separated by flash chromatography using a silicagel column (GraceResolv 80 g), eluted with a gradient of dichloromethane (A), ethyl acetate (B) and methanol (C) at a flow rate of 60 mL/min. The gradient was set as follows: 100% A (0-10 min), followed by an increasing concentration of B (10-60 min) till 100% B; then an increasing concentration of C (60-85 min) till 100% C; finally, this condition was maintained for 10 min (85-100mn). The collected tubes were pooled in 10 fractions (A_{D1}-A_{D10}) based on similarity of their TLC profiles (mobile phase: dichloromethane / ethyl acetate: 7/3 v/v).

The purification of the most active fractions A_{D1} (0.8 g) and A_{D3} (1.2 g) was performed using flash chromatography with a silicagel column (GraceResolv 40 g) eluted with a mixture of *n*-hexane (A), dichloromethane (B) and ethyl acetate (C) at a flow rate of 40 mL/min resulting in the isolation of compounds **1** (4.2 mg), **2** (6.5 mg) and **3** (9.6 mg). The gradient was set as follows: 0-5 min: 100% A, followed by an increasing concentration of B in 30 min (5-35 min: A - 100% B), which was maintained for 10 min (35-45 min: 100% B); then the amount of C was increased during 20 min (45-65 min: B - 100% C); finally, this condition was maintained for 10 min. The isolation and purification of compounds **4** (45.3 mg) from fraction A_{D7} (0.53 g) and **5** (7.4 mg) from fraction A_{D8} (0.62 g) (sample concentration 20 mg/mL; injection volume 400 µL) was achieved on the semipreparative HPLC-MS using a C18 Kinetex column (250 mm × 10.0 mm, particle size, 5 µm) from Phenomenex (Utrecht, The Netherlands) and eluted with a gradient of H_2O + 0.1% formic acid (A) and acetonitrile far UV (B) at a flow rate of 3 mL/min. The DAD spectrum was recorded from 200 to 450 nm, and mass spectra in the ESI (+) and ESI (-) modes, MS scan range: *m/z* 100 to 1000; capillary voltage 3.00 kV, cone voltage 50 V, extractor voltage 3 V, source temperature135 °C, desolvation temperature 400 °C, desolvation gas flow 750 L/h, cone gas flow 50 L/h.

The ethyl acetate fraction (5.0 g) was subjected to flash chromatography using a silicagel column (GraceResolv 80 g), eluted with a gradient of dichloromethane (A), ethyl acetate (B) and methanol (C) at a flow rate of 60 mL/min. The gradient was set as follows: 100% A (0-8 min), followed by an increasing concentration of B (8-40 min) till 100% B, which was maintained for 10 min (40-50 min); then the amount of C was increased till 100% (50-80 min) and this condition was maintained for 10 min (80-90 min). Fractions were pooled in 5 sub-fractions (A_{E1}-A_{E5}) based on the similarity of their TLC and HPLC profiles. A Kinetex C18 (100mm × 2.10 mm, 2.6 µm) (Phenomenex, Torrance, CA, USA) column was used with H2O + 0.1 % formic acid (A) and acetonitrile (B) as the mobile phase. For all fractions a flow rate of 1 mL/mn of the following gradient (min/%B) was used: 0.0/5, 5.0/5, 50.0/95, 55.0/95, 57.0/5, 62.0/5.

Several fractions were subjected to semipreparative HPLC-MS analysis resulting in the purification of fraction A_{E1} (0.6 g) with the following gradient (0-5 min) 30% B, (5-35 min) 50% B, (35−40 min) 50% B, (40−50 min) 95% B, affording compounds **6** (5.0 mg) and **7** (24.1 mg). The purification of fraction A_{E3} (0.81g) with the gradient (0-5 min) 30% B, (5-35mn) 35% B, (35−40 min) 35% B, (40−50 min) 95% B yielded compounds **8** (9.3 mg) and **9** (5.2 mg). Fraction A_{E4} (1.3 g), using the gradient (0-5 min) 20% B, (5-35mn) 30% B, (35−45 min) 95 %

B, resulted in the isolation of compounds **10** (8.4 mg), **11** (4.7 mg), **12** (64 mg) **13** (43.4 mg) and **14** (4.7 mg). During the whole purification process, the flow rate was set at 3.0 mL/min and the sample concentration for all fractions was 20 mg/mL The injection volume for fraction A_{E1} and A_{E3} was 400 µL, while for fraction A_{E4}, 300 µL was injected.

2.5.LC-ESI-MS

The 80% ethanol extract was prepared by dissolving 5 g of *Terminalia albida* root powder in 30 mL of solvent and stirred at room temperature for 24 h. The extract was evaporated under reduced pressure at 35 °C. For the LC-MS analysis the extract was dissolved in 80% MeOH (v/v) at a concentration of 1 mg/mL, which was diluted with water in order to obtain a final concentration of 0.1 mg/mL.

2.6.Biological evaluation

2.6.1.Antibacterial and antifungal activity

The antimicrobial activity of all fractions and pure compounds was evaluated according to Cos et al. (2006) and Baldé et al. (2010). Fractions and pure compounds were tested against the following microorganisms: *Escherichia coli* ATCC8739 (Gram-negative), *Staphylococcus aureus* ATCC 6538 (Gram-positive) and *Candida albicans* ATCC59630 (yeast). The following positive controls were used: flucytosine for *C. albicans* (IC_{50} 0.7 ± 0.01 µM) and doxycycline for *S. aureus* (IC_{50} 0.28 ± 0.2 µM) and *E. coli* (IC_{50} 0.6 ± 0.3 µM). These reference compounds are routinely used in the screening platform and their activities were in the range that is usually observed.

2.6.2.Antiplasmodial and cytotoxicity assays

Antiplasmodial activity and cytotoxicity were assessed as previously described by Cos et al. (2006) and Tuenter et al. (2016). Fractions and pure compounds were tested *in vitro* against the chloroquine-resistant strain *Plasmodium falciparum* K1 using the lactate dehydrogenase assay. The most active compounds were tested in triplicate; mean and standard deviation (SD) were calculated. Tamoxifen was used as the positive control for cytotoxicity on MRC-5 cells (IC_{50} 10.0 ± 1.5 µM), and chloroquine (IC_{50} 0.15 ± 0.10 µM) for *P. falciparum.* These reference compounds are routinely used in the screening platform and their activities were in the range that is usually observed.

3.Results and Discussion

The present study reports the isolation and identification of the active principles of *Terminalia albida* used in Guinean traditional medicine for the management of microbial diseases including malaria. The dichloromethane and ethyl acetate fractions with an IC_{50} of 11.9 µg/mL and 5.9 µg/ml, respectively, against *P. falciparum* (Table 1) were subjected to normal-phase and reverse-phase flash chromatography and semipreparative HPLC with DAD and ESIMS detection to afford 14 compounds. Five compounds were isolated and identified from the dichloromethane extract and nine from the ethyl acetate extract. Chemical structures of the isolated compounds were established by HR-ESI-MS combined with 1D NMR (^{1}H-, ^{13}C-NMR, DEPT 135, DEPT 90) and 2D NMR (COSY, HSQC, HMBC). The ^{1}H- and ^{13}C-NMR spectroscopic as well as mass spectrometric data, of these compounds, available as Supplementary Material, were in agreement with previously published data, allowing the identification of pantolactone (3-hydroxy-4,4-dimethyldihydrofuran-2(3H)-one) (**1**) (Brown et al., 1991), friedelin **(2)** (Akihisa et al., 2002) 3,4,3'-tri-*O*-methyl-ellagic-acid (**3**) (Öksüz et al., 2002), sericic acid **(4)** (Yeo et al., 1998)**,** arjunolic acid (**5)** (Shao et al., 1995), arjungenin (**6**) (Mahato and Kundu, 1994), arjunic acid **(7),** 2α,3β,21β,23-tetrahydroxyolean-12-en-28-oic-acid **(8)** (Ngounou et al., 1999), terminolic acid **(9)** (Li et al., 2002), arjunolic acid 28-*O*-β-D-glucopyranoside (arjunglucoside II) **(10)** (Lalith et al., 1993), chebuloside II (**11)** (Kundu and Mahato, 1993), sericoside **(12)** (Terreaux et al., 1996), arjunglycoside I (**13**) (Xiao-Hong et al., 1992) and calophymembranside B (**14**) (Zou et al., 2005) (Fig. 1). Apart from pantolactone (**1**), an ellagic acid derivative (**3**) and calophymembranside B (**14**), which is a hydroxytyrosol-diglycoside, all isolated compounds were triterpenes, most of them typical for the genus *Terminalia*. It can be noted that sericoside (**12**) is the C-28 glycosyl ester of sericic acid (**4**), that arjunglucoside II (**10**) is the C-28 glycosyl ester of arjunolic acid (**5**), that arjunglycoside I (**13**) is the C-28 glycosyl ester of arjungenin (**6**), and that chebuloside II (**11**) is the C-28 glycosyl ester of terminolic acid (**9**).

None of the isolated compounds showed cytotoxicity up to the highest test concentration of 64 µM. The antiplasmodial activity of all these compounds has been determined and the highest activity was obtained for pantolactone (**1**) (0.6 ± 0.03 µM) (Table 1). A range of other constituents, including 3,4,3'-tri-O-methyl-ellagic acid **(3)**; the triterpenes arjunolic acid **(5),** arjungenin **(6)**, arjunic acid **(7)** and arjunic acid 28-*O*-β-D-glucopyranoside **(10);** and the phenol glycoside calophymembranside-B (**14**), showed IC_{50} values in the range 5 – 15 µM. Apart from

friedelin (**2**) (IC_{50} 31.19 µM) and sericoside (**12**) (IC_{50} 44.4 µM), all other compounds were completely inactive. To best of our knowledge, this is the first report on the *in vitro* antiplasmodial activity of pantolactone (**1**), a γ-butyrolactone derivative. Pantolactone was obtained before from *Pandanus simplex*, an endemic species in the Philippines (Tan et al., 2012); glycosides were found in *Lygopodium japonicum* (Chen et al., 2010) and *Oryza sativa* (rice) (Menegus et al., 1995; Scaglioni et al., 2000).

Most of the isolated compounds in our study were oleanane-type triterpenoids. The structural differences between these 10 oleanane triterpenoids occur at positions C-6, C-19, C-21, C-23 and C-28. As it appeared from the results of the biological assay, the substitution pattern of the triterpenoids, mainly consisting of hydroxyl groups and glucopyranosyl ester substitution at C-28, had an important influence on the antiplasmodial activity. Also the α- or β-configuration of hydroxyls linked to asymmetric carbons had a strong impact on the bioactivity of certain triterpenes. Amongst the tested oleanane terpenoids, arjungenin (**6**) was the most active against *P falciparum* with an IC_{50} value of 5.9 ± 2.2 µM. This activity is much higher compared to that recently reported by Oluyemi et al. (2020) against both *P. falciparum* chloroquine sensitive (D10) and resistant (W2) strains (81 ± 17 and 127 ± 9 µM, respectively).

Based on the activity of arjungenin (**6**), an attempt was made to establish some structure-activity relationships (SAR). It seems that removal of the α-hydroxyl group in position C-19 as in arjunolic acid (**5**) and the addition of a glucosyl moiety in position C-28 to yield arjunglucoside II (**10**), only has a small influence on the antiplasmodial activity. However, when arjungenin (**6**) is only glycosylated as in arjunglycoside I (**13**), the activity is completely lost. Compounds **5**, **6** and **10** all have a hydroxymethyl group (C-23) with α-orientation, but when C-23 is a methyl, as in arjunic acid (**7**), the IC_{50} value remains in the same range, i.e. between 5 and 15 µM. However, both compounds in which C-24 was the hydroxymethyl group (β-orientation), i.e. sericic acid (**4**) (IC_{50} >64 µM) and sericoside (**12**) (IC_{50} 44.4 µM) were completely or almost completely inactive, respectively. Also the C-4 isomer of sericoside (**12**), i.e. arjunglycoside I (**13**), only different in the orientation of the hydroxymethyl group, was inactive. The same is true for several other compounds with a C-23 hydroxymethyl (α-orientation) functionality, such as 2α,3β,21β,23-tetrahydroxyolean-12-en-28-oic-acid (**8**) and terminolic acid (**9**) : apparently, the presence of a 21β-OH or a 6β-OH, respectively, has a negative influence on the activity. The latter observation is in agreement with previous studies which have shown that the

introduction of a hydroxyl group in position C-6 of oleanane-type triterpenoids could decrease their antifungal (Runyoro et al., 2013) or antiplasmodial activity (Oluyemi et al., 2020). Remarkably, glycosylation at C-28 results in a loss of activity of arjungenin (**6**) compared to arjunglycoside I (**13**); whereas arjunolic acid (**5**) and arjunglucoside II (**10**) are equally (moderately) active; and terminolic acid (**9**) / chebuloside II (**11**), as well as sericic acid (**4**) / sericoside (**12**) all are inactive.

In contrast to these polyhydroxylated oleanane-type triperpenes, friedelin is a friedelane-type triterpene with only one hydroxyl group. Friedelin has been previously isolated from several *Terminalia* species including *T. glaucescens T. mollis* and *T. avicennioides* (Mann et al., 2012). The activity of friedelin against *P. falciparum* found in our study (31.2 µM) is lower than previously reported (IC_{50} 7.70 and 7.20 ± 0.5 µM) (Ngouamegne et al., 2008; Bapela et al., 2019).

The phenol glycoside calophymembranside-B (**14**) has previously been isolated from *Calophyllum membranaceum* (Zou et al., 2005) and *Clematis mandshurica* (Shi et al., 2006), but its antiplasmodial activity (IC_{50} 7.60±0.05) is reported here for the first time.

Apart from pantolactone (**1**), calophymembranside-B (**14**) and some of the triterpenes, also 3,4,3'-tri-*O*-methyl-ellagic-acid (**3**) was obtained as one of the active constituents of *T. albida*. Since it is well known that the use of extraction solvents like methanol may contribute to the formation of artefacts such as methyl ethers (Venditti, 2020; Sauerschnig et al., 2018; Maltese et al., 2014), it was checked if compound 3 was present in the genuine plant material. Therefore, an ethanolic extract was prepared from the root and analysed by LC-MS. The ethanolic extract displayed a pseudomolecular ion at *m/z* 343.045 $[M-H]^-$ at the same retention time as the isolated compound 3,4,3'-tri-*O*-methyl-ellagic acid (Fig.2), confirming that 3,4,3'-tri-*O*-methyl-ellagic acid was not an artefact. Although polymethoxylated derivatives of ellagic acid have been reported in other plant species such as *Terminalia macroptera* (Conrad et al., 1998; Silva et al., 2000), *Syzygium aromaticum* (Begum et al., 2014) and *Irvingia malayana* (Jaipetch et al., 2019), to the best of our knowledge, this is the first time that this compound was isolated from *Terminalia albida*, and that antiplasmodial activity was reported for a trimethyl ether of ellagic acid.

Based on the antibacterial and antifungal activity found during our preliminary biological screening (Baldé et al., 2020) the fractions and compounds isolated from the methanolic extract were tested against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Although the methanolic extract was active against *S. aureus* (IC_{50} 12.8 µg/ml) and *C. albicans* (IC_{50} 7.9 µg/ml), none of its fractions and isolated compounds showed antibacterial or antifungal activity. It appears that the initial fractionation of the methanolic extract has led to the loss of the antibacterial and the antifungal activities. In contrast to our result, a previous study carried out by Runyoro et al. (2013) revealed that terminolic acid (**9**) isolated from *Combretum zeyheri* demonstrated activity against *C. albicans strains* MTCC1637 (MIC 125 µg/mL) and ATCC90028 (MIC 62.50 µg/mL). Furthermore, a study carried out by Gossan et al. (2016) demonstrated that terminolic acid exhibited moderate antibacterial activity against *S. aureus* and *E. coli* (minimum inhibitory concentration (MIC) within a range of 64 and 256 µg/mL). Eldeen et al. (2008) have reported the effectiveness of arjunic acid (**7**) against *B. subtilis* (IC_{50} 2.1 µg/mL) *S. aureus* (3.0 µg/mL), *E. coli* (3.5 µg/mL), and *K. pneumoniae* (5.9 µg/mL). Ghosh and Sil (2013) have observed moderate antifungal activity against *Candida albicans*, *C. krusei* and *C. parapsilosis* for a mixture of arjunolic acid and asiatic acid with MIC values between 50 and 200 mg/mL.

It is important to point out that, despite the historical efficacy of bioassay-guided fractionation of plant extracts, loss of activity and failure in isolation of active compounds during the fractionation process could occur (Caesar and Cech, 2019; Nothias et al., 2018) The main reasons of these pitfalls may be the degradation of compounds during the purification process, a low concentration of bioactive compounds making their isolation difficult, and/or potential synergistic effects (Nothias et al., 2018). These may be causes for the loss of activity during our fractionation process. This may be avoided in "synergy-directed fractionation" (Junio et al., 2011) which combines chromatographic separation and synergy testing in combination with a known active constituent in the original extract.

Conclusion

The results of this study support, at least in part, the traditional use of *T. albida* against microbial diseases, more in particular malaria. This study reported the bioassay-guided isolation of constituents from the roots *T. albida*, which are active against microbial diseases including malaria, from the dichloromethane and the ethyl acetate extract. Compounds from different phytochemical classes, including pantolactone, 3,4,3'-tri-*O*-methyl-ellagic-acid, the phenol glycoside calophymembranside-B, and a range of hydroxylated oleanane-type triterpenes,

were found to contribute to the activity against *P. falciparum.* In addition, a series of less active or inactive hydroxylated triterpenes was obtained. Most of the isolated compounds are reported for the first time in this species. Although less active than the reference control (Chloroquine), some of these compounds could constitute a good antiplasmodial scaffold for the discovery of new potential antimalarial drugs.

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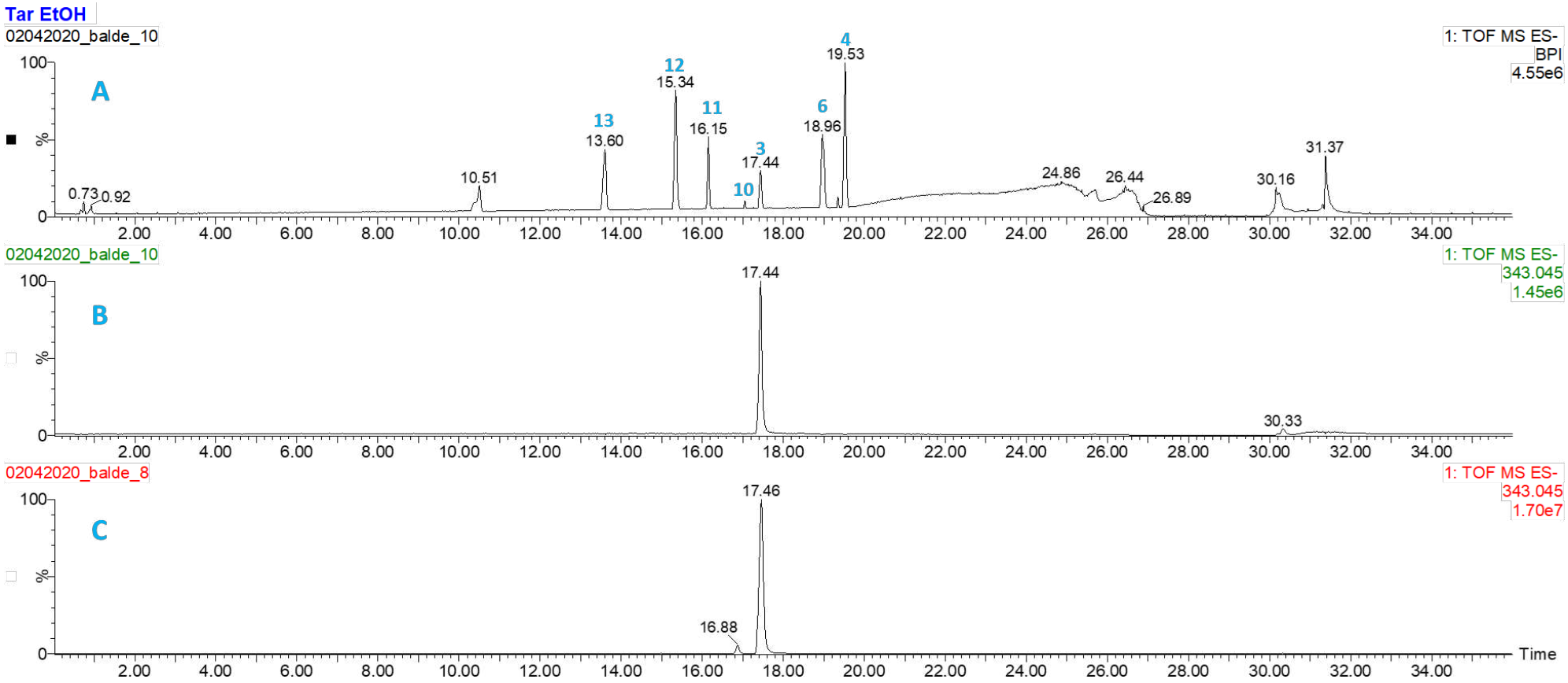
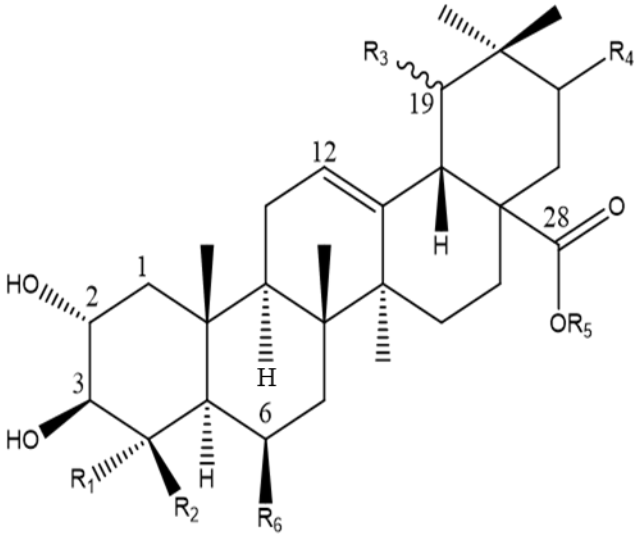


Fig.2 Total Ion Chromatogram (TIC) of the ethanolic extract with major peaks labelled (compound no.) (A**);** Extracted Ion Chromatogram (EIC) of *m/z* 343.045 **(**B**)** and of 3,4,3'-tri-O-methyl-ellagic acid (**3**) (C)

Table 1: *In vitro* antimicrobial, antiplasmodial and cytotoxic activity of extracts, fractions and isolated compounds from *Terminalia albida* root

Fraction and Compound names	**Antibacterial and antifungal activity (IC_{50} µM)**			**Antiprotozoal activity ((IC_{50} µM)**	**Antiprotozoal activity ((IC_{50} µg/ml)**	**Cytotoxicity (CC_{50} µM)**	**Selectivity index**
	S. aureus	***E. Coli***	***C. albicans***	***Pf-K1***	***Pf-K1***	***MRC-5***	***MRC-5/ PfK1***
Methanol 80% extract	12.8	>64.0	7.9	0.8	Nd	21.5	26.8
Dichloromethane fraction	>64.0	>64.0	>64.0	11.9	Nd	28,6	2.3
Ethyl acetate fraction	>64.0	>64.0	>64.0	5.9	Nd	>64,0	>10.8
n-Butanol fraction	>64.0	>64.0	>64.0	>64.0	Nd	>64,0	Nd
Aqueous residue	>64.0	>64.0	>64.0	39.5	Nd	>64.0	>1.6
Pantolactone **(1)**	>64.0	Nd	>64.0	0.60 ± 0.03	0.08	>64.0	>106.6
Friedelin **(2)**	>64.0	Nd	>64.0	31.2	13.3	>64.0	>106.6
3,4,3'-tri-O-methyl-ellagic acid **(3)**	>64.0	Nd	>64.0	5.9 ± 1.0	2.0	>64.0	>12.3
Sericic acid (**4**)	>64.0	Nd	>64.0	>64.0	Nd	>64.0	Nd
Arjunolic acid **(5)**	>64.0	Nd	>64.0	9.9±5.6	4.8	>32.0	>3.2
Arjungenin **(6)**	>64.0	Nd	>64.0	5.9 ± 2.2	2.9	>64.0	>13.4
Arjunic acid **(7)**	>64.0	Nd	>64.0	9.7	4.7	>32.0	>3.3
2α, 3β, 21β, 23 tetra hydroxy-olean-12-en-28 oic-acid **(8)**	>64.0	Nd	>64.0	>64.0	Nd	>64.0	Nd
Terminolic acid **(9)**	>64.0	Nd	>64.0	>64.0	Nd	>64.0	Nd
Arjunglucoside II **(10)**	>64.0	Nd	>64.0	12.2	7.9	>64.0	>5.2
Chebuloside-II **(11)**	>64.0	Nd	>64.0	>64.0		>64.0	Nd

Sericoside **(12)**	>64.0	Nd	>64.0	44.4	29.6	>64.0	Nd
Arjunglucoside I **(13)**	>64.0	Nd	>64.0	>64.0	Nd	>64.0	Nd
Calophymembranside-B **(14)**	>64.0	Nd	>64.0	7.6 ± 0.05	3.5	>64.0	>8.3
Chloroquine				0.15 ± 0.1 µM			
Doxycycline	0.28 ± 0.2 µM	0.6 ± 0.3 µM					
Flucytosine			0.70±0.01 µM				
Tamoxifen						10.0 ± 1.5 µM	



Compounds	R1	R2	R3	R4	R5	R6
4	CH_3	CH_2OH	αOH	H	H	H
5	CH_2OH	CH_3	H	H	H	H
6	CH_2OH	CH_3	αOH	H	H	H
7	CH_3	CH_3	αOH	H	H	H
8	CH_2OH	CH_3	H	OH	H	H
9	CH_2OH	CH_3	H	H	H	OH
10	CH_2OH	CH_3	H	H	1-β-D-Glcp	H
11	CH_2OH	CH_3	H	H	1-β-D-Glcp	OH
12	CH_3	CH_2OH	αOH	H	1-β-D-Glcp	H
13	CH_2OH	CH_3	αOH	H	1-β-D-Glcp	H

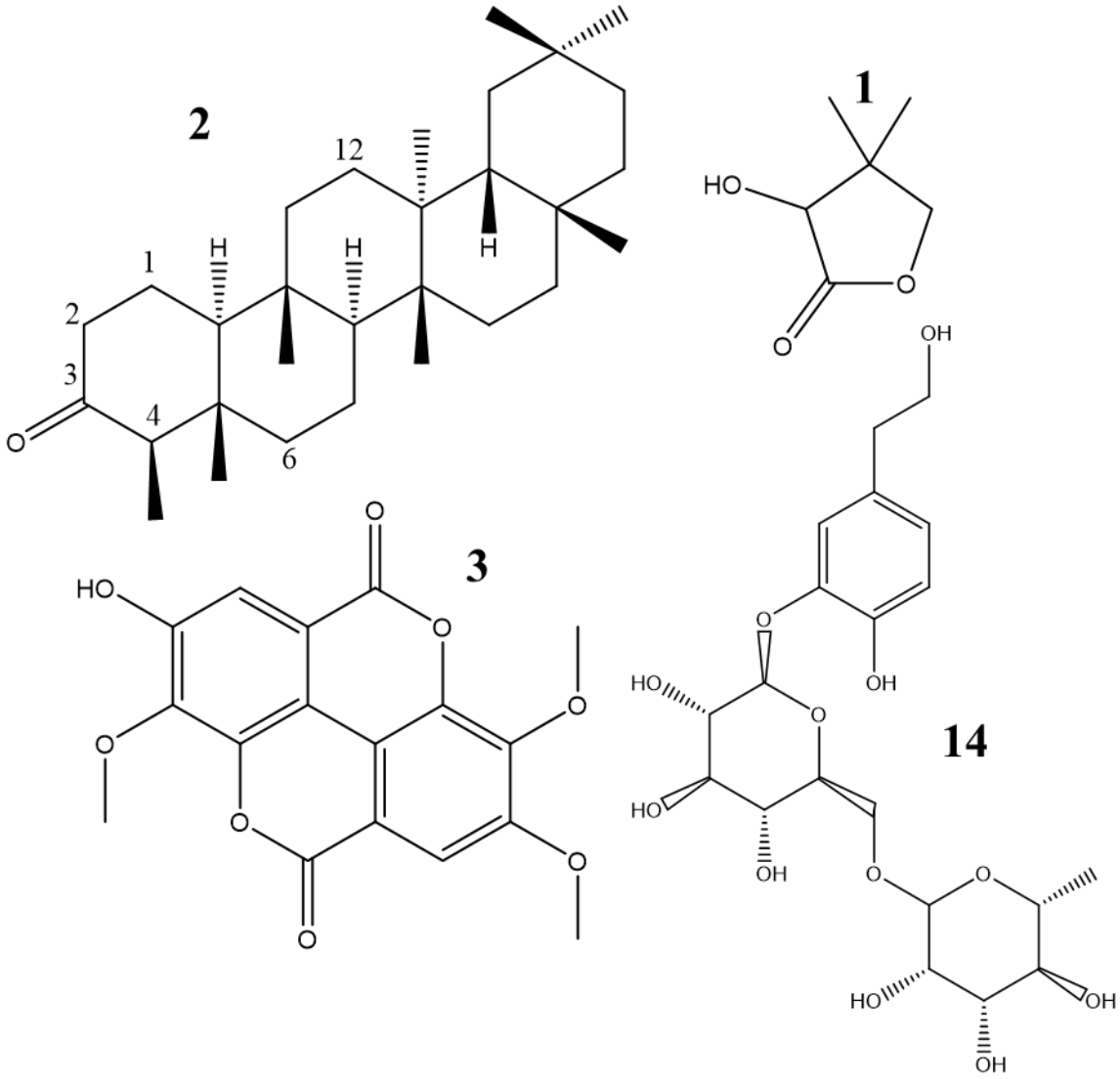


Fig. 1. Compounds **1**–**14** isolated from *Terminalia albida*

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