Research Square

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In vitro antiplasmodial activity and toxicological profile of extracts, fractions and chemical constituents of leaves and stem bark of Dacryodes edulis (Burseraceae)

Kevine Johane Jumeta Dongmo
University of Yaoundé I
Mariscal Brice Tchatat Tali
University of Yaoundé I
Yannick Stéphane Fotsing Fongang (fongangfys@yahoo.fr)
The University of Maroua
Pierre Leonel K. Tafokeu Taguimjeu
University of Yaoundé I
Donald Ulrich Kenou Kagho
University of Yaoundé I
Gabin Thierry Bitchagno
University of Dschang
Bruno Ndjakou Lenta
University of Yaoundé I
Fabrice Boyom Fekam
University of Yaoundé I
Norbert Sewald
Bielefeld University
Silvère Augustin Ngouela
University of Yaoundé I

Research Article

Keywords: Dacryodes edulis, Burseraceae, Antiplasmodial, Acute toxicity, Cytotoxicity, 3,3',4-tri-O-methylellagic acid

Posted Date: November 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2191689/v1

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Abstract

Background

Dacryodes edulis is a plant that belongs to the Burseraceae family. It is widely used traditionally alone or in association with other plants in Cameroonian folk medicine to cure wounds, fever, headaches, and malaria. The aim of this work was to investigate the leaves and stem bark of *D. edulis* with an emphasis on the antiplasmodial and cytotoxic effects on extracts, fractions and isolated compounds.

Methods

Extracts, fractions, and some isolated compounds were subjected to antiplasmodial activity screening *in vitro* against chloroquine-sensitive 3D7 and multidrug resistant Dd2 strains of *Plasmodium falciparum* using a SyBr Green fluorescence-based assay. The cytotoxicity of active extracts, fractions, and compounds was tested against mammalian Raw cell lines using an *in vitro* resazurin-based viability assay. The structures of the compounds were determined based on their NMR and MS data. The *in vivo* toxicity using female BALB/c mice was performed on the most active extract according to the protocol of OECD (2001), guideline 423.

Results

The hydroethanolic extract from the leaves of *D. edulis* displayed good antiplasmodial activity with IC_{50} values of 3.10 and 3.56 μg/mL respectively on sensitive (3D7) and multiresistant (Dd2) strains of *P. falciparum*. Of the sixteen compounds isolated, 3,3',4-tri-*O*-methylellagic acid (**4**) exhibited the highest antiplasmodial activity against *Pf*Dd2 strains with an IC_{50} value of 0.63 µg/mL. All extracts, fractions, and isolated compounds demonstrated cytotoxicity against Raw cell lines with $CC_{50} > 250$ μg/mL. In addition, the most active extract on both strains of *P. falciparum* was nontoxic *in vivo*, with an LD_{50} greater than 2000 and 5000 mg/kg. A phytochemical survey of the stem bark and leaves of *D. edulis* afforded sixteen compounds, including two xanthones (**1**–**2**), three ellagic acid derivatives (**3**–**5**), one phenolic compound (**6**), one depside (**7**), one triglyceride (**8**), one auranthiamide acetate (**9**), one gallic acid derivative (**10**), four triterpenoids (**11**–**14**), and two steroids (**15**–**16**). Compounds **1**, **2**, **5**, **7**, **8**, and **9** were herein reported for the first time from the Burseraceae family.

Conclusion

This work highlight the good antiplasmodial potency of the hydroethanolic extract of the leaves of this plant and the antiplasmodial activity of 2 of its constituents (3,3',4-tri-*O*-methylellagic acid and ethylgallate) *in vitro*. These biological results confirmed the use of *D. edulis* in traditional medicine against malaria.

Background

Emerging and/or re-emerging epidemic diseases represent an ongoing threat to global security [1]. The current situation that the whole world is experiencing with COVID-19 sufficiently illustrates this allegation. Despite the emergence of COVID-19, malaria still remains a major public health concern worldwide, with the African region being disproportionately affected [2]. According to the WHO (2021), among the approximately 1.5 million lives claimed each year by malaria, 96% of deaths occurred in Africa, with children under 5 years of age accounting for about 80% of all deaths [3]. The ravages caused by this disease are felt much more acutely in the developing countries due to the high costs of conventional drugs used in therapy, added to their precarious accessibility first, and second, to the increase in resistance that vectors and parasites developed toward the available medicines. Despite scientific efforts to control the pathogenic agent, *Plasmodium falciparum*, malaria continues to decimate the population mostly in Africa. Faced with these health issues, the search for new active, and more efficient antimalarials with a novel mode of action to combat pathogenic agents and of almost zero toxicity is becoming imperative. To find such agents, medicinal plant-based drug discovery remains a hard-wearing solution. As such, medicinal plants always have a strong historical link with malaria treatment, exemplified by chloroquine and artemisinin isolated from cinchona trees and *Artemisia annua* respectively. Hence, investigating plants widely used in traditional medicine to treat malaria can provide new drugs with an innovative biological mode of action. *Dacryodes edulis* (Burseraceae) is traditionally used alone or in association with other plants in folk medicine to cure wounds, fever, headaches, and malaria [4, 5]. *D. edulis* is a fruit tree that grows naturally in the rainforests of Central Africa, the Gulf of Guinea, and the Congo Basin. Its current range due to culture extends from Central Africa to Uganda in the east and south to Angola [6]. It is a plant largely used for its nutritional and medicinal values and locally called bush butter tree and «safoutier» by English and French speaking, respectively [5]. Previous phytochemical investigation of the stem bark of *D. edulis* led to the identification of several classes of secondary metabolites including alkaloids, steroid/triterpenes, phenols, reducing sugars, cardiac glycosides, flavonoids, saponins, tannins, and anthraquinones [7, 8]. Additionally, analgesic, anti-inflammatory, anti-allergic, anticancer, antimicrobial, and antiplasmodial activities were also reported [9]. Knowing that the phytochemical composition of plants varies dramatically according to their geographical location, country, day, and time place of collection combined with our continuous search for bioactive compounds from Cameroonian medicinal plants, we investigate the leaves and stem bark of *D. edulis* with an emphasis on the antiplasmodial and cytotoxic effects of the extracts, fractions, and isolated compounds.

Methods

General experimental procedures

Column chromatography separations were performed on 230–400 mesh silica gel (Merck, Darmstadt, Germany), and Sephadex LH-20 (Sigma-Aldrich, Munich, Germany). Fractions were grouped using thin-

layer chromatography (TLC) profiles with Merck precoated silica gel sheets (60 F_{254}), and the identification of spots on the TLC plate was carried out by spraying with sulfuric acid reagent solution 20% concentrated and heating the plate at approximately 80ºC. Compounds were visualized under UV light at 254 nm or 365 nm. The 1D and 2D NMR spectra were recorded on Bruker DRX 500 MHz and 600 MHz (Bruker, Rheistetten, Germany) for ^{1}H-NMR and 125 MHz and 150 MHz for ^{13}C-NMR spectrometers, giving the chemical shifts in ppm and the coupling constants in Hertz.

Plant Material

The leaves and stem bark of *D. edulis* were collected in April 2017 in Batcham, West Region of Cameroon. Although, no specific license is required for *D. edulis*, its parts were collected upon oral approval by local authorities and in strict respect of the Cameroonian biodiversity rights and regulations. The plant material was identified by Mr. NANA Victor, botanist at the National Herbarium of Cameroon, Yaoundé by comparison with voucher specimens formally kept at the National Herbarium under the registration number 45713 HNC.

Extraction and isolation

The air-dried and powdered stem bark (4.5 kg) of *D. edulis* was extracted with MeOH (15×4 L) (3 days, repeated two times) at room temperature (approximately 27°C). The extract was freed to solvent under vacuum at low temperature (40°C) to give 239.8 g of crude extract. The crude extract was subjected to liquid-liquid partition using *n*-hexane, dichloromethane, EtOAc and *n*-BuOH to afford four fractions, including the *n*-hexane fraction (DEH) (20.5 g), dichloromethane fraction (DEC) (3.92 g), ethyl acetate fraction (DEA) (35.5 g), and *n*-butanol fraction (DEN) (43.2 g). The *n*-hexane fraction was subjected to flash column chromatography using silica gel (Merck, 230–400 mesh) eluting with *n*-hexane, a mixture of *n*-hexane-DCM gradient (1:0–0:1), a mixture of DCM-EtOAc (1:0–0:1), and EtOAc-MeOH (1:0–0:1) of increasing polarity to yield four main subfractions labeled A (8.9 g), B (7.1 g), C (10.4 g) and D (10.4 g). Subfraction A was subjected to CC over silica gel and eluted with an *n*-hexane/DCM mixture (1:0–0:1) to yield *β*-amyrinacetate (**11**) (5.7 mg) and *β*-amyrin (**12**) (8.4 mg). Subfraction B was also subjected to CC over silica gel and eluted with an *n*-hexane/DCM mixture (1:0–0:1) to yield griseoxanthone C (**2**) (9.4 mg) and a (1:1) mixture of *β*-and *α*- amyrin (**14**) (50.9 mg). Subfraction C was further submitted to CC over silica gel and eluted with *n*-hexane/DCM (1:0–0:1) to afford masticadienonic acid (**13**) (10.5 mg) and confluentic acid (**7**) (6.0 mg). Subfraction D was subjected to CC over silica gel and eluted with an *n*-hexane/DCM mixture (1:0–0:1) to produce a (1:1) mixture of *β*-sitosterol and stigmasterol (**15**).

The DCM fraction was subjected to CC over silica gel (Merck, 230–400 mesh) and eluted with a mixture of *n*-hexane/EtOAc (1:0–0:1) and an EtOAc/MeOH mixture (1:0–0:1) of increasing polarity to afford lichexanthone (**1**) (3.1 mg), 3,3'-*O*-dimethylellagic acid (**3**) (3.7 mg), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**16**) (30.2 mg).

The EtOAc fraction was also subjected to CC over silica gel (Merck, 230–400 mesh) and eluted with an *n*-hexane/EtOAc mixture (1:0–0:1) and an EtOAc/MeOH mixture (1:0–0:1) of increasing polarity.

Subfractions (120) of 100 mL each were collected and combined according to their TLC and LC-MS profiles to afford four subfractions (A-D). Subfraction B (8.5 g) was subjected to silica gel column chromatography (CC) and eluted with DCM-MeOH mixture (1:0-0.8:0.2) to yield 3,3',4-tri-*O*-methylellagic acid (**4**) (50.0 mg). Subfraction C (5.1 g) was also subjected to silica gel column chromatography (CC) and eluted with DCM-MeOH mixture (1:0–1:0) to produce 3,3"-di-*O*-methylellargic acid 4-*O*-(3"-galloyl)-*β*-*D*-xylopyranoside (**5**) (6.7 mg) and 3,4-dihydroxybenzoic acid (**6**) (6.5 mg).

The air-dried and powdered leaves (2.5 kg) of *D. edulis* were extracted with a (7:3) mixture of ethanol-water (2.5×10 L) (three days, repeated two times) at room temperature (approximately 26°C). The extract was then freed from solvent under vacuum at low temperature (40°C) to give 205.5 g of crude extract. The crude extract was then subjected to liquid-liquid extraction with different solvents and gave four fractions including the *n*-hexane fraction (DFH) (25.7 g), dichloromethane fraction (DFC) (17.5 g), ethyl acetate fraction (DFA) (20.3 g), and *n*-butanol fraction (DFN) (102.3 g). The ethyl acetate fraction was assessed by CC over silica gel (Merck, 230–400 mesh) and eluted with a DCM/MeOH mixture (1:0–1:0) of increasing polarity to afford glyceryl-1-tetracosanoate (**8**), auranthiamide acetate (**9**) (4.9 mg), and ethyl gallate (**10**). The structures of these compounds (Fig. 1) were determined by comparison of their spectroscopic data with those reported in the literature.

Bioassay

The *in vitro* and in vivo assays were performed at the Antimicrobial and Biocontrol Agents Unit, Laboratory of Phyto-biochemistry and Medicinal Plants Study, Department of Biochemistry, University of Yaoundé I, Cameroon. *In vivo* studies were performed in the strict respect of animal welfare and the data reported following the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments) [10].

- Plasmodium falciparum **growth inhibition assay**
- ***In vitro cultivation of P. falciparum***

The chloroquine-sensitive *Pf*3D7-(MRA-102) and chloroquine-resistant *Pf*Dd2-(MRA-150) *P. falciparum* strains were maintained as previously described [11] in fresh O^+ human red blood cells at 4% haematocrit in complete RPMI 1640 medium [500 mL RPMI 1640 (Gibco, UK) supplemented with 25 mM HEPES (Gibco, UK), 50% albumax I (Gibco, USA), 1X hypoxanthine (Gibco, USA) and 50 mg/mL gentamicin (Gibco, China)] and incubating at 37°C in a humidified atmosphere with 5% CO_2. The medium was renewed daily to propagate the culture. Giemsa-stained thin blood smears were examined microscopically under immersion oil to monitor cell-cycle transition and parasitemia evolution. O^+ human red blood cells were obtained from a consent volunteer donor (Supplementary material).

- ***Blood specimen***

Whole blood (25 mL) was drawn for a heathy volunteer without a historical contraceptive or anticoagulant therapy using a protocol approved by the Institutional Review Board (IRB No. 001/UY11

BTC/IRBI 2009), Biotechnology Centre, University of Yaoundé I, Cameroon. A consent, approved by Fekam Boyom Fabrice, Professor and Head of the Antimicrobial & Biocontrol Agents Unit (AmBcAU), Laboratory for Phytobiochemistry and Medicinal Plants Studies, University of Yaoundé 1, Cameroon, for collection of blood sample from human volunteer.

- ***Statement on informed consent of the donor***

The volunteer donor was supplied a consent form which informed the purpose of the research project, name and details as well as contact person. Inclusion and exclusion criteria of the donor, volume of blood to be taken, age, sex and time required for the blood sampling. Information's were asked during face to face interview. Blood donor was aware that it sample might be used for research purpose and written consent form was obtained.

- ***Synchronization of P. falciparum parasite culture***

Before each experiment, synchronized ring-stage parasite were obtained by 5% sorbitol (w/v) treatment with respect to Lambros and Vanderberg, 1979 [12]. It is crucial to note that, using cultures over mixed stage cultures can enable the test molecules to interact with all three stages (ring, trophozoite, and schizont) of the 48 hours long life cycle of *P. falciparum* in culture.

- ***Antiplasmodial SYBR green I-based fluorescence assay***

A drug sensitivity assay was carried out in 96-well microtitration plates using a SYBR green I-based fluorescence assay [13]. This assay is specifically based on the ability of SYBR green to give strong fluorescence only in the presence of parasite DNA during cell proliferation.

Shortly, sorbitol-synchronized ring stage parasites (hematocrit: 1%, parasitemia: 2%, 90 μl) under normal culture conditions were incubated in the presence of prediluted extracts, fractions, isolated compounds and reference drug (10 μL) followed by incubation at 37°C for 72 hours. After incubation, 100 μL of SYBR Green I buffer [6 μL of 10,000 × SYBR Green I (Invitrogen) + 600 μL of Red Blood Cells lysis buffer {Tris (25 mM; pH 7.5)} + 360 μL of EDTA (7.5 mM) + 19,2 μL of parasites lysis solution {saponin (0.012%; wt/vol)} and 28,8 μL of Triton X-100 (0.08%; vol/vol)}] was added to each well, mixed twice gently with a multichannel pipette and incubated in the dark at 37°C for 1 hour [11]. Fluorescence was measured using a TECAN M 200 Microplate reader with excitation and emission at 485 and 538 nm, respectively. The fluorescence counts were plotted against the logarithm of the sample concentration and the 50% inhibitory concentration (IC_{50}) was determined by analysis of dose-response curves using GraphPad Prism 5.0. Experiments were performed in duplicate.

- In vitro cytotoxicity assay.

The cytotoxicity profile of extracts, fractions and isolated compounds was assessed using the resazurin-based assay essentially as previously described [14] against RAW 264.7 cells duly cultivated in complete Dulbecco's Modified Eagle's Medium (DMEM) that contain 13.5 g/L of DMEM (Sigma Aldrich), 10% fetal

bovine serum (Sigma Aldrich), 0.2% sodium bicarbonate (w/v) (Sigma Aldrich) and 50 μg/mL of gentamicin (Sigma Aldrich). Globally, macrophages (Raw cells) were seeded into 96-well cell-culture flat-bottomed plates at a density of 10^4 cells in 100 μL of complete medium/well and placed in incubation for 24 hours at 37°C, and 5% CO_2 allowing cell adhesion. Following cell adhesion, ten microliters of each serially diluted test sample solution was added to assay plates and left in incubation for 48 h under the same experimental conditions. Growth control (1% DMSO-100% growth) and positive control wells (podophyllotoxin at 20 μM) were included in the experimental plates. Cell proliferation was checked by adding 10 μL of a stock solution of resazurin (0.15 mg/mL in sterile PBS) to each well followed by an additional incubation of 4 h in the same culture conditions. Fluorescence was then read using Tecan Infinite M200 fluorescence multiwell plate reader (Tecan) at an excitation/emission of 530/590 nm. The results were expressed as 50% cytotoxic concentrations (CC_{50}) and selectivity indices (CC_{50} Mammalian cell/IC_{50} *Pf*3D7) were calculated for each test substance.

- In vivo **acute toxicity**

In vitro studies were performed in the animal house of the Laboratory of the Phyto-biochemistry and Medicinal Plants Study at the University of Yaoundé I, Cameroon, using female healthy BALB/c mice between 6–8 weeks old (20–25 g) maintained in constant and standard laboratory conditions (23–25°C and light/dark cycles i.e. 12/12h) with free access to food and tap water. Experiments with animals were performed according to the ethical standards formulated in the Declaration of Helsinki, and adequate measures were taken to protect animals from pain or discomfort, including sacrifice through injection of a mixture of ketamine (120 mg/Kg) and xylazine (1mg/Kg). The protocol used for animal experimentation received approval from the Institutional Review Board (IRB No. 001/UY11 BTC/IRBI 2009), Biotechnology Centre, University of Yaoundé I, Cameroon.

To assess the safety of *D. edulis* in an animal model, healthy female *BALB/c* mice were randomly divided into three (03) groups of three (03) mice and treated by the oral route with a single dose of hydroethanolic leaf extract of *D. edulis* at doses of 2000 mg/kg and 5000 mg/kg while 20 mL/kg of distilled water was used for the control group. Oral gavage was chosen as a mode of administration to mimic the traditional route of administration as described previously [15]. Oral gavage was achieved as per the guidelines of the Organization for Economic Cooperation and Development (OECD) (OECD, 2002). Following extract administration, animals were observed for 30 min and the first 4 h after treatment to record immediate deaths and once daily for 14 days to record any manifestation of toxicity.

Results And Discussion

Isolated compounds

The methanol and hydroethanolic extracts from the stem bark and leaves of *D. edulis*, respectively, were subjected to silica gel and Sephadex LH-20 column chromatography (CC) to yield 16 known compounds

(**1**–**16**) (Fig. 1). The structures of the isolates were determined from their spectroscopic data (UV, IR, NMR and MS) and by comparison with those of similar compounds reported in the literature.

Lichexanthone **(1)**: white powder, HR-ESI-MS (positive mode, *m/z*): 287.0935 $[M + H]^+$, (calcd 287.0914 for $C_{16}H_{15}O_5$). ^{1}H-NMR (Pyridin-d_5, 500 MHz): 2.91 (3H, s, -CH_3), 3.78 (3H, d, *J* = 1.5 Hz, -OCH_3), 3.81 (3H, d, *J* = 1.5 Hz, -OCH_3), 6.55 (1H, d, *J* = 2.1 Hz, H-2), 6.60 (1H, d, *J* = 2.1 Hz, H-4), 6.79 (1H, d, *J* = 2.4 Hz, H-5), 6.86 (1H, d, *J* = 2.2 Hz, H-7), 13.91 (1H, -OH). ^{13}C-NMR (Pyridin-d_5, 125 MHz): 23.8 (C-15), 56.2 (C-16), 56.2 (C-14), 92.8 (C-4), 97.8 (C-2), 99.5 (C-5), 104.7 (C-10), 113.4 (C-13), 116.3 (C-7), 143.8 (C-8), 157.7 (C-11), 160.0 (C-12), 164.7 (C-6), 164.8 (C-1), 166.8 (C-3), 183.0 (C-9) [16].

Griseoxanthone C (**2**): white powder, HR-ESI-MS (positive mode, *m/z*): 274.0837 for $[M + 2H]^+$, (calcd 274.0836 for $C_{15}H_{14}O_5$).^{1}H-NMR ($CDCl_3$, 600 MHz): 13.39 (1H, OH), 6.68 (1H, s, H-7), 6.66 (1H, s, H-5), 6.35 (1H, s, H-4), 6.31 (1H, s, H-2), 3.86 (3H, s, OCH_3), 2.88 (3H m, -CH_3). ^{13}C-NMR ($CDCl_3$, 150 MHz): 182.6 (C-9), 165.9 (C-3), 163.8 (C-1), 163.7 (C-6), 159.4 (C-11), 156.9 (C-12), 143.5(C-8), 115.6 (C-7), 113.0 (C-13), 104.2 (C-10), 98.5 (C-5), 96.8 (C-2), 92.1 (C-4), 55.7 (C-14), 23.6 (C-15) [17].

3,3'-*O*-Dimethylellargic acid (**3**): white powder, HR-ESI-MS (positive mode, *m/z*): 353.0268 for $[M + Na]^+$, (calcd 353.0260 for $C_{16}H_{10}O_8Na$). ^{1}H-NMR (Pyridin-d_5, 500 MHz): 3.59 (3H, s, -OCH_3), 4.17 (3H, s, -OCH_3), 4.99 (1H, -OH), 8.04 (1H, s) [18].

3,3',4-Tri-*O*-methylellagic acid (**4**): white powder, HR-ESI-MS (positive mode, *m/z*): 367.0425 for $[M + Na]^+$. (calcd 367.0424 for $C_{17}H_{12}O_8Na$). ^{1}H-NMR (Pyridin-d_5, 500 MHz): 3.85 (3H, s, -OCH_3), 4.13 (3H, s, -OCH_3), 4.19 (3H, s, -OCH_3), 7.82 (1H, -OH), 8.03 (1H, s). ^{13}C-NMR (Pyridin-d_5, 125 MHz): 56.4 (-OCH_3), 61.1 (-OCH_3), 61.3 (-OCH_3), 107.8 (C-5'), 111.6 (C-1'), 112.6 (C-1), 112.9 (C-5), 113.6 (C-6'), 114.1 (C-6), 141.1 (C-2), 142.1 (C-3'), 141.8 (C-2'), 142.1 (C-3), 154.1 (C-4'), 154.3 (C-4), 158.9 (C-7'), 159.0 (C-7) [18].

3,3"-Di-*O*-methylellargic acid 4-*O*-(3"-galloyl)-*β*-*D*-xylopyranoside (**5**): white powder, HR-ESI-MS (positive mode, *m/z*): 637.0839 for $[M + Na]^+$, (calcd 637.0806 for $C_{28}H_{22}O_{16}Na$). ^{1}H-NMR (MeOD, 600 MHz): 8.66 (1H, s, H-5), 8.36 (1H, s, H-5'), 7.85 (2H, s, H-2" and H-6"), 6.20 (2H, d, *J* = 7.4 Hz), 5.91 (2H, d, *J* = 9.3 Hz, H-1"), 4.73 (1H, dd, *J* = 11.1; 5.2 Hz), 4.53 (1H, dq, *J* = 9.5, 4.4 Hz), 4.46 (1H, d, *J* = 6.9 Hz), 4.38 (1H, t, *J* = 10.7 Hz), 4.88 (3H, s, OCH_3), 4.87 (3H, s, OCH_3). ^{13}C-NMR (MeOD, 150 MHz): 174.9 (C-7‴), 168.0 (C-7), 167.9 (C-7'), 162.5 (C-4'), 160.5 (C-4), 155.0 (C-3‴/5‴), 151.5 (C-6), 151.2 (C-6'), 150.5 (C-5), 149.7 (C-5'), 147.7 (C-4‴), 129.4 (C-1‴), 124.1 (C-2), 122.4 (C-2'), 122.6 (C-1'), 121.5 (C-1), 121.2 (C-3), 120.6 (C-3'), 118.3 (C-2‴), 118.3 (C-6‴), 110.9 (C-1"), 86.7 (C-3"), 80.7 (C-2"), 76.9 (C-4"), 75.2 (C-5"), 71.2 (OCH_3), 70.5 (OCH_3) [19].

3,4-Dihydroxybenzoic acid (**6**): white powder, ^{1}H-NMR (MeOD, 600 MHz): 7.45 (1H, d, *J* = 2.0 Hz, H-2), 7.44 (1H, d, *J* = 2.1 Hz, H-6), 6.82 (1H, d, *J* = 7.9 Hz, H-3). ^{13}C-NMR (MeOD, 150 MHz): 170.0 (CO), 151.3 (C-4), 145.9 (C-5), 123.6 (C-1), 122.9 (C-3), 117.5 (C-6), 115.5 (C-2) [20].

Confluentic acid (**7**): white powder, HR-ESI-MS (positive mode, *m/z*): 523.2336 for $[M + Na]^+$, (calcd 523.2302 for $C_{28}H_{36}O_8Na$). ^{1}H NMR ($CDCl_3$, 600 MHz): 11.36 (OH), 6.62 (1H, d, *J* = 2.0 Hz, H-5'), 6.56 (1H, d, *J* = 2.1 Hz, H-3"), 6.49 (1H, d, *J* = 2.6 Hz, H-3), 6.32 (1H, d, *J* = 2.6 Hz, H-5), 4.10 (2H, s, H-8), 2.75 (2H, m, H-8"), 2.44 (2H, m, H-10), 1.65 (2H, m, H-9'), 1.55 (2H, m, H-11), 1.36 (4H, m, H-10" and H-11"), 1.22 (2H, m, H-12 and H-13), 0.91 (3H, m, H-12"), 0.85 (3H, t, *J* = 7.1 Hz, H-14). ^{13}C-NMR ($CDCl_3$, 150 MHz) : 207.1 (C-9), 170.5 (C-7"), 169.2 (C-7), 166.6 (C-2), 164.9 (C-4), 157,8 (C-2"), 151.4 (C-4"), 144.5 (C-6"), 138.9 (C-6), 119.9 (C-1"), 115.2 (C-5"), 113.4 (C-5), 104.2 (C-1), 103.1 (C-3"), 100.1 (C-3), 56.4 (-OCH_3), 55.5 (-OCH_3), 51.2 (C-8), 42.6 (C-10), 33.8 (C-8"), 31.6 (C-10"), 31.3 (C-12), 30.7 (C-9"), 23.4 (C-11), 22.5 (C-11"), 22.4 (C-13), 14.0 (C-14), 13.9 (C-12") [21].

Glyceryl-1-tetracosanoate (**8**): white Powder, HR-ESI-MS (positive mode, *m/z*): 465.4543 for $[M + Na]^+$.^{1}H-NMR ($CDCl_3$, 500 MHz) : 4.21 (1H, dd, *J* = 11.7, 4.5 Hz, H-1), 4.15 (1Hb, dd, *J* = 11.7, 6.2 Hz, H-1), 3.93 (1H, tt, *J* = 6.0, 4.2 Hz, H-2), 3.69 (1Ha, dd, *J* = 11.4, 4.0 Hz, H-3), 3.60 (1H, dd, *J* = 11.4, 5.8 Hz, H-3), 2.35 (2H, t, *J* = 7.6 Hz, H-2'), 1.63 (2H, q, *J* = 7.5 Hz, H-3), 1.25 (nH, brs, H-4 /H-23), 0.88 (3H, t, *J* = 7.0 Hz, H-24). ^{13}C-NMR ($CDCl_3$, 125 MHz): 174.4 (C-1), 70.2 (C-2), 65.2 (C-1), 63.6 (C-3), 34.3 (C-2), 24.9 (C-3), 29.7–32.0 (C4 -23), 14.1 (C-24) [22].

Auranthiamide acetate (**9**): white powder, HR-ESI-MS (positive mode, *m/z*): 455.2156 for $[M + H]^+$, (calcd 523.2122 for $C_{27}H_{29}O_4N_2$). ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.71 (1H, s, H-3), 7.52 (1H, s, H-3), 7.44 (2H, dddd, *J* = 8.5, 6.7, 1.6, 1.0 Hz, H-3), 7.25 (1H, d, *J* = 3.2 Hz, H-3), 7.17 (2H, ddt, *J* = 8.0, 6.6, 1.3 Hz, H-3), 6.71 (1H, d, *J* = 7.6 Hz, H-3), 5.90 (1H, d, *J* = 8.6 Hz, H-3), 3.93 (1H, dd, *J* = 11.3, 4.9 Hz, H-1), 3.82 (1H, dd, *J* = 11.3, 4.2 Hz, H-3), 3.22 (1H, dd, *J* = 13.7, 5.9 Hz, H-3), 3.05 (1H, dd, *J* = 13.7, 8.5 Hz, H-3). ^{13}C-NMR ($CDCl_3$, 125 MHz): 170.7 (C-2), 170.2 (C-6), 167.1 (C-9), 136.7 (C-1), 136.6 (C-1"), 132.0 (C-1‴), 129.3 (C-4‴), 129.1 (C-4), 129.1 (C-3‴/5‴), 128.7 (C-2‴/6‴), 128.6 (C-3"/5"), 128.5 (C-2 /6'), 127.1 (C-2"/6"), 127.0 (C-3 /5'), 126.7 (C-4"), 64.5 (C-3), 55.1 (C-7), 49.4 (C-4), 38.3 (C-11), 37.4 (C-10), 20.8 (C-1) [23].

Ethyl gallate (**10**): white powder, ^{1}H-NMR (MeOD, 600 MHz): 7.06 (2H, s), 4.29 (2H, q, *J* = 7.1 Hz), 1.36 (3H, t, *J* = 7.1 Hz). ^{13}C-NMR (MeOD, 150 MHz): 167.1(CO), 145.1 (C-3,5), 138.3 (C-4), 120.2 (C-1), 108.4 (C-2,6), 60.2 (CH_2), 13.0 (CH_3) [20].

β-Amyrinacetate (**11**): white powder.^{1}H-NMR ($CDCl_3$, 600 MHz,): 5.20 (1H, t, *J* = 3.7 Hz,), 4.55–4.50 (1H, m), 2.07 (7H, s), 1.60 (2H, s), 1.15 (3H, s), 0.98 (2×3H, d, *J* = 2.9 Hz), 0.90 – 0.88 (4×3H, m), 0.85 (2H, s). ^{13}C-NMR ($CDCl_3$, 150 MHz,): 171.4 (CO), 145.8 (C-13), 121.8 (C-12), 80.9 (C-3), 55.3 (C-5), 47.6 (C-9), 47.2 (C-18), 46.8 (C-19), 41.7 (C-14), 39.8 (C-8), 38.3 (C-1), 37.7 (C-4), 37.1 (C-22), 36.8 (C-10), 34.8 (C-21), 33.5 (C-17), 32.6 (C-7), 31.2 (C-20), 28.4 (C-28), 28.1 (C-23), 26.9 (C-2), 26.1 (C-15), 26.0 (C-16), 25.9 (C-27), 23.7 (C-11), 23.6 (C-29), 23.5 (C-30), 21.4 (CH_3CO), 18.3 (C-6), 16.8 (C-24), 16.7 (C-25), 15.6 (C-26) [24].

β-Amyrin (**12**): white powder, ^{1}H-NMR ($CDCl_3$, 600 MHz) : 5.63 (1H, dd, *J* = 5.2, 2.9 Hz), 3.46 (1H, dd, *J* = 3.5, 2.3 Hz), 1.27–1.23 (3H, m), 1.15 (6H, d, *J* = 10.6 Hz), 1.09 (3H, s), 1.04 (3H, s), 1.00 (6H, d, *J* = 7.8 Hz),

0.95 (3H, s), 0.85 (3H, s). ^{13}C-NMR ($CDCl_3$, 150 MHz): 141.7 (C-13), 122.0 (C-12), 76.4 (C-3), 49.7 (C-5), 47.5 (C-9), 43.1 (C-18), 40.9 (C-19), 39.3 (C-14), 38.9 (C-8), 37.8 (C-1), 36.1 (C-4), 35.1 (C-22), 34.8 (C-10), 34.6 (C-21), 34.5 (C-17), 33.1 (C-7), 32.4 (C-20), 32.1 (C-28), 32.0 (C-23), 30.4 (C-2), 30.1 (C-15), 28.9 (C-16), 28.3 (C-27), 27.8 (C-11), 25.5 (C-29), 23.6 (C-30), 19.6 (C-6), 18.4 (C-24), 18.2 (C-25), 16.2 (C-26) [24].

Masticadienonic acid (**13**): white powder, HR-ESI-MS (positive mode, *m/z*): 455.3526 for $[M + H]^+$. ^{1}H-NMR ($CDCl_3$, 600 MHz): 6.93 (1H, m, H-24), 5.67 (1H, m, H-7), 2.52 (1H, m, H-2), 0.93 (1H, dd, *J* = 6.4, 1.6 Hz, H-27), 0.83 (1H, d, *J* = 1.6 Hz, H-22). ^{13}C-NMR ($CDCl_3$, 150 MHz): 219.1 (C-3), 173.2 (C-26), 148.9 (C-8), 145.5 (C-24), 126.6 (C-25), 121.2 (C-7), 52.9 (C-17), 52.3 (C-5), 52.0 (C-14), 47.0 (C-4), 45.4 (C-9), 44.0 (C-13), 34.2 (C-2), 18.2 (C-27) [25].

Antiplasmodial activity screening

The *in-vitro* antiplasmodial activity screening of the methanol and hydroethanolic extracts, fractions and isolates of the leaves and stem bark of *D. edulis* against the chloroquine-sensitive (3D7) and the multidrug-resistant (Dd2) strains of *P. falciparum* was evaluated by measuring growth inhibition based on SYBR green fluorescence. Chloroquine (CQ) and artemisinin were used as reference compounds. To classify the antiplasmodial activity expressed as IC_{50} of tested samples, the following criteria were adopted: $IC_{50} \leq 5$ µg/mL: pronounced activity; $5 < IC_{50} \leq 10$ µg/mL: good activity; $10 < IC_{50} \leq 20$ µg/mL: moderate activity; $20 < IC_{50} \leq 40$ µg/mL: low activity; $IC_{50} > 40$ µg/mL: inactive [26]. According to these criteria and as shown in Table 1, both methanol and hydroethanolic extracts and fractions from the leaves and stem bark of *D. edulis* inhibited the growth of both the *P. falciparum* 3D7 and Dd2 strains with IC_{50} values ranging from 1.44 to 23.39 µg/mL. The methanol extract from the stem bark displayed good antiplasmodial activity against both *Pf3D7* and *PfDd2*, with IC_{50} values of 9.62 and 6.32 µg/mL, respectively. The hydroethanolic leaf extract of *D. edulis* exhibited pronounced antiplasmodial activity with IC_{50} values of 3.10 and 3.56 µg/mL, respectively against both the *Pf*3D7 and *Pf*Dd2 strains. Interestingly, the hydroethanolic leaf extract was more potent than the methanol stem bark extract. The difference observed in antiplasmodial activities of both extracts could be attributed to the chemical composition of the extracts or the part of the plant used. Additionally, four fractions obtained from the methanol stem bark extract and five fractions obtained from the hydroethanol leaf extract of *D. edulis* also exhibited good inhibition of *P. falciparum* 3D7 and Dd2 with IC_{50} values ranging from 1.44 to 23.39 µg/mL. Fraction DEA and DEN from the methanol stem bark extract of *D. edulis* exhibited pronounced antiplasmodial activity on both sensitive and resistant strains (DEA: IC_{50}*Pf*3D7: 3.43 and IC_{50}*Pf*Dd2:1.44 µg/mL; DEN: IC_{50}*Pf*3D7: 3.83_{Pf3D7} and IC_{50}*Pf*Dd2: 3.62 µg/mL) (Fig. 2).

Table 1
Results of antiplasmodial screening against *Pf3D7* and *PfDd2* and selectivity on Raw cell lines

	SYBr Green based assay			**Cytotoxicity assay on Raw Cells**		
	IC50 ± SD (µg/mL)		**Resistance Index (RI)**	**CC_{50} (µg/mL)**	**SI**	
	Pf**3D7**	Pf**Dd2**			Pf**3D7**	Pf**Dd2**
Extracts and fractions						
DEM	9.62 ± 0.48	6.32 ± 0.00	0.65	> 250	> 26	> 40
DEF	3.10 ± 0.09	3.56 ± 0.03	1.14	> 250	> 81	> 70
DEH	> 100	23.39 ± 0.30	-	> 250	-	> 11
DEC	1.82 ± 0.72	5.17 ± 0.10	2.84	> 250	> 137	> 48
DEA	3.43 ± 0.01	1.44 ± 0.21	0.41	> 250	> 73	> 174
DEN	3.83 ± 0.17	3.62 ± 0.05	0.94	ND	-	-
DFH	2.70 ± 0.64	2.98 ± 0.22	1.31	> 250	> 93	> 84
DFC	16.92 ± 0.76	10.37 ± 0.19	0.61	> 250	> 15	> 24
DFA	3.84 ± 0.22	8.79 ± 0.10	2.28	> 250	65	> 28
DFN	10.36 ± 0.98	8.47 ± 0.09	0.81	> 250	> 24	> 30
DFM	8.72 ± 1.28	4.56 ± 0.19	0.52	> 250	> 29	> 55
Compounds						
3,3'-di-*O*-methylellargic acid (3)	1.17 ± 0.04	1.36 ± 0.14	1.16	> 100	> 85	> 74
3-oxo-lanosta-7,24-*Z*-dien-26-oid acid	> 25	14.21 ± 0.11	-	> 100	-	> 7

Legend: DEM: Methanolic extract of the stem bark of *D. edulis*, **DEF**: hydroethanolic extract of the leaves of *D. edulis*, **DEH**: Hexane fraction of DEM, **DEC**: DCM fraction of DEM, **DEA**: AcOEt fraction of DEM, **DEN**: *n*-BuOH fraction of DEM. **DFH**: Hexane fraction of DEF, **DFC**: DCM fraction of DEF, **DFA**: AcOEt fraction of DEF, **DFN**: *n*-BuOH fraction of DEF, **DFM**: methanolic fraction of DEF.

	SYBr Green based assay			Cytotoxicity assay on Raw Cells		
Confluentic acid (7)	> 25	> 25	-	> 100	-	-
Mixture of *β*- and *α*-amyrin	> 25	3.14 ± 0.09	-	> 100	-	> 32
Griseoxanthone C (2)	> 25	> 25	-	> 100	-	-
β-amyrinacetate (11)	> 25	> 25	-	> 100	-	-
β-amyrin (12)	> 25	> 25	-	ND	-	-
3,3',4-tri-*O*-methylellagic acid (4)	> 25	0.63 ± 0.27	-	> 100	-	159
3,4-dihydroxybenzoic acid (6)	> 25	17.09 ± 0.06	-	> 100	-	> 6
Ethyl gallate (10)	1.15 ± 0.10	2.86 ± 0.07	2.48	> 100	> 87	> 35
Auranthiamide acetate (9)	> 25	> 25	-	> 100	-	-
Glyceryl-1-tetracosanoate (8)	> 25	> 25	-	> 100	-	-
Artemisinin (nM)	14.66 ± 0.11	18.90 ± 0.13	0.12	-	-	-
Chloroquine (nM)	4.36 ± 0.53	133 ± 0.16	30.5	-	-	-

Legend: DEM: Methanolic extract of the stem bark of *D. edulis*, **DEF**: hydroethanolic extract of the leaves of *D. edulis*, **DEH**: Hexane fraction of DEM, **DEC**: DCM fraction of DEM, **DEA**: AcOEt fraction of DEM, **DEN**: *n*-BuOH fraction of DEM. **DFH**: Hexane fraction of DEF, **DFC**: DCM fraction of DEF, **DFA**: AcOEt fraction of DEF, **DFN**: *n*-BuOH fraction of DEF, **DFM**: methanolic fraction of DEF.

Among fractions from the hydroethanol leaf extract, fraction DFH was the only one showing pronounced antiplasmodial activity on both strains (IC_{50} 2.70_{Pf3D7} and 2.98_{PfDd2} μg/mL). Overall, we can observe that fractionation of both extracts led to some fractions with more potent and pronounced activities than the mother extracts. This observation could be explained by the fact that the phytochemical constituents responsible for the antiplasmodial activity in the mother extract were concentrated in potent fractions during the fractionation process. Of note, fractionation of an extract can positively or negatively change its biological properties by concentrating active ingredients into a fraction, or by sharing them between the various fractions [27]. Among the compounds isolated from both extracts, 3,3'-*O*-dimethylellagic acid (**3**) and ethylgallate (**10**) displayed very good antiplasmodial activities on both *Pf*3D7 and *Pf*Dd2. Interestingly, 3-oxo-7,24-*Z*-tirucalladien-26-oic acid (**13**); a (1:1) mixture of *β*- and *α*- amyrin (**14**), 3,3',4-tri-*O*-methylellagic acid (**4**) and 3,4-dihydroxybenzoic acid (**6**) exhibited pronounced to moderate

antiplasmodial activity only on the multidrug-resistant Dd2 strain of *P. falciparum* with IC_{50} values ranging from 0.63 to 17.09 μg/mL. However, a mixture of β- and α- amyrin (**14**) and 3, 3', 4-tri-*O*-methylellagic acid (**4**) were the most potent compounds with IC_{50} values of 3.14 and 0.63 μg/mL respectively. 3-oxo-7,24-*Z*-tirucalladien-26-oic acid (**13**) and 3,4-dihydroxybenzoic acid (**6**) displayed moderate antiplasmodial activity (Fig. 3). Overall, two hit compounds were identified among the isolates; one from the methanol stem bark extract (3, 3'-*O*-dimethylellargic acid) (**3**) and the other from the hydroethanolic leaf extract (ethyl gallate) (**10**) with very good antiplasmodial potency. These isolates could be interesting starting materials for further structure-activity relationship studies. The results summary is shown in Table 1 below.

In vitro cytotoxicity profiles showed that all active extracts, fractions and isolated compounds were nontoxic with cytotoxic concentrations of 50 (IC_{50}) up to 250 µg/mL for the extract and fractions and > 100 µg/mL for the isolated compounds. In addition, the hydroethanolic extract of *D. edulis* leaf extract at the tested limit doses of 2000 and 5000 mg/kg did not cause any death during the 14 days of experimentation, indicating that the lethal dose 50 (LD50) of this extract is greater than 5000 mg/kg. Therefore, according to the OECD's Globally Harmonized System of Classification (OECD, 2002), *D. edulis* can be classified as category 5 and considered nontoxic once administered orally. Additionally, the recording of body weight during 14 days of observation showed no significant change in treated animals compared to the normal group (Fig. 4). Combining both *in vitro* cytotoxicity and *in vivo* acute toxicity, *D. edulis* is a source of nontoxic molecules suitable for further investigation toward the search for new antimalarial drugs.

The antiplasmodial activities of the methylene chloride/methanol extracts of *Dacryodes edulis* have already been reported by Zofou and colleagues (2013) and showed significant activity against both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum* with IC_{50} values of 4.34 and 6.43 μg/mL, respectively [9]. Additionally, our study provides the antiplasmodial activities of the methanol stem bark extract and hydroethanol leaf extract and underscores the fact that regardless of the solvent or the part of the plant used, *Dacryodes edulis* constitutes a powerful source of antiplasmodial compounds. Nevertheless, in the work conducted by Zofou and colleagues, fractionation of methylene chloride/methanol extract led to the isolation of quercitrin, afzelin, quercetin, methyl 3,4,5-trihydroxybenzoate, and sitosterol 3-*O*-β-*D*-glucopyranoside sterol with antiplasmodial activities ranging from 0.37 to 18.53 μg/mL against both sensitive *Pf*3D7 and resistant *Pf*Dd2 strains of *P. falciparum*. This discrepancy in activities could be explained by the different extraction procedures, the part of plant used and the geographical localization of the plant, which is directly linked to the quantity and quality of secondary metabolites present in the crude extract [28].

Conclusion

This work was conducted the bioguided antiplasmodial investigation the leaves and stem bark of *D. edulis* with an emphasis on the cytotoxic effects on extracts, fractions and isolated compounds. The

hydroethanolic extract from the leaves of *D. edulis* displayed good antiplasmodial activity with IC_{50} values of 3.10 and 3.56 μg/mL on 3D7 and Dd2, respectively. Among the sixteen compounds isolated, 3,3',4-tri-*O*-methylellagic acid exhibited the highest antiplasmodial activity against *Pf*Dd2 strains with an IC_{50} value of 0.63 μg/mL. All extracts, fractions, and isolated compounds demonstrated cytotoxicity against Raw cell lines with $CC_{50} > 250$ μg/mL. in addition, the most active extract on both strains of *P. falciparum* was nontoxic *in vivo* with an LD_{50} greater than 2000 and 5000 mg/kg. The results achieved in the present work showed the antiplasmodial potency of the hydroethanolic extract of the leaves of this plant and the antiplasmodial activity of 3,3',4-tri-*O*-methylellagic acid and ethylgallate. These biological results confirmed the use of *D. edulis* in traditional medicine against malaria, and can therefore contribute to hit optimization studies in antimalarial drug development.

Abbreviations

^{13}C NMR: Carbon-13 nuclear magnetic resonance; ^{1}H NMR: proton nuclear magnetic resonance; CC_{50}: 50% cell cytotoxic concentration; *D. edulis*: *Dacroydes edulis*; DEM: methanol extract of the stem bark of *D. edulis*; DEF: hydroethanolic extract of the leaves of *D. edulis*; DMSO: dimethyl sulfoxide; DCM: dichloromethane; EC_{50}: 50% effective concentration; EDTA: Ethylene diamine tetra acetate; IC_{50}: inhibitory concentration, 50%; NMR: Nuclear Magnetic Resonance spectroscopy; HNC: Herbier National du Cameroun; H_2O: water; RI: resistance index; SD: standard deviation; SI: selectivity index; MeOH: methanol; WHO: World Health Organization.

Declarations

Ethics approval and consent to participate

The collection of plant materials was performed in accordance with the recommendation, relevant guidelines and regulations of the Plant Varieties Protection, National Herbarium of Cameroon. All procedures that were used in this study conformed to the guideline of the care and use of animals and human tissue in research were approved by the Institutional Review Board (IRB No. 001/UY11 BTC/IRBI 2009), Biotechnology Centre, University of Yaoundé I, Cameroon. All the protocols in this study were performed in accordance with the relevant regulations and guidelines for using animals with compliance with the Animal research: Reporting of *in Vivo* Experiments (ARRIVE) guidelines. The study complied with the Helsinki Declaration and the volunteer O+ erythrocytes blood donor. The donor signed the informed consent sheet prior to the blood collection and, was on publication information's in an online open-access publication (Signed consent form is attached as supplementary material).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Competing interest

The authors declare no competing interests.

Funding

This research was funded the German Academic Exchange Service (DAAD) through the Yaoundé-Bielefeld School of Natural Products with Anti-parasitic and Antibacterial Activities [YaBiNaPA Project n° 57316173].

Authors' contribution

Conceptualization, collection of plant material: KJJD, PLTT, YSFF and BLN; Methodology: KJJD, BMTT, FFB, SAN; Validation: BLN, FFB, SAN and NS; Spectroscopic analysis: KJJD, PLTT, DUKK, GTB and YSFF; Antiplasmodial and Cytotoxicity testing: BMTT; Writing-Original draft preparation: KJJD, BMTT and YSFF; Writing-review and editing: DUKK, GTB, YSFF and BLN; Supervision: BLN, FFB and SAN. Project administration: BLN and SAN; Funding acquisition: BLN, SAN and NS. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors are grateful to the National Herbarium of Cameroon through Mr. Nana victor, a retired botanist for plant identification and collection. We are also thankful to all the NMR and MS technicians of Bielefeld University for data recording. We are also thankful to the blood donor.

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Figures

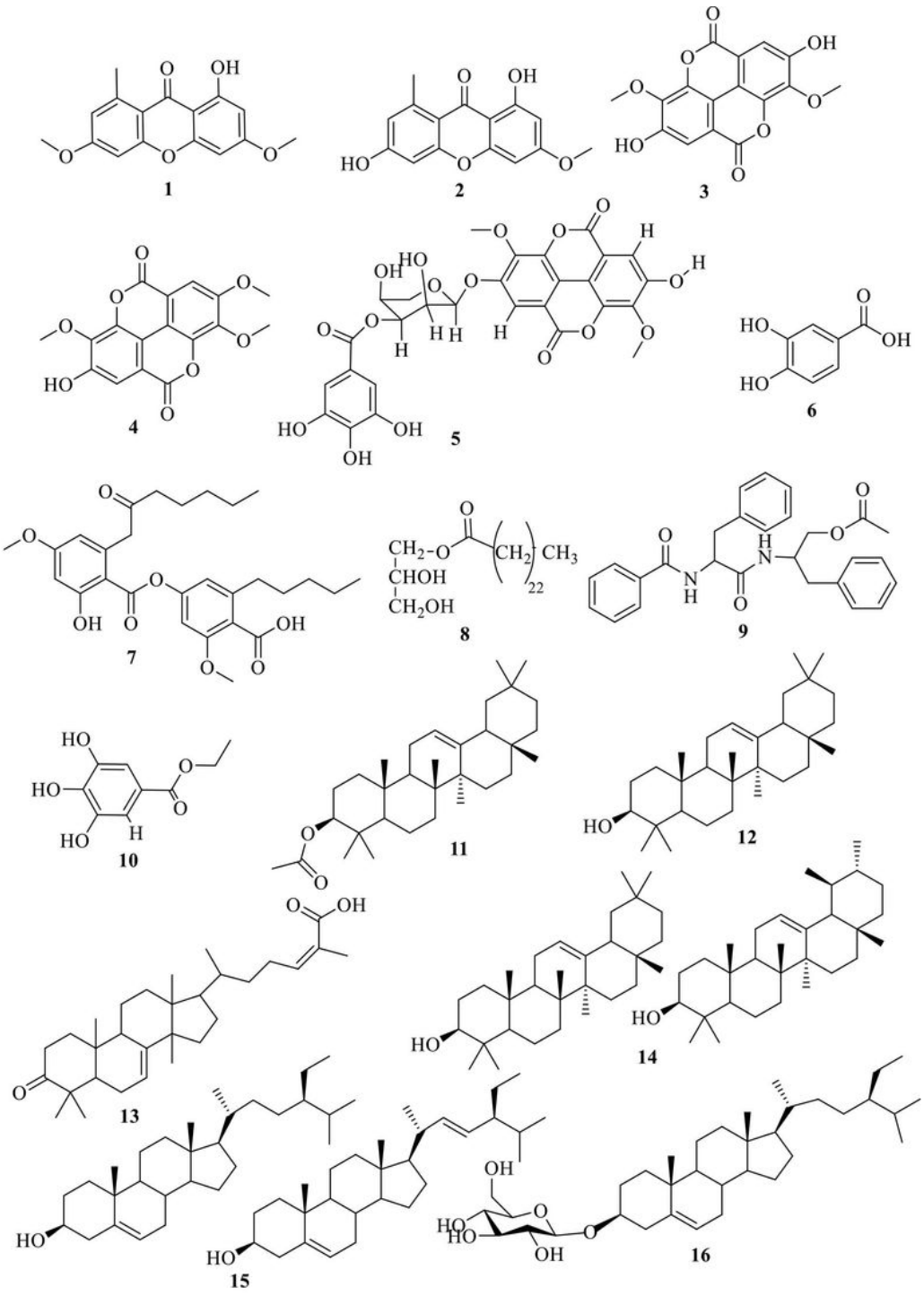


Figure 1

Structures of isolated compounds (**1**-**16**)

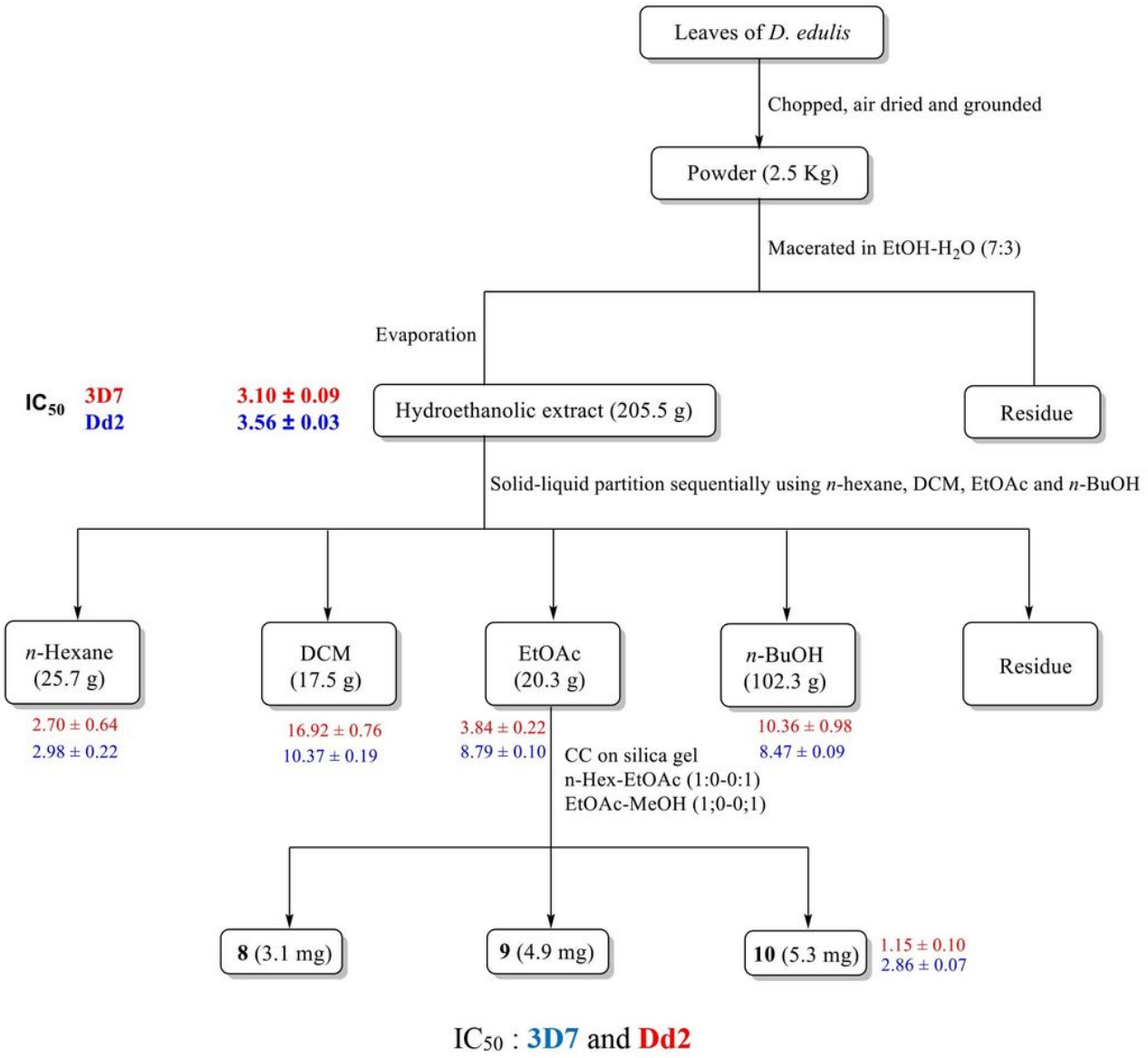


Figure 2

Bio-assay guided isolation of active compounds from the hydroethanolic crude extract of leaf of *D. edulis*

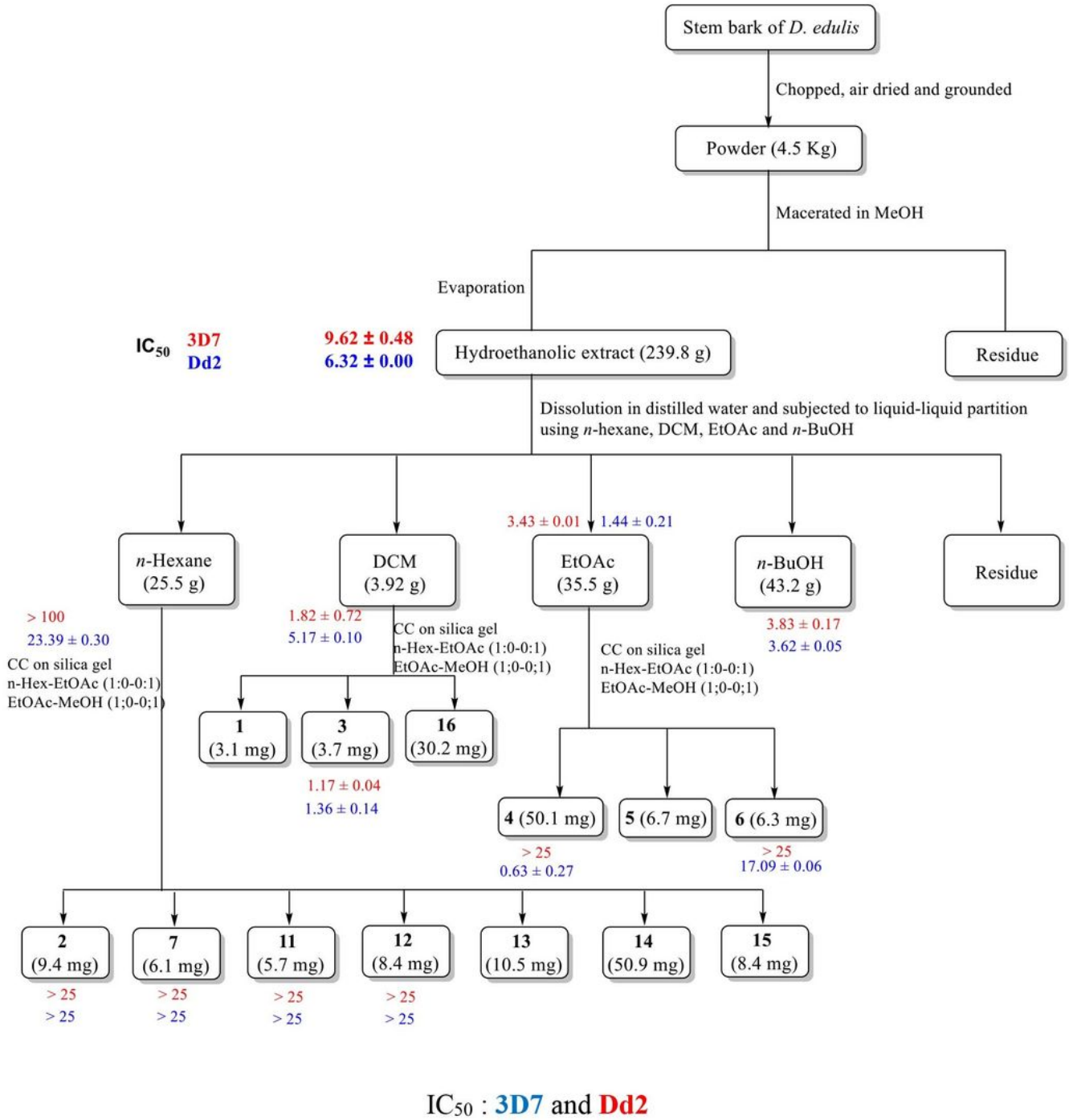


Figure 3

Bio-assay guided isolation of active compounds from the methanol crude extract of the stem bark of *D. edulis*

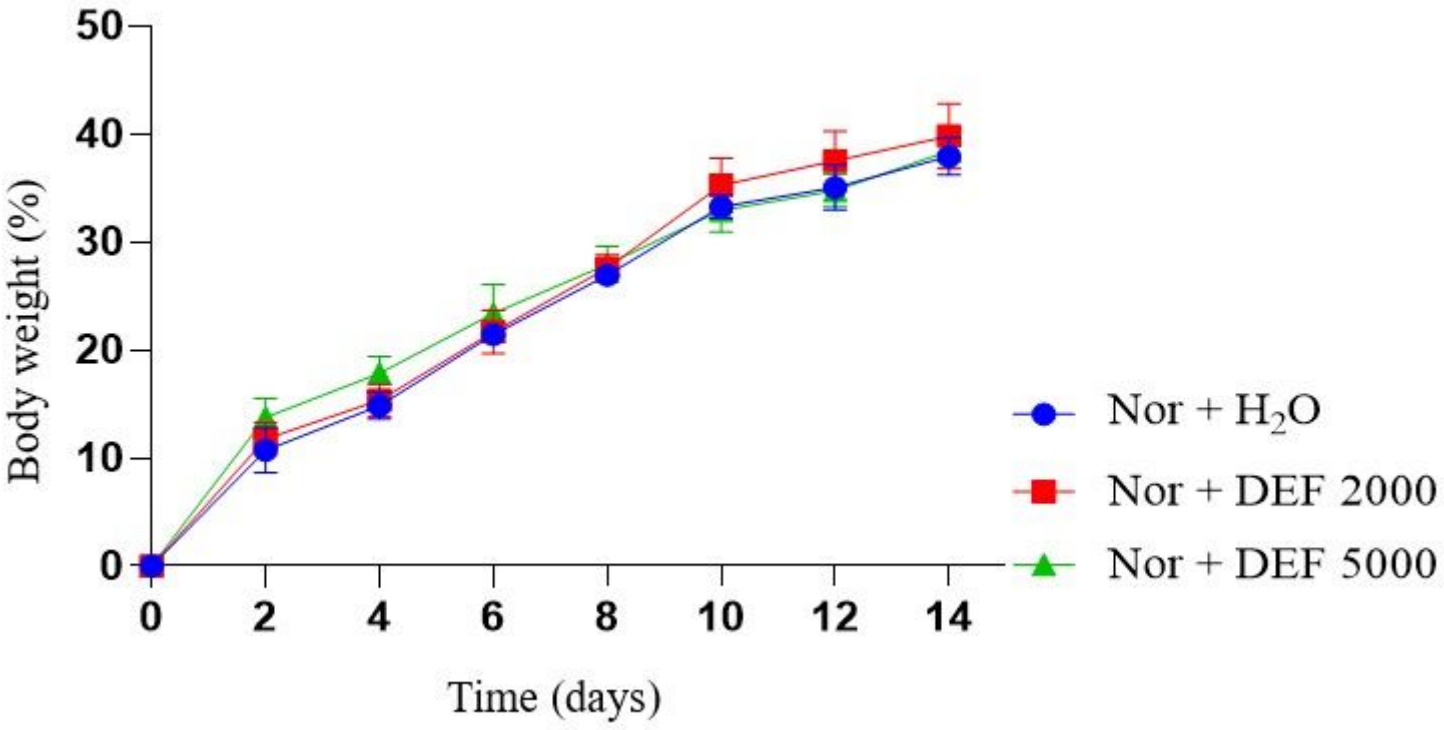


Figure 4

Effects of the aqueous extract of DEF on weight development in acutely toxic rats

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