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Original Research Article

Constituents of the ripe fruits of *Nauclea latifolia* Sm. (Rubiaceae) and their antileishmanial activities

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ABSTRACT

The MeOH extract of the ripe fruits of *Nauclea latifolia* Sm. (Rubiaceae) showed potent antileishmanial activity *in vitro* during preliminary screening (IC_{50} = 2.20 µg/mL) against *Leishmania donovani* 1S (MHOM/SD/62/1S) promastigotes. Three of the four fractions of this extract showed moderate to good activities (7.06 ≤ IC_{50} ≤ 91.21 µg/mL) on the same strain. The purification of the fractions through CC yielded fifteen compounds whose structures were established based on their MS and NMR data. All the isolated compounds were assessed for their antileishmanial activity against *L. donovani* and for their cytotoxicity towards Raw 264.7 macrophage cells. 2,6-Dimethoxybenzoquinone (**1**) and hederagenin (**6**) showed good antileishmanial activity with IC_{50} values of 9.94 and 19.3 µM, respectively. Compound **1** was not selective, while compound **6** displayed a good selectivity towards raw 264.7 macrophage cells (SI > 7.82). These results indicate that the extract from *N. latifolia* fruits could be considered as a source of leishmaniacidal agents.

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1. Introduction

Medicinal and herbal plants are widely employed in a variety of scientific branches, *e.g.*, cosmetics, food industries, and pharmaceutical disciplines (Mohammadhosseini et al., 2021). Natural products from such plants have been shown to possess important biological activities including antileishmanial potency (Ahua et al., 2007; Bapela et al., 2017, Nahar et al., 2021). Leishmaniasis is a neglected tropical disease caused by an intracellular flagellate protozoan parasite belonging to the *Leishmania* genus. About 20 different species of *Leishmania* have been discovered to be pathogenic to humans (Akhoundi et al., 2016), including *Leishmania donovani* that causes the deadliest visceral leishmaniasis. The current chemotherapeutic approaches used to control the disease include the first-line drugs, sodium stibogluconate, meglumine antimoniate and the alternative drugs, amphotericin B, pentamidine, and paromomycin (Singh et al., 2014). However, these drugs face shortcomings such as emerging drug resistance, toxicity and side effects which limit their efficiency in some cases (Croft and Coombs, 2003). Due to the high costs involved in the development and registration of new drugs, the pharmaceutical industry has little interest in research and development of new compounds for the treatment of tropical diseases (WHO, 2000). Therefore, there is an urgent need to support new research on natural products and search for new drugs showing antileishmanial activity (WHO, 2000). Plant extracts from the Rubiaceae families are known to possess antileishmanial activity (Ahua et al., 2007; Sattar et al., 2012; Bapela et al., 2017; Wonkam et al., 2020). Also, these plants are sources

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of compounds with antileishmanial potency (Singh et al., 2014; Lima et al., 2015; Wonkam et al., 2020). *Nauclea latifolia* Sm. (Rubiaceae family, 13,143 species for 620 genera, found in all continents, mostly widespread in tropical and subtropical areas) [syn. *Sarcocephalus latifolius* (Sm.) E.A. Bruce, *S. esculentus* Afzel. ex Sabine, *Cephalina esculenta* (Afzel. ex Sabine) Schumach. & Thonn., *S. russeggeri* Kotschy ex Schweinf., *S. sambucinus* K. Schum., *N. esculenta* (Afzel. ex Sabine) Merr., *S. esculentus* var. *amarissima* A.Chev., and *S. esculentus* var. *velutina* A.Chev.] or the African peach, is a straggling shrub or tree up to 10 m high, sometimes reaching 33 m high. Leaves are broadly oblong-elliptic to obviate or nearly oblong-orbicular; fruits are globosely or ovoid, red-brown pitted with pentagonal scars and edible (Bremer and Eriksson, 2009; Gidado et al., 2005; James and Ugbede, 2011). *N. latifolia* is used extensively in folk medicine in Central Africa (root decoction) and West Africa (infusions and decoctions of the stem, bark and leaves) for body pain, fever, convulsions, malaria, anemia, skin infections and diarrhea (Igoli et al., 2011; Taiwe et al., 2014; Haudecoeur et al., 2018). Moreover, crude extracts from this plant have been reported for their antiplasmodial, antimicrobial, analgesic and antileishmanial activities (Hotellier et al., 1975; Ahua et al., 2007; Haudecoeur et al., 2018). These findings have motivated natural product chemists to carry out phytochemical investigations on this plant, and a variety of secondary metabolites including monoterpene indole alkaloids (Hotellier et al., 1975; Shigemori et al., 2003; Boucherle et al., 2016; Bankeu et al., 2019), pentacyclic triterpenoids (Ngnokam et al., 2003; Bankeu et al., 2019), phenolic compounds (Abreu and Pereira, 2011), fatty acid esters (Fadipe, 2014a; Fadipe et al., 2015), and phthalates (Fadipe et al., 2014b) have been isolated and characterized. To the best of our knowledge, little chemical studies have been reported on the fruits of *N. latifolia* Sm. and this is the first report on the evaluation of its antileishmanial property. In our continuing search for new antileishmanial agents from medicinal plants of Cameroon (Lenta et al., 2007; Lenta et al., 2015; Wonkam et al., 2020), the MeOH extract of the ripe fruits of *N. latifolia* Sm. was found to exhibit good antileishmanial activity during preliminary screening *in vitro* against *L. donovani* 1S (MHOM/SD/62/1S) promastigotes.

2. Experimental

2.1. General experimental procedures

For this study, methanol was used for the extraction of the plant materials; *n*-hexane, dichloromethane, ethyl acetate, acetone, methanol, and water were used as unary, binary or ternary mixtures at different polarities for the fractionation of extracts and purification of compounds. Column chromatography (CC) was carried out on silica gel 230-400 mesh, Merck (Merck, Darmstadt, Germany), 70-230 mesh (Merck) or sephadex LH-20 (Sigma-Aldrich, Munich, Germany). Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel (60 F_{254}) aluminium foil (Merck) and compound spots were detected by spraying with diluted sulfuric acid before heating the plate at about 100 °C or by visual inspection under UV lamp at 254 nm and 365 nm. High resolution mass spectra were obtained with a QTOF Compact Spectrometer (Bruker, Germany) equipped with an ESI source with an HR instrument. The spectrometer was operated in positive and negative modes (mass range: 50-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.4 ppm deviation using sodium formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200 °C. Nitrogen was used as sheath gas (4 L/min). The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX 500 MHz and 600 MHz NMR spectrometers (Bruker Corporation, Brussels, Belgium) in deuterated solvents. Chemical shifts were reported in δ (ppm) while coupling constants (J) were measured in Hz.

2.2. Plant material

The ripe fruits of *N. latifolia* were harvested in June 2017 at Makenene, a locality in the Mbam and Inoubou Division of the Centre Region of Cameroon and identified by Dr. Tacham Walter Ndam, Botanist at the Faculty of Science of The University of Bamenda, Cameroon, and compared with voucher specimens formerly kept at the National Herbarium of Cameroon under the registration number 20144/SFR/Cam.

2.3. Extraction and isolation

The air-dried and powdered fruits of *N. latifolia* (2.1 kg) were extracted thrice at room temperature with MeOH (20 L) (48 h). The extract was concentrated to dryness under vacuum at room temperature to give 459.7 g of a red paste. 439.0 g of this extract was successively and exhaustively partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-butanol (1.5 L each) to give the *n*-hexane fraction (16.4 g), CH_2Cl_2 fraction (18.4 g), EtOAc fraction (28.6 g) and *n*-butanol fraction (91.8 g) along with a solid residue (281.9 g). The *n*-hexane fraction (15.2 g) was subjected to CC over silica gel eluted with *n*-hexane/acetone and acetone/MeOH mixtures of increasing polarity to give one hundred and ninety-three (193) fractions of 125 mL each. These fractions were combined based on their TLC profiles into three subfractions labelled FH1, FH2 and FH3. CC of subfraction FH1 (5.4 g; *n*-hexane/acetone, 1:0-9:1, v/v) led to the isolation of compound **2** (18.3 mg; *n*-hexane/acetone, 9:1, v/v) and compound **3** (4.1 mg; *n*-hexane/acetone, 9:1, v/v). Subfraction FH2 (4.2 g, *n*-hexane/acetone, 8:2-6:4, v/v) was purified using silica gel to give compound **4** (4.6 mg; *n*-hexane/acetone, 8:2, v/v), the mixture of compounds **4** and **5** (5.3 mg; *n*-hexane/acetone, 8:2, v/v) and compound **6** (4.3 mg; *n*-hexane/acetone, 7:3, v/v). Subfraction FH3 (5.6 g, *n*-hexane/acetone, 6:4-0:1; acetone/methanol, 1:0-1.9:1 v/v) was subjected to CC on silica gel and isocratically eluted with *n*-hexane/acetone (6:4, v/v) solvent system, followed by purification on Sephadex LH-20, eluted with CH_2Cl_2/MeOH (3:7, v/v) to afford compound **7** (4.4 mg). Likewise, 17.1 g of the CH_2Cl_2 fraction was subjected

to CC on silica gel eluting with *n*-hexane/CH_2Cl_2 and CH_2Cl_2/acetone mixtures of increasing polarity to give one hundred and ninety-two (192) fractions of 125 mL each. These fractions were also combined on the basis of their TLC profiles into three subfractions labelled FC1, FC2 and FC3. CC of subfraction FC1 (8.4 g; *n*-hexane/CH_2Cl_2, 7:3-0:1; CH_2Cl_2/acetone, 1:0-9:1, v/v) led to the isolation of compound **1** (48.2 mg; *n*-hexane/CH_2Cl_2, 3:7, v/v) and compound **8** (102.6 mg; CH_2Cl_2/acetone, 95:5). Subfraction FC2 (3.4 g; CH_2Cl_2/acetone, 9:1-3:7, v/v) was purified over silica gel followed by Sephadex LH-20, eluting with CH_2Cl_2/MeOH (3:7, v/v) to give compound **9** (3.8 mg; CH_2Cl_2/acetone, 9:1, v/v). Also, 27.3 g of the EtOAc fraction was subjected to CC over silica gel and eluted with CH_2Cl_2/acetone and acetone/MeOH solvent systems of increasing polarities to give one hundred and eighteen (118) fractions of 250 mL each. These fractions were equally combined based on their TLC profiles into three subfractions labelled FA1, FA2 and FA3. CC of subfraction FA1 (6.1 g; CH_2Cl_2/acetone, 1:0-7:3, v/v) led to the isolation of compound **10** (3.8 mg; CH_2Cl_2/acetone, 9:1, v/v). Subfraction FC2 (14.7 g; CH_2Cl_2/acetone, 7:3-3:7; acetone/MeOH, 1:0-1.9:1, v/v) was purified over silica gel to give compound **11** (5.6 mg; CH_2Cl_2/acetone, 9:1, v/v) and compound **12** (1.02 g; CH_2Cl_2/acetone, 8:2, v/v). The non-active *n*-butanol fraction (90.3 g) was subjected to CC on silica gel using EtOAc/MeOH (1.9:1-0:1, v/v) to yield sixty-eight (68) fractions of 500 mL each. They were also combined based on their TLC profiles into four subfractions labelled FB1 to FB4. CC over silica gel of the subfraction FB2 (12.8 g; EtOAc/MeOH, 9:1-6:4, v/v) followed by Sephadex LH-20, eluting with MeOH, yielded compound **13** (17.2 mg; EtOAc/MeOH, 8:2, v/v) and the mixture of compounds **14** and **15** (18.1 mg; EtOAc/MeOH, 7:3, v/v).

2.4. Spectroscopic data of isolated compounds

Compound (**1)** was obtained as a white powder (48.2 mg). HR-ESIMS *m/z* $[2M+Na]^+$ = 359.0788. NMR ^1H (pyridine-d_5, 500 MHz): 5.83 (2H, s, H-3/H-5), 3.80 (6H, s, 2-OMe/6-OMe). NMR ^{13}C (pyridine-d_5, 125 MHz) δ_C 186.8 (C-4), 177.1 (C-1), 157.3 (C-2/C-6); 107.4 (C-3/C-5), 56.5 (OCH_3). The structure was confirmed as it was compared with literature data (Kupchan and Mang, 1960).
Compound (**2**)
White powder (18.3 mg). HR-ESIMS *m/z* $[2M+Na]^+$ = 851.7730. NMR ^1H ($CDCl_3$, 500 MHz) δ_H 5.08 (1H, dd, *J* = 15.2, 8.6 Hz, H-22) and 4.95 (1H, dd, *J* = 15.1, 8.7 Hz, H-23), 3.46 (1H, m, H-3). The structure was confirmed as it was compared with literature data (Habib et al., 2007).
Compound (**3**)
White powder (4.1 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 353.2689. NMR ^1H ($CDCl_3$, 500 MHz) δ_H 4.17 (1H, dd, *J* = 11.7, 6.2 Hz, H-1b), 4.23 (1H, dd, *J* = 11.7, 4.6 Hz, H-1a), 3.93 (1H, m, H-2), 3.62 (1H, dd, *J* = 11.5, 5.8 Hz, H-3b), 3.72 (1H, dd, *J* = 11.5, 4.0 Hz, H-3a), 2.37 (2H, t, *J* = 7.6 Hz, H-2′), 1.28 (br s, $12CH_2$), 0.90 (3H, t, *J* = 7.0 Hz, H-16). NMR ^{13}C ($CDCl_3$, 125 MHz) δ_C 174.4 (C-1′), 70.3 (C-2), 65.2 (C-1), 63.3 (C-3), 34.1 (C-2′) 31.9-22.7 (12 CH_2), 14.1 (C-16′). The structure was confirmed as it was compared with literature data (Xiaoling et al., 2004).
Compound (**4**)
White powder (4.6 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 465.3694. NMR ^1H($CDCl_3$/methanol-d_4, 500 MHz) δ_H 5.24 (1H, t, *J* = 3.7 Hz, H-12), 3.17 (1H, dd, *J* = 10.7, 5.4 Hz, H-3), 2.82 (1H, dd, *J* = 14.1, 4.6 Hz, H-18), 1.14 (3H, s, H-27), 0.96 (3H, s, H-23), 0.92 (3H, s, H-30), 0.91 (3H, s, H-25), 0.89 (3H, s, H-29), 0.79 (3H, s, H-26), 0.76 (3H, s, H-24). NMR ^{13}C($CDCl_3$/methanol-d_4, 125 MHz) δ_C 38.5 (C-1), 27.5 (C-2),.88.5 (C-3), 38.6 (C-4), 55.2 (C-5), 18.2 (C-6), 32.6 (C-7), 39.2 (C-8), 47.6 (C-9), 36.9 (C-10), 23.3 (C-11), 122.2 (C-12), 144.8 (C-13), 41.6 (C-14), 26.6 (C-15), 22.9 (C-16), 46.3 (C-17), 41.2 (C-18), 45.9 (C-19), 30.5 (C-20), 33.7 (C-21), 32.4 (C-22), 27.7 (C-23), 15.3 (C-24), 15.0 (C-25), 15.6 (C-26), 25.6 (C-27), 180.6 (C-28), 32.7 (C-29), 23.1 (C-30). The structure was confirmed as it was compared with literature data (Venditti et al., 2015).
Compound (**5**)
White powder (5.3 mg). NMR ^1H($CDCl_3$/methanol-d_4, 500 MHz). NMR ^{13}C (pyridine-d_5, 500 MHz). δ_C 39.0 (C-1), 28.0 (C-2),.77.3 (C-3), 39.2 (C-4), 54.7 (C-5), 18.5 (C-6), 34.6 (C-7), 40.8 (C-8), 50.7 (C-9), 37.2 (C-10), 20.9 (C-11), 25.8 (C-12), 38.3 (C-13), 42.6 (C-14), 30.9 (C-15), 32.6 (C-16), 56.4 (C-17), 49.5 (C-18), 47.5 (C-19), 151.1 (C-20), 30.0 (C-21), 37.3 (C-22), 28.5 (C-23), 16.1 (C-24), 16.2 (C-25), 16.2 (C-26), 14.6 (C-27), 178.6 (C-28), 109.7 (C-29), 19.2 (C-30). The structure was confirmed as it was compared with literature data (Eder et al., 2008).
Compound (**6**)
White powder (4.3 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 495.3487. NMR ^1H (pyridine-d_5, 500 MHz) δ_H 5.48 (1H, t, *J* = 3.0 Hz, H-12), 4.19 (1H, dd, *J* = 18.0, 7.6 Hz, H-3), 4.17 (1H, d, *J* = 10.1, H-23a), 3.71 (1H, d, *J* = 10.3 Hz, H-23b), 3.29 (1H, dd, *J* = 13.8, 4.0 Hz, H-18), 1.22 (3H, s, H-27), 1.04 (3H, s, H-23), 1.03 (3H, s, H-26), 0.98 (3H, s, H-30), 0.95 (3H, s, H-25), 0.91 (3H, s, H-29). NMR ^{13}C (pyridine-d_5, 125 MHz). δ_C 38.5 (C-1), 27.4 (C-2),.73.4 (C-3), 42.6 (C-4), 48.3 (C-5), 18.3 (C-6), 32.7 (C-7), 39.5 (C-8), 47.9 (C-9), 36.9 (C-10), 23.6 (C-11), 122.3 (C-12), 144.6 (C-13), 41.9 (C-14), 28.1 (C-15), 23.3 (C-16), 46.4 (C-17), 41.7 (C-18), 46.2 (C-19), 30.7 (C-20), 34.3 (C-21), 32.9 (C-22), 67.6 (C-23), 12.9 (C-24), 15.7 (C-25), 17.2 (C-26), 25.9 (C-27), 179.9 (C-28), 32.9 (C-29), 23.5 (C-30). The structure was confirmed as it was compared with literature data (Nkouayeb et al., 2020).
Compound (**7**)
White powder (4.4 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 495.3487. NMR ^1H (acetone-d_6/methanol-d_4 500 MHz) δ_H 5.49 (1H, t, *J* = 3.3 Hz, H-12), 3.87 (1H, ddd, *J* = 11.9, 4.6, 2.9 Hz, H-2), 3.50 (1H, d, *J* = 11.0 Hz, H-23), 3.37 (1H, d, *J* = 11.0 Hz, H-23), δ_H 3.45 (1H, dd, *J* = 10.2, 5.9 Hz, H-3), 2.63 (1H, d, *J* = 11.3 Hz, H-18), 1.12 (3H, s, H-27), 0.95 (3H, d, *J* = 6.4 Hz, H-30), 0.89 (3H, s, H-25), 0.85 (3H, s, H-26), 0.80 (3H, s, H-24). NMR ^{13}C (acetone-d_6/methanol-d_4, 500 MHz). δ_C 41.6 (C-1), 65.6 (C-2),.77.7 (C-3), 42.5 (C-4), 46.9 (C-5), 17.7 (C-6), 32.3 (C-7), 41.0 (C-8), 46.9 (C-9), 37.6 (C-10), 23.3 (C-11), 127.7 (C-12), 138.7 (C-13), 39.7 (C-14), 29.3 (C-15), 25.9 (C-16), 47.9 (C-17), 53.3 (C-18), 72.0(C-19), 41.3 (C-20), 28.2 (C-21), 37.7 (C-22), 70.1 (C-23), 16.3 (C-24), 16.0 (C-25), 16.4 (C-26), 23.7 (C-27), 179.5 (C-28), 26.0 (C-29), 15.5 (C-30). The structure was confirmed as it was compared with literature data (Wandji et al., 2003).
Compound (**8**)

Colorless crystals (102.6 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 257.0649. NMR $^1H(CDCl_3$, 500 MHz) δ_H 3.77 (3H, s, OCH_3), 3.63 (6H, s, OCH_3), 2.84 (2H, d, *J* = 15.6 Hz, H-1a/H-3a), 2.74 (2H, d, *J* = 15.6 Hz, H-1b/H-3b). NMR ^{13}C ($CDCl_3$, 125 MHz) δ_C, 173.8 (C-2′), 170.2 (C-1′/C-1′′), 73.1 (C-2), 53.2 (OCH_3), 52.0 (OCH_3). The structure was confirmed as it was compared with literature data (Kuete et al., 2015).

Compound (**9**)

Orange crystals (3.8 mg). mp: 241-242°C. HR-ESIMS *m/z* $[3M+Na]^+$ = 530.1056. NMR ^1H (methanol-d_4, 500 MHz) δ_H 6.86 (1H, d, *J* = 3.9 Hz, H-3), 6.85 (1H, d, *J* = 3.9 Hz, H-4), 3.88 (3H, s, OCH_3). NMR ^{13}C (methanol-d_4, 125 MHz) δ_C 127.3 (C-2), 115.0 (C-3), 115.1 (C-4), 126.1 (C-5), 162.0 (2-COOH), 161.0 (5-COO), 50.7 (OCH_3). The structure was confirmed as it was compared with literature data (Kostalova et al., 1992).

Compound (**10**)

White powder (3.8 mg). NMR ^1H ($CDCl_3$, 500 MHz) δ_H: 8.13 (4H, s, H-2/H-3/H-5/H-6), 4.71 (6H, s, OCH_3). NMR ^{13}C ($CDCl_3$, 125 MHz) δ_C 134.0 (C-1/C-4), 129.6 (C-2/C-3/C-5/C-6), 165.5 (C-1′), 62.9 (OCH_3). The structure was confirmed as it was compared with literature data (Adeniran and Abimbade, 2015).

Compound (**11**)

Colorless crystals (5.6 g). HR-ESIMS *m/z* $[M+Na]^+$ = 243.0484. NMR ^1H (methanol-d_4, 500 MHz) δ_H 3.63 (6H, s, OCH_3), 2.96 (2H, d, *J* = 15.4 Hz, H-3b/H-1′b), 2.84 (2H, d, *J* = 15.4 Hz, H-3a/H-1′a). NMR ^{13}C (methanol-d_4, 125 MHz) δ_C 175.0 (C-1), 170.4 (C-4/C-2′), 72.8 (C-2), 42.6 (C-3/C-1′), 50.7 (OCH_3). The structure was confirmed as it was compared with literature data (Nkouayeb et al., 2020).

Compound (**12**)

Colorless crystals (1.02 g). HR-ESIMS *m/z* $[M+Na]^+$ = 229.0302. NMR ^1H (methanol-d_4, 500 MHz) δ_H 3.77 (3H, s, OCH_3), 2.93 (2H, d, *J* = 15.6 Hz, H-2b/H-4b), 2.78 (2H, d, *J* = 15.6 Hz, H-2a/H-4a). NMR ^{13}C (methanol-d_4, 125 MHz) δ_C 174.1 (C-1′), 171.9 (C-1/C-5), 73.1 (C-3), 51.6. (OCH_3), 42.8 (C-2/C-4). The structure was confirmed as it was compared with literature data (Nkouayeb et al., 2020).

Compound (**13**)

Chesnut paste (17.2 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 575.1946. NMR ^1H (methanol-d_4, 500 MHz) δ_H 7.42 (1H, s), 5.23 (1H, d, *J* = 5.0 Hz, H-1), 4.68 (1H, d, *J* = 7.9 Hz, H-1′), 4.39 (1H, d, *J* = 7.8 Hz, 1″), 4.08 (1H, m, H-7), 3.69 (3H, s, OCH_3), 3.13 (1H, brq, *J* = 8.2 Hz, H-5), 2.26 (1H, m, H-6a), 2.02 (1H, td, *J* = 8.8, 5.0 Hz, H-9), 1.91 (1H, m, H-8), 1.65 (1H, m, H-6b), 1.13 (3H, d, *J* = 6.9 Hz, H-10). NMR ^{13}C (methanol-d_4, 125 MHz) δ_C 96.9 (C-1), 150.9 (C-3), 112.4 (C-4), 31.0 (C-5), 41.3 (C-6), 73.6 (C-7), 40.9 (C-8), 45.0 (C-9), 12.3 (C-10), 168.2 (C-11), 50.2 (OCH_3), 98.9 (C-1′), 73.7 (C-2′), 76.5 (C-3′), 70.1 (C-4′), 76.6 (C-5′), 68.7 (C-6′), 103.7 (C-1′′), 73.2 (C-2′′), 75.9 (C-3′′), 70.1 (C-4′′), 76.7 (C-5′′), 61.3 (C-6′′). The structure was confirmed as it was compared with literature data (Kanchanapoom et al., 2001).

Compound (**14**)

Chesnut paste (18.1 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 203.0572. NMR ^1H (methanol-d_4, 500 MHz) δ_H 4.19 (1H, d, *J* = 7.8 Hz, H-1), 3.89 (1H, dd, *J* = 11.9, 1.8 Hz, H-6), 3.69 (1H, dd, *J* = 11.9, 5.3 Hz, H-6), 3.18 (1H, dd, *J* = 7.8, 9.1 Hz , H-2), 3.37 (1H, t, *J* = 9.0 Hz, H-3), 3.29 (2H, m, H-4/H-5). The structure was confirmed as it was compared with literature data (Agrawal, 1992).

Compound (**15**)

Chesnut paste (18.1 mg). HR-ESIMS *m/z* $[M+Na]^+$ = 203.0572. NMR ^1H (methanol-d_4, 500 MHz) δ_H 5.13 (1H, d, *J* = 3.6 Hz, H-1), 3.89 (1H, dd, *J* = 11.9, 1.8 Hz, H-6), 3.69 (1H, dd, *J* = 11.9, 5.3 Hz, H-6), 3.18 (1H, dd, *J* = 7.8, 9.1 Hz , H-2), 3.37 (1H, t, *J* = 9.0 Hz, H-3), 3.29 (2H, m, H-4/H-5). The structure was confirmed as it was compared with literature data (Agrawal, 1992).

2.5. Antileishmanial and cytotoxicity assays

The cryopreserved promastigote form of *Leishmania donovani* 1S (MHOM/SD/62/1S) was obtained from Bei Resources and is routinely maintained at the Antimicrobial and Biocontrol Agents Unit, University of Yaoundé 1, in M199 medium supplemented with 10% Heat-Inactivated Fetal Bovine Serum (HIFBS) (Sigma) with 100 IU/mL penicillin and 100 µg/mL streptomycin. The culture is maintained in 75 cm^2 cell culture flask at 28 °C (Khanjani et al., 2015) and checked for growth daily and sub-cultured every 72 h. The antileishmanial effect of *N. latifolia* crude extract, fractions and some isolated compounds on cultured *L. donovani* 1S (MHOM/SD/62/1S) promastigotes was evaluated using the resazurin colorimetric method as described by Siqueira-Neto et al. (2010) with little modifications. For this purpose, the promastigotes from a logarithmic phase culture (4×10^5 cells/mL; 90 µL) were seeded in 96-well microtiter plates and were treated with 10 µL of inhibitors at different concentrations. They were all screened in triplicate at final concentrations of 100-0.16 µg/mL for extracts/fractions and 50-0.08 µg/mL for compounds and test plates were incubated for 28 h at 28 °C, followed by the addition of 1 mg/mL resazurin. The negative and positive controls were 0.1% DMSO and amphotericin B (10-0.016 µg/mL), respectively. After an additional incubation for 44 h, plates were then read on a Magelan Infinite M200 fluorescence multi-well plate reader (Tecan) at an excitation and emission wave lengths of 530 and 590 nm, respectively. The cytotoxicity profile of the crude extract, fractions and compounds was assessed using the Alamar blue assay (Mosmann, 1993) against Raw 264.7 cells duly cultivated in complete Dulbecco's Modified Eagle's Medium (DMEM) containing 13.5 g/L DMEM (Sigma Aldrich), 10% Fetal Bovine Serum (Sigma Aldrich), 0.2% sodium bicarbonate (w/v) (Sigma Aldrich) and 50 µg/mL gentamycin (Sigma Aldrich). Globally, macrophages were seeded into 96-wells cell-culture flat-bottomed plates at a density of 104 cells in 100 µL of complete medium/well and incubated for 24 h at 37 °C, 5% CO_2 to allow cell adhesion. 10 µL of each serially diluted test sample solutions were added and assay plates were then incubated for 48 h in the same experimental conditions. Growth control (0.1% DMSO-100% growth) and positive control wells (Podophyllotoxin at 20 µM) were included in the experiment 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 plates incubation during 4 h. Fluorescence was then read on a Tecan Infinite M200 fluorescence multi-well plate reader (Tecan) at an excitation/emission of 530/590 nm. Results were expressed as 50% cytotoxic concentration

(CC_{50}) and selectivity index (SI) (CC_{50} Mammalian cell/ IC_{50} *Leishmania donovani*) were calculated for each tested substance. The data was subjected to one-way analysis of variance (ANOVA) and results were presented as means ± SD of the replicated values.

3. Results and Discussion

3.1. Antileishmanial activity and cytotoxicity of crude extract and fractions of *N. latifolia*

The MeOH crude extract of *N. latifolia* displayed high antileishmanial activity against *Leishmania donovani* (IC_{50} = 2.20 µg/mL) with good selectivity (SI > 43.35) (Table 1). This dry extract was dissolved again in water and then fractionated using *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol, successively. The four fractions obtained were also screened for their activity against the same strain. The *n*-hexane fraction was the most active fraction with an IC_{50} value of 7.06 µg/mL. The dichloromethane and ethyl acetate fractions exhibited good and moderate activity with IC_{50} values of 30.57 and 91.21 µg/mL, respectively. All the active fractions were selective (SI > 1). The *n*-butanol fraction was inactive with an IC_{50} value > 100 µg/mL (Table 1).

3.2. Isolation and characterization of compounds

Successive column chromatographies of the fractions led to the isolation of fifteen compounds (**1-15)** whose structures were established based on their MS and NMR data and by comparison of their data with those reported in the literature. From the *n*-hexane soluble fraction, six compounds were isolated including, stigmasterol (**2**) (Habib et al., 2007), glycerol palmitate (**3**) (Xiaoling et al., 2004), oleanolic acid (**4**) (Venditti et al., 2015), mixture of oleanolic (**4**) and betulinic acid (**5**) (Eder et al., 2008), hederagenin (**6**) (Nkouayeb et al., 2020), and myrianthic acid (**7**) (Wandji et al., 2003) (Fig. 1). From the dichloromethane soluble fraction, three compounds were isolated namely, 2,6-dimethoxybenzoquinone (**1**) (Jian-Juan et al., 2012), trimethyl citrate (**8**) (Kuete et al., 2015; Sorensen et al., 2010), and monomethyl ester of 1H-pyrrole-2,5-dicarboxylic acid (**9**) (Kostalova et al., 1992) (Fig. 1). From the ethyl acetate soluble fraction, three compounds were isolated namely, 1,4-dimethylbenzene-1,4-dicarboxylate (**10**) (Adeniran and Abimbade, 2015), dimethyl citrate (**11**) (Sorensen et al., 2010), and methyl citrate (**12**) (Nkouayeb et al., 2020) (Fig. 1). In order to contribute to the chemotaxonomy knowledge of this plant, the *n*-butanol fraction, though inactive, was also studied and afforded three compounds namely, loganin 6′-*O*-β-glucopyranoside (**13**) (Kanchanapoom et al., 2001) and mixture of β-D-glucose (**14**) and α-D-glucose (**15**) (Agrawal, 1992) (Fig. 1). To the best of our knowledge, this study is the first report on the isolation of 2,6-dimethoxybenzoquinone (**1**), myrianthic acid (**7**), monomethyl ester of 1H-pyrrole-2,5-dicarboxylic acid (**9**), 1,4-dimethylbenzene-1,4-dicarboxylate (**10**), dimethyl citrate (**11**), and loganin 6′-*O*-β-glucopyranoside (**13**) from plants of the genus *Nauclea*. Monomethyl ester of 1H-pyrrole-2,5-dicarboxylic acid (**9**) and dimethyl citrate (**11**) are isolated here for the first time from the Rubiaceae family, even though these two compounds including trimethyl citrate (**8**) and methyl citrate (**12**) are most likely artifacts; their possible artifact origin being due to the extraction with methanol (Venditti, 2018). Also, dimethyl terephthalate (**10**) isolated from the ripe fruits could either be a contaminant or of anthropic origin (Bianco et al., 2014; Thiemann, 2021). Even though alkaloids are markers of plants of the genus *Nauclea* (Liu et al., 2018; Mai et al., 2021), no alkaloids were detected in the ripe fruits of the sample investigated. In fact, the Dragendorff and Mayer tests used to identify alkaloids were negative.

3.3. Antileishmanial activity and cytotoxicity of isolated compounds of *N. latifolia*

All the isolated compounds were tested for their antileishmanial activity against the same strain of *L. donovani* and their cytotoxicity profiles evaluated. Hederagenin (**6**), coming from the most active *n*-hexane fraction, showed promising activity with IC_{50} value of 19.3 µM and was selective (SI > 5.18). It was observed that the mixture of oleanolic (**4**) and betulinic acids (**5**) (1:1) showed moderated activity (IC_{50} = 45.19 µM), while oleanolic acid (**4**) was inactive (IC_{50} > 50 µM). Myrianthic (**7**) acid showed also moderated activity with IC_{50} value of 46.12 µM (Table 1). Most of the triterpenoids isolated from the *n*-hexane fraction exhibited a certain threshold of antileishmanial activity. The results highlight the antileishmanial potential of this class of secondary metabolites. Some authors suggested that the antileishmanial activity of triterpenoids could be related to the inhibition of leishmanial proteins and nucleic acids synthesis and/or to the inhibition of a membrane-associated calcium-dependent ATPase pump (Goijman et al., 1984; Mishina et al., 2007; Isah et al., 2016). From the dichloromethane fraction, 2,6-dimethoxybenzoquinone (**1**) was the most active compound with IC_{50} value of 9.94 µM. Monomethyl ester of 1H-pyrrole-2,5-dicarboxylic acid (**9**) showed moderate activity with IC_{50} value of 47.98 µM, while trimethyl citrate (**8**) was inactive (Table 1). Unfortunately, compound **1** was toxic and not selective towards RAW 264.7 macrophage cells (SI = 0.29). Except for stigmasterol, oleanolic acid, betulinic acid, hederagenin and myrianthic acid, this is the first report on the antileishmanial activity of compounds **1**, **3**, **8-13** (Fig. 1). The mechanisms involved in cytotoxicity of the quinone derivatives are still largely unknown. So far, it is clear that quinones can alkylate essential proteins or inactivate enzymes either directly or following reduction. However, the most prominent characteristic of quinones is their ability to undergo reversible oxidation-reduction (De Sena et al., 2016; Sun et al., 1997). All the isolated compounds from the ethyl acetate fraction were inactive (Table 1).

From these results, we can conclude that the difference in activity between the crude extract, fractions and the isolated compounds may be due to a synergistic action

Table 1
Antileishmanial activity and selectivity indices of crude extracts, fractions and compounds from *N. latifolia*.

Extract/Fractions/Compounds	Promastigotes IC_{50} ± SD	Macrophages CC_{50} ± SD	Selectivity Index (SI) (CC_{50}/IC_{50})
MeOH fruits crude extract	2.20 ± 0.25	> 100	> 43.35
n-Hexane fraction	7.06 ± 0.09	> 100	> 14.15
Stigmasterol (**2**)	> 50	ND	ND
Glycerol palmitate (**3**)	> 50	ND	ND
Oleanolic acid (**4**)	> 50	ND	ND
Mixture of oleanolic (4) and betulinic acid (**5**) (1:1)	45.19 ± 0.35	ND	ND
Hederagenin (**6**)	19.3 ± 0.61	> 100	> 5.18
Myrianthic acid (**7**)	46.12 ± 0.15	> 100	1.99
CH_2Cl_2 fraction	30.57 ± 0.20	> 100	> 3.27
2,6-dimethoxybenzoquinone (**1**)	9.94 ± 0.17	2.84 ± 0.23	0.29
Trimethyl citrate (**8**)	> 50	ND	ND
Monomethyl ester of 1H-pyrrole-2,5-dicarboxylic acid (**9**)	47.98 ± 0.23	63.45 ± 0.76	7.82
AcOEt fraction	91.21 ± 0.13	> 100	> 1.09
1,4-dimethylbenzene-1,4-dicarboxylate (**10**)	> 50	ND	ND
Dimethyl citrate (**11**)	> 50	ND	ND
Methyl citrate (**12**)	> 50	ND	ND
n-Butanol fraction	> 100	ND	ND
Loganin 6′-*O*-β-glucopyranoside (**13**)	> 50	ND	ND
Mixture of β-D-glucose (**14**) and α-D-glucose (**15**) (1:1)	> 50	ND	ND
Amphotericin B	0.35 ± 0.23	-	-

ND: not determined; Extract, fractions in µg/ml and compounds in µM. Data points are means from triplicate experiments. SD = Standard Deviation; Activity values were obtained from sigmoidal dose-response curves of concentration versus response.

of the isolated compounds. This synergistic effect of the chemical constituents in plant extracts was previously discussed by Caesar and Cech (2019), who demonstrated that the complexity of crude extracts enhances its biological activity on certain therapeutic targets.

4. Concluding remarks

The present study showed that the MeOH crude extract, the *n*-hexane and CH_2Cl_2 fractions of the edible ripe fruits of *N. latifolia* possess potent to good antileishmanial activity on *L. donovani* 1S (MHOM/SD/62/1S) promastigotes with good selectivity towards Raw 264.7 cells. 2,6-dimethoxybenzoquinone and hegeragenin showed good to promising antileishmanial activity. Even though compound 2,6-dimethoxybenzoquinone was not selective, hegeragenin displayed good selectivity towards Raw 264.7 cells. This study enriches the knowledge on phytochemistry of this plant and shows the presence of uncommon metabolites encountered in the Rubiaceae family. The fruit of *N. latifolia* is widely consumed by populations in Cameroon and this shows the evidence of its safety and non-toxicity. The results presented in this study indicate that herbal preparations from the fruits of *N. latifolia* may be developed as an adjuvant therapy for the treatment of visceral leishmaniasis.

Conflict of interest

The authors declare that there is no conflict of interest.

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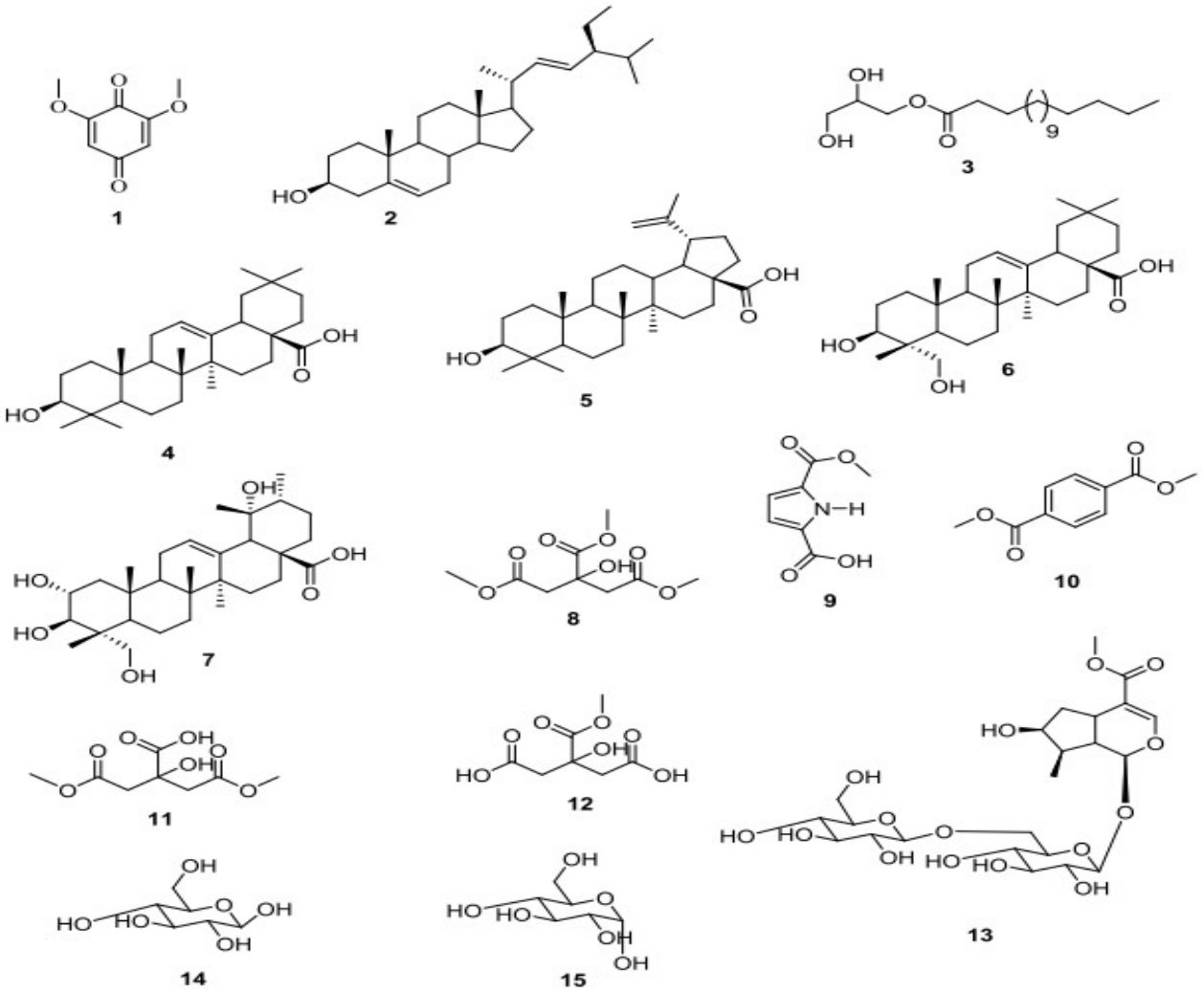

Fig. 1. Structures of isolated compounds from *N. latifolia* ripe fruits (**1-15**).

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