



Antimicrobial, antioxidant, anti-inflammatory activities and phytoconstituents of extracts from the roots of *Dissotis thollonii* Cogn. (Melastomataceae)



R.N. Nono^a, L. Barboni^b, R.B. Teponno^a, L. Quassinti^c, M. Bramucci^c, L.A. Vitali^c, D. Petrelli^d, G. Lupidi^c, A.L. Tapondjou^{a,*}

^a Laboratory of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Box 183, Dschang, Cameroon

^b School of Science and Technology, Chemistry Division, University of Camerino, I-62032 Camerino, Italy

^c School of Pharmacy, University of Camerino, I-62032 Camerino, Italy

^d School of Biosciences and Veterinary Medicine, University of Camerino, I-62032 Camerino, Italy

ARTICLE INFO

Article history:

Received 19 January 2014

Received in revised form 5 March 2014

Accepted 19 March 2014

Available online 13 April 2014

Edited by AK Jäger

Keywords:

Dissotis thollonii

Melastomataceae

Extracts

Compounds

Antimicrobial

Antioxidant

Anti-inflammatory activity

ABSTRACT

Background: *Dissotis thollonii* Cogn. belonging to the Malastomataceae family is used in the West Region of Cameroon for the treatment of inflammation, kidney diseases, pregnancy control and sinusitis. Despite the traditional use of this plant, no scientific report or information was found in the literature regarding neither its biological activity nor its chemical constituents.

Aim of the study: The present work was designed to determine the antimicrobial, antioxidant and anti-inflammatory activities of different extracts of the roots of *D. thollonii* Cogn. as well as the isolation and identification of the chemical constituents of this plant.

Materials and methods: The tests for antimicrobial, antioxidant and anti-inflammatory activities were performed over the MeOH, EtOAc, n-BuOH and aqueous extracts. Compounds were isolated from EtOAc and n-BuOH extracts of the roots of *D. thollonii* Cogn. through column chromatography and their structures were determined by means of NMR and MS analysis, and published data.

Results: According to the antimicrobial and antioxidant assays, the EtOAc and n-BuOH extracts were submitted to further separation and purification. This led to the isolation of twelve compounds identified as 3,3'-di-O-methylellagic acid 4'-O-β-D-xylopyranoside **1**, 3-O-methylellagic acid 4'-O-β-D-arabinopyranoside **2**, casuarinin **3**, betulinic acid **4**, β-sitosterol-3-O-D-glucopyranosyl-6'-mirystate **5**, cellobiosylsterol **6**, β-sitosterol **7**, β-sitosterol-3-O-β-D-glucopyranoside **8**, arjunolic acid **9**, 3,3'-di-O-methylellagic acid **10**, ellagic acid **11**, and 3,3'-di-O-methylellagic acid 4'-O-β-D-glucopyranoside **12**. The EtOAc extract was the only antimicrobial active sample [diameter of the zone of inhibition (DZI) of 10 mm against *Staphylococcus aureus*] among all the tested extracts. The analysis of fractions of this extract revealed the presence of bioactive compounds with a described antimicrobial activity such as β-sitosterol, β-sitosterol-3-O-β-D-glucopyranoside and arjunolic acid. By using Trolox as the standard drug, all extracts showed antioxidant activity against DPPH in the following order of scavenging ability: Trolox > nBuOH > EtOAc > MeOH > WE (water extract). The ABTS⁺ scavenging ability was similar to that found for the DPPH assay, being Trolox > n-BuOH > MeOH > EtOAc > WE. Along with the DPPH and ABTS assays, the FRAP assay showed the scale n-BuOH > MeOH > WE > EtOAc. The phytochemical study of the EtOAc and n-BuOH extracts revealed the presence of important known antioxidant compounds such as ellagic acid derivatives, arjunolic acid, betulinic acid and β-sitosterol. The anti-inflammatory properties of *D. thollonii* extracts were investigated using RAW 264.7 murine macrophage cells. The MeOH extract reduced the stimulated NO production in a concentration-dependent manner. 86% reduction was observed at the highest tested concentration of 100 μg/ml (IC₅₀ = 5.9 μg/ml). The n-BuOH extract showed higher dose dependent reduction of NO formation (IC₅₀ = 6.5 μg/ml) than the EtOAc extract (IC₅₀ = 18.1 μg/ml), whereas the water extract had no significant influence on the NO production. All the extracts did not have any influence on the macrophage viability. The phytochemical investigation of the EtOAc and n-BuOH extracts revealed that the main compounds identified do have potent anti-inflammatory properties.

* Corresponding author. Tel.: +237 75004826; fax: +237 33451735.

E-mail address: tapondjou2001@yahoo.fr (A.L. Tapondjou).

Conclusion: The biological and phytochemical characterization of the root extracts of *D. thollonii* validates the use of this plant for the treatment of inflammation and sinusitis, thus providing evidence that this plant extracts, as well as some of the isolated compounds, might be potential sources of antioxidant and anti-inflammatory drugs.
© 2014 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

African populations are confronted with chronic diseases emergence whose treatment and follow-up constitute for them a major economic problem (Konkon et al., 2008). Of all the alternative modalities, herbal medicine is probably the most popular and the most ubiquitous (Akerele, 1993). The World Health Organization has described traditional herbal medicine as one of the surest means to achieve total health care coverage of the world's population (Okunlola et al., 2007). In traditional herbal practice in Africa, indigenous medicinal plants have been employed in the treatment of several important infections (Taylor et al., 2001). The Melastomataceae are predominantly pantropical plants, including approximately 163 genera and 4300 species (Lais et al., 2011). Many species of this family are known for their different uses in folk medicine as antioxidant, antihypertensive, antihyperglycemic, hemostatic, antihepatitis, and antiarrhythmic drugs (Susanti et al., 2007; Cheng et al., 1993; Amalraj and Ignacimuthu, 1998; Nicholl et al., 2001; Abere et al., 2010). Phytochemical studies of Melastomataceae plants have indicated an abundance of tannins, polyphenols, flavonoids, fatty acids, steroids, and free triterpenoids (Yoshida et al., 1994; Isaza et al., 2001; Calderón et al., 2002; Ndjateu et al., 2014). *Dissotis thollonii* (IPNI, 2012) is used in the West Region of Cameroon for the treatment of inflammation, kidney diseases, pregnancy control and sinusitis. Despite the traditional use of this plant, no scientific report or information was found in the literature regarding neither its biological activity nor its chemical constituents. In our continuous search for bioactive extracts and secondary metabolites from Cameroonian medicinal plants (Nzowa et al., 2013; Fouedjou et al., 2014), and with the aim to support the traditional use of *D. thollonii*, we undertook the present study to investigate the phytochemistry and the antimicrobial, antioxidant and anti-inflammatory activities of the roots extracts of *D. thollonii*.

2. Materials and methods

2.1. Plant material

The roots of *D. thollonii* Cogn. (Melastomataceae) were collected in Bangoua village near Bangangté (West Region of Cameroon) in November 2011. The plant was identified at the Cameroon National Herbarium, Yaoundé, Cameroon where a voucher specimen was deposited under the reference number 28107/SRF/CAM.

2.2. Extraction and isolation

2.2.1. Preparation of extracts

The air-dried and pulverized roots of *D. thollonii* (1.8 kg) were extracted three times (each time for 24 h) by maceration with MeOH (11 l) at room temperature. The filtrate obtained was concentrated under reduced pressure to yield a dark crude extract (211 g). Part of this extract (183 g) was suspended in water (400 ml) and successively extracted with EtOAc and n-BuOH which were concentrated to dryness under reduced pressure to afford EtOAc (21 g) and n-BuOH (28.5 g) extracts, respectively. The aqueous extract was obtained by decoction of 50 g of roots' powder in 200 ml of distilled water and the filtrate obtained was concentrated under reduced pressure to yield 7 g of residue. The EtOAc and n-BuOH extracts were purified using column chromatography.

2.2.2. Isolation of compounds

According to the antimicrobial and antioxidant assays, the EtOAc and n-BuOH extracts were submitted to further separation and purification. Part of the EtOAc extract (18 g) was subjected to column chromatography (CC) over silica gel (hexane-EtOAc with increasing polarity) and EtOAc-MeOH yielding five main fractions (A–E). Fraction A (7.0 g) (hexane-EtOAc 90/10 to 80/20) was rechromatographed on silica gel column using hexane-EtOAc (85/15) to yield compound **7** (40 mg) and **4** (25 mg). Fraction B (300 mg) (hexane-EtOAc 70/30 to 50/50) was rechromatographed using hexane-EtOAc (70/30) to yield **5** (20 mg) and **9** (30 mg). Fraction D (1 g) (EtOAc) was subjected to CC over Sephadex LH-20 gel using MeOH as eluent to yield **1** (15 mg) and **8** (30 mg). Fraction E (5.5 g) was combined mainly on the basis of TLC to a part of n-BuOH extract (23.5 g) and subjected to CC over silica gel (EtOAc-MeOH with increasing polarity) yielding seven main fractions (I–VII). Fraction III (EtOAc) (4.8 g) was rechromatographed on silica gel column using EtOAc as eluent to yield **10** (4.7 mg) and **11** (5 mg). Fraction IV (EtOAc-MeOH 95/5) (5.3 g) was subjected to CC over Sephadex LH-20 using MeOH as eluent to yield **3** (914 mg). Fraction V (EtOAc-MeOH 95/5) (4.9 g) was rechromatographed over silica gel using EtOAc-MeOH-H₂O 9.5/1/0.5 as eluent to yield subfraction Va, Vb, Vc and Vd. Sub-fraction Vc (50 mg) was subjected to Sephadex LH-20 gel CC to yield **6** (8 mg) and **12** (9 mg). Sub-fraction Vd (111 mg) was subjected to silica gel CC using CHCl₃-MeOH (90/10) as eluent to yield **2** (22 mg).

2.3. Structure elucidation and identification of the isolated compounds

ESI mass spectra were carried out on an Agilent Technologies LC/MSD Trap SL (G2445D SL). Samples for NMR experiments were dissolved in deuterated solvents (CD₃OD, CDCl₃, Acetone-d₆, D₂O and DMSO-d₆) on a varian Mercury plus Spectrometer (400 MHz for ¹H and 100 MHz for ¹³C). CC was performed on silica gel 60 merck (0.040–0.063 or 0.063–0.2 mm) and sephadex gel LH-20. Fractions were monitored by TLC using Merck pre-coated silica gel sheets (60 F₂₅₄), and spots were visualized under UV light (254 and 365 nm) and by spraying with 50% H₂SO₄ and heating at 110 °C.

3,3'-Di-O-methylellagic acid 4'-O-β-D-xylopyranoside **1**: yellow powder, ESIMS (negative-ion mode) exhibited a pseudomolecular ion-peak at *m/z* 461.0 [M – H][–], consistent with the molecular formula C₂₁H₁₈O₁₂. ¹³C NMR (DMSO-d₆, 100 MHz): δ 113.2 (C-1), 142.0 (C-2), 142.3 (C-3), 153.3 (C-4), 112.3 (C-5), 114.9 (C-6), 158.8 (C-7), 112.0 (C-1'), 140.5 (C-2'), 141.4 (C-3'), 151.6 (C-4'), 112.0 (C-5'), 111.5 (C-6'), 158.8 (C-7'), 62.0 (3-OMe), 61.4 (3'-OMe) for aglycone; 102.2 (C-1''), 73.5 (C-2''), 76.5 (C-3''), 69.6 (C-4''), 66.2 (C-5'') for sugar moiety. ¹H NMR (DMSO-d₆, 400 MHz) δ 7.52 (1H, s, H-5), 7.76 (1H, s, H-5'), 4.08 (3H, s, 3-OMe), 4.05 (3H, s, 3'-OMe) for aglycone; 5.15 (1H, d, J = 7.2 Hz, H-1'') 3.78 (1H, dd, J = 11.2, 5.1 Hz, H-2''), 3.24 (1H, m, H-3''), 3.39 (1H, m, H-4''), 3.83 (1H, m, H-5'a), 3.38 (1H, m, H-5'b) for sugar moiety.

3-O-methylellagic acid 4'-O-β-D-arabinopyranoside **2**: yellow powder, ¹³C NMR (DMSO-d₆, 100 MHz) δ 140.5 (C-3), 11.2 (C-5), 160.5 (C-7), 150.0 (C-4'), 118.4 (C-5'), 159.5 (C-7'), 60.8 (3-OMe) for aglycone; 106.5 (C-1''), 73.6 (C-2''), 76.8 (C-3''), 69.6 (C-4''), 66.5 (C-5'') for sugar moiety. ¹H NMR (DMSO-d₆, 400 MHz) δ 7.49 (1H, s, H-5), 7.41 (1H, s, H-5'), 3.93 (3H, s, 3-OMe) for aglycone; 4.45 (1H, br, H-1''), 3.15 (1H, m, H-2''), 3.15 (1H, m, H-3''), 3.28 (1H, m, H-4''), 3.75 (1H, m, H-5'a), 3.08 (1H, m, H-5'b) for sugar moiety.

Casuarinin 3: yellowish powder, ESIMS (negative-ion mode) m/z 934.7 $[M - H]^-$ consistent with the molecular formula $C_{41}H_{28}O_{26}$; in this spectrum, we observe the peak at m/z 170.4 $[C_7H_5O_5 + H]^-$ and m/z 301.0 $[C_{14}H_5O_8]^-$. ^{13}C NMR (Acetone- d_6 + D_2O , 100 MHz) δ 66.6 (C-1), 75.9 (C-2), 68.6 (C-3), 73.3 (C-4), 70.3 (C-5), 63.6 (C-6) glucose moiety. 116.1, 119.6, 137.6, 142.8, 145.3 (C-1 HHDP moiety); 104.6, 145.7, 134.2, 115.5, 168.8 (C-3 HHDP (hexahydrodiphenoyl) moiety); 107.5, 144.4, 135.9, 115.5, 167.8 (C-4 HHDP moiety); 119.9, 109.1, 145.1, 138.2, 145.1, 109.1, 164.9 (C-5 galloyl moiety); 114.5, 135.0, 144.5, 168.0 (C-6 HHDP moiety); 163.7, 168.8, 167.8, 164.9, 168.0 (respectively for ester carbonyls in C-2, C-3, C-4, C-5 and C-6). 1H NMR (Acetone- d_6 , 400 MHz) δ 5.62 (1H, d, $J = 5.4$ Hz, H-1), 4.65 (1H, dd, $J = 1.5, 4.9$ Hz, H-2), 5.40 (1H, m, H-3), 5.41 (1H, m, H-4), 5.32 (1H, dd, $J = 2.5, 8.8$ Hz, H-5), 4.04 (1H, m, H-6a), 4.81 (1H, dd, $J = 3.4, 12.2$ Hz, H-6b) glucose moiety; 6.48 (1H, s, C-3 HHDP moiety), 6.53 (1H, s, C-6 HHDP moiety), 6.78 (1H, s, C-4 HHDP moiety), 7.09 (2H, s, galloyl-H).

Betulinic acid 4: white powder. ^{13}C NMR (CD_3OD , 100 MHz) δ 38.5 (C-1), 26.6 (C-2), 78.2 (C-3), 38.6 (C-4), 55.4 (C-5), 18.0 (C-6), 34.2 (C-7), 40.5 (C-8), 50.6 (C-9), 36.9 (C-10), 20.6 (C-11), 25.5 (C-12), 38.3 (C-13), 42.2 (C-14), 30.3 (C-15), 31.9 (C-16), 56.1 (C-17), 46.9 (C-18), 49.1 (C-19), 150.5 (C-20), 29.4 (C-21), 36.7 (C-22), 27.1 (C-23), 14.6 (C-24), 15.2 (C-25), 15.2 (C-26), 13.7 (C-27), 178.5 (C-28), 108.6 (C-29), 18.1 (C-30). 1H NMR (CD_3OD , 400 MHz) δ 0.92 (1H, m, H-1a), 1.68 (1H, m, H-1b), 1.54 (2H, m, H-2), 3.11 (1H, dd, $J = 5.0, 10.9$ Hz), 0.70 (1H, m, H-5), 1.41 (1H, m, H-6b), 1.53 (1H, m, H-6a), 1.39 (2H, m, H-7), 10.31 (1H, m, H-9), 1.42 (1H, m, H-11a), 1.25 (1H, m, H-11b), 1.04 (1H, m, H-12a), 1.70 (1H, m, H-12b), 2.34 (1H, m, H-13), 1.37 (1H, m, H-15a), 1.92 (1H, m, H-15b), 1.40 (1H, m, H-16a), 2.22 (1H, m, H-16b), 1.62 (1H, m, H-18), 3.30 (1H, ddd, $J = 5.0, 16.9, 10.6$ Hz, H-19), 1.43 (1H, m, H-22a), 1.89 (1H, m, H-22-b), 0.95 (3H, s, H-23), 0.75 (3H, s, H-24), 0.97 (1H, s, H-25), 0.86 (3H, s, H-26), 1.00 (3H, s, H-27), 4.58 (1H, m, H-29a), 4.71 (1H, m, H-29b), 1.68 (3H, s, H-30).

β -Sitosterol-3- O - β -D-glucopyranosyl-6'-mirystate 5: white powder. ESIMS (negative-ion mode) m/z 803.5 $[M + H]^-$, 413 $[C_{29}H_{49}O]^-$. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.2 (C-1), 32.0 (C-2), 79.6 (C-3), 38.8 (C-4), 140.2 (C-5), 122.1 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.6 (C-10), 21.1 (C-11), 39.8 (C-12), 42.1 (C-13), 56.7 (C-14), 24.8 (C-15), 28.3 (C-16), 56.1 (C-17), 11.8 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.1 (C-25), 19.8 (C-26), 19.1 (C-27), 23.1 (C-28), 12.0 (C-29) for aglycone; 101.3 (C-1'), 76.1 (C-2'), 73.9 (C-3'), 73.5 (C-4'), 70.1 (C-5'), 63.3 (C-6') for sugar moiety; 174.6 (C-1''), 34.2 (C-2''), 24.25 (C-3''), 26.13 (C-4''-C-13''), 14.13 (C-14'') for mirystate moiety; 1H NMR ($CDCl_3$, 400 MHz) δ 3.53 (1H, m, H-3), 2.25 (1H, m, H-4), 2.35 (1H, m, H-4b), 5.35 (1H, m, H-6), 1.25 (1H, m, H-8), 0.90 (1H, m, H-9), 0.97 (1H, m, H-14) for aglycone; 4.37 (1H, m, H-1'), 3.55 (H-2'), 3.44 (H-3'), 3.35 (H-4'), 3.37 (H-5'), 4.29 (H-6a'), 4.43 (H-6b') for sugar moiety; 2.34 (2H, m, H-2''), 1.60 (2H, m, H-3''), 1.13 (CH₂ group H-4'' to H-13''), 0.67 (3H, m, H-14'') for mirystate moiety.

Cellobiosylsterol 6: white powder. ^{13}C (DMSO- d_6 + D_2O , 100 MHz) δ 37.4 (C-1), 29.3 (C-2), 77.6 (C-3), 141.0 (C-5), 122.0 (C-6), 32.1 (C-7), 50.2 (C-9), 36.1 (C-10), 21.4 (C-11), 39.0 (C-12), 42.53 (C-13), 56.8 (C-14), 28.4 (C-16), 56.0 (C-17), 12.3 (C-18), 19.3 (C-19), 36.8 (C-20), 34.0 (C-22), 45.8 (C-24), 26.0 (C-25), 19.6 (C-26), 19.6 (C-27), 23.2 (C-28), 12.4 (C-29) for aglycone; 100.8 (C-1'), 74.4 (C-2'), 88.5 (C-3'), 70.4 (C-4'), 77.7 (C-5'), 61.7 (C-6'), 104.6 (C-1''), 72.8 (C-2''), 76.7 (C-3''), 69.0 (C-4''), 76.9 (C-5''), 61.1 (C-6'') for sugar moieties. 1H NMR (DMSO- d_6 + D_2O , 400 MHz) δ 3.45 (1H, m, H-3), 5.30 (1H, m, H-6), 1.25 (1H, m, H-8), 0.90 (1H, m, H-9), 0.97 (1H, m, H-14) for aglycone; 4.32 (1H, d, $J = 7.7$ Hz, H-1'), 3.01 (1H, m, H-2'), 3.00 (1H, m, H-4'), 4.29 (1H, d, $J = 7.7$ Hz, H-1''), 3.07 (1H, m, H-2''), 3.13 (1H, m, H-4'') for sugar moieties.

β -Sitosterol 7: ^{13}C ($CDCl_3$, 100 MHz) δ 37.3 (C-1), 31.6 (C-2), 71.8 (C-3); 42.2 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14),

24.3 (C-15), 28.3 (C-16), 56.0 (C-17), 11.9 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.3 (C-27), 23.1 (C-28), 12.2 (C-29). 1H (CD_3Cl , 400 MHz) δ 3.52 (1H, m, H-3), 5.35 (1H, brs, H-6), 0.92 (3H, d, $J = 6.4$ Hz, H-21), 0.81 (3H, d, $J = 6.5$ Hz, H-26), 0.83 (3H, d, $J = 6.5$ Hz), 0.85 (3H, t, H-29).

β -Sitosterol-3- O - β -D-glucopyranoside 8: ^{13}C ($CDCl_3$, 100 MHz) δ 37.3 (C-1), 30.2 (C-2), 78.5 (C-3); 39.2 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.0 (C-17), 11.9 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.3 (C-27), 23.1 (C-28), 12.2 (C-29) for aglycone, 102.5 (C-1'), 75.3 (C-2'), 78.6 (C-3'), 71.6 (C-4'), 78.1 (C-5'), 62.5 (C-6') for sugar moiety. 1H (CD_3Cl , 400 MHz) δ 0.66–1.25 (CH₃ groups), 3.50 (1H, m, H-3), 5.35 (1H, m, H-5) for aglycone; 5.02 (1H, d, $J = 7.5$ Hz, H-1') representing the anomeric proton of the sugar.

Arjunolic acid 9: white powder. ^{13}C (CD_3OD , 100 MHz) δ 47.8 (C-1), 69.6 (C-2), 78.1 (C-3), 43.0 (C-4), 48.3 (C-5), 19.1 (C-6), 33.8 (C-7), 40.7 (C-8), 48.1 (C-9), 38.5 (C-10), 24.0 (C-11), 123.4 (C-12), 145.3 (C-13), 42.7 (C-14), 29.1 (C-15), 24.4 (C-16), 47.6 (C-17), 43.3 (C-18), 44.1 (C-19), 31.6 (C-20), 34.8 (C-21), 33.5 (C-22), 66.2 (C-23), 13.9 (C-24), 17.7 (C-25), 17.5 (C-26), 26.4 (C-27), 181.8 (C-28), 25.3 (C-29), 24.6 (C-30). 1H (CD_3OD , 400 MHz) δ 3.66 (1H, m, H-2), 3.36 (1H, m, H-2), 5.23 (1H, m, H-12), 3.50 (1H, m, H-23a), 3.31 (1H, m, H-23b), 0.69 (3H, s, H-24), 0.81 (3H, s, H-25), 0.91 (3H, s, H-26), 1.17 (3H, s, H-27), 1.03 (3H, s, H-29), 0.94 (3H, s, H-30).

3,3'-Di-*O*-methyllellagic acid 10: yellow powder. ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 112.1 (C-1; C-1'), 141.9 (C-2; C-2'), 140.9 (C-3; C-3'); 152.5 (C-4; C-4'), 112.37 (C-5; C-5'), 112.8 (C-6; C-6'), 159.1 (C-7; C-7'), 61.6 (3; 3'-OMe). 1H NMR (DMSO- d_6 , 400 MHz) δ 4.05 (6H, s, 3; 3'-OMe), 7.50 (2H, s, H-5; H-5').

Ellagic acid 11: yellow powder. ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 112.2 (C-1; C-1'), 136.3 (C-2; C-2'), 139.4 (C-3; C-3'), 148.0 (C-4; C-4'), 110.2 (C-5; C-5'), 107.6 (C-6; C-6'), 159.0 (C-7; C-7'). 1H NMR (DMSO- d_6 , 400 MHz) δ 7.05 (2H, s, H-5 and H-5').

3,3'-Di-*O*-methyllellagic acid 4'- O - β -D-glucopyranoside 12: yellow powder. ^{13}C NMR (DMSO- d_6 , 400 MHz) δ 111.9 (C-1), 140.9 (C-2), 140.5 (C-3), 151.3 (C-4), 112.0 (C-5), 112.8 (C-6), 158.5 (C-7), 114.4 (C-1'), 141.7 (C-2'), 141.8 (C-3'), 154.2 (C-4'), 112.8 (C-5'), 112.9 (C-6'), 158.5 (C-7'), 60.9 (3-OMe), 61.5 (3'-OMe) for aglycone; 101.3 (C-1''), 73.3 (C-2''), 77.2 (C-3''), 69.7 (C-4''), 76.4 (C-5''), 60.5 (C-6'') for sugar moiety. 1H NMR (DMSO- d_6 , 400 MHz) δ 7.51 (1H, s, H-5), 7.81 (1H, s, H-5'), 4.05 (3H, s, 3-OMe), 4.09 (1H, s, 3'-OMe) for aglycone; 5.14 (1H, d, $J = 7.5$ Hz, H-1''), 3.45 (1H, m, H-2''), 3.44 (1H, m, H-3''), 3.26 (1H, m, H-4''), 3.44 (1H, m, H-5''), 3.73 (1H, m, H-6'a''), 3.54 (1H, m, H-6'b'') for sugar moiety.

2.4. Antimicrobial activity

Microorganisms included in this study were: *Staphylococcus S. aureus* ATCC (American Type Culture Collection) 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 24433. Antimicrobial activity was assessed by disc diffusion test as reported by the European Committee for Antimicrobial Susceptibility testing (EUCAST, www.eucast.org). 0.2 mg of each extract was included in paper discs by spotting 10 μ l of extract solution (0.02 mg/ μ l), while the known antimicrobials ciprofloxacin (0.5 μ g/ μ l disc) and fluconazole (2.5 μ g/ μ l disc) were used as a reference against bacteria and fungi, respectively. Activity was determined by measuring the diameter of the growth inhibition zone (inhibition zone diameter, IZD) visible around the paper disc (expressed in mm). Reported IZDs are inclusive of the paper disc diameter (6 mm). Therefore, a 6 mm IZD means no activity. Tests were run in duplicates.

2.5. Antioxidant activity

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl), on a microplate analytical assay according to the procedures described by Srinivasan et al. (2007). The total antioxidant activity of *D. thollonii* extracts was measured by ABTS (2',2'-azinobis(3-ethylbenzothiazline-6-sulfonic acid) radical cation decolorization assay according to the method of Re et al. (1999) described for application to a 96-well microplate assay. Ferric reducing antioxidant capacity assay (FRAP assay) was carried out according to the procedure described by Müller et al. (2011) by monitoring the reduction of Fe^{3+} -2,4,6-tri[2-pyridyl-s-triazine] (TPTZ), to blue-colored Fe^{2+} -TPTZ. The ability of the samples tested to scavenge the different radicals in the assays was compared to trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) used as standard. The activities of *D. thollonii* extracts were expressed as tocopherol-equivalent antioxidant capacity $\mu\text{mol TE/g}$ of product.

2.6. Anti-inflammatory activity

2.6.1. RAW 264.7 cell culture

Murine macrophage cell line RAW 264.7 was cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 2 mM L-glutamine, 100 IU/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, and supplemented with 10% heat-inactivated foetal bovine serum (HI-FBS) (PAA Laboratories GmbH, Austria). Cells were cultured in a humidified atmosphere at 37 °C in the presence of 5% CO_2 . Cells were counted with a hemocytometer, and the number of viable cells was determined by trypan blue dye exclusion (Shin et al., 2004).

2.6.2. Assay for NO production by activated macrophages

RAW 264.7 macrophages were seeded in 96 well plates at a density of 5×10^5 cells/ml in the presence of *D. thollonii* extracts (6.25–100 $\mu\text{g/ml}$). Cells were incubated with 1 $\mu\text{g/ml}$ lipopolysaccharide (LPS), alone or co-incubated with extracts and 1 $\mu\text{g/ml}$ LPS for 24 h. 200 μM aminoguanidine plus 1 $\mu\text{g/ml}$ LPS served as a control for the reduction of NO-production (Koh et al., 2009). NO production was determined by measuring the amount of the primary stable reaction product nitrite with Griess reagent (Huygen, 1970) (1% sulfanilamide, 0.1% naphthylethylenediamine-dihydrochloride, 5% H_3PO_4) by mixing 50 μl of cell culture supernatant with the same volume of reagents. After incubation for 10 min at room temperature, the absorption of the formed diazo dye was measured spectrophotometrically at 540 nm using a Titertek Multiscan microElisa (Labsystems, Helsinki, Finland). The nitrite concentration was determined by comparison with a sodium nitrite standard calibration curve in culture medium (0–100 μM).

The cell viability of the macrophages was determined by MTT assay (Mosmann, 1983). After removal of the cell culture supernatant for nitrite measurement, each well received 5 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (5 mg/ml in phosphate-buffered saline, PBS) and the plates were incubated for 4 h at 37 °C. To dissolve formazan crystals, the MTT solution was removed and 100 μl of DMSO was added to each well. After 10 min incubation at room temperature the absorbance was measured spectrophotometrically at 540 nm using a Titertek Multiscan microElisa (Labsystems, FI-Helsinki). Experiments were conducted in triplicate.

2.6.3. Statistical analysis

All assays were conducted at least three times with three different sample preparations. All data are expressed as the mean \pm standard deviation (SD). Analysis of variance was performed in InStat, GraphPad software, San Diego, CA, USA. A one-way ANOVA with Dunnett's post hoc testing was used for these analyses, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Phytochemical analysis

The structures of the isolated compounds were established using spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments, COSY, HSQC, HMBC, and direct comparison with published information and with authentic samples obtained by our research group for some cases. The twelve compounds isolated from the roots of *D. thollonii* (Fig. 1) were identified as 3,3'-di-O-méthylellagic acid 4'-O- β -D-xylopyranoside **1** (Xing-Cong et al., 1999), 3-O-méthylellagic acid 4'-O- β -D-arabinopyranoside **2**, casuarinin **3** (Morio et al., 2008), betulinic acid **4** (Janeczko et al., 1990), β -sitosterol-3-O- β -D-glucopyranosyl-6'-mirystate **5**, cellobiosylsterol **6** (Bankeu et al., 2010), β -sitosterol **7** (Saeidnia et al., 2011) and β -sitosterol-3-O- β -D-glucopyranoside **8** (Saeidnia et al., 2011), arjunolic acid **9** (Kundu and Mahato, 1993), 3,3'-di-O-méthylellagic acid **10** (Da Silva et al., 2008), ellagic acid **11** (Xing-Cong et al., 1999; Guan et al., 2007), and 3,3'-di-O-méthylellagic acid 4'-O- β -D-glucopyranoside **12** (Guan et al., 2007).

3.2. Antimicrobial activity

The extracts assayed for antimicrobial activity were the MeOH extract (1st step), the EtOAc extract (2nd step), and n-BuOH extract from the aqueous residue of the second step. The activity of the water extract was also assessed. The only measurable activity was observed for the EtOAc extract against the bacterial species *S. aureus* with an inhibition zone diameter of 10 ± 1 mm. The analysis of fractions of this extract revealed the presence of bioactive compounds with a described antimicrobial activity, such as compound **7** (β -sitosterol), compound **8** (β -sitosterol-3-O- β -D-glucopyranoside) (Beltrame et al., 2002; Kim et al., 2003) and arjunolic acid (Thiagarajan et al., 2010). The diameter of the inhibition zone for the EtOAc extract observed in the present work (10 mm) was comparable with that measured by Bumrela and Naik (2011) while studying the methanolic extract from the leaves of *Dipteracanthus patulus*, for which they claimed a direct association between the presence of β -sitosterol and the antimicrobial activity. The activity of β -sitosterol-3-O- β -D-glucopyranoside in a disk diffusion test has never been evaluated. Available data are from minimal inhibitory concentration (MIC) determinations (Kim et al., 2003). β -Sitosterol-3-O- β -D-glucopyranoside was active only against Gram-positive bacteria with MICs of 50–400 mg/l. *S. aureus* was intermediately susceptible with an MIC equal to 200 mg/l. Therefore β -sitosterol-3-O- β -D-glucopyranoside could contribute to the observed antibacterial activity. Conversely, there was not a measurable activity either for the methanolic extract or for the butanolic or the aqueous extracts. The absence of antibiotic activity by the former could be explained by the lower amount of active compounds contained in it, relative to the extracts obtained in the subsequent ethyl acetate step. A proposed mechanism of action for β -sitosterol activity has been the inhibition of the bacterial deformylase (Hoskeri et al., 2012). The bacterial sortase seems to be the molecular target for the action of the β -sitosterol-3-O- β -D-glucopyranoside (Kim et al., 2003).

3.3. Antioxidant activity

The DPPH radical assay is a suitable model for estimating the total antioxidant potential of antioxidants (Huang et al., 2005). Table 1 shows the DPPH radical scavenging activities of different extracts of *D. thollonii*. All extracts have antioxidant activity against DPPH and the reducing power of the n-BuOH extract exhibits the highest scavenging activity. The IC_{50} values, defined as the concentration of samples reducing 50% of free radical DPPH were calculated. According to these IC_{50} values, the DPPH radical scavenging ability was found in the order of Trolox > n-BuOH > EtOAc > MeOH > WE (water extract). ABTS is another widely used synthetic radical for both the polar and non-polar

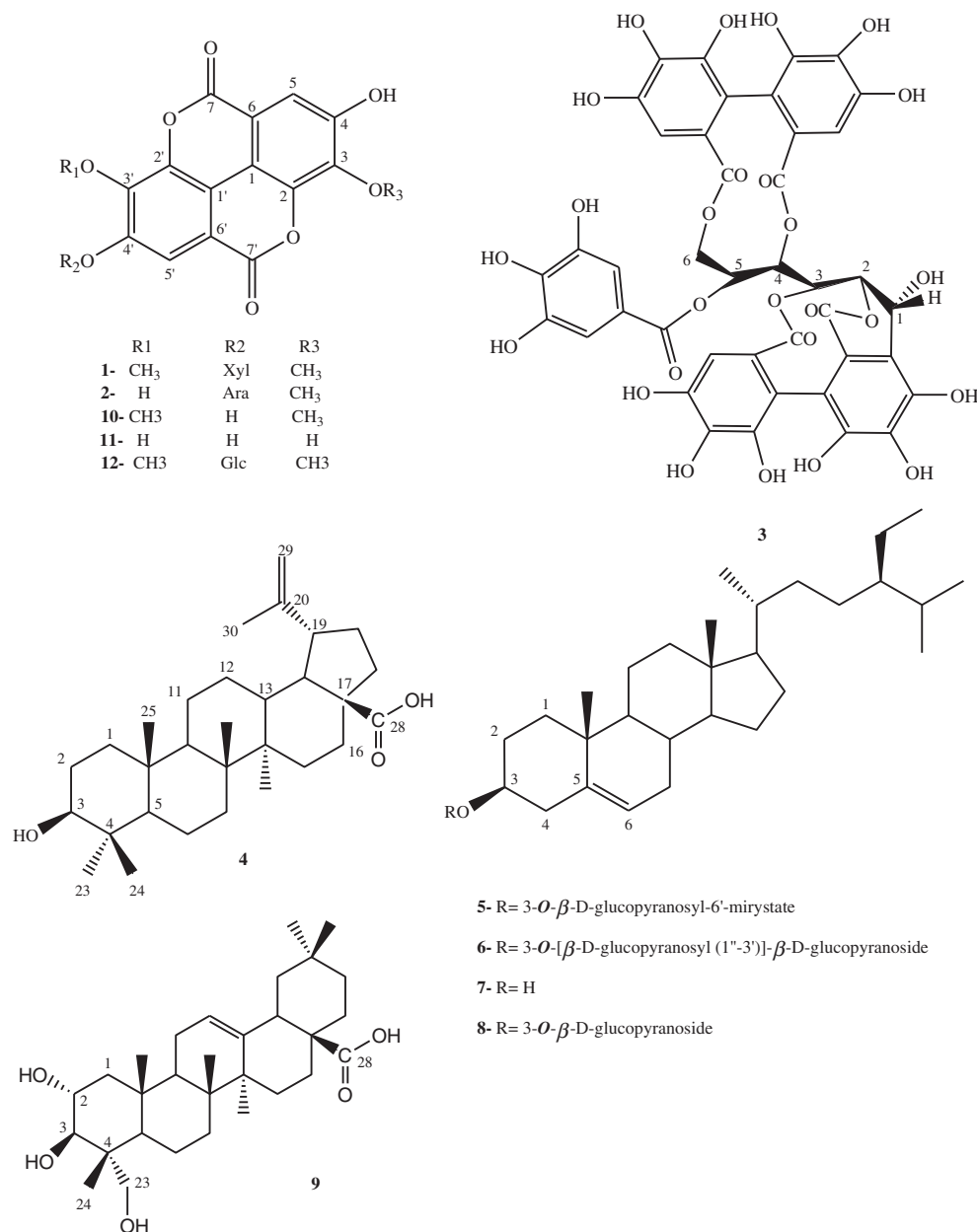


Fig. 1. Chemical structures of compounds isolated from the roots of *D. thollonii* Cogn.

samples (Re et al., 1999). The ABTS⁺ scavenging abilities of the different extracts of *D. thollonii* were also evaluated and reported in Table 1. Also for this assay, the n-BuOH extract exhibits a maximum scavenging activity. The IC₅₀ values of the scavenging activities of the different extracts were evaluated and the order of ABTS radical scavenging ability is similar to that reported for DPPH assay and was Trolox >

n-BuOH > MeOH > EtOAc > WE. The FRAP assay mainly depends on the reducing capacity of Fe³⁺-Fe²⁺ conversion and serves as a significant indicator of its potential antioxidant activity. The antioxidant activities have been attributed to various reactions, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continuous proton abstraction and radical scavenging activity (Baskar et al.,

Table 1
Total antioxidant capacity of crude MeOH, EtOAc, n-BuOH, and water extracts of *D. thollonii* Cogn.

Extract	DPPH		ABTS		FRAP
	TEAC (μmol TE/g)	IC ₅₀ (mg/ml)	TEAC (μmol TE/g)	IC ₅₀ (μg/ml)	TEAC (μmol TE/g)
MeOH	4.45 ± 1.24	5.97 ± 0.09	5.63 ± 0.36	2.79 ± 0.11	1.31 ± 0.08
EtOAc	6.17 ± 0.81	4.12 ± 0.12	1.94 ± 0.10	8.03 ± 0.18	0.19 ± 0.01
n-BuOH	12.07 ± 1.65	2.11 ± 0.10	6.66 ± 0.44	2.36 ± 0.10	2.52 ± 0.25
Water extract	2.26 ± 0.29	11.2 ± 0.16	1.09 ± 0.05	14.32 ± 0.21	0.30 ± 0.01
Trolox	–	(6.35 ± 0.8)10 ⁻³	–	(3.93 ± 0.2)10 ⁻³	–

TEAC = trolox equivalent antioxidant concentration.

2008). As compared to the DPPH and ABTS assays, in the FRAP assay the pattern of reducing capacity of different extracts was found in the order of n-BuOH > MeOH > WE > EtOAc. The differences observed about the behavior of water and EtOAc extracts are quite similar. As earlier indicated, the phytochemical analysis of roots of the EtOAc extract has led to the isolation of compounds **1**, **4**, **5**, **7**, **8** and **9** while in the n-BuOH fractions the compounds isolated were **2**, **3**, **6**, **10**, **11** and **12**. All these secondary metabolites are known compounds present in other plants used also in traditional medicine but herein isolated and reported for the first time in the roots of *D. thollonii*. Their isolation in large amount probably plays an important role on the medicinal properties of this plant material. The antioxidant activity determined on a different extract followed by fractionation and isolation reflects the properties of the main components isolated. The antioxidant properties of the compounds isolated from the EtOAc extract of *D. thollonii* have previously been reported in different other plant extracts. These included 3,3'-di-O-méthylelagic acid 4'-O- β -D-xylopyranoside **1** (Khallouki et al., 2007), betulinic acid **4** (De Souza et al., 2011; Yogeewari and Sriram, 2005; Adesanwo et al., 2013), β -sitosterol 3-O- β -D-glucopyranosyl-6'-mirystate **5** (Wangensteen et al., 2013), β -sitosterol **7** (Rao et al., 2013; Gupta et al., 2011; Rajanandh and Kavitha, 2010), β -sitosterol-3-O- β -D-glucopyranoside **8** (Rajanandh and Kavitha, 2010) and arjunolic acid **9** (Sai et al., 2010). The synergic action of these compounds could contribute to the antioxidant activity observed and we can hypothesized that a large contribution is assigned to the presence of β -sitosterol, betulinic acid and arjunolic acid for which more important properties and biological activities have been reported (Rao et al., 2013; Gupta et al., 2011; Rajanandh and Kavitha, 2010; Sai et al., 2010; Thiagarajan et al., 2010). As reported in Table 1, the highest antioxidant activity was demonstrated in the n-BuOH extract; therefore, the repeated column chromatography of this extract followed by purification on silica gel and sephadex LH-20 columns afforded six known compounds amongst which some have been reported with potent

antioxidant activities namely 3,3'-O-dimethylelagic acid 4'-O- β -D-xylopyranoside **1** (Zengjun et al., 2011), casuarinin **3** (Cheng et al., 2003; Liu et al., 2004) and ellagic acid (Hayes et al., 2011; Fukuda et al., 2003; Soong and Barlow, 2005). Derivatives of ellagic acid and β -sitosterol probably with synergistic or additive effects are also responsible for the total antioxidant activity observed for the n-BuOH extract of *D. thollonii*. It could be deduced from these results that the important antioxidant activities observed in the initial crude MeOH extract are recovered in the n-BuOH part, where important antioxidant components have been characterized; especially casuarinin **3**, which has been obtained in large amount (914 mg) and seems to represent one of the main components of the root of this plant.

3.4. Anti-inflammatory activity

3.4.1. Effects of *D. thollonii* extracts on LPS-induced NO production and cell viability

The potential anti-inflammatory properties of *D. thollonii* extracts were investigated using RAW 264.7 murine macrophage cells. Cells were stimulated by 1 μ g/ml lipopolysaccharide (LPS) and the effect of extracts during a co-incubation period of 24 h was determined by using NO production as final read-out parameter. Validity of the assays was shown by using untreated cells as negative control, LPS-stimulated cells as positive control and additionally a cell group as reduction control group with LPS-stimulated cells, co-incubated together with aminoguanidine, an inhibitor of iNOS (Koh et al., 2009) (Fig. 2). As shown in Fig. 2a, the MeOH extract reduced the stimulated NO production in a concentration-dependent manner of about 86% at the highest tested concentration of 100 μ g/ml ($IC_{50} = 5.9 \mu$ g/ml). During the investigation of other extracts derived from the MeOH extract, it was established that the n-BuOH extract showed the highest dose dependent reduction of NO formation ($IC_{50} = 6.5 \mu$ g/ml; Fig. 2b) compared to the EtOAc extract ($IC_{50} = 18.1 \mu$ g/ml; Fig. 2c) whereas the water

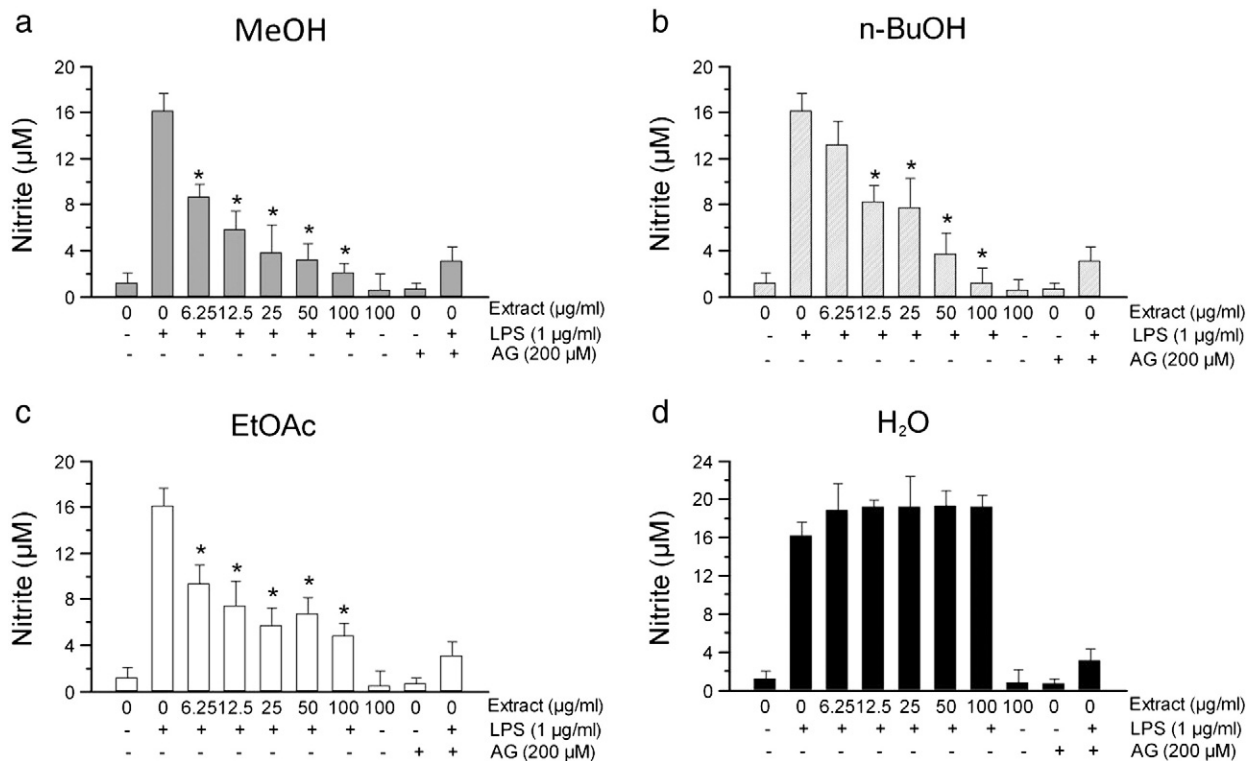


Fig. 2. Influence of different extracts of *D. thollonii* on NO production (determined as nitrite via Griess reaction) of RAW 264.7 macrophages after co-incubation with LPS 1 μ g/ml, and extracts at different concentrations for 24 h. a) Methanol extract; b) n-butanol extract; c) ethyl acetate extract; d) aqueous extract. LPS, lipopolysaccharide. AG, aminoguanidine. Data are means \pm SD of three independent experiments. * $p < 0.01$, sample vs LPS-treated group; Dunnett multiple comparisons test.

extract had no significant influence on the NO production (Fig. 2d). Both the MeOH extract and the other derived extracts did not have any influence on the cell viability of the macrophages within the test concentrations used (data not shown).

The phytochemical analysis of the root extracts of *D. thollonii* led to the isolation and characterization of compounds with previously described anti-inflammatory activity. Indeed, ellagic acid (compound **11**) has been reported as an effective anti-inflammatory agent in the carrageenan-induced rat paw edema, with a prolonged onset and duration of action (Corbett et al., 2010). Another research also revealed that ellagic acid took an important part in the anti-inflammatory, antiedematous and analgesic effects of the extract of *Lafoensia pacari* St. Hil. (Lythraceae) (Rogerio et al., 2006). Moreover, ellagic acid inhibits PGE₂ released in human monocytes (Karlsson et al., 2010) and inhibits NO production in LPS-stimulated RAW 264.7 cells (Yang et al., 2011). Compound **1** (3,3'-di-O-methylellagic acid 4'-O-β-D-xylopyranoside) was shown to exert anti-inflammatory activity as reported by Sgariglia et al. (2013). Compound **10** (3,3'-di-O-methylellagic acid) has been reported as an inhibitor of Hyal and iNOS, reaching the 90% inhibitory concentration (IC₉₀) at values between 2.8 and 16.4 μM, which are higher than the positive controls aspirin (IC₉₀: no reached) and aminoguanidine (IC₉₀: 20.2 μM) (Sgariglia et al., 2013). Compound **3** (casuarinin) inhibits the IκB kinase, which is involved in the activation of NFκB, and the increase of inducible nitric oxide synthase levels in LPS-stimulated RAW 264.7 cells (Pan et al., 2000). Compound **4** (betulinic acid) had anti-inflammatory activities in carrageenan-treated mice and LPS-treated RAW 264.7 cells (Steinkamp-Fenske et al., 2007; Recio et al., 1995). β-Sitosterol **7** and β-sitosterol-3-O-β-D-glucopyranoside **8** have also been reported to exhibit anti-inflammatory activities (Perez, 2001). Moreover, compound **7** (β-sitosterol) down-regulates some pro-inflammatory signal transduction pathway in murine macrophages and decreased disease incidence by reducing the presence and inflammatory activity of macrophages in the brains of treated mice (Valerio and Awad, 2011; Valerio et al., 2011).

4. Conclusion

It is established from this study that most of the isolated compounds from the different extracts of *D. thollonii* have previously revealed potent antioxidant, antimicrobial and anti-inflammatory activities. It could then be concluded that the biological and phytochemical characterization of the root extracts of *D. thollonii* is supportive of the use of this plant for the treatment of inflammation and sinusitis, thus providing evidence that this plant extracts might be potential sources of new antioxidant and anti-inflammatory drugs.

Acknowledgements

This research work was supported by the IFS (International Foundation for Science, Stockholm, Sweden) program through grant to Prof. A. Léon TAPONDJOU (RGA No. F/3976-3F) and the Italian Ministry of Education (MIUR) (COOPERLINK 2011 Prot. CII113PPUC).

References

- Abere, T.A., Okoto, P.E., Agoreyo, F.O., 2010. Antidiarrhoea and toxicological evaluation of the leaf extract of *Dissotis rotundifolia* triana (Melastomataceae). *BMC Complementary and Alternative Medicine* 10, 71.
- Adesanwo, J.K., Makinde, O.O., Obafemi, C.A., 2013. Phytochemical analysis and antioxidant activity of methanol extract and betulinic acid isolated from the roots of *Tetracera potatoria*. *Journal of Pharmacy Research* 6, 903–907.
- Akerele, O., 1993. Nature's medicinal bounty: don't throw it away. *World Health Forum* 14, 390–395.
- Amalraj, T., Ignacimuthu, S., 1998. Evaluation of the hypoglycaemic effect of *Memecylon umbellatum* in normal and alloxan diabetic mice. *Journal of Ethnopharmacology* 62, 247–250.
- Bankeu, K.J.J., Mustafa, A.S.A., Gojayev, A.S., Lenta, D.B., Nongoué, T.D., Ngoula, A.S., Asaad, K., Choudhary, I.M., Prigge, S., Guliyev, A.A., Nkenfack, K.A., Tsamo, E., Shaiqali, M., 2010. Ceramide and cerebroside from the stem bark of *Ficus mucosa* (Moraceae). *Chemical and Pharmaceutical Bulletin* 58, 1661–1665.
- Baskar, R., Lavanya, R., Mayilvizhi, S., Rajasekaran, P., 2008. Free radical scavenging activity of antitumor polysaccharide fractions isolated from *Ganoderma lucidum* (Fr.) P.Karst. *Natural Product Radiance* 7, 320–325.
- Beltrame, F.L., Pessini, G.L., Doro, D.L., Dias, B.P., Bazotte, R.B., Aparício, D., Cortez, G., 2002. Evaluation of the antidiabetic and antibacterial activity of *Cissus sicyoides*. *Brazilian Archives of Biology and Technology* 45, 21–25.
- Bumrela, S.B., Naik, S.R., 2011. Identification of β-carotene and β-sitosterol in methanolic extract of *Dipteracanthus patulus* (Jacq) nees and their role in antimicrobial and antioxidant activity. *International Journal of Ophthalmic Practice* 3, 204–215.
- Calderón, A.I., Terreaux, C., Schenk, K., Pattison, P., Burdette, J.E., Pezzuto, J.M., Gupta, M.P., Hostettmann, K., 2002. Isolation and structure elucidation of an isoflavone and a sesterterpene acid from *Henriettella fascicularis*. *Journal of Natural Products* 65, 1749–1753.
- Cheng, J.T., Hsu, F.L., Chen, H.F., 1993. Antihypertensive principles from the leaves of *Melastoma candidum*. *Planta Medica* 59, 405–407.
- Cheng, H.Y., Lin, T.C., Yu, K.H., Yang, C.M., Lin, C.C., 2003. Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biological and Pharmaceutical Bulletin* 26, 1331–1335.
- Corbett, S., Daniel, J., Drayton, R., Field, M., Steinhart, R., Garrett, N., 2010. Evaluation of the anti-inflammatory effects of ellagic acid. *Journal of PeriAnesthesia Nursing* 25, 214–220.
- Da Silva, S.L., Calgarotto, A.K., Chaar, J.S., Marangoni, S., 2008. Isolation and characterization of ellagic acid derivatives isolated from *Casearia sylvestris* SW aqueous extract with anti-PLA₂ activity. *Toxicol* 52, 655–666.
- De Souza, P.A., Clara, R., Santiago, A.P.S.A., De Lucas, N.C., Leitao, G.G., Filho, A.G., 2011. Antioxidant activity of natural compounds of *Stachytarpheta cayennensis* by scavenger of mitochondrial reactive oxygen species. *Brazilian Journal of Pharmacognosy* 21, 420–426.
- Fouedjoui, R.T., Teppono, R.B., Quassinti, L., Bramucci, M., Petrelli, D., Vitali, L.A., Fiorini, D., Tapondjou, L.A., Barboni, L., 2014. Steroidal saponins from the leaves of *Cordyline fruticosa* (L.) A. Chev. and their cytotoxic and antimicrobial activity. *Phytochemistry Letters* 7, 62–68.
- Fukuda, T., Ito, H., Yoshida, T., 2003. Antioxidative polyphenols from walnuts (*Juglans regia* L.). *Phytochemistry* 63, 795–801.
- Guan, Y., Mingsong, F., Chenggang, H., 2007. Ellagic acid glycosides from the stem bark of *Aphananthe aspera*. *Chemistry of Natural Compounds* 43, 458–459.
- Gupta, R., Sharma, A.K., Dobhal, M.P., Sharma, M.C., Gupta, R.S., 2011. Antidiabetic and antitumor potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. *Journal of Diabetes* 3, 29–37.
- Hayes, J.E., Allen, P., Brunton, N., O'Grad, M.N., Kerry, J.P., 2011. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: olive leaf extract (*Olea europaea* L.), lutein, sesamol and ellagic acid. *Food Chemistry* 126, 948–955.
- Hoskeri, J., Krishna, H., Jignesh, V., Roshan, S., Vijay, S., 2012. In-silico drug designing using β-sitosterol isolated from *Flaveria trinervia* against peptide deformylase protein to hypothesize bactericidal effect. *International Journal of Pharmacy and Pharmaceutical Sciences* 4, 192–196.
- Huang, D., Ou, B., Ronald, L., 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53, 1841–1856.
- Huygen, C., 1970. Reaction of nitro oxide with Griess-type reagents. *Analytical Chemistry* 42, 407–409.
- IPNI, 2012. The international plant names index. Published on the Internet <http://www.ipni.org> (2012). [accessed 16 December 2013].
- Isaza, J.H., Ito, H., Yoshida, T., 2001. A flavonol glycoside-lignan ester and accompanying acylated glucosides from *Monochaetum multiflorum*. *Phytochemistry* 58, 321–327.
- Janeczko, Z., Sendra, J., Kmiec, K., Brieskorn, C.H., 1990. A triterpenoid glycoside from *Menyanthes trifoliata*. *Phytochemistry* 29, 3885–3887.
- Karlsson, S., Nånberg, E., Fjaeraa, C., Wijkander, J., 2010. Ellagic acid inhibits lipopolysaccharide-induced expression of enzymes involved in the synthesis of prostaglandin E₂ in human monocytes. *British Journal of Nutrition* 103, 1102–1109.
- Khallouki, F., Haubner, R., Hull, W.E., Erben, G., Spiegelhalter, B., Bartsch, H., Owensolow, R.W., 2007. Purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of *Anisophyllea dichostyla* R. Br. *Food and Chemical Toxicology* 45, 472–485.
- Kim, S.H., Shin, D.S., Oh, M.N., Chung, S.C., Lee, J.S., Chang, I.-M., Oh, K.B., 2003. Inhibition of sortase, a bacterial surface protein anchoring transpeptidase, by β-sitosterol-3-O-glucopyranoside from *Fritillaria verticillata*. *Bioscience, Biotechnology, and Biochemistry* 67, 2477–2479.
- Koh, E.M., Kim, H.J., Kim, S., Choi, W.H., Choi, Y.H., Ryu, S.Y., Kim, Y.S., Koh, W.S., Park, S.Y., 2009. Modulation of macrophage functions by compounds isolated from *Zingiber officinale*. *Planta Medica* 75, 148–151.
- Konkon, N.G., Adjoungou, A.L., Manda, P., Simaga, D., N'Guessan, K.E., Kone, B.D., 2008. Toxicological and phytochemical screening study of *Mitragyna inermis* (willd.) O krtze (Rubiaceae), anti-diabetic plant. *Journal of Medicinal Plants Research* 2, 279–284.
- Kundu, A.P., Mahato, S.B., 1993. Triterpenoids and their glycosides from *Terminalia chebula*. *Phytochemistry* 32, 999–1002.
- Laís, G.P., Fernanda, M.R., Valdecir, F.X., Anne, L.D., 2011. Antioxidant activity and total phenols from the methanolic extract of *Miconia albicans* (Sw.) Triana leaves. *Molecules* 16, 9439–9450.
- Liu, T.Z., Kuo, T.C., Lu, F.J., Chen, Y.C., Chang-Chien, Y.W., Lin, C.C., 2004. Casuarinin protects cultured MDCK cells from hydrogen peroxide-induced oxidative stress and DNA oxidative damage. *Planta Medica* 70, 1022–1026.
- Morio, Y., Hideyuki, I., Kyoko, M., Tsutomu, H., Shoko, T., Yoshiaki, A., Takashi, Y., 2008. Flavonol glucuronides and C-glucosidic ellagitannins from *Melaleuca squarrosa*. *Phytochemistry* 69, 3062–3069.

- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65, 55–63.
- Müller, L., Fröhlich, K., Böhm, V., 2011. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (α TEAC), DPPH assay and peroxy radical scavenging assay. *Food Chemistry* 129, 139–148.
- Ndjateu, F.S.T., Tsafack, R.B.N., Awouafack, M.D., Wabo, H.K., Tene, M., Tane, P., Eloff, J.N., 2014. Antimicrobial and antioxidant activities of extracts and ten compounds from three Cameroonian medicinal plants: *Dissotis perkinsiae* (Melastomataceae), *Adenocarpus manii* (Fabaceae) and *Barteria fistulosa* (Passifloraceae). *South African Journal of Botany* 91, 37–42.
- Nicholl, D.S., Daniels, H.M., Thabrew, M.L., Grayer, R.J., Simmonds, M.S.J., Hughes, R.D., 2001. In vitro studies on the immunomodulatory effect extracts of *Osbeckia aspera*. *Journal of Ethnopharmacology* 78, 39–44.
- Nzowa, L.K., Teponno, R.B., Taponjhou, L.A., Verotta, L., Liao, Z., Graham, D., Zink, M.-C., Barboni, L., 2013. Two new tryptophan derivatives from the seed kernels of *Entada rheedii*: effects on cell viability and HIV infectivity. *Fitoterapia* 87, 37–42.
- Okunlola, A., Adewoyin, B.A., Odeku, O.A., 2007. Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in South Western Nigeria. *Tropical Journal of Pharmaceutical Research* 6, 661–670.
- Pan, M.H., Lin-Shiau, S.Y., Ho, C.T., Lin, J.H., Lin, J.K., 2000. Suppression of lipopolysaccharide-induced nuclear factor- κ B activity by the aflavin-3,3'-digallate from black tea and other polyphenols through down-regulation of I κ B kinase activity in macrophages. *Biochemical Pharmacology* 59, 357–367.
- Perez, R.M.G., 2001. Anti-inflammatory activity of compounds isolated from plants. *The Scientific World* 1, 713–784.
- Rajanandh, M.G., Kavitha, J., 2010. Quantitative estimation of β -sitosterol, total phenolic and flavonoid compounds in the leaves of *Moringa oleifera*. *International Journal of PharmTech Research* 2, 1409–1414.
- Rao, N., Mittal, S., Sudhanshu, Menghani, E., 2013. Antioxidant potential and validation of bioactive β -sitosterol in *Eulophia campestris* Wall. *Advances in Bioresearch* 4, 136–142.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Journal of Free Radical Biology and Medicine* 26, 1231–1237.
- Recio, M.C., Giner, R.M., Máñez, S., Gueho, J., Julián, H.R., Hostettmann, K., Ríos, J.L., 1995. Investigations on the steroidal anti-inflammatory activity of triterpenoids from *Diospyros leucomelas*. *Planta Medica* 61, 9–12.
- Rogério, A.P., Fontanari, C., Melo, M.C., Ambrosio, S.R., de Souza, G.E., Pereira, P.S., França, S.C., Da Costa, F.B., Albuquerque, D.A., Faccioli, L.H., 2006. Anti-inflammatory, analgesic and anti-oedematous effects of *Lafloensia pacari* extract and ellagic acid. *Journal of Pharmacy Pharmacology* 58, 1265–1273.
- Saeidnia, S., Gohari, A.R., Malmir, M., Moradi-Afrapoli, F., Ajani, Y., 2011. Tryptophan and sterols from *Salvia limbata*. *Journal of Medicinal Plants* 10, 41–47.
- Sai, R.A., Godwin, C.J., Setty, C.R., Thankamani, V., 2010. In vitro study on antioxidant property of arjunolic acid. *IJPI's Journal of Pharmacognosy and Herbal Formulations* 1, 1–7.
- Sgariglia, M.A., Soberón, J.R., Cabanes, A.P., Sampietro, D.A., Vattuone, M.A., 2013. Anti-inflammatory properties of phenolic lactones isolated from *Caesalpinia paraguayensis* stem bark. *Journal of Ethnopharmacology* 147, 63–73.
- Shin, K.-M., Kim, R.-K., Taponjhou, A.L., Lontsi, D., Sondengam, B.L., Choudhary, M.I., Park, H.-J., Choi, J.-W., Lee, K.-T., 2004. In vitro anti-inflammatory activity of 23-hydroxyursolic acid isolated from *Cussonia bancoensis* in Murine Macrophage RAW 264.7 cells. *Planta Medica* 70, 803–807.
- Soong, Y.Y., Barlow, P.J., 2005. Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longan* Lour.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. *Food Chemistry* 97, 524–530.
- Srinivasan, R., Chandrasekar, M.J.N., Nanjan, M.J., Suresh, B., 2007. Antioxidant activity of *Caesalpinia digyna* root. *Journal of Ethnopharmacology* 113, 284–291.
- Steinkamp-Fenske, K., Bollinger, L., Xu, H., Yao, Y., Horke, S., Förstermann, U., Li, H., 2007. Reciprocal regulation of endothelial nitric-oxide synthase and NADPH oxidase by betulinic acid in human endothelial cells. *Journal of Pharmacology and Experimental Therapy* 322, 836–842.
- Susanti, D., Sirat, H.M., Ahmad, F., Ali, R.M., Aimi, N., Kitajima, M., 2007. Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food Chemistry* 103, 710–716.
- Taylor, J.L.S., Rabe, T., McGaw, L.J., Jäger, A.K., Van, S.J., 2001. Towards the scientific validation of traditional medicinal plants. *Plant Growth Regulation* 23–37.
- Thiagarajan, H., Sivasami, P., Chidambaram, B., Bhakthavatsalam, M.M., Rengarajulu, P., 2010. Arjunolic acid: a novel phytochemistry with multifunctional therapeutic applications. *Indian Journal of Experimental Biology* 48, 238–247.
- Valerio, M., Awad, A.B., 2011. β -Sitosterol down-regulates some pro-inflammatory signal transduction pathways by increasing the activity of tyrosine phosphatase SHP-1 in J774A.1 murine macrophages. *International Immunopharmacology* 11, 1012–1017.
- Valerio, M., Liu, H.B., Heffner, R., Zivadinov, R., Ramanathan, M., Weinstock-Guttman, B., Awad, A.B., 2011. Phytosterols ameliorate clinical manifestations and inflammation in experimental autoimmune encephalomyelitis. *Inflammation Research* 60, 457–465.
- Wangenstein, H., Klarpås, L., Alamgir, M., Samuelsen, A.B.C., Malterud, K.E., 2013. Can scientific evidence support using Bangladeshi traditional medicinal plants in the treatment of diarrhoea? A review on seven plants. *Nutrients* 5, 1757–1800.
- Xing-Cong, L., Hala, N.E., Charles, D.H., Alice, M.C., 1999. NMR assignments of ellagic acid derivatives. *Magnetic Resonance in Chemistry* 37, 856–859.
- Yang, Z.G., Jia, L.N., Shen, Y., Ohmura, A., Kitanaka, S., 2011. Inhibitory effects of constituents from *Euphorbia lunulata* on differentiation of 3T3-L1 cells and nitric oxide production in RAW264.7 cells. *Molecules* 16, 8305–8318.
- Yogeeswari, P., Sriram, D., 2005. Betulinic acid and its derivatives: A review on their biological properties. *Current Medicinal Chemistry* 12, 657–666.
- Yoshida, T., Arioka, H., Fujita, T., Chen, X.M., Okuda, T., 1994. Monomeric and dimeric hydrolysable tannins from two Melastomataceous species. *Phytochemistry* 37, 863–866.
- Zengjun, G., Ying, X., Ling, H., Xiaoqian, B., Chen, H., Lei, N., 2011. Antioxidant and cytotoxic activity of the acetone extracts of root of *Euphorbia hylonoma* and its ellagic acid derivatives. *Journal of Medicinal Plants Research* 5, 5584–5589.