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

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**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 purpose of the present work is 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-methylgallic acid 4-O-β-D-xylpyranoside 1, 3-O-methylgallic acid 4′-O-β-D-arabinopyranoside 2, casarzinin 3, betulinic acid 4, β-sitosterol-3-O-D-glucopyranosyl-6′-methyl ester 5, cellobiosylsterol 6, β-sitosterol-3-O-β-D-glucopyranoside 7, arjunolic acid 8, β-D-arabinopyranosyl-3,3′-di-O-methylgallic acid 9, ellagic acid 10, 3,3′-di-O-methylgallic acid 4′-O-β-D-glucopyranoside 11 and 3,3′-di-O-methylgallic 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.

**Keywords:**

* Dissotis thollonii
* Melastomataceae
* Extracts
* Compounds
* Antimicrobial
* Antioxidant
* Anti-inflammatory activity

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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 (Akerele, 1993). The World Health Organization has described traditional herbal medicine is probably the most popular and the most ubiquitous in the treatment of several important infections (Taylor et al., 2001).

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 9.5-1.0-0.5 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 (11 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/MS Trap SL (G2445D SL). Samples for NMR experiments were dissolved in deuterated solvents (CD3OD, CDC13, 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 (400 F 254), 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-methyllellagic acid 4′-O-β-D-xyllopyranoside 1: yellow powder, ESIMS (negative-ion mode) exhibited a pseudomolecular ion-peak at m/z 461.0 [M – H]−, consistent with the molecular formula C12H12O12. 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), 158.7 (C-7), 112.0 (C-8), 140.5 (C-9), 141.4 (C-10), 151.6 (C-11), 122.0 (C-12), 111.5 (C-13), 158.8 (C-14), 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-methyllellagic acid 4′-O-β-D-arabinopyranoside 2: yellow powder, 13C NMR (DMSO-d6, 100 MHz) δ 140.5 (C-5′), 112.0 (C-5), 160.5 (C-7), 150.0 (C-4′), 118.4 (C-5′), 159.5 (C-5′), 60.8 (3-Ome) for aglycone; 106.8 (C-1′), 73.6 (C-2′), 76.8 (C-3′), 69.4 (C-4′), 48.3 (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.

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 sinussitis, 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.
**Casuarinin:** yellowish powder, ESIMS (negative-ion mode) m/z 934.7 [M − H]− consistent with the molecular formula C_{29}H_{49}O_{26}. In this spectrum, we observe the peak at m/z 170.4 [C_{4}H_{3}O_{2}]− and m/z 301.0 [C_{6}H_{10}O_{5}]−.

**13C NMR (Acetone-d_{6}, D_{2}O, 100 MHz)** at 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 (hexahydroxyphenyl) 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 carbons in C-2, C-3, C-4, C-5 and C-6).

**1H NMR (Acetone-d_{6}, D_{2}O, 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, d, 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:** white powder.

**13C NMR (CD_{3}OD, 100 MHz)** at δ 38.5 (C-1), 26.6 (C-2), 78.2 (C-3), 83.6 (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), 156.0 (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), 181.1 (C-30).

**1H NMR (CD_{3}OD, 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, 16.8, 10.6 Hz, H-19), 1.43 (1H, m, H-22a), 1.89 (1H, m, H-22b), 0.95 (3H, s, H-23), 0.75 (3H, s, H-24), 0.97 (1H, m, 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′-mirtystate:** white powder.

**ESIMS (negative-ion mode) m/z 803.5 [M − H]−, 413 [C_{39}H_{64}O_{15}]^{−}**

**13C NMR (CDCl_{3}, 100 MHz)** at δ 73.2 (C-1), 32.0 (C-2), 79.6 (C-3), 38.4 (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), 24.3 (C-13), 26.0 (C-14), 24.3 (C-15), 28.3 (1H, m, H-16), 3.44 (1H, t, J = 7.7 Hz, H-17), 0.78 (3H, m, H-16), 1.07 (3H, s, H-5, H-6 and H-7) visible around the paper disc used as a reference against bacteria and fungi, respectively. Activity was measured by determining 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-ethylbenzothiazoline-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 Fe3+-2,4,6-tri[2-pyridyl]-3,5-diphenyl-tetrazolium (TPTZ), to blue-colored Fe2+-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 μmol 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 μg/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% CO2. 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 × 105 cells/ml in the presence of D. thollonii extracts (6.25–100 μg/ml). Cells were incubated with 1 μg/ml lipopolysaccharide (LPS), alone or co-incubated with extracts and 1 μg/ml LPS for 24 h. 200 μM aminoguanidine plus 1 μg/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% naphthylethylene diamine-dihydrochloride, 5% H3PO4) by mixing 50 μl of extracts with Griess reagent (Huygen, 1970) (1% sulfanilamide, 0.1% naphthylethylene diamine-dihydrochloride, 5% H3PO4) and measuring the amount of the primary stable reaction product nitrite as the absorbance at 540 nm using a Titertek Multiscan microElisa (Labsystems, FI-Helsinki). The absorbance was measured spectrophotometrically at 540 nm using a Titertek Multiscan microElisa (Labsystems, FI-Helsinki). 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 ± 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-méthyléllagic acid 4′-O-β-D-xylopyranoside 1 (Xing-cong et al., 1999), 3′-O-méthylléllagic acid 4′-O-β-D-arabinoxyranoside 2, casuarinin 3 (Morio et al., 2008), betulinic acid 4 (Janeczko et al., 1990), β-sitosterol-3-0-β-D-glucopyranosyl-6′-miryistate 5, cellubiosylsterol 6 (Bankeu et al., 2010), β-sitosterol 7 (Saeddina et al., 2011) and β-sitosterol-3-0-β-D-glucopyranoside 8 (Saeddina et al., 2011), arjunic acid 9 (Kundu and Mahato, 1993), 3,3′-di-O-méthyléllagic acid 10 (Da Silva et al., 2008), ellagic acid 11 (Xing-cong et al., 1999; Guan et al., 2007) and 3,3′-di-O-méthylléllagic 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-3-0-β-D-glucopyranoside) (Beltrame et al., 2002; Kim et al., 2003) and arjunic 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-3-0-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-3-0-β-D-glucopyranoside was active only against Gram-positive bacteria with MICs of 50–400 mg/ml. S. aureus was immediately susceptible with an MIC equal to 200 mg/ml. Therefore β-sitosterol-3-0-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-3-0-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 IC50 values, defined as the concentration of samples reducing 50% of free radical DPPH were calculated. According to these IC50 values, the DPPH radical scavenging ability was found in the order of Tropolon > n-BuOH > EtOAc > MeOH > WE (water extract). ABTS is another widely used synthetic radical for both the polar and non-polar
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 IC$_{50}$ 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 Fe$^{3+}$-Fe$^{2+}$ 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.,

![Chemical structures of compounds isolated from the roots of D. thollonii Cogn.](image)

**Table 1**

| 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 ± 1.63 | 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 | 1.69 ± 0.05 | 14.32 ± 0.21 | 0.30 ± 0.01 |
| Trolox          | - | $(6.35 ± 0.8) \times 10^{-3}$ | - | $(3.93 ± 0.2) \times 10^{-3}$ | - |

TEAC = trolox equivalent antioxidant concentration.
antioxidant activities namely 3,3′-diformylxylellagic 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 μg/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 μg/ml (IC50 = 5.9 μg/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 (IC50 = 6.5 μg/ml; Fig. 2b) compared to the EtOAc extract (IC50 = 18.1 μg/ml; Fig. 2c) whereas the water extract (Fig. 2d) did not reduce NO production, this is probably due to the low antioxidant activity as measured in the FRAP assay (Table 1).

**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 μg/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 ± 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 Lofenisia pacari St. Hil. (Lythraceae) (Rogerio et al., 2013). Moreover, ellagic acid inhibits PGE2 released in human monocytes (Karlsson et al., 2010) and inhibits the isolation and characterization of compounds with previously de-
tions used (data not shown).

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