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Characterization, anti-oxidative and anti-inflammatory effects of Costa Rican noni juice (*Morinda citrifolia* L.)

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ABSTRACT

Aim of the study: Noni fruit (*Morinda citrifolia* L.) juice has been used for more than 2000 years in Polynesia as a traditional folk medicine. The aim of the present study was to finely characterize noni juice from Costa Rica and to evaluate its anti-oxidative and anti-inflammatory activities.
Materials and methods: A microfiltrated noni juice was prepared with Costarican nonis. HPLC-DAD and Electro Spray Ionization Mass Spectrometric detection (HPLC-ESI-MS) were used to identify phenolic compounds and iridoids. The anti-oxidative activity of noni juice was measured in vitro by both Oxygen Radical Absorbance Capacity (ORAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging methods. The anti-inflammatory effects of noni juice were investigated in vitro by: measuring its effect on nitric oxide and prostaglandin E2 production by activated macrophages, evaluating its inhibitory activities on cyclooxygenase (COX)-1 and -2 and in vivo on a carrageenan-induced paw oedema model in rats.
Results: Several polyphenols belonging to the coumarin, flavonoid and phenolic acid groups, and two iridoids were identified. Noni juice demonstrated a mean range free radical scavenging capacity. Furthermore, it also reduced carrageenan-induced paw oedema, directly inhibited cyclooxygenase COX-1 and COX-2 activities and inhibited the production of nitric oxide (NO) and prostaglandins E_2 (PGE_2) in activated J774 cells, in a dose dependent manner.
Conclusions: This study showed that noni's biological effects include: (1) anti-oxidant properties probably associated with phenolic compounds, iridoids and ascorbic acid and (2) anti-inflammatory action through NO and PGE_2 pathways that might also be strengthened by anti-oxidant effects.

1. Introduction

Morinda citrifolia L. (Rubiaceae) is a small tropical tree that grows widely in Polynesia. Commonly called noni, its fruits have been used as a folk medicine for thousands of years for the treatment of many diseases including diabetes, high blood pressure, inflammation or cancer (Chan-Blanco et al., 2006). Currently, the noni juice market is continuously growing for its possible biological activities despite poor existing evidence. In 2002, the noni juice was even accepted in the European Union as a novel food. In response to this international demand for noni juice, some countries such as Costa Rica have increased noni cultivation and harvesting.

It has been described that noni fruits contain some active components such as phenolic compounds, in particular coumarins and flavonoids, and iridoids (Potterat et al., 2007). Phenolic compounds are plant secondary metabolites known for theirs anti-oxidative and anti-inflammatory properties. That is the case of flavonoids, in particular with quercetin and kampferol derivatives already described for theirs in vitro and in vivo anti-inflammatory properties (Morikawa et al., 2003; Blonska et al., 2004; Wang et al., 2008) and known to be natural antioxidants exhibiting inhibition of various enzymes associated with production of radical oxygen species (Pietta, 2000). Another group of polyphenols, coumarin group with in particular scopoletin and esculetin have also been described for theirs anti-oxidant, by free radicals-scavenging activities (Ng et al., 2003; Lin et al., 2008), and for theirs anti-inflammatory activities in various models (Tubaro et al., 1988; Silvan et al., 1996; Kang et al., 1999). Iridoids are plants metabolites based on a monoterpene structure with a cyclopenta[c]puranoid skeleton. They are present in various medicinal plants (Van Wyk, 2008; Adams et al., 2009) and some of them present a biological activity. Indeed, some iridoids have been described to possess antioxydative properties;

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this is the case of genipine that scavenge hydroxyl radicals (Koo et al., 2004). Others iridoids from Teucrium chamaedris (Pacifico et al., 2009) and from *Globularia alypum* L. (Es-Safi et al., 2007a) have also been described for theirs anti-oxidant properties. This activity seems less significant than that of polyphenols and could be correlated to the number of hydroxyl group present in the molecule (Es-Safi et al., 2007a). In the other hand, the number of publications on anti-inflammatory activity of iridoids has increased significantly in recent years. For instance, lamiide (Delaporte et al., 2002), ipolamiide (Schapoval et al., 1998), genipin (Koo et al., 2004), scrovalentinoside (Bas et al., 2007), and hydrolyzed aucubin (Park and Chang, 2004) have been described for theirs anti-inflammatory activities in various models.

Recent studies emphasise that recurrent or chronic inflammations associated with an oxidative stress have been implicated in various diseases such as cancer, diabetes, asthma and autoimmune diseases. The development of strategies for reducing inflammation and oxidation status could lead to effective treatments for these diseases. In this way, some natural products containing biological active molecules could participate to the prevention or the treatment of some of these diseases.

In regards to the wide use of noni juice and to its particular composition, the aim of this study was therefore to finely analyse our noni juice and to correlate this composition to their antioxidant and anti-inflammatory capacities.

2. Materials and methods

2.1. Plant material

The noni fruits used in this study were obtained from an experimental plantation established by EARTH University in the humid tropical region of Limón (Costa Rica).

2.2. Sample preparation

The fresh fruits were washed and disinfected using chloride solution (100 ppm). Noni juice puree was submitted to an enzymatic treatment with 150 ppm of Klerzyme®-DSM for 150 min at 35 °C to reduce the viscosity and suspended solids content of the juice. The noni puree was then pressed with a press cloth using a 25 T hydraulic rack. Tangential microfiltration was performed according to Vaillant et al. (1999). The pilot-scale microfiltration unit used featured a tubular ceramic membrane (Membralox® 1P19-40, Pall Exekia, Bazet, France) with the following attributes: 19 channels with internal diameter ($d = 4 \times 10^{-3}$ m), length ($L = 1.02$ m), average pore diameter ($\theta = 0.2$ μm) and total effective filtration area ($A = 0.22$ m^2). The process conditions were a flow velocity of 5 m/s, temperature 35 °C and an applied transmembrane pressure of 150 kPa. Tangential microfiltration represents an alternative to high temperature treatment. Microfiltration allows the production of a microbiologically stabilized clarified juice (Vaillant et al., 1999). The noni juice was stored at −20 °C until used.

Microfiltered noni juice was successively partitioned with hexane, ethyl acetate and *n*-butanol. 50 mL of noni juice was firstly delipidated with 3× 50 mL of hexane and then, successively extracted with 3× 50 mL of ethyl acetate (ethyl acetate extract, EAE) and 3× 50 mL of butanol (butanol extract, BE). Extracts were finally evaporated to dryness in a rotavapor and redissolved in methanol–chloroform (1:1, v/v).

2.3. Chemicals

All solvents were HPLC grade, purchased from Carlo Erba (Val de Reuil, France). Folin–Ciocalteu reagent was purchased from Carlo Erba (Val de Reuil, France). Ascorbic acid, quercetin, vanillin, vanillic acid, scopoletin, Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and metaphosphoric acid (MPA), fluorescein, 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxilic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), LPS from *Escherichia coli* 055:B5, indomethacin and carrageenan were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). Rutin, kaempferol and loganic acid were purchased from Extrasynthese (Genay, France). 2-2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, USA). RPMI 1640 with glutaMAX®, foetal bovine serum, streptomycin and penicillin were purchased from Gibco, Invitrogen (Cergy Pontoise, France). Recombinant murine IFN-γ was purchased from Genzyme (Tebu-Bio, Le Perray en Yvelines, France).

2.4. Physico-chemical analysis

Noni juice dry weight (DW) was determined by gravimetry after a 3 h period at 50 °C in drying chamber followed by a 12 h period at 60 °C in a steamroom under vacuum. Total soluble solids were measured in the juice with an Atago refractometer (Japan) at 20 °C. The results were reported as Brix degree. The pH was measured with a Schott pH-meter. Titratable acidity was determined by titration with 0.1 N NaOH to pH 8.2. The results were expressed as citric acid equivalent per 100 mg fresh weight (FW).

Sugar analysis was performed on a DX-600 ion chromatograph (Dionex, France) equipped with a GP50 pump and an ED50 electrochemical detector. The separation was performed using a 4 mm × 250 mm CarboPac MA1 analytical column (Dionex, France). The solvents were water for solvent A and NaOH 0.8 M for solvent B. Sugars were analyzed using the following gradient: 100% B for 10 min, from 100 to 75% B in 10 min, from 75 to 100% B in 10 min and 100% B for 10 min at the flow rate of 0.4 mL/min. The injection volume was 10 μL. Identification was achieved via comparison with standards: sucrose, fructose, and glucose. The analysis was performed in triplicate.

2.5. Total polyphenol content

The total polyphenol content in noni juice was determined by the Folin–Ciocalteu method optimized by George et al. (2005). The results were expressed as mg gallic acid equivalent per 100 g FW. The analysis was performed in triplicate.

2.6. Ascorbic acid and dehydroascorbic acid content

Ascorbic acid (AA) was measured by the HPLC method. Noni juice was diluted to 1/5 in a solution of 4% metaphosphoric acid to stabilize the ascorbic acid. A calibration curve was established with ascorbic acid in a dose range of 10–200 mg/L diluted in 4% metaphosphoric acid. The samples were then filtered through a 0.45 μm filter (Millipore). The HPLC analysis was carried out on a Dionex liquid chromatograph equipped with model P680 pumps, an ASI 100 autosampler and a UVD 340U diode array detector coupled to a HP ChemStation (Dionex, France). The separation was performed at 30 °C using a 250 mm × 4.6 mm, 5 μm particle size, endcapped reversed-phase Lichrospher ODS-2 (Interchim, Montluçon, France) in isocratic mode with 0.01% sulphuric acid solution (pH 2.6) as mobile phase. The flow rate was 0.8 mL/min. The injection volume was 20 μL and detection was carried out at 245 nm.

The dehydroascorbic acid (DHAA) was quantified according to Wechtersbach and Cigić (2007) with some modifications. After reduction of DHAA to AA by the Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) solution at 10 mM in 4% metaphosphoric acid (MPA), the AA was quantified. The DHAA content was calculated

by the difference between the peak area of AA with and without reduction. The analysis was performed in triplicate.

2.7. HPLC-DAD-MS^n analysis of noni juice compounds

Samples were filtered through a 0.45 μm filter (Millipore). The HPLC analysis was carried out on a Waters 2690 HPLC system equipped with a Waters 996 DAD (Waters Corp., Milford, MA) and Empower Software (Waters). The separation was performed at 30 °C using a 250 mm × 4.6 mm i.d., 5 μm, endcapped reversed-phase Lichrospher ODS-2 (Interchim, Montluçon, France). The solvents were water/acetonitrile/formic acid (99.195:0.8:0.005, v:v:v) for solvent A and 100% acetonitrile for solvent B. Phenolic compounds and standards were analyzed using the following gradient: 5% B for 5 min, from 5 to 35% B in 40 min, from 35 to 100% B in 3 min and 100% B for 10 min at a flow rate of 0.7 mL/min. The injection volume was 10 μL, and detection was carried out between 200 and 600 nm. After passing through the flow cell of the diode array detector, the column eluate was split and 0.25 mL/min was directed to an LCQ ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA). Experiments were performed in both negative and positive ion modes. The scan range was 100–2000 and the scan rate 1 scan/s. The desolvation temperatures were 250 and 300 °C in positive and negative ion mode, respectively. High spray voltage was set at 5000 V. Nitrogen was used as the dry gas at a flow rate of 75 mL/min. MS^2 and MS^3 were carried out using helium as the target gas, and the collision energy was set at 25–35 and 50%, respectively. Identifications were made based on the ion molecular mass, MS^n and UV–visible spectra.

2.8. HPLC-DAD quantification of noni juice compounds

Phenolic compounds and iridoids were quantified in EAE and BE extracts. Samples and standards (rutin, scopoletin, kaempferol, quercetin, isoquercitrin, vanillin, vanillic acid, loganic acid) were filtered through a 0.45 μm filter (Millipore). The HPLC analysis was carried out on a Dionex liquid chromatograph equipped with model P680 pumps, an ASI 100 autosampler and a UVD 340U diode array detector coupled to a HP ChemStation (Dionex, France), with the same column as described above. The separation was performed at 30 °C. The solvents were water/acetonitrile/formic acid (99.195:0.8:0.005, v:v:v) for solvent A, and acetonitrile 100% for solvent B. Phenolic compounds and standards were analyzed using the following gradient: 5% B for 7 min, from 5 to 35% B in 38 min, from 35 to 100% B in 3 min and 100% B for 2 min at a flow rate of 1 mL/min. The injection volume was 20 μL and detection was carried out at 240, 280 and 330 nm. In order to quantify the amount of each compound in both extracts, calibration curves were prepared with the standards: rutin, scopoletin, quercetin, vanillin, vanillic acid, kaempferol, isoquercitrin and loganic acid dissolved in methanol–chloroform (1:1, v/v). Conjugated forms of quercetin and kaempferol glycosides were quantified as their corresponding aglycones. All calibration curves showed good linearity in the studied concentration range.

2.9. Antioxidant capacity determination

2.9.1. ORAC assay

ORAC assays were performed according to Huang et al. (2002). We used a TECAN Infinite 200 microplate spectrofluorometer (TECAN Austria GmbH) in 96-well polypropylene plates. Briefly, the excitation and emission wavelengths were 485 ± 9 and 520 ± 20 nm, respectively. The solutions were all prepared with 75 mM phosphate buffer (pH 7.4). Each well was filled with 160 μL of a 78.75 nM fluorescein solution and 20 μL of buffer, standard 0–40 μM Trolox solution or diluted noni juice. The plate was incubated at 37 °C for 15 min before 20 μL of 178 mM AAPH solution were added. After the AAPH addition, the fluorescence decay was measured every minute for 60 min. The final values were calculating by using a regression equation between the trolox concentration (5–40 μM) and the net area under the fluorescein decay curve. The ORAC values were expressed as micromole Trolox equivalents per 100 g FW.

2.9.2. DPPH scavenging

The scavenging activity of noni juice on the DPPH free radical was measured according to Cheng et al. (2006) with some modifications. DPPH in radical form has an absorbance at 517 nm that disappears with the acceptance of an electron from an antioxidant compound to become a stable molecule.

Briefly, 0.2 mL of different juice dilutions or trolox solution in distilled water was added to 1.8 mL of 10^{-4} M DPPH solution in ethanol. After 1 h incubation in darkness, the decrease in absorbance at 517 nm was measured. The DPPH radical scavenging activity was expressed as micromole Trolox equivalent per 100 g FW.

2.10. Anti-inflammatory assays

2.10.1. In vitro assay

The ability to inhibit ovine cyclooxygenase 1 and 2 (COX-1, COX-2) of noni juice (1.5 mg of DW/mL) and indomethacin (0.2×10^{-6} and 1.3×10^{-6} M for COX-1and COX-2, respectively) was determined using an enzyme immuno assay kit: COX (ovine) Inhibitor Screening Assay® (Cayman Chemical, Spi Bio, Montigny-le-Bretonneux, France) that measures prostaglandins $F_{2\alpha}$ by $SnCl_2$ reduction of COX-derived prostaglandins H_2 produced in the COX reaction. Briefly, 20 μL of noni juice or indomethacin was incubated with COX-1 or COX-2 enzyme in buffer in the presence of heme at 37 °C. The substrate of COXs: arachidonic acid was then added and the level of prostaglandins $F_{2\alpha}$ was dosed. In the presence of COX inhibitors, the level of prostaglandins $F_{2\alpha}$ decreased.

2.10.2. Cell culture assay

The J774 macrophage cell line was obtained from the American Type Culture Collection (ATCC, TIB67; Rockville, MD). Cells were cultured in RPMI 1640 + glutamax® medium supplemented with streptomycin (20 μg/ml), penicillin (100 Units/ml) and 10% heat inactivated foetal bovine serum (complete RPMI medium). Cells were incubated at 37 °C in a humidified incubator containing 5% CO_2.

2.10.3. Cell viability by MTS assay

The cell proliferation assay was conducted using the CellTiter 96 aqueous non-radioactive cell proliferation assay (Promega France, Charbonnières, France) using a novel tetrazolium compound, 3-(4,5dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt (MTS) and an electron-coupling reagent: phenazine methosulphate (PMS). Briefly, 10^5 cells were seeded in a 96-well plate (100 μL/well) in complete RPMI medium and incubated for 1 h at 37 °C for adherence. After 1 h, the cells were treated with different noni juice concentrations ranging from 0.23 mg/mL to 3.6 mg/mL diluted into 100 μL in RPMI complete medium for 20 h. After 20 h, 20 μL of MTS/PMS were added to each well. The plate was incubated at 37 °C for another 4 h and the absorbance was read at 490 nm. Absorbance was proportional to mitochondrial respiration, and therefore to cell viability. The percentage of viable cells was calculated as follows: % viability = absorbance of sample/absorbance of control × 100.

2.10.4. Nitrite measurement

J774 cells were seeded onto a 24-well culture plate at density 5×10^5 cells per well with 500 μL of culture medium and incubated for 1 h. The cells were then pre-treated with various concentrations of noni juice for 4 h before stimulation with LPS + IFN (1 ng/mL + 10 UI/mL, respectively) for 24 and 48 h. The presence of nitrite, a stable oxidized product of nitric oxide (NO), was determined in cell culture media using Griess reagent. Briefly, 100 μL of supernatant was removed and combined with 100 μL of Griess reagent in a 96-well plate, followed by spectrophotometric measurement at 550 nm using a microplate reader (Molecular Devices) as previously described by Boudard et al. (1994). Nitrite concentration was determined by comparison with a sodium nitrite standard curve.

2.10.5. EIA for prostaglandin E2

J774 cells were seeded onto a 24-well culture plate at density 5×10^5 cells per well in 500 μL of RPMI complete medium and incubated for 1 h at 37 °C. The cells were then treated with various concentrations of noni juice or indomethacin at 100 μM for 4 h before stimulation with LPS + IFN (1 ng/mL + 10 UI/mL, respectively) diluted into 500 μL complete RPMI medium and incubated at 37 °C for another 20 h. Culture supernatants were stored at −20 °C until use. Prostaglandins E2 (PGE_2) were measured using an EIA kit (Cayman, Spi Bio, Montigny-le-Bretonneux, France).

2.10.6. In vivo assay: carrageenan-induced paw oedema in rat

The method of Winter et al. (1962) was employed in this experiment. Male Wistar rats (90 g ± 10 g) were purchased from Janvier® (France) and maintained under constant conditions (temperature: 22 °C ± 1 °C, 12 h light/12 h darkness cycle).

The rats were randomly divided into six groups of eight animals. Two doses (37 mg/kg and 7.4 mg/kg) of noni juice were administered by intraperitoneal injection and two doses (37 mg/kg and 14.8 mg/kg) of noni juice were administered by gavage to the test groups. A positive control group was given indomethacin (10 mg/kg in ethanol) per os and a control group was assessed.

After the administration of treatment, acute paw oedema was induced in the right hind paw by subplantar injection of 2% carrageenan suspension in saline, 0.05 mL per rat. The paw volume was measured with a plethysmometer (Ugo Basile) immediately after and 1, 2, 3, 4 and 24 h post carrageenan injection. The percentage inhibition was determined as follow:

$$\text{Inhibition}\ (\%) = \frac{[(V_t - V_0)\ \text{control} - (V_t - V_0)\ \text{treated}]}{(V_t - V_0)\ \text{control}}$$

where V_t = volume of right-hind paw at time t; V_0 = volume of right-hind paw immediately after carrageenan injection.

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Our laboratory practice was approved by the "Comité Régional d'Ethique pour l'Expérimentation Animale du Languedoc-Roussillon".

2.11. Statistical analysis

Values were given as means ± standard error of the mean (sem). Statistical analysis was performed using an unpaired Student's *t*-test. Differences were considered significant at $p \leq 0.05$

Table 1
Physicochemical characteristics of noni juice.

Characteristics	
Dry weight[a]	7.37 ± 0.06
Soluble solids[b]	5.8 ± 0.0
pH	3.4 ± 0.1
Titratable acidity[c]	1.76 ± 0.01
Glucose[d]	2.07 ± 0.01
Fructose[d]	2.44 ± 0.02
Total polyphenol[e]	47.6 ± 2.0
Total Vitamin C[f]	97.1 ± 2.3
Ascorbic acid[f]	71.1 ± 1.4
Dehydroascorbic acid[f]	26.0 ± 0.8

Values are means ± sem of three analyses.
[a] %/fresh weight (fw).
[b] Brix degree.
[c] g of citric acid/100 g fw.
[d] g/100 g fw.
[e] mg of equivalent gallic acid/100 g fw.
[f] mg/100 g fw.

3. Results and discussion

3.1. Chemical analysis

The physicochemical characteristics of noni juice are presented in Table 1. The major dry weight (DW) components (7.4% of the juice) were sugars, with glucose and fructose at 2.07 and 2.44 g/100 g FW, respectively.

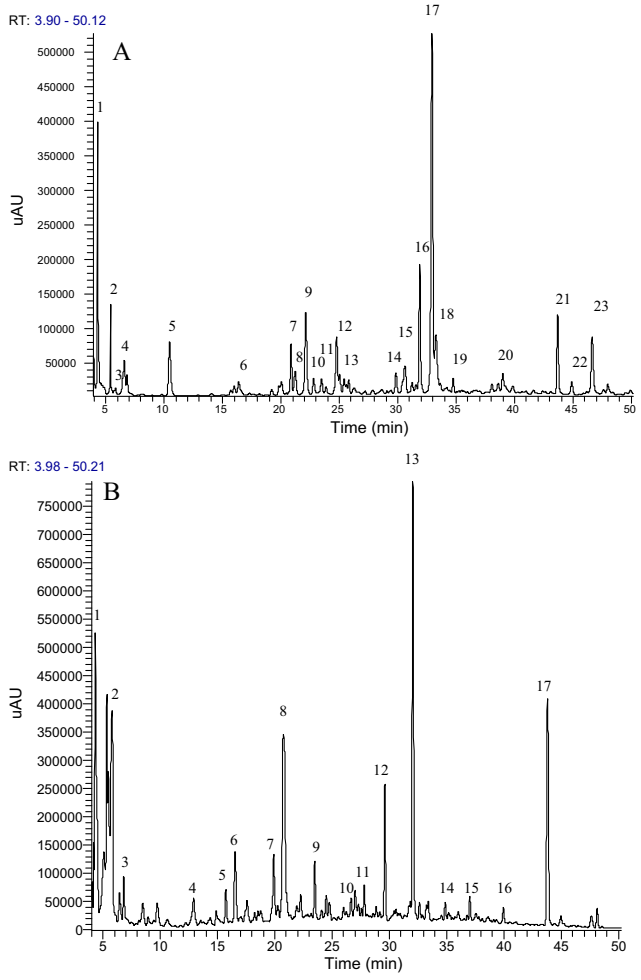

Fig. 1. 4–50 min segment of PDA-LC-DAD chromatograms of ethyl acetate extract (A) and butanol extract (B). For peak annotations refer to Table 3.

Table 2
Identification of compounds in ethyl acetate (A) and butanol extract (B).

Peak	Rt (min)	UV data (nm)	MS	MS^2/MS^3	Tentative identification
A					
1	4.3	241	nd[a]	nd	Not identified
2	5.4	282	nd	nd	Not identified
3	6.6	nd	nd	nd	Not identified
4	6.8	269	nd	nd	Not identified
5	10.5	241, 293	nd	nd	Not identified
6	16.4	259, 293	nd	nd	Protocatechuic acid
7	20.8	236	431 (−)	269, 251	Asperulosidic acid
8	21.2	262	nd	nd	Not identified
9	22.1	254	nd	nd	Vanillin
10	22.8	262	nd	nd	Not identified
11	23.4	262	nd	nd	Not identified
12	24.8	260, 292	167 (−)	nd	Vanillic acid
13	25.0	225, 298(sh)[b], 345	177 (−)	nd	Esculetin
14	29.8	263	nd	nd	Not identified
15	30.6	nd	nd	nd	Not identified
16	31.9	256, 354	609 (−)	301/179, 151	Rutin
17	32.9	229, 297(sh)[b], 342	191 (−)	nd	Scopoletin
18	33.3	nd	463 (−)	301/179,151	Isoquercitrin
19	34.7	265, 345	593 (−)	285	Kaempferol rutinoside
20	39.0	263	nd	nd	Not identified
21	43.7	273	nd	nd	Not identified
22	44.9	269	nd	nd	Not identified
23	46.6	255, 370	301 (−)	179,151	Quercetin
B					
1	4.3	237	nd	nd	Not identified
2	5.8	235	389 (−)	227,209	Desacetylasperulosidic acid
3	6.8	215, 271	nd	nd	Not identified
4	12.9	252, 286	nd	nd	Not identified
5	15.7	234	nd	nd	Not identified
6	16.5	218, 278	nd	nd	Not identified
7	19.8	228	nd	nd	Not identified
8	20.7	233	431 (−)	269, 251	Asperulosidic acid
9	23.5	226, 287 (sh)[b], 338	nd	nd	Not identified
10	26.6	235	nd	nd	Not identified
11	27.8	236	681 (−)	nd	Not identified
12	29.6	255, 353	741 (−)	609, 301	Quercetin derivative
13	32.0	254, 352	609 (−)	301	Rutin
14	34.9	265, 345	593 (−)	285	Kaempferol rutinoside
15	37.0	nd	nd	nd	Not identified
16	40.0	nd	nd	nd	Not identified
17	43.8	265	nd	nd	Not identified

[a] nd: not detected.
[b] sh: maximum of the shoulder in the spectrum; (−) negative mode.

HPLC-DAD-MS^n was used for analysing the minor components of noni juice. Fig. 1 shows the LC-DAD chromatograms of EAE and BE extracts. UV–visible characteristics and LC–MS data are given in Table 2. Protocatechuic acid, vanillin, vanillic acid, quercetin, kaempferol, rutin, scopoletin and isoquercitrin were formally identified by co-injection of the corresponding standard compounds. UV–visible characteristics, LC–MS and the MS^n fragmentations of the predominant positive and negative ions were used to confirm these identifications and to identify some aglycone derivatives. Identification of the individual peaks was performed according to data presented in Table 2.

The MS spectrum of peak 23 in EAE extract showed an $[M-H]^-$ at m/z 301 with MS^2 data at m/z 179 and m/z 151. These data are characteristics of quercetin (Mullen et al., 2003). The identification was confirmed by co-injection with authentic standard.

UV spectra of peak 16 in EAE extract and peaks 12 and 13 in BE extracts are similar to those of quercetin glycosides. Peak 16 (EAE) and peak 13 (BE) showed an $[M-H]^-$ at m/z 609 with MS^2 data at m/z 301 and MS^3 data at m/z 179 and m/z 151. The MS^n fragmentation pattern matched a quercetin derivative. MS^2 showed a loss of 308 mass units from the parent compound that might represent the loss of a hexose and a deoxyhexose, probably rutinose. The identification as rutin was confirmed by co-injection with the authentic standard. Peak 12 in BE extract showed $[M-H]^-$ at m/z 741 with MS^2 data at m/z 609 and MS^3 data at m/z 301. The loss of 132 mass units could represent a pentose, and the loss of 308 mass units a hexose and a deoxyhexose. The attachment position of the sugars could not be determined. In the same way, peak 18 in EAE extract (m/z 463) showed a fragmentation pattern matching a quercetin derivative. MS^2 showed a loss of 162 mass units from the parent compound, potentially represented by a hexose. Peak 18 (EAE) was identified as isoquercitrin by coinjection with the authentic standard.

UV spectra of peak 14 in BE extract was similar to those of kaempferol glycosides. Peak 14 showed an $[M-H]^-$ at m/z 593 with MS^2 data at m/z 285, the MS^2 fragmentation matching a kaempferol rutinoside. Indeed, MS^2 showed a loss 308 of mass units from the parent compound that could represent the loss of an [hexose + rhamnose] group. Even if the strong hypsochromic shift at 345 nm betrays substitution at position 4′ or 3, the attachment position of sugars could not be unambiguously determined.

UV spectra of peaks 13 and 17 in EAE extract were characteristic of a coumarin derivative. Peak 13 showed an $[M-H]^-$ at m/z 177 and peak 17 at m/z 191. Identification as esculetin and scopoletin was established by co-injection with the authentic standards. Identification of peaks 5, 9 and 12 in EAE extract was confirmed by co-injection with authentic standards as being protocatechuic acid, vanillin and vanillic acid, respectively. Previous reports from

Table 3
Quantification[a] (mg/100 g FW) of compounds in noni juice.

Compounds	Noni juice
Rutin	4.63 ± 0.04[a]
Quercetin	0.29 ± 0.01[a]
Quercetin derivative	0.46 ± 0.02[a]
Kaempferol derivative	tr[b]
Scopoletin	1.32 ± 0.02[a]
Esculetin	0.20 ± 0.01[a]
Vanillin	0.35 ± 0.01[a]
Vanillic acid	0.26 ± 0.00[a]
Protocatechuic acid	tr[b]
Isoquercitrin	tr[b]
Asperulosidic acid	71.6 ± 4.1[a]
Desacetylasperulosidic acid	159.1 ± 8.1[a]

[a] Values are means ± sem of three independent determinations.
[b] tr: traces (<0.1 mg/100 g FW).

the literature described the presence in noni of rutin (Akihisa et al., 2007), scopoletin (Potterat et al., 2007), quercetin and vanillin (Deng et al., 2007).

UV spectra of peak 7 in EAE extract and peaks 2 and 8 in BE extract are similar to iridoid UV spectra (~240 nm) (Chen et al., 2007). Peaks 7 in EAE extract and 8 in BE extract were similar and showed an $[M-H]^-$ at *m/z* 431 with MS^2 data at *m/z* 269 and *m/z* 251. This loss of 162 mass units represented the loss of glucose $[M-Glc]^-$and the loss of 180 mass units represented $[M-Glc-H_2O]^-$. Those weight losses are found in iridoid compounds (Es-Safi et al., 2007b). This data showed that peaks 7 (EAE) and 8 (BE) could be asperulosidic acid. Peak 2 in BE extract showed an $[M-H]^-$ at *m/z* 389 with MS^2 data at *m/z* 227 and *m/z* 209. Again, this loss of 162 mass units represented the loss of glucose $[M-Glc]^-$ and the loss of 180 mass units represented $[M-Glc-H_2O]^-$. This data showed that peak 2 could be deacetylasperulosidic acid. Those iridoids are commonly reported in noni juice (Akihisa et al., 2007; Potterat et al., 2007).

Twelve compounds were identified and 9 were quantified. We established the presence of phenolic compounds: coumarins (scopoletin, esculetin), flavonoids (rutin, quercetin, quercetin derivative, isoquercitrin and kaempferol rutinoside), phenolic acid (vanillic acid), vanillin and iridoids (asperulosidic acid and deacetylasperulosidic acid). Deacetylasperulosidic acid and asperulosidic acid were the major compounds in noni juice, with 159.1 and 71.6 mg loganic acid equivalent/100 g FW, respectively. The major polyphenolic compound was rutin, with 4.63 mg/100 g FW, followed by scopoletin with 1.33 mg/100 g FW. The quantification of phenolic compounds and iridoids is presented in Table 3.

3.2. Antioxidative activity

The in vitro evaluation of the antioxidant effect of noni juice was performed using two methods: (i) DPPH assay where radical scavenging activity measures the reduction of the DPPH free radical by electron transfer by the anti-oxidant and (ii) the ORAC assay where oxygen radical absorbance capacity measures the antioxidant activity of chain-breaking antioxidants against the peroxyl radical. ORAC values in noni juice: 955.5 ± 93.9 μM of trolox equivalent/100 g fw, were lower than in pomegranate juice, red wine and blueberry juice but higher than in orange juice, iced teas and apple juice (Seeram et al., 2008). DDPH scavenging activity values in noni juice: 329.5 ± 8.9 μM of trolox equivalent/100 g fw, were lower than blackberry and strawberry, but higher than tomato, passion fruit and physalis (Vasco et al., 2008).

This activity can be explained by the amount of phenolic compounds and vitamin C found in noni juice: 47.6 mg/100 g FW and 97.1 mg/100 g FW, respectively. In comparison with other juices, this total phenolic content was lower than in red wine, blueberry juice, pomegranate juice, blackberries and strawberries, but higher than in orange juice, iced tea, apple juice and tomato (Seeram et al., 2008; Vasco et al., 2008). Vinci et al. (1995) showed that orange, lemon, lychee and kiwi did not exceed 70 mg per 100 g FW of ascorbic acid, which was lower than that of noni juice.

3.3. Anti-inflammatory activity

The anti-inflammatory activity of noni juice was studied in vitro and in vivo, analysing their inhibitory effects on COX-1 and -2 activity, on chemical mediators released from activated macrophages and on carrageenan-induced paw oedema.

Activation of J774 macrophages triggered by LPS associated with IFNγ, induced proinflammatory mediators such as NO and PGE_2. The inhibition of the activated macrophages resulted in decreased LPS associated with IFN-stimulated production of NO and PGE_2.

NO produced by the enzyme iNOS, which is inducible during inflammation, is known to be an important mediator in inflammation (Coleman, 2001). NO is a signalling molecule that plays a key role in the pathogenesis of inflammation, and is considered as a pro-inflammatory mediator due to overexpression in abnormal situations. Transcription of iNOS, and thus NO production, is increased in activated macrophages (Alley et al., 2005). We first measured noni juice potential cytotoxic activity on J774 cells by MTS assay. The results demonstrated that noni up to 1.8 mg of DW/mL did not affect cell viability (data not shown). In this study, NO production was assessed by measuring the accumulation of nitrites, a stable metabolite of NO, in the media by colorimetric assay based on the Griess reaction. For noni juice, we observed an NO production decrease through reduced nitrites in the supernatant, in a dose dependent manner; this effect was moderate with a maximum inhibition of 30% for the concentration at 1.8 mg of DW/mL and was more pronounced after 48 h of culture (Fig. 2).

During the inflammation, PGE_2 is produced by the enzyme COX-2 (which is inducible during the inflammatory phenomenon). COX-2 is a key enzyme in the inflammatory phenomenon, and so PGE_2 is an important marker of inflammation. In vitro, noni juice at 1.5 mg of DW/mL can decrease COX-1 and COX-2 activity by 32.7 ± 9.3% and 23.1 ± 4.0%, respectively. In comparison, indomethacin (0.2 μM for COX-1 and 1.3 μM for COX-2) decrease COX-1 and COX-2 activity by 26.8 ± 6.7% and 89.6 ± 0.5%, respectively (data not shown). Noni juice inhibits the COX pathway by directly inhibiting the activity of both COX. These results are consistent with those published by Li et al. (2003) who tested noni juice's inhibitory effect on COX-1 activity (COX-2 activity not tested, Li et al., 2003). Wang et al. (2002) showed a selective COX-2 inhibitory activity of Tahitian Noni Juice while Deng et al. (2007) showed a selective COX-2 inhibitory activity of quercetin from noni juice.

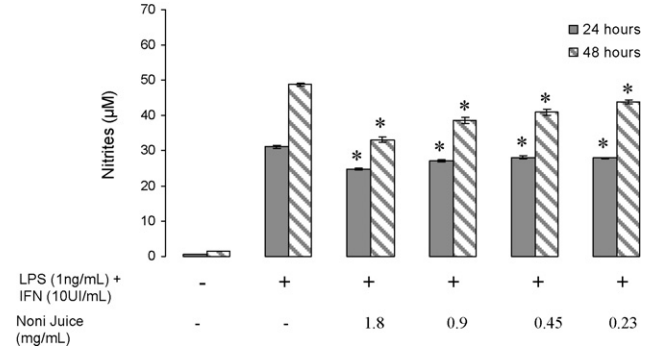

Fig. 2. Effect of noni juice on nitric oxide production for 24 and 48 h in LPS/IFN-stimulated J774 macrophages. Nitrites (stable NO metabolite) was measured in the cell culture media by the griess reaction assay. Values are means ± sem of three determinations. Data are from a representative experiment.

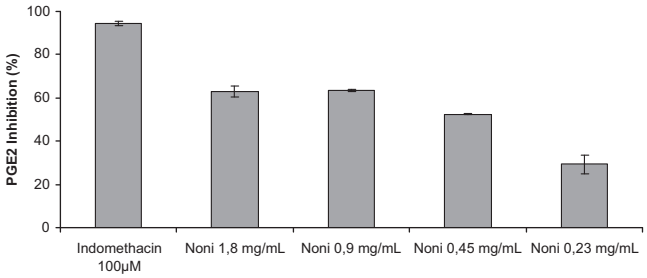

Fig. 3. Effect of noni juice and indomethacin on PGE_2 production for 24 h in LPS/IFN-stimulated J774 cells. PGE_2 was measured in the cell culture media by EIA. Values are means of inhibition percentage ± sem of three determinations. Data are from a representative experiment.

These two studies highlight a selective COX-2 inhibitory activity contrary to our results and those of the literature indicating a COX-1 and COX-2 inhibitory activities of quercetin (Al-Fayez et al., 2006; Warren et al., 2009). Following the discovery of this COX inhibitory activity, we evaluated the effect of noni juice on the production of PGE_2 by activated J774 macrophages. We have shown that noni juice inhibited PGE_2 production in a moderate but dose dependent manner (Fig. 3). Noni juice contains some major constituents such as fructose and glucose witch have no activity on PGE2 production. This biological activity may be due to minor noni constituents such as polyphenols.

This anti-inflammatory effect of noni juice can be explained by the presence of our identified molecules. Indeed, in vitro anti-inflammatory effects have been shown for flavonoids such as kaempferol and quercetin aglycones and glycosides (Blonska et al., 2004; Wang et al., 2008) and coumarins such as scopoletin and esculetin (Silvan et al., 1996; Kang et al., 1999) by inhibition activity on NO and PGE_2 production by activated macrophages. Asperulosidic acid and deacteylasperulosidic acid have so far not be described to have an anti-inflammatory activity while others iridoids showed possess this activity (Schapoval et al., 1998; Delaporte et al., 2002; Koo et al., 2004; Bas et al., 2007; Park and Chang, 2004). Moreover the roots of Paederia scandebs that contains asperulosidic acid, have been used in folk medicine for its anti-inflammatory activity (Quang et al., 2002). Asperulosidic acid and deacteylasperulosidic acid may therefore contribute to this activity.

Since noni juice possessed a moderate NO and PGE_2 inhibitory activities on J774 cells, the inhibitory activity of noni juice on carrageenan-induced rat paw oedema was further investigated. Carrageenan-induced rat paw oedema is a model of acute inflammation. Acute inflammation is a short-term process characterized by swelling, redness, pain, heat generation and infiltration of leukocytes at the inflammatory site. Local injection of carrageenan induced an acute inflammation in the rats' paws. During this inflammation, two phases can be characterized by the release of different mediators (DiRosa, 1972). An initial phase for the first 1 h is caused by the release of mediators including histamine, serotonin and bradykinins. A late phase, which is at its peak in the third hour after carrageenan injection, is mediated by an eicosanoid like PGE_2 and by neutrophils infiltration (Cuzzocrea et al., 1998). During the late phase of the oedema, NO is also produced through iNOS. This production is directly related to the inflammation found in carrageenan-induced paw oedema, as inhibition of NO production by iNOS reduces oedema (Salvemini et al., 1996; Cuzzocrea et al., 1998). In this model, as shown in Fig. 4, noni juice administered at 37 mg/kg and 7.4 mg/kg intraperitoneally and 37 mg/kg orally, significantly decreased paw oedema 3 h after carrageenan injection by 31%, 32% and 19%, respectively, and showed a similar effect to the standard drug indomethacin (10 mg/kg, p.o., 25% inhibition at 3 h).

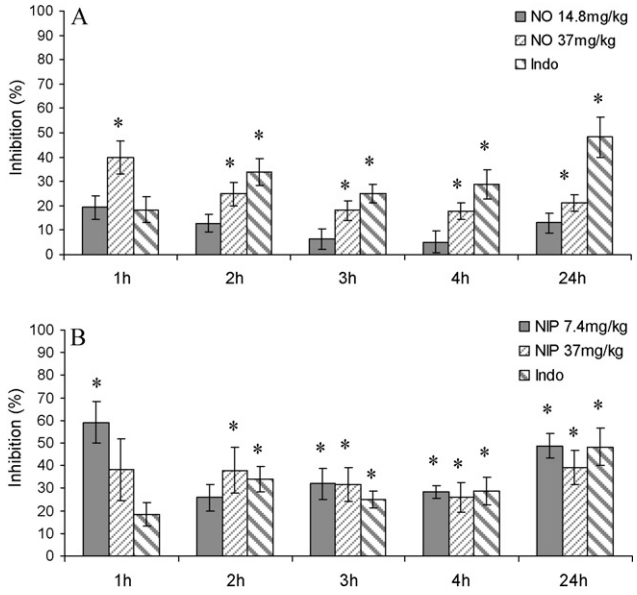

Fig. 4. Effect of noni juice and indomethacin on a carragennan induced paw oedema. Indomethacin (indo)was administrated orally at the dose of 10 mg/kg. Noni juice was administrated by gavage at the dose of 14.8 mg of DW/kg (NO 14.8 mg/kg) or 37 mg of DW/kg (NO 37 mg/kg) (A) or by intraperitoneal injection at the dose of 7.4 mg of DW/kg (NIP 7.4 mg/kg) or 37 mg of DW/kg (NIP 37 mg/kg) (B). Values are means of percentage of inhibition ± sem of eight determinations. $^*p < 0.05$ compared with the control group.

This inhibitory effect started at 1 h and lasted for 24 h. Our in vivo results showed an inhibitory effect on carrageenan-induced paw oedema from 1 h up to 24 h. Noni juice could deal in the initial and late phases. Those results were in accordance with those of McKoy et al. (2002) who found an inhibitory effect of an aqueous extract of noni on carrageenan-induced paw oedema.

This in vivo anti-inflammatory effect could be explained by the presence of flavonoids and coumarins in the juice. Indeed Morikawa et al. (2003) showed that quercetin, isoquercitrin and rutin inhibit carrageenan-induced paw oedema. Esculetin also possesses an in vivo anti-inflammatory effect, reducing croton oil-induced ear oedema (1988).

4. Conclusions

Noni juice from Costa Rica demonstrated anti-inflammatory and anti-oxidative effects in vitro and in vivo. The presence of flavonoids, coumarins, iridoids and vitamin C can partially explain these activities. However, considering our dataset with regard to the literature, since the effects observed were similar to reference drugs, some putative compound combinations might boost the pharmacological potential of noni molecules. To this end investigations are currently being performed to better identify compounds and/or compound combinations bearing potential therapeutic activity with lower toxicity than reference drugs on the market. This research will provide better understanding of noni pharmacology and positive health impact through characterization of the nutritional and therapeutic potential of this tropical fruit.

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References

Adams, M., Berset, C., Kessler, M., Hamburger, M., 2009. Medicinal herbs for the treatment of rheumatic disorders—a survey of European herbals from the 16th and 17th century. Journal of Ethnopharmacology 121, 343–359.

Akihisa, T., Matsumoto, K., Tokuda, H., Yasukawa, K., Seino, K.I., Nakamoto, K., Kuninaga, H., Suzuki, T., Kimura, Y., 2007. Anti-inflammatory and potential cancer chemopreventive constituents of the fruits of *Morinda citrifolia* (noni). Journal of Natural Products 70, 754–757.

Al-Fayez, M., Cai, H., Tunstall, R., Steward, W.P., Gescher, A.J., 2006. Differential modulation of cyclooxygenase-mediated prostaglandin production by the putative cancer chemopreventive flavonoids tricin, apigenin and quercetin. Cancer Chemotherapy and Pharmacology 58, 816–825.

Alley, E.B., Murphy, W.J., Russell, S.W., 2005. A classical enhancer element responsive to both lipopolysaccharide and interferon-γ augments induction of the iNOS gene in mouse macrophages. Gene 158, 247–251.

Bas, E., Recio, M.C., Abdallah, M., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Ríos, J.L., 2007. Inhibition of the pro-inflammatory mediators' production and anti-inflammatory effect of the iridoid scrovalentinoside. Journal of Ethnopharmacology 110, 419–427.

Blonska, M., Bronikowska, J., Pietsz, G., Czuba, Z.P., Scheller, S., Krol, W., 2004. Effects of ethanol extract of propolis (EEP) and its flavones on inducible gene expression in J774A.1 macrophages. Journal of Ethnopharmacology 91, 25–30.

Boudard, F., Vallot, N., Cabaner, C., Bastide, M., 1994. Chemiluminescence and nitrite determinations by the Malu macrophage cell line. Journal of Immunological methods 174, 259–268.

Chan-Blanco, Y., Vaillant, F., Perez, A.M., Reynes, M., Brillouet, J.M., Brat, P., 2006. The noni fruit (*Morinda citrifolia* L.): a review of agricultural research, nutritional and therapeutic properties. Journal of Food Composition and Analysis 19, 645–654.

Chen, J., Song, Y., Li, P., 2007. Capillary high-performance liquid chromatography with mass spectrometry for stimultaneous determination of major flavonoids, iridoid glucosides and saponins in *Flos Lonicerae*. Journal of Chromatography A 1157, 317–326.

Cheng, Z., Moore, J., Yu, L., 2006. High-throughput relative DPPH radical scavenging capacity assay. Journal of Agricultural and Food Chemistry 54, 7429–7436.

Coleman, J.W., 2001. Nitric oxide in immunity and inflammation. International Immunopharmacology 1, 1397–1406.

Cuzzocrea, S., Zingarelli, B., Hake, P., Salzman, A.L., Szabo, C., 1998. Antiinflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Radical Biology and Medicine 24, 450–459.

Delaporte, R.H., Sánchez, G.M., Cuellar, A., Giuliani, A., Palazzo de Mello, J.C., 2002. Anti-inflammatory activity and lipid peroxidation inhibition of iridoid lamiide isolated from *Bouchea fluminensis* (Vell.) Mold. (Verbenaceae). Journal of Ethnopharmacology 82, 127–130.

Deng, S., Palu, A.K., West, B.J., Su, C.X., Zhou, B.N., Jensen, J.C., 2007. Lipoxygenase inhibitory constituents of the fruits of noni (*Morinda citrifolia*) collected in Tahiti. Journal of Natural Products 70, 859–862.

DiRosa, M., 1972. Biological properties of carrageenan. Journal of Pharmacy and Pharmacology 24, 89–102.

Es-Safi, N.E., Kollmann, A., Khlifi, S., Ducrot, P.H., 2007a. Antioxidative effect of compounds isolated from *Globularia alypum* L. structure–activity relationship. LWT—Food Science and Technology 40, 1246–1252.

Es-Safi, N.E., Kerhoas, L., Ducrot, P.H., 2007b. Fragmentation study of iridoid glucosides through positive and negative electrospray ionization, collision-induced dissociation and tandem mass spectrometry. Rapid Communication in Mass Spectrometry 21, 1165–1175.

George, S., Brat, P., Alter, P., Amiot, M.J., 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. Journal of Agricultural and Food Chemistry 53, 1370–1373.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A., Prior, R.L., 2002. Highthroughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. Journal of Agricultural and Food Chemistry 50, 3122–3128.

Kang, T.H., Pae, H.O., Jeong, S.J., Yoo, J.C., Choi, B.M., Jun, C.D., Chung, H.T., Miyamoto, T., Higuchi, R., Kim, Y.C., 1999. Scopoletin: an inducible nitric oxide synthesis inhibitory active constituent from *Artemisia feddei*. Planta Medica 65, 400–403.

Koo, H.J., Song, Y.S., Kim, H.J., Lee, Y.H., Hong, S.M., Kim, S.J., Kim, B.C., Jin, C., Lim, C.J., Park, E.H., 2004. Antiinflammatory effects of genipin, an active principle of gardenia. European Journal of Pharmacology 495, 201–208.

Li, R.W., Myers, S.P., Leach, D.N., Lin, G.D., Leach, G., 2003. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. Journal of Ethnopharmacology 85, 25–32.

Lin, H.C., Tsai, S.H., Chen, C.S., Chang, Y.C., Lee, C.M., Lai, Z.Y., Lin, C.M., 2008. Structure-activity relationship of coumarin derivatives on xanthine oxidase-inhibiting and free radical-scavenging activities. Biochemical Pharmacology 75, 1416–1425.

McKoy, M.L., Thomas, E.A., Simon, O.R., 2002. Preliminary investigation of the anti-inflammatory properties of an aqueous extract from *Morinda citrifolia* (noni). Proceedings of the Western Pharmacology Society 45, 76–78.

Morikawa, K., Nonaka, M., Narahara, M., Torii, I., Kawaguchi, K., Yoshikawa, T., Kumazawa, Y., Morikawa, S., 2003. Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. Life Sciences 74, 709–721.

Mullen, W., Yokota, T., Lean, M.E.J., Crozier, A., 2003. Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC–MS^n. Phytochemistry 64, 617–624.

Ng, T.B., Liu, F., Lu, Y., Cheng, C.H., Wang, Z., 2003. Antioxidant activity of compounds from the medicinal herb *Aster tataricus*. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 136, 109–115.

Pacifico, S., D'Abrosca, B., Pascarella, M.T., Letizia, M., Uzzo, P., Piscopo, V., Fiorentino, A., 2009. Antioxidant efficacy of iridoid and phenylethanoid glycosides from the medicinal plant *Teucrium chamaedris* in cell-free systems. Bioorganic and Medicinal Chemistry 17, 6173–6179.

Park, K.S., Chang, I.M., 2004. Anti-inflammatory activity of aucubin by inhibition of tumor necrosis factor-alpha production in RAW 264.7 cells. Planta Medica 70, 778–779.

Pietta, P.G., 2000. Flavonoids as antioxidants. Journal of Natural Products 63, 1035–1042.

Potterat, O., von Felten, R., Dalsgaard, P.W., Hamburger, M., 2007. Identification of TLC markers and quantification by HPLC–MS of various constituents in noni fruit powder and commercial noni-derived products. Journal of Agricultural and Food Chemistry 55, 7489–7494.

Quang, D.N., Hashimoto, T., Tanaka, M., Dung, N.X., Asakawa, Y., 2002. Iridoid glucosides from roots of Vietnamese *Paederia scandens*. Phytochemistry 60, 505–514.

Salvemini, D., Wang, Z.Q., Bourdon, D.M., Stern, M.K., Currie, M.G., Manning, P.T., 1996. Evidence of peroxynitrite involvement in the carrageenan-induced rat paw oedema. European Journal of Pharmacology 303, 217–220.

Schapoval, E., Winter de Vargas, M.R., Chaves, C.G., Bridi, R., Zuanazzi, J.A., Henriques, A.T., 1998. Antiinflammatory and antinociceptive activities of extracts and isolated compounds from *Stachytarpheta cayennensis*. Journal of Ethnopharmacology 60, 53–59.

Seeram, N.P., Avuram, M., Zhang, Y., Henning, S.M., Feng, L., Dreher, M., Heber, D., 2008. Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. Journal of Agricultural and Food Chemistry 56, 1415–1422.

Silvan, A.M., Abad, M.J., Bermejo, P., Villar, A., 1996. Antiinflammatory activity of coumarins from *Santolina oblongifolia*. Journal of Natural Products 59, 1183–1185.

Tubaro, A., Del Negro, P., Ragazzi, E., Zampiron, S., Della Loggia, R., 1988. Anti-inflammatory and peripheral analgesic activity of esculetin *in vivo*. Pharmacological Research Communications 20, 83–85.

Vaillant, F., Millan, P., O'Brien, G., Dornier, M., Decloux, M., Reynes, M., 1999. Cross-flow microfiltration of passion fruit juice after partial enzymatic liquefaction. Journal of Food Engineering 42, 215–224.

Van Wyk, B.E., 2008. A broad review of commercially important southern African medicinal plants. Journal of Ethnopharmacology 119, 342–355.

Vasco, C., Ruales, J., Kamal-Eldin, A., 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chemistry 111, 816–823.

Vinci, G., Botrè, F., Mele, G., Ruggieri, G., 1995. Ascorbic acid in exotic fruits: a liquid chromatographic investigation. Food Chemistry 53, 311–314.

Wang, M.Y., West, B.J., Jensen, C.J., Nowicki, D., Su, C., Palu, A.K., Anderson, G., 2002. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta Pharmacologica Sinica 23, 1127–1141.

Wang, G.J., Chen, Y.M., Wang, T.M., Lee, C.K., Chen, K.J., Lee, T.H., 2008. Flavonoids with iNOS inhibitory activity from *Pogonatherum crinitum*. Journal of Ethnopharmacology 118, 71–78.

Warren, C.A., Paulhill, K.J., Davidson, L.A., Lupton, J.R., Taddeo, S.S., Hong, M.Y., Carroll, R.J., Chapkin, R.S., Turner, N.D., 2009. Quercetin may suppress rat aberrant crypt foci formation by suppressing inflammatory mediators that influence proliferation and apoptosis. Journal of Nutrition 139, 101–105.

Wechtersbach, L., Cigi, B., 2007. Reduction of dehydroascorbic acid at low pH. Journal of Biochemical and Biophysical Methods 70, 767–772.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced oedema in hind paw of the rat as an assay for antiinflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine 111, 544–547.