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Preparative Isolation, Fast Centrifugal Partition Chromatography Purification and Biological Activity of Cajaflavanone from *Derris ferruginea* Stems

Sylvie Morel,^a Anne Landreau,^{b*} Van Hung Nguyen,^b Séverine Derbré,^a Philippe Grellier,^c Patrice Le Pape,^d Fabrice Pagniez,^d Marc Litaudon^e and Pascal Richomme^a

ABSTRACT:

Introduction – The *Derris* genus is known to contain flavonoid derivatives, including prenylated flavanones and isoflavonoids such as rotenoids, which are generally associated with significant biological activity.

Objective – To develop an efficient preparative isolation procedure for bioactive cajaflavanone.

Methodology – Fast centrifugal partition chromatography (FCPC) was optimised to purify cajaflavanone from *Derris ferruginea* stems in a single step as compared to fractionation from the cyclohexane extract by successive conventional solid–liquid chromatography procedures. The purification yield, purity, time and solvent consumption per procedure are described. The anti-fungal, anti-bacterial, anti-leishmanial, anti-plasmodial, anti-oxidant activities and the inhibition of advanced glycation end-products (AGEs) by cajaflavanone accumulation are described.

Results – FCPC enabled cajaflavanone purification in a single separation step, yielding sufficient quantities to perform *in vitro* biological screening. Interestingly, cajaflavanone had an inhibitory effect on the formation of AGEs, without displaying any *in vitro* anti-oxidant activity.

Conclusion – A simple and efficient procedure, in comparison with other preparative methods, for bioactive cajaflavone purification has been developed using FCPC.

Keywords: Fast centrifugal partition chromatography; cajaflavanone; *Derris ferruginea*; Fabaceae

Introduction

Derris ferruginea (Roxb.) Benth., a liana species exhibiting densely rust-colored pubescent branchlets, originates from India, but it is also found in Laos and Vietnam (Subba Rao and Seshadri, 1946). *Derris ferruginea* roots, which are known to contain rotenone and rotenoids (Subba Rao and Seshadri, 1946), are traditionally used as fish poison and pesticide in Assam State, India (Lamba, 1970; Moretti and Grenand, 1982). Moreover, leaf decoctions of this species are used orally to treat gastrointestinal diseases (Zheng and Xing, 2009). In spite of the fact that the biological activities of *Derris* species have been widely described, i.e. cytotoxic, anti-bacterial, anti-fungal and anti-oxidant properties (Laupattarakasem *et al.*, 2003; Khan *et al.*, 2006; Cheenpracha *et al.*, 2007), and that major secondary metabolites in the genus are known to be flavonoids, including prenylated flavanones and isoflavonoids such as rotenoids (Mahabusarakam *et al.*, 2004; Yenesew *et al.*, 2005; Ranga Rao *et al.*, 2009; Tewtrakul *et al.*, 2009), very little phytochemical information is available on *D. ferruginea* (Subba Rao and Seshadri, 1946). Cajaflavanone (1), the main compound – which was obtained for the first time from the cyclohexane extract of

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Abbreviations: AAPH: 2,2'-azobis (2-methylpropionamide) dihydrochloride; AGEs: advanced glycation endproducts; CFU: colony forming unit; DPPH: 1,1-diphenyl-2-picrylhydrazyl; FCPC: fast centrifugal partition chromatography; HPLC: high performance liquid chromatography; MICs: minimum inhibitory concentrations; MPLC: medium pressure liquid chromatography; ORAC: oxygen radical absorbance capacity; TLC: thin layer chromatography.

the stems of this species – was first isolated from another fabaceous species, namely *Cajanus cajan* (Bhanumati *et al.*, 1978). The compound has shown some anti-plasmodial and anti-dermatophytic activity (Khaomek *et al.*, 2008; Ribeiro *et al.*, 2008). It is also an inhibitor of the Epstein–Barr virus (EBV; Itoigawa *et al.*, 2002). Two separation techniques were applied to isolate this compound from cyclohexane extracts from *D. ferruginea* stems. Conventional isolation of **1** requires medium pressure liquid chromatography (MPLC) over silica gel, followed by size-exclusion chromatography on LH-20 Sephadex gel and filtration over silica gel, leading to high solvent consumption. In contrast with this solid/liquid partition, **1** could be quickly obtained in a one-step high-yield separation procedure using fast centrifugal partition chromatography (FCPC). This method, was first developed by Ito and Bowman (1970), and has recently been described for the purification of flavonoid compounds (Marston and Hostettmann, 2006; Pauli *et al.*, 2008), particularly prenylated flavanones (Maver *et al.*, 2005). Consequently, FCPC can facilitate direct high-yield isolation of **1** from the aforementioned crude extract for the purpose of screening its biological activities in a set of different assays, including inhibition of advanced glycation end-product (AGE) accumulation, as well as anti-oxidant, anti-fungal, anti-microbial and anti-parasitic activities.

Experimental

Reagents

Solvents used for plant extractions and centrifugal partition chromatography separation were of analytical grade (Carlo Erba reactif, Val de Reuil, France). HPLC grade solvents were purchased from VWR international (Fontenay-sous-Bois, France). Deionized water with a resistivity of 18 M Ω cm or more was used for HPLC/UV. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (L'Isle d'Abeau Chesnes, France). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox[®]) and 5'-caffeoylquinic acid (chlorogenic acid), 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH) and fluorescein (FL) were purchased from Acros Organics (Noisy-Le-Grand, France). TLC analyses were performed on silica gel 60 F254 (Merck, Darmstadt, Germany).

Plant

Derris ferruginea (Roxb.) Benth. was collected at Ha Tinh, Vietnam, in 1998. The plant was identified by Dr. Nguyen Tien Hiep from the Hanoi National Herbarium, where a voucher specimen is kept under reference VN-0452.

Preparation of the crude extracts

Dried and ground stems (1200 g) of *D. ferruginea* were successively extracted with cyclohexane, dichloromethane, ethyl acetate and methanol (8 L), in a Soxhlet apparatus (72 h), yielding four extracts that were named DfS1–4, respectively.

Apparatus

Preparative centrifugal partition chromatography was performed using a FCPC 200 (Kromaton, Angers, France) with a total cell volume of 275 mL. A valve incorporated in the FCPC apparatus allowed operation in descending or ascending mode. The system was equipped with a gradient pump, a UV-vis detector, a Rheodyne valve with a 10 mL sample loop and a fraction collector (Kromaton, Angers, France).

¹H-NMR, ¹³C-NMR and 2D-NMR spectra were recorded in deuterated chloroform on a Bruker Avance DRX 500 MHz (Bruker, Wissembourg,

France) spectrometer. Mass spectra were recorded on an Esquire 3000 PLUS apparatus (Bruker).

HPLC analysis

The HPLC system consisted of a Waters 2695[®] separation module coupled to a Photodiode Array Detector Waters[®] 2996 using the Empower software package. Twenty microlitres of each sample were injected onto a Hypersil C₁₈-column (250 × 4.6 mm, 5 μ m, Thermo Electron Corporation) using the following gradient: initial mobile acetonitrile:water phase 10:90 reaching 95:5 (v/v) in 60 min, with a flow rate of 1 mL/min. Purity and yield were measured at 254 nm.

Isolation procedure using solid–liquid chromatography. Seven grams of the crude cyclohexane extract were processed by MPLC (column diameter and length 7 × 45 cm, silica gel 60 G (Merck): 550 g). Elution was completed with mixtures of cyclohexane:ethyl acetate (90:10 to 10:90 in 5% stepwise) then chloroform:methanol (99:1 to 90:10 in 1% then 5% stepwise). Sixty-five 500-mL fractions were collected. After TLC analysis, fractions 19 to 27 eluted with cyclohexane:ethyl acetate (85:15) were combined and concentrated under reduced pressure, yielding fraction DfS1-6 (535.9 mg). DfS1-6 was finally purified on LH-20 Sephadex gel (2.4 × 35 cm, 30 g LH-20, elution; dichloromethane 100% to methanol 100%) followed by filtration on a silica gel column (1.0 × 13.5 cm, 3 g of silica gel, elution: cyclohexane:ethyl acetate 70:30 to 0:100) yielding **1** (1.5 mg, Fig. 1; *t*_R: 56.5 min; yield: 0.02%; HPLC purity: 86.8%), which was identified by NMR and mass spectroscopy analysis.

Selection of the two-phase solvent system for FCPC. The two-phase solvent system was selected according to an evaluation of the partition coefficient (*K*). The latter is defined by the ratio of the solutes of interest distributed between two non-miscible phases. The *K* value was estimated by TLC analysis as follows: the same amount of the cyclohexane extract was dissolved in a mixture of 1 mL (upper phase) and 1 mL (lower phase) of a two-phase solvent system. The solution was then mixed thoroughly. After phase separation and identification the distribution of **1** between the upper and lower phases was investigated by TLC (Hostettmann *et al.*, 1998). After HPLC analysis (Fig. 2) the *K* value was then calculated according to the following equation: $K = A/A'$ lower phase/upper phase, where *A* and *A'* represent the absorbances of cajaflavanone at 254 nm (AUC). The solvent system exhibiting a *K* value closest to 1 (equal content of **1** in each phase) was finally selected. The quaternary Arizona solvent system, consisting of heptane, ethyl acetate, methanol and water, was selected according to the literature (Maver *et al.*, 2005). With reference to the latter, preliminary TLC experimental system U [heptane:ethyl acetate:methanol:water (4:1:4:1, v/v)] was proposed as an optimized solvent system for FCPC separation of the crude cyclohexane extract of *D. ferruginea*. This was also confirmed by HPLC determination of the *K* value of **1**, which was $K = 0.91$ at 254 nm. Before conducting the FCPC experiment, upper and lower phases of solvent system U were mixed in a separation funnel at room temperature. After phase separation, the upper organic phase was used as the mobile phase, whereas the lower was employed as the stationary phase and the consecutive FCPC experiment was conducted in ascending mode.

FCPC isolation of cajaflavanone. The instrument (275 mL) was filled in ascending mode with stationary phase using a KP100 pump at a flow rate of 40 mL/min, at a rotation speed of 400 rpm. Then the upper phase (mobile phase) was introduced at a flow rate of 10 mL/min, at 900 rpm. Solvent equilibrium was reached once the mobile phase emerged from the FCPC. Retention of the stationary phase was calculated as 60%. Then 10 mL of the sample (5 g/10 mL), dissolved in a 1:1 (v/v) mixture of each phase, was injected through a Rheodyne injection valve. The separation was performed in ascending mode at a flow-rate of 10 mL/min, at 900 rpm until 550 mL of the upper phase were collected. Then the extrusion phase was initiated using a lower phase solvent as the mobile phase (360 mL, 12 mL/min, 200 rpm). This was done to ensure that any residual extract was recovered from the machine.

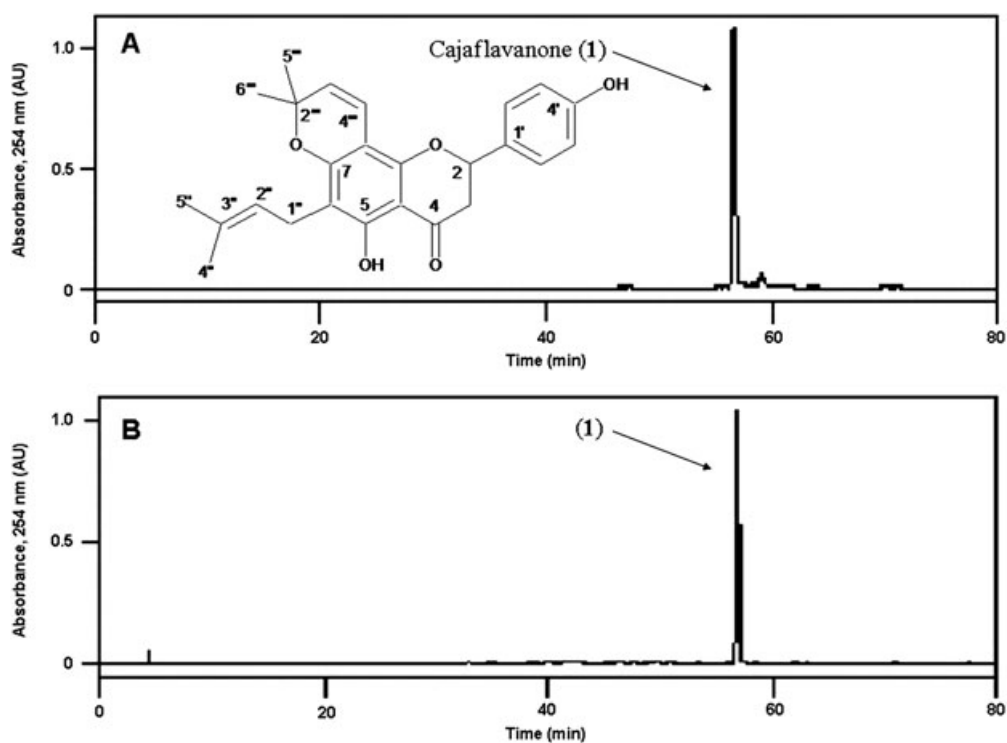


Figure 1. HPLC chromatograms at 254 nm (for conditions see Experimental section) of cajaflavanone (1) obtained by (A) solid-liquid procedure and (B) FCPC. The peak corresponding to cajaflavanone is indicated by an arrow.

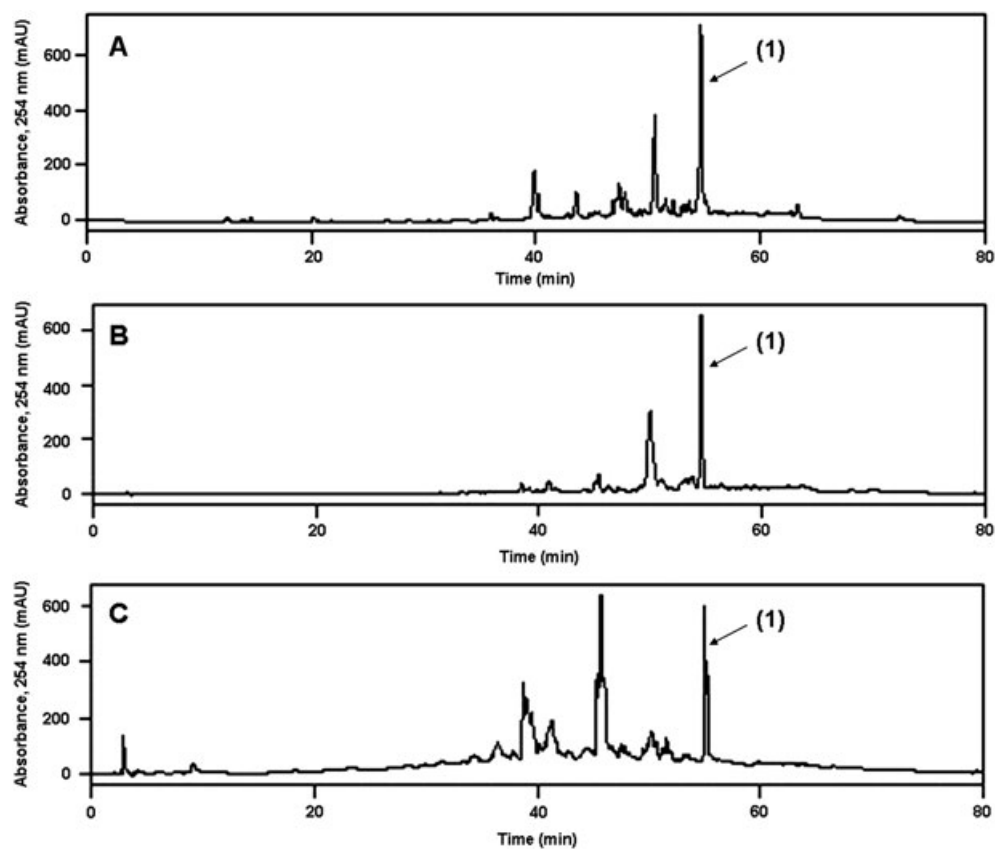


Figure 2. HPLC chromatograms at 254 nm (for conditions see Experimental section) of cyclohexane crude extract of (A) *Derris ferruginea*, (B) upper phase and (C) lower phase. The peak corresponding to cajaflavanone (1) is indicated by an arrow.

After TLC analysis and according to the FCPC chromatogram (Fig. 3), fractions 34 to 44 (10 mL per fraction) were combined and concentrated under reduced pressure, yielding 11.7 mg (0.2%) of **1** (Fig. 1; t_R : 56.5 min, HPLC purity 86.9%).

Antifungal activity. The bioassays were performed by a paper disc diffusion method (Morel *et al.*, 2001) using the following fungi: *Candida albicans* (ATCC 66–390), *Candida glabrata* (LMA 9061085) and *Aspergillus fumigatus* (CBS 113–26). Fungi were cultivated on yeast extract peptone dextrose agar (YPDA), for 48 (yeasts) or 72 h (*Aspergillus*) at 37°C. Test compounds were dissolved in dimethyl sulphoxide (DMSO) and 250 µg aliquots were applied to 12-mm-diameter paper discs (rf 06234304, Prolabo 33173 Gradigan). After evaporating the solvent, discs were placed in the centre of 90-mm-diameter casitone agar petri dishes previously inoculated with 10 mL of the spore suspension. The yeast suspensions were obtained, after the incubation period, by incorporating one colony in 10 mL of sterile distilled water (colony forming unit (CFU) *C. albicans* 3×10^6 , CFU *C. glabrata* 5×10^6). The *Aspergillus* suspension was prepared by fragmenting the culture in 10 mL sterile distilled water with a ground-glass grinder, according to the National Committee For Clinical Laboratory Standard (NCCLS) guidelines for filamentous fungi (M38-P). The fungal suspensions were finally adjusted spectrophotometrically to an A_{450} of 0.6. An amphotericin B paper disc was used as positive control, with drug-free DMSO as negative control. After 48 h incubation for the yeasts, or 72 h for *A. fumigatus*, diameters of growth inhibition zones (mm) were measured around the paper discs.

Antibacterial activity. Bacteriostatic activities were evaluated on 21 bacterial strains obtained from the bacteriology laboratory of the University Hospital of Angers: seven strains of *Acinetobacter baumannii* (RCH, SAN008, 12, AYE, CIP7034, CIP107292, CIP5377), five of *Staphylococcus aureus* (ATCC25923, two methicillin sensitive clinical isolates, two methicillin resistant clinical isolates), two of *Escherichia coli* (ATCC25922 and a clinical isolate), three of *Pseudomonas aeruginosa* (ATCC27853 and two clinical isolates), and one clinical isolate of *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella oxytoca* and *Salmonella enteritidis* (phage type 4). Tests were performed using a methodology described in the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM, www.sfm.asso.fr). In short, a stock solution of each compound was prepared at 20 mg/mL in DMSO under sterile conditions. Each extract was tested at two concentrations: 10 and 100 µg/mL in 20 mL of Mueller Hinton agar (Merck, Germany) transferred onto petri plates. Then, about 2×10^4 bacteria suspended in sterile NaCl

(0.15 M) were inoculated onto the different petri plates using the multipoint inoculator (AQS, England). After 24 h incubation at 37°C, the minimum inhibitory concentration (MIC; µg/mL) of each extract against each bacterial strain was determined. The MIC was the lowest concentration leading to bacterial growth inhibition.

Antileishmanial activity. *Leishmania major* (MHOM/II/81/BNI) was cultured at 26°C in Schneider's insect medium (Sigma, St Quentin Fallavier, France) supplemented with 15% fetal bovine serum (FBS) (Sigma), penicillin (100 IU/mL) and streptomycin (50 µg/mL). Exponentially growing cells were maintained at 26°C. Promastigote susceptibility testing was performed with the previously described Uptiblu[®] micromethod (Le Pape *et al.*, 2003). Briefly, 100 µL of a 10^6 promastigote/mL suspension were placed into wells of a 96-well microplate (Nunc[®]). The cultures were exposed for 96 h at 26°C to the anti-leishmanial drugs at the concentrations used above. Four hours before measurement, 10 µL of Uptiblu[®] were added. The fluorescence was measured at 590 nm with an excitation wavelength of 550 nm.

Antiplasmodium activity. The *Plasmodium falciparum* strain (FcB1/ Columbia) was cultivated by continuous culture on human erythrocytes in RPMI 1640 medium with heat-inactivated human serum under an atmosphere of 3% CO₂, 6% O₂, 91% N₂, at 37°C, as described in the literature (Trager and Jensen, 1976). Drug susceptibility assays were performed using a modification of the semi-automated microdilution technique of the Desjardins method (Desjardins *et al.*, 1979) based on the uptake of [G-³H]hypoxanthine as an index of parasite growth. Drug solutions were diluted with 100 µL culture medium in 96-well plates. Each extract was evaluated in duplicate at 10 µg/mL. Chloroquine served as the positive control and drug-free DMSO as the negative control. The growth inhibition for each extract was determined by comparing the detected radioactivity present in the treated culture with those of the negative control culture on the same plate.

Cytotoxic evaluation. Cytotoxic activities were evaluated on MRC5 cells in DMSO at 10 and 1 µg/mL based on the method described by Morel *et al.* (2009).

Scavenging activity of diphenyl-picrylhydrazyl radicals. Radical-scavenging activity was evaluated using DPPH free radicals according to the method of Abdel-Lateff *et al.* (2002) with some modifications. In its radical form, DPPH[•] has an absorption band at 517 nm, which disappears upon reduction by an anti-radical compound. Tested compounds and standards were diluted in absolute ethanol at different concentrations

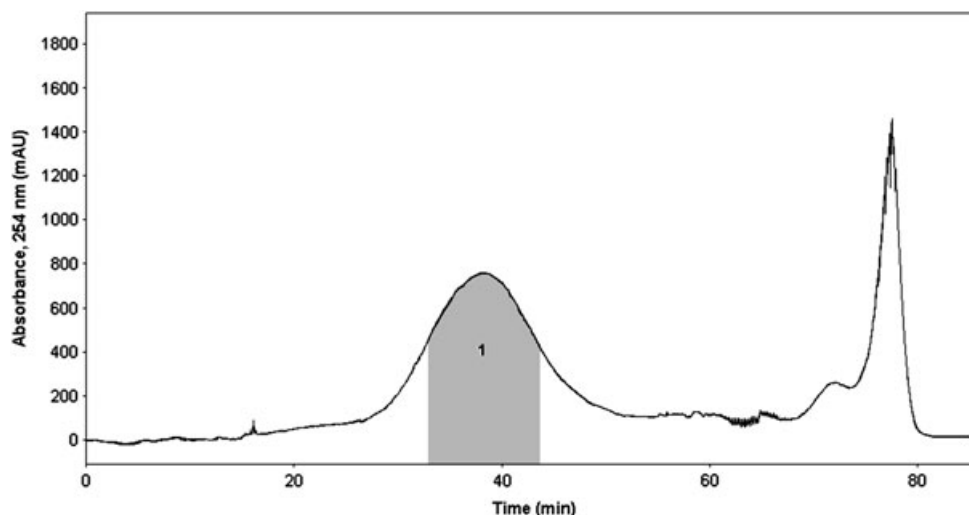


Figure 3. FCPC chromatogram of cajaflavanone (**1**) from the cyclohexane extract of *Derris ferruginea* stems (for FCPC and chromatographic conditions see Experimental section). Elution first with organic phase of Arizona system U. Phase of extrusion was initiated at 55 min to remove all the crude extract.

from stock solutions of 1 mg/mL in DMSO. Aliquots (100 μ L) of these diluted solutions were placed in 96-well plates in triplicate for each concentration tested. The reaction was initiated by adding 25 μ L of freshly prepared DPPH solution (1 mM) and 75 μ L of absolute ethanol using the microplate reader injector (Infinite[®] 200, Tecan, France) to obtain a final volume of 200 μ L/well. After 30 min in the dark at room temperature, absorbance was determined at 517 nm. Ethanol was used as a blank, and 10, 25, 50 and 75 μ M of Trolox (hydrophilic α -tocopherol analogue) were used as calibration solutions. A sample of 0.02 mg/mL chlorogenic acid was used as quality control. The DPPH-scavenging activity of the tested compounds was compared to that of the Trolox calibration curve. The results were expressed as Trolox equivalent (micromoles of Trolox equivalents per gram of dry matter).

Measurement of oxygen radical absorbance capacity. Oxygen radical absorbance capacity (ORAC) assays were carried out according to the method of Huang *et al.* (2002) with some modifications. This assay measures the ability of anti-oxidant compounds to inhibit the decline in fluorescein (FL) fluorescence induced by a peroxy radical generator – AAPH. The assay was performed in a 96-well plate. The reaction mixture contained 100 μ L of 75 mM phosphate buffer (pH 7.4), 100 μ L of freshly prepared FL solution (0.1 μ M in phosphate buffer), 50 μ L of freshly prepared AAPH solution (51.6 mg/mL in phosphate buffer), and 20 μ L of sample per well. Samples were analysed in triplicate and diluted to different concentrations (25 μ g/mL, 12.5 μ g/mL, 6.25 μ g/mL and 3.12 μ g/mL) using a stock solution of 1 mg/mL in DMSO. Fluorescein, phosphate buffer and samples were preincubated at 37°C for 10 min. The reaction was started by the addition of AAPH using the microplate reader injector (Infinite[®] 200, Tecan, France). Fluorescence was then measured and recorded for 40 min at 485 nm excitation and 520 nm emission. The 75 mM phosphate buffer solution was used as a blank, and 12.5, 25, 50 and 75 μ M of Trolox (hydrophilic α -tocopherol analogue) were used as calibration solutions. An 8.8 μ M chlorogenic acid sample was used as quality control. The final ORAC values were calculated using a regression equation between the Trolox concentration and the net area under the FL decay curve and expressed as micromoles of Trolox equivalents per gram of dry matter. The AUC was calculated using Magellan[™] data analysis software (Tecan, France).

Inhibition of AGE accumulation. The assay involved incubating bovine serum albumin (BSA, 10 mg/mL) with D-ribose (0.5 M) and the tested compound (3×10^{-3} M) in a phosphate buffer, 50 mM, pH 7.4 (Na₂PO₄, 0.02%). Solutions (100 μ L) were incubated in 96-well microtitre plates at 37°C for 24 h in a closed system before AGE fluorescence measurement. To avoid quenching phenomena, fluorescence resulting from the incubation, under the same BSA conditions (10 mg/mL) and the tested compound (3×10^{-3} M), was subtracted for each measurement. Tests were performed in triplicate. Wells solely containing BSA, facilitating 100% inhibition of AGE formation, were used as a negative control. Wells containing BSA (10 mg/mL) and D-ribose (0.5 M) served as a positive control. The final volume assay was 100 μ L. AGE fluorescence (λ_{exc} 370 nm; λ_{em} 440 nm) was measured using a microplate spectrofluorometer Infinite M200 (Tecan, Lyon, France) and Magellan (Tecan) software.

Results and Discussion

Comparison of purification procedures

Medium pressure liquid chromatography of the cyclohexane extract (7 g) of *D. ferruginea*, followed by size exclusion chromatography (LH-20 Sephadex gel) and filtration over silica gel, yielded 1.5 mg of **1**, which was identified by NMR and mass spectroscopy analysis as cajaflavanone, also called erythrisedenone (Khaomek *et al.*, 2008). As larger amounts of pure **1** were required to evaluate its biological activities, an optimized FCPC methodology was developed. Fast centrifugal partition chromatography is an efficient method for the purification of natural products. This technique has many advantages such as no

irreversible adsorption to the stationary phase, no loss of injected sample, low risk of degradation of sensitive material, as well as a significant decrease in solvent consumption (Marston and Hostettmann, 2006; Schinkovitz *et al.*, 2008). Several papers describing high-yield isolation strategies for natural compounds (Yang *et al.*, 2010; Guo *et al.*, 2010; Zhai and Zhong, 2010), in particular flavonoids (Berthod *et al.*, 2009; Sutherland and Fisher, 2009), have outlined the efficiency of FCPC. The Arizona system of solvents is one of the most commonly used systems for natural product purification (Berthod *et al.*, 2009). It has been used for compounds such as **1** and others of similar structure (Maver *et al.*, 2005). A two-phase solvent system was therefore selected according to a TLC-based pre-evaluation of suitable solvent compositions (Hostettmann *et al.*, 1998) and *K* value determination by HPLC analysis (Fig. 1). System U from the Arizona range of solvents was therefore chosen for the purification (*K* = 0.91 for cajaflavanone at 254 nm). Consequently 5 g of the cyclohexane crude extract were processed by FCPC, yielding **1** (11.7 mg) in a one-step separation procedure. It is particularly worth mentioning that the overall solvent consumption was only 140 mL/g of crude extract, i.e. much lower than levels consumed in conventional column chromatography (5 L/g of crude extract) (Table 1). Likewise, a large amount of material was irreversibly adsorbed by the silica gel during the first purification step. Only 5.5 g out of 7 g (<80%) could be recovered after MPLC. Conversely, no significant loss of extract was observed for the FCPC isolation. Both methods yielded **1** at similar purity (Fig. 2): 86.8% (MPLC) vs. 86.9% (FCPC), but a tenfold higher yield could be achieved by FCPC. The FCPC-based approach therefore appears superior in comparison to a classic solid-liquid isolation strategy in terms of solvent and time consumption, as confirmed previously (Pinel *et al.*, 2007). This method allows quick isolation of a biologically active compound at a high yield in a one-step purification procedure.

Biological evaluation of cajaflavanone

Cajaflavanone has been reported to have anti-dermatophytic activity (Ribeiro *et al.*, 2008) and to prevent EBV activation (Itoigawa *et al.*, 2002). However, **1** did not present anti-bacterial or anti-fungal activity during our biological screening (data not shown). On one hand, the results revealed (Table 2) that the anti-parasitic activity of this compound was moderate, with an IC₅₀ of 40 ± 2 μ g/mL on *L. major* and 55.4% inhibition on *P. falciparum* at 10 μ g/mL. These values were in total agreement with those reported on another chloroquino-resistant strain (K1) (Khaomek *et al.*, 2008). On the other hand, **1** significantly inhibited AGE formation, with an IC₅₀ of 0.54 mM vs. 10 mM for the reference aminoguanidine and without any cytotoxicity on MRC-5 cells (0% inhibition at 10 μ g/mL). *In vivo* formation of

Table 1. Comparison of FCPC/conventional procedures

	Required time (h)	Solvent consumption	Yield (%)	Purity (%)
Conventional method	120	5 L/g of crude extract	0.02	86.8
FCPC	3	140 mL/g of crude extract	0.2	86.9

Table 2. Biological activity of cajaflavanone

Activity	Cajaflavanone	Positive control
Anti-leishmanial (IC ₅₀ (μg/mL ± SD))	40 ± 2	Pentamidine 28 ± 1
Anti-plasmodium (<i>Plasmodium falciparum</i>) (mean % inhibition at 10 μg/mL (n = 2))	55.4	Chloroquine > 80
Inhibition of AGEs IC ₅₀ (mM)	0.54	Aminoguanidine 10

AGEs from proteins and sugars, and their involvement in the pathogenesis of diabetes as well as cardiovascular, neurological and age-related diseases, has been clearly demonstrated (Singh *et al.*, 2001; Derbré *et al.*, 2010). Molecules capable of inhibiting their formation or inducing their decay are the most interesting new drug candidates. Anti-AGE molecules are anti-oxidants, α, β dicarbonyl scavengers or breakers (Reddy and Beyaz, 2006). The anti-AGE potential of numerous flavonoids has been reported previously (Matsuda *et al.*, 2003) and in most cases those activities seemed closely related to anti-oxidant (i.e. radical scavenging) activity (Matsuda *et al.*, 2003; Wu and Yen, 2005). Nevertheless, these correlations are not valid in some exceptional cases. Concerning **1**, no significant anti-oxidant activity could be identified in DPPH or ORAC bioassays (data not shown). According to Matsuda, the anti-AGE activity of flavonoids increased with the number of free hydroxyl groups in positions 3', 4', 5- and 7. However, although its oxygen in position 7 is integrated within a dihydropyran ring, **1** exhibited inhibition of AGE formation to a similar extent as naringenin (0.6 mM in the same assay; Derbré *et al.*, 2010), which bears a free OH group in position 7. These findings suggest that **1**, with its prenyl groups, exerts its anti-AGE properties through mechanisms that differ from radical scavenging. The abilities to chelate divalent metal ions (Jomova *et al.*, 2010), to catch (di-)carbonyl compounds (Pashikanti *et al.*, 2010) or to react with amino groups may also explain the lower formation of fluorescent AGEs induced by BSA and ribose. This mechanism warrants further studies.

Structural identification

Cajaflavanone or 5,4'-dihydroxy-6-(3'''-méthyl-2'''-butenyl)-2'',2'-dimethyl pyrano-[5'',6'':7,8]-flavanone, was identified by MS, ¹H-, ¹³C- and 2D- (COSY, HMQC, HMBC) NMR analysis and by comparison with previously published data (Bhanumati *et al.*, 1978; Khaomek *et al.*, 2008).

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