



American Journal of
**Biochemistry and
Molecular Biology**

ISSN 2150-4210



Academic
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Anti-inflammatory and Antioxidant Activities of a Fraction I1 of Male Inflorescences of *Borassus aethiopum* Mart (Arecaceae)

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ABSTRACT

In previous studies, using a bioassay-guided fractionation procedure; five fractions (E1F1, E2F2, E3F3, E4F4 and E5F5) from powdered *Borassus aethiopum* male inflorescences were extracted. Among these, the dichloromethane methanol extract E2F2 was found to exert significant anti-inflammatory and antipyretic activities and pro apoptotic effect. It seemed important to pursue the investigations to understand the mechanism of the anti-inflammatory activity. The anti-inflammatory activity was studied, C Reactive Protein (CRP) level in mice blood was immunoturbidimetry after inflammation induction and antioxidant activity was studied using 1,1 diphenyl picrylhydrazyl (DPPH). Phytochemical screening was carried out according to the methodology for chemical analysis for vegetable drugs. Among 3 fractions (I1; I2, I3) of E2F2, I1 was the most active with a percentage of inhibition (PI) of 80%. This anti-inflammatory activity was twice high than indometacin (PI = 40%). The I1 fraction cause significant decline of concentration of CRP compared with indometacin. The radical scavenging activities of I1 were approximately 4 times lower than ascorbic acid. Phytochemical analyses of *Borassus aethiopum* extracts revealed the presence of terpenoids, steroids and saponins which all have been shown to be potent anti-inflammatory and antioxidants. The present study confirmed the anti-inflammatory and antioxidant potential of *Borassus aethiopum* extracts with results comparable with those of standard compounds such as indometacin. Further studies are needed to isolate, purify and identify the chemical structure of the compounds responsible for anti-inflammatory and antioxidant activity.

Key words: *Borassus aethiopum*, anti-inflammatory, antioxidant, CRP

INTRODUCTION

Borassus aethiopum Mart (Arecaceae) is a tropical plant widely spread in Africa. This plant is used in Burkina Faso traditional medicine for the treatment of inflammatory diseases (Cassou *et al.*, 1997). In previous studies, using a bioassay-guided fractionation procedure; five fractions (E1F1, E2F2, E3F3, E4F4 and E5F5) from powdered *Borassus aethiopum* male inflorescences were extracted. Among these, the dichloromethane methanol extract E2F2 was found to exert significant anti-inflammatory and antipyretic activities (Sakande *et al.*, 2004a-b) and

pro apoptotic effect (Sakande *et al.*, 2011). It seemed important to pursue the investigations because other studies on *Borassus flabellifer* flowers a plant belonging to the same family reported the isolation of molecules with therapeutic interest (Revesz *et al.*, 1999; Yoshikawa *et al.*, 2007). Furthermore, the potential herbal sources of future drugs effective in oxidant-related diseases are reported (Hasani-Ranjbar *et al.*, 2009; Rahimi *et al.*, 2010; Kayode and Kayode, 2011; Malekirad *et al.*, 2011). The present study aimed at progressing towards the isolation of the active substance by bioassay-guided fractionation of E2F2 and to understand the mechanism of the anti-inflammatory activity.

MATERIALS AND METHODS

Vegetable drug: *Borassus aethiopum* male inflorescences.

Animals: Male NMRI mice weighing between 30 and 40 g were used. These animals receiving food and water *ad libitum* were stabilized in an atmosphere in 22°C with 70% of humidity. The photoperiod was 12-24 h.

Chemicals:

- Extraction solvents: dichloromethane and methanol from Prolabo (France)
- Phytochemical screening reagents from Prolabo (France)
- DPPH (1,1 diphenyl picrylhydrazyl) from Sigma-Aldrich (France)
- Carrageenan from Sigma-Aldrich (France)
- Ascorbic acid, Sigma-Aldrich (France)
- Indometacin, Sigma-Aldrich (France)
- C Reactive Protein (CRP) from Roche Diagnostics (Germany)

Equipment:

- Column of chromatography (Kieselgel 63-160 μ , Prolabo, France)
- Lyophilisator alpha chris 1-2
- Preparative Thin layer chromatography (TLC) plate (silica gel G6F254), Prolabo, France
- Spectrophotometer Cobas c111 (Roche diagnostic)
- Rotary evaporator (Rotavapor)
- Plethysmometer (Ugo Basile)

Plant material and extraction: *Borassus aethiopum* male inflorescences were collected and identified by Professor Sita Guinko, Institute of natural products research of Ouagadougou (Vegetal Biology and Ecology Laboratory of UFR SVT). A voucher specimen AA1522 was deposited at the Herbarium.

The inflorescences were air-dried in the shade and powdered. The powder was exhaustively extracted by percolation with 3 L of dichloromethane-methanol (50:50). The fraction obtained (E2F2 extract) was evaporated under reduced pressure to obtain 10 g of residue.

The extract E2F2 was split by preparative TLC in 3 fractions I1, I2 and I3. The pharmacological screening of the anti-inflammatory activity by the method of Winter *et al.* (1962) allowed to retain

the most active which was I1. Phytochemical screening was carried out according to the methodology for chemical analysis for vegetable drugs (Ciulei, 1982; Bankole *et al.*, 2011; Oseni and Akindahunsi, 2011).

Carrageenan-induced mouse paw oedema: The method of Winter *et al.* (1962) was used with slight modification to induce inflammation (Hajarolasvadi *et al.*, 2006; Bala *et al.*, 2011). Inflammation of mouse paw was induced by injecting 0.025 mL of carrageenan prepared in distilled water (1%, w/v) into the subplantar surface of the right hind paw. Male mice were divided into six groups of six animals each. The mice were deprived of food and water during the experiment. Mice paw were measured before oedema induction. The control group was given 0.025 mL of Isotonic Saline Solution (ISS) by intraperitoneal (i.p.) injection. The reference group received indometacin (1 mg kg⁻¹, i.p.) and 4 groups receiving i.p. injection of *Borassus aethiopum* extracts (E2F2: 100 mg kg⁻¹, I1: 100 mg kg⁻¹, I2: 100 mg kg⁻¹, I3: 100 mg kg⁻¹). These drugs were injected into mice immediately before the injection of carrageenan. The doses of plant extracts and indometacin were chosen according to Choi *et al.* (2004). The degree of oedema was measured at 0 and 3 h after injection. Volumes of right hind paw of mice were measured with a plethysmometer (Ugo Basile). The percentage of inhibition of the inflammatory was determined for each animal by comparing with control and calculated by the following formula used by Karaca *et al.* (2009):

$$PI \% = [(1-(dt/dc)] \times 100$$

where, dt is difference in paw volume in the drug treated group and dc the difference in paw volume in the control group.

The median effective dose (ED₅₀) of the most active fraction I1 was determined using the same method of Winter *et al.* (1962). Male mice were divided into seven groups of six animals each. A study on series of five concentrations of I1 (mg kg⁻¹): 100; 50; 25; 12.5; 6.25 allowed to determine the ED₅₀.

Effect of I1 on the kinetics of C reactive protein (CRP): Inflammation of mice paw was induced by the method of Winter *et al.* (1962). Male mice were divided into 4 groups of twenty five animals each. The control group was given 0.025 mL of ISS by intraperitoneal (i.p.) injection. The reference group received indometacin (1 mg kg⁻¹, i.p.) and 2 groups receiving i.p. injection of *Borassus aethiopum* extracts (E2F2: 100 mg kg⁻¹, I1: 100 mg kg⁻¹). Before oedema induction blood were collected from 5 mice of each group (T0). Drugs were injected into mice immediately before the injection of carrageenan. After carrageenan injection blood were collected at 3 h (T3), 9 h (T9), 24 h (T24) and 48 h (T48) from 5 mice from each group. Mice were anesthetized by dichloromethane and the blood was collected by cardiac draining in dry tubes. The serum was separated by centrifugation at 2000 g during 5 min and kept at -20°C until the dosage.

CRP was measured by immunoturbidimetry using a spectrophotometer Cobas C111 (Roche Diagnostics).

Measurement of DPPH (1,1 diphenyl picrylhydrazyl) radical scavenging activity: The method of DPPH (Everette and Islam, 2012; Hemalatha *et al.*, 2012) was used for the determination of free radical scavenging activity of the extracts. Five hundred microliter of extracts, were introduced into 3 mL of solution of DPPH (1 mM). The mixture was shaken and incubated in dark for 10 min. The decrease in absorbance at 517 nm was then measured using

spectrophotometer. Ascorbic acid was used as reference. All analysis were performed in duplicate and the ability to scavenge the DPPH radical was calculated using the following formula:

$$\text{Scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where, A₀ is the absorbance of the control, A₁ is the absorbance in the presence of the sample of extract or reference.

A curve of calibration allowed determining the concentration of extracts corresponding to a decrease of 50% of absorbance (IC₅₀).

Statistical analysis: All data were represented as Mean±SD or as percentage. Data were analyzed by ANOVA test using SPSS software. The differences were considered as significant for p<0.05. Curves were drawn by MS Excel 2000 software.

RESULTS

Phytochemical screening: The results of phytochemical screening (Table 1) showed the presence of sterols, triterpenes and saponins in E2F2 extract and its fraction I1. A Chromatogram of E2F2 and its fractions after revelation with Liebermann-Buchard reagent showed 3 spots of E2F2 fractions: I₁ (Rf = 68), I₂ (Rf = 57), I₃ (Rf = 43). The I1 fraction with a yield of 8% reported to 750 g of dry powder of *Borassus aethiopus* male inflorescences was the main compound.

Carrageenan-induced mouse paw oedema: The results of anti-inflammatory activity of *Borassus aethiopus* extracts are presented in Table 2. Bioassay-guided fractionation procedure using Winter *et al.* (1962) method allowed to show that the fraction I1 was the most active with 80% of oedema inhibition. This anti-inflammatory activity was twice high than indometacin (PI = 40%) used as reference substance. The anti-inflammatory activity of I1 fraction was less important than E2F2 extract (PI: 82%). The median effective dose (ED50) value of I1 fraction was 33 mg kg⁻¹.

Table 1: Chemical screening of *Borassus aethiopus* extracts

Substances	E ₂ F ₂	I1	Reactions
Sterol/triterpene	+++	+++	Liebermann-Burchard
Flavonoids	-	-	Shibata
Saponosides	++	+++	Hemolysis
Alkaloids	-	-	Dragendorff and Mayer
Anthra-cenosids	-	-	Borntrager

+++ : Moderate quantity, +++ : High quantity, - : Absent

Table 2: Anti-inflammatory activity of *Borassus aethiopus* fractions

Groups	Dose (kg ⁻¹)	Mean increase in paw volume (mL)	Inhibition in paw volume (%)
ISS	0.025 mL	0.160±0.010	-
Indometacin	1 mg	0.140±0.020	40
E2F2	100 mg	0.119±0.020	82*
I1	100 mg	0.120±0.010	80*
I2	100 mg	0.140±0.010	40
I3	100 mg	0.140±0.010	40

Values are Mean±SEM, n = 6 animals in each group, ED50 of I1 was 33 mg kg⁻¹, *p<0.05 when compared to Indometacin group

Table 3: Effect of extracts on the kinetic of CRP (mg L⁻¹)

Time (h)	ISS	Indometacin	E2F2	I ₁
T0	2.10±0.40	2.40±0.50	3.68±0.80	3.50±1.10
T3	22.50±1.04	7.34±2.50*	14.68±3.30	18.35±5.40
T9	19.80±1.14	8.42±3.44*	13.75±2.04*	16.51±1.95*
T24	22.90±3.04	6.42±0.90*	8.26±3.24*	2.75±1.35*
T48	24.10±2.54	3.67±1.14*	7.28±2.40*	1.83±1.14*

*p<0.05 when compared to isotonic saline solution (ISS) group

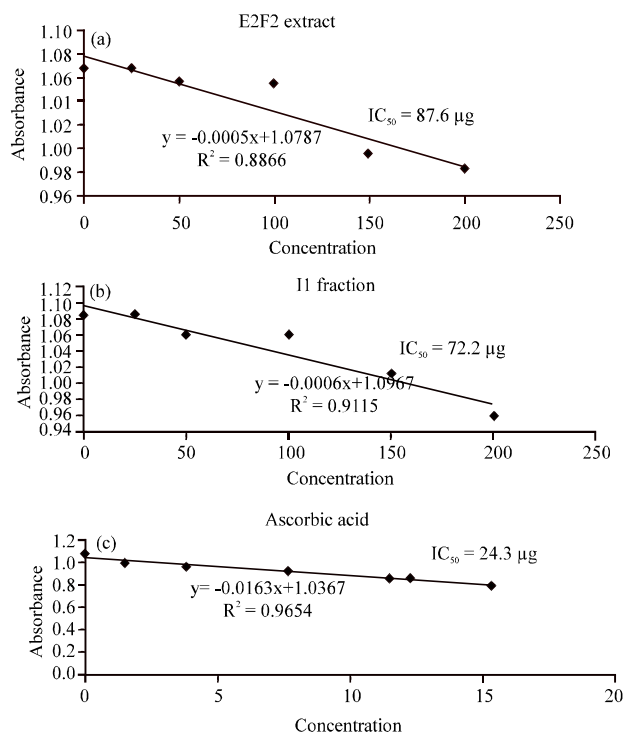


Fig. 1(a-c): Antioxidant activity of (a) E2F2 extracts, (b) I1 fraction and (c) Ascorbic acid using DPPH method. The linear regressions showed that radical scavenging activities of extracts were dose dependant

Effect of I1 on the kinetics of CRP: The Table 3 presents the effect of *Borassus aethiopum* extracts on the kinetics of protein C reactive (CRP). These extracts cause significant decline of concentration of this protein of acute inflammation phase compared with indometacin. From 24 h the decline of CRP was more important with I1 fraction than indometacin.

Measurement of DPPH (1,1 diphenyl picrylhydrazyl) radical scavenging activity: Figure 1 presents the antioxidant activity of E2F2 extracts, I1 fraction and ascorbic acid used as reference. E2F2 linear regression equation was $y = -0.005x + 1.0787$ with a correlation coefficient: $R^2 = 0.8866$, I1 linear regression equation was $y = -0.0006x + 1.0967$ with correlation coefficient $R^2 = 0.9115$. The anti-oxidizing activity of the ascorbic acid linear regression equation was $y = -0.0163x + 1.0367$ with correlation coefficient $R^2 = 0.9654$. These linear regressions showed that radical scavenging activities of extracts were dose dependant. Decreases of 50% of absorbance

(IC50) values were: E2F2 (87.6 µg), I1 (72.2 µg) and ascorbic acid (24.3). The radical scavenging activities of extracts were approximately 4 times lower than ascorbic acid.

DISCUSSION

The results showed that dichloromethane-methanol extract of *Borassus aethiopum* E2F2 exercises an anti-inflammatory activity. The percentage of inhibition (PI) on mice paw oedema treated with E2F2 was 82% against 40% of inhibition obtained with indometacin. The interesting activity of E2F2 extract led to its fractionation in order to progress towards the isolation of the active principle. I1 fraction was the most active with a percentage of inhibition of 80%. The decline of activity of I1 (80%) compared to E2F2 (82%) from which it arises shows that there would be a synergy of action between the constituents of E2F2 (I1, I2 and I3). The anti-inflammatory activity of *Borassus aethiopum* extracts are comparable to other plants reported by authors (Okokon *et al.*, 2008; Usman *et al.*, 2008; Karaca *et al.*, 2009; Gill *et al.*, 2011). The phytochemical screening allowed attributing the anti-inflammatory activity to the group of sterols, triterpenes and saponins. The anti-inflammatory activity of these compounds was reported by many authors (Sawadogo *et al.*, 2006; Kawabata *et al.*, 2011; Wu *et al.*, 2011; Zha *et al.*, 2011; Zeng *et al.*, 2011).

To understand better the mechanism of the anti-inflammatory activity of extracts, we studied their effect on CRP, an acute phase protein of inflammation. Indeed CRP rises from the 6th hour of the inflammation, is openly pathological 24 h after the beginning of the inflammation and quickly normalizes after its disappearance (7-14 days). So, carrageenan -induced mice paw oedema in group receiving ISS, increased CRP level from 2.1 to 22.5 mg L⁻¹ after 3 h and this level was 24.1 mg L⁻¹ after 48 h. In group treated with extracts a decrease of CRP was observed after 24 h of treatment with I1 (CRP = 2.75 mg L⁻¹). Decreasing effects of hydro alcoholic extract of plant (*Urtica dioica*) on IL-6 and hs-CRP levels in patients with type 2 diabetes is also reported (Namazi *et al.*, 2011). The administration of indometacin (Anti-inflammatory drug of reference) exhibit a decrease of CRP (6.42 mg L⁻¹) after 24 h.

The role played by non-steroidal anti-inflammatory drugs (NSAIDs) on CRP kinetics is known. After 2 weeks of treatment with flurbiprofen (200 mg day⁻¹) and with ibuprofen to subjects presenting a rheumatoid arthritis, a significant reduction of CRP was observed (Cush *et al.*, 1990). The decline of CRP led by the extracts of *Borassus aethiopum* opens a way for the clarification of the mechanism of action. Indeed the replication of the CRP mRNA by hepatocytes is stimulated by interleukin-6 which is stimulated in his turn by tumor necrosis factor (TNF-α) and interleukin 1 Mostafa *et al.* (2005) and Salari and Abdollahi (2011). Furthermore, it was observed that serum C-reactive Protein (CRP) positively correlates with serum concentrations of IL-6 and TNF-α in healthy (Yudkin *et al.*, 2000). Present study is the first that demonstrated effects of *Borassus* extract on inflammatory indicators as CRP, so exact mechanism on inflammatory cytokine is unknown. More studies are essential to discover mechanism of I1 fraction. The dosage of these cytokines is necessary to understand the mechanism. The role of extracts on CRP could present an interest in the treatment of cardiovascular diseases. Indeed an influencing role of atorvastatin on C-Reactive protein profile is reported (Anand *et al.*, 2009).

The study of the anti-oxidizing activity of *Borassus aethiopum* extracts had for objective to understand the anti-inflammatory mechanism. Indeed several works on the extracts of plants showed that the anti-inflammatory mechanism was bound to their antioxidant power (Karaca *et al.*, 2009; Alam *et al.*, 2011; Karou *et al.*, 2011; Sombie *et al.*, 2011). The study showed a weak dose dependant radical scavenging activities of extracts. Phytochemical screening of

Borassus aethiopum extracts revealed the presence of terpenoids, steroids and saponins. All of these compounds have been shown to be potent antioxidants (Chanwitheesuk *et al.*, 2005; Hafidh *et al.*, 2009; Arora *et al.*, 2011; Roy *et al.*, 2011). This weak anti-oxidizing activity obtained allows saying that the anti-inflammatory activity of *Borassus aethiopum* extracts fear to be bound to an anti-free radical mechanism but would not be the only explanation.

CONCLUSION

The present study confirmed the anti-inflammatory and antioxidant potential of *Borassus aethiopum* extracts with results comparable with those of standard compounds such as indometacin. Further studies are needed to isolate, purify and identify the chemical structure of the compounds responsible for anti-inflammatory and antioxidant activity.

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