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journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)Original article <http://dx.doi.org/10.1016/j.apjtb.2016.10.009>Antioxidant capacity and genoprotective effect of ethanol fruit extract from *Detarium microcarpum* Guill. and Perr. (Caesalpinaceae)Ablassé Rouamba<sup>1</sup>, Maurice Ouedraogo<sup>2</sup>, Martin Kiendrebeogo<sup>1\*</sup><sup>1</sup>Laboratoire de Biochimie et Chimie Appliquées (LABIOCA), UFR-SVT, Université de Ouagadougou, 03 BP 7021, Ouagadougou 03, Burkina Faso<sup>2</sup>Laboratoire de Physiologie Animale (LAPA), UFR-SVT, Université de Ouagadougou, 09 BP 848, Ouagadougou 09, Burkina Faso

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## ABSTRACT

**Objective:** To evidence the ability of ethanol fruit extract from *Detarium microcarpum* (*D. microcarpum*) to preserve DNA integrity against oxidative genomic damage.**Methods:** Ethanol extract from *D. microcarpum* fruit pulp was analyzed for its antioxidant capacity using ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azinobis-3-ethyl-ethylbenzothiazoline-6-sulphonate, superoxide anion, deoxyribose degradation and lipid peroxidation models. The genoprotective activity was assessed *ex vivo* by comet assay, on liver cells of NMRI female mice using cyclophosphamide (CP) as genotoxic agent.**Results:** Ethanol extract from *D. microcarpum* fruit pulp exhibited interesting antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl, deoxyribose degradation and lipid peroxidation assays. The extract did not present any genotoxic effect but protected DNA against CP-induced damages with a dose-dependent manner. The genoprotective effect observed was related to the antioxidant molecules of the fruit that scavenged the hydroxyl radical (generated by the metabolism of CP) as well as the peroxy and alkoxy radicals issued from lipid peroxidation. Other mechanisms such as inactivation of CP metabolism to genotoxic end products, induction of the expression of antioxidant and DNA repair enzymes have been discussed.**Conclusions:** Our results suggest that the wild edible fruit from *D. microcarpum* could be beneficial on consumer's health by its antioxidant and genoprotective effects, particularly during chemotherapies exhibiting genotoxic effects like CP in cancer treatment.

## 1. Introduction

*Detarium microcarpum* Guill. and Perr. (Caesalpinaceae) (*D. microcarpum*) is a well-known wild edible fruit species growing

in Saharan and sub-Saharan countries. Its fruits are traditionally consumed as food source and for medicinal purpose [1]. Fruit pulp of *D. microcarpum* has a remarkable nutritional value with important content of protein and vitamins [2,3], carbohydrates and mineral nutrients [4,5]. Clerodane diterpenes molecules isolated from the pulp showed antifungal activity and inhibition of the enzyme acetylcholinesterase implicated in Alzheimer's disease [6]. Fruit pulp of *D. microcarpum* is also rich in flavonoid and polyphenols exhibiting hence a strong antioxidant capacity [7].

Direct relationship was found between consumption of fruits rich in antioxidant compounds and the reduction of oxidative stress-related diseases such as cancer [8], diabetes [9] and neuronal disorders [10]. Epidemiological studies also highlight health benefits of natural antioxidant nutrients when consumed within their food matrices (fruits, vegetables and grains) instead of being extracted, concentrated and consumed in food supplements [11].

In living cells, reactive oxygen species (ROS) are continuously produced as a consequence of normal metabolism or

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All experimental procedures involving animals were conducted in accordance to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, publication number 85-25, revised 1996) and approved by the University of Ouagadougou regarding the internationally accepted standard ethical guideline for laboratory animals use and care as described in the European committee guidelines (EEC directive 86/609/EEC, 24th November, 1986).

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external factors [12,13]. ROS can oxidize DNA leading to several types of genomic damage including oxidized bases [14], single and double-strand breaks [15]. Genomic damage plays a major role in mutagenesis, carcinogenesis and aging. ROS can also easily initiate peroxidation of membrane lipids causing damage of their phospholipids and lipoprotein by propagating a chain reaction cycle [16].

Given the high antioxidant capacity of *D. microcarpum* fruits pulp, we are focusing in this paper on its genoprotective effect against cyclophosphamide (CP) induced oxidative stress.

## 2. Materials and methods

### 2.1. Plant material and extraction

Fresh fruits from *D. microcarpum* Guill. and Perr. (Caesalpiniaceae) were harvested in January 2013 at Gampela (25 km east of Ouagadougou, Burkina Faso). Botanical identity was assessed by Professor Jeanne Millogo-Rasolodimby from Laboratory of Ecology and Vegetable (University of Ouagadougou, Burkina Faso) where a voucher specimen (CI: 15928) was deposited. Fresh fruits were washed with distilled water and pulp was scraped prior to soaking in ethanol (24 h, 25 °C, continuous stirring). Extract was filtrated, concentrated to dryness in a vacuum evaporator and stored at 4 °C for further investigations.

### 2.2. Animals handling

NMRI female mice (7–8 weeks old, 25–35 g body weight), provided by the animal housing facility of the University of Ouagadougou, were used. Mice were kept in an environmentally controlled breeding room (25 °C, 12 h photoperiod), fed with standard laboratory food and water *ad libitum*. All experimental procedures involving animals were conducted in accordance to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, publication number 85-25, revised 1996) and approved by the University of Ouagadougou regarding the internationally accepted standard ethical guideline for laboratory animals use and care as described in the European committee guidelines (EEC directive 86/609/EEC, 24th November, 1986) [17].

### 2.3. Chemicals

Chemicals were from analytical grade. Gallic acid, quercetin, sodium phosphate dibasic, sodium phosphate monobasic, thiobarbituric acid, CP, Trizma base, Triton-X, ethylenediaminetetraacetic acid (EDTA), agarose, propidium iodide, Hank's balanced salt solution, potassium persulfate, Trichloroacetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), deoxyribose, hydrogen peroxide and lecithin were purchased from Sigma-Aldrich (St. Louis, USA). Ascorbic acid, sodium hydroxide, sodium chloride, iron sulfate and iron trichloride were supplied by Labosi (Paris, France). Potassium hexacyanoferrate, 2,2'-azobis-3-ethyl-ethylbenzothiazoline-6-sulphonate (ABTS), diethyl ether and ethanol were purchased from Prolabo (Paris, France).

### 2.4. Antioxidant capacity measurements

#### 2.4.1. DPPH radical scavenging assay

Ability of extract to scavenge the DPPH radical was measured at 517 nm as described by Akomolafe *et al.* [18]. Scavenging activities were plotted against sample concentrations and results

were expressed as concentration ( $\mu\text{g/mL}$ ) scavenging 50% of DPPH radicals ( $\text{IC}_{50}$ ). Quercetin and Gallic acid were used as positive controls.

#### 2.4.2. ABTS radical cation scavenging assay

Capacity of extract to scavenge the ABTS radical cation was evaluated at 734 nm as described by Compaoré *et al.* [19]. Results were expressed as  $\mu\text{mol}$  Trolox equivalent per gram ( $\mu\text{mol TE/g}$ ) of extract against a calibration curve ( $y = -72.384x + 54.57$ ,  $r^2 = 0.998$ ). Quercetin and gallic acid were used as positive controls.

#### 2.4.3. Superoxide radical anion scavenging assay

Scavenging activity of the superoxide anion radical was determined at 560 nm according to Sasikumar *et al.* [20]. Results were expressed as scavenging percentage (%) of superoxide radical. Quercetin was used as positive control.

#### 2.4.4. Ferric reducing/antioxidant power assay

The ability of fruits extract to reduce iron was assessed at 700 nm according to Sudha *et al.* [21]. Ascorbic acid was used to generate a calibration curve ( $y = 0.00944x + 0.0825$ ,  $r^2 = 0.998$ ) and reducing power was expressed as mg ascorbic acid equivalent per gram (mg AAE/g) of extract. Quercetin and gallic acid were used as positive controls.

#### 2.4.5. Deoxyribose degradation assay

Inhibition of deoxyribose degradation was measured at 532 nm following the procedure described by Perjési and Rozmer [22]. Results were expressed as inhibitory percentage (%) of deoxyribose degradation. Quercetin was used as positive control.

#### 2.4.6. Inhibition of lipid peroxidation

Inhibition of lipid peroxidation was determined by measuring malondialdehyde formation at 532 nm according to the thiobarbituric acid method as described by Molehin and Adefegha [23]. Results were expressed as inhibitory percentage (%) of lecithin peroxidation. Quercetin was used as positive control.

### 2.5. Genoprotective effect evaluation

#### 2.5.1. Experimental design

Experimental design was inspired from Sathya *et al.* [24]. Fruit ethanol extract of *D. microcarpum* was suspended in vehicle (5% DMSO in distilled water) to get an 80 mg/mL suspension that was administrated orally at various doses of 500, 1000 and 2000 mg/kg body weight. CP was injected intraperitoneally with vehicle at a single dose of 20 mg/kg body weight.

Twenty-four randomly selected mice were divided into 8 groups as following: control group received only the vehicle for 7 consecutive days; CP group received the vehicle for 7 days and a single dose of CP was administered on the 7th day; *D. microcarpum* 500 group received fruits extract at a dose of 500 mg/kg body weight for 7 days; *D. microcarpum* 500 + CP group received fruits extract at a dose of 500 mg/kg body weight for 7 consecutive days and a single dose of CP was administered on the 7th day; *D. microcarpum* 1000 group received fruits extract at a dose of 1000 mg/kg body weight for 7 days; *D. microcarpum* 1000 + CP group received fruits extract at a dose of 1000 mg/kg body weight for 7 consecutive days and a single dose of CP was administered on the 7th day; *D. microcarpum* 2000 group received fruits extract at a dose of 2000 mg/kg body weight for 7 days; *D. microcarpum* 2000 + CP group received fruits extract at a dose of 2000 mg/kg

body weight for 7 consecutive days and a single dose of CP was administered on the 7th day.

On Day 8, animals were anaesthetized using diethyl ether and sacrificed by cervical dislocation. Liver samples were rapidly removed and quickly rinsed and suspended in chilled homogenization buffer (cold 1× Hank's balanced salt solution containing 25 mmol/L EDTA and 10% DMSO).

### 2.5.2. Single cell gel electrophoresis

Comet assay was performed according to Singh *et al.* [25] with slight modifications. Liver samples were chopped in chilled homogenization buffer and centrifuged. Supernatants (100 µL) containing isolated cells were mixed with an equal volume of low melting point agarose (1%) for slides preparation on microscope slides pre-coated with normal melting point agarose. Following cells lysis (24 h at 4 °C in a 2.5 mol/L NaCl supplemented with 100 mmol/L EDTA, 1 mol/L NaOH and 1% Triton X-100) and electrophoresis (30 min, 25 °C, 0.56 V/cm electric field), solutions of DNA were stained with propidium iodide.

### 2.5.3. Comet analysis

Nuclei were observed at 400× magnification with a fluorescence inverted microscope (Carl Zeiss, Germany) equipped with a camera connected to a computer. Images of 150 randomly selected nuclei (50 nuclei from three replicated slides) for each treatment were analyzed using CometScore software (version 1.5 of TriTek Corporation) according to Kumaravel and Jha [26]. Percentage DNA in tail (%) and olive tail moment [arbitrary unit (AU)] were considered to assess the level of DNA damages in nucleus.

### 2.6. Statistical analysis

Experiments were performed in triplicate and data were presented as mean ± SD. GraphPad Software (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analyses. Fischer test was used to compare the antioxidant activities of extract to positive controls. The One-way ANOVA for repeated measures followed by Newman–Keuls post-test was used to verify the impact of treatments on DNA damages. *P* value < 0.05 was considered as being significant.

## 3. Results

### 3.1. Antioxidant capacity

Six antioxidant models [DPPH, ferric reducing antioxidant power (FRAP), ABTS, deoxyribose degradation, superoxide

anion and lipid peroxidation], involving different antioxidant mechanisms were used to assess the antioxidant capacity of ethanol fruit extract of *D. microcarpum* (Table 1). As shown, fruit extract of *D. microcarpum* exhibited interesting antioxidant activities in DPPH, deoxyribose degradation and lipid peroxidation models. On the contrary, a weak reducing power on FRAP model as well as a weak scavenging activity on ABTS radical cation and superoxide anion were recorded. Though the scavenging activity on DPPH radical is quite low, compared to that of the potent antioxidant quercetin and gallic acid used as standards, inhibition of lipid peroxidation and deoxyribose degradation is less than two-fold lower than that of quercetin. Moreover, the weak superoxide anion scavenging activity demonstrated was similar to that of the flavonoid quercetin.

### 3.2. Genoprotective effect

To investigate the effect of *D. microcarpum* fruit ethanol extract on DNA integrity (genotoxicity), mice were treated with vehicle (control) or fruit extract (500–2000 mg/kg body weight) and comet parameters were reported (Table 2). Considering the percentage of DNA in tail (2.34% ± 0.29%) and the olive tail moment [(0.32 ± 0.05) AU] reported in control group, a weak DNA damage was observed. Furthermore, *D. microcarpum* fruit ethanol extract did not affect significantly (*P* > 0.05) the integrity of DNA compared to control group. These results suggested that *D. microcarpum* fruit ethanol extract (500–2000 mg/kg body weight) did not exhibit any genotoxic effect on mice hepatocytes when administered for 7 consecutive days.

To assess the genoprotective effect of *D. microcarpum* fruit ethanol extract, DNA damage was induced by a single dose of CP after 7 consecutive days of vehicle or fruit extract treatment (Table 2). As expected, a significant DNA damage on mice hepatocyte (*P* < 0.001) was observed in CP group [percentage DNA in tail: 20.60% ± 1.19%; olive tail moment: (10.64 ± 0.71) AU] compared to control [percentage DNA in tail: 2.34% ± 0.29%; olive tail moment: (0.32 ± 0.05) AU]. In the groups of mice treated with *D. microcarpum* fruit ethanol extract (500–2000 mg/kg body weight) before genotoxicity induction, the level of DNA damage decreased significantly (*P* < 0.001) compared to the CP group, suggesting a genoprotective effect against genomic damages induced by CP. Any statistical difference wasn't found (*P* > 0.05) between control [percentage DNA in tail: 2.34% ± 0.29%; olive tail moment: (0.32 ± 0.05) AU] and group pretreated with 2000 mg/kg body weight of *D. microcarpum* fruit ethanol extract prior to CP injection [percentage DNA in tail: 4.26% ± 0.43%; olive tail moment:

**Table 1**

Antioxidant capacity of *D. microcarpum* ethanol fruit extract.

Antioxidant activities	Samples		
	Fruit extract	Quercetin	Gallic acid
DPPH radical scavenging activity (IC <sub>50</sub> : µg/mL)	49.87 ± 2.88 <sup>c</sup>	0.11 ± 0.01 <sup>b</sup>	0.05 ± 0.00 <sup>a</sup>
ABTS cation radical scavenging activity (µmol TE/g)	159.55 ± 2.35 <sup>b</sup>	2573.17 ± 15.53 <sup>a</sup>	2572.50 ± 23.52 <sup>a</sup>
Superoxide anion radical scavenging activity (% at 100 µg/mL)	19.01 ± 2.31 <sup>a</sup>	21.64 ± 3.30 <sup>a</sup>	ND
FRAP (mg AAE/g)	12.01 ± 0.47 <sup>c</sup>	889.89 ± 23.78 <sup>b</sup>	1058.58 ± 43.14 <sup>a</sup>
Deoxyribose degradation inhibitory activity (% at 100 µg/mL)	69.06 ± 1.67 <sup>b</sup>	90.40 ± 1.41 <sup>a</sup>	ND
Lipid peroxidation inhibitory activity (% at 100 µg/mL)	49.36 ± 0.01 <sup>b</sup>	98.02 ± 0.01 <sup>a</sup>	ND

Values are expressed as mean ± SD (*n* = 3 independent experiments). Scavenging activities of DPPH radical are expressed as concentration (µg/mL) scavenging 50% of DPPH radicals (IC<sub>50</sub>) by plotting scavenging activities (%) versus samples concentrations (µg/mL). Results from ABTS and FRAP models are given respectively in µmol TE/g (10<sup>-6</sup> mol TE/g of extract) and mg AAE/g (10<sup>-3</sup> g AAE/g of extract) using Trolox or ascorbic acid calibration curves. <sup>abcd</sup>: Values within each line with different superscripted letters differ significantly (*P* < 0.05) as determined by ANOVA. ND: Not determined.

**Table 2**Genoprotective activity of *D. microcarpum* ethanol fruit extract.

Treatments		DNA in tail (%)	Olive tail moment (AU)
Treatments without genotoxicity induction	Vehicle (control)	2.34 ± 0.29 <sup>a</sup>	0.32 ± 0.05 <sup>a</sup>
	<i>D. microcarpum</i> extract (500 mg/kg body weight)	2.94 ± 0.28 <sup>a</sup>	0.32 ± 0.05 <sup>a</sup>
	<i>D. microcarpum</i> extract (1000 mg/kg body weight)	2.31 ± 0.23 <sup>a</sup>	0.28 ± 0.01 <sup>a</sup>
	<i>D. microcarpum</i> extract (2000 mg/kg body weight)	2.60 ± 0.24 <sup>a</sup>	0.27 ± 0.02 <sup>a</sup>
Treatments after genotoxicity induction by CP (20 mg/kg body weight)	Vehicle (CP control)	20.60 ± 1.19 <sup>d</sup>	10.64 ± 0.71 <sup>d</sup>
	<i>D. microcarpum</i> extract (500 mg/kg body weight)	9.80 ± 0.84 <sup>c</sup>	1.39 ± 0.18 <sup>c</sup>
	<i>D. microcarpum</i> extract (1000 mg/kg body weight)	8.24 ± 0.77 <sup>b</sup>	0.90 ± 0.12 <sup>b</sup>
	<i>D. microcarpum</i> extract (2000 mg/kg body weight)	4.26 ± 0.43 <sup>a</sup>	0.52 ± 0.07 <sup>a</sup>

Values are expressed as mean ± SD ( $n = 3$  independent experiments). <sup>abcd</sup>: Values within each column with different superscripted letters differ significantly ( $P < 0.05$ ) as determined by ANOVA.

(0.52 ± 0.07) AU], demonstrating a total protective effect of *D. microcarpum* fruit ethanol extract at 2000 mg/kg body weight against CP induced genotoxicity.

#### 4. Discussion

In biological environment, numerous ROS are naturally produced in small amount [27]. Peroxyl and alkoxy radicals issue from lipid peroxidation, hydroxyl radical, iron ion and nitric oxide species produced by cell metabolism leading to genomic damages [28]. The weak DNA damage observed in the control group (receiving only vehicle) is in accordance with literature [24] and could be considered as DNA baseline damage in normal metabolism of hepatocytes.

Mice receiving CP exhibited a strong DNA degradation compared to control ( $P < 0.001$ ). Under oxidative stress conditions, huge amount of endogenous ROS is generated with important alteration of biological molecules (protein, lipid and nucleic acid) leading to important genomic damage [15] and cells death [28]. In the *in vivo* CP induced oxidative stress model, CP is metabolized through the hepatic mixed function oxygenases system into 4-hydroxycyclophosphamide that exists as aldophosphamide. Aldophosphamide further metabolizes in a non-enzymatic way to form hydroxyl radical, cytotoxic (acrolein and phosphoramidate mustard) and non-cytotoxic (4-ketocyclophosphamide, carboxyphosphamide and aldophosphamide) DNA alkylation agents responsible for DNA strands breakage [24].

Fruit pulp of *D. microcarpum* exhibited significant genoprotective activity in CP induced oxidative stress model, along with interesting antioxidant activities in DPPH, deoxyribose degradation and lipid peroxidation models. The antioxidant capacity of *D. microcarpum* fruit pulp we pointed out is in accordance with previous studies [7] and could justify the genoprotective effect observed.

Hydroxyl radical, formed as well as *in vivo* or *in vitro* by Fenton reaction, reacts directly with DNA by addition to form oxidized bases or by removing hydrogen from DNA sugar moiety leading to thiobarbituric products conducting to DNA strand breakage [29]. Hydroxyl radical also affects indirectly DNA through polyunsaturated lipids peroxidation end products (lipid hydroperoxides, alkoxy radicals, peroxyl radicals, malondialdehyde, 4-hydroxy-2-alkenals and 2-alkenals) forming DNA adducts and therefore DNA strand breakage [11]. Free radical trapping is a plausible mechanism in genomic protection against oxidative stress [30]. By scavenging the hydroxyl radical, *D. microcarpum* fruit pulp inhibits initiation of lipid peroxidation and deoxyribose degradation, reducing hence the

production of DNA strand breaking species. Meanwhile, peroxyl and alkoxy radicals produced by lipid peroxidation may be scavenged, impeaching their genotoxicity.

Other mechanisms could also explain the genoprotective effect of fruit pulp of *D. microcarpum*. Antioxidant compounds from the fruit extract may inhibit the cytochrome P450 enzymes involved in the activation of CP [14]. Antioxidant compounds from the fruit extract may induce the expression of antioxidant enzymes [31] such as catalases, superoxide dismutase and glutathione peroxidase (first line of antioxidant defense associated to DNA protection) or stimulate DNA repair enzymes [14] (second line of DNA protection). Indeed, food antioxidant compounds like anthocyanin have demonstrated stimulation effect on the production of DNA repair enzymes and antioxidant enzymes by regulating the expression of the transcription factor Nrf2 [14].

Our study clearly shows that *D. microcarpum* fruit pulp ethanol extract possesses antioxidant compounds with genoprotective properties. The use of *D. microcarpum* fruit pulp as food could therefore confer benefit on consumer's health, particularly during chemotherapies exhibiting genotoxic effects like CP in cancer treatment. Therefore, future investigations are necessary to isolate and characterize the genoprotective compounds of *D. microcarpum* fruit pulp.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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