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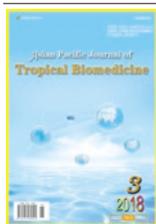
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### Antioxidant and antiglycation properties of two mango (*Mangifera indica* L.) cultivars from Senegal

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#### ABSTRACT

**Objective:** To evaluate the total phenolic contents, antioxidant and antiglycation activities of leaves, barks, roots and kernels from two cultivars of *Mangifera indica* (Anacardiaceae).

**Methods:** Total phenolic contents were determined by using Folin-Ciocalteu's method. The antioxidant activities were assessed by three different protocols including DPPH, oxygen radical absorbance capacity and iron (II) chelation assays. In addition, *in vitro* bovine serum albumin/D-ribose assay was chosen to evaluate the antiglycation properties of the extracts.

**Results:** All the investigated extracts were found to contain high level of total phenols as well as potent antioxidant activities. Kernel extracts showed the highest total phenol contents and DPPH radical scavenging activities whereas higher oxygen radical absorbance capacity values were observed for leave, root and bark extracts. Besides, extracts from leaves, roots and barks from both cultivars exhibited potent inhibitory effects against the formation of advanced glycation end products, with IC<sub>50</sub> values lower than the standard positive control aminoguanidine. **Conclusions:** The potent antiglycation and antioxidative activities of these two *Mangifera indica* cultivars suggest a possible role in targeting aging, diabetic complications and oxidative stress related diseases.

## 1. Introduction

*Mangifera indica* (*M. indica*) L. (Anacardiaceae) is a large tree native from tropical Asia. Its leaves are spirally arranged on branches and its fruit is a popular edible drupe that contains a solitary seed covered by a fibrous endocarp[1]. Of interest, fruits, seeds, pulp, bark, leaves and roots are widely employed as traditional

medications. For instance, seeds are employed as astringent to the bowels and leaves are used to treat piles. Besides, the ripe fruit and the bark are respectively used to treat constipation and diarrhea[2]. In African traditional medicine, water infusion of *M. indica* leaves can

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also be employed for its antiplasmodial and antipyretic properties[3]. Of note, numerous biological activities have been reported for this plant including antidiarrheal[4], immunomodulatory[5], bactericidal[6], antiviral[7] and anti-inflammatory properties[8].

The chemical composition of *M. indica* has been widely investigated over the past and numerous terpenoid constituents have been reported including sterols, triterpenes and carotenoids. In addition, phytochemical analyses of this species have led to the characterization of a wide diversity of phenolic components including flavonoids, phenolic acids, gallotannins, benzophenones as well as xanthenes such as mangiferin[9].

It is now well established that phenolic constituents are highly implicated in the health benefits of plant food products consumption[10]. Owing to their hydroxyl substituents and aromatic rings, they exert a major role as antioxidants and are capable of protecting human organism against the deleterious effect of reactive oxygen species and free radicals[11]. Overproduction of such species can result in oxidative stress which is contributing to the development of numerous degenerative diseases including chronic inflammation and several type of cancers[12]. Increasing attention has been thus directed towards antioxidant capacity of natural phenolics compounds because of their potential nutritional and therapeutic value[13]. It has to be noted that several studies have also highlighted that some phenolic compounds can be regarded as promising agents for the prevention of Advanced Glycation End products (AGEs) formation[14]. AGEs can be defined as altered proteins that become non-enzymatically glycosylated after reaction with aldose sugars[15]. By inducing protein dysfunction and cell damages, AGEs accumulation is involved in the course of ageing. In addition, AGEs are also increased and play a key role in the development of atherosclerosis, neurodegenerative diseases as well as diabetic complications[16]. Inhibition of AGEs formation represents thus an attractive preventive and therapeutic target.

It has been clearly shown that qualitative and quantitative phenolic composition of mangoes strongly differs among cultivars, parts and environmental conditions[17]. In addition, there is a lack of chemical and biological data about numerous Senegalese varieties of *M. indica*. Therefore, the present study aimed at evaluating total phenolics as well as antioxidant and antiglycation activities of four different parts (leaves, stem barks, roots and kernels) of Sewe and Bouka varieties, two major mango cultivars grown in Senegal.

## 2. Materials and methods

### 2.1. Reagents

Methanol, Folin-Ciocalteu's reagent, bovine serum albumin (BSA), D-ribose, aminoguanidine hydrochloride, gallic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Trolox, fluorescein, 2,2'-azobis(2-methylpropionamide) dihydrochloride, Iron (II) chloride and ferrozine were bought from Sigma-Aldrich (Saint-Quentin Fallavier, France). Ethylenediaminetetraacetic acid disodium salt (EDTA) was

purchased from Fisher Chemical (Illkirch, France).

### 2.2. Plant materials

Leaves, stem barks, roots and kernels of two varieties (Sewe and Bouka) of *M. indica* L. were obtained from cultivated trees at Diender, Senegal, in July 2016. A sample of each part of the investigated plants was deposited in a laboratory herbarium (Laboratory of Pharmacognosy, Faculty of Medicine, Pharmacy and Odontology from University Cheikh Anta Diop of Dakar, Senegal). Plant material was shade dried with ventilation for six weeks, then powdered using a mechanical grinder.

### 2.3. Preparation of extracts

For each extract, 100 g of plant material were extracted twice with methanol (500 mL) for 48 h at room temperature and under magnetic agitation. After filtration, methanol was removed under reduced pressure and the dried extracts were stored at 4 °C before analyses.

### 2.4. Total phenolic content (TPC)

TPC was evaluated according to the method of Folin and Ciocalteu[18], with slight modifications as previously reported[19]. A standard curve of gallic acid in the range of 30 µM to 470 µM was performed ( $R^2 = 0.9979$ ,  $y = 4.3937x + 0.021$ ). Total phenolic content was expressed as mg of gallic acid equivalents per g (mg GAE/g) of extract. All analyses were performed in triplicate and results were indicated as means±SEM.

### 2.5. Antioxidant activity

#### 2.5.1. DPPH radical-scavenging activity assay

DPPH scavenging activity was evaluated as previously described by Meda *et al*[19]. A standard curve of Trolox in the range of 0.1 mM to 6 mM was constructed ( $R^2 = 0.9978$ ,  $y = 1101.1x + 1.5196$ ) and results were indicated as µmol of Trolox equivalents per g (µmol TE/g) of extract.

#### 2.5.2. Oxygen radical absorbance capacity (ORAC) assay

The assay was done in 96-well plates with a final volume of 200 µL as previously reported[19]. ORAC values were determined using the respective area under the curve (AUC) and the regression equation between Trolox equivalents and the net AUC (concentration of Trolox in the range of 3 µM to 100 µM,  $R^2 = 0.9904$ ,  $y = 35.63x + 11.26$ ). The results were presented as µmol TE/g of extract.

#### 2.5.3. Iron (II) chelating activity

Metal chelating activities were measured following the protocol of Wang *et al*[20]. A standard curve of EDTA in the range of 8 µM to 135 µM was performed ( $R^2 = 0.986$ ,  $y = 6648x + 10.252$ ). The results were indicated as µg of EDTA equivalents per g (µg EDTAE/g) of

extract.

## 2.6. Advanced glycation end products (AGEs) assay

Inhibition of AGEs formation was evaluated as previously described by Derbré *et al*[21], with slight adjustments. Reaction solution (100 µL) was prepared by mixing 20 µL of each plant extract (0.05 to 1 mg/mL), 40 µL of 25 mg/mL BSA and 40 µL of 120 mM D-(-)-ribose in a phosphate buffer (50 mM, pH = 7.4). This mixture was incubated at 37 °C for 24 h in the dark in 96-well microtiter plates before AGEs fluorescence evaluation. AGEs fluorescence was monitored on a microplate reader (TECAN infinite F200 PRO) using 370 and 440 nm as the excitation and emission wavelengths, respectively. Aminoguanidine was employed as positive control and results were presented as IC<sub>50</sub> values in µg/mL.

## 3. Results

### 3.1. Total phenolic content

As shown in Figure 1, substantial TPC was determined for all the studied extracts. With respective values of (546±1) mg GAE/g and (489±3) mg GAE/g of extract, Sewe kernel extract (SKE) and Bouka kernel extract (BKE) were shown to possess the highest phenolic contents, indicating that kernel is the richest source of phenolics for both varieties.

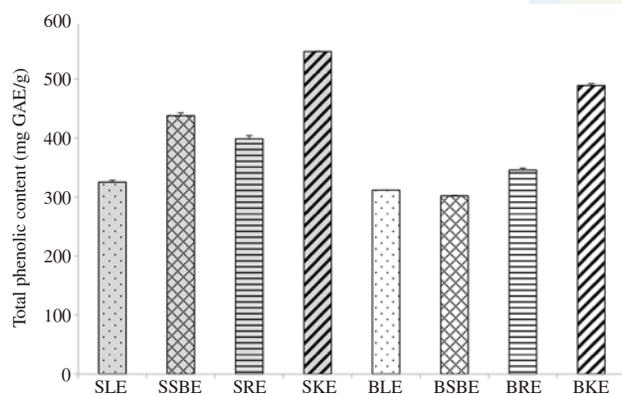


Figure 1. TPC of different extracts (means±SEM).

Table 1

DPPH radical scavenging activity, ORAC value, iron (II) chelating activity and extraction yield of extracts from Sewe and Bouka cultivars.

Extracts	DPPH scavenging activity (µmol TE/g)	ORAC value (µmol TE/g)	Iron (II) chelating activity (µg EDTAE/g)	Extraction yield (%)
SLE	2 139±146	6 335±176	3 827.7±0.3	12.24
SSBE	2 913±115	5 920±59	10 593.0±4.0	19.52
SRE	2 582±9	5 638±35	5 374.0±0.5	17.36
SKE	4 980±50	1 604±67	4 870.8±0.2	15.33
BLE	1 704±69	5 310±61	4 275.7±0.1	9.38
BSBE	1 702±108	4 383±23	3 555.6±0.4	21.22
BRE	2 098±52	4 478±169	2 617.6±0.4	13.20
BKE	5 510±6	1 257±26	6 267.6±0.4	11.65

Antioxidant values are presented as means±SEM and extraction yields are expressed as percentage.

SLE: Sewe leaves extract, SSBE: Sewe stem bark extract, SRE: Sewe root extract, SKE: Sewe kernel extract, BLE: Bouka leaf extract, BSBE: Bouka stem bark extract, BRE: Bouka root extract and BKE: Bouka kernel extract.

## 3.2. Antioxidant activity

### 3.2.1. DPPH radical–scavenging assay

As indicated in Table 1, DPPH scavenging activity of the extracts ranged between (1 702±108) and (5 510±6) µmol TE/g. Once again, kernels exhibited the highest activities with values of (4 980±50) µmol TE/g and (5 510±6 µmol) TE/g for SKE and BKE, respectively. Conversely, extracts from leaves and stem bark from Bouka were shown to be the least effective ones with values lower than 2 000 µmol TE/g.

### 3.2.2. ORAC assay

As shown in Table 1, sample ORAC values varied noticeably from (1 257±26) to (6 335±176) µmol TE/g. Of interest, the highest activity was exerted by Sewe leaves extract (SLE) whereas SKE and BKE were found to possess the lowest ORAC values.

### 3.2.3. Iron (II) chelating activity

Iron (II) chelating activity of the different extracts was presented in Table 1. With a value higher than 10 000 µg EDTAE/g, Sewe stem bark extract (SSBE) was shown to possess very potent Fe<sup>2+</sup> chelating ability [(10 593±4) µg EDTAE/g]. On the contrary, roots from Bouka cultivar were shown to induce only moderate metal chelating effects [(2 617±0.4) µg EDTAE/g].

## 3.3. AGEs assay

In this study, a BSA/D-ribose model was adopted to assess antiglycation effects of *M. indica* extracts. Our data indicated that roots, leaves and stem barks extracts of both varieties exerted noticeable antiglycation effects, with IC<sub>50</sub> values lower than the standard positive control aminoguanidine [(259±7) µg/mL]. Of interest, SSBE and Sewe root extracts (SRE) displayed the most potent inhibitory activities, with respective IC<sub>50</sub> values of (145±5) and (147±3) µg/mL. As attested by their respective IC<sub>50</sub> values of (165±2) and (185±10) µg/mL, leave extracts from Sewe and Bouka cultivars were also shown to strongly inhibit the formation of AGEs. By contrast, with IC<sub>50</sub> values higher than 500 µg/mL, kernel extracts of the two studied varieties were found to be almost ineffective in blocking AGEs formation.

#### 4. Discussion

The present results demonstrate that all parts from the two investigated varieties of *M. indica* possess high contents of phenolic compounds. Phenolic constituents are well known to be main contributors to antioxidant capacities in plant extracts and are considered as the most predominant antioxidant phytochemicals[22]. Owing to their reactivity as electron or hydrogen-donating agents and metal ion chelating activities, these compounds can exert positive effects on oxidative stress[23,24]. The potent antioxidant properties of the studied extracts were highlighted by three different spectrometric assays. For both varieties, kernels were shown to exert the highest DPPH radical scavenging activity. Conversely, leaves, stem barks and roots of the two varieties possessed higher ORAC values. In addition, potent Fe<sup>2+</sup> chelating effects were observed for all studied parts including stem bark of Sewe cultivars which had remarkable high activity.

Discrepancies between antioxidant potency of the different organs found with the three methods can be largely explained by the different principle of the assays. DPPH radical scavenging is one of the most widely employed antioxidant method for plant samples. This assay is mainly based on single electron transfer of antioxidants to neutralize DPPH radical[25]. The reaction leads to the discoloration of the purple-colored DPPH radical which is an indicator of the antioxidant efficacy[26]. ORAC assay is regarded as a relevant protocol for evaluating antioxidant activity of biological samples and foodstuffs[27]. By contrast with DPPH assay, deactivation of radical species is considered to be related to a hydrogen atom transfer mechanism[28]. DPPH and ORAC can be thus regarded as distinct and complementary evaluations that reflect the two major mechanisms leading to radical deactivation, single electron transfer and hydrogen atom transfer. Besides these two radical scavenging assays, metal chelating capacity can be also used as an indicator of antioxidant activity. Indeed, Fenton reaction, which involves transition-metal ions such as Fe<sup>2+</sup>, is an important source of hydroxyl radical, a highly reactive oxygen species[29]. Furthermore, differences in the observed activities can be also explained by disparities in the chemical composition of the organs. Indeed, previous chemical analyses of various *M. indica* parts indicated that benzophenone and xanthone derivatives represent the major phenolics in leaves as well as in bark, iriflophenone 3-*C*-β-D-glucoside and mangiferin being the two most abundant compounds in the majority of the studied varieties[30]. Conversely, it has been reported that *M. indica* kernels mostly contain gallotannin derivatives, with penta-*O*-galloyl-glucoside as the major one. Of interest, this compound has been previously shown to only exert moderate radical scavenging activities when submitted to ORAC evaluation[30] while mangiferin and iriflophenone 3-*C*-β-D-glucoside are both known to give excellent results with that assay[31]. Taken together, these data tend to explain why, in the present study, kernel extracts possess the lowest ORAC values despite being the richest source of phenolic compounds.

It is now well established that AGEs have a significant role in ageing process as well as in numerous degenerative diseases[32]. Inhibition of the formation of these harmful products is now regarded as an attractive preventive or therapeutic target[33] and

increasing attention is recently being given to the evaluation of plant extracts and phytochemicals as antiglycating agents. Of interest, a substantial number of natural phenolic compounds have been identified as potent inhibitors of AGEs formation[21,34,35]. However, it has to be noted that only limited data are available regarding the antiglycation activity of *M. indica*. Flesh and peel extracts of mango[36,37] have been reported to possess significant anti-AGEs properties. In addition, the antiglycation potential of mango leaves has also been recently documented[38]. However, to our knowledge, no data are available concerning kernels, bark and roots. Furthermore, the present study constitutes the first evaluation taking into account intraspecific variability of *M. indica*. By using a BSA/D-ribose system, our experiments established that kernels of both cultivars induced only weak inhibitory effect on AGEs formation. Conversely, leaves of Sewe and Bouka cultivars possess strong anti-AGEs activities with IC<sub>50</sub> values lower than the reference compound aminoguanidine. The present data also demonstrate for the first time the pronounced interest of *M. indica* roots and bark as antiglycating agents, as attested by the particularly low IC<sub>50</sub> value of Sewe cultivar bark extract. It is important to note that xanthone and benzophenone derivatives have been reported to exert potent anti-glycation effects[38]. They might be thus be regarded as important contributors to the anti-AGEs properties of the studied extracts. Such assumption is further supported by the weak effects of kernel extracts. Indeed, several studies have previously shown that this organ only contains traces of such kind of phenolic compounds[30].

The present study attests that the two investigated *M. indica* cultivars are substantially rich in phenolic constituents and exhibit powerful antioxidant effects. In addition, it demonstrates that their roots, leaves and barks also exert potent antiglycation activities. These results thus suggest that Sewe and Bouka cultivars of *M. indica* can be regarded as potential nutraceutical resources to prevent oxidative stress and carbonyl stress related disorders.

#### Conflict of interest statement

The authors declare that there is no conflict of interest.

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