In vitro antioxidant activity and phenolic contents of different fractions of ethanolic extract from *Khaya senegalensis* A. Juss. (Meliaceae) stem barks

Lombo Marius¹,², Traoré Rakiatou¹,², Ouédraogo Noufou¹,²*, Kini Félix¹, Tibiri André¹,⁴, Duez Pierre³ and Guissou I. Pierre¹,²

¹Département de médecine-pharmacopée traditionnelle/pharmacie (IRSS/CNRST) 03 BP 7192 Ouagadougou 03, Burkina Faso.
²Laboratoire de pharmacologie et toxicologie, UFR/SDS, Université de Ouagadougou 03 BP 7021 Ouagadougou 03, Burkina Faso.
³Service de Chimie Thérapeutique et de Pharmacognosie, Université de Mons, Bât. Mendeleiev Avenue Maistriaux 19; 7000 Mons, Belgique, Burkina Faso.
⁴Unité Mixte Internationale-Environnement, Santé, Sociétés (UMI 3189, ESS) CNRS/UCAD/USTTB/CNRST/UGB, Burkina Faso.

Received 17 March, 2016; Accepted 25 May, 2016

*Khaya senegalensis* A. Juss (Meliaceae) is a medicinal plant used in folk medicine of Burkina Faso. Its stem barks are used to treat several diseases such as inflammation, arthritis, infections, ulcer, malaria, fever and dermatosis. The antioxidant activity of aqueous ethanol extract and fractions of *Khaya senegalensis* stem bark was evaluated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH•), 2,2'-azino-bis (ABTS•⁺), ferric reducing antioxidant power (FRAP) and lipid peroxidation methods. Total phenolic, tannins, flavonoids and flavonol contents of extract and fractions were determined. Butanol fraction had the highest value with IC⁵₀ = 1.76 ± 0.19 µg ml⁻¹ (ARP = 0.56) with DPPH⁺ assay, however n-hexan fraction showed the highest capacity to scavenge ABTS⁺⁺; FRAP values varied from 13.04 ± 0.25 to 13.60 ± 0.09 mmol Trol Equivalent per gram (mmoll TE g⁻¹) of extract or fraction. Ethyl acetate fraction presented the best activity (70.30 ± 0.40%, 100 µg ml⁻¹) using lipid peroxidation inhibition method. Aqueous fraction contained the highest of total phenolics and tannins contents with, respectively 3.68 ± 0.11 and 2.65 ± 0.18 g TA/100 g of dry weight (dw) of plant material. Aqueous fraction also showed the highest of total flavonoids (0.04 ± 0.01 g QE/100 g dw) and flavonol (0.10 ± 0.01 g QE/100 g dw) contents. *K. senegalensis* possesses a potential antioxidant effect and contains phenolic compounds. These results provide scientific evidence that validates the use of *K. senegalensis* in traditional medicine.

**Key words:** *Khaya senegalensis*, antioxidant, phenolic, flavonoids, tannins

**INTRODUCTION**

Plants play an important role in human life since thousands of years; they provide humanity food, energy (coal and firewood), building material and medicine. Plants have formed the basis of traditional medicine and provide new remedies through new compounds isolated and used as drugs (Gurib-Fakim, 2006). Secondary
metabolites production by plants are responsible for the therapeutic properties of medicinal plants. Secondary metabolites have been known to be synthesized by plants in response to infectious attack and environmental conditions (Parvin et al., 2015; Ghasemzadeh and Jaafar, 2013). There is a variety of these compounds found in plants such as phenolic compounds that exhibit a wide range of biological properties, including anti-inflammatory, antioxidant, antimicrobial, anticancer, hypoglycemicant (Wen et al., 2015).

Many drugs possessing antioxidant property are used to treat oxidative stress. Medicinal plants play a vital role in the production of the antioxidant defense system by providing antioxidant plant phenol (phenolic compounds and flavonoids) (Willcox et al., 2012).

Reactive oxygen species (ROS) and other free radicals produced during metabolism arise from a necessary and normal process that contributes to the defense system of organism. However excessive production of free radical is harmful to the organism, leading to oxidative stress which is associated with the pathogenesis of chronic diseases including cancer, diabetes, cardiovascular and neurodegenerative diseases, arthritis, obesity, and autoimmune disorders (Willcox et al., 2012; Pham-Huy et al., 2008).

Khaya senegalensis A. Juss (Meliaceae) is a medicinal plant used in folk medicine of Burkina Faso. The leaves, stem barks, seeds, and roots of this plant are used to treat several diseases such as inflammation, arthritis, infections, ulcer, malaria, fever, dermatosis. Literature reported that ROS production plays important role in the pathogenesis of inflammation, arthritis, ulcer and malaria (Percário et al., 2012; Mirshafiey and Monireh, 2008). Previous studies had reported the antioxidant property of extracts from stem bark of K. senegalensis (Lombo et al., 1998; Lombo et al., 2007). Limonoids were identified and isolated in the leaves and stem bark of K. senegalensis (Zhang et al., 2009; Yuan et al., 2012).

The aim of the present study was to evaluate the antioxidant activity of aqueous ethanol extract and its fractions (n-hexan, ethyl acetate, n-butanol and aqueous) of K. senegalensis stem barks, and this study was to determine total phenolic, tannins, flavonoids and flavonol contents in the extract and its fractions.

MATERIALS AND METHODS

Chemicals and reagents

ABTS (2, 2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), trolox, quercetin, sodium acetate, Folin-Ciocalteu reagent (FCR 2N), polyvinylpolypyrrolidone, aluminum chloride and potassium persulfate were purchased from Sigma (St Louis, USA). Trichloroacetic acid and 2-thiobarbituric acid were from Fluka chemica. Potassium hexacyanoferrate [KFe(CN)₆] were purchased from Prolabo (Paris, France).

Plant

The present study was undertaken on the stem bark of K. senegalensis, which were collected in May, 2011 at Samogohiri, in Kenedougou district (West region of Burkina Faso). The plant was identified by Dr Ouédraogo Amadé, a Botanist at the Department of Forest of INERA/CNRST-Burkina Faso. A voucher specimen was deposited at the National Herbarium of CNRST with number ID16879 and GPS data (10°39’14.25 N; 4°39’52.96 W).

Preparation of plant extract and fractions

Five hundred grams (500 g) of powder of stem bark of K. senegalensis were macerated with 2.5 L of 80% (v/v) of aqueous ethanol (96%) for 24 h at 25°C. The resulting mixture was filtered using paper Whatman (N°1) and then was evaporated to dryness under reduced pressure in a rotary evaporator (BUCHI 461, Switzerland) at 45°C to yield crude aqueous ethanol extract (69 g). Aqueous ethanol extract (AEE) (34.5 g) suspended in water (500 ml) was partitioned with n-hexan (3 × 200 ml), ethyl acetate (3 × 200 ml) and n-butanol (3 × 200 ml) to obtain a n-hexan fraction (0.61 g), an ethyl acetate fraction (1.88 g), n-butanol fraction (1.51 g) and aqueous fraction (13.1 g).

Antioxidant activity determinations

DPPH+ assay

DPPH+ radical scavenging activity was done according to Kim et al. (2003). 10 µl of extract or fractions or standard was added to 200 µl of DPPH methanolic solution (0.04 mg ml⁻¹) in a 96-well microtiter plate and vortexed. After 30 min incubation in the dark at room temperature, the absorbance was measured at 490 nm using spectrophotometer BioRad (model 680, Japan). Each determination was carried out in triplicate. Antiradical activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% and expressed as antiradical power (ARP = 1/EC₅₀).

DPPH assay on thin layer chromatography (DPPH-TLC)

Aqueous ethanol extract and fractions of K. senegalensis stem bark were applied using Silica gel 60 F₂₅₄ plates (Merck). The mobile phase was butanol-glacial acetic acid-water (60:20:20; V/V/V). Sample (10 mg ml⁻¹, 10 µl) were directly deposited as spot onto the TLC plates. After deposition of sample, the plates were dried and placed in migration chamber previously containing eluent. On the plate, the distance of the eluent path was 80 mm from the point of deposit spot. After migration, the plates were removed and dried at room temperature for 30 min. Detection of antioxidant compounds was achieved by spraying plates with a DPPH in methanol. The presence of antioxidant compounds was detected by yellow spots.

---

*Corresponding author. E-mail: arnoufou2@yahoo.fr, ouednouf@gmail.com. Tel: 00226 25363215/ (00226) 78087450.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
RESULTS AND DISCUSSION

Antioxidant activity of aqueous ethanolic extract (AEE) of stem bark of *K. senegalensis* and its fractions was measured using DPPH•, ABTS•⁺, FRAP and Lipid peroxidation (LPO) methods. Liquid partition was allowed to obtain four fractions from aqueous ethanolic extract, using solvents such as n-hexan, ethyl acetate, n-butanol and water. Polar and non-polar fractions of *K. senegalensis* could be worthwhile in order to find a correlation between the antioxidant and the phenolic contents. The antioxidant activity using five different methods (DPPH, DPPH-TLC, ABTS, LPO, FRAP) of aqueous ethanolic extract and fractions are summarized in Table 1.

DPPH• radical scavenging activity was evaluated in terms of percentage inhibition of a pre-formed free radical...
Table 1. Antioxidant activity of aqueous ethanol extract and fractions of K. senegalensis stem bark.

<table>
<thead>
<tr>
<th>Samples</th>
<th>ABTS (TEAC)</th>
<th>FRAP (mmol TE/g)</th>
<th>Lipid peroxidation inhibition (%)</th>
<th>DPPH IC50 (µg ml⁻¹) (ARP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous ethanol extract</td>
<td>3 ± 0.05*</td>
<td>13.40 ± 0.05*</td>
<td>57.08 ± 1.06*</td>
<td>2.3 ± 0.2 (0.43)*</td>
</tr>
<tr>
<td>n-Hexan fraction</td>
<td>8478 ± 0.3*</td>
<td>---</td>
<td>49.65 ± 1.61</td>
<td>170.3 ± 0.2 (0.006)*</td>
</tr>
<tr>
<td>Ethylacetate fraction</td>
<td>166 ± 0.2*</td>
<td>13.04 ± 0.25*</td>
<td>70.30 ± 0.40*</td>
<td>7.6 ± 0.15 (0.13)*</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>4 ± 0.2*</td>
<td>13.60 ± 0.09*</td>
<td>58.70 ± 0.80*</td>
<td>1.76 ± 0.2 (0.56)*</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>3.01 ± 0.2*</td>
<td>13.55 ± 0.10</td>
<td>61.72 ± 0.00*</td>
<td>2.05 ± 02 (0.49)*</td>
</tr>
<tr>
<td>Quercetin</td>
<td>---</td>
<td>---</td>
<td>47.92 ± 0.001*</td>
<td>1.06 ± 0.13 (0.94)</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>---</td>
<td>---</td>
<td>43.14 ± 0.43*</td>
<td>---</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for triplicate; *: P < 0.05 significant from control (one-way ANOVA analysis followed by Dunnett’s test); ARP (antiradical power) = 1/IC50, TEAC: trolox equivalent antioxidant capacity. TE: trolox equivalent.

Figure 1. TLC plates photography of extract, fractions and standards before (A) and after (B) sprayed with DPPH solution. HA: aqueous ethanol extract, AE: ethyl acetate fraction, But: n-butanol fraction, Aq: aqueous fraction, AT: tannic acid and Q: quercetin.

by antioxidants compounds in extract and fractions. Aqueous ethanolic extract and fractions exhibited free radical scavenging effect in concentration dependent manner. The IC50 values ranged from 1.76 ± 0.19 to 170.30 ± 0.20 µg ml⁻¹. Butanol fraction had the highest value with 1.76 ± 0.19 µg ml⁻¹ (ARP = 0.56) against n-hexan fraction with 170.30 ± 0.20 µg ml⁻¹ (Table 1). Ibrahim et al. (2014) reported that ethanol extract of K. senegalensis stem bark contained polyphenols and possessed antioxidant activity (IC50 = 1.99 ± 0.87 µg ml⁻¹) using DPPH assay. This value is not significantly different than obtained value. Comparing these results with others species of Khaya genus, K. senegalensis possess high antioxidant activity than methylene chloride/methanol (1:1 v/v) extract of Khaya grandifoliola C. DC (Meliaceae) stem barks with IC50= 4.54 ± 0.28 µg ml⁻¹ using DPPH assay (Njayou et al., 2015).

TLC-DPPH assay revealed the antioxidant compounds while DPPH assay using spectrophotometer gave the antioxidant activity of whole extract. TLC-DPPH assay revealed the antioxidant activity of whole extract. TLC-DPPH tests, reported in literature, focus on phenolic compounds such as flavonoids, phenolic acid, tannins (Ciesla et al., 2012).

Like DPPH assay, ABTS⁺ radical scavenging activity of extract and fractions was expressed in TEAC values which ranged from 8478 ± 0.3 to 3 ± 0.05. N-hexan fraction showed the highest capacity to scavenge ABTS⁺ (Table 1). Variation of ABTS⁺ radical scavenging activity may be associated to the different constituents in each extract; aqueous ethanolic extract activity could be due to the tannins contents (Khan et al., 2012) and n-hexan fraction activity could be due to the presence of lipophilic compounds (Osman et al., 2009).

FRAP values varied from 13.04 ± 0.25 to 13.60 ± 0.09 mmol Trolox Equivalent per gram (mmol TE/g) of extract or fraction (Table 1). The highest FRAP value was obtained with ethyl acetate fraction (13.04 ± 0.25 mmol TE g⁻¹); however there is not significant difference between FRAP values of fractions and extract. At a
concentration of 100 μg ml⁻¹, the crude extract and its fractions showed by the lipid peroxidation test inhibition values ranged from 49.65 ± 1.61 to 70.30 ± 0.40%. Ethyl acetate fraction presented the highest activity (70.30 ± 0.40%) and the lower activity was given by n-hexan fraction (49.65 ± 1.61). In addition, inhibitor effect of ethyl acetate fraction against lipid peroxidation was more than the standard compounds gallic acid (43.14 ± 0.43%) and quercetin (47.92 ± 0.01%).

In pathological conditions, the excessive production of free radical provokes the induction of lipid peroxidation leading to cell damaging. Lipid peroxidation inhibition allows the prevention of cell lysis inhibiting free radical. The test of lipid peroxidation inhibition method allowed to obtain percentage inhibition varied from 49.65 ± 1.61 to 70.30 ± 0.40% at same concentration (100 μg ml⁻¹). Ethyl acetate fraction presented the best activity (70.30 ± 0.40%) and lower activity was n-hexan fraction (49.65 ± 1.61%). In addition, inhibitory effect of ethyl acetate fraction against lipid peroxidation was more than standard compounds such as gallic acid (43.14 ± 0.43%) and quercetin (47.92 ± 0.01%).

The literature for antioxidant activity of *K. senegalensis* using ABTS, FRAP, and LPO methods has not been found, however the antioxidant activity of *K. senegalensis* was measured using deoxyguanosine, hydroxyl radical (HRS) and Nitric oxide (NO) radical scavenging models (Atawodi et al., 2009; Ibrahim et al., 2014).

One method is not sufficient to evaluate that antioxidant capacity but it takes more than one method to take into account different modes of action of antioxidants (Dudonné et al., 2009). This study showed that the most active fraction depends on the method used; n-hexan fraction was more active than other fractions in ABTS⁺ assay; however, in DPPH⁺ assay, n-butanol fraction was more active. This could be due to different mechanisms involved in the steps of oxidation process and antioxidant composition such as secondary metabolites (Conforti et al., 2009). The study found that n-hexan fraction containing lipophilic compounds was more active with ABTS⁺ assay. According to Prior et al. (2005), hydrophilic and lipophilic compounds act against ABTS⁺ radical. In addition, the antioxidant activity depends on the amount of compounds that react with the free radical formed in each method used.

The total phenolics, tannins, total flavonoids and flavanol contents of extract and fractions are shown in Table 2. Aqueous ethanolic extract of *K. senegalensis* contains total phenolic, tannins, total flavonoids and flavanol. Among fractions, aqueous fraction had the highest of total phenolics and tannins contents with, respectively 3.68 ± 0.11 and 2.65 ± 0.18 g TAE/100 g of dry weight (dw) of plant material, followed by n-butanol fraction. Aqueous fraction also showed the highest of total flavonoids (0.04 ± 0.01 g QE/100 g dw) and flavanol (0.10 ± 0.01 g QE/100 g dw) contents. Phenolic contents have already been reported in stem barks extracts of *K. grandifoliola* (Njayou et al., 2015) and *K. senegalensis* (Ibrahim et al., 2014).

The antioxidant effect of substances is important to prevent, to delay or to treat oxidative stress involved in pathogenesis of many chronic pathologies including cancer, cardiovascular diseases, arthritis, diabetes. Several study has reported the antioxidant activity of phenolic compounds such as polyphenolic, tannins and flavonoids. Antioxidant activity of these compounds is due to their oxidation-reductive property, which play an important role in the adsorption and neutralization of free radical (Manish et al., 2011; Ouédraogo et al., 2012).

Previous studies had reported a strong correlation between antioxidant activity and phenolic compounds present in the extracts from medicinal plants (Wang et al., 2016; Dudonné et al., 2009). The analysis of data significantly revealed a correlation observed between DPPH⁺ method and total phenolic (R² = 0.98, p < 0.05) (Figure 2) and flavonols (R² = 0.98, p < 0.05). The antioxidant activity of *K. senegalensis* stem bark is due to the synergic action of different compounds which act by direct free radical scavenging, chelation of transition metal and direct inhibition of lipid peroxidation.

**Table 2. Total phenolics, tannins, flavonoids and flavonols contents of *K. senegalensis* stem bark.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic (g TAE/100 g dw)</th>
<th>Tannins (g TAE/100 g dw)</th>
<th>Total flavonoid (g QE/100 g dw)</th>
<th>Flavonol (g QE/100 g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous ethanol extract</td>
<td>9.36±0.53*</td>
<td>5.78±0.44*</td>
<td>0.11±0.02*</td>
<td>0.36±0.04*</td>
</tr>
<tr>
<td>n-Hexan fraction</td>
<td>0.01±0.001*</td>
<td>0.01±0.001*</td>
<td>0.001±0.001*</td>
<td>-</td>
</tr>
<tr>
<td>Ethylacetate fraction</td>
<td>0.33±0.04*</td>
<td>0.04±0.001**</td>
<td>0.01±0.001*</td>
<td>0.001±0.001</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>0.49±0.03*</td>
<td>0.25±0.02*</td>
<td>0.01±0.001*</td>
<td>0.01±0.001*</td>
</tr>
<tr>
<td>Aqueous Fraction</td>
<td>3.68±0.11*</td>
<td>2.65±0.18*</td>
<td>0.04±0.01</td>
<td>0.10±0.01*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. for triplicate; *: P < 0.05 significant from control (one way ANOVA analysis followed by Dunnett’s test); TAE: tannin acid equivalent; QE: quercetin equivalent; dw: dried weight.
fractions obtained of stem bark of *K. senegalensis* A. Juss (Meliaceae). Stem bark of *K. senegalensis* contains total phenolic, tannins and flavonoids. These results provide scientific evidence that validates the use of *K. senegalensis* in traditional medicine.

**Conflict of interests**

The authors have not declared any conflict of interests.

**ACKNOWLEDGMENTS**

This work was supported in 2010 by the Program of C.U.D (Belgium) for Post-doctoral research. This research was carried on both in Belgium and in Burkina Faso in the Department of Traditional Medicine of IRSS/CNRST-Burkina Faso. The author wish to thank Dr Amadé OUEDRAOGO to plant identification.

**REFERENCES**


