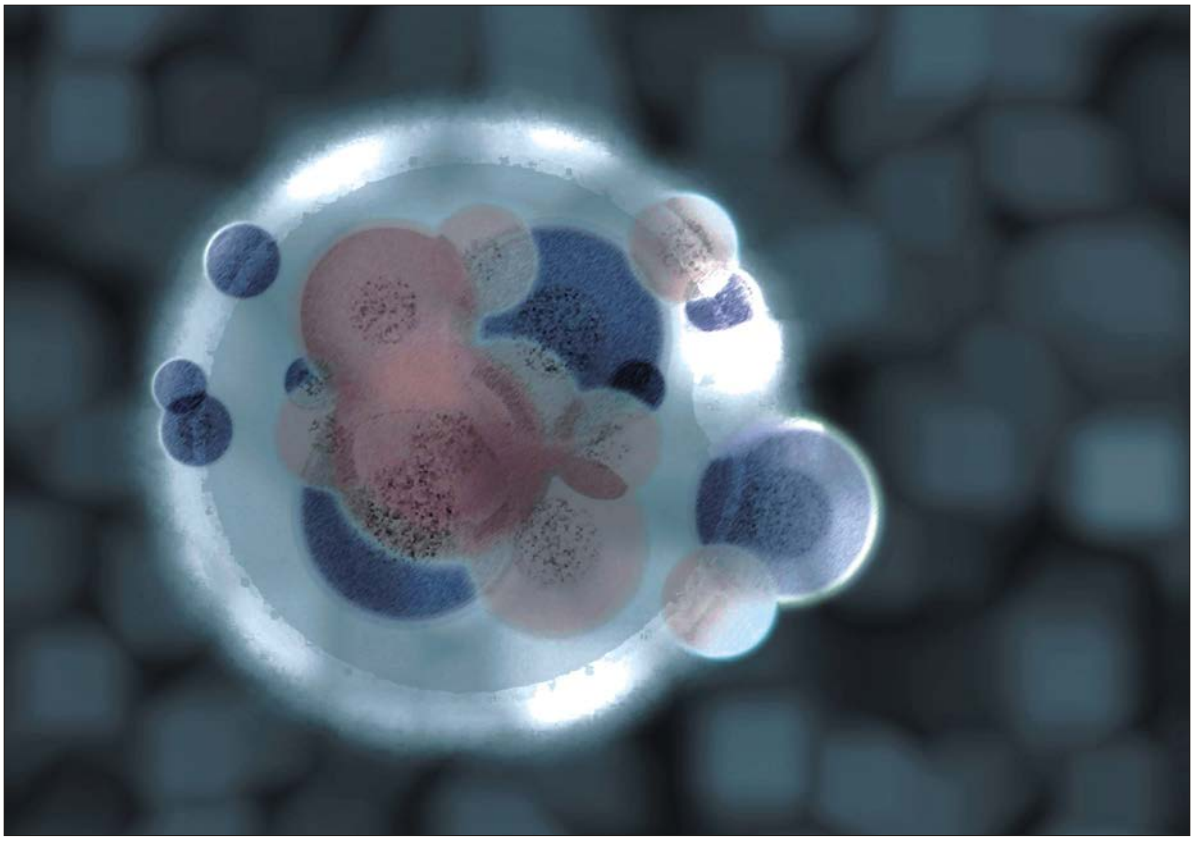

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 7 (4): 677-680, 2007
ISSN 1727-3048

In vitro Preliminary Study of Free Radical Scavenging Activity of Extracts from *Khaya senegalensis* A. Juss. (Meliaceae)

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Abstract: Aqueous and ethanolic crude extracts and fractions of *Khaya senegalensis* stem barks were investigated for their antioxidant activities. Their ability to act as radical scavengers have not been previously reported and was investigated using 1,1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical in comparison with Rutin and Quecetin as antioxidant standards. The study shows that all extracts and fractions are able to scavenge significantly free radicals of DPPH. The IC_{50} are, respectively 9.82 μg $mL^{\bullet 1}$ for defatted extract; 7.42 μg $mL^{\bullet 1}$ for Rutin (Standard); 7.33 μg $mL^{\bullet 1}$ for lyophilised aqueous extract; 6.01 μg $mL^{\bullet 1}$ for the fraction F_1 of lyophilised aqueous extract; 4.70 μg $mL^{\bullet 1}$ for ethanolic extract; 4.37 μg $mL^{\bullet 1}$ for the fraction F_2 from methanolic crude extract of stem barks and 2.58 μg $mL^{\bullet 1}$ for Quercetin (Standard). Lyophilised aqueous extract activity is closely related to that of Rutin but less than Quercetin. Fractions can play a pivotal role in the antioxidant activity of crude lyophilised aqueous and alcoholic extract of stem barks of *Khaya senegalensis*.

Key words: *Khaya senegalensis*, aqueous extract, alcoholic extract, antioxidant activity, free radical scavenger

INTRODUCTION

Khaya senegalensis A. Juss. (MELIACEAE) is a tall savannah tree that has been used in folk medicine in Burkina Faso to treat many pathologies like inflammatory diseases (Lompo, 1993), fever and gastrointestinal pain (Kerharo and Adam, 1974). Some studies (Gamez *et al*., 1998; Waffo Teguo *et al*., 1998; Ng *et al*., 2000; Gaboriau *et al*., 2002; Gil *et al*., 2003; Groussard *et al*., 2003) have related the importance of the oxidative stress in numerous human diseases. Free radicals and lipid peroxidation have been suggested as potentially important causative agents of aging and several human disease (Zhou and Zheng, 1991; Ng *et al*., 2002).

We reported previously (Lompo, 1993, 1998, 1999) the anti-inflammatory activity of *K. senegalensis* but up to now, it's anti-oxidative action has not been established. The anti-oxidant effect has not been investigated. Thambi *et al.* (2006) suggested that anti-inflammatory activity of the ethanol extract of *Tabemaemontana coronaria* was possibly attributed to it's free radical scavenging properties.

The aim of the present research is to investigate the anti-oxidant activity which can complete the mechanism of anti-inflammatory effect of *Khaya senegalensis* and therefore to look for natural products from plants against free radical-induced pathological status.

MATERIALS AND METHODS

Chemicals: 1-1-diphenyl-2-picryl-hydrazyl (DPPH) and Quercetin dihydrate were obtained from Sigma Chemicals Co.,St Louis. Rutin trihydrate was obtained from Fluka Biochemika. Methanol HPLC Gradient Grade and Diethyl Ether Anhydrous were obtained from J.T. BAKER Holland, Ethyl Alcohol anhydrous for HPLC was obtained CARLO ERBA, Petroleum benzine extra pure (50-70°C) was obtained from MERCK. Dimethylsulfoxyde (DMSO) Analytical reagent was obtained from LAB-SCAN.

Plant material: *K. senegalensis* stem barks were collected in April 2002 in Koubri about 25 km. In the south of Ouagadougou in Burkina Faso and identified by the Department of Traditional Medicine and Pharmacopoeia in the Institute of Health Sciences Research. The barks were air-dried in shade and powdered.

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Preparation of the extracts

Aqueous extract: One hundred and fifty grams of the powder were macerated 24 h and extracted with 800 mL of distillate water at 22°C. The extract was filtered and centrifuged at 3000 rpm for 15 min and the supernatant was lyophilised giving 22 g of powder.

Ethanolic extract: One hundred grams of the powder were macerated 24 h at 22°C with 200 mL of ethanol 96°. The extract was filtered and centrifuged at 3000 rpm for 15 min and the supernatant was evaporated under reduced pressure with a rotavapor Büchi at 35°C 9.6 g of residue was obtained.

Defatted extract from lyophilised aqueous extract: Three grams of lyophilised extract were shacked 90 min with 30 mL of extra pure petroleum benzine. The extract was filtered and evaporated under reduced pressure at 35°C. The obtained residue was 2.4 mg. The marc (defatted lyophilised extract) was dried and used for experiments.

Fraction F_1 from lyophilised aqueous extract: Defatted extract was solubilized and shacked 3 h with 300 mL of Methanol. The mixture was filtered with Whatman paper filter 40. The marc was dried at the room temperature. Three hundred milliter of anhydrous Diethyl Ether was added to the filtrate. The fraction F_1 precipitated in the alcoholic solution according to the methods described by Barbouche *et al.* (2001). The supernatant was removed and the fraction of Saponosids was evaporated 45 min in the automatic Environmental Speed Vac® AES 1010 and yield 330.1 mg of residue.

Fraction F_2 from the powder of the stem barks: Three hundred grams of powder of stem barks were defatted with 500 mL of petroleum benzine. The filtered extract was evaporated under reduced pressure at 35°C with the Rotavapor Büchi and yield 438.3 mg of residue. The marc was then shacked 3 h with 500 mL of methanol. The mixture was filtered with Whatman paper filter 40. The filtrate was evaporated under reduced pressure at 35°C and the residue was treated 3 times with 25 mL of Diethyl Ether and filtered again. The residue was dried at room temperature and yield 20.26 g. Four grams of this residue were dissolved in 440 mL of Methanol. The same volume of Diethyl Ether was added to the solution and mixed.

Fraction F_2 precipitated. The supernatant was removed and the precipitates were dried 45 min in the Automatic Environmental Speed Vac® AES 1010 and yield 208 mg of residue.

***In vitro* antioxidant activity: DPPH method:** The hydrogen-donating ability of extracts was examined using the method of Blois (1958) described by Kim *et al.* (2003) in the presence of DPPH stable radical. Used as reagent, DPPH evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants (Cao *et al.*, 1997; Kim *et al.*, 2003).

The antioxidant activity of the plant extract and the standards were assessed on the basis of the radical scavenging effect of the DPPH free radical (Bang *et al.*, 2001) in (Badami *et al.*, 2003). A total of 10 μL of extracts (from 21 mg $mL^{\bullet 1}$ to 1.28 μg $mL^{\bullet 1}$ in DMSO solution or standard was added to 200 μL of DPPH in methanol solution (100 μM) in 96-well microtitre plate. After incubation at 37°C for 30 min, the absorbance of each solution was determined at 492 nm using Elisa microtitre plate Labsystem iEMS Reader MF Type 1401. Measurements were performed at least in triplicate for each concentration for any experiment. The corresponding blank was calculated. IC_{50} value is the concentration of sample required to scavange 50% DPPH free radicals. Each value of IC_{50} is the mean of at least three independent experiments.

Statistical analysis: Data are expressed as X±SE (n = 5). Significant differences were determined by the student t-test from Sigma Plot software program.

RESULTS AND DISCUSSION

All the extracts and fractions exhibited a significant antioxidant activity. Free radical scavenging effects on DPPH are evaluated according to the values of the IC_{50} in Table 1.

Average of at least three independent experimentations in which every concentration has done four times when compared by IC_{50}, the free radical scavenging potency of all extracts, fractions and standards has following range: Defatted lyophilsed aqueous extract < Rutin < Lyophilised aqueous extract < Fraction F_1 from lyophilised aqueous extract < Ethanolic extract < Fraction F_2 from barks methanolic extract < Quercetin.

The difference in activity between Quercetin used as a standard and the other extracts and fractions is significant at $p<0.05$. But there is no significant difference between Rutin used as a positive reference drug, lyophilised aqueous extract and the Fraction F_1 from lyophilised aqueous extract. The IC_{50} are, respectively 7.42, 7.33 and 6.01 μg $mL^{\bullet 1}$. Fraction F_1 appears to play a pivotal role in the activity of lyophilised aqueous extract.

Table 1: Effect of different extracts of *Khaya senegalensis* stem barks on free radical generation *in vitro*

Test material	IC_{50}(μg mL^{-1}) ±SE[a]
Defatted *Lyophilised aqueous* extract	9.82±0.60*
Rutin	7.42±0.62*
Lyophilised aqueous extract	7.33±0.98*
Fraction F_1 from *Lyophilised aqueons* extract	6.01±1.33*
Ethanolic extract	4.70±0.92*
Fraction F_2 from barks methanolic extract	4.37±0.46*
Quercetin	2.58±0.70

[a]Average of at least five independent experimentations in which every concentration has been done four times. *Different at $p<0.05$ vs Querection

The activities of the Fraction F_2 from barks methanolic extract, the ethanolic extract and Fraction F_1 from lyophilised aqueous extract are quite similar at $p<0.05$. The IC_{50} are, respectively 4.37, 4.70 and 6.01 μg mL^{-1}.

The activity of lyophilised aqueous extract decreases significantly at $p<0.05$ about 25%. There is extensive evidence to implicate free radicals in the development of degenerative diseases (Kim *et al*., 2003), inflammatory bowel disease (Peluso *et al*., 2002), skin inflammation (Fuchs *et al*., 1994). The consequences of oxidative stress are serious and in many cases are manifested by increased activities of enzymes invovled in oxygen detoxification (Kim *et al*., 2003). Therefore, the identification of new antioxidants may reduce the risk of various chronic diseases caused by free radicals. Our future studies will concern the identification of these compounds in *K. Senegalensis*.

ACKNOWLEDGMENTS

This research was supported by research grant from the Cooperation Office (Projet d'Initiative Propre: P.I.P.) of Free University of Brussels.

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