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Research Article

## *Sterculia setigera* hydroethanolic extract protects brain tissues *ex vivo* against lipid peroxidation and possesses *in vitro* antioxidant and anti-inflammatory properties

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### Abstract

Medicinal plants remain good sources of remedies both in traditional and modern medicine. The present study aimed to evaluate *ex vivo* and *in vitro* neuroprotective effects of *Sterculia setigera*, a medicinal plant of Togolese flora. Inhibition of lipid peroxidation in rat brain homogenate was assayed *ex vivo* using the combination of ascorbic acid and FeCl<sub>2</sub> as prooxidants. *In vitro* anti-inflammatory tests consisted in the evaluation of inhibition of albumin denaturation and red blood cells hemolysis. DPPH tests, FRAP test and Total antioxidant capacity (TAC) of the hydroethanolic extract of *S. setigera* were realized. Quantitative determination of total phenols, flavonoids and tannins was also performed. *S. setigera* hydroethanolic extract protected significantly brain homogenates against lipid peroxidation (-78.28 % of MDA reduction at 200 µg/mL, P<0.001). DDPH radical was scavenged by extract with IC<sub>50</sub> of 21.25 ± 0.41 µg/mL, close to that of the standard ascorbic acid (20.60 ± 0.26 µg/mL). The TAC of *S. setigera* hydroethanolic extract expressed as equivalent of standard ascorbic acid was 102.44 ± 19.48 µg AAE/mg. The IC<sub>50</sub> found for *S. setigera* (139.68 ± 0.36 µg/mL) when inhibiting RBCs hemolysis is very close to that of Diclofenac (128.41 ± 0.21 µg/mL). Among polyphenolic compounds quantified, flavonoids were well represented with concentration of 172.38 ± 8.36 µgRE/mg. The presence of these polyphenolics may explain good antioxidant and anti-inflammatory activities recorded, confirming its suitable usage in the local pharmacopoeia and making *S. setigera* a good candidate for neuroprotective drugs discovery.

**Keywords:** *Sterculia setigera*, neuroprotection, lipid peroxidation, antioxidant, anti-inflammatory.

## INTRODUCTION

Despite decades of active research, the identification of neuroprotective molecules/treatment remains a priority objective to provide therapeutic solutions to the millions of people worldwide suffering from neurodegenerative diseases. Considering that oxidative stress and inflammation remains major deleterious process in brain degeneration<sup>1,2</sup>, plants which often contain antioxidants and anti-inflammatory compounds may represent good candidates for neuroprotection studies. In a previous ethnopharmacological survey, we found that in Togo, local populations used *Sterculia setigera* Del. (Sterculiaceae) to manage central nervous system (CNS) disorders in general and neurodegenerative diseases in particular<sup>3</sup>. This plant is a wild tree commonly found in the Sudano-Sahelian zone of Africa. In Togo, *S. setigera* is more present in the savannas of the north where its name in Moba is “Natouféglique”. It is called “ponsemporgo” in Mooré (Burkina-Faso), “kukkuki” in Hausa (Niger, Nigeria), “kôgurani” in Bambara (Mali) and “mbep” in Wolof (Senegal). Ethnobotanical studies report the use of this gum for the treatment of snake bites, jaundice and bronchitis, when several studies have detected the presence of active

metabolites in stem barks of *S. setigera* such as tannins, saponins, glycosides and anthraquinone<sup>4</sup>. Traditional healers have mentioned the use of *S. setigera* leaves extracts for the treatment of epileptic conditions<sup>5</sup> and for the treatment of memory loss and dementia<sup>3</sup>. However, the potential neuroprotective properties of *S. setigera* have not been tested. The aim of this study was to investigate the neuroprotective potential of *S. setigera* leaves hydroethanolic extract in *ex vivo* and *in vitro* conditions.

## MATERIALS AND METHODS

### Plant material and extraction

#### Plant material

*Sterculia setigera*'s leaves were harvested in Dapaong (TOGO) in the month of October 2021. A voucher specimen was deposited, after identification, in the herbarium of the Laboratory of Botany and Plant Ecology (University of Lomé-TOGO) under the number 08655TG.

## Extraction

400 g of the powder were macerated in a volume of 4 L of an ethanol-distilled water mixture (80:20, v/v) for 72 hours with an intermittent manual stirring. The macerate obtained was filtered and evaporated under vacuum at 45°C using a rotary evaporator. The evaporated extract was lyophilized to obtain a dry extract stored in the refrigerator at 4°C until use.

## Ex vivo neuroprotection by inhibition of lipid peroxidation

### Brain homogenates

Animals (wistar rats) were anesthetized with ether before being decapitated. Brains were quickly removed, rinsed in an ice-cold normal saline solution (NaCl 9 g/L). 4 mg of brain tissues were then ground in 1 mL of 150 mM Tris-HCl solution pH=7.4 for lipid peroxidation assay, and in 0.2 M Tris-HCl solution pH = 8.2 for Glutathione assay. The homogenates were stored at -20°C until use.

### Ascorbic acid/Fe<sup>2+</sup> (AA-FeCl<sub>2</sub>) induced lipid peroxidation in rat brain homogenate

The anti-lipid peroxidation effect of *S. setigera* extract was studied using colorimetric method<sup>6</sup> of Kpemissi et al. (2019). The investigational tubes contained 500 µL of brain homogenate, 200 µL of Tris-HCl buffer (pH 7.4), 100 µL of 0.1 mM ascorbic acid, 100 µL of 4 mM FeCl<sub>2</sub> and 100 µL of different amount of *S. setigera* extract or quercetin. The samples were kept at 37 °C for one hour. After the incubation time, the malondialdehyde (MDA) concentration in the samples was estimated as previously described using 1,1,3,3-tetra-methoxypropane to make a standard curve (Kpemissi et al., 2019) at concentration points: 0.625; 1.25; 2.5; 5 and 10 nM. Absorbances were read at 586 nm using a UV-VIS spectrophotometer (Genesys 20, Thermo Scientific).

## In vitro anti-inflammatory tests

### Inhibition of egg albumin denaturation

This assay was realized using egg albumin denaturation method<sup>7</sup>. The reaction mixture was composed of 0.2 mL of fresh chicken's egg albumin, 2.8 mL phosphate buffer saline (pH 6.4) and 2 mL of various concentrations (25 - 400 µg/mL) of CM extract or standard drugs. All the samples were set aside at 37 °C for 25 minutes followed by heating for 5 minutes at 70 °C. The cooled solutions were centrifuged at 3,000 rpm for 10 minutes. Then the absorbance of supernatant solutions was measured at 660 nm. The % of inhibition and IC50 values were calculated using the equation:

$$\% \text{ inhibition} = ((A_0 - A_e) * 100) / A_0.$$

Where A<sub>0</sub> represents the absorbance of control tube without extract or Diclofenac, and A<sub>e</sub> represents the absorbance in the presence of the extract or Diclofenac. The tests were repeated three times.

### Inhibition of red blood cells hemolysis

The anti-inflammatory activity was assessed using red blood cells (RBCs) membrane stability method<sup>8</sup> according to the protocol of Shinde et al. (1999). The equal volume of rat blood and 0.9% w/v normal saline was taken in heparinized tubes. The blood was centrifuged for 10 min at 3,000 rpm. Then the suspended cells were washed carefully with saline three times. Finally, 10% v/v cell suspension was prepared using saline and used in heat-induced hemolysis assay. Briefly, the assay mixture contained 1mL of RBCs suspension and 1mL *S. setigera* extract or standard drug solutions (50- 1,000 µg/mL). The tube samples were kept at 70 °C in the bath for 30 minutes. After cooling and centrifuging for 5 minutes at 3,000 rpm, the absorbance was measured at 560 nm using UV

spectrophotometer (Dadoriya et al., 2020). In addition, the % inhibition of hemolysis and IC50 values were calculated using the same equation described in the assay of egg albumin denaturation.

## In vitro antioxidant tests

### DPPH radical scavenging activity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) test was realized<sup>9</sup> according to the method of Sen M and Dastida (2010). To 1.5 mL of the DPPH solution (100 µmol/L), 0.25 mL of methanol (white) or 0.25 mL of methanolic solution of *S. setigera* (25; 50; 100 and 200 µg/mL) were added into tubes. Ten minutes (10 min) after incubation at room temperature, absorbances were read at 517 nm. Ascorbic acid (200 µg/mL) served as reference antioxidant. The percentage inhibition of the DPPH radical was calculated using the equation:

$$\% \text{ inhibition} = ((A_0 - A_e) * 100) / A_0.$$

Where A<sub>0</sub> represents the absorbance of DPPH alone, and A<sub>e</sub> represents the absorbance in the presence of the extract or ascorbic acid. The tests were repeated three times.

### Ferric ions reducing power assay

The ability to reduce ferric ions was measured using the method described by Nisha et al. (2012) and slightly modified<sup>10</sup>. The FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ (tripyridyl triazine) solution, and 20.0 mM FeCl<sub>3</sub>.6H<sub>2</sub>O solution in a 10:1:1 volume ratio. *S. setigera* at different concentrations (25; 50; 100; 200; 500 µg/mL) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. The higher the absorbance of the reaction mixture, the greater the reducing power. Ascorbic acid was used as a positive control. The tests were repeated three times.

### Determination of total antioxidant capacity (TAC)

The phosphomolybdenum method was used to access the total antioxidant capacity of the extracts<sup>11</sup>. A 0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (Genesys 20, Thermo Scientific) against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The total antioxidant activity is expressed as the number of microgram equivalents of ascorbic acid or vitamin E used as reference antioxidants. The calibration curve was prepared by mixing ascorbic acid or vitamin E (0; 25; 50; 100; 200, 500 and 1000 µg/mL) with methanol.

## Quantitative dosage of phytochemicals

### Quantitative determination of total flavonoids

The determination of total flavonoids was carried out according to the colorimetric method, basing on their ability to chelate metals including aluminum<sup>12</sup>. To 2 mL of the ethanolic solution of *S. setigera* (1 mg/mL) or of rutin (1 mg/mL), were added 2 mL of aluminum chloride (20 mg/mL) and 6 mL of sodium acetate (50 mg/mL). The blank was made in the same way without *S. setigera*, replaced by 2 mL of ethanol. After an incubation time of 150 minutes, absorbances were read at 440 nm. The total flavonoid content of *S. setigera* was determined from the linear regression equation of the calibration curve established with rutin (0-100 µg/mL) and expressed in µg of rutin equivalent per milligram of dry extract (µg Eq/mg of

extract). Rutin served as a reference compound. The tests were repeated three times.

#### Quantitative determination of total phenols and tannins

The determination of total phenols was based on the Folin-Ciocalteu method<sup>13</sup>. Total phenols were assayed after fixing the tannins with PVPP (polyvinylpolypyrrolidone) following two steps. Firstly, to tubes containing 10 mg of PVPP diluted in 500  $\mu$ L of methanol, 500  $\mu$ L of *S. setigera* 1 mg/mL were added. The mixture thus obtained was homogenized and incubated on ice for 30 minutes. After centrifugation, 200  $\mu$ L of the supernatant were transferred into dry tubes for the assay with the Folin-Ciocalteu reagent. The blank didn't contain *S. setigera*, replaced by 500  $\mu$ L of methanol. Secondly, Folin-Ciocalteu (200  $\mu$ L) was added to 200  $\mu$ L of *S. setigera* (1mg/mL) or to 200  $\mu$ L of the Gallic Acid solutions (200, 150, 100, 50, 25 and 0  $\mu$ g/mL) or 200  $\mu$ L of the solution obtained during the previous step (extract + PVPP). The mixtures were incubated at room temperature for 15 minutes. After incubation, 800  $\mu$ L of sodium carbonate solution (700 mM)

were added to the mixtures. The blank was prepared with 200  $\mu$ L of Folin-Ciocalteu, 200  $\mu$ L of methanol and 800  $\mu$ L of the sodium carbonate solution (700 mM). Absorbances were read at 735 nm. The quantity of total phenol is expressed in mg equivalent of Gallic Acid/g of extract. The total amount of tannin was calculated using the formula:

$$DOT = DO_{Ext} - (DO_{Ext} + PVP)$$

Where DOT = DO tannins; DO<sub>Ext</sub> = DO extracted; DO<sub>Ext</sub> + PVP = DO extracted + PVP

## RESULTS

#### Lipid peroxidation inhibition

Peroxidation of lipids contained in brain homogenates have significantly raised in the concomitant presence of Ascorbic acid and FeCl<sub>2</sub> (AA-FeCl<sub>2</sub>) as shown by Figure 1. Incubation with total hydroethanolic extract of *S. setigera* or standard antioxidant Quercetin have reversed the lipoperoxidation (-78.28 % and -86.23 % at 200  $\mu$ g/mL, P<0.001 respectively).

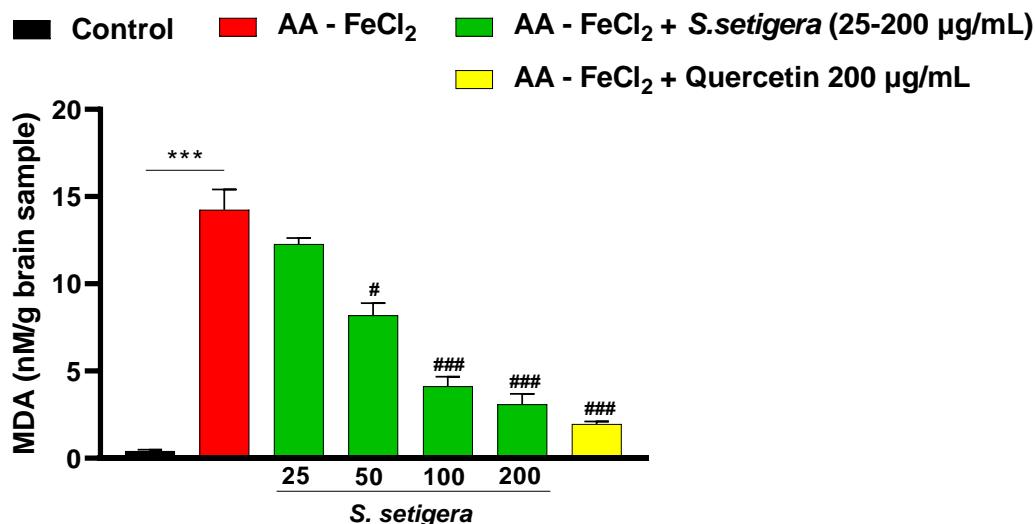


Figure 1: Inhibition of lipid peroxidation in brain homogenates by *S. setigera*

Values are expressed as Means  $\pm$  SEM, n = 3. Units: nM/mg brain samples. \*\*\*P<0.001: vs Control; # P<0.05 and ###P<0.001: vs AA-FeCl<sub>2</sub>.

#### Anti-inflammatory activities

Table 1 shows that the extract inhibited the denaturation of chicken egg albumin and stabilized the red blood cell

membrane against hemolysis induced by heat. The IC<sub>50</sub> found for *S. setigera* ( $139.68 \pm 0.36$   $\mu$ g/mL) when inhibiting RBCs hemolysis is very close to that of Diclofenac ( $128.41 \pm 0.21$   $\mu$ g/mL).

Table 1: Inhibition of albumin denaturation and RBCs hemolysis by *S. setigera*

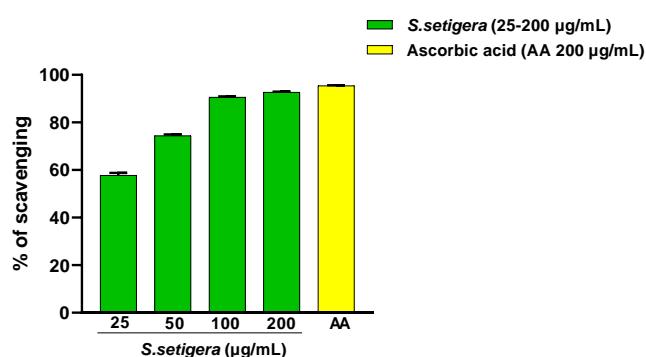
Inhibition of albumin denaturation		Inhibition of RBCs hemolysis		
<i>S. setigera</i>	Diclofenac	<i>S. setigera</i>	Diclofenac	
IC <sub>50</sub> ( $\mu$ g/mL)	$790.28 \pm 2.72$	$386.00 \pm 2.02$	$139.68 \pm 0.36$	$128.41 \pm 0.21$

Values are expressed as Means  $\pm$  SEM, n = 3. Units: IC<sub>50</sub> are expressed as  $\mu$ g/mL of *S. setigera* ( $\mu$ g/mL).

#### DPPH radical scavenging activity

Figure 2 shows the free radical scavenging activity of the hydroethanolic extract of *S. setigera* and standard Ascorbic acid (AA). Among these two compounds, Ascorbic acid activity

( $95.48 \pm 0.08$  %) was higher than that of the extract ( $92.67 \pm 0.17$  %) at the same concentration of 200  $\mu$ g/mL. The IC<sub>50</sub> of *S. setigera* extracts ( $21.25 \pm 0.41$   $\mu$ g/mL) closely resembled that of the standard ( $20.60 \pm 0.26$   $\mu$ g/mL).

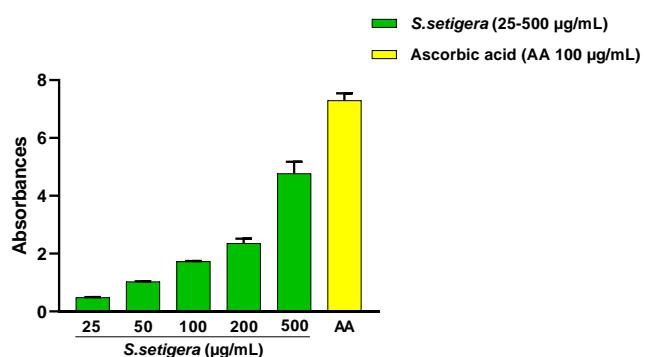


**Figure 2: Determination of DPPH radical scavenging activity of *S. setigera***

Values are expressed as Means  $\pm$  SEM, n = 3. Units: µg/mL

#### Ferrous reducing antioxidant capacity

A higher absorbance indicates a higher Ferrous reducing power (FRAP). Figure 3 indicates moderate to high FRAP with increased concentration of the extracts. At 500 µg/mL, the absorbance of *S. setigera* hydroethanolic extract was  $4.77 \pm 0.40$ .



**Figure 3: Absorbance of FRAP of *S. setigera* extract at different concentrations**

Values are expressed as Means  $\pm$  SEM, n = 3. Units: µg/mL.

#### Total antioxidant capacity

The TAC of *S. setigera* hydroethanolic extract expressed as equivalent of standards used (Vitamin E and Ascorbic acid) are presented in Table 2.

**Table 2: TAC of *S. setigera* extract**

	Vitamin E Equivalent (µg Vit.EE/mg)	Ascorbic acid Equivalent (µg AAE/mg)
TAC	$96.21 \pm 5.56$	$102.44 \pm 19.48$

Values are expressed as Means  $\pm$  SEM, n = 3. Units: TAC are expressed as Vitamin E Equivalent (µg Vit.EE/mg) and Ascorbic acid Equivalent (µg AAE/mg) (µg/mL).

#### Total phenolic, flavonoid and tannins contents

Table 3 shows the total polyphenols, flavonoid and tannins contents in the hydroethanolic extracts of *S. setigera* expressed as Gallic acid Equivalent (GAE) and Rutin Equivalent (RE). Flavonoids ( $172.38 \pm 8.36$  µgRE/mg) are well represented.

**Table 3: Polyphenol contents of *S. setigera* extracts**

Compounds	<i>S. setigera</i>	Reference compound
Total phenols	$101.93 \pm 3.07$ µgGAE/mg	Gallic acid
Flavonoids	$172.38 \pm 8.36$ µgRE/mg	Rutin
Tannins	$10.93 \pm 0.72$ µgGAE/mg	Gallic acid

Values are expressed as Means  $\pm$  SEM, n = 3. Units: total phenols and tannins are expressed as µg Gallic acid equivalent/mg of *S. setigera* (µgGAE/mg), while flavonoids are expressed as µg Rutin equivalent/mg of *S. setigera*.

## DISCUSSION

Free radicals' production is one of the well-known outcomes of brain's high consumption of oxygen and glucose<sup>14,15</sup>. This important demand of oxygen in particular for ATP production, as neuronal activity fails quickly when privates in transient ischemia, leads unfortunately to the rise of free radical and non-radicals, notably superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ) and hydroxyl ( $.OH$ )<sup>16</sup>. Such species, usually considered to constitute the "dark side" of  $O_2$  biochemistry, can initiate disruptive peroxidation reactions with various substrates important to the survival of cells such as proteins, lipids and nucleic acids. Lipids peroxidation especially may then be used as a good biomarker of brain oxidation status. In our *ex vivo* studies, ascorbic acid/ $Fe^{2+}$ -induced lipid peroxidation in wistar rat's brain homogenates was significantly reduced by *S. setigera* hydroethanolic extract. Malondialdehyde (MDA), the end-product of lipid peroxidation, was significantly lowered by concomitant incubation of brain homogenates with extract at doses ranging from 50 to 200 µg/mL. This significant antioxidant neuroprotection afforded by *S. setigera* hydroethanolic extract could be linked to its antioxidant capacities, as confirmed by *in vitro* antioxidant tests. In fact, the extract showed a strong scavenging activity, with an inhibitory concentration ( $IC_{50}$ ) of DPPH free radicals very close to that of the standard antioxidant used, ascorbic acid. *S. setigera* also demonstrated its capability to reduce ferric ions, well known as prooxidant. Through Fenton-type reactions, the conversion of  $Fe^{2+}$  into  $Fe^{3+}$  can generate free radicals, leading to increased peroxidation and lipids damages<sup>17</sup>. Then, by reducing ferric ions in FRAP test, *S. setigera* confirms its good antioxidant capacities and gives credence to its folkloric usage.

Moreover, the *S. setigera* hydroethanolic extract has proved in this study it's *in vitro* anti-inflammatory capacities. The capability of this extract to counteract RBCs hemolysis in particular was remarkable, with an  $IC_{50}$  close to that of standard anti-inflammatory drug Diclofenac. Elsewhere, Henneh et al. (2018) also showed that the stem bark extract of *Sterculia setigera* (30, 100 and 300 mg/kg, p.o.) exhibits *in vivo* anti-inflammatory activity by reducing rats paw oedema in the carrageenan and prostaglandin E2-induced inflammation tests<sup>18</sup>. These antioxidants and anti-inflammatory activities should be linked to its phytochemical composition, namely the presence of flavonoids in high concentration in our study. Flavonoids are natural components that received great attention in the last decades. They are well known to prevent oxidative stress and cell death by scavenging ROS, chelating metal ions and quenching singlet oxygen<sup>19</sup>. Catechin for example, an abundant flavonoid, has been shown to have an important antioxidant and anti-inflammatory<sup>20</sup>. As oxidative stress and inflammatory processes are among the most deleterious in neurodegeneration, our results showing antioxidant, anti-inflammatory and antilipoperoxidative

effects, make *S. setigera* a good candidate for neuroprotective studies and neuroactive drug discovery.

## CONCLUSION

*S. setigera* hydroethanolic extract protected the brain homogenate against lipid peroxidation induced by ascorbic acid/Fe<sup>2+</sup> mixture *ex vivo*. The plant extract also exhibited *in vitro* antioxidant and anti-inflammatory properties. These preliminary observations confirm the use of this plant in traditional medicine strategies against central nervous system disorders in general and neurodegenerative diseases in particular. Further *in vivo* investigations are needed to reveal its neuroprotective effects in models of animal's neurodegeneration and its chemical composition for potential drug discovery.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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