



Journal of  
**Pharmacology and  
Toxicology**

ISSN 1816-496X



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## Brain Protective and Erythrocytes Hemolysis Inhibition Potentials from Galls of *Guiera senegalensis* J.F. Gmel (Combretaceae)

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### ABSTRACT

The purpose of the present study was to investigate the protective effects of the extracts against H<sub>2</sub>O<sub>2</sub>-induced hemolysis of erythrocytes and the neuroprotective potential from galls of *Guiera senegalensis*. Ethyl acetate fraction of aqueous decoction extract (EAF/ADE) significantly ( $p < 0.05$ ) inhibited lipid peroxidation in rat's brain homogenate *in vitro* ( $37.79 \pm 0.93\%$  at  $1.25 \text{ mg mL}^{-1}$ ) when compared to the gallic acid and quercetin used as positive controls and has the best anti-acetylcholinesterase activity ( $64.02 \pm 4.07\%$  at  $100 \text{ } \mu\text{g mL}^{-1}$ ). Under the oxidative action of H<sub>2</sub>O<sub>2</sub>, the extracts from galls of *G. senegalensis* showed significant protection of the erythrocyte membrane from hemolysis. The Aqueous Decoction Extract (ADE) contains the highest amount of total tannin ( $25.3 \pm 1.55 \text{ mg TAE/100 mg of extract}$ ) content. The erythrocytes hemolysis inhibitory property from galls of *G. senegalensis* seems to be related weakly to its total tannin content ( $p < 0.05$ ). The present study thus suggested that the galls from *G. senegalensis* could be used as a new potential source of natural neuro-protective and antioxidant components.

**Key words:** *Guiera senegalensis*, galls, brain protective potential, erythrocytes hemolysis inhibition, tannin content

### INTRODUCTION

Plants materials are a rich source of phytochemicals such as phenolic compounds, vitamins, anthocyanins which scavenge free radicals. Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers and other degenerative diseases (Lim and Murtijaya, 2007). The researchers have showed that free radicals can lead to cell degeneration, especially in the brain (Obboh, 2008). Neurons are the first cells to be affected by a shortage of antioxidants and are most susceptible to oxidative stress because the brain has limited access to the bulk of antioxidants produced by the body (Obboh, 2009).

In the healthy brain, acetylcholinesterase (AChE) is the most important enzyme regulating the level of acetylcholine (Adersen *et al.*, 2007). The inhibition of this enzyme is considered as a promising approach for the treatment of Alzheimer's Disease (AD) and for the other possible therapeutic applications in the treatment of Parkinson's disease, ageing and myasthenia gravis (Ahmad *et al.*, 2003).

Erythrocytes became an useful general model to evaluate the effects of Reactive Oxygen Species (ROS) and antioxidants on a very accurate cellular system among the different models proposed. Erythrocytes represent an important component of the antioxidant capacity of blood, comprising the glutathione system and intracellular enzymes (Suwalsky *et al.*, 2006; Rodriguez *et al.*, 2006). The erythrocyte is considered as an *in vitro* excellent model to study the oxidant/antioxidant interaction since its membrane contains abundant polyunsaturated fatty acids (LH) which are extremely susceptible to free radical-mediated peroxidation (Liu *et al.*, 2002; Magalhaes *et al.*, 2009). It is an excellent model for the study of biomembrane toxicity *in vitro* (Singh and Rajini, 2008). A potential source of AChE inhibitors and antioxidant compounds are certainly provided by the abundance of plants in nature.

*Guiera senegalensis* (Combretaceae) is a well known medicinal plant and have been used in Burkina Faso as antioxidant and anti-inflammatory agent since ancient times (Sombie *et al.*, 2011). Clinically, *G. senegalensis* is used to treat fatigue, depression, anemia, nervous system disorders and bacterial diseases. The aim of this study was to assess brain protective potential and erythrocytes hemolysis inhibition activity from galls of *Guiera senegalensis* J.F. Gmel (Combretaceae). The contribution of total tannin content to brain protective effect and erythrocytes hemolysis inhibition activities was studied using linear regression analysis.

## MATERIALS AND METHODS

**Chemical and reagents:** Acetylthiocholine iodide (ATCI), Acetylcholinesterase (AChE), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma. Tris-HCl, tannic acid, gallic acid, quercetin, trichloroacetic acid, 2-thiobarbituric acid, bovine serum albumin (BSA) were purchased from Sigma-Aldrich Chemie, Steinheim (Germany), ferrous chloride (FeCl<sub>2</sub>) were from Prolabo, Paris (France). All others reagents were of analytical and HPLC grades.

**Sample preparation:** The plant material is constituted of galls of *Guiera senegalensis* and collected in August 2009. The galls were dried and ground to powder. The obtained powder was extracted with acetone 80% via maceration (hydroacetic extract) and distilled water via decoction (aqueous decoction extract). The hydroacetic and the aqueous decoction extracts obtained were respectively dissolved in distilled water and successively extracted with the ethyl acetate and butanol. Each extract was dried to give: Ethyl Acetate Fraction (EAF), butanol fraction (BF) and final Water Fraction (WF). The obtained extracts were stored in a refrigerator at +4°C until use.

**Inhibition of erythrocyte hemolysis in rat blood:** The assay carrying out based on the procedure reported by Su *et al.* (2009). The blood sample was obtained from rat and made of 0.5% erythrocyte suspension for the assay. The reaction mixture was consisted of 1.0 mL of erythrocyte suspension (0.5%), 1.0 mL of extract (0.1 mg mL<sup>-1</sup>) and 0.1 mL of H<sub>2</sub>O<sub>2</sub> (100 mM). The mixture was incubated at 37°C for 60 min and then 8.4 mL of distilled water was added to the mixture and centrifuged at 1000 rpm for 10 min. The absorbance of the supernatant was read at 415 nm.

The percentage of erythrocyte hemolysis inhibition effect was calculated according to the following equation:

$$\text{Inhibition rate\%} = [1 - (A_1 - A_2) / A_0] \times 100$$

where,  $A_0$  is the absorbance of the supernatant without extract,  $A_1$  is the absorbance of the extract addition and  $A_2$  is the absorbance of extract solution.

### Brain protective potential

**Inhibition of lipid peroxidation in rat brain homogenate:** The inhibitory activity of extracts or fractions on lipid peroxidation (LPO) was determined according to the thiobarbituric acid method (Su *et al.*, 2009).  $\text{FeCl}_2\text{-H}_2\text{O}_2$  was used to induce the liver homogenate peroxidation according to the procedure of Sombie *et al.* (2011). In this method, 0.2 mL of extract or fraction at the concentration of  $1.25 \text{ mg mL}^{-1}$  was mixed with 1.0 mL of 1% brain homogenate (each 100 mL homogenate solution contains 1.0 g of rat brain), then 50  $\mu\text{L}$  of  $\text{FeCl}_2$  (0.5 mM) and 50  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  (0.5 mM) were added. The mixture was incubated at  $37^\circ\text{C}$  for 60 min, then 1.0 mL of trichloroacetic acid (15%) and 1.0 mL of 2-thiobarbituric acid (0.67%) was added and the mixture was heated up in boiled water for 15 min. The absorbance was recorded at 532 nm. Quercetin and gallic acid were used as the positives controls. The percentage of inhibition effect was calculated according to following equation:

$$\text{Inhibition rate\%} = [1 - (A_1 - A_2)/A_0] \times 100$$

where,  $A_0$  is the absorbance of the control (without extract),  $A_1$  is the absorbance of the extract addition and  $A_2$  is the absorbance without brain homogenate.

**Acetylcholinesterase inhibitory activity:** The inhibitory effect of extracts or fractions from galls of *Guiera senegalensis* on acetylcholinesterase (AChE) activity was evaluated using the procedure reported by Lopez *et al.* (2002) with some modifications. Briefly, 100  $\mu\text{L}$  of extract ( $0.1 \text{ mg mL}^{-1}$  in 50 mM Tris-HCl, pH 8 buffer, 10% methanol) was mixed with 100  $\mu\text{L}$  of AChE ( $0.22 \text{ U mL}^{-1}$  in 50 mM Tris-HCl, pH 8 buffer, 0.1% BSA) and 200  $\mu\text{L}$  of buffer (50 mM Tris-HCl, pH 8, 0.1% BSA). The mixture was incubated for 5 min at  $30^\circ\text{C}$  in a 1 mL cuvette. Subsequently, 500  $\mu\text{L}$  of DTNB (3 mM in Tris-HCl, pH 8 buffer, 0.1 M NaCl, 0.02 M  $\text{MgCl}_2$ ) and 100  $\mu\text{L}$  of ATCI (15 mM in water) were added. A blank was also prepared by replacing AChE with 100  $\mu\text{L}$  of buffer (50 mM Tris-HCl, pH 8 buffer, 0.1% BSA). The reaction was monitored for 5 min at 405 nm and velocity ( $V_0$ ) recorded. Buffer (0.1% in 50 mM Tris-HCl, pH 8, 10% methanol) was used as negative control. Antiacetylcholinesterase activity (I%) was calculated following the equation:

$$I (\%) = \frac{V_{0\text{Control}} - V_{0\text{Sample}}}{V_{0\text{control}}} \times 100$$

All the reactions were performed in triplicate and data were presented as Mean $\pm$ Standard deviation.

**Determination of tannins contents in the extracts:** The method of reference of the European Commission (1984) was used to determine the total tannin content. The total tannin was determined using tannic acid as standard.

Two hundred microliters of extract or fraction was mixed with 1000  $\mu\text{L}$  of water, 200  $\mu\text{L}$  of ferric ammonium citrate ( $3.5 \text{ g L}^{-1}$ ) prepared freshly and 200  $\mu\text{L}$  of ammoniac ( $8 \text{ g L}^{-1}$ ). The absorbance

of the mixture was measured at 525 nm. The results were expressed in mg tannic acid equivalent (TAE) per 100 mg of extract or fraction (mg TAE/100 mg of extract or fraction).

**Statistical analysis:** Statistical analyses were performed using ANOVA one way (Fisher LSD). All data were expressed as Mean $\pm$ SD of at least three different determinations.

## RESULTS AND DISCUSSION

**Erythrocytes hemolysis inhibition activity from galls of *Guiera senegalensis*:** The *in vitro* oxidative hemolysis of rat erythrocytes was used here as a model to study the free radical-induced damage of biological membranes and the protective effect of extracts from galls of *G. senegalensis*. The amount of hemolysis of H<sub>2</sub>O<sub>2</sub>-induced was reduced in the presence of the different extracts and fractions used in our study.

Table 1 shows the value of the erythrocytes hemolysis inhibitory percent of various extracts and fractions of *G. senegalensis*. The aqueous decoction extract showed the best protective activity against the erythrocytes hemolysis (83.13 $\pm$ 1.27%) at a concentration of 250  $\mu$ g mL<sup>-1</sup>. There was a significant difference between hemolytic reduction using Aqueous Decoction Extract (ADE) and the others fractions from galls of *G. senegalensis* excepted the hydroacetonic extract (HAE). However, percentages of hemolysis inhibition of different extracts and fractions were not significantly higher than ascorbic acid used as positive control in terms of hemolysis reduction.

The amount of hemolysis was reduced in a dose-dependent manner in the presence of the aqueous decoction extract and ascorbic acid used as positive reference. The aqueous decoction extract (ADE) reduced the erythrocytes hemolysis more than the ascorbic acid in all the tested concentration (200-1200  $\mu$ g mL<sup>-1</sup>). Figure 1 shows the relationship between the inhibitory percentage and the corresponding concentration of aqueous decoction extract from galls of *G. senegalensis*.

Accordingly, it is quite possible that the location of the extracts components into the membrane bilayer and the resulting restriction on its fluidity might hinder the diffusion of H<sub>2</sub>O<sub>2</sub> and its consequent damaging effects (Singh and Rajini, 2008).

At concentrations of 125  $\mu$ g mL<sup>-1</sup>, the aqueous decoction extract from galls of *Guiera senegalensis*, inhibited the erythrocytes hemolysis (76.46 $\pm$ 1.38) more than those of *Pinus koraiensis*

Table 1: Erythrocytes hemolysis inhibition potential from galls of *G. senegalensis*

Extract/Fraction	Erythrocytes hemolysis inhibition (%)
HAE	80.53 $\pm$ 2.26 <sup>a</sup>
EAF/HAE	35.54 $\pm$ 2.41 <sup>b</sup>
BF/HAE	30.56 $\pm$ 2.54 <sup>c</sup>
WF/HAE	31.70 $\pm$ 1.04 <sup>c</sup>
ADE	83.13 $\pm$ 1.27 <sup>a</sup>
EAF/ADE	36.69 $\pm$ 1.46 <sup>b</sup>
BF/ADE	29.20 $\pm$ 2.36 <sup>c</sup>
WF/ADE	25.60 $\pm$ 1.45 <sup>d</sup>
Ascorbic acid	80.85 $\pm$ 1.65 <sup>a</sup>

Data are Mean $\pm$ SEM (n = 3). HAE: Hydroacetonic extract, EAF/HAE: Ethyl acetate fraction from HAE, BF/HAE: Butanol fraction from HAE, WF/HAE: Water fraction from HAE, ADE: aqueous decoction extract, EAF/ADE: Ethyl acetate fraction from ADE, BF/ADE: Butanol fraction from ADE, WF/ADE: Water fraction from ADE, Erythrocytes hemolysis inhibition potential expressed as percentage of inhibition at a concentration of 250  $\mu$ g mL<sup>-1</sup> of extract/fraction or ascorbic acid. Values showing the same letter are not significantly different (p>0.05)

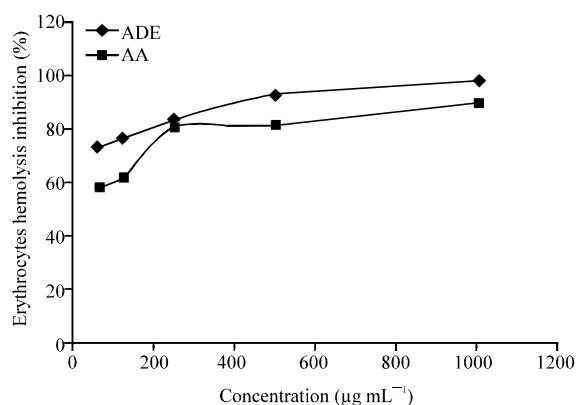


Fig. 1: Dose dependent manner of erythrocytes hemolysis inhibition (ADE); ADE: Aqueous decoction extract and AA: Ascorbic acid

Table 2: Brain protective effect from galls of *Guiera senegalensis*

Extracts and fractions	Anti-AChE activity (%)	Brain LPO inhibition (%)
HAE	28.80±01.22 <sup>b</sup>	37.31±0.80 <sup>a,b</sup>
EAF/HAE	14.40±05.50 <sup>c,d</sup>	35.00±1.69 <sup>b</sup>
BF/HAE	32.40±03.62 <sup>b</sup>	32.49± 2.37 <sup>d,e</sup>
WF/HAE	18.01±03.23 <sup>c,d</sup>	34.36±0.38 <sup>c,d</sup>
ADE	20.39±04.23 <sup>c</sup>	28.39 ±1.03 <sup>f</sup>
EAF/ADE	64.02±4.07 <sup>a</sup>	37.79±0.93 <sup>a</sup>
BF/ADE	10.38±6.91 <sup>d</sup>	34.51±1.12 <sup>c,d</sup>
WF/ADE	10.79±03.09 <sup>d</sup>	31.24±1.39 <sup>e</sup>
Gallic acid	Nd	27.00±2.90 <sup>f</sup>
Quercetin	Nd	28.75±0.10 <sup>f</sup>

Data are mean±SEM (n = 3). HAE: Hydroacetonic extract, EAF/HAE: Ethyl acetate fraction from HAE, BF/HAE: Butanol fraction from HAE, WF/HAE: Water fraction from HAE, ADE: aqueous decoction extract, EAF/ADE: Ethyl acetate fraction from ADE, BF/ADE: Butanol fraction from ADE, WF/ADE: Water fraction from ADE, Acetylcholinesterase (ACHE) inhibitory activity expressed as percentage of inhibition at a concentration of 100 µg mL<sup>-1</sup>, Nd: Not determined, Anti-Lipid peroxidation (LPO) of rat brain expressed as percentage of inhibition at a concentration of 1.25 mg mL<sup>-1</sup> of extract or fraction, Values showing the same letter are not significantly different (p>0.05) from one other in the same columns

seed extract (59%) tested at the concentration of 200 µg mL<sup>-1</sup> (Su *et al.*, 2009). These results in support with those obtained by Su *et al.* (2009) suggest that the erythrocytes treatment with plants extracts particularly with aqueous decoction extract from galls of *G. senegalensis* showed dose dependent protection against the injurious effects of hydrogen peroxide.

It can be concluded that all the extracts and fractions tested have the anti-oxidative potential in the H<sub>2</sub>O<sub>2</sub>- induced hemolysis. The extracts from galls of *G. senegalensis* would not be toxic for the biomembrane but would thus play a protective role for erythrocytes.

### Brain protective potentiel

**Anti-Lipid peroxidation activity of brain rat's homogenate:** The process of peroxidation can result in damage to the biomembrane (liver, kidney and especially brain) and also is considered to be involved with aging and several clinically significant disorders, such as Parkinson's, Alzheimer's disease (Candan and Tuzmen, 2008).

The ability from galls of *G. senegalensis* to prevent lipid peroxidation in rat brain tissue

homogenate is shown in Table 2. All the extracts and positives controls were analyzed on what concerns their inhibition activity of lipid peroxidation at a concentration of  $1.25 \text{ mg mL}^{-1}$ . Values oscillating between  $27 \pm 2.29$  and  $37.79 \pm 0.93\%$  were obtained.

The ethyl acetate fraction from aqueous decoction extract (EAF/ADE) gave the significant best inhibition of lipid peroxidation of brain rat's homogenate among all the extracts and fractions except of the hydroacetonetic extract (HAE). All the extracts of *G. senegalensis* except the Aqueous Decoction Extract (ADE) significantly inhibits the lipid peroxidation of brain rat's homogenate when compared to gallic acid and quercetin. At the concentration of  $4 \text{ mg mL}^{-1}$ , the percentage inhibition of EAF/ADE was  $49.45 \pm 0.82\%$ . It inhibited in a dose-dependent manner lipid peroxidation of in brain tissue (Fig. 2).

$\text{FeCl}_2 \cdot \text{H}_2\text{O}_2$  system was used to induce lipid peroxidation in rat brain homogenate. The Fe (II) catalyzes one electron transfer reactions that generate reactive oxygen species, such as the reactive OH which is formed from  $\text{H}_2\text{O}_2$  through the Fenton reaction. The decrease of lipid peroxidation in the rat brain homogenates in the presence of the extracts could be as result of the ability of the extracts to chelate  $\text{Fe}^{2+}$  and/or scavenge free radicals produced by the Fe .<sup>2</sup>Previous studies indicated that the extracts from galls of *Guiera senegalensis* suppression of extracts contain polyphenols (flavonoids ) compounds that can scavenge free radicals and chelate metal ions (Sombie *et al.*, 2011). The *G. senegalensis* protective potential against lipid peroxidation of liver is higher when compared to the brain lipid peroxidation (Sombie *et al.*, 2011). This result could be explained by the fact that the Fe (II) is a potent initiator of LPO in brain (Oboh, 2008).

The extracts and fractions from galls of *Guiera senegalensis* showed a protective effect in brain homogenate against toxicity induced by hydrogen peroxide and iron.

**Acetylcholinesterase (AChE) inhibitory activity:** Inhibition of AChE serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease (Mukherjee *et al.*, 2007).

Table 2 shows the inhibitory effects of the extracts on AChE activity. All the extracts showed inhibitory effects. Extracts and fractions inhibitory activity were in order of efficacy: EAF/ADE>BF/HAE>HAE>ADE>WF/HAE>EAF/HAE>WF/ADE. Ethyl acetate fraction from aqueous decoction extract (EAF/ADE) showed strong inhibition of acetylcholinesterase with a percentage value of  $64.02 \pm 4.07$  at a final concentration of  $100 \text{ } \mu\text{g mL}^{-1}$ . Butanol fraction from

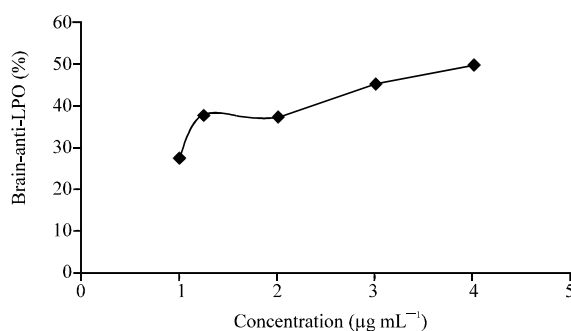


Fig. 2: Brain anti-lipid peroxidation of EAF/ADE from galls of *G. senegalensis*; EAF/ADE: Ethyl acetate fraction from aqueous decoction extract and LPO: Lipid peroxidation

aqueous decoction extract (BF/ADE) showed the weak inhibition of acetylcholinesterase with a percentage value of  $10.38 \pm 6.91$ . The percentage inhibition of EAF/ADE is significant higher than those of others extracts and fractions. The extracts from roots of *Stephania suberosa* Forman. and *Tabernaemontana divaricata* (L.) R.Br. Ex Roem and Schult showed high AChE inhibitory activity ( $91.93 \pm 10.80$  and  $93.50 \pm 0.37$ , respectively) at the concentration of  $100 \mu\text{g mL}^{-1}$  comparatively to the extracts from galls of *Guiera senegalensis* (Ingkaninan *et al.*, 2003).

A weak but significant correlation was found between the acetylcholinesterase inhibition activity and erythrocytes hemolysis inhibition potential ( $R^2 = 0.1894$ ;  $p < 0.034$ ) supporting a possible role of oxidative stress in erythrocytes as a peripheral marker of Alzheimer disease (Posser *et al.*, 2006).

From these results, it can be postulated that the ethyl acetate fraction from aqueous decoction extract (EAF/ADE) has a potential AChE inhibitory activity and could be used for the treatment of Alzheimer's disease.

**The contribution of total tannin content of brain protective and erythrocytes hemolysis inhibition activities:** Table 3 shows the total tannin content in the extracts and fractions from galls of *G. senegalensis*. The amount of total tannin content ranged from  $4.12 \pm 0.035$  mg TAE to  $25.3 \pm 1.55$  mg TAE. The Aqueous Decoction Extract (ADE) contain the highest of total tannin ( $25.3 \pm 1.55$  mg TAE/100 mg).

The participation of the total tannin content from galls of *G. senegalensis* in the brain protective potential and erythrocytes hemolysis inhibition activity was studied using a linear regression analysis. The correlations obtained between the total tannin content and acetylcholinesterase inhibitory activity were found to be very weak, less than 0.5 (data not shown). No significant correlation was obtained between the total tannins content and anti-lipid peroxidation of rat's brain homogenate.

These lows correlations values suggest that the total tannin content don't take part to the brain anti-lipid peroxidation effect and anti-acetylcholinesterase activity from galls of *G. senegalensis*. Galls of *Guiera senegalensis* contain probably some compounds which have protective effects on Fe (II) induced lipid peroxidation in rat brain and anti-AChE activity.

Candan and Tuzmen (2008) showed that the tannic acid protects against Al and Pb induced lipid peroxidation in brain tissues. Inhibition of peroxidation is one of the most important activities

Table 3: Total tannins content from galls of *G. senegalensis*

Extract/Fraction	Total tannins (mgTAE/100 mg of extract)
HAE	$22.43 \pm 0.058^b$
EAF/HAE	$20.30 \pm 0.14^d$
BF/HAE	$21.25 \pm 0.07^{c,d}$
WF/HAE	$21.50 \pm 0.28^{bc}$
ADE	$25.30 \pm 1.55^a$
EAF/ADE	$4.12 \pm 0.035^f$
BF/AD	$4.193 \pm 0.234^f$
WF/ADE	$18.97 \pm 0.61^e$

Data are mean  $\pm$  SEM (n = 3). HAE: Hydroacetic extract, EAF/HAE: Ethyl acetate fraction from HAE, BF/HAE: Butanol fraction from HAE, WF/HAE: Water fraction from HAE, ADE: aqueous decoction extract, EAF/ADE: Ethyl acetate fraction from ADE, BF/ADE: Butanol fraction from ADE, WF/ADE: Water fraction from ADE. Values showing the same letter are not significantly different ( $p > 0.05$ ) from one other in the same columns



underlying the health effects of tannins. Tannins at low concentration sometimes enhance the activities of enzymes, although they usually inhibit the activities of enzymes at moderate or high concentrations (Okuda, 2005). Our results don't support this report. The ethylacetate fraction from aqueous decoction extract (EAF/ADE) which possess the low tannin content has exhibited the highest anti-acetylcholinesterase activity. The aqueous decoction extract which possess the highest total tannin content has inhibited slightly the acetylcholinesterase activity. The anti-acetylcholinesterase activity from galls of *Guiera senegalensis* probably depend to tannin structures.

A weak but significant correlation ( $R^2 = 0.3817$ ,  $p = 0.001$ ) was obtained between the total tannin content and the erythrocytes hemolysis inhibition activity.

This report indicates that the total tannins content takes part weakly in the inhibition of erythrocytes hemolysis.

The molecular mechanisms of the action of tannin in the inhibition of erythrocytes hemolysis have not yet been fully elucidated. Fedeli *et al.* (2004) studied the influence of some tannin on the rate of hemolysis in stressed trout erythrocytes. Their results indicate that tannic acid accelerates the hemolytic event while gallic and ellagic acids have no significant effect. The presence of tannins mainly derived from gallic acid (gallic acid, 3-O-, 4-O-, 5-O-, 3,4-di-O-, 4,5-di-O-, 3,5-di-O-, 3,4,5-tri-O-and 1, 3, 4, 5-tetra-O-galloylquinic acids) in the galls extracts of *Guiera senegalensis* (Bouchet *et al.*, 1996; Lamien *et al.*, 2005) probably justifying the low participation of tannins content to the erythrocytes hemolysis inhibition.

## CONCLUSION

In conclusion, the results of this study represent the first evidence that extracts from galls of *G. senegalensis* possess effective anti-acetylcholinesterase, antilipid peroxidation in rat's brain homogenate and erythrocytes hemolysis inhibitory activities. Brain protection potential from galls of *G. senegalensis* does not seem more likely related to its total tannin content.

*G. senegalensis* extracts also play an antioxidative role to protect brain against  $\text{FeCl}_2 \cdot \text{H}_2\text{O}_2$ -induced oxidation and erythrocytes hemolysis induced by  $\text{H}_2\text{O}_2$ .

Future clinical investigations on this medicinal plant should be encouraged. The antioxidative mechanism of various extracts of GS in  $\text{FeCl}_2 \cdot \text{H}_2\text{O}_2$ -induced lipid peroxidation in rat's brain will be further studied in detail, but the obtained information may be useful in the clinical usage of *G. senegalensis*. More detailed phytochemical studies of the ethyl acetate fraction from aqueous decoction extract are thus necessary to identify the active principle (s) responsible for the acetylcholinesterase inhibitory.

## ACKNOWLEDGMENT

The Chinese embassy in Burkina Faso gratefully acknowledged for financial assistance to the first author. The first author also thanks Dr Lamien for the contribution to his formation in scientific research.

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