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Antioxidant and Anti-inflammatory Activities from Galls of *Guiera senegalensis* J.F. Gmel (Combretaceae)

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ABSTRACT

The galls of *Guiera senegalensis* J.F. Gmel (Combretaceae) are used in Burkina Faso traditional medicine to treat some microbial and metabolic diseases such as malaria, dysentery, diabetes and hypertension. The purpose of the present study was to investigate the phytochemical analysis, the antioxidant and anti-inflammatory inhibitory activities from the galls of *Guiera Senegalensis*. Phytochemical assay was conducted with following standard protocols. The method of DPPH, FRAP, β -carotene assay, anti-lipid peroxidation (according to the thiobarbituric acid method) studies in vitro were employed to assess the antioxidant efficacy. The xanthine oxidase and lipoxygenase inhibition properties of extracts and fractions were also evaluated to assess the anti-inflammatory activity from galls of *Guiera senegalensis*. Galls extracts of *G. senegalensis* are rich in totals polyphenol and in totals flavonoid content. The hydroacetic extract showed the most potent antioxidant activity in FRAP method (10.88 ± 0.86 mmol AEAC g^{-1}) and anti-lipid peroxidation activity ($75.06 \pm 2.42\%$) at a concentration of 1.25 mg mL^{-1} . The water fraction of hydroacetic extract showed the most potent antioxidant activity (6.012 ± 0.13 mmol AEAC g^{-1}) in the DPPH radical scavenging method and butanol fraction of hydroacetic extract in the β -carotene bleaching method ($59.00 \pm 3.27\%$) at a concentration of 100 μg mL^{-1} . The highest antioxidant activity can be positively correlated with the highest total phenolics content and also with the highest anti-inflammatory activity of the tested extract/fraction. The present study thus provides a scientific rationale for the traditional use of this plant product to the treatment of metabolic diseases.

Key words: *Guiera senegalensis*, galls, antioxidant activity, xanthine oxidase, lipoxygenase

INTRODUCTION

Guiera senegalensis (GS) is a traditional plant distributed widely in western Africa. In Burkina Faso traditional medicine, this plant is used to treat some diseases like cough, dysentery, malaria, diabetes and hypertension. The galls of *G. senegalensis* are used for the treatment of fowlpox and have antiseptic, antifungal activities. In addition, recent studies demonstrate that an aqueous

acetone extract from galls of *Guiera senegalensis* inhibits *in vitro* FowlPox Virus growth in secondary CES cells (Lamien *et al.*, 2005). Further pharmacological activities (antimicrobial properties for example) of leaves of *Guiera senegalensis* have also been shown (Kudi *et al.*, 1999; Fiot *et al.*, 2006). The antiplasmodial activity of infusion, decoction and chloroformic extract of stems and leaves of *Guiera senegalensis* has been demonstrated on two strains of *Plasmodium falciparum* (Benoit *et al.*, 1996; Fiot *et al.*, 2006). The chloroformic extract of roots and the isolated alkaloids harman and tetrahydroharman exhibited a significant antimalarial activity associated with a low cytotoxicity (Ancolio *et al.*, 2002). Aniagu *et al.* (2005) demonstrate that the aqueous extract of *Guiera senegalensis* protect the gastric mucosa of rats against ethanol-induced acute mucosal damage with a reduction of the ulcer index; so indicating that *Guiera senegalensis* could be an effective gastroprotective agent. The central sedative properties of *Guiera senegalensis* have already been demonstrated (Amos *et al.*, 2001). Various chemical constituents are found in the extracts from galls of *Guiera senegalensis* such as tannins, flavonoids, anthocyanidins, alkaloids and steroids (Lamien *et al.*, 2005).

Recently, a great interest has been given to naturally occurring antioxidants, which may play important roles in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes (Nsimba *et al.*, 2008). Edible plants contain a wide variety of chemicals such as flavonoids and other phenolic compounds that have potential antioxidant activity through a number of different mechanisms. The proposed mechanisms for their action include (1) direct radical scavenging, (2) inhibition of enzymes, such as NO-synthase, xanthine oxidase, cyclooxygenase and lipoxygenase, (3) iron chelation and (4) direct inhibition of lipid peroxidation (Xanthopoulou *et al.*, 2009). Several methods are available to evaluate antioxidant activities of natural compounds in foods or biological systems. Methods commonly used in antioxidant activity assays are the DPPH (2, 2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), β -carotene bleaching (BCB) procedures.

The DPPH method is based on the reduction of alcoholic DPPH solutions at 517 nm in the presence of an antioxidant that donate hydrogen or electron (Kulisic *et al.*, 2006). DPPH takes normally several hours for the reaction to be completed and colour interference of the DPPH assay with samples that contain anthocyanins leads to under-estimation of antioxidant activity (Teow *et al.*, 2007). DPPH method is independent of the substrate polarity. The BCB method is usually used to evaluate the antioxidant activity of compounds in emulsions, accompanied with the coupled oxidation of β -carotene and linoleic acid. Ascorbic acid, is a well known antioxidant polar compound, its antioxidant activity was not proved by this method. This can be explained by a phenomenon formulated as the »polar paradox«: non-polar antioxidants exhibit stronger antioxidative activities in emulsions because they concentrate at the lipid/ air surface, thus ensuring high protection of the emulsion itself (Kulisic *et al.*, 2006). FRAP is used in assay to assess the metal (iron exclusively) ions binding ability (Nsimba *et al.*, 2008).

The reducing power is generally associated with the presence of reductones, which exerts antioxidant action by breaking the free radical chain by donating a hydrogen atom (Prasad *et al.*, 2010). Xanthine Oxidase (XO) catalyzes oxypurines (hypoxanthine and xanthine) to uric acid in the purine catabolic pathway. Inhibition of XO activities decreases the uric acid levels and results in an anti-hyperuricemic effect (Zhu *et al.*, 2004). The XO plays an important role in various ischemic tissues, vascular injuries, inflammatory diseases, chronic heart failure, is a major source of free radicals (superoxide), its activity contributes significantly to the degree of oxidative stress, brain edema, thermal stress, respiratory syndrome, viral infection and hemorrhagic shock *in vivo* and it is also associated with gout (Naoghare *et al.*, 2010).

The products of 5-lipoxygenase including leukotrienes (LTs) constitute an important class of inflammatory mediators of asthma and various inflammatory diseases, contribute to vascular changes in inflammation (Li *et al.*, 2003).

The ethnomedicinal uses activities from the galls of GS suggest that the galls might possess antioxidant activities. The aim of the present study was to screen the phytochemical analysis and the antioxidant activities from galls of GS. This was performed by assessing the lipoxygenase (LOX) and the Xanthine Oxidase (XO) activities.

MATERIALS AND METHODS

Plant material: The galls of *Guiera senegalensis* were collected in kadiogo (province of Burkina Faso) in August 2009 and authenticated by professor Millogo-Rasolodimby, botanist at Ouagadougou University.

Chemical and reagents: Folin-Ciocalteu reagent, aluminium trichloride (AlCl_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), xanthine, gallic acid, quercetin, allopurinol, trichloroacetic acid, tween 40, 2-thiobarbituric acid, Xanthine oxidase (E.C. 1.1.3.22), Urethane were purchased from Sigma-Aldrich Chemie, Steinheim (Germany). Sodium carbonate, potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$], ascorbic acid, ferric chloride (FeCl_3), ferrous chloride (FeCl_2) were from Prolabo, Paris (France). Solvents used were supplied by Fluka Chemie, Buchs (Switzerland). β -Carotene type I, Linoleic acid, (+)- α -tocopherol from vegetable oil type V were from sigma (USA). 15-Lipoxygenase (EC 1.13.11.12) type I-B (Soybean) was purchased from Sigma (St. Louis, MO). All other reagents were of analytical and HPLC grades.

Animals: A group of 5 adult wistar rats (155-201 g) of either sex were used. The animals were obtained from the Laboratory of institute of research in health science (IRSS, Burkina Faso). The animals had free access to food and water and kept in a regulated environment at $25 \pm 1^\circ\text{C}$ under 12 h light/12 h dark conditions during 2 days for acclimatization. Rats were subjected to urethane anesthesia (1.5 g kg^{-1} of b.wt.). Liver was removed, washed with cold saline, quickly blotted and weighed. Liver was stored at -70°C for estimation of extracts/fractions inhibition of lipid peroxidation. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals.

Extraction and fractionation: The galls were dried and ground to powder. The obtained powder was extracted with selected solvents: acetone 80% and distilled water.

The first extraction was processed, using 50 g of powder in 500 mL of acetone/water (80/20) during 48 h under mechanical agitation at room temperature.

Fifty gram of powder were also extracted with 500 mL of distilled water by heating (100°C) for 30 min. The filtrate obtained using whatman filter paper was concentrated under reduced pressure in a rotary evaporator and lyophilized using a freeze drying system to give the hydroacetic extract (HAE) and aqueous decoction extract (ADE).

The hydroacetic and the aqueous decoction extracts were respectively dissolved in distilled water and successively extracted with ethyl acetate, 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 $+4^\circ\text{C}$ until use.

Determination of totals phenol, totals flavonoid content in the extracts: The total phenol content of extracts and fractions was determined as described by Singleton *et al.* (1999) using gallic acid as standard. The diluted aqueous solution of each extract or fraction (0.25 mL) was mixed with 1.25 mL of Folin Ciocalteu reagent (0.2 N). This mixed solution was allowed to stand at room temperature for 5 min and then 1 mL of sodium carbonate solution (75 g L⁻¹) was added. After 2 h incubation, the absorbance was measured at 760 nm against blank. A standard curve was plotted using gallic acid (0-200 mg L⁻¹). The results were expressed as mg of Gallic Acid Equivalents (GAE) per 100 mg of extract or fraction (mg GAE/100 mg extract or fraction).

Totals flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand *et al.* (1994). For each extract, 1 mL of methanolic solution (100 µg mL⁻¹) was mixed with 1 mL of aluminium trichloride (AlCl₃) in methanol (2%). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a 1 mL of methanol and 1 mL of plant extract without AlCl₃. The totals flavonoid content was determined on a standard curve using quercetin as a standard. The mean of three readings was used and expressed as mg of quercetin equivalents (QE) per 100 mg of extract or fraction (mg QE/100 mg extract or fraction).

Antioxidant activity

Iron (III) to iron (II) reduction activity: The ability of the extracts or fractions to reduce iron from the form (III) to the form (II) was assessed with the method of Hinneburg *et al.* (2006). A 0.5 mL aliquot of each extract dissolved in water was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of a 1% aqueous potassium hexacyanoferrate [K₃Fe (CN)₆] solution. After 30 min incubation at 50°C, 1.25 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 2000 g for 10 min.

A 1.25 mL aliquot of the upper layer was mixed with 1.25 mL of water and 0.25 mL of aqueous FeCl₃ (0.1%) and the absorbance was recorded at 700 nm. Iron (III) reducing activity was determined as mmol ascorbic acid equivalents per gram of extract or fraction (mmol AEAC g⁻¹ extract or fraction). The values are presented as the means of triplicate analyse.

Free radical-scavenging activity *in vitro*: The ability of the extracts or fractions to scavenge the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical was evaluated as described by Lamien-Meda *et al.* (2008).

Extracts or fractions were dissolved in methanol and 0.75 mL of each was mixed with 1.5 mL of a 0.02 mg mL⁻¹ solution of DPPH in methanol. The mixtures were left for 15 min at room temperature and the absorbance was measured at 517 nm. The blank sample consisted of 0.75 mL of extract or fraction solution with 1.5 mL of methanol. The antioxidant content was determined using standard curves for ascorbic acid (0-10 mg L⁻¹). The means of three values were obtained, expressed as mmol of ascorbic acid equivalent per g of extract or fraction antioxidant content (mmol AEAC g⁻¹ extract or fraction).

Antioxidant assay using the β-carotene linoleate model system: The antioxidant activity of extracts and fractions was evaluated by the method of Shon *et al.* (2003) with some modifications. A solution of β-carotene was prepared by dissolving 8 mg of β-carotene in 10 mL of chloroform. The 0.5 mL of this solution was pipetted into a 100 mL round-bottom flask. After the chloroform was removed at 40°C under vacuum. Forty seven microliter of linoleic acid, 362 µL of Tween 40 emulsifier and 100 mL of distilled water were added to the flask with vigorous shaking.

Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of the extracts. The tubes were shaken and incubated at 50°C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Absorbance readings were then recorded at 20 min intervals until the control sample had changed colour. A blank, devoid of β -carotene, was prepared for background subtraction. α -Tocopherol, quercetin and gallic acid were used as positive controls. Antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity} = \left(\frac{\beta\text{-carotene content after 2 h of assay}}{\text{Initial } \beta\text{-carotene content}} \right) \times 100$$

The assays were carried out in triplicate and the results expressed as mean values \pm standard deviations.

Inhibition of lipid peroxidation in rat liver homogenate: The inhibition activity of extracts or fractions on lipid peroxidation (LPO) was determined according to the thiobarbituric acid method. $\text{FeCl}_2\text{-H}_2\text{O}_2$ was used to induce the liver homogenate peroxidation to the method of Su *et al.* (2009) with slightly modification. In this method, 0.2 mL of extract or fraction at the concentration of 1.25 mg mL⁻¹ was mixed with 1.0 mL of 1% liver homogenate (each 100 mL homogenate solution contains 1.0 g rat liver), then 50 μ L of FeCl_2 (0.5 mM) and 50 μ L of H_2O_2 (0.5 mM) was added. The mixture was incubated at 37°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:

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

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 liver homogenate.

Anti-inflammatory activity

Xanthine oxidase inhibitory: The XO activities with xanthine as the substrate were measured spectrophotometrically using the procedure reported by Filha *et al.* (2006) with some modifications. The assay mixture consisted of 50 μ L of extract or fraction solution at final concentration of 100 μ g mL⁻¹, 150 μ L of 1:15 M phosphate buffer (pH 7.5) and 50 μ L of enzyme solution (0.28 U mL⁻¹ in buffer). After pre-incubation of the mixture at 25°C for 1 min, the reaction was initiated by adding 250 μ L of xanthine substrate solution (0.6 mM) and the absorbance was measured for 120 sec. A negative control was prepared without extract. Allopurinol, a known inhibitor of XO, quercetin and gallic acid were used as positive controls, in a final concentration of 100 μ g mL⁻¹ in the reaction mixture.

Lipoxygenase inhibitory activity: Lipoxygenase inhibitory activity was measured by slightly modifying the spectrometric method as developed by Malterud and Rydland (2000).

Four hundred microliter of lipoxygenase solution (167 U mL^{-1}), $100 \mu\text{L}$ of the sample solution ($50 \mu\text{g mL}^{-1}$ at final concentration) were mixed and incubated for 1 min at 25°C . The reaction was initiated by the addition of $500 \mu\text{L}$ of linoleic acid substrate solution ($134 \mu\text{M}$) and the absorption change at 234 nm with the formation of (9Z, 11E)-13S)-13-hydroperoxyoctadeca-9, 11-dienoate was followed for 3 min. All the reactions were performed in triplicate. Quercetin and gallic acid were used as positive control for lipoxygenase inhibition.

Statistical analysis: Chemical and enzymatic analyses of individual samples were performed in triplicates. The results presented are the mean and standard deviations of the obtained values. Data manipulation was performed by means of Microsoft Excel. Data were analyzed using ANOVA test (Fisher). The $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Extraction yields: All extracts were analyzed for polyphenols compounds content. The yield of aqueous decoction and hydroacetic extracts was determined. The yield extraction is 17.6% for the aqueous decoction and 16.9% for the hydroacetic (80:20) extraction. We obtained almost the same yield for the two types of extractions.

Determination of total phenol and flavonoid content in the extracts: Table 1 shows the totals phenol, totals flavonoid content in the extracts and fractions from galls of *G. senegalensis*.

The hydroacetic extract (HAE) exhibited the highest total phenolic content ($73.9 \pm 2.34 \text{ mg GAE/100 mg}$ of extract). This extract is significantly higher than when compared to the others extract and fractions. The result of hydroacetic extract is higher than that obtained by Lamien *et al.* (2005) on the same extract. This result could be explained by the fact that the total phenol content of the plants varies as a function of the plant part collected and the season (De Sousa Araújo *et al.*, 2008). The amounts of total phenol contents were increased by polarity of the extraction solvent. Polyphenols are secondary plant metabolites that are present in many plant and plant product. Many of the phenolics have been shown to contain high levels of antioxidant activities.

Table 1: Totals phenolic and totals flavonoid content

Extract/Fraction	Totals phenolic (mg GAE/100 mg of extract)	Totals flavonoid (mg EQ/100 mg of extract)
HAE	69.67 ± 2.39^b	2.458 ± 0.008^d
EA/HAE	60.83 ± 2.86^c	2.574 ± 0.08^e
BF/HAE	63.00 ± 0.10^c	5.390 ± 0.05^b
WF/HAE	73.9 ± 2.34^a	0.440 ± 0.027^f
ADE	61.33 ± 0.58^c	1.990 ± 0.045^e
EA/ADE	19.87 ± 2.68^e	0.220 ± 0.01^e
BF/AD	22.93 ± 3.06^e	8.440 ± 0.05^a
WF/ADE	52.20 ± 0.66^d	0.270 ± 0.022^f

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

The butanol fraction of aqueous decoction (BF/ADE) contains the significant highest of flavonoid (8.44±0.05 mg QE/100 mg of fraction). Totals flavonoid content did not change according to the polarity of the extraction solvents. Flavonoids are good free radical scavengers that donate hydrogen and have lipid peroxidation, anti-mutagenic and anti-inflammatory activities (Tunalier *et al.*, 2007). The totals flavonoid content of HAE and ADE are also higher than these obtained by Lamien *et al.* (2005) on the same extracts (respectively 1.61±0.01 mg QE/100 mg for HAE and 0.79±0.00 mg QE/100 mg for ADE).

The galls of *Guiera senegalensis* contain high polyphenols content than that six species of acanthaceae from Burkina Faso (Sawadogo *et al.*, 2006).

Antioxidant activity: The principle of the antioxidant activity is the availability of electrons to neutralize any so-called free radicals. Since, antioxidant mechanisms are diverse, a variety of in vitro techniques has been developed. It is better to use different assays based on different mechanisms to evaluate the antioxidant capacity. In this study, the antioxidant activity of the extracts and fractions was evaluated using DPPH scavenging, FRAP and β-carotene bleaching assays.

The results of antioxidant activity of extracts from *Guiera senegalensis* are summarized in Table 2.

The FRAP assay measured the ability of antioxidant components to reduce Fe⁽³⁺⁾ to Fe⁽²⁺⁾. Related FRAP values ranged from 04.73±0.10 to 10.88±0.86 mmol AEAC g⁻¹ extract or fraction. Hydroacetic extract show the strongest and significantly activity to reduce Fe⁽³⁺⁾ to Fe⁽²⁺⁾ than the others fractions except the aqueous decoction extract and water fraction of hydroacetic extract.

The radical-scavenging activities of the extracts and fractions from galls of *G. senegalensis* were estimated by comparing the inhibition of formation of DPPH radicals by the extracts and those of ascorbic acid. The water fraction of hydroacetic extract (WF/HAE) from galls of *G. senegalensis* (6.012±0.13 mmol AEAC/g extract) which has the best free radical scavengers is significantly higher than aqueous decoction extract and its fractions (WF/ADE, EAF/ADE and BF/ADE). The

Table 2: Antioxidant and anti-lipid peroxidation activity

Antioxidant activity	FRAP (mmol AEAC g ⁻¹ of extract)	DPPH (mmol AEAC g ⁻¹ of extract)	β-Carotene (%)	Anti-LPO (%)
HAE	10.88±0.86 _c	5.47±0.16 _{c,d}	31.94±2.1 _g	75.06±2.42 ^b
EA/HAE	09.24±0.07 _{d,e}	5.45±0.28 _{c,d}	31.15±1.76 ^f	44.9±2.28 ^e
BF/HAE	09.29±0.22 _{d,e}	5.63±0.18 _{c,d}	59.00±3.27 ^a	56.53±2.31 ^c
WF/HAE	10.58±0.18 _{c,d}	6.012±0.13 _c	40.98±1.03 ^{d,e}	32.15±2.8 ^e
ADE	10.07±0.24 _{c,d,e}	5.05±0.1 _d	45.84±0.89 ^{b,c}	84.94±1.067 ^a
EA/ADE	04.87±0.13 _f	01.4±0.17 _e	35.64±1.22 ^f	32.47±1.76 ^f
BF/ADE	04.73±0.10 _f	01.9±0.11 _e	42.79±1.15 ^{c,d}	37.36±2.52 ^f
WF/ADE	08.95±0.4 _e	04.77±0.15 _d	38.95±2.74 ^{e,f}	87.25±1.27 ^a
Quercetin	14.33±0.99 _b	13.76±0.26 _b	-----	37.38±1.01 ^d
Gallic acid	18.84±0.46 _a	29.45±0.26 _a	-----	50.63±0.41 ^f
α-tocopherol	-----	-----	45.30±2.39 ^b	-----

Data are Mean±SEM (n = 3). HAE: Hydroacetic extract, EAF/HAE: Ethyl acetate fraction from HAE, BF/HAE: Butanol fraction from HAE, W/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. β-Carotene bleaching expressed as percentage of inhibition at a concentration of 100 µg mL⁻¹. Anti-Lipid peroxidation (Anti-LPO) of rat liver expressed as percentage of inhibition at a concentration of 1.25 mg mL⁻¹ of extract or fraction. Values showing the same letter are not significantly different (p>0.05) from one other in the same columns

ability to scavenge DPPH radicals of various solvent extracts from GS was in the order of water fraction (HAE)>butanol fraction (HAE)>hydroacetic extract>ethyl acetate fraction (HAE)>Aqueous decoction extract>Water fraction (ADE)>butanol fraction (ADE)>Ethyl acetate fraction (ADE).

The antioxidant activities of GS extracts and fractions were also evaluated by the β -carotene bleaching method, in which the oxidation of β -carotene in the presence of linoleic acid takes place. The percentage inhibition of β -carotene values varied from 31.15 \pm 1.76 to 59.00 \pm 3.27%. β -Carotene method demonstrated the significant higher antioxidant activity for the butanol fraction of hydroacetic extract. This activity is significant higher than α -tocopherol antioxidant activity which is a reference inhibition of β -carotene compound. The antioxidant activity of carotenoids is based on the radical adducts of carotenoids with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β -carotene models. The presence of different antioxidants can hinder the extent of β -carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (Suja *et al.*, 2005).

Total phenols content versus the antioxidant activity: In this study, the correlation between the antioxidant activity and totals phenolic content of extracts and fractions was studied using a linear regression analysis. As demonstrated in Fig. 1, the good and significant correlation coefficient between totals phenolic and FRAP values ($R^2 = 0.9702$, $y = 0.1127x + 2.4818$, $p = 0.003$), total phenolic and DPPH radical scavenging ($R^2 = 0.9519$, $y = 0.712x - 1.6532$, $p = 0.006$) were found. The correlation coefficient between totals phenol and β -carotene assay ($y = 0.0583x + 37.634$; $R^2 = 0.019$, $p > 0.05$) was found to be very weak, less than 0.5 (Fig. 1).

An almost linear correlation between DPPH radical-scavenging activity and concentrations of totals phenolic compounds in various vegetable and fruits have been reported (Lamien-Meda *et al.*, 2008; Bakasso *et al.*, 2008). This low correlation values between totals phenolic content and the β -carotene assay suggest that the totals phenol from galls of *G. senegalensis* do not take part in the inhibition of β -carotene oxidation. We can also suggest that the strong free radical-scavenging and iron (III) to iron (II) reduction capacities of these extracts and fractions were due to their high phenolic contents. This last assertion is confirmed by the correlation studies between the total phenolic and antioxidant activities (DPPH and FRAP). Sofidiya *et al.* (2006) also reported a strong relationship between total phenol content and reducing power.

High and significant correlation ($R^2 = 0.93$, $p < 0.05$, $y = 0.712x - 1.6532$) was found between data from the DPPH and FRAP antioxidant assays. However, found no correlation between the FRAP

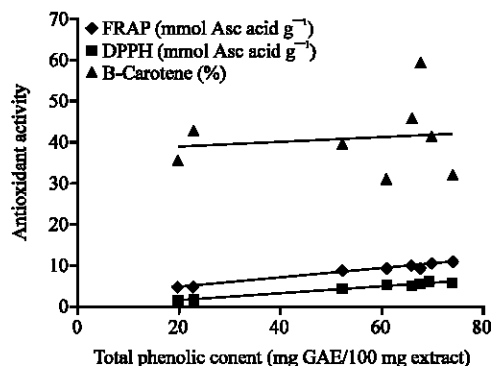


Fig. 1: Correlation between antioxidant activity and totals phenolic content

and β -carotene assays or DPPH and β -carotene assays on extracts from galls of *G. senegalensis*. Present results indicate that 93% of the radical scavenging compounds from galls of *Guiera senegalensis* also take part in iron (III) to iron (II) reduction activity and don't contribute to β -carotene bleaching assays. On the contrary Hinneburg *et al.* (2006) did not find any correlation with the DPPH and the iron chelation assay.

Anti-lipid peroxidation activity: The percent inhibition of lipid peroxidation was quantified by measuring the reduction of TBARS production with respective controls in the presence of extracts or fractions. Quercetin and gallic acid were used as standard antioxidant. The role of peroxidative processes in disease is a subject of intense research interest. Lipid peroxidation of cell membranes is associated with various pathological events such as atherosclerosis, inflammation and liver injury (Roome *et al.*, 2008). $\text{FeCl}_2\text{-H}_2\text{O}_2$ system was used to induce lipid peroxidation in rat liver homogenate.

Water fraction (WF/ADE) from the galls of GS demonstrated a strong anti-lipid peroxidative effect ($87.25 \pm 1.27\%$) at a concentration of 1.25 mg mL^{-1} . All the extracts or fractions except water fraction (WF/HAE) and ethyl acetate fraction (EAF/ADE) produced greater inhibition as compared to the quercetin. Hydroacetic extract, butanol fraction (BF/HAE), aqueous decoction extract and water fraction (WF/ADE) produced greater inhibition as compared to gallic acid. The lipid peroxidation inhibition activity of extracts and fractions from galls of *G. senegalensis* is stronger when compared to the findings of study concerning *Pinus koraiensis* seed extract (Su *et al.*, 2009).

The involvement of iron in lipid peroxidation is well established. In fact, ferrous ions can precipitate the formation of oxygen radicals and either initiate or take part in peroxidative process (Dixit and Kar, 2009). The low correlation values between extracts or fractions activities to reduce $\text{Fe}^{(3+)}$ to $\text{Fe}^{(2+)}$ and anti-lipid peroxidation ($R^2 = 0.265$; $p > 0.05$, $y = 0.0538x + 5.5479$), DPPH radical-scavenging activity and anti-lipid peroxidation ($R^2 = 0.1684$; $p = 0.805$, $y = 0.0317x + 2.6705$), β -carotene bleaching assay and lipid peroxidation ($R^2 = 0.0063$, $p > 0.005$, $y = 0.0307x + 39.055$) were obtained (Fig. 2). The anti-peroxidative effects of the extracts were not also correlated with their totals phenolic and totals flavonoid contents.

The low correlation obtained between antioxidant and anti-lipid peroxidation activities suggest that the anti-peroxidation activity from galls of *Guiera senegalensis* is not related to their radical scavenging and ferric reduction activities. Ozgova *et al.* (2003) did not find correlation between IC_{50} values with the quantitatively assayed chelation potency of different polyphenols. Decrease

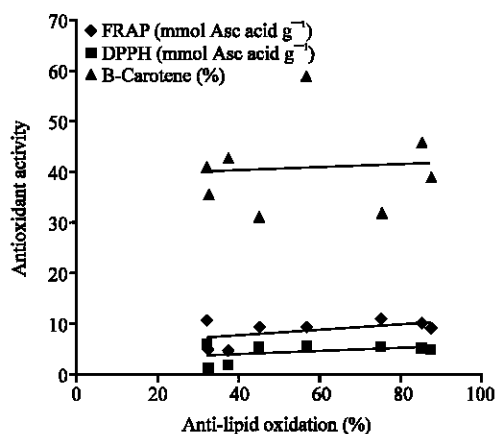


Fig. 2: Correlation between antioxidant activity and anti-lipid peroxidation activity

Table 3: Xanthine oxidase (XO) and of soybean lipoxygenase (LOX) inhibitory activity

Extracts or fractions	XO inhibition (%)	LOX inhibition (%)
HAE	50.90±6.91 _c	98.95±1.11 ^a
AE/HAE	45.88±4.48 _c	65.43±4.09 ^f
BF/HAE	36.56±4.30 _d	79.81±4.11 ^b
WF/HAE	43.73±9.15 _{c,d}	79.19±2.42 ^b
ADE	45.88±2.71 ^e	69.41±4.19 ^c
EA/ADE	1.08±1.08 ^f	16.51±4.51 ^e
BF/ADE	4.30±1.08 ^f	19.03±0.72 ^e
W/ADE	20.79±4.07 ^e	48.27±1.27 ^d
Quercetin	80.74±1.29 ^b	53.11±0.65
Gallic acid	23.21±0.43 ^e	35.24±0.6
Allopurinol	95.70±1.52 ^a	22.80±0.38

Data are Mean±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. For XO inhibitory activity extracts, fractions and references were tested at a concentration of 100 µg mL⁻¹. For LOX inhibitory activity extracts and fractions were tested at a concentration of 50 µg mL⁻¹ references were tested at a concentration of 25 µg mL⁻¹

in lipid peroxidation by extracts from galls of *Guiera senegalensis* may be a result of it scavenging OH produced by FeCl₂-H₂O₂ and H₂O₂ in the reaction system (Wang *et al.*, 2008). Our extracts reduced lipid peroxidation in liver, the primary target organ of drug metabolism. These results suggest that the extracts of *G. senegalensis* may not cause hepatotoxicity; but acts as protective agent by preventing oxidative damage.

Anti-inflammatory activity

Xanthine oxidase and of soybean lipoxygenase inhibitory activity: Xanthine oxidase inhibitors are known to be therapeutically useful for the treatment of gout, hepatitis and brain tumor (Song *et al.*, 2003). At a concentration of 100 µg mL⁻¹ of hydroacetic extract, uric acid formation was partially suppressed (50.90±6.91%).

Hydroacetic extract may contain bioactive substances useful in the treatment of gout or other XO-induced diseases justifying the traditional use of this specie as diuretic, depurative. Table 3 shows the inhibitory effects of the extracts and fractions from galls of GS on Xanthine Oxidase (XO) activities. Allopurinol (95.7±1.52%), the positive control is similar to published data at a concentration of 100 µg mL⁻¹ (Umamaheswari *et al.*, 2007). These clearly showed that hydroacetic extract from galls of *G. senegalensis* has the best inhibitory activities of the xanthine oxidase enzyme. Ethyl acetate fraction (ADE) showed any inhibitory effects. Umamaheswari *et al.* (2009) obtained a better inhibitory activity (84.75±0.54%) of the chloroform fraction of *Erythrina stricta* on the xanthine oxidase at a concentration of 100 µg mL⁻¹.

The 5-LOX pathway generates an important class of inflammatory mediators, such as leukotrienes (LTs), which plays a major part in the inflammatory process (Li *et al.*, 2006).

Table 3 also shows the inhibitory effects of the extracts and fractions on lipoxygenase (LOX) activity at a concentration of 50 g mL⁻¹. All the extracts showed LOX inhibitory effects, enzyme involved in generating free radicals. According to the inhibition percentage values, the most effective LOX inhibitory extracts were, in order of efficacy, HAE>BF (HAE)>WF(HAE)>ADE >EAF(HAE) WF (ADE)>BF (ADE) and EAF (ADE). Hydroacetic extract from galls of *Guiera senegalensis*(HAE) showed stronger inhibitory activity towards lipoxygenase at a concentration of 50 µg mL⁻¹ comparatively of ethanol extracts from nine vine plants used in traditional Chinese medicine at a concentration of 340 µg mL⁻¹ (Li *et al.*, 2003).

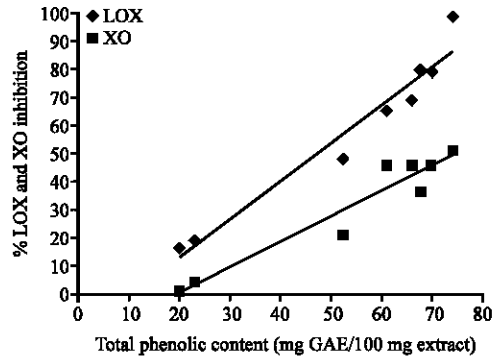


Fig. 3: Correlation between LOX, XO inhibition and totals phenolic content

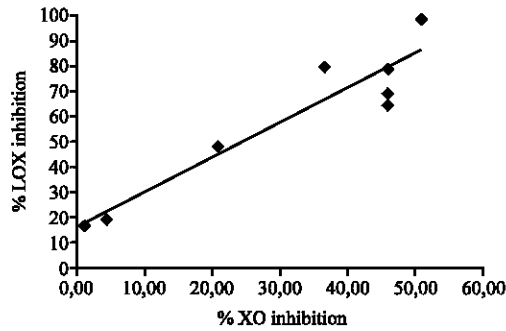


Fig. 4: Correlation between LOX inhibition and XO inhibition activity

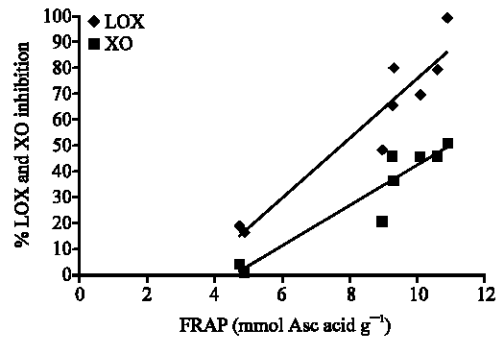


Fig. 5: Correlation between LOX, XO inhibition and FRAP

Regression equations and coefficients of correlation between the totals phenolic and the inhibition of XO and LOX activities by extracts and fractions are represented on Fig. 3.

These results suggest that totals phenolic of extracts from galls of GS contribute for more than 90% to the inhibition of the XO and the LOX.

A good correlation ($R^2 = 0.8931$, $p = 0.008$) between the ability of galls extracts to inhibit lipoxygenase action and their ability to inhibit xanthine oxidase was obtained (Fig. 4).

The anti-inflammatory effect could be also related to antioxidant activity. All the extracts from galls of GS possess anti-inflammatory activity correlated to its high antioxidant/free radical-scavenging capacities (Fig. 5, 6). Maiga *et al.* (2006) also show a correlation between the antioxidant and anti-inflammatory activity of the extracts.

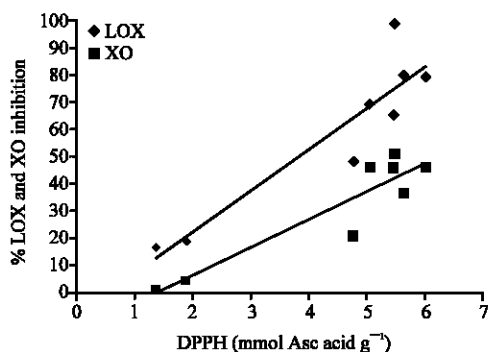


Fig. 6: Correlation between LOX, XO inhibition and DPPH

Low correlations were obtained between the antioxidant activity by the β -carotene bleaching method and the activities of inhibition of the xanthine oxidase and the lipoxygenase.

The non-polar antioxidants which exhibit stronger antioxidative activities in emulsions do not take part in xanthine oxidase and lipoxygenase inhibition activities.

CONCLUSION

In this study HAE, BF (HAE), WF (HAE) and ADE extracts derived from galls of *Guiera senegalensis* demonstrated pronounced antioxidant potential. These extracts contain polyphenols compounds that can scavenge free radicals, chelate metal ions and inhibit the lipid peroxidation activity. The antioxidant activity is correlated with the total phenolic content. However, extracts from galls of *Guiera senegalensis* demonstrated effective anti-inflammatory activity which probably be related at least in part to its antioxidant activity that justifies its traditional use against inflammatory and xanthine oxidase-induced diseases. It also provides useful information for pharmacological activities associated with free radicals. However, there is no correlation between total phenolic content and anti-lipid peroxidation activity. More detailed phytochemical studies are thus necessary to identify the extracts active principle(s) responsible for the β -carotene and the anti-lipid peroxidation activities.

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