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***In vitro* Antioxidant, Xanthine Oxidase and Acetylcholinesterase Inhibitory Activities of *Balanites aegyptiaca* (L.) Del. (Balanitaceae)**

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Abstract: The present study aimed to test the validity of *Balanites aegyptiaca* remedies used for the treatment of rheumatism and mental disorders by examining the antioxidant, xanthine oxidase and acetylcholinesterase inhibitory activities of galls and leaves extracts and fractions. The total phenolics and flavonoids were measured using Folin-Ciocalteu and AlCl₃ reagents, respectively. Two methods i.e., FRAP and ABTS were used to estimate the total antioxidant capacity of the plant materials. The FRAP and ABTS antioxidant activities showed that among all extracts and fractions tested, the best antioxidant activities were found with the galls dichloromethane and the leaves ethyl acetate fractions. The antioxidant activities did not correlate significantly with the total phenolic and flavonoid contents. The study also showed that *B. aegyptiaca* galls and leaves fractions exhibited a moderate xanthine oxidase inhibitory activity comparatively to the acetylcholinesterase which was weakly inhibited by the tested extracts and fractions.

Key words: *Balanites aegyptiaca*, total phenolics, total flavonoids, antioxidant, rheumatism, mental disorders

INTRODUCTION

Balanites aegyptiaca (L.) Delile (Balanitaceae) is widely distributed along the tropical belt of Africa (Mohamed *et al.*, 1999) and is a small spinescent evergreen savanna tree, growing up to 4.5-6 m with dark brown stem (Koko *et al.*, 2000).

Almost all the parts (leaves, galls, thorns, root bark, stem bark and fruit) of that plant are traditionally used in several folk medicines. In Egypt, the fruits are used as oral hypoglycemic drug (Kamel, 1998). The seed oil is used in Nigeria against skin disease and rheumatism (Obidah *et al.*, 2009). The stem bark is reputed for treatment of tape worm infection, stomach trouble, amoebic dysentery and less frequently for malaria in Sudan (Koko *et al.*, 2000). In Burkina Faso, the galls of *B. aegyptiaca* are used to treat the cattle brucellosis (Tamboura *et al.*, 1998), sickle cell disease, whooping cough and mental disorders (Nacoulma, 1996) while leaves are used to treat wounds, dermatosis, bilharziosis, cold and rheumatism. Furthermore, pharmacological and phytochemical investigations carried out on *B. aegyptiaca* evidenced fasciolocidal activity in the stem

bark (Koko *et al.*, 2000) and nematocidal compounds in seed fixed oil (Gnoula *et al.*, 2007).

Despite the widespread use of *B. aegyptiaca* in traditional medicine, there is currently no data on the inhibitory activity of xanthine oxidase. In addition, there is a limited literature on the acetylcholinesterase inhibitory activity of *B. aegyptiaca* (Gnoula *et al.*, 2007). It is well known that xanthine oxidase is an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues that are involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging (Sweeney *et al.*, 2001). Moreover, the endogenous formation of free radicals can contribute to the development of the Alzheimer's disease (Orhan *et al.*, 2007). The antioxidant activity is also relevant in the treatment of Alzheimer's disease. It was demonstrated that the absence of the natural antioxidant, vitamin E, enhanced AD in a mouse model. The endogenous formation of free radicals can contribute to the inflammatory processes (Hernandez *et al.*, 2010).

In traditional practices, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases and gout.

Ethnopharmacological approach and bioassay-guided isolation have provided a lead in identifying potential xanthine oxidase and acetylcholinesterase inhibitors from plant sources (Filha *et al.*, 2006; Umamaheswari *et al.*, 2007; Mukherjee *et al.*, 2007).

The present study aimed to test the validity of *B. aegyptiaca* remedies used for the treatment of rheumatism and mental disorders by examining the antioxidant, xanthine oxidase and acetylcholinesterase inhibitory activities of galls and leaves extracts and fractions. The identification of phenolic compounds from *B. aegyptiaca* galls and leaves by HPLC-MS method have been previously studied. Two methods i.e., FRAP (ferric reducing antioxidant capacity) and ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) were used to estimate the total antioxidant capacity of the plant materials.

MATERIALS AND METHODS

Plant materials: The galls and leaves of *B. aegyptiaca* (L.) Del. were collected in November 2008 at Gampella, 25 km East from Ouagadougou (Burkina Faso). The plant was botanically identified by Prof. Millogo-Rasolodimby from the plants Biology Department of the University of Ouagadougou. A Voucher specimen (MR_01) was deposited in the herbarium of the Laboratoire de Biologie et d'Ecologie Végétales, UFR/SVT of the University of Ouagadougou.

Chemicals: All reagents were of analytical grade: Folin Ciocalteu-reagent, NaH_2PO_4 , Na_2HPO_4 , sodium carbonate, aluminium trichloride, gallic acid, quercetin, xanthine, xanthine oxidase (OX) from bovine milk, allopurinol, galanthamine, acetylthiocholine iodide (ATCI), acetylcholinesterase (AChE) and 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). 2,2-diphenylpicrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS, trichloroacetic acid, potassium persulfate, methanol, acetone, n-hexane, n-butanol, dichloromethane and ethyl acetate were supplied by Fluka Chemie (Buchs, Switzerland). Potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$] was from Prolabo (Paris, France) and ascorbic acid was from Labosi (Paris, France).

Extraction and fractionation: The dried and powdered galls and leaves of *B. aegyptiaca* (50 g) were extracted with 500 mL of acetone 80% for 24 h under mechanical agitation (SM 25 shaker, Edmund BÜHLER, Germany), at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI

Rotavapor R-200, Switzerland) at 40°C. The aqueous extracts were subjected to sequential liquid-liquid extraction with n-hexane, dichloromethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain n-hexane fraction (n-HF), dichloromethane fraction (DCMF), ethyl acetate fraction (EAF) and n-butanol fraction (n-BF).

Determination of total phenolics and total flavonoids

Total phenolics: Total phenolics of each extract and fraction were determined by Folin-ciocalteu reagent method (Lamien-Meda *et al.*, 2008). After dilution, 125 μL of each extract or fraction was mixed with 625 μL of Folin Ciocalteu reagent (0.2 N). This mixture was allowed to stand at room temperature for 5 min and then, 500 μL of aqueous sodium carbonate solution (75 mg mL^{-1}) was added. After 2 h of incubation, the absorbencies were measured at 760 nm against a water blank using a spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The standard calibration curve was plotted using gallic acid ($y = 0.0092x + 0.021$; $R^2 = 0.999$). The mean of three readings was used and the results expressed as mg of Gallic Acid Equivalents (GAE)/100 mg of extract or fraction.

Total flavonoids: The total flavonoids were evaluated the aluminium trichloride method (Lamien-Meda *et al.*, 2008). Briefly, 625 μL of extracts or fractions (100 $\mu\text{g mL}^{-1}$) was mixed with 625 μL of aluminium trichloride (AlCl_3) in methanol (2%). The absorbencies reading at 415 nm were taken after 10 min against a blank consisted in 625 μL of extracts or fractions and 625 μL of methanol without AlCl_3 . Quercetin was used as reference compound to produce the standard curve ($y = 0.0289x - 0.0036$; $R^2 = 0.9998$) and the results were expressed as mg of quercetin equivalent (QE)/100 mg of extract or fraction.

Antioxidant activity determination

ABTS radical cation decolorization assay: The radical scavenging capacity of antioxidants for the ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical cation was determined as described by Lamien-Meda *et al.* (2008). $\text{ABTS}^{\bullet+}$ was generated by mixing a 7 mM aqueous solution of ABTS with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 12 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using spectrophotometer.

For the study, 10 μL of the diluted extracts or fractions (100 $\mu\text{g mL}^{-1}$ in methanol) was allowed to react

with 990 μL of fresh ABTS*⁺ solution and the absorbance was taken 15 min after initial mixing. Ascorbic acid was used as standard ($y = -0.0342x + 0.634$; $R^2 = 0.9996$) and the capacity of free radical scavenging was expressed as μmol ascorbic acid equivalent (AAE)/g of extract or fraction.

Iron (III) to iron (II) reduction activity (FRAP): The FRAP assay was done according to the method of Hinneburg *et al.* (2006). Briefly, 0.5 mL of extracts or fractions ($100 \mu\text{g mL}^{-1}$) were mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$] solution (1%). After 30 min incubation at 50°C , 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at $3000\times g$ for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared FeCl_3 solution (0.125 mL, 0.1%). Absorbencies were read at 700 nm and Ascorbic acid was used to produce the calibration curve ($y = 0.008x - 0.0081$; $R^2 = 0.9999$). The iron (III) reducing activity determination was performed in triplicate and expressed in μmol Ascorbic Acid Equivalent (AAE)/g of extract or fraction.

Xanthine oxidase (XO) inhibitory activity: The XO activities were measured spectrophotometrically using the procedure of Filha *et al.* (2006) with some modifications. The extracts or fractions were directly dissolved in phosphate buffer-MeOH (1%) and screened for XO inhibitory activity at final concentration of $100 \mu\text{g mL}^{-1}$. The assay mixture consisted of $100 \mu\text{L}$ of extracts or fractions solution, $300 \mu\text{L}$ of phosphate buffer (pH 7.49) and $100 \mu\text{L}$ enzyme solution (0.28 U mL^{-1} in phosphate buffer). The mixture was incubated at room temperature for 2 min. Then, the reaction was initiated by adding $500 \mu\text{L}$ of xanthine solution (0.15 mM in phosphate buffer) and the change in absorbance recorded at 295 nm for 2 min at room temperature. Allopurinol, a well known inhibitor of XO, was used as a positive control at a final concentration of $100 \mu\text{g mL}^{-1}$.

The results were expressed as percent inhibition of xanthine oxidase, calculated as $(1-B/A)\times 100$, where A is the change in absorbance of the assay without the plant extract ($\Delta\text{abs. with enzyme} - \Delta\text{abs. without enzyme}$) and B is the change in absorbance of the assay with the plant extract ($\Delta\text{abs. with enzyme} - \Delta\text{abs. without enzyme}$).

AChE inhibitory activity: The AChE inhibitory assay and inhibition kinetics analysis were conducted according to the protocol described by López *et al.* (2002) with some modifications. Briefly described, the assay mixture consisted of $200 \mu\text{L}$ of Tris-HCl 50 mM pH8, 0.1% BSA buffer, $100 \mu\text{L}$ of extracts or fractions solution (final concentration: $100 \mu\text{g mL}^{-1}$) was dissolved in buffer-

MeOH (10%) and $100 \mu\text{L}$ of AChE (0.22 U mL^{-1}). The mixture was incubated at room temperature for 2 min before the addition of $500 \mu\text{L}$ of DTNB (3 mM) and $100 \mu\text{L}$ of substrate (ATCI 15 mM). The developing yellow color was measured at 405 nm after 4 min. Galanthamine was used as a positive control at a final concentration of $0.2 \mu\text{g mL}^{-1}$ in the assay mixture.

AChE inhibitory activity was expressed as percent inhibition of AChE, calculated as $(1-B/A) \times 100$, where A is the change in absorbance of the assay without the plant extract ($\Delta\text{abs. with enzyme} - \Delta\text{abs. without enzyme}$) and B is the change in absorbance of the assay with the plant extract ($\Delta\text{abs. with enzyme} - \Delta\text{abs. without enzyme}$).

Statistical analysis: The data are expressed as the Means \pm Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p < 0.05$ and linear regression) was carried out with XLSTAT 7.1.

RESULTS AND DISCUSSION

Phenolic and flavonoid contents: The total phenolics content per 100 mg of galls extract and fractions ranged from 8.35 to 34.77 mg GAE and that of the leaves extract and fractions were 8.43 to 30.66 mg GAE (Table 1). The highest content of total phenolics in galls was detected in DCMF with 34.77 mg GAE followed by EAF (27.45 mg GAE). The lowest total phenolics were obtained in n-HF (8.35 mg GAE). In the leaves material the highest total phenolics content was measured in EAF followed by n-BF with 29.73 and 18.80 mg GAE, respectively. Present finding was that the aqueous acetone extract of leaves contains more phenolics content (30.66 mg GAE) than aqueous acetone extract of galls (8.72 mg GAE) and these compounds are more extractable by dichloromethane and ethyl acetate.

Table 1: Total phenolics and flavonoids in galls and leaves extracts and fractions

Samples	Total phenolics (mg GAE/100 mg)	Total flavonoids (mg QE/100 mg)
Galls		
n-HF	8.35 \pm 0.35 ^d	0.63 \pm 0.19 ^b
DCMF	34.77 \pm 0.81 ^a	2.47 \pm 0.06 ^a
EAF	27.45 \pm 0.52 ^b	2.40 \pm 0.12 ^a
n-BF	16.85 \pm 0.40 ^c	2.35 \pm 0.04 ^a
AAE	8.72 \pm 0.66 ^d	0.58 \pm 0.08 ^b
Leaves		
n-HF	8.43 \pm 0.03 ^c	1.76 \pm 0.26 ^d
DCMF	13.37 \pm 0.45 ^d	3.78 \pm 0.26 ^c
EAF	29.73 \pm 0.43 ^a	15.69 \pm 1.83 ^a
n-BF	18.80 \pm 0.20 ^c	10.93 \pm 0.30 ^b
AAE	30.66 \pm 5.34 ^a	6.07 \pm 0.20 ^c

n-HF: n-hexane fraction; DCMF: Dichloromethane fraction; EAF: Ethyl acetate fraction; n-BF: n-butanol fraction; AAE: Aqueous acetone extract. Values are Mean \pm SD (n = 3). Different letters in the same column indicate significant difference ($p < 0.05$) for galls and leaves

The total flavonoid content per 100 mg of galls extract and fractions varied from 0.58 to 2.47 mg QE and 1.76 to 15.69 mg QE in leaves extract and fractions (Table 1). The highest values of total flavonoid content were found in the DCMF (2.47 mg QE) and EAF (15.69 mg QE) of galls and leaves, respectively. Aqueous acetone extract of galls (0.58 mg QE) and n-HF of leaves (1.76 mg QE) contained the lowest quantities of flavonoids compounds. The total flavonoids were significantly higher ($p < 0.05$) in the leaves extract and fractions than those of the galls.

Indeed, the galls and the leaves of *B. aegyptiaca* were previously disclosed to be a rich source of phenolics compounds such as gentisic, *p*-coumaric, ferulic and sinapic acids, hyperoside, isoquercitrin, quercitrin and rutin. In this study it's well established that the leaves are rich in phenolic compounds than galls considering the standard phenolic compounds used.

Antioxidants: In the ABTS assay, the antioxidant capacities of the extracts and fractions ranged from 15.98 to 65.02 $\mu\text{mol AAE g}^{-1}$ for the galls and 6.58 to 43.17 $\mu\text{mol AAE g}^{-1}$ for the in leaves (Fig. 1a). Among all fractions, the strongest ABTS radical cation scavenging activities was found in DCMF (galls) and EAF (leaves) with 65.02 and 38.95 $\mu\text{mol AAE g}^{-1}$, respectively. The lowest activities were obtained in n-HF of galls (15.98 $\mu\text{mol AAE g}^{-1}$) and leaves (6.58 $\mu\text{mol AAE g}^{-1}$). The ABTS radical cation scavenging was significantly higher in the galls than in the leaves ($p < 0.05$).

Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Hinneburg *et al.*, 2006). The FRAP assay measured the ability of phenolics to reduce Fe(III) to Fe(II). The FRAP activity of the extracts and fractions varied from 259.75 to 1836.08 $\mu\text{mol AAE g}^{-1}$ for the galls and 492 to 1572.02 $\mu\text{mol AAE g}^{-1}$ for the leaves (Fig. 1b). The strongest ferric reducing ability was found in DCMF (1836.08 $\mu\text{mol AAE g}^{-1}$) followed by EAF (1620.29 $\mu\text{mol AAE g}^{-1}$) both obtained from aqueous acetone extract of galls. Concerning the leaves extract, the EAF showed the best ferric reducing activity (1572.02 $\mu\text{mol AAE g}^{-1}$) followed by DCMF (1166.00 $\mu\text{mol AAE g}^{-1}$). While no significant difference was observed between galls and leaves fractions one noted that DCMF of galls significantly reduced Fe(III) to Fe(II) than DCMF of leaves ($p < 0.05$).

Several studies have revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors

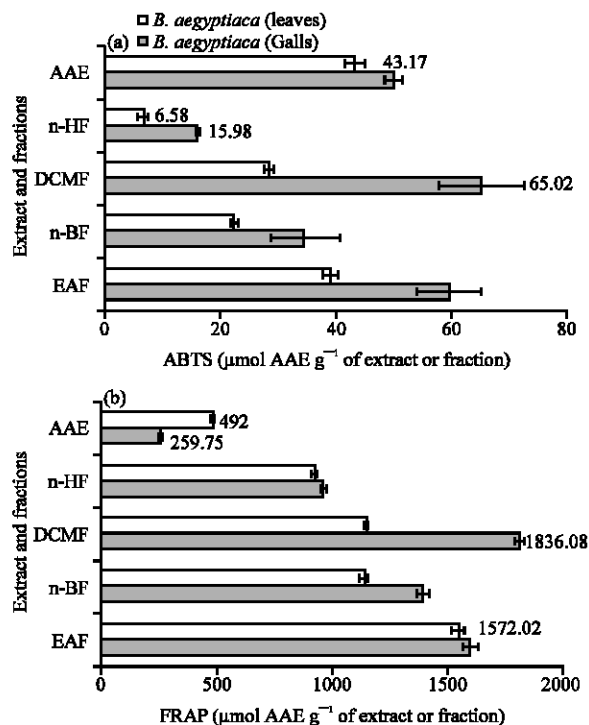


Fig. 1: Antioxidant activities obtained using the (a) ABTS and (b) FRAP methods on *B. aegyptiaca* galls and leaves extracts and fractions. n-HF: n-hexane fraction; DCMF: dichloromethane fraction; EAF: ethyl acetate fraction; n-BF: n-butanol fraction; AAE: aqueous acetone extract. Values are Mean \pm SD (n = 3)

and singlet oxygen quenchers (Ismail *et al.*, 2010). The antioxidant activity of phenolic compounds identified in *B. aegyptiaca* galls and leaves is well documented. Ferulic acid prevents the lipid oxidation in food and free-radical-induced diseases such as cancer and atherosclerosis or aging caused by oxidative tissue degeneration (Anselmi *et al.*, 2005) and *p*-coumaric acid is able to protect rabbit corneal-derived cells from UVB-induced oxidation damage (Lodovici *et al.*, 2009). Gentisic acid is suggested to possess an antioxidant effect on LDL oxidation (Ashidate *et al.*, 2005). Furthermore hyperoside was shown to possess cytoprotective properties against oxidative stress by scavenging intracellular reactive oxygen species (Piao *et al.*, 2008) and quercitrin prevents the lipids peroxidation *in vitro* (Wagner *et al.*, 2006).

The antioxidant activity of many compounds of botanical origin is proportional to the phenolic contents, suggesting a causative relationship between total phenolics content and antioxidant activity (Lamien-Meda *et al.*, 2008; Couliadiati *et al.*, 2009;

Table 2: Xanthine oxidase and acetylcholinesterase inhibitory activities of galls and leaves extract and fractions

Samples	Inhibitory activities (%)	
	Xanthine oxidase	Acetylcholinesterase
Galls (100 µg mL ⁻¹)		
n-HF	Not active	Not active
DCMF	33.33±2.56 ^b	20.30±0.66 ^b
EAF	40.56±1.27 ^a	11.88±1.33 ^b
n-BF	23.06±2.1 ^c	13.79±1.15 ^b
AAE	6.06±2.78 ^d	Not active
Leaves (100 µg mL ⁻¹)		
n-HF	Not active	Not active
DCMF	21.94±0.48 ^b	3.83±0.89 ^c
EAF	31.67±1.44 ^a	21.07±0.66 ^b
n-BF	20.28±0.48 ^b	11.49±1.15 ^b
AAE	Not active	Not active
Allopurinol (100 µg mL ⁻¹)	96.42±0.62	Not determined
Galanthamine (20 µg mL ⁻¹)	Not determined	50.00±1.21

n-HF: n-hexane fraction; DCMF: Dichloromethane fraction; EAF: Ethyl acetate fraction; n-BF: n-butanol fraction; AAE: Aqueous acetone extract. Values are Mean±SD (n = 3). Different letters in the same column indicate significant difference (p<0.05) for galls and leaves

Ciz *et al.*, 2010; Céspedes *et al.*, 2010). In this study, the Total Phenolic (TP) values significantly correlated with the ABTS and FRAP assays: 0.744 (TP-ABTS) and 0.934 (TP-FRAP) for fractions obtained from leaves aqueous acetone extract; 0.916 (TP-ABTS) and 0.954 (TP-FRAP) for fractions obtained from galls aqueous acetone extract. Thus, the antioxidant activity of *B. aegyptiaca* could be attributed to the phenolic compounds identified and quantified in galls and leaves.

Enzymatic activities: Xanthine Oxidase (XO) is the enzyme responsible for the formation of uric acid from the purines hypoxanthine and xanthine and is responsible for the medical condition known as gout (Filha *et al.*, 2006). Aqueous acetone extracts of galls and leaves and their fractions were assayed for XO inhibitory activity at a concentration of 100 µg mL⁻¹ in the assay mixture. The results are shown in Table 2. Of the aqueous acetone extracts and their fractions, it was evident that EAF exhibited the greater inhibitory activity (40.56%) followed by DCMF (33.33%) in galls. The same trend was found in leaves with 31.67% in EAF and 21% in DCMF. These results show that the xanthine oxidase inhibitory activity is better in galls extract and fractions than leaves extract and fractions and these activities are moderate compared to allopurinol (96.42%) at 100 µg mL⁻¹. Minimal or no inhibition was found with n-HF and aqueous acetone extracts of galls (6.06%) and leaves (2.20%).

Flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity (Costantino *et al.*, 1992). In fact, rutoside previously identified in the plant is an efficient suppressor of oxygen radical overproduction by rheumatoid arthritis neutrophils (Ostrakhovitcha and

Afanas'ev, 2001) and decreases serum urate levels in hyperuricemic mice (Zhu *et al.*, 2004). The p-coumaric acid has been reported to have xanthine oxidase inhibitory activity (Owen and Johns, 1999). Moreover, positive correlations were found between total flavonoids and xanthine oxidase inhibitory activity in galls fractions (R² = 0.526) and leaves fractions (R² = 0.4688). Hence, the presence of these phenolic compounds in aqueous acetone extracts of galls and leaves would have contributed towards xanthine oxidase inhibition. The galls and leaves of *B. aegyptiaca* could then contain bioactive substances useful in the treatment of gout or other xanthine oxidase induced diseases, justifying the popular use of this specie in rheumatism or arthritis in Burkina Faso folk medicine.

The acetylcholinesterase inhibitory activity is, usually, the first of a number of requirements for the development of medicines for treating some neurological disorders, such as Alzheimer's disease (Pereira *et al.*, 2009). Aqueous acetone extracts of galls and leaves and their fractions were also tested for their *in vitro* AChE inhibitory activity at a concentration of 100 µg mL⁻¹ in the assay mixture using galanthamine as a positive control. The results are summarized in Table 2. Among these fractions the galls DCMF (20.30%) and the leaves EAF (21.07%) exhibited the best AChE inhibitory activities, but these values are lowest compared to galanthamine inhibitory activity (50%) at 0.2 µg mL⁻¹. No activity was found in aqueous acetone extract and n-HF of galls and leaves.

Despite the fact that the fractions containing high levels of total phenolics presented the best acetylcholinesterase inhibitory activities, one can notice that extracts and fractions of galls and leaves of *B. aegyptiaca* were not good AChE inhibitors to be used in the treatment of Alzheimer disease. Gnoula *et al.* (2007) investigated the AChE inhibitory activity of *B. aegyptiaca* using the seed extracts and fractions. None of the tested extracts and fractions was inhibitory to AChE. This plant would be certainly used in traditional medicine to treat other mental disorders.

CONCLUSIONS

Aqueous acetone extracts and fractions from galls and leaves of *B. aegyptiaca* were analysed for their antioxidant, xanthine oxidase and acetylcholinesterase inhibitory activities. The results obtained in this study showed an interesting antioxidant activity of galls and leaves of *B. aegyptiaca* through the *in vitro* ABTS and FRAP assay. This activity is correlated to total phenolics content. The best antioxidant activities were found in

DCMF and EAF obtained from galls and leaves aqueous acetone extracts, respectively. The acetylcholinesterase inhibitory activities of galls and leaves fractions obtained are weak compared to the activity of galanthamine recognized as a potential inhibitor of this enzyme. Present finding was that the fractions of aqueous acetone extracts of galls and leaves materials exhibited a moderate XO inhibitory activity and therefore may contain bioactive constituents useful in the treatment of gout or other XO induced diseases. Future studies aim to isolate and identify these active constituents that exhibit significant XO inhibitory activity through bioassay-guided.

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