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Original Research Article

Antioxidant activity of *Allanblackia floribunda* trunk bark ethanol extract on alcohol-induced hypertensive rats

Danielle Claude Bilanda[1, ✉] , Alice Louise Woguia[2], Paul Désiré Djomeni Dzeufiet[1], Pierre Kamtchouing[1] and Théophile Dimo[1]

[1]Department of Animal Biology and Physiology, Laboratory of Animal Physiology, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon
[2]Department of Biochemisty, Laboratory of phytochemistry, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon

ABSTRACT

Oxidative stress is involved in many chronic diseases. The ethanol extract of *Allanblackia floribunda* (*A. floribunda*) was investigated for the antioxidant and antihypertensive activities on alcohol hypertensive rat's model. The free radical scavenging and antioxidant activities of plant extracts were evaluated *in vitro* by DPPH and ABTS assays, the ferric reducing antioxidant power assay and chelating ability on ferrous ions. Alcohol-induced hypertensive rats (AHR) were obtained by oral administration of ethanol (3 g/kg/day during 8 weeks) followed by 4 weeks of concomitant treatment with *A. floribunda* (100 and 200 mg/kg) and ethanol. Hemodynamic parameters were evaluated using the direct cannulation method. The effects of the extract on lipid profile, kidney and liver functions as well as oxidative stress markers were evaluated using colorimetric method. *A. floribunda* significantly reduced the mean blood pressure. The plant extract protected against the deterioration in lipid profile, reduced kidney and liver impairment, malondialdehyde (MDA) and increased the reduced glutathione (GSH) concentrations in organs. The total phenolic content of *A. floribunda* extract was found to be 76772.5 µg equivalent catechin/g. Antioxidant potential of the extract was higher with DPPH assay as compared to ABTS assay. These results suggest that the protective effect of the ethanol extract of *A. floribunda* in alcohol-induced hypertension was in part due to its antioxidant properties.

1. Introduction

Oxidative stress is mainly involved in the mechanisms of vascular injury of chronic diseases such as hypertension, atherosclerosis, and other forms of cardiovascular diseases (Sorriento et al., 2018). In hypertension, atherosclerosis, heart failure, and other contexts of vascular damage, increased reactive oxygen species (ROS) production leads to endothelial dysfunction and lipid peroxidation (Small et al., 2018). Markers of systemic oxidative stress are increased in both experimental and human hypertension (Montezano et al., 2015; Bilanda et al., 2018). Treatment with antioxidants may lower blood pressure and improve vascular structure and function in hypertension

(Sorriento et al., 2018). Many extracts from medicinal plants are able to prevent the formation of ROS and minimize their injuries with the huge amount of natural antioxidants (Banerjee et al., 2018; Kalantari et al., 2018; Lin et al., 2018). Bioactive phytochemical molecules have long been recognized as essential in maintaining healthy body systems. The antioxidant activity of some phytochemicals is even exploiting in cosmetic to increase skin firmness and prevent or slow down the ageing process (Sarker and Nahar, 2018; Mohammadhosseini et al., 2019). For these reasons, it has been suggested that treatment with antioxidants could be helpful in patients with chronic diseases as hypertension. Alcohol-induced hypertension is a suitable model to study the antioxidant properties of a compound on chromic

✉ Corresponding author: Danielle Claude Bilanda
Tel: +237 699966243; Fax: +237 699966243
E-mail address: bilandad@yahoo.com, dbilanda@uy1.uninet.cm

stress. In fact, chronic ethanol ingestion produces an increase in blood pressure that is related to the increased in vascular inflammation and oxidative stress., increased angiotensin II levels, induction of NADPH oxidase (causing endothelial injury), depletion of superoxide dismutase, down-regulation of endothelial NO generating system, impaired vascular relaxation, an imbalance of the nervous system, stimulation of the renin-angiotensin-aldosterone system, increased cortisol levels and increased intracellular calcium levels (Husain et al., 2014; Bilanda et al., 2018).

Allanblackia floribunda Oliv. (Clusiaceae) is a medicinal plant from the African rain forest on the mountains, and used in Cameroonian traditional medicine to treat hypertension. The antihypertensive as well as the improvement on oxidative status of animals treated with the aqueous extract of the trunk bark of *A. floribunda* has been examined (Bilanda et al., 2010, 2018). The *in vitro* antioxidant activity of some polysaccharides from *A. floribunda* had already been demonstrated (Boudjeko et al., 2013, 2015). Moreover, antioxidants play an important role to protect the body against injuries caused by reactive oxygen species (Small et al., 2018). In addition, ethanol extract of *A. floribunda* has shown to be more potent than aqueous extract (Boudjeko et al., 2015) probably because it contains more alkaloids, flavonoids, anthraquinones, cardiac glycosides and phenolic compounds than aqueous extract. Furthermore, among the flavonoids identified by HPLC, chlorogenic acid, *p*-coumaric acid, ferulic acid, quercitrin and kaempfrol were present in ethanol extract and absent in aqueous extract (Kada et al., 2013). Therefore, it was of great interest to understand the antioxidant activity of *A. floribunda* ethanol extract on hypertension. The present study was designed to evaluate the antioxidant potential of *A. floribunda* trunk bark ethanol extract on alcohol-induced hypertensive rats.

2. Experimental

2.1. Plant collection and extraction

The trunk bark of *A. floribunda* was collected in January 2016, in the centre region of Cameroon. The plant material was identified at the National Herbarium, Yaoundé where a voucher specimen N.1380/HNC was deposited. The bark was dried at room temperature (27 to 32 °C for one week and reduced to powder using an electric grinder "Moulinex, France". Ethanol extract was obtained after two days of maceration of 1.0 kg of the bark power in 2.0 L of aqueous ethanol 80%. The solution was filtered and concentrated to dryness under reduced pressure in a rotary evaporator at 90 °C. After the complete evaporation of ethanol at room temperature, 160.0 g (16% yields) of brown powder was obtained.

2.2. Phytochemical screening

Phytochemical analyses of the ethanol extract were done following the procedure described by Odebiyi and Sofowora, (1999). Chemical groups tested were alkaloids, saponins, flavonoids, cardiac glycosides, anthraquinones, phenols, glycosides and lipids.

2.3. Determination of polyphenolic concentration in the extract

The polyphenolic contents in the extract were measured using the Folin-Ciocalteu reagent (Sigma chemical Co., St. Louis, Mo), according to the method of Singleton and Rossi (1965). Plant extract (60.0 μL) was mixed with 2 mL of Folin-Ciocalteu reagent diluted 10 times for the determination of free polyphenolic content. The extract concentrations were from 0.05 to 4 mg/mL (seven different concentrations). The absorbance was read at 750 nm after 30 minutes using a spectrophotometer (UV-Shimadzu). Catechin (Sigma) and ferulic acid were used as standard and each concentration was replicated three times. The catechin concentrations were from 10.0 to 200.0 μg/mL.

2.4. Effect of scavenging 1,1-diphenyl 2-picrylhydrazyl (DPPH) radicals of the extract of *A. floribunda*

The free radical scavenging activity of the extract was measured by the DPPH using the method described by Mediesse-Kengne et al. (2014). Briefly, 4000 μL of the extract concentrations [50-600 μg/mL] were introduced into test tubes and 1000 μL of the freshly prepared solution of DPPH (400 μM) in methanol was added. The final concentrations of the extract in different tubes were 0.05, 0.1, 0.25, 0.5, 1, 2.5 and 4 mg/mL. The mixture was shaken vigorously and left to stand for 30 minutes in the dark. The absorbance was measured at 517 nm using a UV-VIS 1605 Shimadzu spectrophotometer. The scavenging ability was calculated as follows:

scavenging ability (%) = [(ΔA of control - ΔA of sample)/ ΔA of control] x 100   (Eqn. 1)

The EC_{50} value (mg/mL), the effective concentration at which the DPPH radicals were scavenged by 50% was calculated with the equation from the curve. Ascorbic acid (Asc) and catechin (Cat) were used as positive controls.

2.4.1. Determination of antioxidant potential by the 2,2'-azino-bis-3-éthylbenzylthiazoline-6-sulphonic acid (ABTS) free radicalscavenging assay of the extract of *A. floribunda*

The effect of the extract on ABTS radical was evaluated according to the method of Fadilah et al. (2018) with some modifications. To each 20 μL of

different extracts (0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 4.0 mg/mL), 1.0 mL of ABTS reagent was added. The mixture was agitated and kept in the dark for 30 minutes. The absorbance was measured at 734 nm using UV-VIS 1605 Shimadzu spectrophotometer. Catechin was used as a positive control.

2.4.2. Chelating ability on ferrous ions of the extract

The chelating ability was determined according to the method of Yasar et al. (2017). One millilitre of the sample (0.05-4.0 mg/mL) was reacted with 0.1 mL of $FeCl2$ (2.0 mM) for 1 minute, and then 0.2 mL of ferrozine (5.0 mM) was added. After 10 min at room temperature, the absorbance of the mixture was read at 562 nm against a blank (distilled water). A lower absorbance indicates a higher chelating power. The EC_{50} value (mg/mL) is the effective concentration at which ferrous ions were chelated by 50%. Citric acid (Sigma) and ethylene diaminetetraacetic acid (EDTA, Sigma) were used for comparison.

2.4.3. Antioxidant potential by ferric reducing antioxidant power (FRAP) of the extract

Extract antioxidant activity was measured by FRAP assay as described previously by Apak et al. (2010). Briefly, ferric to ferrous ion reduction at low pH forms a colored ferrous-2,4,6-tri(2-pyridyl)-s-triazine complex. 2.0 mL of FRAP reagent, containing 2,4,6-tripyridyl-striazine (TPTZ) 10.0 mM, $FeCl_3$ (10.0 mM) and acetate buffer (300 mM; pH=3.6) was mixed with 75 μL of extract or solvent (blank) to evaluate the free antioxidant capacity. The absorbance was read at 593 nm after 12 min of incubation. Catechin, vitamin C and α-tocopherol were used for calibration from 0.05 to 4.0 mg/mL as the extract.

2.4.4. *In vivo* antioxidant activity

2.4.4.1. Animals

Male Wistar rats of 4-6 weeks old, weighing between 100 and 120 g at the beginning of the experiment were used. They were maintained under standard laboratory conditions with a 12 h light and dark cycle, with free access to standard laboratory rat food and tap water. The standard animal diet was made up with corn (40.0%), wheat (20.0%), fish flour (24.0%), palm oil (7.0%), groundnut (3.0%), bones flour (2.0%), edible salt (1.0%), cotton edible crab (2.0%) and vitamin complex (1.0%). Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethical Committee (Reg. N°FWAIRD 0001954). To determine the antihypertensive and antioxidant activity of *A. floribunda*, alcohol-induced hypertensive rats (AHR) models were used.

2.4.4.2. Experimental design

Rats were randomly divided into two groups and allocated to each group according to their weight. The first group (of 5 rats) received distilled water by a gavage throughout 8 weeks and constituted the control normotensive rat (NTR). The second group (20 rats), the alcohol hypertensive rats (AHR), were obtained from normal rats by giving orally using by a gavage during 8 weeks, once a day, a 35° ethanol (3 g/kg). At the end of these 8 weeks, the rats of the second group were hypertensive (Bilanda et al., 2018). The first group (NTR) continued to receive distilled water, while the second group (AHR) was randomly divided into 4 groups of 5 rats each. All the 4 groups continued to receive ethanol concomitantly with distilled water (group 2), *Allanblackia floribunda* ethanol extract (100 and 200 mg/kg chosen from preliminary studies) groups 3 and 4 respectively, or nifedipine (10 mg/kg, p.o.) group 5, for 4 weeks. Throughout the experiment, body weight was evaluated twice a week. At the end of the respective treatments, arterial blood pressure and heart rate of all rats were recorded as previously described by Bilanda et al. (2010). Briefly, the rat was anesthetized using an intraperitoneal injection of urethane (1.5 g/kg). The trachea was exposed and cannulated to facilitate spontaneous breathing. The arterial blood pressure was measured from carotid artery *via* an arterial cannula connected to a pressure transducer coupled with a hemodynamic recorder Biopac Student Lab. (MP35) and computer. After blood pressure and heart rate recording, rats were sacrificed by decapitation and arteriovenous blood was collected. Serum was separated to determine serum total cholesterol, high
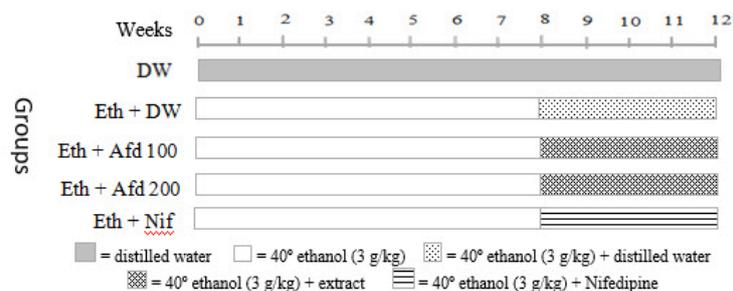

Fig. 1. Diagram of experimental design.

Table 1

Effect of *A. floribunda* ethanol extract on mean arterial blood pressure and heart rate.

	Water	Alcohol+water	Al+Ee100mg/kg	Al+Ee200mg/kg	Al+nif10mg/kg
Mean Blood pressure (mmHg)	105.23±4.92	156.42±3.98[c]	120.90±3.37[bα]	116.36±4.40[ω]	113.21±2.49[β]
Heart Rate (beat/minute)	330.06±18.18	348.49±25.77	347.46±24.11	336.14±22.09	346.16±17.65

Each value represents means ± SEM. N=5 rats; [b]P< 0.01, [c]P< 0.001, significantly different compared to normal rats. [α]P < 0.05, [β]P< 0.01, [ω]P< 0.001, significantly different compared to hypertensive rats. Water 10 mL/kg, alcohol (Al): ethanol 3 g/kg, Ee100: ethanol extract 100 mg/kg, Ee200: ethanol extract 200 mg/kg; nif: nifedipine 10 mg/kg.

Table 2

Total phenolic content and ferric reducing antioxidant power (FRAP) assay of the extract compared to standards.

Compounds	Catechin equivalence (µg/g extract)	Ascorbic acid equivalence (µg/g extract)	Vitamin E equivalence (µg/g extract)	Phenolic compound contain (mg eq catechin/g extract)
Extract	6636.3 ± 44.64	176.53 ± 1.77	66.40 ± 1.10	76.77 ± 9.20

Each value represents means ± SD (n=3)

density lipoprotein cholesterol (HDL-cholesterol), triglycerides, urea, alanine and aspartate transaminases (ALT and AST, respectively) activity and bilirubin levels using commercial diagnostic kits, Fortress, UK. Atherogenic index was calculated using the following formula (Wakayashi and Kobaba, 2002):

Atherogenic index = total cholesterol-HDL-cholesterol/ total cholesterol   (Eqn. 2)

Then, some organs (heart, aorta, liver and kidney) were removed, washed and weighed. Homogenates of 20% were prepared in Mc Even solution for aorta and heart or in Tris-HCl buffer (50 mM) for liver and kidney. Homogenates were centrifuged at 10,000 × g for 30 min. Serum and homogenate supernatant were stored at -20 °C. Reduced glutathione (GSH) was determined using Misra and Fridovish (1972) method. The end product of lipid peroxidation, malondialdehyde (MDA) was determined using the procedure of Wilbur et al. (1949).

2.5. Statistical analysis

Results are expressed as the mean ± SD for the *in vitro* activity and as mean ± SEM for the *in vivo* activity. The difference between the groups was compared using one-way analysis of variance (ANOVA) followed by the Dunnett's post hoc test, using Graphpad Prism version 5.03 software. A value of P < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Effect of *A. floribunda* on hemodynamic parameters

The effect of *A. floribunda* ethanol extract on blood pressure and heart rate of the experimental animals is summarized in Table 1. Administration of alcohol resulted in an increase in mean blood pressure of the rats. The increase in blood pressure was of 48.63% for the alcohol untreated group compared with normal rats. The blood pressure of treated animals was significantly lower as compared with the untreated rats. The reduction in blood pressure produced by the plant extract was of 22.70 and 25.60%, respectively, at the doses of 100 and 200 mg/kg and 27.62% with nifedipine (10 mg/kg) as compared with the alcohol hypertensive rats. No significant change in heart rate was observed in all the groups at the end of treatment.

The decrease in blood pressure on the AHR treated with the plant extract may be attributed to its antihypertensive properties (Bilanda et al., 2018). In fact, the aqueous extract of the stem back of *A. floribunda* has already proved to prevent and treat ethanol as well as sucrose-induced hypertension rat (Bilanda et al., 2010; Bilanda et al., 2018). In addition, ethanol extract possesses more alkaloids, flavonoids, anthraquinones, cardiac glycosides and phenolic compounds than aqueous extract. Furthermore, among the flavonoids identified, chlorogenic acid, *p*-coumaric acid, ferulic acid, quercitrin, kaempfrol were present in ethanol extract and absent in aqueous extract (Kada et al., 2013). Therefore, a better activity with ethanol compared to aqueous extract can be explained (Boudjeko et al., 2015). However, by considering the other parameters of the study, we could also suggest the implication of the antioxidant activity in blood pressure reduction (Sorriento et al., 2018).

3.2. Phytochemistry and antioxidant activities *in vitro*

Among the chemical groups tested anthraquinones, phenols, flavonoids, alkaloids, cardiac glycosides, glycosides, lipids and tannins were present, whereas saponins were absent. As shown in Table 2, the total phenolic content of *A. floribunda* extract was of 76.77 mg equivalent catechin/g extract. This value is confirmed by the FRAP results of the extract in relation to catechin, ascorbic acid and vitamin E equivalence. The
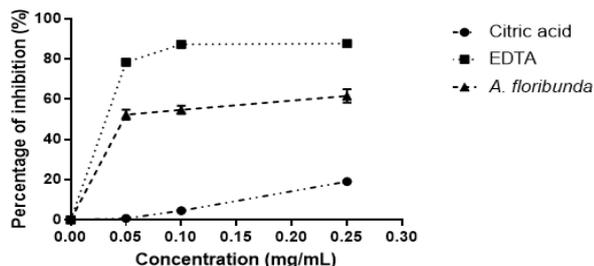
Fig. 2. Reducing power of ethanol extract of *A. floribunda* and control standards (Each point is expressed as means ± SD (n=3). PI eth: ethanol extract of *Allanblackia floribunda*).

Table 3

Antioxidant potential of *A. floribunda* ethanolic extract by ABTS and DPPH assays compared with some standards.

| Compounds	DPPH (IC$_{50}$ mg/mL)	ABTS (IC$_{50}$ mg/mL)
Catechin	0.27 ± 0.01	1.14 x 10^{-5}
Ascorbic Acid	0.82 ± 0.02	0.006
Vitamin E	2.00 ± 0.08	0.15 ± 0.003
Ethanolic extract	1.06 ± 0.02	0.20 ± 0.02

Each value is expressed as means ± SD (n=3)

antioxidant activity obtained from the ethanol extract of *A. floribunda*, 6636.3 ± 44.64 catechin equivalence µg/g extract shows that its activity is closer to that of catechin as compared to the other standards used (Table 2).

Ferrous chelating ability assay is based on the competition between ferrozine and the bioreactive components of the plant extract in trapping ferrous ions (Yasar et al., 2017). This is translated by a reduction in the absorbance of the ferrozine (Fe^{2+}) complex. One of the assays of antioxidative action is chelation of transition metal, thus preventing catalysis of hydroperoxide decomposition and reactions with genetic material. As shown in Fig. 2, EDTA has an excellent chelating ability of 86.51% at 0.25 mg/mL whereas chelating ability of citric acid was less, 19.0% at equal concentration. *A. floribunda* extract chelated ferrous ions by 53-60.3% at the same concentration (Fig. 2). It is at that remarkable concentration that *A. floribunda* ethanol extract reached its highest chelating ability level. The IC$_{50}$ of extract (0.025 ± 0.002 mg/mL) was lower than the citric acid one (0.58 ± 0.03 mg/mL), showing its good chelating ability for ferrous ions.

To determine the free radical scavenging activity of the plant extract, DPPH and ABTS assays were performed and results are shown in Table 3. With DPPH test, *A. floribunda* ethanol extract radical scavenging activity was lower as compared to catechin and ascorbic acid, but higher than vitamin E. The plant extract was relatively less potent than ascorbic acid and relatively more potent than vitamin E. with ABTS test. However, vitamin E became more potent than the plant extract, showing that the extract had more affinity with the DPPH radical as compared to the ABTS radical.

The free radical scavenging effects obtained with the *in vitro* test may be linked to the presence of flavonoids and tannins as polyphenols in the ethanol extract. Flavonoids are phenolic compounds and as well-known compounds many of those have strong antioxidants or free radical scavenging activity (Banerjee et al., 2018; Lin et al., 2018). The great amount of phenolic compounds (76.77 mg equivalent catechin/g) in *A. floribunda* ethanol extract is in same line with the observations made by some authors (Boudjeko et al., 2013). It is well-established that total phenolic content has a great influence on antioxidant capacity (Sorriento et al., 2018). The antioxidant capacity increases with the phenolic content (Lin et al., 2018). A high antioxidant activity was obtained from the plant extract (6636.3 ± 44.64 catechin equivalence µg/g extract). Compared to the other standards used in the test, the antioxidant activity of *A. floribunda* was closer to the one of catechin. Many plants are sources of natural antioxidants that are used in the treatment of many diseases or as a dietary supplement (Banerjee et al., 2018; Kalantari et al., 2018). Currently, there is continuing research on screening of antioxidant therapy in hypertension and other areas (Sarker and Nahar, 2018; Sorriento et al., 2018). *A. floribunda* had appeared to possess *in vitro* antioxidant and vascular protective activity (Banerjee et al., 2018; Bilanda et al., 2018). With DPPH test, the ethanol extract radical scavenging activity was lower as compared to catechin and ascorbic acid, but higher than vitamin E. with ABTS test. However, vitamin E became more potent than the plant extract, showing that the extract had more affinity with the DPPH radical as compared to the ABTS radical. In any case, the two tests clearly showed that the plant extract possessed a strong free radical scavenging activity. The scavenging activity of *A. floribunda* ethanol extract was even higher than that of vitamin E with DPPH test. A similar observation was reported with the *in vitro* antioxidant activities of root extract of *Asparagus racemosus* Linn (Karuna et al., 2018). However, some antioxidants with ABTS scavenging activity may not have DPPH scavenging activity, as found in phenolic compounds from *Salvia officinalis* L (Wang et al., 1998). The difference in the two assays could be explained by the different mechanisms in scavenging ABTS and DPPH radicals. In the DPPH assay, the antioxidant effect was likely to be due to the hydrogen donating ability of the extract (Conforti et al., 2005), while ABTS assay measures the activity of the antioxidant in scavenging proton radicals through a donation of electrons. The results of the present study implied that ethanol extract of *A. floribunda* might mostly contain compounds with hydrogen donating ability. The involvement of free radicals, specifically their increased production, appears to be a feature of cardiovascular diseases (Small et al., 2018). Therefore *A. floribunda* ethanol extract may be particularly important in fighting these diseases through affording protection against free radical damages to cellular DNA, lipids and proteins. That action of those
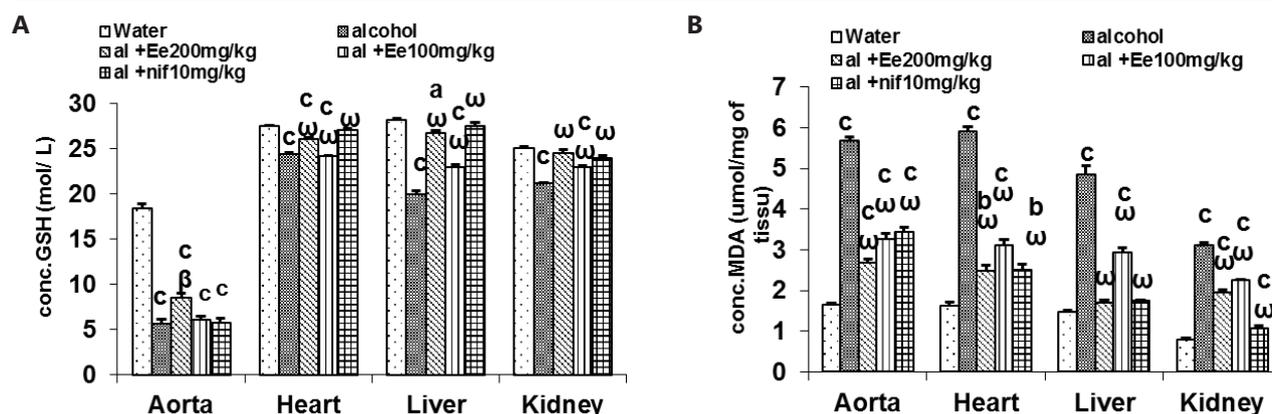
Fig. 3. Effect of *Allanblackia floribunda* on GSH (A) and MDA (B) levels of ethanol induced hypertensive rats after 4 weeks of treatment. Each value represents means ± S.E.M. of 5 rats; .aP< 0.05, bP< 0.01, cP< 0.001, significantly different compared to normal rats. ωP< 0.001, significantly different compared to hypertensive rats. Water 10 mL/kg, Alcohol (Al): Ethanol 3 g/kg, Ee100: ethanol extract 100 mg/kg, Ee200: Ethanol extract 200 mg/kg Nif: nifedipine (10 mg/kg).

compounds may in turn be involved in the protection of the organs against free radicals injuries. The radical scavenging potential of the extract was confirmed by its strong ability in chelating the transition metal, thus preventing catalysis of hydroperoxide decomposition and phenotype reactions. In metal chelating ability, the IC_{50} of the extract (0.025 ± 0.002 mg/mL) was lower than the one of citric acid (0.58±0.03 mg/mL), showing its good chelating ability for ferrous ions.

3.3. *Allablanckia floribunda in vivo* antioxidant activity

Chronic feeding with alcohol resulted in a significant increase of MDA and reduction of reduced glutathione levels in aorta, heart, liver and kidney (Fig. 3) as compared with the NTR. The additions of *A. floribunda* ethanol extract or nifedipine to these treatments markedly reduced the MDA (Fig. 3B) and increased reduced glutathione (Fig. 3A) levels as compared with the AHR. MDA was significantly increased by 246.3% in the aorta, 265.4% in the heart, 227% in the liver and 295.1% in the kidney in AHR as compared to NTR. *A. floribunda* extract significantly (P<0.001) reduced MDA by 42.60% and 52.81% in the aorta, 47.63% and 58.10% in the heart, 39.25% and 65.12% in the liver and 27.43% and 36.83% in the kidney at the doses of 100 and 200 mg/kg respectively as compared to alcohol hypertensive rats (AHR).

The reduction of GSH was of 69.30% in the aorta, 10.98% in the heart, 29.05% in the liver and 15.56% in the kidney in AHR as compared with normotensive rats. *A. floribunda* significantly increased GSH levels in the investigated organs as compared to AHR. That increase was of 7.06% and 50.88% in the aorta, of 14.78% and 33.96% in the heart and 8.50% and 15.97% in the kidney at the doses 100 and 200 mg/kg, respectively as compared to AHR. The increase in reduced glutathione (GSH) was of 10.53% in the heart, 37.36% in the liver and 13.42% in the kidney of the group treated with nifedipine (10 mg/kg) as compared with the AHR.

The antioxidant activity of the extract was evident in vivo by the protective effects of *A. floribunda* ethanol extract ability to prevent lipid peroxidation. That was shown with the low levels in MDA on different organs of AHR treated with the plant extract. Inhibition of lipid peroxidation and production increase of GSH may confirm the implication of the antioxidant effect of the plant extract in the treatment of hypertension. Similar results were obtained with *Capparis spinosa* L extract when studying its hepatoprotective activity related to its antioxidant potential (Kalantari et al., 2018). An increase in organs GSH levels in rats treated with the plant extract may be attributed to the antioxidant effect of the extract to scavenge ROS, and then minimized the amount of GSH used (Kukongviriyapan et al., 2007). We could therefore correlate the antioxidant activities by

Table 4

Effect of *A. floribunda* on lipid profile of alcohol-induced hypertension in rats.

	Water	Alcohol	Al + Ee100	Al + Ee200	Al + Nif10
Triglycerides (mg/dL)	56.01 ± 3.07	107.63 ± 4.12[c]	98.81 ± 4.56[c]	69.25 ± 5.75[aω]	76.98 ± 2.91[aω]
Total cholesterol (mg/dL)	71.90 ± 4.53	163.93 ± 4.20[c]	89.15± 4.58[ω]	69.08 ± 2.98[ω]	84.06 ± 5.29[ω]
HDL cholesterol (mg/dL)	30.37 ± 4.47	59.22 ± 3.33[c]	56.93 ± 3.10[β]	38.41 ± 3.58[b]	39.45 ± 3.48[β]
Atherogenic Index (mg/dL)	71.48 ± 4.53	163.56 ± 4.23[c]	88.72 ± 4.59[ω]	68.25 ± 3.00[ω]	83.57 ± 5.35[ω]

Each value represents means ± S.E.M. of 5 rats; [a]P< 0.05, [b]P< 0.01, [c]P< 0.001, significantly different compared to normotensive rats (NTR). [β]P< 0.01, [ω]P< 0.001, significantly different compared to hypertensive rats. Water 10 mL/kg, Alcohol (Al): Ethanol 3 g/kg, Ee100: Ethanol extract 100 mg/kg, Ee200: Ethanol extract 200 mg/kg Nif: nifedipine10 mg/kg.

Table 5

Effect of *A. floribunda* on liver and kidney function parameters.

	Water	Alcohol	Al + Ee100	Al + Ee200	Al + Nif10
Urea(mg/dL)	**45.56**±1.00	**70.65**±2.02[c]	**52.32**±1.79[aω]	**44.83**±1.51[ω]	**45.84**±1.12[ω]
Bilirubin(mg/dL)	**1.13**±0.05	**1.48**±0.07[a]	**1.27**±0.09	**1.23**±0.05	**1.25**±0.05
ALT(UI)	**179.70**±4.00	**273.92**±4.25[c]	**201.55**±3.76[aω]	**178.30**±3.74[ω]	**197.84**±7.32[ω]
AST(UI)	**96.08**±5.15	**156.84**±5.98[c]	**104.14**±3.69[ω]	**91.69**±4.08[ω]	**81.43**±3.55[ω]

Each value represents means ± S.E.M. of 5 rats; [a] $P< 0.05$, [b] $P< 0.01$, [c] $P< 0.001$, significantly different compared to normal rats. [ω] $P< 0.001$, significantly different compared to hypertensive rats. Water 10 mL/kg, Alcohol (Al): Ethanol 3 g/kg, Ee100: ethanol extract 100 mg/kg, Ee200: Ethanol extract 200 mg/kg Nif: nifedipine10 mg/kg.

DPPH, ABTS and FRAP assays with the levels of MDA and GSH. Moreover, the antioxidant activity of the plant extract can also be linked with the improvement of hemodynamic parameters of AHR. In fact, it was observed that the extract strongly scavenged the free radicals with radical quenching tests as well as ferric reducing power. The strong antioxidant potential of ethanol extract of *A. floribunda* as shown in vitro can then explain the protective effect of the extract on liver and kidney as well as the effect on lipid profile (Elks et al., 2011).

3.4. Effect of *A. floribunda* on lipid profile, liver and kidney functions

The administration of ethanol during twelve weeks increased significantly the level of triglycerides, total cholesterol and atherogenic index by 91.81%, 127.99% and 128.81%, respectively as compared to the control normotensive rats (NTR). The concomitant treatment with ethanol and *A. floribunda* extract (100 and 200 mg/kg) or nifedipine (10 mg/kg, p.o.) has decreased the serum total cholesterol level and the atherogenic index and increased serum HDL-cholesterol. However, there was no significant change in the concentration of triglyceride in the group treated with *A. floribunda* (100 mg/kg), while triglycerides decreased with the dose of 200 mg/kg of the extract and with nifedipine treatment (Table 4).

As shown in Table 5, the levels of AST, ALT, bilirubin and urea in serum were significantly increased (P < 0.001) in AHR as compared to control NTR. *A. floribunda* ethanol extract has significantly and dose-dependently decreased the level of these parameters as compared to AHR.

The protection of the extract against lipid profile deterioration might be due to its hypotensive activity as well as its antioxidant capacity (Boudjeko et al., 2015; Banerjee et al., 2018). The results of the present study showed that the plant extract really protected liver and kidney by reducing the peroxidation of cell membranes. The plant extract was also able to reduce the markers of liver and kidney function impairment. In fact, many authors have already linked the improvement of these markers to the protection of the function of these organs (Elks et al., 2011; Bilanda et al., 2018; Kalantari

et al., 2018). The lipid profile's improvement by *A. floribunda* ethanol extract suggests that the extract has hypolipidemic activity. These results are consistent with those of some authors (Aikawa et al., 2008) who have shown that vascular functions and some indices of oxidative stress can be improved with lipid lowering. Collectively, these results suggest a strong antioxidant capacity that may be involved in the treatment of hypertension induced by the ethanol of the trunk bark of *A. floribunda*.

4. Concluding remarks

The findings of the present study suggest that ethanol extract of *A. floribunda* could be a potential source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing down oxidative stress related degenerative diseases. This antioxidant activity is due mostly to compounds with hydrogen donating ability which combined with its good chelating ability for ferrous ions contributing to protect vital organs. However, further studies are necessary to examine underlying mechanisms of antioxidant effect and to isolate the bioactive compounds responsible for these pharmacological activities as well as its *in vitro* effects on blood vessels.

Conflict of interest

The authors declare that there is no conflict of interest to declare.

References

Aikawa, M., Sugiyama, S., Hill, C.C., Voglic, S.J., Rabkin, E., Fukumoto, Y., Schoen, F.J., Witztum, J.L., Libby, P. 2008. Lipid lowering reduces oxidative stress and endothelial cell activation in rabbit atheroma. Circulation 106, 1390-1396.

Apak, R., Güçlü, K., Ozyürek, M., Bektaşoğlu, B., Bener, M., 2010. Cupric ion reducing antioxidant capacity assay for antioxidants in human serum and for hydroxyl radical scavengers. Methods Mol. Biol. 594, 215-239.

Kasrati, A., Alaoui Jamali, C., Abbad, A., 2017. Antioxidant properties of various extract from selected wild Moroccan aromatic and medicinal species. Trends Phytochem. Res. 1(4), 175-182.

Banerjee, J., Das, A., Sinha, M., Saha, S., 2018. Biological efficacy of medicinal plant extracts in preventing oxidative damage. Oxid. Med. Cell Longev. ID 7904349.

Bilanda, D.C., Dimo, T., Djomeni, D.D.P., Bella, T.M.N., Aboubakar, O.B.F., Nguelefack, B.T., Tan, V.P., Kamtchouing, P., 2010. Antihypertensive and antioxidant effects of *Allanblackia floribunda* Oliv. (Clusiaceae) aqueous in alcohol- and sucrose-induced hypertensive rats. J. Ethnopharmacol. 128, 634-640.

Bilanda, D.C., Dzeufiet, D.P.D., Bopda, A.M., Kamtchuoing, P., Dimo, T., 2018. *Allablanckia floribunda* hypotensive activity on ethanol induced hypertension in rat. J. Phytopharmacol. 7(2), 146-151.

Boudjeko, T., Kanemoto, N.J.E., Woguia, A.L., Njintang, Y.N., 2013. Partial characterization, antioxidative properties and hypolipidemic efects of oil seed cake of *Allanblackia floribunda* and *Jatropha curcas*. BMC Complement. Altern. Med. 13, 352- 362.

Boudjeko, T., Megnekou, R., Woguia, A., Kegne, F.M., Ngomoyogoli, J.E., Tchapoum, C.D., Koum, O., 2015. Antioxidant and immunomodulatory properties of polysaccharides from *Allanblackia floribunda* Oliv stem bark and *Chromolaena odorata* (L.) King and H.E. Robins leaves. BMC Res. Notes. 8, 759-768.

Conforti, F., Loizzo, M.R., Statti, G.A., Menichini, F., 2005. Comparative radical scavenging and antidiabetic activities of methanolic extract and fractions from *Achillea ligustica*. All. Biol. Pharm. Bull. 28(9), 1791-1794.

Elks, C.M., Reed, S.D., Mariappan, N., Shukitt-Hale, B., Joseph, J.A., Ingram, D.K., Francis, J.A., 2011. Blueberry-enriched diet attenuates nephropathy in a rat model of hypertension via reduction in oxidative stress. PLOS ONE 6(9), 1-10.

Fadilah, F., Arsianti, A., Yanuar, A., Andrajati, R., Paramita, R.I., Purwaningsih, E.H., 2018. Structure activity relationship analysis of antioxidant activity of simple benzene carboxylic acids group based on multiple linear regression. Orient. J. Chem. 34(5), 1214-1224.

Husain, K., Ansari, R., Ferder, L., 2014. Alcohol-induced hypertension: Mechanism and prevention. World. J. Cardiol. 6, 245-252.

Ipek, Y., Ozen, T., Demirtas, I., 2017. Extraction, purification, characterization and antioxidant activities of heat-resistance phenolic compounds from lemon pulp. Trends Phytochem Res. 1(1), 199-206.

Kada, A.S., Miegueu, P., Bilanda, D.C., Nya Ngassam, F.M., Watco, P., Dzeufiet, D.P.D., Kamtchouing, P., 2013. Ejaculatory activities of *Allanblackia floribunda* stem back in spinal male rats. Pharm. Biol. 51(8), 1014-1020.

Kalantari, H., Foruozandeh, H., Khodayar, M.J., Siahpoosh, A., Saki, N., Kheradmand, P., 2018. Antioxidant and hepatoprotective effects of *Capparis spinosa* L. fractions and quercetin on tert-butyl hydroperoxide- induced acute liver damage in mice. JTCM 8(1), 120-127.

Karuna, D.S., Dey, P., Das, S., Kundu, A., Tejendra, B., 2018. *In vitro* antioxidant activities of root extract of *Asparagus racemosus* Linn. JTCM 8(1), 60-65.

Kukongviriyapan, U., Luangaram, S., Leekhaosoong, K., Kukongviriyapan, V., Preeprame, S., 2007. Antioxidant and vascular protective activities of *Cratoxylum formosum*, *Syzygium gratum* and *Limnophila aromatica*. Biol. Pharm. Bull. 30, 661-666.

Lin, H-Y., Chang, T-C., Chang, S-T., 2018. A review of antioxidant and pharmacological properties of phenolic compounds in *Acacia confuse*. JTCM 8, 443-450.

Mediesse-Kengne, F., Woguia, AL., Fogue, P., Atogho-Tiedeu, B., Simo, G., Boudjeko, T., 2014. Antioxidant properties of cell wall polysaccharides of *Stevia rebaudiana* leaves. J. Cost Life Med. 2(12), 962-969.

Misra, H., Fridovish, I., 1972. Determination of the level of superoxide dismutase in whole blood. New Haven, Yale University Press, pp. 101-109.

Mohammadhosseini, M., Venditti, A., Sarker, S.D., Nahar, L., Akbarzadeh, A., 2019. The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities - A review. Ind. Crops Prod. 129, 350-394.

Montezano, A.C., Dulak-Lis, M., Tsiropoulou, S., Harvey, A., Briones, A.M., Touyz, R.M., 2015. Oxidative stress and human hypertension, vascular mechanisms, biomarkers, and novel therapies. Can. J. Cardiol. 31(5), 631-641.

Odebiyi, A., Sofowora, A.E., 1999. Phytochemical screenings of Nigerian medicinal plants part. Lyodia. A.E. 44, 234-246.

Sarker, S.D., Nahar, L., 2018. Phytochemicals and phyto-extracts in cosmetics. Trends Phytochem. Res. 2(4), 185-186.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144-158.

Small, H.Y., Migliarino, S., Czesnikiewicz-Guzik, M., Guzik, T.J., 2018. Hypertension: Focus on autoimmunity and oxidative stress. Free Radic. Biol. Med. 125, 104-115.

Sorriento, D., De Luca, N., Trimarco, B., Iaccarino, G., 2018. The antioxidant therapy, new insights in the treatment of hypertension. Front. Physiol. 9, 258-265.

Wakayashi, I., Kobaba, W.R., 2002. Effet de l'âge sur le rapport entre le boire et les rapports arthérosclérotiques. Gerontology 48, 151-156.

Wang, M., Li, J., Rangarajan M., Shao, Y., Lavoie, EJ., Huang, TC., Ho, C.T., 1998. Antioxidative phenolic compounds from Sage (*Salvia officinalis*). J. Agric. Food Chem. 46, 4869-4873.

Wilbur, K.M., Bernheim, F., Shapiro, O., 1949. Determination of lipid peroxidation. Arch Biochem. 24, 305-310.