

Asian Journal of Research in Biological and Pharmaceutical Sciences

Journal home page: www.ajrbps.com



IN VITRO ANTIOXIDANT ACTIVITY, CYTOTOXICITY AND GENOTOXICITY OF EXTRACTS FROM THE LEAVES AND BARKS OF ANTHOCLEISTA SCHWEINFURTHII GILG (LOGANIACEAE)

Steve Valdi Djova^{2*}, Maximilienne Ascension Nyegue¹, Alian Desire Afagnigni², Francois-Xavier Etoa¹

^{1*}Department of Microbiology, University of Yaounde I, PO Box 812 Yaounde, Cameroon.

²Department of Biochemistry, University of Yaounde I, PO Box 812 Yaounde, Cameroon.

ABSTRACT

The present study evaluated *in vitro* antioxidant activity, cytotoxicity and genotoxicity of aqueous extracts from *Anthocleista schweinfurthii*. Polyphenols content was determined by the Folin Ciocalteu method. The antioxidant activities were evaluated using ABTS and β -carotene assays. Cytotoxicity was investigated by the MTT method while genotoxicity was ascertained using the *Salmonella typhimurium* test strains TA98 and TA100. Standard methods were used for phytochemical screening. The aqueous extract of the bark is rich in total polyphenols of 985 ± 32 mg EAA/g of dry weight. Aqueous extract of *Anthocleista schweinfurthii* barks had a high antiradical activity with IC_{50} of $153 \pm 3 \mu\text{g/mL}$ and high reducing activity with IC_{50} of $2.3 \pm 0.12 \mu\text{g/mL}$. Toxicity study indicated that all extracts are cytotoxic and non genotoxic. Phytochemical screening revealed the presence of alkaloids, saponins and flavonoids in both extracts. Lack relatively toxicity suggest that this plant is probably safe for use, however futher studies are needed for longer.

KEYWORDS

Anthocleista schweinfurthii, Aqueous extracts, Antioxidant, Cytotoxicity and Genotoxicity.

Author for Correspondence:

Steve Valdi Djova,
Department of Biochemistry,
University of Yaounde I,
PO Box 812 Yaounde, Cameroon

Email: djova.stevevaldi@yahoo.fr

INTRODUCTON

The human body produces in a normal metabolic process free radicals known as oxygen reactive species¹. Oxidative stress is an imbalance between the production of free radicals and defense antioxidants in favor of the production of free radicals². Several acute and chronic diseases such as certain cancers, diabetes, inflammation, arthritis, atherosclerosis and various other metabolic disorders result from oxidative stress^{3,4}. Thus, a balance between oxidation and antioxidation must be maintained for a healthy biological system^{1,5}.

This can be accomplished by exploiting biomolecules with antioxidant activities⁶. Indeed, the search for natural antioxidants is the subject of several research studies and a new approach is being developed in the exploration of secondary metabolites in general and polyphenols in particular for the prevention of degenerative diseases and for their use in the agri-food industry^{7,8}.

A. schweinfurthii is a plant of the Loganiaceae family. In Cameroon and Gabon, *A. schweinfurthii* is used for its galactogenic properties. In Congo, *A. schweinfurthii* stem-bark decoction is taken to treat hernia⁹. A root decoction is used to treat stomach upset in women, ovarian diseases, venereal diseases, hernia, bronchitis and fever; a purgative and used to trigger delivery. In Tanzania, the root decoction is taken against malaria, hard abscesses. Juice of young leaves, root powder or bark pulp are used to treat wounds, abscesses, as a hemostatic and for cicatrizing. Its juice is used to treat otitis and ophthalmia⁹. In Nigeria, *A. djalonensis* is used in traditional medicine to treat breast cancer¹⁰. The *in vitro* evaluation of the antioxidant activity of several species belonging to the genus *Anthocleista* has already been carried out¹¹.

A Greek pharmacist and physician demonstrated that plants do not only contain beneficial medicinal constituents, but they also have toxic substances¹². Several studies have led to the validation of some traditional remedies. However, research has demonstrated that natural products are potentially toxic, carcinogenic and teratogenic in both *in vitro* and *in vivo* assays, thus they should be used cautiously¹³⁻¹⁵. Concerns have been raised about the toxicity and side effects of medicinal plants in long term use. The WHO continues to encourage the use of medicinal plants in developing countries to supplement their healthcare program provided that they are proven to be non-toxic¹⁶. The potential toxicity effects of some of the more popularly used herbal remedies are a cause for concern. It is thus necessary to assess the cytotoxicity and genotoxicity of traditional medicinal plants to ensure their relative safety as it would be dangerous to assume that all plant extracts are safe to use.

To date, to the best of our knowledge, very few pharmacological data exist on the species *A. schweinfurthii*. Therefore, the aim of the present

study was to evaluate the *in vitro* antioxidant activity, cytotoxicity and genotoxicity of extracts from leaf and bark of *A. schweinfurthii*.

MATERIAL AND METHODS

Plant material and extraction procedure

A. schweinfurthii plant was collected at Loum in the Littoral region of Cameroon in August 2016. The plant identification was done at the Cameroon National Herbarium by comparison with specimen number 52349/HNC of *A. schweinfurthii* Gild (Loganiaceae).

The leaves and barks of *A. Schweinfurthii* were air-dried for one week at room temperature and weighed. The samples were then ground to fine powder in a mortar and 500g of dried powder of each sample was soaked for 48 hours in water. The mixing was filtered through Whatman No.1 filter paper and lyophilized using the lyophilizer Biobase. Then, the aqueous extracts of *A. Schweinfurthii* was collected in Eppendorf tubes and preserved in a refrigerator at 4°C for further use.

Determination of total polyphenols content

The total polyphenols were evaluated according to the spectrophotometric method using the Folin-Ciocalteu reagent as described by Chew *et al*¹⁷. In this method, 1817 µL of distilled water were introduced into the test tube, 115 µL of Folin-Ciocalteu diluted 1:10 and 345 µL of 15% sodium carbonate (Na₂CO₃) were added. Tubes were vortexed and incubated for 2 hours, and the absorbance measured at 765 nm. Calibration was performed using a freshly prepared aqueous solution of ascorbic acid.

ABTS radical scavenging assay

The ABTS scavenging activity assay was performed as described by Re *et al*¹⁸. The discoloration of ABTS was monitored to evaluate the antioxidant capacity of the extracts. For this, 7mM of stock solution of the cationic radical ABTS was prepared. This stock solution was diluted with methanol until an absorbance of 0.7 (± 0.02) at 734 nm was obtained with the spectrophotometer JENWAY 6305. The stock solution diluted constituted the working solution. Tubes containing 20 µL of 10 times diluted extracts to which 2 mL of the ABTS working solution was added were used and after 30

minutes of incubation, the absorbance was read at 734 nm against a blank constituted of 20 μ L of methanol replacing the extract. The tests were carried out in triplicate and the reference molecule used was ascorbic acid.

β -carotene bleaching coupled with the auto-oxidation of linoleic acid assay

The reductive capacity of extracts was estimated by the method described by Moure *et al*¹⁹. A quantity of 2 mg of β -carotene is dissolved in 10 mL of chloroform. Then, 1 mL of this solution was taken from a vial containing 200 mg of Tween 20 and 20 μ L of linoleic acid. This solution was evaporated by rotary evaporator until the odor of chloroform disappeared. Then, a volume of 100 mL of the dilute hydrogen peroxide was added to the flask and the resulting mixture was stirred vigorously. In screw tubes, the β -carotene/linoleic acid emulsion of 4 mL was added to 200 μ L of the methanolic solution of the extract or the synthetic antioxidant BHA of different concentrations. After stirring properly, the absorbance was measured immediately at 470 nm, which corresponded to $t = 0$ min against the blank containing the emulsion without β -carotene. The well-closed tubes were placed in a water bath at 50°C for 120 minutes. Then, the absorbance of each extract is measured at 470 nm at $t = 120$ min. For positive control, the sample was replaced by BHA. The negative control consists of 200 μ L of methanol instead of the extract for the synthetic antioxidant. All analyses were repeated in triplicate.

***In vitro* cytotoxicity testing of biologically active extracts using the MTT assay**

The MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay according to Mosmann²⁰ was used to evaluate viability of cells after their exposure to the test substances. The biologically active extracts were tested for cytotoxicity against the Vero (African green monkey kidney) cell line obtained from the Department of Veterinary Tropical Diseases, University of Pretoria, South Africa. The Vero cells were cultured in sterile Minimal Essential Medium (MEM, Sigma) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (FCS, Sigma) in a 75 cm² flask incubated at 37 °C in 5% CO₂. After one week of incubation, the medium was decanted,

and the cells adhering to the bottom of the flask were washed twice with 20 mL of phosphate buffered saline (PBS, Sigma). Following the washing step, trypsin (3 mL) was added to the cells which were then incubated at 37°C for up to 20 min to allow detachment of cells from the flask. When the cells had detached from the flask, MEM containing FCS was added to stop the enzymatic action of the trypsin. The cells were poured into centrifuge tubes and centrifuged for 2 min at 200 x g. A Neubauer haemocytometer was used to count the number of cells per mL. Trypan blue was added to determine the number of viable cells as viable cells extrude the trypan blue and appear clear while dead cells are blue. The cells were resuspended to a concentration of 1×10^5 cells/mL and 100 μ L were added to each well of columns 2 to 11 of 96-well microtiter plates at a final concentration of 10 000 cells per well. Minimal Essential Medium (200 μ L) was added to wells of columns 1 and 12 to maintain humidity. The microplates were incubated overnight at 37°C in a 5% CO₂ incubator until the cells were in the exponential phase of growth, to allow cell attachment to the bottom of the microplates. The attached cells in 96 well microplates were used for the cytotoxicity assay.

The bioactive plant extracts were resuspended in ethanol/DMSO to a concentration of 100 mg/mL and filter sterilized before being diluted in fresh MEM to the required concentrations. The concentrations tested ranged from 0.0075 to 1 mg/mL. The plant extract dilutions (100 μ L) were pipetted into wells of the microplates in quadruplicate. Doxorubicin hydrochloride (Sigma) was used as a positive control and solvent controls were also included. The microplates were incubated at 37°C in a 5% CO₂ incubator for 48 h. Following incubation with test extracts, the cells were examined using an inverted microscope to detect cytopathic effects (CPE). Minimal Essential Medium containing plant extracts was removed and each well was rinsed with PBS immediately, and fresh MEM (200 μ L) without test substance was added to the wells. A concentration of 5 mg/mL of MTT in PBS was prepared, and 30 μ L of the solution were then added to each well, and the plates were incubated for a further 4 h at 37 °C in 5% CO₂ incubator. The medium was removed from

the wells after 4 h incubation and 50µL of DMSO were added to each well to dissolve the formazan crystals. The plates were gently shaken until the solution was dissolved prior to reading. The amount of MTT reduction was evaluated by measuring the absorbance at a wavelength of 570 nm (reference wavelength of 630nm) in a microplate reader. The wells without cells which contained only the medium were used to blank the plate reader. The results were interpreted as a percentage of the control wells and the LC₅₀ (lethal concentration 50) values were calculated. The selectivity index (SI) values for each extract were calculated using the following formula modified and adapted by Al-Musayelo²¹. A SI value higher than 1 was considered effective non-toxic.

$$SI = LC_{50} \text{ mg/mL} / IC_{50} \text{ } \mu\text{g/mL}.$$

Where LC₅₀ stands for the concentration of a given agent (plant extract) which is lethal to 50% of the cells, and IC₅₀ stands inhibitory concentration of ABTS.

Genotoxicity assay

The potential genotoxic effects of the leaves and barks of *Anthoclesita schweinfurthii* were investigated using the *Salmonella typhimurium* test strains TA98 and TA100²². Briefly, 0.1 mL of bacterial stock will be incubated in 20 mL of Oxoid Nutrient broth (Fluka) for 16h at 37°C on a rotative shaker. Of this overnight culture, 0.1 mL was added to 2.0 mL of top agar (containing traces of biotin and histidine) together with 0.1 mL test solution (test sample, solvent control or positive control) and 0.5 mL phosphate buffer. The top agar mixture was poured over the surface of a minimal agar plate and incubated for 48 h at 37°C. After incubation the number of revertant colonies (mutants) was counted. The positive control used in this study was 4-nitroquinoline 1-oxide (4-NQO) (Sigma) at concentrations of 2 and 1µg/mL for *S.typhimurium* TA98 and TA100 respectively. All cultures were made in triplicate (except the solvent control where five replicates were made).

Preliminary phytochemical analysis of screened extracts

Phytochemical screening makes it possible to identify the different families of bioactive compounds present in the extracts This was done

using the methods described by Harborne²³ and Sofowora²⁴.

Statistical analysis

Data were combined and analyzed by analysis of variance (ANOVA). The ANOVA was performed with SPSS software (version 23). The significant differences (p<0.05) were estimated by Tukey and values were expressed as mean ± SD.

RESULTS

Phenolic content

The determination of the total polyphenols by the Folin Ciocalteu method was among those used to determine the polyphenol content and the results are summarized in Table No.1. The aqueous extract of *A. schweinfurthii* bark had a higher quantity of polyphenols with a polyphenol content equal to 985± 32 mg Equivalent Ascorbic Acid/g dw (EAA/g) extracted with respect to the aqueous extract of the leaves (260±10 mg EAA/g dw). It was also noted that the extracts had a higher amount of antioxidant chelator (681.91±25.68 mg EAA/g dw and 113.49±2.47 mg EAA/g dw) respectively for the aqueous extract of the barks and leaves, than the true antioxidants (84.513±9.112 mg EAA/g dw and 13.52±2.47 mg EAA/g dw) respectively for the aqueous extract of the barks and leaves of *A. schweinfurthii*.

Legend

Primary antioxidants or antiradicals also called true antioxidants which are molecules capable of interrupting the autocatalytic chain by blocking lipid free radicals by transfer of a hydrogen radical. Secondary or preventive antioxidants that act on other oxidation factors. They are able to delay the oxidation of lipids by indirect mechanisms such as oxygen reduction or complexation of metal ions. The values that had the same letters were statistically identical while those with different letters were statistically different with a significance level P <0.05.

In vitro evaluation of the antiradical activity of *A. schweinfurthii*, ascorbic acid and BHT

In vitro test 2, 2-azino-bis (3-ethylbenzothiazoline) -6-sulfonic radical (ABTS⁺)

Figures No.1 and 2 showed that, the aqueous bark extract with an IC₅₀ equal to 153±3 µg/mL was more active than the aqueous leaf extract with an

IC₅₀ of 185±2 µg/mL. Also the two extracts had an antiradical activity lower than the two reference molecules namely ascorbic acid with IC₅₀ of 1.4±0.09 µg/mL and BHT so the IC₅₀ was 1.1±0.06µg/mL. All of these results are summarized in Table No.2.

Legend

The values that had the same letters were statistically identical while those with different letters were statistically different with a significance level p<0.05.

To evaluate the *in vitro* antiradical activity of aqueous extracts of *A. schweinfurthii* and reference molecules, we used two tests: the DPPH test and the ABTS test. At the end of these two tests, statistically, ascorbic acid and BHT had the same antiradical activities and activity greater than the two extracts evaluated. The aqueous extract of *A. schweinfurthii* barks had a high antiradical activity with IC₅₀ of 120±11 µg/mL compared to the aqueous extract of leaves. With the ABTS test, the aqueous extract of *A. schweinfurthii* barks, with an IC₅₀ of 153±3µg/mL has an antiradical activity greater than that of the aqueous extract of the leaves (IC₅₀ of 185±2 µg/mL).

Evaluation of the reductive activity of *A. schweinfurthii*, ascorbic acid and BHT

β-carotene bleaching coupled with the auto-oxidation of linoleic acid assay

Figure No.3 showed that, the aqueous extract of bark of *A. schweinfurthii*, ascorbic acid and BHT had the same reductive activity (p <0.05) with IC₅₀ of 2.3±0.1 µg/mL; 2±0.03 µg/mL and 2±1.5µg/mL respectively (Table No.3). Aqueous extract of leaves of *A. schweinfurthii* with an IC₅₀ of 3.2±0.1µg/mL had a reducing activity lower than the aqueous extract of stem-barks of *A. schweinfurthii*, ascorbic acid and BHT.

Legend

The values that have the same letters are statistically identical while those with different letters are statistically different with a significance level p<0.05.

MTT assay

The cytotoxicity extracts was determined using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay on the Vero monkey kidney cell line. The results of the

cytotoxicity showed that aqueous extract of bark and leaves had a lethal concentration (LC₅₀) of 0.131 mg/mL and 0.145 mg/ml respectively With a selectivity index (0.85 and 0.78 respectively for the barks and leaves extract) lower than 1, the aqueous extracts from *A. schweinfurthii* has been shown that they are cytotoxic on the Vero monkey kidney cell line. For more in depth investigation, subsequent tests should be conducted with other method such as the lactate dehydrogenase (LDH) leakage, protein quantification or neutral red. However, high toxicity intended for cancerous cells is essential.

Genotoxicity assay

The Ames test without metabolic activation is designed only for direct mutagen detection. To designate a substance as a mutagen, a positive response in any single bacterial strain either with or without metabolic activation is sufficient. TA98 and TA100 bacterial strains are often used as they detect the great majority of mutagens. Positive results from *Salmonella typhimurium* strain TA98 detects frame-shift mutations based on spontaneous reversion of the *Salmonella typhimurium* from His- to His+ caused by crude plant extracts. Positive results caused by TA100 indicate base-pair substitution. The evaluated extracts must exhibit a dose-dependent increase in the number of revertants in order to be considered as genotoxic. Furthermore, the number of revertant colonies of the extracts must be equal to or greater than two times that of the negative control (Table No.4). However, in our study none of the tested extracts demonstrated a dose-dependent increase or revertant colonies that were equal to or greater in number than twice those of the negative control. Therefore, the tested plant extracts lacked direct genotoxic compounds. In all cases, the values of the negative as well as the positive control were within normal limits and in accordance with literature.

Legend

4 NQO: positive control, water: negative control.

Phytochemical screening

The present study revealed that the aqueous extracts of leaf and bark of *A. schweinfurthii* contained alkaloids, flavonoids, phenols, saponins, steroids, tannins catechic, triterpene (Table No.5). However, phenols were detected only in bark extracts of *A. schweinfurthii* parts and tannins

catechic were found in the bark extract compared to leaf extracts. Leaf and bark extracts had a high number of alkaloids with a high degree of precipitation (++). Steroids, triterpenes and polyphenols were determined to be present in lesser amount (+).

Legend

(+) Presence of the compound, (++) Abundance of the compound, (-) Absence of the compound.

DISCUSSION

Extensive progress in scientific research has been focused on medicinal plants and their extracts with antioxidant, cytotoxicity and genotoxicity properties in recent years. The properties are commonly postulated to play an important role in preventing diseases caused by oxidative stress, such as cancer, coronary arteriosclerosis, and ageing processes^{3,4}. The present study aimed to develop the phytochemical screening, antioxidant, cytotoxicity and genotoxicity activities of extracts from leaves and stem-barks of *A. schweinfurthii* Gild (Loganiaceae). A rich literature study is available showing that the antioxidant potential is mainly due to presence of polyphenol content^{25,26}. The data indicate the highest polyphenol content of 985±32 mgEAA/g dw and 260±10 mg EAAA/g respectively for the aqueous extract of barks and leaves of *A. schweinfurthii*. Polyphenols are the major group of compounds that contribute to the antioxidant properties²⁷. The antioxidant activity of polyphenols is due to the reactivity of phenol moiety (hydroxyl group on aromatic ring)^{28,29}. Polyphenols have the ability to scavenge free radicals via hydrogen donation or electron donation. Antioxidants play a vital role in the control of reactive oxygen species (ROS), produced during cell metabolism. ROS are implicated in the main pathogenesis of diabetes, rheumatic joint pain, atherosclerosis and hypertension^{30,31}. To evaluate the antioxidant activity of the aqueous extracts of *A. schweinfurthii*, two methods were used: the first to evaluate the scavenging activity through the ABTS assay. The evaluation of the antiradical activity with the ABTS method showed that the aqueous extract of *A. schweinfurthii* barks with an inhibitory concentration of 153±3.3µg/mL had a high antiradical activity compared with the aqueous

extract of the leaves. Therefore, the inhibitory concentration was 185±2 µg/mL. According to Emad *et al*³², this is because ABTS had the advantage of being soluble in aqueous and organic solvents and therefore is mostly used in evaluating the antiradical activities of aqueous extracts. With the scavenging assay, the barks aqueous extract of *A. schweinfurthii* exhibited significant scavenging activity which can be related to their highly phenolic content²¹. The evaluation of antioxidant activity also consisted of evaluating the reducing activity of the extract using the β-carotene assay. Statistically, the aqueous extract of the barks had the same reducing activity as the reference compounds tested namely ascorbic acid and BHT. The results showed that the content of secondary antioxidants in the extracts was higher than the primary antioxidant content, which also explains the fact that the extracts studied had a reducing activity greater than the antiradical activity. Indeed, by their action as metal chelators or oxygen reducers, they are preventive and act on the other oxidation factors³³. The results of the phytochemical screening showed the presence of terpenes, steroids, saponins, flavonoids and an abundance of alkaloids in the aqueous extracts of the leaves and barks of *A. schweinfurthii*. Mbougouere *et al*³⁴ showed that the methanolic extracts of the roots of *A. schweinfurthii* are rich in steroids. In most countries there is no universal regulatory system insuring the safety and activity of natural products and they had not been sufficiently investigated analytically or toxicologically³⁵. Herbal medicines can be potentially toxic to human health. In this way, scientific research has shown that. Based on their long-term use by humans, many plants used in traditional and folk medicine are potentially cytotoxic, genotoxic, mutagenic, and carcinogenic^{36,37}. In order to ensure the extracts were not toxic to the mammalian cells, the cytotoxicity of the aqueous extracts of *A. schweinfurthii* was evaluated on wit Vero monkey kidney cells with LC₅₀ of 0.131mg/mL and 0.145mg/mL respectively for the barks and leaves aqueous extract of *A. schweinfurthii*. Many extracts have been shown to contain potentially harmful substances that could impact adversely on human health when consumed³⁸. Our study suggests that

the aqueous extract of *A. schweinfurthii* was relatively cytotoxic on Vero monkey kidney line cell. Cell line. For more in depth investigation, subsequent tests should be conducted with other method such as the lactate dehydrogenase (LDH) leakage, protein quantification or neutral red. However, high toxicity intended for cancerous cells is essential²⁰. Assessment of the potential genotoxicity of traditional medicines is indeed an important issue as damage to the genetic material may lead to critical mutations and therefore also to an increased risk of cancer and other diseases. This is true also when evaluating the potential DNA damaging effects of plant extracts containing a plethora of more or less potent bioactive compounds³⁹.

In our study none of the tested extracts demonstrated a dose-dependent increase or revertant colonies that were equal to or greater in number than twice those of the negative control. Therefore, the tested plant extracts lacked direct genotoxic compounds. The possible ways in which inhibitors of genotoxic agents can act include the inhibition of interaction between genes and biochemically reactive genotoxic agent and the inhibition of metabolic activation of indirectly acting toxicants⁴⁰.

Table No.1: Summary of total polyphenol content in *A. schweinfurthii* extracts

Samples	Q _{Polyphénols}			True antioxidant			Chelant antioxidant		
	(mg)	EAA/g	dw)	(mg)	EAA/g	dw)	(mg)	EAA/g	dw)
Aqueous bark extract									
<i>A. schweinfurthii</i>	985 ± 32 ^a			84.513 ± 9.112			681.91 ± 25.68		
Aqueous leaf extract									
<i>A. schweinfurthii</i>	260 ± 10 ^b			13.52 ± 2.47			113.49 ± 2.47		

Table No.2: Summary of *in vitro* antiradical activity by the ABTS test

S.No	Samples	IC ₅₀ at µg/mL
1	Aqueous leaf extract <i>A. schweinfurthii</i>	0.1850 ± 2 ^c
2	Aqueous Bark extract <i>A. schweinfurthii</i>	0.153 ± 3 ^b
3	Ascorbic acid	1.4 ± 0.09 ^a
4	BHT	1.1 ± 0.06 ^a

Table No.3: Summary of *in vitro* reducing activity by the β -carotene assay

S.No	Samples	IC ₅₀ (µg/mL)
1	Aqueous leaf extract <i>A. schweinfurthii</i>	3.2 ± 0.1 ^b
2	Aqueous extract Bark <i>A. schweinfurthii</i>	2.3 ± 0.1 ^a
3	Ascorbique acid	2 ± 0.03 ^a
4	BHT	2 ± 1.5 ^a

Table No.4: Summary of genotoxicity assay

Samples	Concentrations (mg/mL)	<i>Salmonella typhimurium</i> strains	
		TA 98	TA 100
Aqueous extract Bark	5	32± 0.000	113±2.60
<i>A. schweinfurthii</i>	0.5	35±0.33	50.33±1.66
	0.005	32±0.33	97.66±2.66
Aqueous extract leaves	5	29± 0.01	120±3.40
<i>A. schweinfurthii</i>	0.5	32±0.58	60.33±2.56
	0.005	36±0.33	87.66±1.46
4NQO		133±0.57	95.66±0.54
Water		22.00±0.57	104.00±2.00

Legend: 4NQO: positive control, water: negative control

Table No.5: Summary of phytochemical screening of aqueous extracts of leaves and barks of *A. schweinfurthii*

	Aqueous extract <i>A. schweinfurthii</i> Leaf	Aqueous extract <i>A. schweinfurthii</i> Bark
Tests		
Phenols	-	+
Alkaloids	++	++
Steroids	+	+
Tannins Cathechic	-	++
Triterpenes	+	+
Saponines	+	+
Polyphenols	+	+
Flavonoids	+	-

Legend: (+) Presence of the compound; (++) Abundance of the compound; (-) Absence of the compound.

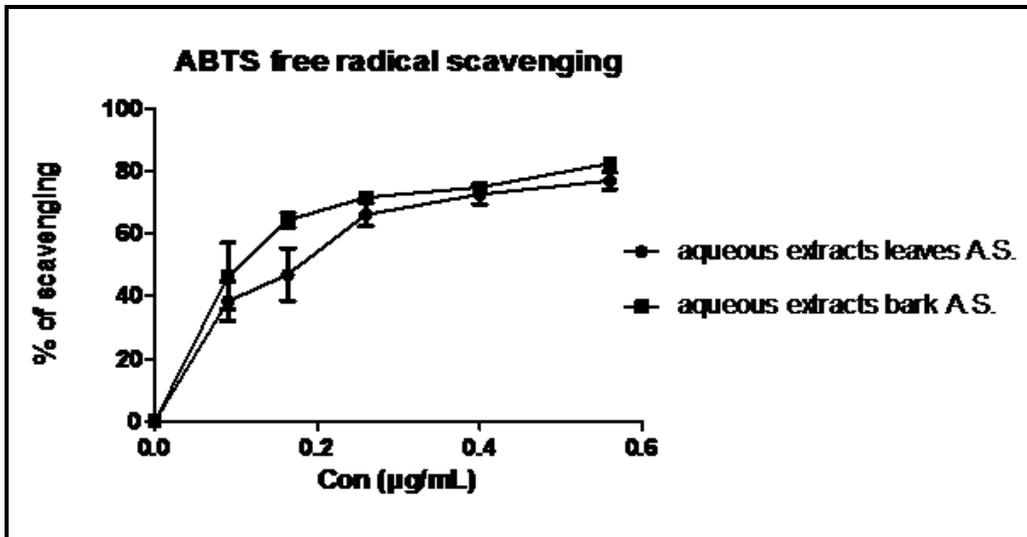


Figure No.1: Curve of variation of the percentage of trapping of the ABTS as a function of the concentration of the aqueous extracts of the barks and leaves of *A. schweinfurthii*

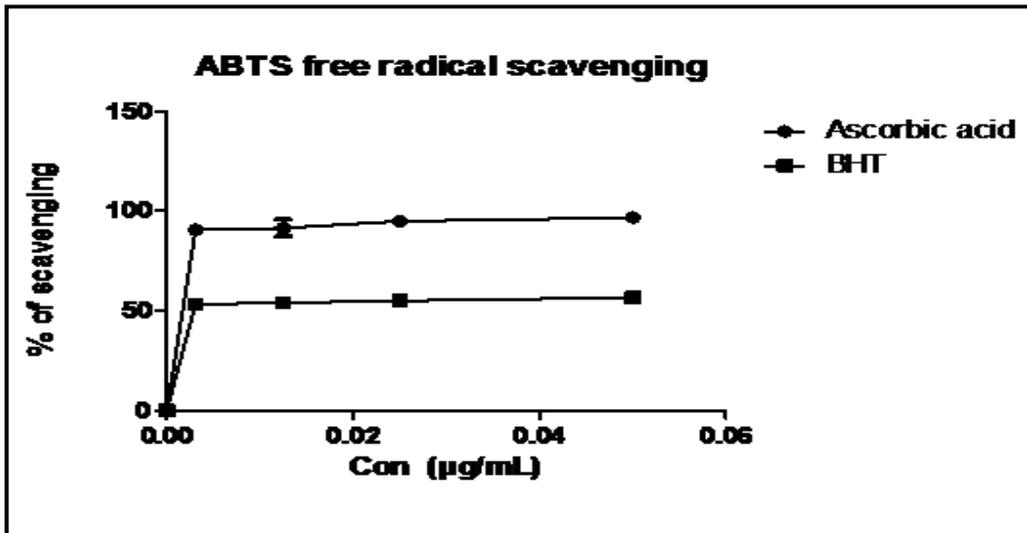


Figure No.2: Curve of variation of the percentage of trapping of ABTS as a function of the concentration of ascorbic acid and BHT

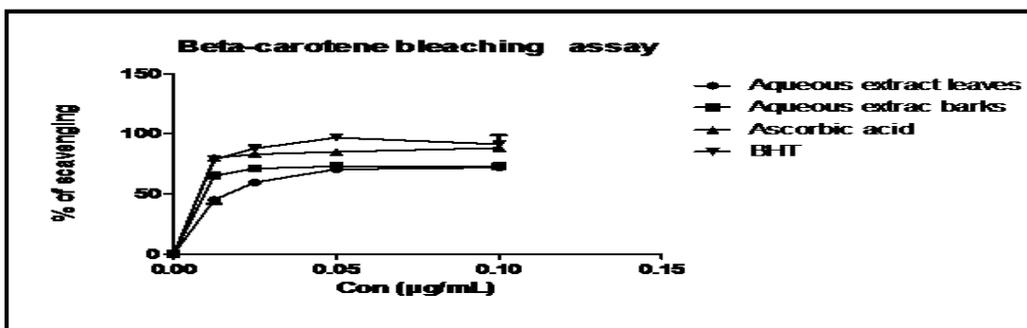


Figure No.3: Curve of variation of the percentage of entrapment of beta-carotene as a function of the concentration of the aqueous extracts of the barks, leaves of *A. schweinfurthii*, ascorbic acid and BHT

CONCLUSION

In vitro antioxidant activity, cytotoxicity and genotoxicity of extracts from the leaves and stem-barks of *A. schweinfurthii* Gilg (Loganiaceae). At the end from the current study, it can be concluded that the aqueous extract of bark contained more antioxidants compared to the aqueous extract of the leaves, the aqueous extract of the barks had the same scavenging activity with ABTS as the reference molecules BHT and ascorbic acid. The antioxidant activity was justified by their polyphenol content, so the aqueous extract of the bark had a high polyphenol content compared to the aqueous extract of the leaves. The aqueous extract of the bark was richer in chelating antioxidants than in true antioxidants and also non-toxic and non-genotoxic to normal cells, under reserve for *in vivo* toxicity studies to define the exact efficient non-toxic dose. It can be recommend the use of aqueous extracts of *Anthocleista schweinfurthii* bark in the prevention of non-communicable diseases like cancers, cardiovascular diseases, diabetes and various other metabolic disorders common among populations.

ACKNOWLEDGEMENT

Authors are very grateful to prof Lyndy MacGaw who provided the facilities for the study, to Cameroon National Herbarium (Yaounde) for identification of the plant. They are also thank the African-German Network of Excellence in Science (AGNES), the German Federal Ministry of Education and Research (BMBF) and the Alexander Von Humboldt Foundation (AvH) through AGNES Mobility Grant (Intra-Africa) for Junior Researchers for their financial assistance.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Sruthi D and John Zachariah T. *In vitro* antioxidant activity and cytotoxicity of black pepper (*Piper nigrum* L.) and *Piper* species, *International Food Research Journal*, 24(1), 2017, 75-85.
2. Sayre L M, Moreira P I, Smith M A, Perry G. Inorganic ion and oxidative protein modification in neurological diseases”, *Ann Ist Super Sanita*, 41(2), 2008, 143-164.
3. Favier A. The stressor oxidant, Experimental and conceptual interest in understanding disease mechanisms and therapeutical potential, *Chem. News*, 2003, 108-115.
4. Saha K, Lajis N H, Israf D A, Hamzah A S, Khozirah S, Khamis S. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants, *J Ethnopharmacol*, 92(2-3), 2004, 263-267.
5. Hossain M A, Sbah M D, Gnanaraj C, Iqbal M. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah, *Asian Pac J Trop Med*, 4(9), 2011, 717-721.
6. Agbor A G, Vinson A J, Oben E J, and Ngogang Y J. Comparative analysis of the *in vitro* antioxidant activity of white and black pepper”, *Nutrition Research*, 26(12), 2006, 659-663.
7. Prior R I, Wu X L, Schaich K. Standard methods for the determination of antioxidant and phenolic in foods and dietary supplements, *J. Agric. Food Chem*, 53(10), 2005, 4290-4302.
8. Sawadogo W R, Le Douaron G, Maciuk A, Bories C, Loiseau P M, Figadere B. *In vitro* antileishmanial and antitrypanosomal activities of five medicinal plants from Burkinafaso, *Parasitol Res*, 110(5), 2012, 1779-1783.
9. Ruitjter A. *Anthocleista schweinfurthii* Gilg. In Schmelzer, G.H and Gurib-Fakim, A. (Editors), *PROTA (Plant Resources of Tropical Africa)*, Wageningen, Netherlands, 2007.
10. Gbadosi I T and Erinoso S M. A review of twenty ethnobotanicals used in the management of breast cancer in Abeokuta, Ogun State, Nigeria, *Afi. J. pharm. Pharmacol*, 10(27), 2016, 546-564.
11. Guessom K O, Miaffo D, Kodjio N, Oumar Kuate J R, Kamanyi A. *In vitro* antioxidant

- properties of aqueous and methanol extracts of the stem bark of *Anthocleista Vogeli* Planch (Loganiaceae), *J. pharm., Chem. Biol. Sci*, 4(2), 2016, 271-280.
12. Ifeoma O, Oluwakanyinsola S. Screening of herbal medicines for potential toxicities. Screening of Herbal Medicines for Potential Toxicities. In: GOWDER, S., Pharmacology, Toxicology and Pharmaceutical Science "New Insights into Toxicity and Drug Testing, InTech, Rijeka, 2013, 1-252.
 13. Elgorashi E E, Taylor J L S, Maes A, Van Staden J, D E Kimpe N, Verschaeve L. Screening of medicinal plants used in South African traditional medicine for genotoxicity effects, *Toxicol Let*, 143(2), 2003, 195-207.
 14. Verschaeve L, Kestens V, Taylor J L S, Elgorashi E E, Maes A, Van Puyvelde L, D E Kimpe N, Van Staden J. Investigation of the antimutagenic effects of selected South African medicinal plants, *J. Toxicol In Vitro*, 18(1), 2004, 29-35.
 15. Santin J R, Silveira A, Muller E, Claudino V D, Cruz A B, Bürger C, D E Freitas R A, Malheiros A. Evaluation of the acute toxicity, genotoxicity and mutagenicity of ethanol extract of *Piper aduncum*, *J of Med Plants Research*, 5(18), 2011, 4475-4480.
 16. Hong C E, Lyu S Y. Genotoxicity detection of five medicinal plants in Nigeria, *The J. Toxicol Sci*, 36(1), 2011, 87-93.
 17. Chew Y L, Goh J K, Lim Y Y. "In vitro from the Leguminosae family in Peninsular Malaysia", *J. Food Chem*, 116(1), 2009, 13-18.
 18. Re S, Link E J, Bui H H, Wang R. Quantitative structure-activity relationship analysis of phenolic antioxidant", *Free Radic Biol Med*, 26(3-4), 1999, 285-294.
 19. Moure A, Cruz J M, Daniel F, Dominguez M, Sineiro J, Dominguez H, Nunez M J. Natural antioxidants from residual sources, *Fd chem*, 72(2), 2001, 145-171.
 20. Mosmann T. Rapid colorimetric assay for cell growth and survival: application to proliferation and cytotoxicity assays, *J. of Immuno Methods*, 65(1-2), 1983, 55-63.
 21. Al-Musayeib N M, Mothana R A, Matheussen A, Cos P, Maes L. *In vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsular region, *BMC Complement Altern Med*, 12(49), 2012, 1-7.
 22. Maron D M, Ames B N. Revised methods for the *Salmonella* mutagenicity test, *Mutation Research*, 113(3-4), 1983, 173-215.
 23. Harbone J B. Phytochemical method, a guide to modern technique of plants: 0412-57260-5, 3rd Edition, 1998, 302.
 24. Sofowora E A. Phytochemical Screening: Medicinal Plant and Traditional Medicine in Africa, *Spectrum Books Ltd, Ibadan, Nigeria*, 2nd Edition, 1993, 320.
 25. Bruneton J. Pharmacognosy: Phytochemistry, Medicinal Plants, Lavoisier Techniques and Documentation, Paris, 2nd Edition, 1999, 310-313.
 26. Firuzi O, Lacanna A R, Petrucci, Marrosu G, Saso L. Evaluation of the antioxidant activity of flavonoids by ferric reducing antioxidant power assay and cyclic voltammetry, *Biochim Biophys Acta*, 1721(1-3), 2005, 174-184.
 27. Aynehchi Y. Pharmacognosy and medicinal plants of Iran, *Tehran: Tehran University Press*, 1986.
 28. Sannigrahi S, Mazumder U K, Pal D, Mishra M, Maity S. Flavonoids of *Enhydra Fluctuans* exhibits analgesic and anti-inflammatory activity in different animal models", *Pak J Pharm Sci*, 24(3), 2011, 369-375.
 29. Collier H, Dinneen L, Johnson C A, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse, *Br J Pharmacol Chemother*, 32(2), 1986, 295-310.
 30. Afonso V, Champy R, Mitrovic D, Collin P, Lomri A. Reactive oxygen species and superoxide dismutases: role in joint diseases, *Joint Bone Spine*, 74(4), 2007, 324-329.
 31. Halliwell B. Free radicals, reactive oxygen species and human disease: a critical

- evaluation with special reference to atherosclerosis, *Br J Exp Pathol*, 70(6), 1986, 737-757.
32. Emad A S and Sanaa M M. Antiradical and antioxidant activities of different *Spirulina platensis* extracts againsts DPPH and ABTS radical assays, *J Mar Biol Oceanogr*, 2(1), 2013, 8.
33. Margaret E Sears. Chelation: Harnessing and Enhancing Heavy Metal Detoxification-A Review”, *The Scientific World Journal*, 13, 2013.
34. Mbouangouere R N, Tane P, Ngamga D, Khan S N, Choudhary M I, Ngadjui B T. A new steroids and α -glucosidase inhibitors from *Anthocleista schweinfurthii*, *J Med. Plant*, 1(3), 2008, 106-111.
35. Valerio Jr G, Gonzales G F. Toxicological aspects of the South American herbs cat's claw (*Uncaria tomentosa*) and maca (*Lepidium meyenii*): a critical synopsis, *Toxicological Reviews*, 24(1), 2005, 11-35.
36. Mengs U. Toxic effects of sennosides in laboratory animals and *in vitro*, *Pharmacology*, 36(1), 1988, 180-187.
37. Ferreira-Machado S C, Rodrigues M P, Nunes A P. Genotoxic potentiality of aqueous extract prepared from *Chrysobalanus icaco* L. leaves, *Toxicology Letters*, 151(3), 2004, 481-487.
38. Watt J M, Breyer-Brandwijk M G. The medicinal and poisonous plants of Southern and Eastearn Africa, *London: Livingstone*, 2nd Edition, 1962.
39. Demma J, Engidawork E and Hellman B. Potential genotoxicity of plant extracts used in Ethiopian traditional medicine, *Journal of Ethnopharmacology*, 122(1), 2009, 136-142.
40. Tulay A C Potential Genotoxic and cytotoxic effects of plant extracts, 2012, 234-250.

Please cite this article in press as: Steve Valdi Djova et al. *In vitro* antioxidant activity, cytotoxicity and genotoxicity of extracts from the leaves and barks of *Anthocleista schweinfurthii* gilg (loganiaceae), *Asian Journal of Research in Biological and Pharmaceutical Sciences*, 6(2), 2018, 58-69.