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Phytochemical Profile and Antioxidant and Antihemolytic Activities of *Garcinia lucida* Vesque (Clusiaceae)

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Abstract Background: *Garcinia lucida*, known as "ESSOK" in Ewondo (Cameroon), is distributed in the tropical zones of West Africa and in the continental region of Equatorial Guinea, specifically in Gabon and Cameroon. Its seeds and trunk bark are used to treat various gastric and intestinal disorders and thus can serve as an antacid. A decoction of fruit and leaves is used in the manufacture of many traditional curries to treat many ailments.
Objective: The aim of this study was to evaluate the phytochemical profile and the antioxidant and antihemolytic activities of the leaves and trunk bark of *Garcinia lucida* Vesque (Clusiaceae).
Methods: We determined the antioxidant, antihemolytic and total phenolic content, and performed phytochemical screening of leaf and trunk bark extracts of *Garcinia lucida* using standard procedures.
Results: With the exception of steroids, polyphenols, anthocyanes, saponins and tannins were present in all the extracts. The aqueous bark extract had the highest total polyphenol content (468 mg/g) followed by the ethanol leaf extract (445 mg/g) with the ethanol bark extract having the least (10 mg/g).The results of the antioxidant properties showed that at the concentration of 250 µg/ml, between 56.65 and 61.61% of DPPH (2,2 diphenyl-1-pycrilhydrazyl) radicals were inhibited by the extracts and the extracts showed appreciable FRAP (ferric reducing antioxidant power) activity. The 50% Inhibitory concentration (IC_{50}) ranged from 40.83 ± 0.5 to 94.020 ± 0.7 µg/ml, depending on the type of extracts. The results indicated that all extracts exhibited antioxidant properties and antihemolytic activity with a percent inhibition ranging from 21.32 to 100% from 500 to 250 µg/ml with no significant difference in biochemical and hematologic parameters compared to the control. The cytotoxicity test carried out at concentrations ranging from 125 to 500 µg / ml showed that the hydro-ethanolic leaf extract, the hydro-ethanolic bark extract and the ethanol bark extract were not toxic to the red blood cell membrane and exerted a protective effect on the erythrocyte membrane (antihemolytic effect) against oxidative stress induced by hydrogen peroxide.
Conclusions: The extracts of *Garcinia lucida* presents potential antioxidant properties (a good ability to scavenge DPPH radical and to reduce ferric oxide). The hydro-ethanolic leaf extract, the hydro-ethanolic bark extract and the ethanol bark extract were non-cytotoxic whereas the ethanol leaf extract and aqueous bark extract were cytotoxic on the Vero cell The high total polyphenol content the extracts of *Garcinia lucida* provides this plant with pharmacological properties thereby supporting its traditional use for the relief of various diseases.

Keywords Antioxidants, Reducing power, Free radicals, antihemolytic, Polyphenols, *Garcinia lucida*

Introduction

Many developing countries such as Cameroon have long been affected by infectious and cardiovascular diseases, which are among the current public health problems [1]. The treatment of these pathologies by modern medicine is very expensive for the majority of the population. Thus, the population is turning more and more towards traditional medicine. According to who, more than 80% of African populations use traditional medicine and pharmacopoeia [2-3]. Cardiovascular diseases are the leading cause of death worldwide accounting for 17.3 million deaths representing 1/3 of all deaths [4]. On the other hand, cancer is the second leading cause of death, accounting for 7.6 million deaths worldwide [5].

This is not surprising, because the excessive production of reactive oxygen species is responsible for significant cellular damage including the induction of breaks and mutations in the DNA, the modification of protein structures, the lipid peroxidation, inactivation of various enzymes and oxidation of sugars [6]. The metabolism imbalance leads to increased production of reactive oxygen species that can cause damage to many cells in the body including red blood cells. Indeed, red blood cells are normally destroyed physiologically after 120 days. Nevertheless, during oxidative stress, free radicals can denature the erythrocyte membrane, which will cause early aging of red blood cells and promote their abnormal destruction. This pathological hemolysis may eventually cause more serious diseases [7].

Fight against oxidative stress is therefore important for the body. As a result, the body disposes a powerful anti-oxidative stress assembly using antioxidant defenses. There are two types of antioxidant defenses: the endogenous and exogenous defenses. The endogenous enzymatic antioxidant defenses are dominated by superoxide dismutase (SOD), catalase, glutathione peroxidase (GP), complex thioredoxin / thioredoxin reductase [8]. The endogenous non-enzymatic defenses systems include glutathione, uric acid, bilirubin, melanin, melatonin and transition metal chelating proteins such as haptoglobin and ferritin, albumin and cereuplasmin [8]. The exogenous defenses are provided by the diet, which include vitamin E (tocopherol), vitamin C (ascorbic acid), vitamin Q (ubiquinone), trace elements (selenium, copper, zinc, manganese), carotenoids, polyphenols, phytates and flavonoids [9]. However the nutritional deficit in antioxidant, the endogenous overproduction of inflammatory origin, the environmental exposure to pro-oxidant factors such as pollution, smoking, immoderate exposure to the sun, the practice of high-level sport, lead to alteration of defensive systems [10].

As a result, synthetic antioxidants such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), propylgallate, and tertiary butylhydroxyquinone have been proposed in the food industry to improve food preservation. Unfortunately, these synthetic antioxidants have been shown to be toxic or carcinogenic [11].

Recent studies of medicinal plants have been conducted to extract natural and inexpensive antioxidants to replace synthetic antioxidants [11]. Medicinal plants like *Garcinia lucida* constitute indeed an important source of secondary metabolites such as polyphenols, flavonoids, carotenoids and tannins. These secondary metabolites are natural antioxidants that can reduce the excess of oxidants and other deleterious molecules by their ability to capture free radicals, activate antioxidant enzymes, inhibit oxidases such as lipoxygenases and cyclooxygenases, and protect blood cells [12]. *Garcinia lucida* Vesque (Clusiaceae), is a medicinal plant used by Cameroonian traditional healers for the treatment of various diseases such as gastric and intestinal disorders, decreased appetite, steatosis, stocking, mosquito bites [13].

The objective of the present research work was to study the phytochemical profile and the antioxidant and antihemolytic properties of the ethanol, hydroethanolic and aqueous extracts of *Garcinia lucida* as a contribution to the valorisation of Cameroonian medicinal plants.

Materials and Methods

Collection of plant material and identification

The whole plant was used for the plant material. The plant was harvested from the hills of MBAM MINKOM, a locality of Yaoundé and was identified with the Cameroon National Herbarium (Yaoundé) as *Garcinia lucida* Vesque (Clusiaceae) in comparison with that of Etugue M.4110 registered under the number 62835 / HNC.

Extract Preparation

The plant material (leaves and trunk bark, roots) was air dried in the barn at room temperature (26 °C) for two weeks, and subsequently was ground into a powder. Finely ground powder (500 g) was macerated in 1500 ml of 95 % ethanol for the ethanolic extract; then 500 mg of finely ground powder was macerated in 1500ml of 95% ethanol and water in the proportion (70/30) for the hydroethanolic extract; finally 500 mg of finely ground powder were macerated in 1500 ml of distilled water for the aqueous extract. Regular stirring for 72 hours and solvent replacement every 24 hours were observed for each extract. The macerates were filtered using No.1 Whatman filter paper. The filtrate was concentrated using a rotary evaporator (Heidolph WB 2000). The filtrate was evaporated to dryness in a 60 °C water bath. Then, the crude extract of each plant material was stored at 4 °C. A series of dilutions (640, 320, 160, 80, 40, 20, 10 µg/ml) were prepared for each extract for the determination of different tests. Vitamin C, gallic acid used as a control was prepared in the same conditions [9,14].

Phytochemical Assay

Qualitative phytochemical tests were performed to identify certain bioactive components such as alkaloids, tannins, saponins, flavonoids, glycosides and cardiac polyphenols in each extract using different standard methods.

Alkaloid Test

The sample (0.5 g) was stirred with 5 ml of 1% aqueous HCl on a steam bath and then filtered. A total of 1 ml of the filtrate was treated with a few drops of a solution of Mayer reagent; and another 1 ml of the filtrate was treated with Dragendorff Reagent. Turbidity or precipitation with either of these reagents could indicate the presence of alkaloids in the extract.

Tannin Test

Dry extract (0.5 g) was stirred with 5 ml of distilled water and then filtered. A solution of chloric ferric ($FeCl_3$) reagent was added to the filtrate. A blue-black precipitate indicated the presence of tannins.

Saponin Test

The plant extract (0.5 g) was mixed with water before shaking in a test tube. Persistent foam upon warming was used as evidence of the presence of saponins.

Flavonoid Test

In 2 ml of extract solution, 2 ml concentrated HCl and a pink magnesium were added. The appearance of the red tomato color indicated the presence of flavonoids.

Cardiac Glycoside Test

Glacial acetic acid (1 ml), $FeCl_3$ (1 ml) and concentrated H_2SO_4 (1 ml) were respectively added in 2 ml of the extract solution. A green-blue color indicated the presence of cardiac glycosides.

Polyphenols Test

Plant extract (2 ml) was heated for 30 minutes in a water bath. About 1 ml of 1% $FeCl_3$ was added to the mixture and then to 1 ml of 1% potassium ferrocyanide solution. The mixture was filtered and a green-blue color indicated the presence of polyphenols.

Determination of the Antioxidant Property

The DPPH free radical scavenging assay

The DPPH free radical scavenging assay was performed to measure the free radical scavenging capacity of the extracts. DPPH is a molecule containing a stable radical substance. In the presence of an antioxidant, which can give an electron to DPPH, DPPH disintegrates radically causing a change in absorbance usually measured at $\lambda = 517$ nm.

In 3 ml of each diluted extract, 1 ml of methanol in a solution of DPPH (0.1 mmol/L) was added. The mixture was kept in the dark at room temperature for 30 minutes and the absorbance was measured at 517 nm against a blank.

The following equation was used to determine the percentage of the radical scavenging activity of each extract:

Percentage of Radical Elimination Activity = [(sample with OD control) / OD check] X 100

Where DO is the optical density.

The IC_{50} (µg / ml) is the effective concentration at which the DPPH radicals were captured at 50% and its value was obtained by extrapolation from the linear regression analysis.

Determination of TPC (Phenol Compound Content)

Phenolic compound content (TPC) was determined by Folin Ciocalteu reagent. A diluted extract (30 µl) was mixed with Folin Ciocalteu reagent (1 ml, diluted 1:10 with distilled water) and aqueous Na_2CO_3 (4 mol/l). After 10 minutes, the absorbance of the mixture was measured at 765 nm [15].

Total Antioxidant Activity by FRAP *(Ferric Reducer Antioxidant Potency Assay)*

The FRAP method (ferric reducing antioxidant power) was used to determine the antioxidant capacity to reduce the Fe^{3+} ion to Fe^{2+} ion in the presence of antioxidant compounds. The FRAP reagent contains 500 ml of acetate buffer (300 mmol / L at pH 3.6), 50 ml of 2,4,6-Tris (2-pyridyl) -s-triazine (10 mmol / L) and 50 ml of $FeCl_3 + 6H_2O$ (50 mmol / L). For the assay, 75 µl of each extract was mixed with 2 ml of FRAP reagent and the optical density was read after 2 min at 593 nm against the blank. [15;16].

Statistical Analyses

Each test was performed in triplicates and the results were expressed as mean standard deviation. All data in this study are represented as average of two or three trials. We compared the results obtained during this work by using comparison tests of the averages with the Excel 2013 software of the Microsoft program and the determination of the inhibitory concentrations 50 with the software STATGRAPHICS plus 5.0. The differences were considered significant at the threshold of a calculated probability $P < 0.05$. The correlation between antioxidant, antihemolytic capacity and total phenol content was established using the Pearson correlation product moment. IC_{50} was determined with multiple regression analysis.

Results

Phytochemical screening of plant extracts

Table 1 describes the results of phytochemical analysis. While polyphenols, anthocyanes, saponins and tannins were present in all the extracts, steroids were absent in the extracts of *G. lucida*. On the other hand, while flavonoids were only detected in ethanol and hydro-ethanolic bark extracts, alkaloids were only present in the aqueous bark extract. In addition, quinones were present in all the extracts except in the ethanol and aqueous bark extract. Moreover, it is interesting to notice that triterpenes and glycosides were present only in the leaf extracts irrespective of the solvent used (Table 1).

Total polyphenols content (TPC)

Figure 1 describes the total polyphenol content of the extracts. Results indicate that the aqueous bark extract had the highest total polyphenol content (468 mgEAG/g) followed by the ethanol leaf extract (445 mgEAG/g) with the ethanol bark extract having the least (10 mgEAG/g).

The DPPH free radical scavenging Activity

Results described in figure 2 reveal that at the concentration of 250 µg/ml, the scavenging activity of all extracts ranged between 56.65 and 61.61% with the aqueous bark extract having highest inhibition percentage (61.61%) and the ethanol leaf extract registering the least (56.65%). The inhibition performance of the aqueous bark extract, hydroethanolic bark extract and hydroethanolic leaf extract was closer to that of the control (vitamin C).

Table 1: Families of metabolites present in extracts of *G. lucida*

Phytochemicals	Test	Ethanol leaf extract	Hydroethanolic leaf extract	Aqueous leaf extract	Ethanol leaf extract	Hydroethanolic bark extract	Aqueous bark extract
Po	10%Fe (III) chloride	+	+	+	+	+	+
Fl	SHINODA	-	-	-	+	+	-
Al	MAYER	-	-	-	-	-	+
TC	STIASNY	+	+	+	+	+	+
TG	STIASNY	+	+	+	+	+	+
Sa	Agitation Test	+	+	+	+	+	+
QL	Sodium hydroxide +ammoniac	+	+	+	-	+	-
Tr	Lieberman-bouchard	+	+	+	-	-	-
An	Hcl+ammoniac	+	+	+	+	+	+
Gl	(A+B) Fehling solution	+	+	+	-	-	-

Legends: + : presence ; - : absence, Po : polyphenols ; Te : Terpenoids ; St : steroids ; Sa : Saponins ; Ta : Tannin ; Fl : Flavonoids ; Al : Alcaloides ; Gl : Glycosides. An : Anthocyane, tr : triterpenes, Ql : free quinones, TG : gallic tannins, TC : catechin tannins

Ferric Reducing Antioxidant Power (FRAP)

Figure 3 below shows the variation of the reducing power (OD at 505 nm) of the different extracts. The ethanol leaf extract had the greatest ferric reducing antioxidant power (FRAP) even higher than the control (NH_2OH) with an optical density of 2.3 at 500 µg/ml. In contrast, the aqueous leaf extract showed the lowest reducing capacity for Fe^{3+} ions with OD just above 0.5.

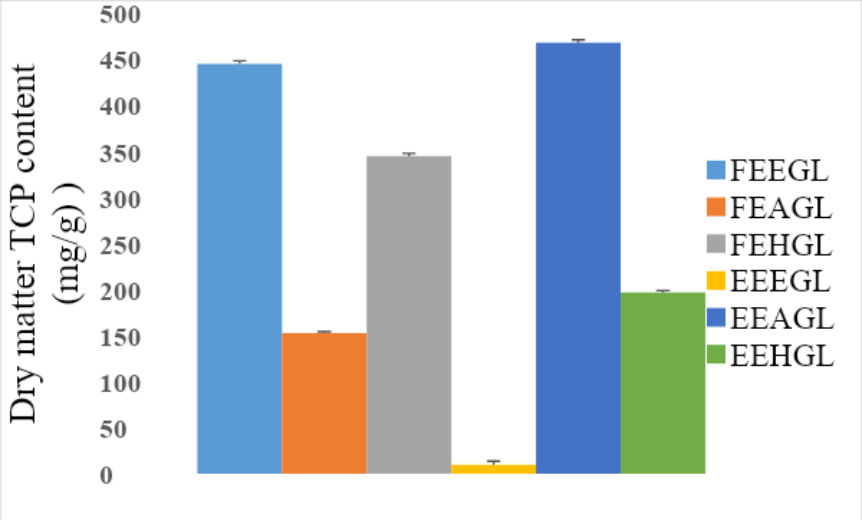

Figure 1: Histogram of phenol content of extracts of Garcinia lucida Vesque (Clusiaceae)

TCP: Total Polyphenol content; FEHGL: hydroethanolic leaf extract; FEEGL: ethanol leaf extract; EEHGL: hydroethanolic trunk bark extract; EEAGL: aqueous trunk bark extract; EEEGL: ethanol trunk bark extract.

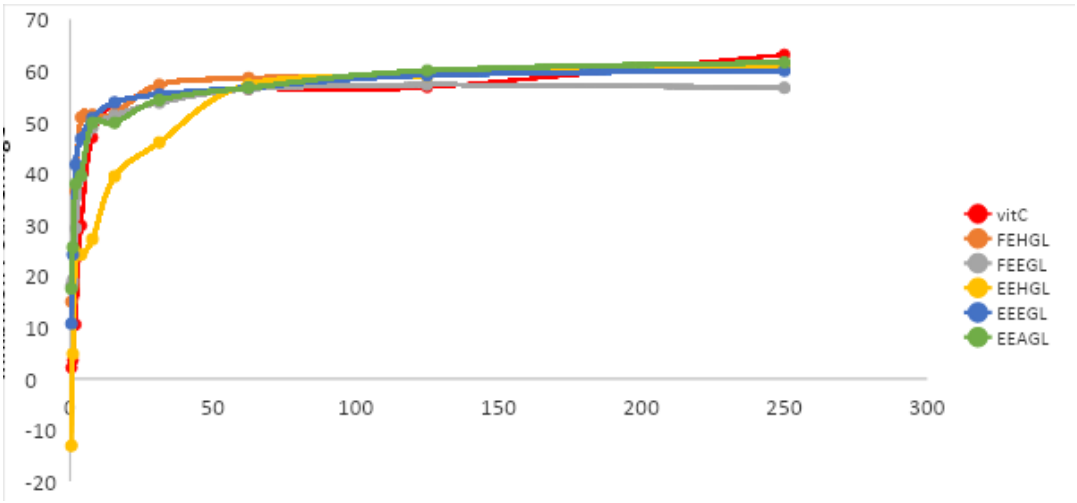

Figure 2: DPPH curve representative of the inhibition percentages of our extracts as a function of the concentration.

VitC: vitamin C; FEHGL: hydroethanolic leaf extract; FEEGL: ethanol leaf extract; EEHGL: hydroethanolic trunk bark extract; EEAGL: aqueous trunk bark extract; EEEGL: ethanol trunk bark extract.

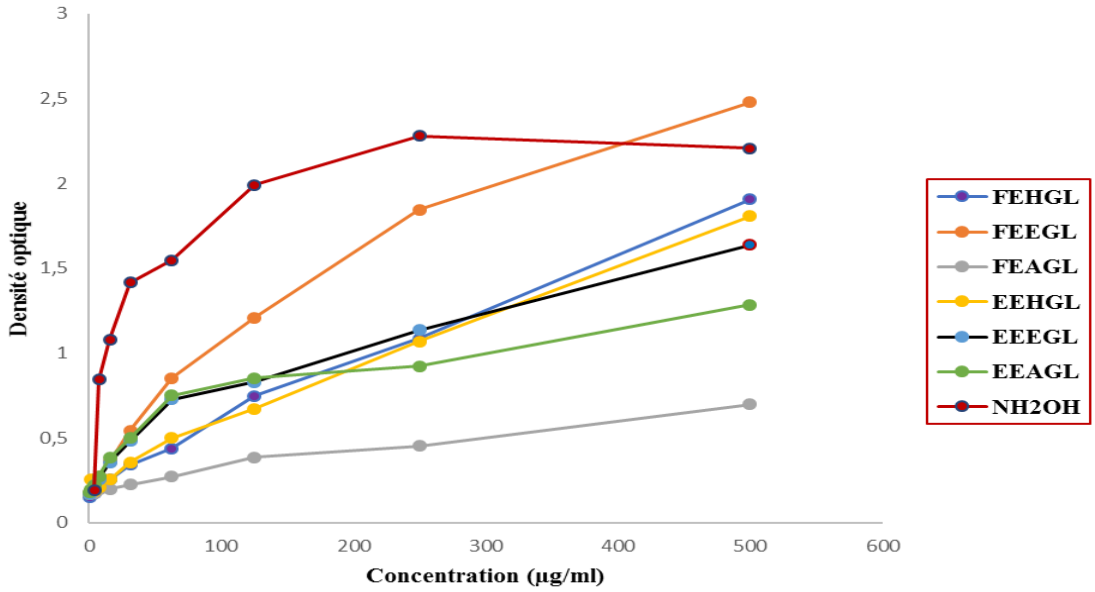

Figure 3: Reducing powers (Optical Density) of crude extracts as a function of their concentrations

NH_2OH: hydroxylamine; FEHGL: hydroethanolic leaf extract; FEEGL: ethanol leaf extract; EEHGL: hydroethanolic trunk bark extract; EEAGL: aqueous trunk bark extract; EEEGL: ethanol trunk bark extract.

Antihemolytic test

Results in Figure 4 show that the hemolysis rates induced by the plant extracts on the Vero cells varied between 20 and 110% corresponding to concentrations of 500 to 125 µg/ml. At the concentration of 500 µg/ml, the aqueous bark extract had the greatest rate of hemolysis (110%) followed by the hydro-ethanoic bark extract (Close to 80%) with the ethanol leaf extract registering the least percentage of hemolysis (19.73%).

The protective effect of extracts of *Garcinia lucida Vesque (Clusiaceae)* was investigated against the hemolysis of red blood cells induced by H_2O_2. The extracts of *Garcinia lucida* exhibited concentration dependent antihemolytic effect. The percentages of inhibition varied from 19.73-71.09% for the ethanol leaf extract, 50.57-59.00% for the

hydro-ethanoic leaf extract, 21.32-27.41% for the ethanol the trunk bark extract; 18.05-104.91% for the aqueous the trunk bark extract and 77.12-100% for the ethanol the trunk bark extract (Data not shown).

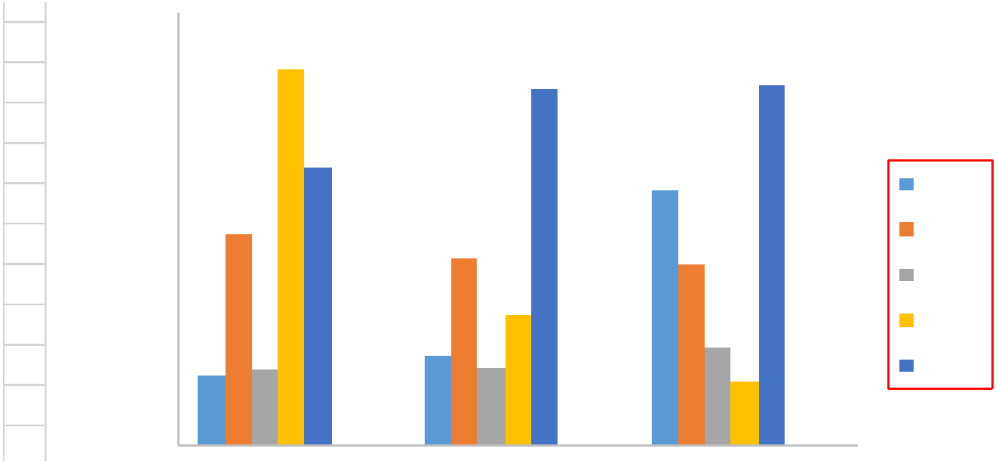

Figure 4: histogram of hemolysis rate (%) of RBCs induced by extracts of Garcinia lucida Vesque (Clusiaceae) FEHGL: hydro-ethanoic leaf extract; FEEGL: ethanol leaf extract; EEHGL: hydro-ethanoic trunk bark extract; EEAGL: aqueous trunk bark extract; EEEGL: ethanol trunk bark extract.

Cytotoxicity test

The table 2 represents the CC_{50} of the extracts of *Garcinia lucida* Vesque.

Table 2

Extracts	FEEHGL	FEEGL	EEHGL	EEEGL	EEAGL
CC_{50} (µg/ml)	>1000	453.9±1.37	>1000	>1000	572.6±1.39

FEHGL: hydro-ethanoic leaf extract; FEEGL: ethanol leaf extract; EEHGL: hydro-ethanoic trunk bark extract; EEAGL: aqueous trunk bark extract; EEEGL: ethanol trunk bark extract; CC_{50}: cytotoxic concentration 50.

Table 2 summarises the cytotoxic activity (CC_{50}) of the extracts on Vero cells. Results reveal that the hydro-ethanolic leaf extract, the hydro-ethanolic bark extract and the ethanol bark extract had CC_{50} values > 1000 µg/ml whereas the ethanol leaf extract and aqueous bark extract recorded CC_{50} values of 453.9 ±1.37 µg/ml 572.6 ±1.36 µg/ml, respectively.

Discussion

The present work was aimed at evaluating the antioxidant and anti-hemolytic properties of leaf and trunk bark extracts of *Garcinia lucida* Vesque.

The trunk bark powder content was 5.5% while that of the leaf was 14.38%. Thus, for both leaf and trunk bark extracts, the powder content is greater than 10 % owing to the fact that the drying time was insufficient; however, the powders from the different extracts were usable for the study. For a plant material to be considered dry, its water content after drying must be less than 10% [9].

The extraction yields of extracts varied between 3.80% and 12.32% depending on the solvent and the part of the plant used This result differs from the work carried out by Djacbou *et al*. (2014) who obtained a yield of 8.90% with the ethanol leaf and barks extract of *Garcinia lucida*. The differences in solvent, parts of the plant used and the geographical locations could explain this variation [17].

Phytochemical tests on the plant extracts of *Garcinia lucida* revealed the presence of polyphenols, anthocyanes, saponins and tannins in all the extracts except steroids which were noticeably absent in the extracts of *G. lucida*. These results are to certain extent consistent with the work of Olayinka *et al*. (2015) who detected the presence of polyphenols, saponins and tannins in all the extracts of this same plant (*Garcinia lucida*). However, while Djacbou *et al*. (2014) and Oy Tabi *et al*. (2012) discovered the presence flavonoids and alkaloids in all the extracts, the present study conformed the availability of these metabolites only in the ethanol and hydro-ethanolic bark extracts

and the aqueous bark, respectively. Another difference resides in the absence of steroids in all the extracts in the present study whereas Djacbou *et al*. (2014) detected steroids in the fruits, bark and leaves of *Garcinia lucida*. [17;18].

The values of TPC ranged between 10 and 468 milligrams of catechin equivalent per gram extract depending on the extract and part of plant used. These results are not consistent with the work of Djacbou *et al*. (2014) who reported that the TPC values ranged between 54.58 and 165.81 milligrams of catechin equivalent per gram extract. Both the present work and that of Djacbou *et al*. (2014) agree that the bark extract of this plant had the highest TPC content. The aqueous bark extract had the highest total polyphenol content (468 mgEAG/g) followed by the ethanol leaf extract (445 mgEAG/g). Therefore, it is evident from the present data that water and ethanol are the best solvents for fractioning polyphenol constituents, due to their polarity index and the best solubility for the type of metabolites in *Garcinia lucida.* [17].

The antiradical activity of the extract was measured by the ability to remove free radicals from DPPH and was compared with standard catechin. It has been observed that all the extracts tested have a higher scavenging activity (Figure 2). The inhibition performance of the aqueous bark extract, hydroethanolic bark extract and hydroethanolic leaf extract was closer to that of the control (vitamin C).

The difference observed in the scavenging activity between the extracts is either due to the type of free radical or the presence of several bioactive compounds in extracts such as phenols and flavonoids capable of producing proton or hydrogen atoms to stabilise free radicals.

Since the reduction activity could serve as a significant indicator of the antioxidant potential, we evaluated this property by measuring the ability of the extracts to transform Fe^{3+} into Fe^{2+} and make an electron donation. The extracts registered IC_{50} values ranging from 40.83 to 94.02 µg/ml which means that more than 50% of Fe^{3+} at a concentration of 250 µg/ml of the extracts would reduce more than 50% of Fe^{2+}. The ethanol leaf extract had the greatest Ferric reducing antioxidant power (FRAP) even higher than the control (NH_2OH) with an optical density of 2.3 at 500 µg/ml. It should be noted that the higher the OD, the more Fe^{3+} present in the medium is converted into Fe^{2+}, which indicates a strong reducing capacity of the extracts. The results are in agreement with the work done by Djacbou *et al*. (2014) who reported that at IC_{50} value of 640 µg/ml, *Garcinia lucida* extract (bark and leaves) recorded the greatest reduction capacity (optical density = 2.317, 0.105). The ability of Fe^{3+} reducing of the extracts could be attributed to either the reducing agents such as phenol groups or the number or / and position of the hydroxyl molecule on these groups. [17].

Based on CC_{50} values, the hydro-ethanolic leaf extract, the hydro-ethanolic bark extract and the ethanol bark extract are non-cytotoxic whereas the ethanol leaf extract and aqueous bark extract are cytotoxic on the Vero cell. These results are consistent with the work of Omokhua *et al.* (2018) presenting the cytotoxicity test of the extract of *Garcinia hombroniana* at concentrations between 10 and 100 µg/ml against MCF-7, DBTRG, PC-3 and U2OS. [18].

Conclusion and Perspectives

The phytochemical profile and antioxidant and antihemolytic activities of leaf and trunk bark extracts suggest that *Garcinia lucida* is a plant relatively rich in active metabolites with extraction yields ranging from 5.5% to 14.38%. Phytochemical profile revealed the presence of polyphenols, anthocyanes, saponins and tannins in all the extracts except steroids which were noticeably absent in the extracts of *G. lucida*. The rich polyphenol and flavonoids content of the plant extract may explain the antioxidant power of *Garcinia lucida*. The aqueous bark extract had the highest total polyphenol content (468 mgEAG/g) followed by the ethanol leaf extract (445 mgEAG/g). The inhibition performance of the aqueous bark extract, hydroethanolic bark extract and hydroethanolic leaf extract was closer to that of the control (vitamin C). The hydro-ethanolic leaf extract, the hydro-ethanolic bark extract and the ethanol bark extract were non-cytotoxic whereas the ethanol leaf extract and aqueous bark extract were cytotoxic on the Vero cell. The high total polyphenol content the extracts of *Garcinia lucida* provides this plant with pharmacological properties thereby supporting its traditional use for the relief of various diseases.

Finally, given the results obtained in the present study, as future perspective, we intend to evaluate some biological activities such as anti-cancer, anti-inflammatory, antimicrobial activities as well as quantify the phytochemicals present in the extracts including their toxicological profile beyond the present study site.

Conflict of interest statement

We declare that we have no conflict of interest.

References

1. Aviram M."Review of Human Studies on Oxidative Damage and Antioxidant Protection Related to Cardiovascular Diseases". Free Radic Res. 2000; 33 Suppl. 85-97.
2. Bidié A., N'guessan BB. Activités Antioxydantes De Dix Plantes Médicinales De La Pharmacopée Ivoirienne. Sciences & Nature. 2011; 8(1-2): 1-12.
3. Guedje M., Onana J. *Garcinia Lucida Vesque* (*Clusiaceae*): Des Utilisations Traditionnelles A La Monographie Pharmacopée Pour Un Développement Emergent De Médicaments A Base De Plantes. Journal of Applied Biosciences. 2014; 109: 10594-608.
4. Reiter R. Oxidative Processes and Antioxidative Defense Mechanisms in The Aging Brain. FASEB. 1995; 526-33.
5. Christen Y. "Oxidative Stress and Alzheimer Disease". The American Journal of Clinical Nutrition. 2000; 71 (2): 621-29.
6. Lenaz G. The Mitochondrial Production of Reactive Oxygen Species: Mechanisms and Implications in Human Pathology. IUBMB Life. 2001; 159-64.
7. Mintzer DM, Billet SN, Chmielewski L. Drug-Induced Hematologic Syndromes. Advances In Hematology. (2009). 27(4): 153-8
8. Odebeyi O.O. Sofowara F.H. Antimicrobial Alkaloids From A Nigérian Chewing Stick (*Fagara Zanthoxyloides*). Planta Medica, 1978; 36:204-207.
9. Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales. (Ed.) Lavoisier, Tec et Doc. 3 -ème édition. Paris. 1999; 1120 p.
10. Rahal A., Kumar A. Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. Biomed Research International. 2014.
11. Iverson F. Phenolic Antioxidants: Health Protection Branch Studies on Butylated Hydroxyanisole. Cancer Letter. 1995;. 93 (1): 49-54.
12. Gutowski M. Et Kowalczyk S. A Study of Free Radical Chemistry: Their Role and Pathophysiological Significance. Acta Biochimica Polonica. 2013; 60(1): 27-39
13. Harris, D.J.« A Taxonomic Revision and An Ethnobotanical Survey of The Irvingiaceae In Africa. » PhD Dissertation, University of Oxford, Oxford. (1993).
14. Harbone J.B (1973) Phytochemical Methods: A guide To Modern techniques Of Plant Analysis. London, Chapman and Hall Ltd 1973; 116p.
15. Boizot N. & Charpentier J.P. Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le cahier des techniques de l'INRA. 2006 : 79-82.
16. Popovici C., Saykova I. et Tylkowski B. *Evaluation De L'activité Antioxydant des Composés Phénoliques Par La Réactivité Avec Le Radical Libre DPPH.* E-Revue De Génie Industriel.2010; (4). Disponible Sur Internet: Http://Www.Revue-Genie- Industriel. Info/Document.Php951. ISSN 1313-8871.
17. Djacbou S., Pieme C. Comparison of *In Vitro* Antioxidant Properties of Extracts from Three Plants Used for Medical Purpose in Cameroon: *Acalypha Racemosa, Garcinia Lucida And Hymenocardia Lyrata*. Journal of Applied Biosciences. 2014; 4(Suppl. 2): 625-632.

18. Oy Tabi, Charles Fokunang, Mv Tsague. Activites Antioxydantes Et Biologiques In Vitro Des Extraits Hydroethanoliques De UAPACA PALUDOSA. Health Sciences and Diseases. The Journal of Medicine and Health Sciences. 2012; Vol. 13 N°3.
19. Omokhua AG, Abdalla MA, Van Staden J, McGaw LJ. A comprehensive study of the potential phytomedicinal use and toxicity of invasive Tithonia species in South Africa. BMC Complementary and Alternative Medicine 2018; 18: 272.