The Pharmaceutical and Chemical Journal, 2019, 6(4):46-54

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Phytochemical Screening, Evaluation of Cytotoxicity, Antioxidant Activities, Anti-Haemolytic Activities of *Bridelia micrantha*

Mekena K. C. Mirabelle[1], Chelea Matchawe[2], Kouamouo Jonas[1], Fekam Fabrice[3], Ngoupayo Joseph[4]*

[1]Université des Montagnes, Bagangté, Cameroon
[2]Institute of Medical Research and Medicinal Plants (IMPM), Yaoundé, Cameroon
[3]Faculty of Sciences, University of Yaoundé 1, Cameroon
[4]Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Cameroon
*Corresponding author: Ngoupayo Joseph, ngoupayo@gmail.com

Abstract Background: The use of plants in our immediate environment in primary health care is a common practice; even the modern medical system uses more than 25% of both plant-based and plant-derived medicines. The more and more, our society is increasingly faced with the emerging of new oxidative stresses-diseases related *Bridelia micrantha* is traditionally used to treat ulcers, joint pain, eye pain, stomach pain, diarrhea and as an aphrodisiac.

Objective: The purpose of this study was to identify some secondary metabolites, to evaluate the cytotoxicity, antioxidant and anti-hemolytic properties of hydroethanolic, ethanol and aqueous extracts of *Bridelia micrantha* widely used in traditional medicine.

Method: The hydroethanolic, ethanol and aqueous extracts of *Bridelia micrantha* were obtained by maceration in ethanol-water (70/30 v: v), ethanol, and water for 72 hours, then filtered using the No. 4 coffee filter paper before oven-drying at 40 °C. DPPH and iron (III) reduction methods were used to evaluate the antioxidant activity of the extracts. The cytotoxic profile of the extracts was determined by evaluating the inhibition percentage of normal cell growth (Vero cells). Their anti-hemolytic activity was evaluated by the protection percentage of erythrocytes against hemolysis.

Results: The extraction yields of the hydroethanolic, ethanol and aqueous extracts were respectively 18.23%, 13.75% and 12.27%. The phytochemical screening based on the modified protocols described by Harborne (1976), Odebeyi and Sofowara (1978), Trease and Evans (1989), and Sofowora, (1993) revealed the presence of polyphenols, flavonoids, tannins, and saponins. The extracts showed an antioxidant activity usin the DPPH radical scavenging method at CI_{50} = 2.420 µg / ml for the aqueous extract, 1.877 µg / ml for the hydroethanolic extract and 4.693 µg / ml for the ethanolic extract. Based on the iron reduction method, the antioxidant activity was shown at concentrations 100, 200 and 400 µg / mL corresponding respectively to the FRAP values 0.55, 1.25 and 1.63 µM for ethanol extract; 0.59, 1.43 and 1.74 for hydroethanolic extract and 0.59, 1.29 and 1.54 for the aqueous extract. In addition, cytotoxicity was exhibited at CC_{50} greater than 1000 µg/mL for the hydroethanolic extract, equal to 381.1 µg/mL for the ethanolic extract and 340.25 µg / mL for the aqueous extract. Finally, the anti-hemolytic activity of concentrations 125 µg/mL, 250 µg/mL and 500 µg/mL% recorded the respective protection percentages 55.6%, 62%, and 70%.

Conclusion: These results could be considered as starting point for the applications of this plant in association or not for the treatment of certain diseases.

Keywords *Bridelia micrantha*, phytochemical screening, antioxidant, anti-haemolytic, Vero Cells

Introduction

Oxidative stress is an abnormal situation sometimes experienced by the cells or tissues when they undergo endogenous or exogenous production of oxygen free radicals that exceed their antioxidant capacities [1]. The excess of free radicals not neutralised by the immune defenses system can be damaging for the cell's essential macromolecules, resulting in abnormal expression of genes and membrane receptors, proliferation or cell death, immune disorders, mutagenesis, protein or lipofuscin deposition in the tissues [2]. Many human or animal diseases are associated with oxidative stress, local or general in their pathogenesis as well as the inflammation. In several serious diseases, especially those related to aging such as cancers, ocular pathologies (cataracts and macular degeneration), neurodegenerative diseases (ataxias, lateral sclerosis, Alzheimer's disease), oxidative stress is the original triggering factor [2]. In many other diseases, oxidative stress is secondary to the establishment of the pathology, but contributes to its immune or vascular complications. This is evident in infectious diseases such as AIDS or septic shock, diabetes, Parkinson's disease or kidney failure [2].

Hyper-hemolysis most commonly associated with oxidative stress is responsible for hemolytic anemia, which remains a major public health problem in recent decades worldwide, despite the remarkable improvement in living conditions [2]. It has been classified by the WHO as one of the most serious problems in the world [3]. To combat this anemia and oxidative stress, synthetic drugs and synthetic antioxidants are generally used; unfortunately, these drugs are not only costly but are associated with side effects for developing countries. In fact, a long-term use of synthetic antioxidants is thought to be teratogenic, mutagenic and carcinogenic [4].

Faced with this situation, the search for medicinal plants with natural antioxidant potentials remains one of the best options because plants have long been a source of inspiration for new drug molecules. Almost all ancient civilisations and cultures depended entirely or partially on phytotherapy because of its effectiveness, accessibility, availability and dose-related low toxicity [5]. For instance, in rural African communities, the high cost and limited availability of conventional drugs have led to continued dependence on traditional therapies. About 75 to 90% of the world's population still depends on plants and plant extracts as a source of primary health care [6].

As improving strategies for traditional medicine, several phytochemical investigations have been carried out to provide a scientific basis for the use of medicinal plants [7]. Studies on secondary metabolites present in plants such as flavonoids have revealed some of their biological activities including antioxidant and anti-hemolytic activities. This triggered our interest in assessing the phytochemical, cytotoxic, anti-hemolytic and antioxidant activities of *Bridelia micrantha* extracts. It is a small to medium-sized tree widely used in traditional medicine in the tropics for its antimalarial, antihelminthic, antitussive and laxative properties, to relieve headaches and to treat wounds [8].

Material and Methods

The plant material was made up of the trunk bark of *Bridelia micrantha*. The plant was harvested in the city of Yaoundé, Mfoundi division, Simbok-Mendong district. Mr. NGANSOP identified the Plant material at the National Herbarium as *Bridelia micrantha* (hochst.) Bailt (Euphorbiaceae) in comparison with that of B. SATABIE (No.823) registered to the National Herbarium under No.52652 HNC.

Drying and grinding of plant material

The trunk bark of *Bridelia micrantha* was harvested, cut and dried at room temperature in the dark. The samples were reduced to powder in an electric mill.

Extract preparation

First, the trunk bark was roughly pulverized, then 500 g of powder were macerated for 72 hours at room temperature in 2L of ethanol, 2L of water-ethanol solution and 2 L of water. During the maceration process, the solvent was

renewed every 24 hours. The filtrate obtained was over dried at 40 °C using the No.4 coffee filter paper. After drying, the crude extracts were weighed in order to calculate the extraction yields using the following formula: Rd = 100X P'P where P '= weight of the powder and P = mass of dry extract.

Phytochemical Screening

Phytochemical screening of the different extracts was carried out to assess the presence of secondary metabolites such as alkaloids, tannins, phenolic compounds, flavonoids, saponins, coumarins, steroids, and terpenoids following the modified protocols described by Harborne (1976), Odebeyi and Sofowara (1978), Trease and Evans (1989), Sofowora, (1993) [9-12].

Evaluation of antioxidant activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging method

The extracts were diluted to obtain the following final concentrations of 250 extracts; 125; 62.5; 31.25; 15.625; 7.8125; 3.90625; 1.953125 and 0.9765625µg/mL. In a 96-well microplate, 100 µl of ethanol were introduced into all the wells except that of line 1. Thereafter, 200 µl of the various extracts we introduced into the wells of line 1 and then 100 µl of each extract of the line 1 were removed to make second-order dilutions of concentrations ranging from (250 to 0.97µg / mL) from line 2 to 10. Line 11 was the negative control (without extract). Each dilution (25µl) was added to a new microplateto which 75µl of the 0.01% DPPH solution were added. Optical densities were read at 517 nm after 30 minutes of incubation in the dark and at room temperature. The negative control consisted of DPPH without extract and the positive control of ascorbic acid considered as extract.

Feric Reducing Antioxidant Power (FRAP) method

In a 96-well microplate, 100 µL of solvent (ethanol) was introduced into all the wells except those of the first line 1. Then, 200 µL of our extracts were introduced into the wells of line 1; a serial dilution was carried out until line 10. Then, 25µl of each dilution were introduced into a new microplate with 25µl of Fe^{3+} solution at a concentration 1.2 mg/ml; the mixture was incubated for 15min. After incubation, 50 µl of 0.2% orthophenantroline solution were added and the plate was re-incubated for 20 minutes still at room temperature. At the end of this incubation, the optical densities of the wells were read at 505 nm. The negative control representing 0% reduction was made up of the solvent, Fe^{3+} and orthophenantroline while the positive control corresponding to 100% reduction was made up of the hydroxylamine acting as an extract. The results were expressed as reducing power (OD) as a function of the concentration of extract.

Evaluation of cytotoxic activity

Cell culture

Vero cells were maintained in culture in 75 cm^2 T-Flask containing DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) and 1% streptomycin-penicillin antibiotic, standard condition with 5% CO_2 at 37 °C. The medium was renewed every 3 days after cultivation.

The cells were detached by introducing 5 ml of 0.25% trypsin-EDTA into the T-Flask after triple rinsing with PBS (Phosphate Buffer Saline). After 5 minutes of incubation at 37 °C, the detached cells were centrifuged at1800 rpm for 3 minutes and the resulting pellets were suspended in 1 ml of medium. The suspension (20 µl) was added to 20 µl of trypan blue solution in order to evaluate cell viability and the calibrated cell load via the Neubauerhematimeter.

Evaluation of cytotoxicity at 1000 µg/mL

In a 96-well microplate, 100 µl of cell suspension loaded with 5×10^4 cells / wells were introduced into all the wells. At the end of a 24h incubation period, the medium contained in the wells was removed and 90 µl of a new medium were introduced, then 10 µl of the extracts at a concentration of 1000 µg /ml were also added. The plates were incubated for 48 h under a humidified atmosphere at 37 °C and 5% CO_2. The positive control consisted of 90 µl of cell solution plus 10 µl of DMSO (10%) while the negative control consisted of 100 µl of the cell solution. At the

end of 48 hours of incubation, 20 µl of MTT solution (Sigma) were introduced into each well and the whole mixture was homogenised and incubated for 4 hours at 37 °C. At the end of this incubation period, the content of the wells was carefully drained, replaced with 100 µlof DMSO in order to dissolve the formed formazan. The latter was quantified by reading the optical density at 570 nm using the TECAN plate reader (Infinite M200).

Determination of the cytotoxic concentration 50 (*CC_{50}*)

The cytotoxic extracts at 1000 µg/mL were diluted to obtain the following final concentrations of extracts: 500; 250; 125; 62.5; 31.25; 15.625µg/ml.

In a 96-well microplate, 100 µl of culture medium were introduced into all the wells except for line 1. Then, 180 µl of culture medium plus 20 µl of extracts were introduced into the wells of line 1; then, 100 µl of the content of line 1 were removed to make second-order dilutions of concentrations ranging from (500 to 15.625µg/ml) from line 2 to 7.

Evaluation of anti-haemolytic activity

Preparation of erythrocytes

Before the rats were sacrificed, they were first exposed to ether for a few seconds for stunning purposes. The rats were then sacrificed and blood was collected from the ventricle with a syringe. The collected blood was distributed into sterile heparinised tubes and centrifuged at 1500 rpm for 5 min. After centrifugation, the supernatant was discarded and the erythrocytes were washed three times with 0.9% NaCl solution. A suspension of red blood cells was made from the erythrocytes in order to adjust the hematocrit to 10%. The suspension was kept at refrigeration temperature (4 °C).

Anti-haemolysis experiment

Before starting the experiment, serial of dilutions of the extracts were performed. It was a second order dilution to achieve the following concentrations: 1000; 500; 250; 125; 62.5 µg/ml. Thus, at 0.5 ml of each of extract and at different concentrations, 0.25 ml of the erythrocyte suspension (10% hematocrit) was added and incubated for 5 min at room temperature. After incubation, we 0.25ml of 0.3% H_2O_2 solution was added and then incubated again for 4 hours at 37 °C. After incubation, the mixture was centrifuged at 2500 rpm for 10 min and then 100 µl of the supernatant were transferred to a microplate and the absorbance corresponding to the release of hemoglobin was measured at 540 nm. The negative control consisted of 0.9% sodium chloride and erythrocyte while the positive control consisted of 0.9% sodium chloride, erythrocyte and hydrogen peroxide.

Results

Extraction Efficiency

Table 1 below shows extraction efficiency of extracts with the hydro-ethanolic extract having the highest yield (18.23%) followed by the ethanol extract (13.75). The smallest yield was registered by the aqueous extract (12.27%).

Table 1: Extraction yields and physical characteristics of bark extracts

Plant part	**Weight of powdered extract (g)**	**Weight of dried extract (g)**			**Yield (%)**			**Physical characteristics**
Trunk bark	500	EAEB	EHEB	EEEB	EAEB	EHEB	EEEB	Light brown Shape of crystals
		61.35	91.12	68.75	12.27	18.23	13.75	

Phytochemical Screening

The phytochemical profile of the extracts (Table 2) reveal the presence of polyphenols, tannins and saponins in all the extracts while flavonones, coumarins, alkaloids and terpenes/sterols were not detected in any extract at all. Moreover, while flavones were present only in the aqueous extract, flavonols were only absent in the aqueous extract.

Table 2: Phytochemical composition of crude bark extracts of *Bridelia micrantha*

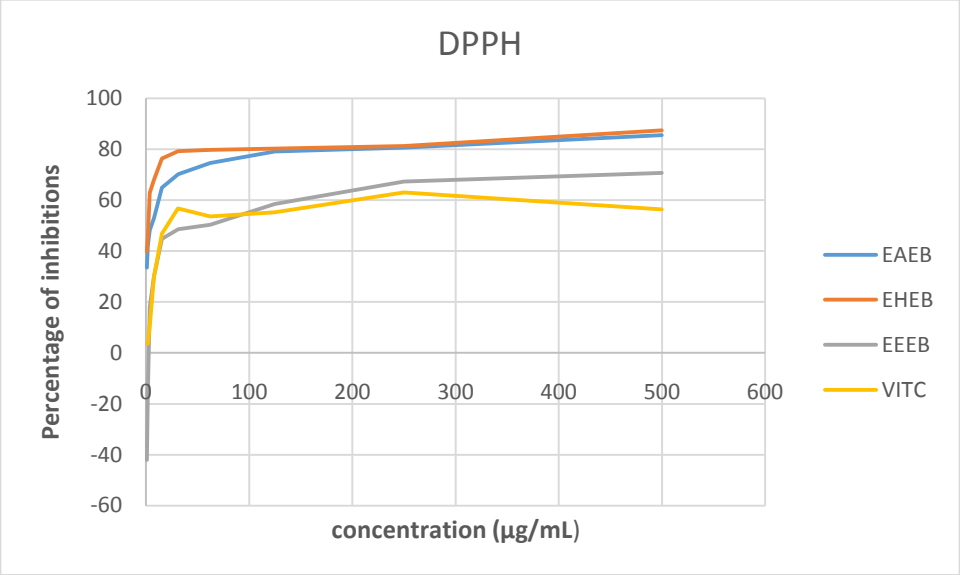
Class of metabolites		Ethanol extract	Hydroethanolic extract	Aqueous extract
Polyphenols		+	+	+
Flavonoids	Flavones	-	-	+
	Flavonols	+	+	-
	Flavonones	-	-	-
Tannins		+	+	+
Coumarins		-	-	-
Alkaloids		-	-	-
Saponins		+	+	+
Triterpenesandsterols		-	-	-

+:Present -: Absent

The antioxidant activity of plant extracts

Free radical sequestration by DPPH

Figure 1 below describes the anti-radical activity of the plant extract. This Figure indicates that the inhibition percentage varied with the concentrations of extracts with the hydro-ethanolic and aqueous extracts having the highest IC_{50} values and the least was recorded by the control (vitamin C).



EAEB = aqueous extract of *Bridelia* bark

EHEB = hydroethanolic extract of *Bridelia* bark

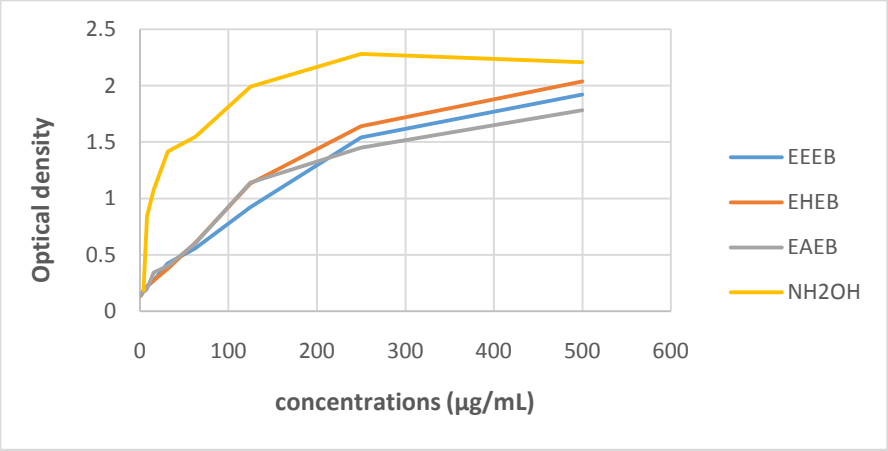
EEEB= ethanol extract of *Bridelia* bark

VITC = vitamin C

Figure 1: The IC_{50} values (Inhibitory concentration 50) of the extracts and vitamin C

Fe^{3+} Reduction activity

The Fe^{3+} reducing activity of the extracts (Figure 2) was determined by increased absorbance of the Fe^{2+}-orthophenantroline complex, proportionally to the concentration of the extracts.



EEEB: ethanol extract of *Bridelia* bark
EAEB: aqueous extract of *Bridelia* bark
EHEB hydroethanolic extract of *Bridelia* bark
NH_2OH: hydroxylamine

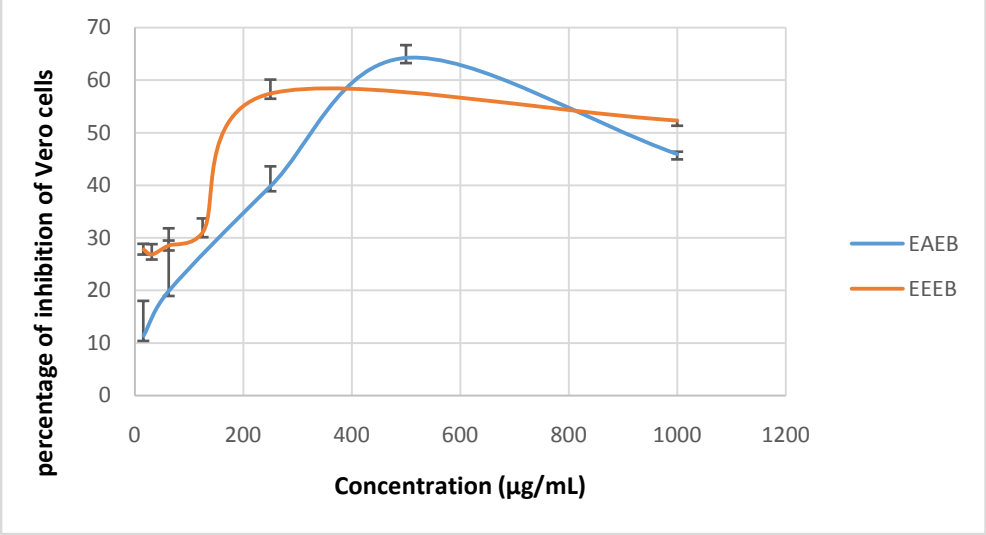
Figure 2: Absorbance of the Fe^{2+}-orthophenantroline complex as a function of the concentration of extract

Results in Figure 2 indicates that the hydro-ethanolic extract had the highest the Fe^{2+} reducing activity with optical density of 2.03 very close to that of the control (2.2) at a concentration of 500 µ/ml followed by the ethanol extract. The aqueous extract registered the small Fe^{2+}reducing activity with optical density of1.78. Thus, a high absorbance reflects the presence of high level of Fe^{2+} -orthophénantroline complex thereby indicating a strong reducing activity of extracts.

Cytotoxic activity

The cytotoxic profile of plant extracts was determined by the inhibition percentage of normal cell growth (Vero cells).

Results (Figure 3) reveal that at a concentration of 1000µ/ml, no plant extracts were toxic except the hydroethanolic extract. Moreover, the results of the serial dilutions of plant extract capable of lysing 50% of cell are still described in Figure 3.



EAEB: aqueous extract of *Bridelia* bark
EEEB: ethanol extract of *Bridelia* bark

Figure 3: Percentage inhibition of cell growth by the different extracts

The curves profile in Figure 3 shows that the inhibition percentage increased with the concentration of extract.
On the other hand, cytotoxic concentration (CC_{50}) of aqueous and ethanol extracts are reported in Table 3 below. Results indicate that the hydro-ethanolic extract had the highest CC_{50}value (> 1000µg/ml) followed by the ethanol and then aqueous extracts (381.1 ± 34.79 and 340.25± 12.94µg/ml, respectively).

Table 3: Cytotoxic concentration of aqueous and ethanol extracts

Plant part	Type of extract	CC_{50} (µg/ml)
Bark	Aqueous extract	340.25± 12.94
	Ethanol extract	381.1 ± 34.79
	Hydro-ethanolic extract	> 1000

Anti-hemolytic activity

The protection ability of the extracts on the red blood cell membrane are presented in Figure 4 below.

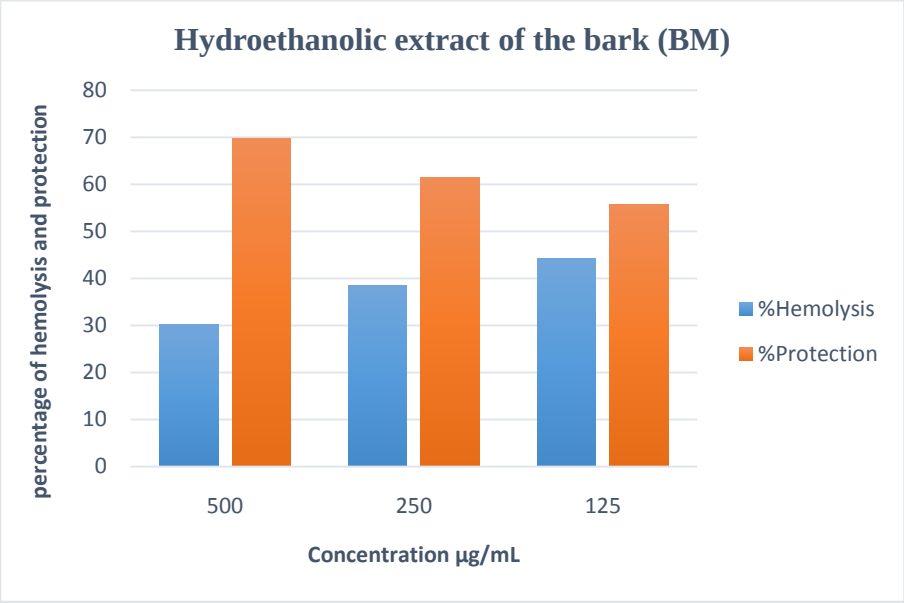


Figure 4: Protection and hemolysis percentages of the hydroethanolic extract

Results in Figure 4 indicate that the protection percentages of the plant extracts at concentrations 125, 250, 500 ranged between 55.6 and 70% and that the lysis percentage decreased with increase in concentrations while the protection percentage increased with the concentrations of extracts.

Discussion

The hydroethanolic extract of *Bridelia micrantha* recorded the highest extraction yield (18.23%) followed by the ethanol extract (13.75%). This result differs from the work of Adefuye et *al*. (2011) who reported 3.19% with the ethanol stem bark extract [13]. This observed variation could be explained by the differences in the plant parts used and the geographical location of the plant material.

Like in the case of ethanol extraction, the extraction yield recorded by Onoja et al. (2014) with the hydro-methanolic stem bark extract of *Bridelia micrantha* was slightly lower (15.5%) than that of the present study [14]. Similarly, this difference could be attributed to the differences in extraction solvent, the plant parts used and the sampling site.

Phytochemical analysis revealed the presence of tannins, saponosides, terpenoids and flavones in all the bark extracts of *Bridelia micrantha* irrespective of the extraction method. These results are consistent with the work of Waiganjo et *al*. (2009). [15] who detected the presence of terpenoids, saponins, tannins and flavones in the aqueous extract and those conducted by Okeleye et *al* (2011) [16] who revealed the presence of flavonoids, tannins and

saponins in the ethyl acetate extract. On the other hand, Okeleye et al revealed the presence of steroids that was absent in all our extracts. This could be explained by the differences in the extraction reagents and the sampling site.

The DPPH scavenging activities of hydroethanolic, aqueous, ethanol extracts were 83%, 82% and 65% respectively at a concentration of 400 µg/mL. These results are lower than that of Nwaehujor et *al*. (2011) who reported the DPPH scavenging activity of 98% for ethyl acetate extract at a concentration of 400 µg/mL [17]. This variation in results could be attributed to the differences in the extraction solvents used, plant part used and the sampling site as well.

The FRAP values of the ethanol extract, hydroethanolic extract, and aqueous extract were 1.63, 1.74µM and 1.54µM, respectively at concentrations of 400 µ/mL. These results are superior to those of Nwaehujor *et al* (2011) who obtained for the same concentrations the FRAP values of 1.382µMwith the ethyl acetate leaf extract of *Bridelia micrantha* [17]. This difference could be due to differences in the method of extraction, the plant parts of and the geographical location of the plant under study.

The cytotoxicity of plant extracts were shown at CC_{50} greater than 1000 µg/mL for the hydroethanolic extract, 340.25 µg / mL for the aqueous extract and 381.1 µg / mL for the ethanolic extract. These results are superior to those of Steenkamp *et al* (2009) who tested the cytotoxicity of bark extract of *Bridelia micrantha* on MCF-12A cells of the human breast at CC_{50}values of 8.9µg/mL and 24.2 µg/mL [18]. The differences in the extraction method and the sampling location might explain these observed variations.

Results of the present study reveal that the percentage of anti-hemolytic effect of ethanol bark extracts increased with decrease in concentrations. The Maximum anti-hemolytic activity (70%) was exhibited by the hydro-ethanolic bark extract at concentration 125µg/mL(Figure 4). The protective activityof ethanol bark extract may be attributed to the presence of secondary metabolites such as flavonoids and polyphenols present in the extract; in fact, several studies have shown that some phenolic compounds, particularly flavonoids possess anti-radical properties thereby neutralising or capturing free radicals [19]. In addition, polyphenols are known as transition metal chelators such as Fe^{2+}, thus reducing the rate of Fenton reaction. They can then prevent oxidations caused by the hydroxyl radicals and the passage of H_2O_2 through the erythrocyte membrane and the generation of free radicals [19].

Conclusion

The presence of secondary metabolites such as of polyphenols, flavonoids, tannins, and saponins in the trunk bark of the plant may be responsible for the antioxidant and anti-hemolytic activities of *Bridelia micrantha*.

While the hydroethanolic extract did not show cytotoxicity, the ethanol and the aqueous extracts exhibited cytotoxicity to Vero cells at a concentration of 1000 µg/ml. However, based of their CC_{50} values (> 30 µg/ml), the extracts failed to toxic.

Generally, the plant extracts had a great antioxidant power with the hydroethanol extract exhibiting the most important DPPH scavenging and chelating capacity. Moreover, the hydroethanolic extract appeared to be the best extract to protect the cell membrane integrity due to its high anti-haemolytic activity.

The abundant presence of secondary metabolites of great importance in the extracts of *Bridelia micrantha* provides this plant with pharmacological properties thereby supporting its use in traditional medicine.

Finally, given the results obtained in the present study, as future perspective, we intend to evaluate some biological activities such as isolation of active compounds for evaluating their respective activities such as the in vivo toxicity for the formulation of an antigastric therapeutic molecule.

Conflict of Interest Statement

We declare that we have no conflict of interest.

References

[1]. Sies H. (1991) In: Oxidative stress, oxidants and antioxidants. Londres: Academic Press.

[2]. Delattre J. (2006) Anales pharmaceutiques françaises. De l'oxygène moléculaire au stress oxydant et à la biochimie radicalaire. 64(6),. [Consulté le 17 nov 2018]. Disponible : https://www.sciencedirect.com/science/article/pii/S0003450906753305#!

[3]. Deflaoui K., Ouamara K. (2018) Activité anti-hémolytique *in vitro* des extraits de *Tamussp* [mémoire en biochimie]. wilaya, Belgique : Université de Bejaia ;.

[4]. Amel M.B. (2016) Etude de l'activité antioxydante de l'extrait hydroacetonique des racines de l'*Arbutusunedo* de la region de Tlemcen [Thèse en biologie]. Tlemcen, Algerie: Université Abou BekrBelkaidde Tlemcen;

[5]. Koudaly S., Benemasaoud., Left D.A., Essaqui A., zerbouti M., Azzi M., Benaissa M. (2014) Study of antioxidant activity and anticorrosion of the methanol extracts of drawf palm leaves (*Chamaerops humilis L.*) from morocco.; 5(3) :887-898.

[6]. Sofowora A., Ogunbodede E., Onayade A. (2013) The role and place of medicinal plants in the strategies for disease. Afr J Tradit Complement Altern Med. 10(5):210-229.

[7]. N'Guessan K., Kadja B., Zirihi G., Traoré D., Aké-Assi L.(2009) Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). Sciences & Nature.; 6(1).

[8]. Bosch C.H. (2012) *Brideliamicrantha* (Hochst.) Baill. [Internet]. [Consulté le 18 nov 2018]. www.prota4u.org/search.asp.

[9]. Harborne J.B. (1976). Phytochemical Methods. A Guide of modern techniques of plants analysis. Chapman and Hall, London, 150.

[10]. Odebeyi O.O., Sofowora F.H. (1978) Antimicrobial alkaloids from a Nigerian Chewing Stick *(Fagarazanthoxyloides).* Planta Medica.; 36: 204-207.

[11]. Sofowara A. (1993) Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Spectrum Books Limited, Ibadan, Nigeria, 97-145.

[12]. Trease G.E., Evans W.C. (1989) Pharmacognosy. 13th edition, Bailliere Tindall, London. 683-684.

[13]. Adefuye A.O., Samie A., Ndip R.N. (2011) *In vitro* evaluation of the antimicrobial activity of extracts of *Bridelia micrantha* on selected bacterial pathogens. Jounal of Médicinal plants Research.; 5(20): 5116-5122.

[14]. Onaja S.O., Ukwueze C.O., Ezeja M.I., Udeh N.E. (2014) Antinociceptive and antioxidant effects of hydromethanolic extract of *Bridelia micrantha* stem bark. Original Research; 4(4): 273-277.

[15]. Waiganjo N., Ochanda H., Yole D. (2013) Phytochimical analysis of the selected five plant extracts. Chemistry and Materials Research. ; 9(3): 2224 – 3224.

[16]. Okeleye B.I., Bessong P.O., Ndip R.N. (2011) Preliminary phytochimical screening and *in vitro* anti-helicobacter pylori activity of *Bridelia micrantha* (Hochst., Baill., euphorbiaceae). Molecules. ; 16(8): 6193-6205

[17]. Nwaehujor C.O., Udeh N.E., (2011) Screening of acetate extract of *Bridelia micrantha* for hepatoprotective and anti-oxidant activities on wistar rats. Science Direct.; 10(4): 796-798.

[18]. Steenkamp V., Mokoele T.L., Rensburg V.C.E.J. (2009) Toxicity testing of two medicinal plants, *Bridelia micrantha* and *Antidesma venosum*. The Open Toxicology Journal; 3:35-38.

[19]. Deflaoui K., Ouamara K. (2018) Activité anti-hémolytique in vitro des extraits de *Tamussp*. [Mémoire en biochimie appliqué]. Wilaya, Algérie: Université de Bejaia..