International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Phytochemical constituents, total phenolics and flavonoids contents and vermicidal activity of the hydroethanolic extract of the bark of *Bridelia ferruginea* Benth. (*Euphorbiaceae*)

Timoléon Andzi Barhé[1]*, Gabin Carel Mboukou Ntontele[1], Ange Brel Boukongo[1], Papy Ngoualali[1,2], Pascal Robin Ongoka[1]

[1]Laboratoire de Recherche en Chimie Appliquée (LARCA), Ecole Normale Supérieure (ENS), Département des Sciences Exactes, Université Marien Ngouabi, Brazzaville-CONGO, [2]Laboratoire de Chimie des Biomolécules organiques et de Pharmacodynamique. Institut National de Recherche en Sciences de la Santé (IRSSA). Département de la Pharmacopée et Médecine Traditionnelle Cité Scientifique de Brazzaville, Congo

Abstract

This article aims to determine the chemical composition, measure the total polyphenol and flavonoid contents and to evaluate the vermicidal activity of the hydroethanolic extract of the barks of *Bridelia ferruginea* Benth (*Euphorbiaceae*). Phytochemical screening, was used to determine its composition as secondary metabolites. A separation on a polyamide column followed a dosage by visible UV spectrometry at 366 nm, to quantify the total polyphenols (PPT) and the flavonoids (FVT). 50g of sterilized sand was mixed with 17.5 mL of an extract solution and earthworms were used to determine the vermicidal activity. This study showed the presence of alkaloids, flavonoids, tannins and saponosides in our extract. The thin layer chromatographic profiles remain dominated by polyphenol compounds in the bark of the plant. The polyphenol and flavonoid contents in the extract are 495.02 mgEQt/100gMS and 1000 mgEAG/100gMS, respectively. The contents of total flavonoids in the fractions vary between 500.85 and 959.1 mgEQt/100gMs and those in polyphenols between 1248.6 and 2340.0 mgEAG/100gMs in the fractions. Vermicidal activity on earthworms (*Lombrics terrestris*) shows that the total paralysis time and the mortality rate are based on a dependent dose. The 50% lethal concentrations (LC50) are 0.41, 0.66, 0.68, 1 and 0.71 mg/ml respectively for the fractions F3, F4, F2, F1 and the extract. The results, which correlate with the content of phenolic compounds, justify the use of this plant in the traditional pharmacopoeia.

Keywords: Chemical screening, polyphenols and flavonoids content, *Bridelia ferruginea* Benth, vermicidal activity, *Lumbricus terrestris*

Full length article **Corresponding Author*, e-mail: andzibarhe@gmail.com

1. Introduction

The people in developing countries often pay a heavy tribute against diseases caused by parasitic worms in the digestive tract [1]. The World Health Organization (WHO) reports that more than two billion people suffer from parasitic worms that infect them. Livestock and crops are also affected by them. The same trends are observed in food production with a significant economic impact. These parasitic diseases cause serious morbidity; it is the case of several illnesses such as: lymphatic filariasis, onchocerciasis, blindness and schistosomiasis. These conditions can have economic and social consequences for most populations in endemic areas [2]. Although the majority of worm infections are generally limited to tropical regions, they can also happen to travelers who visit certain endemic areas. Despite the significant increase in the production of certain synthetic drugs known to be anthelmintic, vermicide and dewormer; research on anthelmintics remains poor due to the increasing resistance of anthelmintics, side effects (vomiting, dizziness and diarrhea) and also the environmental impact of conventional anthelmintics.

It is important to investigate on the alternatives as well as the strategies against gastrointestinal nematodes. Herbal medicine could be one of the main options for controlling these conditions. These parasitic worms are a public health problem in Africa, with around 200.000 deaths recorded each year. These helminthiasis are often responsible for intestinal injuries and obstructions, skin

lesions, blood loss causing anemia. They are also responsible for nutritional deficiencies which can have serious consequences for children and women of reproductive age as well as pregnant women. These diseases undermine the intellectual growth, cognitive development and academic performance of these children. They also lead to loss of efficiency in work, whose consequences are social decline and imbalance [1]. Traditional healers offer a rich repertoire of medicinal plants used by populations for the treatment of parasitic worms. Thanks to that we are interested in the study of *Bridelia ferruginea* Benth (*Euphorbiacea*), a plant from the Congolese pharmacopoeia used in the treatment of intestinal worms. The aim of this work is to carry out a phytochemical study and evaluate the vermicidal activity of the hydro-ethanolic extract of the barks of *Bridélia ferrugenia Benth*. A chemical screening of the plant will be handled in order to determine its composition in secondary metabolites. The latter will be followed by the spectrophotometric determination of total polyphenols and flavonoids, finally there will be the evaluation of the vermicidal activity of the extract on earthworms.

2. Materials and methods

2.1. Vegetable matter

Bridelia ferruginea Benth is a medicinal plant which belongs to the Euphorbiaceae family. It is a small tree of 1m to 8m high, with cracked bark often bright red in section, very widespread in the savannahs of the trees and in the open forests of Africa [3, 4]. It is used in the treatment of gastrointestinal [5], anthelmintic and haemostatic [6], malaria, rheumatism, inflammation and in the treatment of syphilis [7]. It also has healing properties [8], antibacterial, antifungal and anti-inflammatory properties [9]. The stem bark of *Bridelia ferruginea* was collected in the sub-prefecture of Mindouli in the Pool department in Congo. The identification was done at the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) and a specimen was registered at the National Herbarium.

2.2. Animal material

The in vivo evaluation of the vermicidal effect was carried out on adult earthworms (*Lumbricus terrestris*) whose anatomy is very close to certain intestinal parasites. These earthworms were collected at the edge of the river "Tsiéme" which crosses the city of Brazzaville. They were washed in distilled water and stored in petri dishes containing sterilized sand. The worms were kept under these conditions for 72 hours before the experiment.

2.3. Preparation of extracts

The bark was dried at room temperature, for about a week. The dry vegetable matter is ground with an IKA-WERKE Gmbh-CO-KG, D-79219, Staufen-type device, with a sieve of granulometry 0.25mm. To make the measurement, hydro-ethanolic (EtOH-H_2O, 50:50 v/v) extract is obtained on mixing 100 g of vegetable matter with 2 × 500 ml of solution. The mixture is shaked up during 72 hours, then filtered. The filtrate obtained dried concentrated with a rotary evaporator is kept in a cool place (+4°C) awaiting for its analysis.

2.4. Chemical screening and qualitative analysis

EtOH-H_2O (50:50 v/v) is screened for their classes of bioactive compounds using standard procedures [10-15]. The extracts were tested qualitatively for the presence of chemical constituents such as tannins, saponins, flavonoids, polyphenols, anthocyanins, alkaloids and reducing sugar. Testing for these chemical families was done according to the methods described by Békro et al. [10].

2.5. Fractionation of the extract

1g of the hydroethanolic extract was chromatographed on an open column of Polyamide 6 (Fluka) 1.5 mm in diameter and 50 cm long. The elution is carried out with a water-ethanol mixture with decreasing polarity, in the proportions: 100% H_2O, EtOH-H_2O (30:70 v/v), EtOH-H_2O (70:30 v/v), and 100% EtOH. The different fractions collected are monitored by TLC analysis with silica gel on an aluminum support. The plates are visualized firstly in the UV (λ=254 and 366 nm) then revealed by the reagent from NEU [11] followed by another visualization in the UV-366 nm.

2.6. Measurement of total polyphenols

The reagent of Folin-Ciocalteu was used for the evaluation of total phenols of aqueous, hydroethanolic and ethanolic extracts. Folin-Ciocalteu is a mixture of phosphotungstene acid ($H_3PW_{12}O_{40}$) and phosphomolybdène ($H_3PMo_{12}O_{40}$) of yellow color. The method is based on the oxidation of the phenolic compounds by this reagent. This oxidation draws the formation of a new complex molybdenum tungsten of blue color that absorbs to 725 nm. The evaluation of TP is done by comparison of the optic density (D.O) observed to the one obtained from a stallion of known acid Gallic concentration. The total phenol compounds are measured as follows: 0.1ml of the extract hydroethanolic is introduced in an Eppendorff tube of 2 ml, the extract is diluted with 0.9 ml of distilled water. 0.9ml of the reagent of Folin-Ciocalteu (1N) is immediately added after addition of 0.2 ml of Na_2CO_3 (20%) solution. The resultant mixture is hatched to the ambient temperature during 40 minutes safe from light. The absorbance is measured with the spectrophotometer at 725 nm against a solution of ethanol used like white (control). A right of standardization achieved previously with the Gallic acid in the same conditions that the samples to analyze, permitted to calculate the total phenols contain. The results are expressed in mg equivalent to Gallic acid by gram of dry matter (mg E GA/gMs).

2.7. Measurement of total flavonoids (FVT)

The colorless solutions of sodium nitrite ($NaNO_2$, 5%) and aluminum chloride ($AlCl_3$, 10%) have been used for the evaluation of total flavonoids in aqueous, hydroethanolic and ethanolic extracts. The method is based on the oxidation of the flavonoids by these reagents; oxidation that draws the formation of a brownish complex that absorbed at 510 nm. The comparison of the optic density (D.O) observed to the one obtained from a stallion of known concentration quercetin permits to value the total content in flavonoids by colorimetric effect. In a ball of 10 ml are introduced 250 µl of extract and 1 ml of distilled water successively. At the initial time (0 minute) are added 75 µl of a $NaNO_2$ (5%) solution. After 5 min, 75µl of $AlCl_3$ (10%) are added; 6 minutes later, 500µl of NaOH (1N) and 2.5 ml of distilled water are added successively to the mixture. A curve of standardization is elaborated with solutions standards of Quercetin prepared at different concentrations. The results are expressed in mg equivalent Quercetin by gram of dry matter (mg EC/gMs).

2.8. Evaluation of the vermicides activity

The vermicidal effect of the hydro-ethanolic extract and the bark fractions of *Bridelia ferruginea* were evaluated according to the method advocated by Ongoka [12]. 50 g of sterilized sand, 17.5 mL of an extract solution and fractions (F1, F2, F3 and F4) are mixed in a petri dish in increasing concentrations: 3.12; 6.25; 12.5 and 25 mg/ml and 5 adult earthworms previously washed in distilled water. The mixture is vigorously shaken to allow the earthworms to get in contact with the pasty mixture. The time of paralysis was noted after immobility of the earthworms except when they were strongly shaken. Mortality is noted after total immobility following an external mechanical stimulus. The results were compared with those of Levamisole and albendazole (Reference Compounds) prepared at the same concentrations as the extract and the fractions. The water was considered as negative control. The mortality rate is calculated using the formula:

$$TM = \frac{\text{Number of dead worms}}{\text{Total number of worms}} \times 100$$

The behavior of earthworms was observed for 24 hours upto 72 hours and the experiment was repeated five times.

3. Results and discussion

3.1. Chemical screening

The experimental result on the major chemical families is shown in Table 1. Table 1 shows that flavonoids (Anthocyanins and Free) are in very high quantities followed by saponosides and tannins. The reducing sugar is in average quantity and the alkaloids in very small quantities. It should especially be noted that the bark of *Bridelia ferruginae* has a high concentration of polyphenolic compounds. These results agree with Semie [13]. Polyphenols are of capital importance in the plant and have antioxidant, anti-diarrheal, antiseptic, vasoconstrictor, anti-tumor, anti-carcinogenic, anti-inflammatory, anti-oxidant, antiallergic, antiulcerous, antiviral, antimicrobial, hypotensive and diuretic [14-18].

3.2. Fractionation and chemical analysis by TLC

The chromatographic profiles of the hydro-ethanolic extract and the various fractions (F1, F2, F3 and F4) in the barks of *Bridelia ferruginae* are presented in Fig. 1. A series of spots of different colors appears after spraying the TLC plate by the Neu reagent and visualization at 366 nm. The spots reflect the presence of several chemical families. The orange-yellow fluorescence with frontal retention=0.9, highlighted in the F3 and F4 fractions, could be attributed to aglycones of a flavonol ortho-di-hydroxylated in position 3'and 4' [11]. These compounds have been demonstrated in the plant by Nene Bi [13]. The bluish-green fluorescence spots present in the F2 and F3 fractions can be attributed to hydro cinnamic derivatives and the blue fluorescence spots present in the F1 and F2 fractions, with frontal retention = 0.8, materialize the presence of a phenolic acid derivative of type C6-C1 (gallic acid). All of these results are reported in Table 2.

3.3. Dosage of total polyphenols (PPT) and total flavonoids (FVT)

The calibration curve of gallic acid (Y=3.9089x+0.1257) is used for the determination of total polyphenols (PPT) and that of quercetin (Y=1.6954x+0.2816) for the dosage of total flavonoids (FVT). The results are expressed in mg gallic acid equivalent per gram of dry matter (mgEGa/gMS) for polyphenols and in mg catechin equivalent per gram of dry matter (mg EQt/gMS) for flavonoids. The two calibration curves are established with correlation coefficients (R^2) of 0.998 and 0.99 respectively. The results of the quantitative analysis, by UV-visible spectrophotometer, of the hydro-ethanolic extract of *Bridelia ferruginea*, are presented in Fig. 2. It appears that the barks are quantitatively richer in total polyphenols than in total flavonoids. This trend is confirmed in the four fractions F1, F2, F3, and F4. It should be noted that in the fractions, the amounts of PPT and FVT are inversely proportional to the polarity of the elution solvent, since the largest amounts are obtained in the fraction F3. In addition, the fractions are richer than the total extract. The polyphenol and flavonoid contents in the extract are 495.02 mgEQt/100gMS and 1000 mgEAG/100gMS, respectively. The contents of total flavonoids in the fractions are 500.85; 580.0; 959.1 and 589.0 mgEQt/100gMs and those in polyphenols are 1301.8; 1248.6; 2340.0 and 1800.0 mgEAG/100gMs respectively for the fractions F1, F2, F3, and F4 (Fig. 2). It should be emphasized that, total polyphenols are quantitatively superior to total flavonoids.

Overall, it can be observed that the contents of total polyphenols and total flavonoids are high in the fraction F3 followed by F4. The fraction F1 generally remains less rich in polyphenolic compounds. This indicates enrichment in polyphenolic compounds during the separation. The decreasing polarity gradient used would promote the richness of the fractions in phenolic compounds. The high contents of polyphenolic compounds could explain the high use of plant extracts in the African pharmacopoeia. These results confirm those of the qualitative analysis and they show that polyphenols and flavonoids play a significant role in the different pharmacological property attributed to the plant [5, 6, 8, 9].

3.4. Vermicide activity

After a short contact time between the earthworms and the fractions or extract, the earthworms gradually lose their mobility. This paralysis or immobility is based on a dependent dose (Fig. 3) and extends to the loss of response following an external stimulus and gradually to death. This observation has been made by many authors on certain earthworms and intestinal worms [19-23]. These authors attribute the paralysis effect to the chemical substances present in the plant (polyphenols, saponosides, alkaloids, etc.). It appears that the time of paralysis (Fig. 3) decreases with increasing concentration of the extract. This effect is more marked with the F3, F4 and F2 fractions. It is 35, 45 and 50 min respectively at the highest concentration (1.4 mg/mL). This can be attributed to the high concentration of PPT and FVT in these fractions. These results corroborate those obtained during the determination of PPT and FVT (Fig. 2).

The percentage lethality as a function of the logarithm of the concentration, the hydro-ethanolic extract and the four fractions (F1, F2, F3 and F4) obtained after fractionation on a polyamide column after two hours is presented on Figure 4. This figure shows that the F3 fraction gives a more significant percentage of mortality followed by the F4, F2 fractions and the extract. It should be noted that F1 corresponds to the least significant fraction. It is observed that at a concentration of 1.1 mg/ml, the percentage of mortality is: 50, 70, 80, and 96% respectively for the fractions F1, F2, F4, and F3. Only the F3 and F4 fractions reach the percentage of mortality of 100% at the highest concentration (1.4 mg/mL), while the F2 fraction and the total extract give a percentage of mortality which does not exceed 80% at the same concentration. At this concentration, earthworms show heavy bleeding, sharper segmentation and necrotic spots (Figure 5).

The same observation was made by Wahid A Mulla et al [19]. Subsequently, the lethal concentration 50 (LC_{50}) (Figure 6) of the samples used, varies with the nature of the fractions. Indeed, F3 gives a higher LC_{50} (0.41 mg/mL) followed by F4 (0.66 mg/mL), F2 (0.68 mg/mL) and EHE (0.71 mg/mL). F1 gives a lower LC_{50} (1 mg/mL). The activity of the F3 and F4 fractions could be attributed to their richness in total polyphenolic and flavonoic compounds highlighted during screening, dosage and fractionation in TLC. Indeed, some authors attribute the vermicidal effect to derivatives phenolic acids of types C6-C3, to flavonoids and tannins [24-26]. The flavonol derivatives ortho di-hydro in position 3' and 4' and the tannins have been identified and quantified, specifically for flavonoids, at fairly high levels in the F3, F4, and F2 fractions. These results are more significant compared to those of Albendazole because the latter remains inactive after 2 hours at all concentrations. It takes 72 hours to register 50% of deaths. This compound would be used for roundworms of the genus Hookworm. Levamisole shows a strong reactivity because we note 100% of deaths at the lowest concentration and this after 30 minutes. These results are very significant since no deaths are recorded after 72 hours with distilled water which was considered as a negative control.

Table 1: Phytochemical screening of the stem bark extract of *Bridelia ferruginae*

Chemical Families		Hydro-ethanolic Extract
Alkaloids		+
Tannins		+++
Flavonoids	Anthocyanins	++++
	Free	++++
Reducing Sugars		++
Saponosides		+++

++++: very strong presence, +++: strong presence, ++: weak presence, +: very weak presence

Table 2: Qualitative analysis of the extract and fractions by TLC

Samples	Developers NEU reagent UV-365 nm Eluent:9,5/0,25/0,25	Probable structures
EHE	-	-
F1	FB (0,8)	Acid phenols (derived from gallic acid)
F2	FB (0,8)*	Acid phenols (derived from gallic acid)
	FJV (0.2)*	Flavonoids (Flavonol) derived from quercetin
	FVB (0,3 et 0,6)	Phenolic acids (hydroxycinamic)
F3	FJO (0,2 et 0,95)*	Flavonoids (Flavonol) derived from quercetin
	FVB (0,2; 0,4; 0,6 et 0,7)	Acid phenols (derived from gallic acid)
F4	FJO (0.95)*	Flavonoids (Flavonol) derived from quercetin

FJO: Orange Yellow Fluorescence; FVB: Bluish Green Fluorescence; (): frontal retention; *: poor resolution. F: Fraction; F1, F2, F3 and F4, EHE. Hydro-ethanolic extract. (*): good resolution

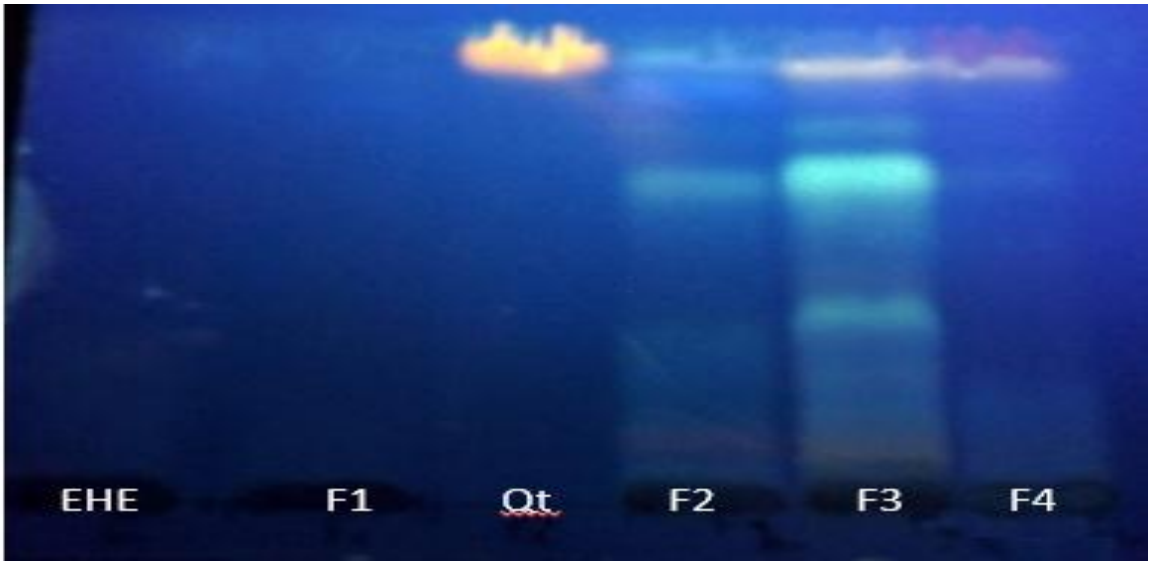

Figure 1: Chromatographic profiles of the extract fractions and reference compounds.
Eluent Ethyl acetate/Formic acid/Water (9.5/0.25/0.25);
Developer: Neu; Observation: UV-366 nm. Reference compound: Q: Quercetin, Hydroethanolic Extract: EHE

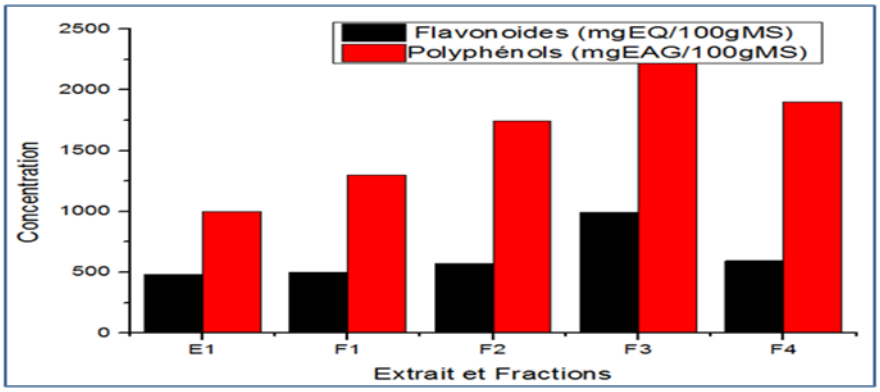

Figure 2: Content of total polyphenols and total flavonoids

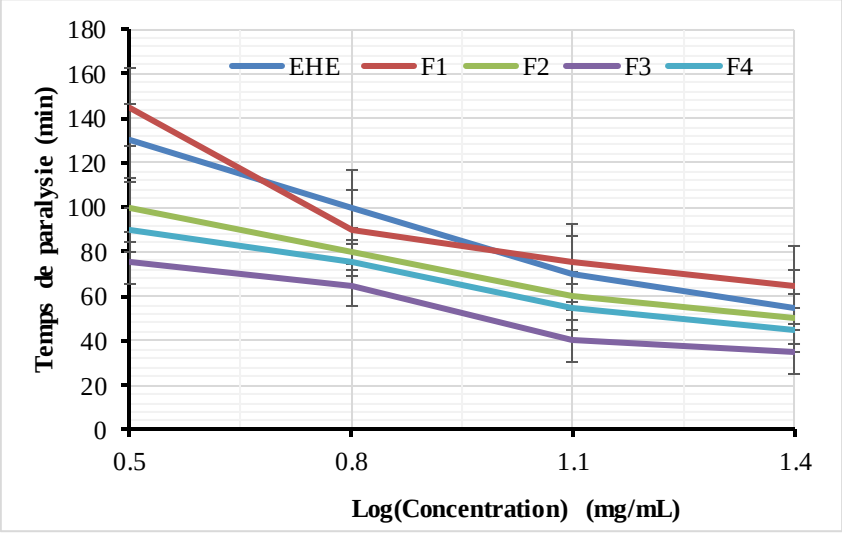

Figure 3: Earthworm paralysis time as a function of concentration

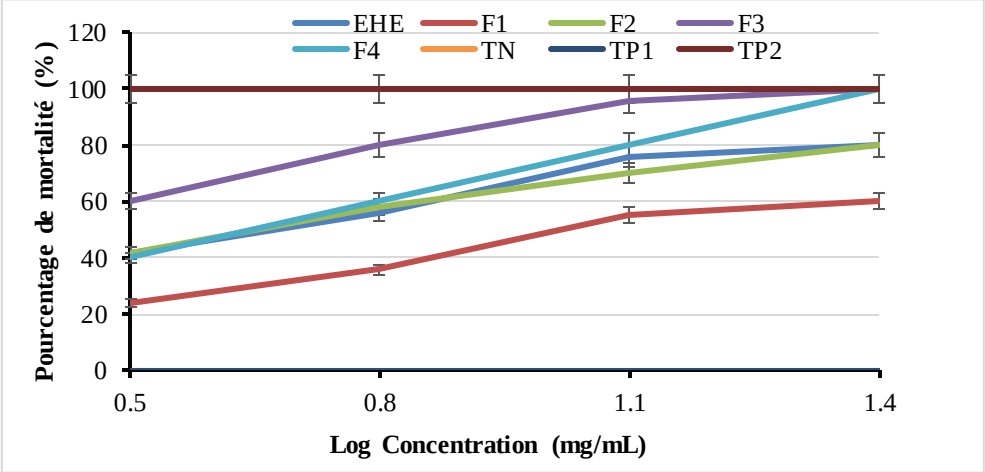

Figure 4: Percentage of mortality according to concentration
TN: negative controls; TP: positive controls

Figure 5: State of earthworms after 6 hours

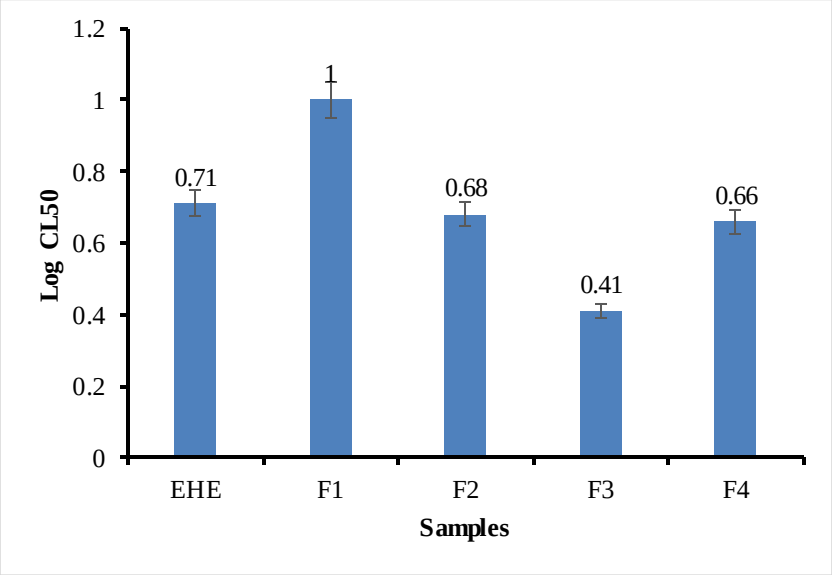

Figure 6: Lethal concentration 50 (LC_{50}) of the extract and fractions

4. Conclusions

The phytochemical study of the hydroethanolic extract of the barks of *Bridelia ferruginea Benth* has permitted to highlight the presence of the secondary metabolites responsible for the various pharmacological activities recognized by this plant. The dosage, after fractionation of the total extract, shows that the amount of total polyphenols is higher than that of flavonoids, both in the crude extract and in the four fractions. The polyphenol and flavonoid contents in the extract are 495.02 mgEQt/100gMS and 1000mgEAG/100gMS, respectively. The contents of total flavonoids in the fractions were between 500.85 and 959.1 mgEQt/100gMs and those in polyphenols between 1248.6 and 2340.0 mgEAG/100gMs in the fractions. The chromatographic profiles on TLC of the extract and the four fractions highlight the yellow, yellow-orange and light green fluorescence which can materialize the presence of flavonols and hydroxycinamic derivatives (C6-C3). The importance of this polyphenolic content is confirmed by the vermicidal activity observed for F3 and F4 fractions evaluated at 100% mortality at a concentration of 1.4 mg/mL. Furthermore, a higher LC_{50} is observed for the same fractions. The results observed correlate with the phenolic content and justify the use of this plant in the Congolese pharmacopoeia particularly in the treatment against intestinal worms instead of synthetic compounds.

References

[1] L. Savioli, M. Albonico, D. Engels, A. Montresor (2004). Progress in the prevention and control of schistosomiasis and soil transmitted helminthiasis. Parasitology International. 53: 103-113.

[2] S. Tagboto and S. Townson (2001). Antiparasitic properties of medicinal and other naturally occurring products. Advances in Parasitology. 50: 199-295.

[3] E.J. Adjanohoun, L. Ake Assi, J.J. Floret, S. Guinko, M. Koumare, A.M.R. Ahyi et J. Raynal (1979). Agence de Coopération Culturelle et Technique (ACCT.), Paris.

[4] Adjanohoun E.J., Aké Assi L. (1979). Contribution au recensement des plantes médicinales de Côte d'Ivoire. Côte d'Ivoire, université d'Abidjan, Centre national de floristique, 358 p.

[5] T. Hernández, M. Canales, J.G. Avila, A. Duran, J. Caballero, A. Romo de Vivar. and R. Lira (2003). Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (Mexico). Journal of Ethnopharmacology. 88 (2-3): 181-188.

[6] J.M. Watt, M.G. Breyer-Brandwijk, (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa, E & S Livingstone, Edinburgh.

[7] Lagnika Latifou (2005). Etude phytochimique et activité biologique de substances naturelles isolées de plantes beninoises. These de Doctorat. Université Louis Pasteur, Strasbourg, France.

[8] L.E. Esmeraldino, A.M. Souza and S.V. Sampa (2005). Evaluation of the effect of aqueous extract of *Croton urucurana* Baillon (*Euphorbiaceae*) on the hemorrhagic activity induced by the venom of Bothrops jararaca, using new techniques to quantify hemorrhagic activity in rat skin. Phytomedicine. 12 (8): 570-576.

[9] M.H. Mavar, D. Brick, D.E.P. Marie, J. Quetin-Leclercq (2004). In vivo anti-inflammatory activity of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (*Euphorbiaceae*). Journal of Ethnopharmacology. 92 (2-3): 209-214.

[10] Y.A. Békro, A. Janat, M. Békro, B. Boua, F.H. Trabi and E.E. Ehouan (2007). Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (*Caesalpiniaceae*). Sciences &Nature. 4(2): 217-225.

[11] H. Wagner and S. Bladt (1996). Plant Drug Analysis, a Thin Layer Chromatography Atlas, 2nd Ed, Springer, NY, USA.

[12] P.R. Ongoka (2005). Etude ethnobotanique, pharmacologique et chimique des plantes anthelminthiques du Congo "Thèse d'Etat, Université Marien Ngouabi, CONGO.

[13] N. Semi, T. Flavien, Z. Ouga, Y. Tianga (2008). Composition chimique d'un extrait aqueux de B*ridelia ferruginea* benth. (*Euphorbiaceae*) et études de ses effets toxicologique et pharmacologique chez les mammifères. Afrique Science. 4(2): 287-305.

[14] Z. Mohammedi (2006). Etude du pouvoir Antimicrobien et Antioxydant des Huile Essentielles et flavonoïdes de quelques plantes de la région de Tlemcen. Mémoire de Magister Université de Tlemcen. Algerie. pp. 155.

[15] B. Halliwell and J.M.C. Gutteridge (2007). Free Radicals in Biology and Medicine. 4th Ed. Oxford University Press. New York.

[16] S.Y. Tang and B. Halliwell (2010). Medicinal plants and antioxidants: What do we learn from cell culture and Caenorhabditis elegans studies?. Biochemical and Biophysical Research Communications. 394: 1-5.

[17] T. Bahorun (1997). Substances naturelles actives : la flore mauricienne, une source d'approvisionnement potentielle. Food and agricultural resarch council, Réduit, Mauritus. 83-94.

[18] A. Benguerba (2008). Etude phytochimique et de la phase butanolique de l'espèce inula crithmoides. Mémoire de Magister, Université Mentouri. Algerie, pp.110.

[19] W.A. Mulla, V.S. Thorat, R.V Patil, K.B. Burade (2010). Anthelmintic activity of leaves of *Alocasia indica* Linn. International Journal of PharmTech Research. 2(1): 26-30.

[20] B. Githiori, J. Hoglund, J. Waller, L. Baker (2002). Anthelminthic activity of preparations derived from *Myrsine africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus* of sheep. Journal of Ethnopharmacology. 80: 187-191.

[21] Y.M. Shivkar and V.L. Kumar (2003). Anthelmintic activity of latex of *Calotropis procera*. Journal of Pharmaceutical Biology. 41: 263-265.

[22] T. Sollmann (1918). Anthelmintics: Their efficiency as tested on earthworms. Journal of Pharmacology and Experimental Therapeutics. 12: 129-133.

[23] R.J. Martin (1985). Y-Aminobutyric acid and Piperazine activated single channel currents from *Ascaris suum* body muscle. British Journal of Pharmacology. 84:445-461.

[24] G. Waghorn, W.C. McNabb (2003). Consequences of plant phenolic compound for productivity and health of ruminants. Proceedings of the Nutrition Society. 62: 383-392.

[25] G. Waghorn (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-progress and challenges. Animal Feed Science and Technology. 147: 116-139.

[26] T.S. Waghorn, A.L. Molan, M. Deighton, R.A. Alexander, D.M. Leathwick, W.C. McNabb, L.P. Meagher (2006). In vivo anthelmintic activity of *Dorycnium rectum* and grape seed extract against *Ostertagia (Teladorsagia) circumcincta* and *Trichostrongylus colubriformis* in sheep. New Zealand Veterinary Journal. 54: 21-27.