Original Research Article

Polyphenol content and antioxidant activity of bark hydroethanolic extract of *Piliostigma reticulatum* (DC) Hochst and its fractions

Abstract

Objective: The purpose of this study was to evaluate the total phenol content and the antioxidant capacity of the hydro-alcoholic bark extract of *Piliostigma reticulatum* and its ethyl acetate, methanol and aqueous fractions.

Material and Methods: Powdered barks were extracted with ethanol (80%). From the crude extract, 3 fractions were obtained after fractionation by column chromatography with three different solvents (ethyl acetate, methanol and water). The polyphenol content was determined with Folin Denis reagent. Antioxidant activity was performed using DPPH (2, 2-diphenyl-picrylhydrazyl), ABTS (2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) and FRAP (ferric reducing antioxidant potential) assays.

Results: The total extract had higher polyphenol contents with 12.55 ± 0.12 mg tannic acid equivalent/ g of dried extract (TAE/g). For ethyl acetate, methanol and water fractions, respective polyphenol content of 7.67 ± 0.4 - 9.01 ± 0.05 and 5.56 ± 0.2 mg TAE / g were obtained. The DDPH test had revealed that the methanol fraction was more active (IC_{50}: 30.83±0.22 µg/ml), while for ABTS assay the ethyl acetate fraction had shown better activity (IC_{50}: 29.08±0.44 µg/ml). For the FRAP test, at all tested concentrations, the methanol fraction revealed highest efficient to reduce ferric ion with percentages of reduction varing from 38 ± 0.73% at 7.81 µg/ml to 93.21 ± 0.24% at 250 µg/ml.

Conclusion: These studies showed that the methanol fraction exhibited the best antioxidant activity and that this activity may be related partially to its polyphenols content.

Keywords: *Piliostigma reticulatum*, bark, fractions, polyphenol, antioxidant.

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1- INTRODUCTION

Plants play an important place in traditional medicine. Out of their use as ornament or shade, they constitute the first means in the health systems of populations in developing countries. They arouse a great medical interest in these countries and in developed countries. Plants are today the main source of raw material for the pharmaceutical and cosmetics industries. They are used directly as drugs or indirectly as in the semisynthesis of drugs.

Given the sometimes high cost and adverse effects associated with the use of so-called modern drugs, some populations make increasingly resorting of plants to cure oneself. In fact, although modern medicine is well represented in our cities, more than 70% of the African population use medicinal plants to treat themselves [1].

Today, we note an upsurge chronic diseases whose among which, oxidative stress is often involved. This is particularly the case of cancer, neurodegenerative diseases, atherosclerosis, irreversible damage of some organism components such as lipids, proteins and DNA among others [2].This situation may be linked to an overproduction of free radicals even if they are naturally produced by the human body. The life in an oxygen rich environment depends on a vital balance between the physiological production of free radicals and the body's ability to eliminate it. However, in some cases, an imbalance may occur following a significant increase in the production of free radicals thus causing a state of oxidative stress. This is often linked to certain behaviors such as sedentary, consumption of alcohol, tobacco etc. Moreover the use of natural products rich in polyphenols is involved in the reduction of oxidative stress related diseases [3]. Antioxidant supplementation could have benefit health effect. That's why in the past decade, several studies to evaluate the antioxidant activity of plants have been conducted in several countries around the world [4,5].

It is in this context that we are interested in the study of the antioxidant activity of *Piliostigma reticulatum* (DC) Hochst, (Synonym: *Bauhinia reticulata*). It's a common plant of Sahelo-Sudanian zone, from Senegal to Sudan. The traditional healers of Senegal use its leaves and bark. Our study focuses on bark because they are more exploited in traditional Senegalese medicine than leaves. *P. reticulatum* bark is often prescribed against many diseases such as ulcers, boils, wounds, syphilitic cancer, toothache, gingivitis and diarrhea [6,7]. Phytochemical analysis of the acetone bark extract showed the presence of polyphenols [8].

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The aim of this study was to evaluate the total polyphenol content and the antioxidant capacity of the hydro-ethanolic bark extract of *Piliostigma reticulatum* and its ethyl acetate, methanol and water fractions.

2- MATERIAL AND METHODS

2-1. Plant material

Bark of *Piliostigma reticulatum* was collected in August 2015 in the forest of Diourbel, a region in central Senegal. The plant has been identified in the Laboratory of Pharmacognosy and Botanical of the Faculty of Medicine, Pharmacy and Odontology of the University Cheikh Anta Diop of Dakar. The herbarium number of this plant is 1641. The barks were washed, dried at ambient temperature in an airy room of the laboratory and then reduced to powder. The latter was kept away from sunlight before handling before treatment.

2-2. Extraction and fractionation

A moderate decoction under reflux of 600 g of powder was carried out with 6 L of water-ethanol mixture (20v / 80v) for 30 minutes. After filtration with Whatmann No. 1 filter paper, the resulting filtrate was evaporated on a rotary evaporator at 60 ° C to obtain a dry extract.

Fractionation of the dry extract thus obtained on a silica column was carried out according to the modified method of Labourel and Péaud-Lenoel [9].

A cylindrical glass column 3 cm of diameter was filled with 100 g of silica (Scharlau GE 0048, 60A- 0.04-0.06 mm). After being well packed with a pump (50Hz), the silica was washed three times with 200 ml of ethyl acetate. Then 15 g of dry extract triturated before with 15 g of silica, were gently deposited at the top of the stationary phase. The elution process was done by polarity gradient with successively 1.5 L of ethyl acetate; 1.5 L of methanol and 1.5 L of distilled water with an average flow of 12.3 ml/min. Fractions thus obtained were evaporated and dried by the same method as the crude extract.

2-3. Total polyphenol content

Total polyphenol contents were evaluated according to the method described by Elgailani and Ishak [10]. To 1 ml of each dry extract in water (250 mg / L), 1 ml of Folin-Denis reagent was added shaking. Then after 3 minutes, 1 ml of 12% sodium carbonate was added and the whole is incubated in the dark for 2 hours at room temperature.

The absorbances of the samples were read at 725 nm against a blank without extract after centrifugation of the tubes at 3000 rpm for 5 minutes. The tests were repeated three times for

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each sample. A calibration line was established with tannic acid at different concentrations (0.01-0.015-0.02-0.03-0.045 mg/ml). The results were expressed in mg TAE/g.

2-4. Antioxidant activity

2-4-1. DPPH assay

The scavenging activity of free radicals of the samples was evaluated by the DPPH (2, 2-diphenyl-picrylhydrazyl) assay according to the method of Molyneux [11]. An ethanolic solution of $DPPH^{\cdot}$ was prepared by dissolving 4 mg of this of DPPH reagent in 100 ml of ethanol followed by a cool incubation between 4-8 ° for at least 16 hours. Then, at 50 µL of the sample (extract, fractions or ascorbic acid) at different concentrations (250 - 125 - 62.5 - 31.25 - 15.62 - 7.81 µg / ml) were added 950 µL of the diluted DPPH solution.

Absorbances were measured at 517 nm after 30 min of incubation in the dark at room temperature. Three tests were performed for each concentration. The antioxidant activity related to the DPPH free radical scavenging effect, is expressed in IC_{50} (Inhibiting Concentration 50% of free radicals) using Statgraphics Plus 5.0 software

2-4-2. ABTS assay

The method of Leong and Shui [12] was used to evaluate the antiradical activity of the samples by the ABTS test. A 7 mM ABTS (2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) solution was prepared with distilled water. To obtain a solution of radical ABTS+•, were mixed at equal volumes, a solution of ABTS at 7 mM and a solution of potassium persulfate at 2.5 mM. This solution $ABTS^{+\bullet}$ thus obtained was diluted with ethanol to obtain an absorbance of 0.700 ± 0.02 to 734 nm before use.

Then, 1.5 ml of the $ABTS^{+\bullet}$ solution was mixed with 50 µL of the sample (extract, fractions and Vitamin C) at different concentrations (250 - 125 - 62.5 - 31.25 - 15.62 - 7. 81 µg / ml). Absorbances were measured at 734 nm after incubation for 10 minutes in the dark and at room temperature. Three tests were performed for each concentration and the results were expressed as IC_{50} as described above for the DPPH test.

2-4-3. Ferric Reducing Antioxidant Power assay (FRAP)

The reducing power of the crude extract or fractions as well as that of the reference was determined by the FRAP method according Bassène [13]. Thus, 400 µL of each sample at different concentrations is mixed with 1 ml of phosphate buffer (0.2 M, pH = 6.6) and 1 ml of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$). After 30 minutes of incubation at 50 ° C., 1 mL of 10% trichloroacetic acid was added before the tubes were centrifuged at 3000 rpm for 10 min.

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Then, 1 ml of the supernatant from each tube was mixed with 0.2 ml of freshly prepared 0.1% ferric chloride solution. The tubes were incubated thereafter at room temperature away from light for 15 minutes before measuring the absorbances at 700 nm against a blank without sample. The results are expressed as percentage of reduction (PR) according to the following formula:

$$PR = [(A_X - A_B)/A_B] \times 100$$

Ax: absorbance sample tested; A_B: Blank absorbance

2-4-4. Statistical analyses

Significance tests were performed by the Fisher test using the StatView software. A value of $p < 0.05$ was considered statistically significant. Data were expressed as mean ± SD.

3- Results

3-1. Extraction and fractionation

From 600 g of bark powder, 70 g of dry hydroethanolic extract were obtained representing a yield of 11.5%. Fractionation of 15 g of crude extract gave the fractionation yields mentioned in Table I.

Table I : Yields of different fractions of bark hydroethanolic extract of *P. reticulatum.*

	Weight (g)	Yield (%)
Hydroethanolic extract	**15**	
Ethyl acetate fraction	0.6	**4**
Methanol fraction	11.16	**74.4**
Aqueous fraction	0.41	**2.73**

3-2. Total polyphenol content

The polyphenol contents of the crude extract and its fractions were obtained from a tannic acid standard curve (*y = 24.90x - 0.13; R² = 0.9994*). The total extract was richer in polyphenols with 12.55 ± 0.12 mg TAE / g. Among the fractions, methanol had the highest content at 9.01 ± 0.05 mg TAE / g. For the ethyl acetate and aqueous fractions, respective contents of 7.67 ± 0.4 mg TAE / g and 5.56 ± 0.2 mg TAE / g were obtained (see Fig. 1).

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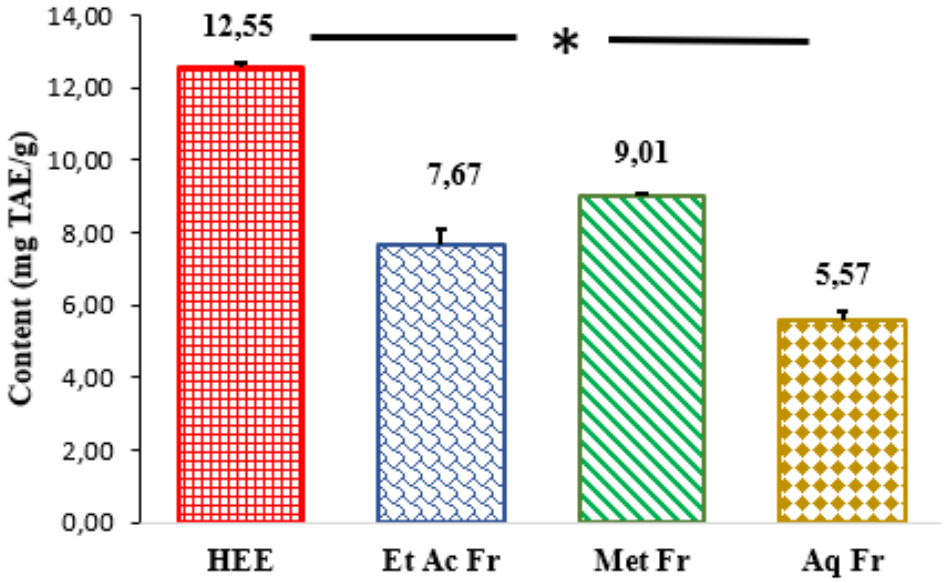

Fig. 1 : Total phenol content of samples

HEE : Hydroethanolic extract; Et Ac Fr: Ethyl acetate fraction; Met Fr : Methanol fraction; Aq Fr: Aqueous fraction; * : $p < 0.05$

3-3. DPPH assay

The root hydro-ethanolic extract of *P. reticulatum* had an IC_{50} of 19.92 ± 1.01 µg/ml. Among the fractions, the methanol had shown the lowest IC_{50} value (30.83 ± 0.22 µg/ml) comparatively to ethyl acetate fraction (IC_{50} : 52.33 ± 0.36 µg/ml) and aqueous fraction (IC_{50} : 103 ± 0.54 µg/ml). Ascorbic acid had an IC_{50} value of 18.6 ± 1.01 µg/ml (Fig. 2).

3-4. ABTS assay

ABTS test revealed that the lower IC_{50} value was obtained with ethyl acetate fraction (29.08 ± 0.44 µg/ml). The methanol and aqueous fractions had respectively IC_{50} values of 48.83 ± 0.74 µg/ml and 58 ± 1.46 µg/ml, while that of crude extract was at 78.17 ± 0.80 µg/ml. Ascorbic acid had an IC_{50} value of 19.66 ± 1.18 µg/ml.

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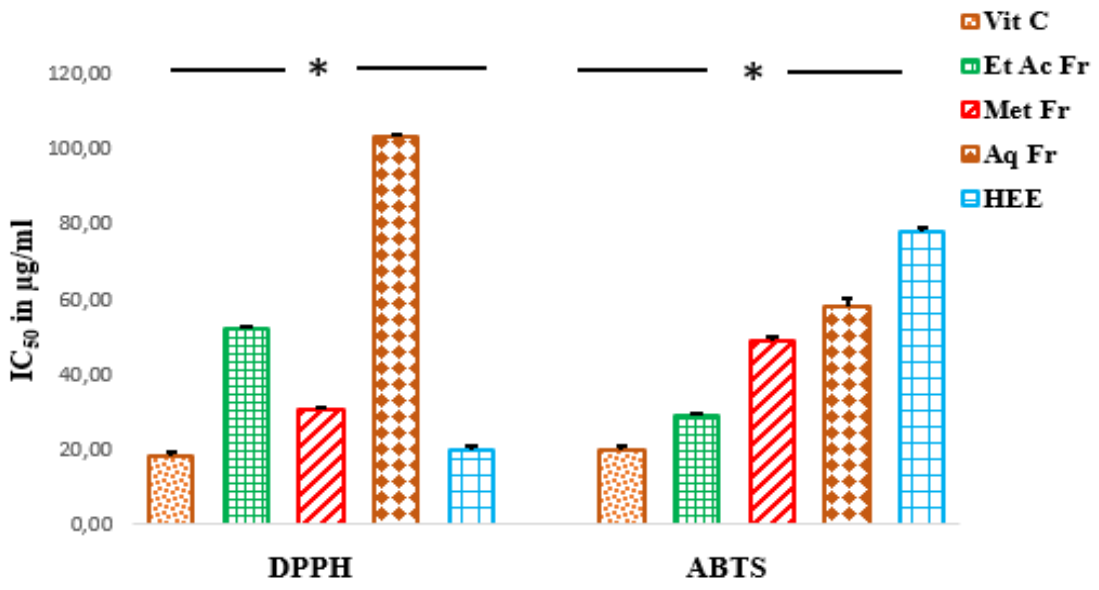

Fig. 2 : IC_{50} of samples by DPPH and ABTS assays

Vit c : Vitamin C ; HEE: Hydroethanolic extract; Et Ac Fr: Ethyl acetate fraction; Met Fr : Methanol fraction ; Aq Fr: Aqueous fraction; * : $p < 0.05$

3-5. FRAP assay

Among all fractions, at all the concentrations tested, the methanol fraction showed the best ability to reduce ferric iron with PR from 38 ± 0.73% to 93.21 ± 0.24% respectively from concentrations of 7.81 µg/ml to 250 µg/ml (Fig.3). It is followed respectively by the ethyl acetate fraction (PR: 25.74 ± 0.21% and 87.21 ± 0.01% respectively at 7.81 µg/ml and 250 µg/ml), the hydroethanolic extract (PR : 14.45 ± 2.66 % and 88.78 ± 0.51% respectively at 7.81 µg/mL and 250 µg/ml) and aqueous fraction (PR: 14.17 ± 1.12% and 83.2 ± 0.15% respectively at 7.81 µg / mL and 250 µg/ml). Vitamin C, showed the best activity of all samples tested (PR: 88.42 ± 0.01% and 97.09 ± 0.01 % respectively at 7.81 µg/ml and 250 µg/ml).

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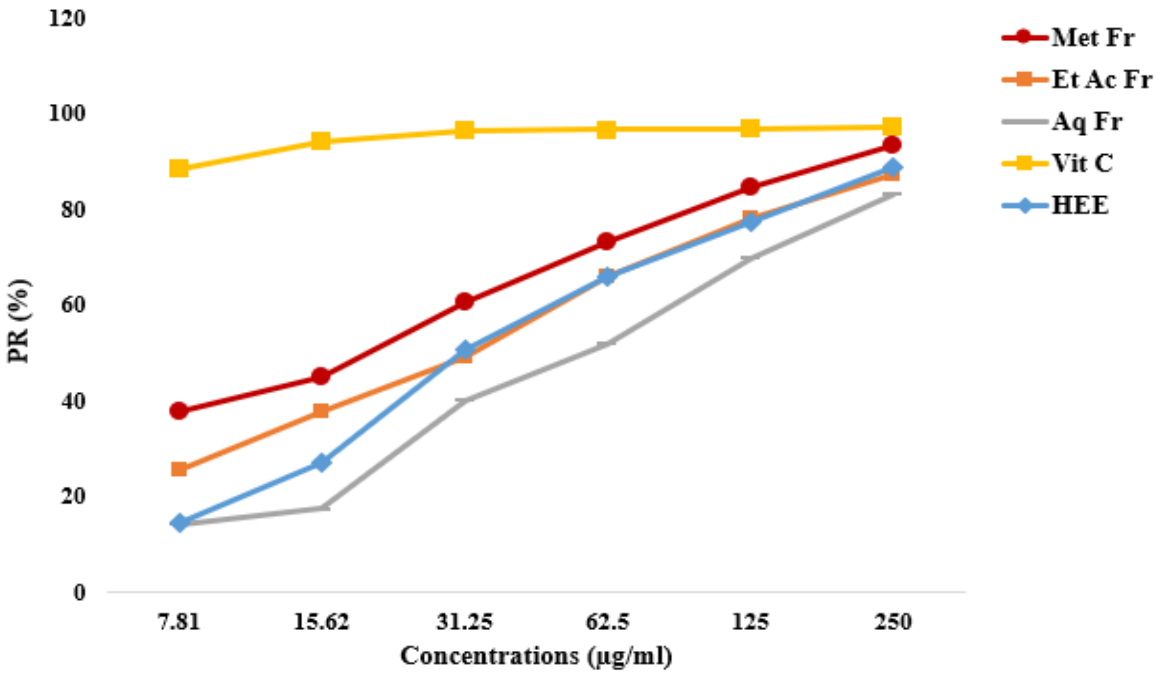

Fig. 3 : Percentage reduction of different samples by FRAP

Vit c : Vitamin C ; HEE: Hydroethanolic extract; Et Ac Fr: Ethyl acetate fraction; Met Fr : Methanol fraction ; Aq Fr: Aqueous fraction

4. DISCUSSION

The hydroethanolic extraction of *Piliostigma reticulatum* bark was aimed to extract the maximum of polar compounds such as polyphenols because according to some studies [13,14], a compound tends to dissolve better in a solvent of the same polarity. Fractionation of the crude extract showed that the methanolic fraction presented the best yield (74.4%). This suggests that the majority of polar compounds have been eluted by methanol.

This seems to be confirmed by the total polyphenol contents of the different fractions with 9.06 ± 0.05 mg EAT / g dry extract for the methanolic fraction against 7.67 ± 0.4 and 5.56 ± 0.2 mg EAT / g for the ethyl acetate and aqueous fractions respectively ($p < 0.0001$). Compared with the Dieng studies [4], these results show that the methanolic and ethyl acetate fractions contain more polyphenolic compounds than the ethanolic bark extract of *Piliostigma thonningii* (*Caesapiniaceae*) which had a content equal to 6.16 mg EAT / g dry extract.

Polyphenols are among the major natural compounds presenting antioxidant activity [15,16]. It's the case of some plants such as green and black tea that have strong antioxidant activity

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due to their high polyphenol content [1] .Thus, they could be partly responsible for the activity of the different fractions. With the DPPH test, the methanolic fraction had the best antiradical activity with an IC_{50} of 30.83 ± 0.38 µg/ml whereas for the ABTS test, the activity was better with the ethyl acetate fraction (29.08 ± 0.76 µg/ml) with a statistically significant difference from the other fractions for both methods ($p < 0.0001$).

Both DPPH an ABTS tests have similar mechanisms of action but with different wavelengths. The DPPH test is simple and rapid but often poses a problem of interpretation because some compounds may have overlapping absorbances at 517 nm like caratenoids [17]. While the ABTS test eliminates most of these interferences because of its 734 nm reading wavelength [5]. This could partly explain why the methanol fraction, although more rich in polyphenols, exhibited a lower activity than the ethyl acetate fraction by the ABTS test. Moreover, natural antioxidants can act differently *in vitro* with respect to the radicals used or oxidizing sources because they have different chemical characteristics. Furthermore, many antioxidants react more slowly with DPPH or may even be inactive [17].

The antioxidant power of the samples, by their ability to reduce oxidants such as iron, seems to be corollary of the antiradical activity and their polyphenol content. The methanolic fraction presented, at all concentrations tested, the best reducing activity with a significant difference ($p < 0.0001$) compared to the other fractions. This activity is probably related to its polyphenols content because they are known for their radical scavenging and antioxidant properties [16,18]. Several plants or parts of plants are used for several reasons. Recent studies have shown a relationship between the use or consumption of products rich in polyphenols and prevention of many diseases related to oxidative stress such as cancer, coronary heart disease, inflammation, thrombosis and other [19]. This is linked in part by their ability to inhibit free radicals and thus the oxidation of some compounds such as LDL-cholesterol [7, 20]. This could explain the fairly frequent use of *P. reticulatum* bark extracts in Senegalese traditional medicine.

5-Conclusion

These studies showed that the methanol fraction exhibited the best antioxidant activity and that this activity could be partially linked by the presence of polyphenols. A subsequent study aimed to evaluate the anti-inflammatory, antimicrobial activity and isolation and identification of the compounds of the methanol fraction could be considered.

Ethical approval and consent are not applicable.

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