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Research Article

Condensed Tannins Content and their Influence on the Antioxidant Activity of Bark Hydroethanol Extract of *Piliostigma reticulatum* (Dc) Hochst and its Fractions

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ABSTRACT

Background: Consumption of natural products from plants is implicated in the reduction of the occurrence of diseases related to oxidative stress. *Piliostigma reticulatum* is a plant well known to traditional practitioners in Senegal where leaves and bark are often used against many diseases, such as ulcers, boils, syphilitic cancer, toothache, gingivitis and diarrhea. **Aim:** This study compared hydroethanol extract from the plant bark and its fractions by assessing their total phenol contents, antioxidant activity and the influence of condensed tannins on their activity. **Method:** barks were extracted by a moderate decoction with ethanol-water followed by a silica column fractionation with successively ethyl acetate, methanol and water. For this study, assays were carried out before and after precipitation of tannins by BSA and PVPP tests. Total phenol and condensed tannins of hydroethanolic extract and its fractions were performed by Folin Ciocalteu and hydrolysis methods. Antioxidant activity was evaluated by DPPH and CUPRAC tests. **Results:** Tannins precipitation leads a light decrease of total phenol and condensed tannins contents of samples. Total phenol content of hydroethanolic extract was 51.2 mg GAE/g *vs* 3.2 after BSA test and 1.7 after PVPP test whereas condensed tannins content obtained 72.2% *vs* 4.2% and 2.3% after precipitation. Antiradical activity was lost following the elimination of tannins with IC_{50}: 5.33 ± 0.04 mg/l *vs* 78.86 ± 0.92 after BSA and > 500 after PVPP. **Conclusion:** This results showed the condensed tannins would be in charge of antiradical and reducing activities of plant barks and PVPP precipitated much better the tannins from those extracts than BSA.

Key words: *Piliostigma reticulatum*, Bark, Tannins, Precipitation, Antioxidant.

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INTRODUCTION

Researchers around the world are increasingly concerned about the development of natural plant-based products used in the health field. Indeed, more and more people are interested in herbal medicine to the detriment of synthetic medicines. Several reasons are often raised to explain this behaviour and the main cause among, them are the high cost of treatments, the health benefits, but also complications or side effects often associated with so-called modern or allopathic medicine. Otherwise, plants are an inexhaustible source of raw material with significant exploitation potential linked to their diversity. That's why today, phytotherapy market is growing well supported by a lot of scientific evidence justifying most of the therapeutic activities granted to medicinal plants. Today, 391,000 plant species have been described worldwide, of which 30,000 plants have at least a documented use and the majority of which are medicinal plants with 17810 species used as a source of medicines.[1] For example, in some countries, such as France, medicinal plants fall under the pharmacists' monopole and are sold only in pharmacies except for a few used in common place.[2]

The prevalence of chronic diseases related to oxidative stress including cancer, neurodegenerative diseases, atherosclerosis, rheumatoid arthritis is increasing year after year. According to several studies, this situation is linked to an overproduction of free radicals of the body as a result of bad lifestyle habits such as sedentary lifestyle, alcohol consumption, tobacco etc.[3-8] Hence, in recent decades, several studies to assess the antioxidant activity of plants have been carried out.[9-11] In addition, the consumption of products from of the plants, such as fruits and vegetables, is implicated in the reduction of the occurrence of diseases directly related to oxidative stress.[12,13]

In Senegal, the use of plants for treatment is a common practice in both rural and urban areas. Traditional medicine, particularly herbal medicine, is the primary care remedy for almost the majority of the population. Senegal possesses an important flora with many species including *Piliostigma reticulatum*, constitutting a richness provided by its value.[14]

Piliostigma reticulatum (DC) Hochst (*Caesalpiniaceae* ; Synonym: *Bauhinia reticulata*) is a plant that grows in the Sahel-Sudan area, from Senegal to Sudan. It is a plant well known to traditional practitioners in Senegal. In Africa, among its organs, leaves and bark are the most used. These are often used against many diseases, such as ulcers[15], boils, sores, syphilitic cancer, toothache, gingivitis and diarrhea.[16,17] The leaves are used for their

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antimicrobial and anti-inflammatory activities.[18,16] But in traditional Senegalese medicine, it is the barks that are much more popular with a wider spectrum of indications, such as hemostatic activity, secretory, antidiarrheal, antoseptic, antibacterial activities.[19-22]

The objective of this study was to compare hydroethanol extract from the plant bark and fractions by assessing their total phenol levels, antiracal and reducing activities and the influence of condensed tannins on their activity.

MATERIAL AND METHODS

Plant material

The barks of *Piliostigma reticulatum* were harvested in Diourbel, central Senegal. The plant has been identified at the Pharmacognosy and Botany Laboratory of Medicine, Pharmacy and Odontology Faculty of the Cheikh Anta Diop University in Dakar. The identification herbarium number of this plant is 1641. The barks were washed for about 15 minutes with tap water and then dried at room temperature for two weeks in the dark in an airy room of the laboratory before being reduced to powder by a grinder (Brabender®). The powder was then stored in a dry place at room temperature.

Reagents and solvents

The solvents and reagents that have been used are: Ethanol (VWR Chemicals BDH, France), Methanol (Carlo ERBA, France), Ethyl acetate (VWR Chemicals BDH, France), distilled water , hydrochloric acid (VWR Chemicals BDH, France), silica gel (Scharlau GE 0048, 60A-0.04-0.06 mm), Folin-Ciocalteu reagent (Merck KGaA, Germany), sodium carbonate ($NaCO_3$, Sigma-Aldrich, USA), gallic acid monohydrate (Sigma-Aldrich, USA), Milli-Q water (Purelabo Classic), 2.2-diphényl-1-picrylhydrazyle (DPPH, Sigma-Aldrich, USA), Trolox (Sigma-Aldrich, USA), Bathocuproinedisulfonic acid disodium salt (BCS, Sigma-Aldrich, Austria), copper sulfate ($CuSO_4$, Sigma-Aldrich, United Kingdom), Ethylene diamine tetraacetic (EDTA, Sigma-Aldrich, Germany).

Extraction

The hydroethanol extract (HEE) of the barks was obtained by moderate decoction under reflux of the powder at about 70° C for 30 minutes. Thus 600 g (4x150 g) of powder was extracted with 6 liters (4x1.5 L) of ethanol-water mixture v/v (80:20). After cooling and filtration on Whatman No 1 filter paper, the filtrate was concentrated in rotavapor at 60° C and then dried to dry in a stove at 45°C. The resulting dry extract was powdered and sealed in a jar.

Silica column fractionation

The fractionation was done by adapting the method of Labourel et Péaud-Lenoel.[23] A cylindrical glass column 3 cm in diameter was filled with 100 g of silica mixed with 300 ml of ethyl acetate while avoiding trapping air. The silica was then washed three times in a row with 200 ml of ethyl acetate generating a separation height of 31 cm with a dead volume of 195 ml. Then 2 g of dry extract powder and 2 g of silica homogeneously mixed by triturating with methanol before evaporating it in the boil at 40° C, were deposited at the head of the column so that the surface is well horizontal, then protected with cotton to avoid that the elution creates depressions in places. Elution was made with successively 0.5 L of ethyl acetate, 0.5 L of methanol and 0.5 L of Milli-Q water with an average flow of 12.3 ml/min. The dead volume was eliminated with each change of solvent. The three fractions thus collected (Methanol Fraction, FM; Ethyl acetate fraction, FAE; Water Fraction, FA) have been evaporated and dried by the same process as the EHH previously described.

Total phenol content

The total phenol contents of the extracts were evaluated by the colorimetric method of Folin Ciocalteu according to the modified protocol of Magalhães et *al.*[24] In a microplate of 96 wells, the samples (hydroethanolic extract and fractions) were treated in quadriplicate (n = 4) by mixing in each well, 20 µl of sample at 100 mg/L, 10 µl of Folin-Ciocalteu reagent and 170 µl of Na_2CO_3 at 2.36%. The microplate was thereafter stirred for 10 seconds by the reader and incubated at 45° C for 45 minutes. The absorbances were measured at 760 nm against a blank with methanol on a BMG Labtech Spectrostar Nano spectrophotometer. A range of calibrations performed by Gallic acid at different initial concentrations (11 -22 - 33 - 44 - 55 - 66 - 77 - 88 - 99 - 110 mg/l) was treated in the same way as the samples in order to obtain a calibration straight. The results were expressed in mg equivalent of gallic acid per gram of dry extract (mg GAE/g) ± SEM (Standard Error of the Mean).

Condensed tannin content

The condensed tannin content of the samples was evaluated by an adaptation of the Waterman et Mole method.[25,26] Samples at 1 mg/ml, were first diluted with water so as to obtain after hydrolysis, an absorbance of less or equal 0.200. For each sample, two hemolysis tubes were used, one as a control (unheated tube), the other for testing (heated tube). In each tube was added successively 2 ml of the diluted sample, 1 ml of water and 3 ml of concentrated hydrochloric acid (12 N). Then the tubes for the test are incubated in the bath at 100 °C for 30 minutes, while the control tubes are placed at the same time in the crushed ice. After heating, the tubes are recovered and cooled in crushed ice before adding 0.5 ml of ethanol to all tubes (Control and test). After agitation at the vortex for 10 seconds the absorbance is read to 550 nm on a Shimadzu UV1800 spectrophotometer. The tests were repeated 3 times for each sample (triplicate, n = 3) and the results were expressed as a percentage of condensed tannins - SEM (Standard Error of the Mean).

DPPH test (2.2-diphényl-1-picrylhydrazyl)

It was carried out according to the adapted method of Tabart et *al.*[27] A radical DPPH• solution was prepared by dissolving 4.8 mg of DPPH in 50 ml of ethanol for 2 hours under magnetic agitation. The resulting solution has been protected from the light and kept cool until it is used. The absorbance of the DPPH solution (Ao) was adjusted to a value between 0.9-1.1 by diluting it with methanol or evaporating it prior to sample analysis. On a microplate of 96 wells, a series of dilution of the mother solutions of the samples (50 mg/l) was carried out with methanol for a final volume of 150 µl. Then it was added in each well, 150 µl of previously DPPH• solution giving final concentrations of 25 - 20 - 16.67 - 13.33 - 8.33 - 5.00 - 3.33 and 1.67 mg/l of samples. The Absorbance A was read at 516 nm after 40 minutes of reaction on a BMG Labtech Spectrostar Nano spectrophotometer. Trolox has been used as a reference.

The DPPH test was conducted in quadruplicate (no = 4) and the antiradical activity associated with the trapping effect of the radical DPPH• will be expressed as a percentage of inhibition (PI) according to the following formula:

$$PI = (1 - \frac{A}{A0}) * 100$$

A0 : absorbance of DPPH to T_0; A : absorbance after 40 minutes of incubation

From PI, the IC_{50} (inhibitory concentrations at 50% of free radicals) were calculated using Statgraphics Plus 5.0 software and expressed in mg/l ± SEM.

CUPRAC Test (CUPric Reducing Antioxidant Capacity)

The reducing power of the extracts was assessed by the CUPRAC-BCS test using the slightly modified method of Campos et *al.*[28] Solutions of BCS (BathoCuproineDisulfonic acid Disodium Salt, 4,2 mg in 30 ml of Milli-Q water), $CuSO_4$ (Copper Sulfate, 18.7 mg in 30 ml Milli-Q water) and EDTA (Ethylene Diamine Tetra Acetic, 43,8 mg in 30 ml Milli-Q water) have been prepared. In a 96 wells microplate were mixed in each well, 40 µl of samples at 6 different concentrations, 160 µl of BCS solution and 50 µl of CuSO4 solution. After 15 minutes incubation at room temperature, 50 µl of EDTA solution was added to stop the reaction. The absorptions were measured at 490 nm against blank (40 µl of water instead of the sample) on a BMG Labtech Spectrostar Nano spectrophotometer.

The test was on triplicate (n: 3) and the Trolox was used as a reference. The results were first expressed in percentage of reduction (PR) according to the following formula :

$$PR\ (\%) = \frac{A - Ab}{Ab} * 100$$

A_b *: Absorbance of blank; A: Absorbance of sample*

The IC_{50} values were determined from PR using Statgraphics Plus 5.0 software and expressed in mg/l ± SEM.

Precipitation tests of Condensed tannins

The condensed tannins were precipitated using the following two precipitation tests: BSA test using bovine albumin protein (BSA) as a matrix and PVPP test using a non-protein polymer Polyvinylpolypyrolidone (PVPP).

PVPP test

The extracts to be analyzed were solubilized in methanol (1 mg/ml). An aliquot from each sample was treated with PVPP in triplicate using the modified method of Peng et *al.*[29] Thus 5 ml of each sample were mixed with 5 ml of water and 500 mg of PVPP. After vortexing for 30 seconds, the samples are placed at 4°C for 15 minutes then they are stirred again for 30 seconds before being centrifuged at 4000 rpm, 3000 g for 10 minutes. The supernatant is collected and the contents of condensed tannins, total phenols and antiradical activity are evaluated according to the protocols described above.

BSA test

The method described by Harbentson et *al.*[30] was used with some modifications. A 1 mg/ml BSA solution is prepared with an acetic acid buffer solution (200 mM) and NaCl (170 mM) at pH 4.9. Thus 1 ml of BSA solution and 500 µl of extract at 1 mg/ml were vortexed well for 10 seconds and then incubated at room temperature for 15 minutes. The samples were subsequently centrifuged for 5 minutes to 13500 g. The supernatant was collected to evaluate of condensed tannin content, total phenols content and antiradical activity using the same protocols described above. All samples were tested in triplicate (no. 3).

Statistical analyses

The significative analyses was carried out by Fisher's test using StatView version 4.55 software. A value of *p*: 0.05 was considered to be statistically significant.

RESULTS

Extraction and fractionation

From 600 g of bark powder, 70 g of dry hydroethanolic extract were obtained representing a yield of 11.66%. Fractionation of 2 g of hydroethanolic extract gave the fractionation yields mentioned in Table 1.

Total phenol contents

The total phenol contents of the hydroethanolic extract and the three fractions were calculated using the equation of the calibration line (y= 0.0623x - 0.0034; R^2 = 0.999) obtained with gallic acid (Figure 1).

The results show that hydroethanolic extract, as well as ethyl acetate and methanol fractions, were rich in total phenols as shown in Table 2.

Condensed tannin contents

Evaluation of condensed tannin contents showed that hydroethanol extract and methanol fraction were more concentrated in condensed tannins than the ethyl acetate and aqueous fractions with respectively 72.18% ± 0.01 and 56.58% ± 0.01 against 26.57% ± 0.02 and 3.30% ± 0.00 (Figure 2).

DPPH test

The PI found showed at all concentrations tested that hydroethanolic extract and methanol fraction had similar activities as shown in Figure 3. The water fraction exhibits very low activity whereas the ethyl acetate fraction had at fairly important activity at concentrations 10 and 12.5 mg/ml.

The IC_{50} found confirmed that of all the fractions tested, the methanol fraction had very interesting antiradical activity with a CI_{50} = 5.49 ± 0.14 mg/l. More the IC_{50} is low, more activity is better. Its activity is statistically identical to that of the hydroethanolic extract with an IC_{50} = 5.32 ± 0.04 mg/l (Table 3). As for the ethyl acetate fraction its IC_{50} is 11.19 ± 0.28 mg/ml whereas the aqueous fraction had the lowest activity on the radical DPPH with an IC_{50} = 50 mg/ml.

CUPRAC test

The evaluation of the reducing power of extracts showed that the hydroethanolic extract reduced more effectively the CUPRAC reagent than its fractions at all concentrations tested with PR ranging from 37.9% ± 3.46 to 78.4% ± 0.33 (from 0.18 to 2.67 mg/l). The methanol and ethyl acetate fractionS had PR respectively from 18.7% ± 0.55 to 72.8% ± 0.34 and 24.08% ±1.51 to 65.2% ± 0.47. The aqueous fraction had the lowest activity with a very low PR of 0 to 4.8% (Figure 5).

Table 1: Yields of ethyl acetate, methanol and aqueous fractions.

	EAF	MF	AF
Dry extract (g)	0.044	1.616	0.209
Yield (%)	2.19 ± 0.1	80.80 ± 3.3	10.47 ± 0.13

Table 2: Total phenol contents of hydroethanolic extract and its fractions.

Extracts	Total phenol contents (mg GAE/ g dry extract)
HEE	51.19 ± 0.76
EAF	45.45 ± 0.46
MF	44.49 ± 0.32
AF	7.85 ± 0.48

Table 3: IC_{50} of DPPH radical by hydroethanolic extract, its fractions and of trolox.

Samples	IC_{50} (mg/l)
HEE	5.33 ± 0.04
EAF	11.19 ± 0.28
MF	5.49 ± 0.14
AF	>50
Trolox	3.86 ± 0.04

The IC_{50} of the various extracts and the reference (Trolox) are mentioned in Table 4.

Condensed tannin, total phenols contents and antiradical activity after tannin precipitation.

Condensed tannins and total phenols contents

The total phenols content and residual condensed tannins after precipitation of the tannins are presented in Figure 6.

DPPH test

After tannins precipitation, the determination of antiradical activity from the supernatant of the extracts tested gave the IC_{50} mentioned in Table 5 according to the tests used.

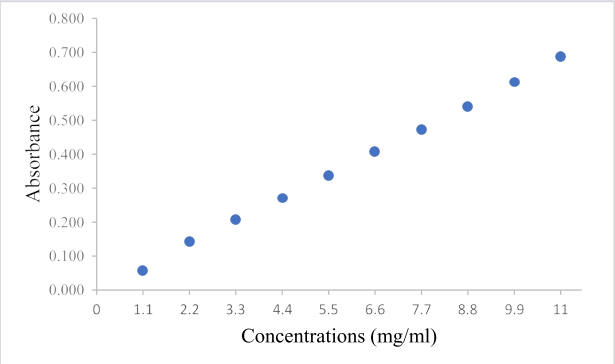

Figure 1: Calibration line made with gallic acid.

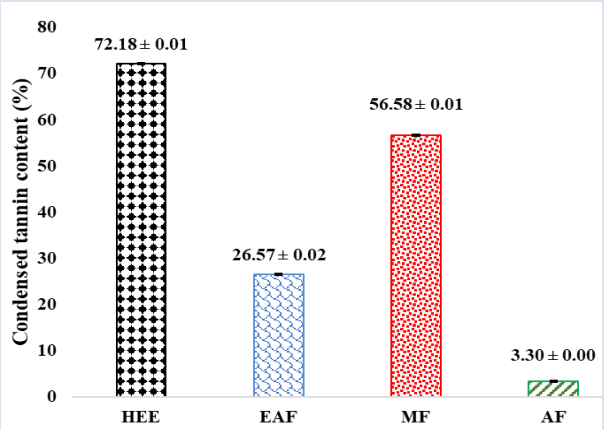

Figure 2: Condensed tannins contents of hydroethanolic extract and its fractions.

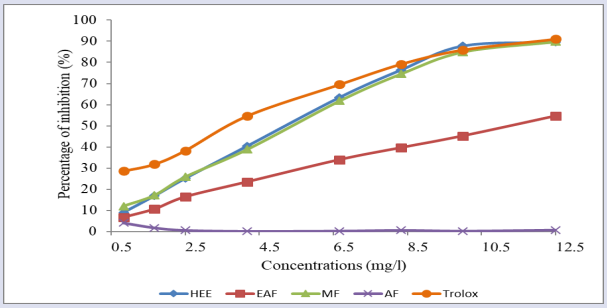

Figure 3: Percentage of inhibition of DPPH radical by hydroethanolic extract, its fractions and of Trolox.

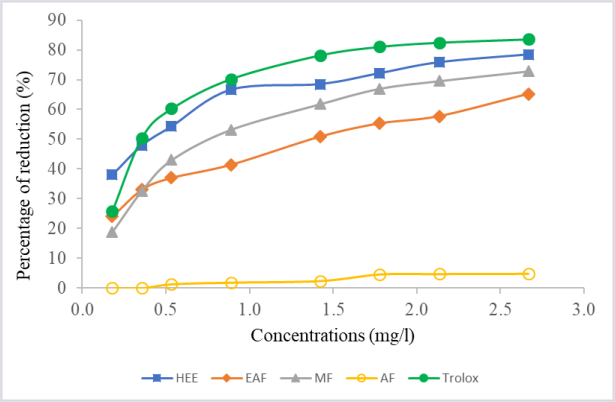

Figure 4: Percentage of reduction of CUPRAC reagent by Trolox, hydroethanolic extract and its fractions.

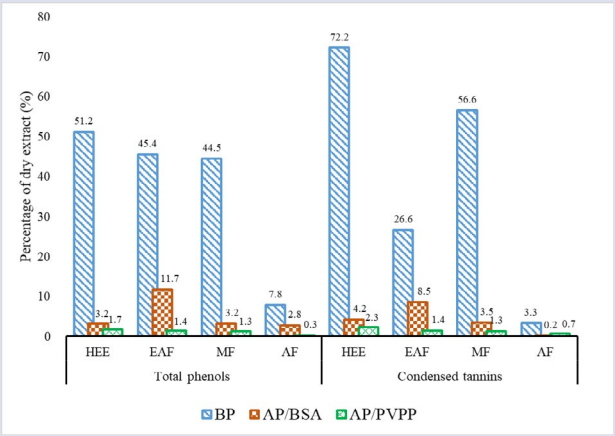

Figure 5: Total phenols and condensed tannins contents before and after precipitation assays by BSA and PVPP tests.

BP: Before precipitation; AP: After precipitation

Table 4: IC_{50} of Trolox, of hydroethanolic extract and its fractions by CUPRAC test.

Samples	IC_{50} (mg/l)
HEE	0.46 ± 0.05
EAF	1.35 ± 0.11
MF	0.77 ± 0.01
AF	>25
Trolox	0.37 ± 0.01

Table 5: IC_{50} (mg/ml) of extracts by DPPH test after précipitation assays by BSA and PVPP tests.

	Inhibitory concentrations 50% (IC_{50}, in mg/l)		
Sample	BP	AP by BSA test	AP by PVPP test
HEE	5.33 ± 0.04	78.86 ± 0.92	> 500
AEF	11.19 ± 0.28	26.69 ± 0.48	489.07 ± 23.30
MF	5.49 ± 0.14	88.74 ± 1.99	> 500
AF	> 50	> 100	> 500

BP: Before precipitation; AP: After precipitation

DISCUSSION

Extraction is considered to be the fundamental step in the process of analysis of plant materials. The type of extraction and the choice of solvents are determining according to the objective of the analysis and the nature of the compounds to be extracted.[31] For this study, extraction was carried out in order to extract the maximum of compounds, mainly polar compounds such as polyphenols, to assess their impact

on extract activity. A given compound has a good affinity for solvents of similar polarity and the solvation step during its extraction is also facilitated. However ethanol even being a polar solvent can extract both hydrophilic compounds and some apolar compounds.[32] Moreover, it was discovered that polymers insoluble in ethanol and water could be dissolved in water-ethanol mixtures.[33] In Senegal, barks are often used as a aqueous extract after maceration. Thus, the water-ethanol solvent system has been used to be closer to the traditional conditions of use of bark and for a best optimization of the extraction. This choice led us to obtain a hydroethanol extract rich in total phenols with 51.19 mg ± 0,8 GAE/g of dry extract. Compared to other plants known for their antioxidant activity, the plant's hydroethanol extract is richer in phenols than the hydro-acetonic extract from *Pterocarpus erinaceus* bark, *Fabaceae* with respectively 51.19 ± 0.79 *versus* 40.8 ± 1.75 mg GAE/g extracted.[34] On the other hand, the extract is 6 times less rich in phenols than the hydroethanol extract from *Cassia sieberiana* barks, *Caesalpiniaceae* with 345.04±3.16 *versus* 51.19 ± 0.79 AGE/g extract respectively.[35]

Total phenol content of a sample is often correlated with its total antioxidant capacity.[36] However, the Folin-Ciacolteu reagent is not specific to phenolic compounds. Many non-phenolic compounds such as ascorbic acid, saccharides, ferrous iron can also react with reagent.[37] It was then important to split the hydroethanolic extract with different solvents. The solid-liquid fractionation (SLF) method on a column method is a very precise purification method that can separate the compounds unitary. Thus the ESL technique on silica gel column was used to purify and fractionate the extract. This is a technique whose separation is based on interactions between analytes and silica.[31] The compounds of the extract, which are mainly polar, tend to be retained by silica. They had to be elected with solvents of increasing polarity: ethyl acetate, methanol and water.

Ethyl acetate and methanol fractions gave statistically similar total phenols contents of 45.45 ± 0.46 and 44.49 ± 0.32 mg GAE/g of extract respectively. There are therefore as many polyphenols in these two fractions. The nature of the phenols in hydro-ethanol extract is therefore particularly broad. The ethyl acetate fraction should be richer in more lipophilic phenolic compounds, despite their polar character, than the methanol fraction.

The nature of bark polyphenols is mainly condensed tannins (72.18%) and more than half of these tannins are concentrated in the methanol fraction (56.58%). The hydroethanolic extract and the methanol fraction have very high antiradical activities similar because statistically having the same activity (with a low IC_{50} : 5.33 ± 0.04 *vs* 5.49 ± 0.14 mg/l; p = 0.452). These results suggest that fractionation has no effect on the antiradical capacity of the extract since the FM fraction generally retains an identical IC_{50}. Therefore, the compounds capable of trapping the DPPH radical found in the hydroethanol extract are likely also present in the FM fraction, and weakly in the FAE fraction.

Moreover, their reducing powers by the CUPRAC test are quite close (0.46 ± 0.05 vs. 0.77 ± 0.01 mg/l; p = 0.003) respectively. The crude extract and its methanol fraction show a close antioxidant activity (antiradical and reductive). This is explained by the fact that the methanol fraction accounts for 81% of the crude extract. The compounds found mainly in the methanol fraction, are strongly involved in the antioxidant activity of the crude extract. Thus, the correlation test carried out showed that there is a strong correlation between condensed tannin content and antiradical activity and the reducing activity of the crude extract and its fractions with respectively coefficients of correlation r = 0.99 and r = 0.98.

The antioxidant activity of the plant barks would be strongly linked to the presence of condensed tannins, as has already been demonstrated for wine[36] and maritime pine bark marketed under the name Pycnogenol.[38] The latter had shown a strong correlation (r = 0.90) between the proanthocyanidin content of the barks of *Pinus densiflora, Pinaceae*, and antiradical activity. Otherwise, the proanthocyanidins of maritime pine are polymers of flavan-3-ol type with the majority monomer (+)-catechin.[38] The influence of condensed tannins is supported by the results observed during the evaluation of the anti-oxidant activity of extracts after precipitation and elimination of tannins (Table 5). In fact, the antiradical activity of supernatant obtained after precipitation of tannins from the ethyl acetate and methanol fractions is very low, whereas it was very important before. Condensed tannins are effective protein precipitation agents. The complex power of the latter was assessed by both tests: PVPP and BSA tests. PVPP is a synthetic polymer that precipitates condensed tannins better than BSA (Figure 6). Tannins being polyphenolic polymers, form insoluble complexes with proteins depending on the pH of the medium. With BSA, the precipitation of tannins is optimal at pH 4.9 with hydrophobic bonds.[39] On the other hand, with PVPP, the precipitation of tannins is not too affected by the pH of the reaction medium which can vary between 3 and 7.[40] Besides, PVPP has a strong affinity for polyphenols, in particular, flavane derivatives as flavan-3-ol mono and dimers such as catechin and procyanidine B3 and propheldinine B3.[41-43] However, our extracts were composed mainly of condensed tannins which are polymers of flavan3ols units. Thus these two points, namely the pH and affinity with flavan3ols derivatives, would explain why PVPP precipitates the tannins of extracts better than the BSA. Similar results were found by Muetzel and Becker[39] who had demonstrated that PVPP precipitated more than 77% of total phenols from hydro-acetone extract from the leaves of three plants against 24% for BSA.

Tannins play an important role in human health because they have interesting therapeutic activities such as their astringent, anti-inflammatory[44,45] and antioxidant properties as evidenced by our results with the DPPH and CUPRAC tests.

CONCLUSION

The barks of *Piliostigma reticulatum* are very rich in polyphenols made up mostly of condensed tannins. The latter would be responsible for their strong antioxidant activity. It would thus be interesting to evaluate the biological activity of the plant's barks as anti-inflammatory, antimicrobial activities. It would also be important to study the nature of tannins for better therapeutic use for the manufacture of traditional medecines improved (TMI).

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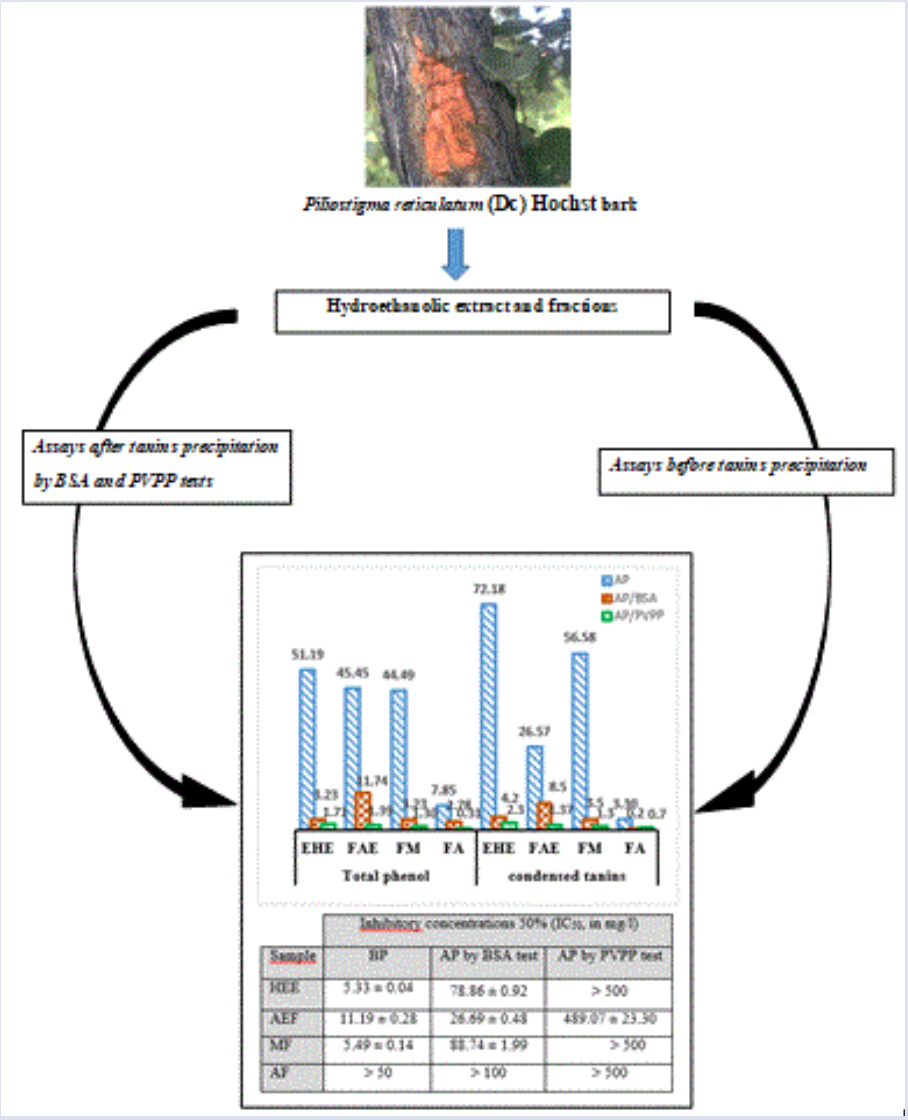
GRAPHICAL ABSTRACT



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