

Phytochemical Screening, Total Phenolic and Antioxidant Activity of Numerous Extracts from Leaves and Bark of *Bauhinia rufescens* Lam

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Abstract

Researches for natural antioxidants that play an important role in the prevention of diseases linked to free radicals have increased in recent years. In this study, antioxidant activity of *Bauhinia rufescens* Lam which is a medicinal plant was investigated. The methanol extract, acetone extract and water extract of leaf and trunk bark had antioxidant activity. Three methods were used: 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric ion reducing power (FRAP) by spectrophotometry. Phytochemical screening was carried out followed by the quantification of the total phenolic by the Folin-Ciocalteu method. The analysis of variance (ANOVA) with the STATISTICA 7.1 software revealed significant differences ($p < 0.05$). Percentage inhibition (PI) and IC_{50} of the extracts were obtained using the OriginPro 8.5 software. These tests show that the bark is as rich in total phenolic as the leaves. The DPPH test shows that at 2.5 mg/ml, the bark and the leaf have similar activity. Bark has a percentage inhibition (PI) of $86.55\% \pm 0.026\%$ compared to the leaf which PI is $85.63\% \pm 0.02\%$. As for the ABTS test, the bark extract reached its maximum activity at 2.5 mg/ml with a PI of $99.81\% \pm 0.012\%$ compared to the leaf extract which has a PI of $99.61\% \pm 0.025\%$. The best IC_{50} of the extracts obtained with the DPPH radical is 0.139 ± 0.001 mg/ml (hydro-acetonic), 0.354 ± 0.001 mg/ml (hydro-methanolic) and 0.840 ± 0.001 mg/ml (aqueous) on the bark. With ABTS test, it was obtained 0.351 ± 0.001 mg/ml (hydro-acetone; bark), 0.403 ± 0.001 mg/ml (hydro-acetone; leaves) and 0.474 ± 0.001 mg/ml (aqueous; bark). The reducing power of the leaves is slightly higher than that of the bark. Standard of ascorbic acid, has a PI of $94.86 \pm 0.008\%$ with an IC_{50} of 0.213 ± 0.001 mg/ml. Thus, it is easy to conclude that the bark of *Bauhinia rufescens* has a better activity than the leaves and the alcoholic extracts have given better re-

sults than the aqueous extract.

Keywords

Bauhinia rufescens Lam, Total Phenolic, Antioxidant Activity

1. Introduction

The use of plants by humans is very old, to fight against diseases linked to free radicals such as asthma, cancer and diabetes and degenerative diseases [1] [2]. Plants, especially medicinal herbs, have been used for the prevention and/or treatment of several diseases since very old times [3]. The use of herbal medicine as a primary source for healthcare is the highest in the developing countries, which constitute about 80% of the world population [4]. It constitutes a medicine and a traditional pharmacopoeia. Among all the chemical constituents of plants, phenolic compounds occupy an important place with several properties such as antioxidant activity. The high cost of medicines, the inaccessibility of health care and the effectiveness and availability of medicinal plants are pushing populations towards herbal medicine [5]. In addition, many diseases including diabetes are treated satisfactorily and at a lower cost by medicinal plants thanks to the active substances they contain [6]. Antioxidant is a family of substances neutralizing the free radical and thus preventing the occurrence of diseases associated with oxidative stress [7]. These radicals are involved in oxidative stress through the antioxidant defense mechanism [8]. The best-known natural antioxidant is ascorbic acid, phenolic compounds and atocopherol [9]. Traditional herbal remedies and a balanced diet were the main sources of antioxidant that can fight free radical damage [10]. In this context, we are interested to assess an antioxidant activity of the hydro-methanolic, hydro-acetonic and aqueous extracts of the leaves and bark of *Bauhinia rufescens* Lam due to their known properties, their accessibility for medical treatments for certain diseases such as diabetes [11]. The active peptides isolated from the seeds of *Bauhinia rufescens* are used against toxicities induced by snake venom [12]. The plant was used in traditional medicine in order to control gastro-intestinal worms because of resistance developed by helminths with regard to synthetic anthelmintics available and their high cost [13]. It is a plant belonging to the Saharan savan that is used in the treatment of pathologies involving oxidative stress such as diabetes [14]. According to that, investigations are conducted to assess their antioxidant activity. This has been achieved in order to complete information and to project possible applications.

2. Materials and Methods

2.1. Collection and Identification of Plant

Leaves and bark of *Bauhinia rufescens* Lam were collected in May 2019, the flo-

wering period, at Diourbel region (Senegal). The plant was identified and authenticated in botany-biodiversité laboratory, plant biology department of Cheikh Anta Diop University in Dakar. Plant leaves and bark were washed with distilled water and air-dried at room temperature in the laboratory. Dried leaves and bark were ground to a fine powder using an electric grinder (Kenwood, France). The sifted powder with 1 mm mesh was stored at 4°C in airtight jars. Thus, the powders are then subjected to two extraction methods: by infusion and by Soxhlet under reflux.

2.2. Extraction

Infusion was made in triplicate with boiling distilled water. The ratios were 10 g (± 0.01 g) of powder in 100 mL of solvent. After cooling, the infused product was filtered under vacuum and then stored in a sterile glass bottle tightly closed at 4°C.

Extraction under reflux

The extraction was carried out with methanol (70% v/v), (99.98%, Scharlau Chemie SA, SPAIN) and acetone (70% v/v), (99.5%, Scharlab SL, SPAIN) on an out-let of 10 g (± 0.01 g) of vegetable powder brought into contact with the solvent for two hours. After cooling, the mixture was separated using a centrifuge (Hittich, Universal 16 A, France) at 3000 rpm for 10 minutes then vacuum filtered on Wattman No.1 paper in order to obtain on the one hand the cake and on the other hand the filtrate consisting of the extract and the solvent. In the extracts, traces of solvent were removed using a rotary evaporator (IKA, RV10-German).

2.3. Qualitative Phytochemical Screening

Standard phytochemical analysis methods were used to test for the presence of phytoconstituents in the extracts. Chemical tests were carried out on hydro-acetone, hydro-methanolic and aqueous extracts using standard methods for the detection of tannins (Stiasny test followed by ferric chloride test), flavonoids (Shibata's test) [15], steroids and triterpenoids (Liebermann-Buchard test) [16] alkaloids (Valser-Mayer and Dragendorff's reagents tests), saponins (foaming index) [17].

2.4. Determination of Total Phenolic Content

Total phenolic content (TPC) of the sample was estimated by the Folin-Ciocalteu reagent colorimetric method as described elsewhere slightly modified [18]. In brief, 50 μ L of sample diluted to 450 μ L with distilled water and standard was added to 2500 μ L of ten times diluted Folin-Ciocalteu reagent. Then, after adding 2.5 mL of 0.75% sodium carbonate, (all from Sigma-Aldrich Chemie, Germany), the mixtures were vortexed and incubated at 50°C in a water bath for 15 minutes. The samples prepared above were kept the samples in the dark at room temperature for 30 minutes. Thereafter, the absorbance of each sample was read at 760 nm against the blank. Gallic acid was used for calibration

of a standard curve. TPC was determined as gallic acid equivalents (GAE) and values were expressed as mg GAE/g dry matter (DM) of plant extract.

2.5. Evaluation of Antioxidant Activity

Three methods were used for the purpose: 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2'-azino-bis-acid-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric ion reducing power (FRAP).

2.5.1. DPPH Radical Scavenging Assay

The antioxidant activity was evaluated with DPPH (2, 2-diphenyl-1-picrylhydrazyl, Sigma, chemical company, USA) by making some modifications to the method of Oliveira *and al.* [19]. The method is based on the ability of an extract to donate a singular electron to the radical free DPPH of dark purple color to stabilize it in DPPH of yellow-green color (Figure 1). Thus, 100 µl of extracts were introduced into a test tube containing 1300 µl of DPPH (0.004% prepared in methanol). The negative control is prepared by mixing 100 µl of methanol with 1300 µl of the methanolic solution of DPPH. The positive control is represented by a solution of a standard antioxidant, ascorbic acid (AnalaR NORMAPUR, Leuven, Belgium) tested at different concentrations (0.039, 0.078, 0.156, 0.313, 0.625, 1.2, and 2.5 mg/ml) the absorbance of which was measured under the same conditions as the samples and for each concentration. The samples were kept in the dark for 15 minutes at room temperature and absorbance was measured at 517 nm on a UV/visible light spectrophotometer (Spectronic Genesys 8, Rochester, USA). All determinations were carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$I(\%) = 100 \left(\frac{A_{co} - A_{ex}}{A_{co}} \right) \quad (1)$$

$$I(\%) = 100((A_{co} - A_{ex})/A_{co}).$$

$I(\%)$: Antioxidant activity as a percentage of inhibition of DPPH.

A_{co} : absorbance of blank sample ($t = 0$ min).

A_{Ex} : absorbance of tested extract solution ($t = 15$ min).

2.5.2. ABTS Radical Cation Decolourisation Assay

The anti-free radical activity was also evaluated by ABTS, 2,2'-azinobis(3ethyl-benzothiazoline-6-sul-fonic acid)diammonium salt (Sigma-Aldrich Chemie,

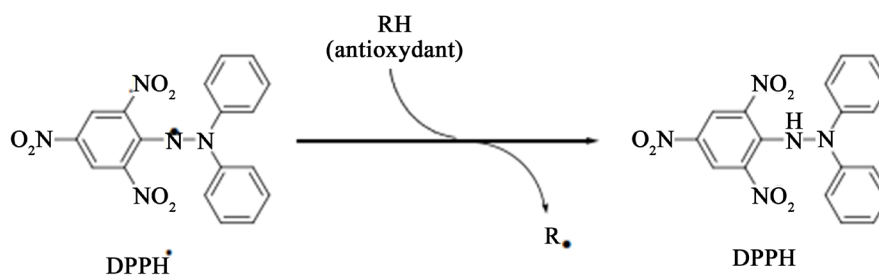


Figure 1. DPPH formation reaction from DPPH Radical and an antioxidant.

Steinheim, Germany) radical cation decolourisation test using spectrophotometric method [20]. ABTS was dissolved in distilled water to a concentration of 7 nM. The solution of the ABTS cation radical was obtained by incubating for 12 to 16 h in the dark and at room temperature a mixture of equal volumes of the stock ABTS solution with a solution of potassium persulfate (99%, Scharlab SL, Spain) at 2.45 nM (Figure 2). ABTS cation solution was diluted with ethanol to an absorbance of 0.700 ± 0.02 at 734 nm before use. Then 1500 μl of ABTS cation solution were mixed with 50 μl of extract solution or reference (ascorbic acid) at different concentrations (same of the DPPH). The samples were kept in the dark for 15 minutes at room temperature and absorption was measured at 734 nm on a spectrophotometer. All determinations were carried out in triplicate. Results were expressed as percentage inhibition (PI) as previously described for DPPH test.

2.5.3. Ferric Reducing Antioxidant Power assay (FRAP)

The ferric reducing power was determined according to the described method [21]. An aliquot of 0.4 ml of each sample at different concentrations (0.039, 0.078, 0.156, 0.313, 0.625, 1.2 and 2.5 mg/ml) is mixed with 1 ml of phosphate buffered saline (0.2 M; pH 6.6; Scharlab S.L, Spain) and 1 ml of 1% potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$; 99.8%, AppliChem GmbH, Germany). The mixture was incubated at 50°C for 30 min and 1 ml of 10% trichloroacetic acid (99.5%, Scharlab S.L, Spain) was added. After shaken for 5 minutes, 1ml of the mixture was mixed with 0.2 ml of ferric chloride (0.1%) and left to stand in the dark for 30 minutes.

Absorbance was measured at 700 nm; ascorbic acid was used as positive control. All determinations were carried out in triplicate. The antioxidant activity linked to the ferric reducing power of the extracts is expressed in reducing power (RP) using the following formula:

$$RP(\%) = 100 \left(\frac{A_{Ex} - A_{Co}}{A_{Ex}} \right) \quad (2)$$

A_{Ex} : absorbance of tested extract solution.

A_{Co} : absorbance of blank sample ($t = 0$ min).

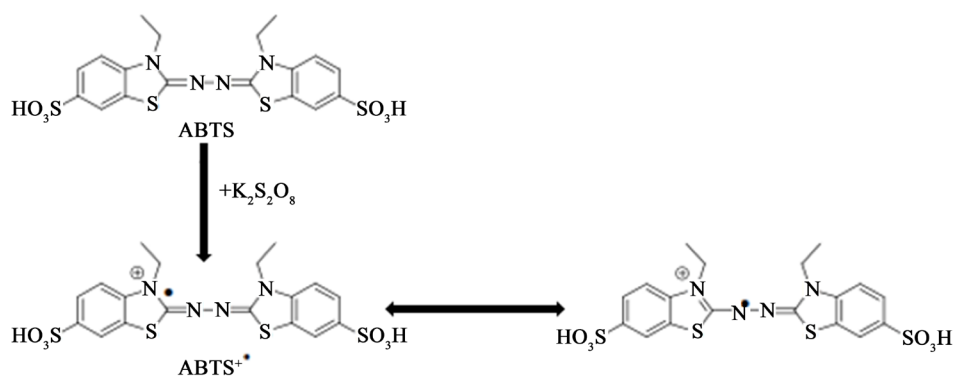


Figure 2. ABTS^{•+} Radical formation from ABTS.

2.6. Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) with STATISTICA 7.1 to determine the significance. Means and standard errors (SE) of all data were calculated based on three replicates ($n = 3$). Comparisons among means were carried out using Tukey's HSD test at a significance level of $P < 0.05$. The IC₅₀ was obtained using the Origine Pro 8.5 software.

3. Results and Discussion

3.1. Phytochemical Screening

The phytochemical screening of the various extracts revealed a significant presence of tannins, flavonoids, sterols and traces of saponosides but there is a low alkaloid content in the leaves and bark of *Bauhinia rufescens* Lam. These secondary metabolites were known to show medicinal activities as well as exhibiting physiological activities [22]. The presence of these phytochemical compounds in the plant materials suggests the potential medicinal value of their extracts in the prevention and/or cure of specific diseases [23]. The presence of these metabolites is revealed in the ethanolic extracts bark with the presence of quinones [13] and also in methanol stem bark extract of *Bauhinia rufescens* Lam [24] [25]. These metabolites are also revealed in *Sclerocarya birrea* [26] *Sebastiania chamaelea* (L.) [27] and *Emilia sonchifolia* (L.) [28]. These compounds are frequently encountered in plant extracts such as *Ziziphus mauritiana* Lam, *Bauhinia purpurea* L. and *Bauhinia variegata* L. [29] [30]. These metabolites, particularly phenols and flavonoids, are the main and most common components of plants with antioxidant activity [31].

3.2. Total Phenolic Content

Total phenolics constituted one of the major groups of compounds acting as primary antioxidants, it was reasonable to determine their total amount in leaves and bark extracts [16]. Gallic acid dilution series at different concentration (0.02 - 0.04 - 0.06 - 0.08 - 0.1 - 0.12 - 0.14 mg/ml) was used for calibration curve in the same way as the extracts. The content of phenolic compounds (mg GAE/g DM) in all extracts, determined from regression equation of calibration curve ($y = 3.120x + 0.069$, $R^2 = 0.98$) and expressed in gallic acid equivalents (GAE). The results showed that the total phenolic contents vary significantly between the extracts of the different plant parts studied as well as the extraction solvents (Figure 3). It showed that the alcoholic bark extracts have higher phenolic contents than the leaves on all the extracts with respective values of 46.75 ± 0.002 mg GAE/g.MS (Hydromethanol), 38.31 ± 0.002 mg GAE/g MS (hydro-acetone) and 12.64 ± 0.002 mg GAE /g MS (aqueous). On the other hand, the infused extract of the leaves had the lowest phenolic content with a value of 8.67 ± 0.003 mg EAG/g DM. As a result, the hydro-alcoholic extracts are richer in polyphenols compared to the aqueous extract. Our results were in line with those of Aliyu *and al.*, found in the methanolic extract of the leaves of *Bauhinia rufescens* Lam

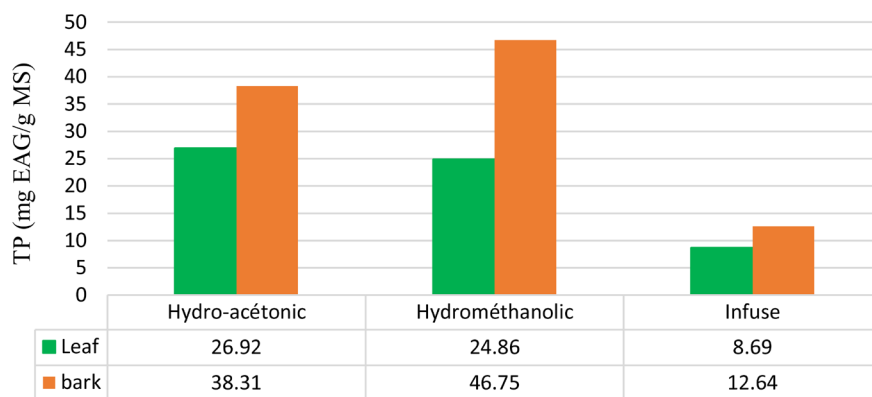


Figure 3. Total phenolic (PT) content in leaf and bark extracts.

(68.40 ± 0.003 mg EAG/g) [32]. The significant presence of total phenolic in bark was found in hydro-ethanolic extracts from the leaves and bark of *Piliostigma thonningii* Schumach [21] and in the same extract of *Sclerocarya birrea* [26]. This disparity of polyphenols in different organs of the same plant was a phenomenon reported by several authors, on several Saharan medicinal plants such as *Anvillea radiata* Coss. & Dur and *Rumex vesicarius* L. [33].

3.3. Antioxidant Activity

The results of the DPPH and ABTS radical tests were obtained by following the evolution of the percentage inhibition as a function of the variation in the concentration of the extracts.

3.3.1. DPPH Radical Scavenging Assay

The inhibition percentages showed that at all the concentrations tested, our extracts inhibit the DPPH radical in a dose-dependent manner as in the case of hydro-ethanolic extracts from the leaves and bark of *Piliostigma thonningii* Schumach [21]. In all the extracts tested, the bark exhibits greater activity than the leaves. Indeed, at a concentration of 2.5 mg/ml, the bark extracts have a percentage inhibition of $86.55\% \pm 0.026\%$, $85.12\% \pm 0.025\%$ and $84.48\% \pm 0.020\%$ compared to the leaves of which the PI are $85.63\% \pm 0.02\%$, $82.3\% \pm 0.03\%$ and $79.52\% \pm 0.016\%$ (hydro-acetonic, hydromethanolic and aqueous extracts, respectively). Ascorbic acid inhibited the DPPH radical of $94.86\% \pm 0.01\%$ at 1.25 mg/ml then its activity leveled up to 2.5 mg/ml (Figure 4). The percentages of inhibitions obtained are greater than those presented in the acetone extract (71.76% at 0.825 mg/ml) of *Foeniculum vulgare* [34]. Thus, in order to better compare the free radical activity of the various plant extracts, the IC_{50} was determined. At all solvents, bark extract showed the best IC_{50} than leave extract with values of 0.139 mg/ml (hydromethanolic), 0.354 mg/ml (hydro-acetone) and 0.84 mg/ml (aqueous) versus 0.834 mg/ml, 0.834 mg/ml and 0.927 mg/ml and 0.998 mg/ml respectively. Ascorbic acid had an IC_{50} of 0.056 mg/ml. The hydro-alcoholic extracts of the bark showed the best results obtained for the PI and the IC_{50} by approaching the values obtained for the reference antioxidant.

3.3.2. ABTS Radical Cation Decolourisation Assay

From **Figure 5** it can be seen that regardless of the extraction solvent, the extracts from the leaves and bark significantly inhibited the ABTS cation radical. The inhibition percentage of the various extracts is close to ascorbic acid ($99.82\% \pm 0.014\%$ at 2.5 mg/ml). Indeed, at this concentration the bark extract reached its maximum activity with a PI of $99.81\% \pm 0.012\%$ (hydro-methanolic) compared to the leaf extracts which have a PI of $99.61\% \pm 0.025\%$ (Hydro-acetonic). At the highest concentration tested (2.5 mg/ml) and whatever the extraction solvent, the bark extract has the greatest PI with $99.81\% \pm 0.012\%$ on the ABTS radical and $86.55\% \pm 0.026\%$ on the DPPH radical against ascorbic acid $99.82\% \pm 0.014\%$ on the ABTS radical and $94.86\% \pm 0.012\%$ on the DPPH radical. Our results are better than those obtained in the methanolic extract of the leaves of *Salvia sclarea* ($92.9\% \pm 0.4\%$) and *Salvia glutinosa* ($91.5\% \pm 0.5\%$) [16]. The determination of the IC_{50} confirms a better activity of the bark compared to the leaves in the various extracts. In fact, the bark extracts exhibit IC_{50} of 0.472; 0.35 and 0.474 mg/ml versus 0.403; 0.566 and 0.937 mg/ml for the leaves (hydromethanolic, hydro-acetonic and aqueous respectively). At the same time,

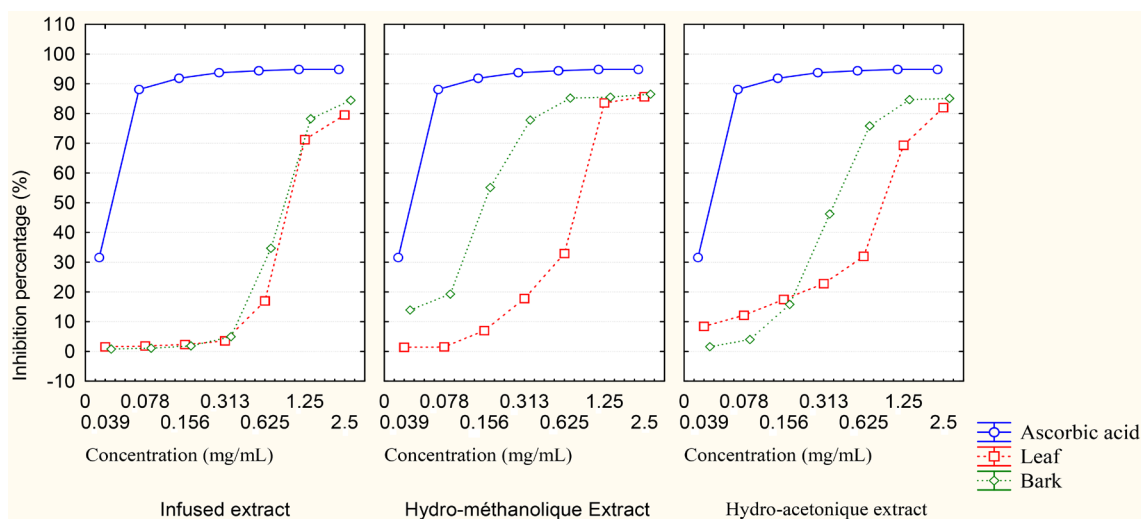


Figure 4. Inhibition percentage evolution on DPPH radical in leaf, bark and ascorbic acid.

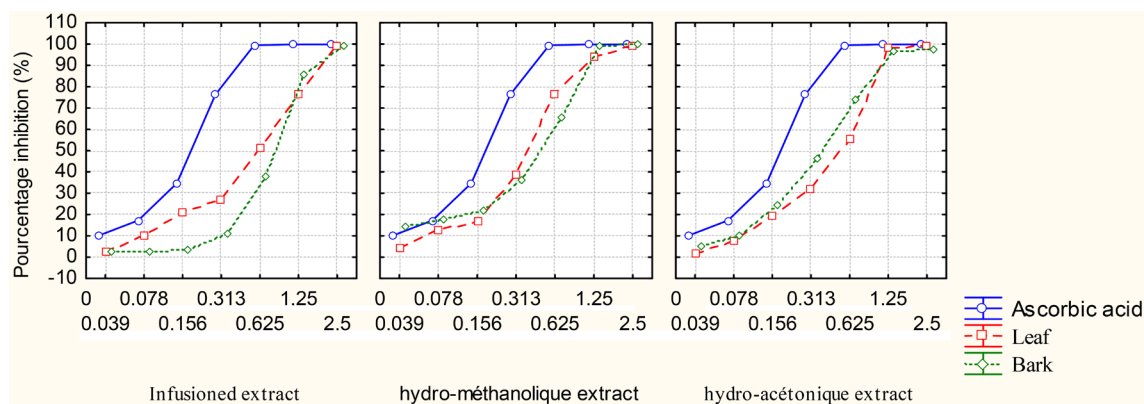


Figure 5. Inhibition percentage evolution on ABTS radical in leaf, bark and ascorbic acid.

ascorbic acid gives an IC_{50} of 0.217 mg/ml. By comparing the two methods, the PI and the IC_{50} show that the bark extracts are more active than the leaf extracts. However, ascorbic acid inhibits the ABTS radical ($99.81\% \pm 0.014\%$) more than the DPPH radical ($94.86\% \pm 0.008\%$). The result showed that ABTS had the highest activity when compared with all other parameters investigated. They were a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. A high proportion of antioxidants in our diet prevent the body from any oxidative damage in order to maintain a better healthy condition and also to slow down aging process. Numerous studies have shown that antioxidants have protective effects on health problems. It was reported that antioxidant prevents the occurrence of diseases, like cancer and diabetes, aging and that they can also interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavenger [35]. These results agree with those found on hydro-ethanolic extracts from the leaves and bark of *Piliostigma thonningii* Schumach [21].

3.3.3. FRAP Test

In analogy with the inhibitory power, the evolution of the reducing power as a function of the concentration of extracts (Figure 6) shows a strong activity of the plant. Indeed, the extracts significantly reduced the ferric ion. The hydro-methanolic, hydro-acetonic and aqueous extracts, at the maximum concentration tested (2.5 mg/ml) gave reducing powers (PR) of the leaves and bark respectively of $61.54\% \pm 0.01\%$ - $60.71\% \pm 0.01\%$ - $67.90\% \pm 0.015\%$ and $79.29\% \pm 0.004\%$ and $60.02\% \pm 0.026\%$ - $57.14\% \pm 0.02\%$ - $52.73\% \pm 0.025\%$. At the same concentration as the extracts, ascorbic acid has a strong reducing power on ferric ion with a value of $90.70\% \pm 0.02\%$. Our reduction values are close to that observed (66.67%) in the ethanolic extracts of leaves of *Boerhavia elegans* Choisy [36].

The results of phytochemical screening showed presence of secondary metabolites which were known to show medicinal activities. The results of total phenolic and antioxidant activity of *Bauhinia rufescens* Lam extracts on all three

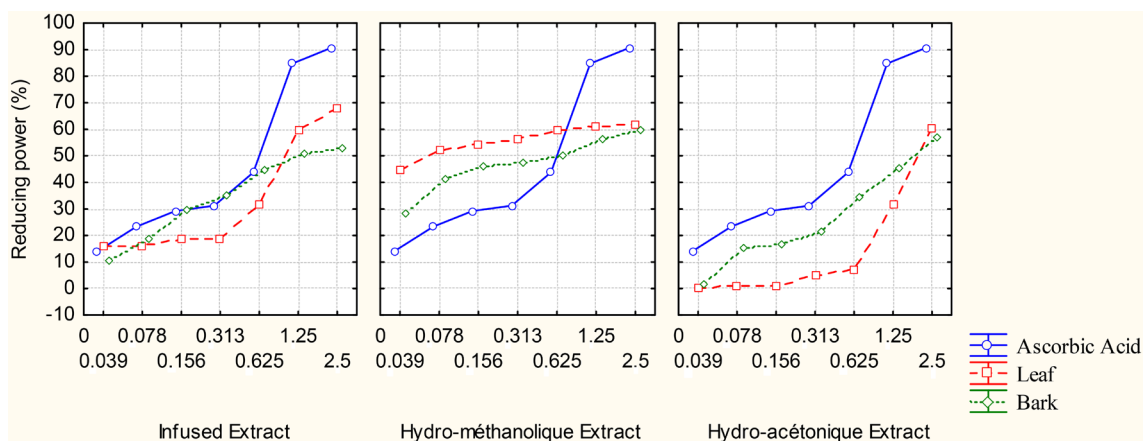


Figure 6. Reducing power evolution in leaf, bark extracts and ascorbic acid.

tests carried out show that the hydro-alcoholic extracts of the bark are more active. Indeed, they showed higher PI and lower IC₅₀ in the different extracts. Thus, by these 3 tests, we see that the antioxidant activity of the bark is stronger than that of the leaves. The result showed that ABTS had the highest activity when compared with all other parameters investigated. The ABTS was a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. In addition, the DPPH and the FRAP test confirmed the activity of the plant.

4. Conclusion

The present study has demonstrated the antioxidant activity of aqueous and hydro-alcoholic extracts of *Bauhinia rufescens* Lam by different methods. It shows that the hydro-alcoholic extracts of the bark have a strong antioxidant activity compared to the leaves and water. These results could constitute a solid scientific basis for the research of new natural molecular compounds, complementary to the existing chemical therapy. In fact, following the presence of secondary metabolites, the evaluation of the antioxidant activity made it possible to partially support their effect on hyperglycemia. In view of this work, biological tests with hydromethanolic and hydro-acetone extracts should provide further evidence of the hypoglycemic activity of *Bauhinia rufescens* Lam. A subsequent study with the aim of characterizing phenolic compounds, isolating and identifying molecules involved in antioxidant activity could provide a better understanding of the nature of the active ingredients.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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