

Phytochemical Screening and Assessment of the Antioxidant Activity of the Mucilage Contained in Baobab Leaves and Bark (*Adansonia Digitata L*) of Senegal

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Abstract

The present work involved determining the chemical composition and assessing the antioxidant activity of the mucilage contained in baobab leaves and bark. The mucilage from each organ was obtained separately in two beakers by extraction with ethanol and acetone. Phytochemical tests revealed the presence of various families of compounds such as alkaloids, flavonoids and tannins in the organic extracts. Antioxidant power was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH-) radical scavenging method, based on determination of the 50% inhibitory concentration (IC₅₀). This study shows that the solvent and the plant organ affect antioxidant activity. In fact, for ethanolic and acetic extracts of leaves, the IC₅₀ value varied respectively between 3.58 and 3.98 mg/mL then between 3.83 and 5.32 mg/mL; while for ethanolic and acetic extracts of bark, the values found varied respectively between 6.49 and 8.12 mg/mL then between 5.51 and 9.11 mg/mL. These results therefore show that organic leaf extracts have a greater DPPH- radical scavenging capacity than organic bark extracts. However, the most powerful antioxidant power is observed in the ethanolic leaf extracts, where the values found are relatively lower and remain below those of ascorbic acid, the reference reducing compound (IC₅₀ = 4.12 mg/mL). A statistical analysis showed that only the leaves had an influence on antioxidant activity, so baobab leaves could be used as a food additive.

Keywords: *Adansonia digitata* L.; Mucilage; Leaves; Bark; DPPH- radical; Antioxidant activity.

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1. Introduction

Many plants are used as remedies by the vast majority of rural populations, particularly in developing countries, because of their effectiveness, but also because conventional medicines are expensive and inaccessible. This is the case, for example, with baobab, which has long been used in traditional medicine, using all its parts: leaves, fruit, bark and roots.

Baobab tree (*Adansonia digitata*) is a deciduous massive, majestic tree up to 25m high which may live for hundreds of years. It has thick angular, wide spreading branches which attains 10-14cm or more in girth and often become deeply eluded. The fruits pulp probably is the most important food stuff. It is dried and used in cool and hot drinks. Pulp can be dissolved in water or milk and the liquid is used as a drink, as food, as fermentation agent in local brewing or as a substitute for cream or tartar in baking.

Energy value of pulp is similar to that of baobab leaves. The leaves of baobab tree are a stable food source for rural population. In many part of Africa, especially the central regions of the continent. They are eaten both fresh and as a dry powder. The leaves of *Adansonia digitata* are importance source of protein completing the amino acid profile aid improving the protein quality of the diet. Flowers can be eaten raw or used as flavor in drinks.

The seeds are characterized as a Potential source of protein and roasted seeds are used as coffee substitute in some areas. The widest use in tradition medicine comes from the baobab bark as a substitute for quinine in case of fever or as a prophylactic.

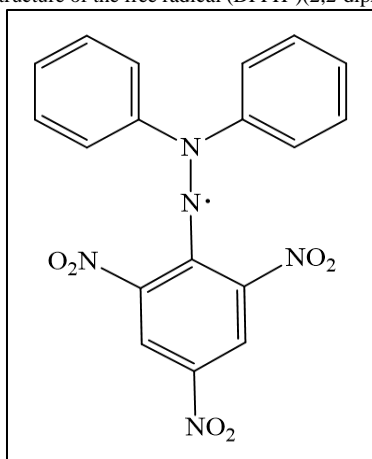
In Indian medicine, baobab bark is used internally as arefrigerant, antipyretic and antiperiodic [1]. More over, the bark contains a white, semi-fluid gum that can be obtained from bark wounds and is used for cleansing sores [2]. A Sufferers of malaria in Africa, India, SriLanka and west Indies are said to consume a mash containing dried baobab bark as a febrifuge in order to treat the fever associated with this illness [3, 4]. Fruit Pulp and seeds are widely used for anti-pyretic properties [2]. The antimicrobial activity of the stem bark of the baobab tree is as a result of some metabolites present in it which are tannin, Flovonoid, alkaloid, and steroids. The activity exhibited by extracts may be related to the presence of tannins in addition to Flovonoid that reported to be responsible for anti microbial properties of some ethno-medicinal plants [4]. these metabolites have been reported to posses antimicrobial activity. In particular the Flovonoid were reported to be responsible for antimicrobial activity associated with some ethnos medicinal plant [4].

This tree grows wild in Senegal and is a good source of mucilage. This substance is water-soluble, sticky and gummy. In plants, it acts as a membrane thickener and a food reserve. It is present in almost all parts of various categories of plants in relatively small proportions [5] and consists mainly of natural polymers. Natural polymers have been used in various pharmaceutical formulations. They are readily available, non-toxic, biodegradable and cost-effective for use as pharmaceutical excipients [6, 7]. In recent years, polymers derived from plants, such as mucilage's, have attracted a great deal of interest because of their various pharmaceutical applications, such as diluents, binders, disintegrates in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppositories. They are also used in cosmetics, textiles, paints and paper manufacture. These natural hydrocolloid gums and mucilage's are biocompatible, inexpensive and easy to obtain. They are preferred to semi-synthetic and synthetic excipients because of their low toxicity, low cost, easy availability, soothing action and non-irritating nature. Demand for these substances is growing and new sources are being developed [8]. The use of synthetic antioxidant molecules is currently being called into question because of the potential toxicological risks. New plant sources of natural antioxidants are now being sought [9, 10]. Polyphenols are natural compounds that are widespread in the plant kingdom and are becoming increasingly important, not least because of their beneficial effects on health [11]. Their role as natural antioxidants is attracting increasing interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [12]. They are also used as additives in the food, pharmaceutical and cosmetics industries [9].

The aim of this work is firstly to identify the chemical compounds present and then to evaluate the antioxidant activity of the mucilage obtained from ethanolic and acetonic extracts of baobab leaves and bark. Antioxidant power can be determined from the IC50 parameter. This parameter expresses the quantity of antioxidant required to reduce the concentration of the DPPH- radical by 50%. The lower the IC50 value, the greater the antioxidant activity of a compound [13].

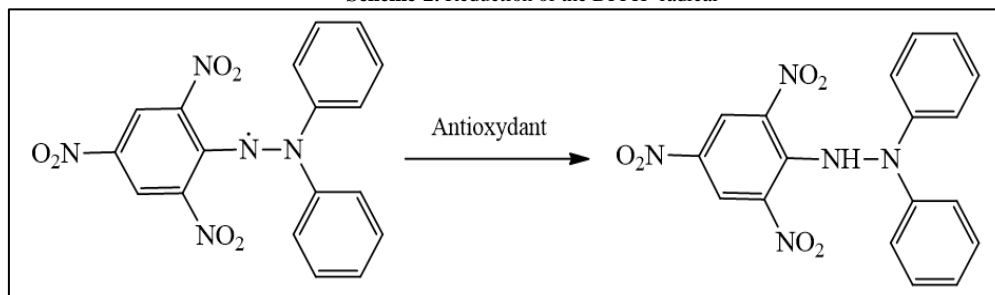
To carry out this work, we used the method of trapping the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH-). This purplish-coloured compound absorbs in the UV-visible at a wavelength of 517nm [14]. It was one of the first free radicals used to study the antioxidant activities of phenolic compounds [15, 16]. It has an unpaired electron on a nitrogen bridge atom (Scheme 1). Because of this delocalization, the radical molecules do not form dimers. The (DPPH-) radical remains in its relatively stable monomeric form at room temperature [17].

Scheme-1. Chemical structure of the free radical (DPPH-)(2,2-diphenyl-1-picrylhydrazyl).



In this test, the substrate is a stable radical which, on reacting with an antioxidant molecule, is transformed into 2,2-diphenyl-1-picrylhydrazine (DPPH-H) with a loss of its characteristic absorbance at 517 nm (Diagram 2). Reactions take place at room temperature and in an ethanolic medium, which allows good solubilization of most antioxidants [18]^[14]. This test is widely used because it is quick, easy and inexpensive [18].

Scheme-2. Reduction of the DPPH- radical



2. Experiences

2.1. Vegetable Material

To carry out this work, we used baobab leaves and bark from three different sites.

2.2. Solvents

Three organic solvents (acetone, ethanol and methanol) and tap water were used in this study. The organic solvents were all of spectroscopic quality and were supplied by Aldrich.

2.3. Equipment

In this work, we used a UV-visible absorption spectrophotometer (UNICAM UV-2), an electric grinder, an oven, a glass rod and a desiccator. Beakers, flasks, a mortar, a pestle, petri dishes and a muslin cloth were also used.

2.4. Preparation of Plant Extract Solutions and DPPH

A stock solution was prepared with 30 mg of extract in 2 ml of methanol to give a concentration of 15 mg/mL. Daughter solutions with concentrations of 7.5, 3.75, 1.875, 0.9375 and 0.4687 mg/mL were prepared by dilution.

To prepare the DPPH- radical, we dissolved 5.2 mg of DPPH in 200 ml of methanol (to give a concentration of 0.026 mg/mL). The resulting solution was then stirred magnetically in the dark for 1 hour. To characterize each type of compound contained in the extracts, we prepared a 1% solution of the extract by dissolving 0.4g of the extract in 20 mL of distilled water.

2.5. Experimental Procedures

2.5.1. Obtaining Leaf or Bark Powder

The harvested plant organs were carefully washed with tap water and then dried at room temperature for 15 days, protected from light, moisture and dust. After drying, they were ground separately using an electric grinder. The powder obtained was stored at room temperature in small bottles.

2.5.2. Extraction Method

A mass of 10 g of powder (leaves or bark) was added to 100 ml of water. The resulting mixture was filtered through a muslin cloth. The resulting filtrate was collected separately in two small beakers. Acetone and ethanol were added separately to these two beakers in a quantity corresponding to three times the volume of the total filtrate. The precipitated mucilage from the two beakers was removed by gently winding it with a glass rod and collected separately in two different Petri dishes. The mucilage was then dried by holding the dishes in an oven (at 50°C). The dried mucilage powder was scraped and ground using a mortar and pestle and weighed. The resulting powder was then stored in a desiccator [19]¹.

2.5.3. Phytochemical Characterization of Extracts

To characterize alkaloids, flavonoids, tannins, terpenoids, saponins and reducing compounds, we carried out tests using the method described by Trease and Evans [20].

2.5.3.1. Characterization of Alkaloids

The tests are carried out by precipitation reactions with Mayer and Wagner reagents. 1ml of each extract is divided equally between two test tubes, one containing 0.5ml of Mayer's reagent and the other 0.5ml of Wagner's reagent. The appearance of a white or brown precipitate, respectively, reveals the presence of alkaloids [21].

2.5.3.2. Characterization of Flavonoids

In a test tube, mix 1 ml of the extract to be tested with 1 ml of hydrochloric acid (HCl) and saturate with 3 magnesium chips. The appearance of a red or yellow color indicates the presence of flavonoids [21].

2.5.3.3. Characterization of Tannins

To 1 ml of extract, 0.5 ml of an aqueous solution of FeCl₃ (1%) is added. The presence of tannins is indicated by a greenish or bluish-black color [21].

2.5.3.4. Characterization of Terpenoids

5ml of extract was added to 2ml of chloroform and 3ml of concentrated sulfuric acid. The formation of two phases and a brown color at the interphase indicate the presence of terpenoids [21].

2.5.3.5. Characterization of Saponins

Saponins were characterized using the foam test. In a test tube, 10ml of the extract to be tested was shaken for a few seconds and then left to stand for 15min. An obstinate moss height (around 1cm) indicates the presence of saponins [21].

2.5.3.6. Characterization of Reducing Compounds

To 1ml of extract, add 2ml of Fehling's liqueur (1ml reagent A and 1ml reagent B) then boil the mixture in a water bath for 8 min. The appearance of a brick-red precipitate indicates the presence of reducing compounds [21].

2.5.4. Assessment of Antioxidant Activity by the DPPH- Test

The method described by Şenay Akkuş Çetinus was used. A solution of DPPH was prepared by solubilizing 9.8 mg DPPH in 100 ml methanol (ethanol for mucilage), different concentrations of sample and control solutions were added to 1ml of the DPPH solution. After incubation for 30 min in the dark at room temperature room temperature, absorbances were measured [22]. The results can be expressed as: percentage of anti-free radical activity or percentage of DPPH remaining or can also be expressed using the IC₅₀ parameter [23]. The antioxidant power is determined in such a way that a quantity of the extract with a well-defined concentration neutralizes 50% of the radical.

The results were calculated from curves showing the variation in the PI% inhibition percentage as a function of the concentration of each extract and then expressed as IC₅₀. It should be remembered that the lower the IC₅₀ value, the greater the antioxidant activity of the extracts [13]. The inhibition power was calculated by applying the following formula:

PI (%) = (Abs negative control - Abs sample)/(Abs negative control) x 100 where the negative control is methanol and DPPH.

The value of the IC₅₀ parameter is then determined graphically from the curve or by calculation from the equation of the straight line obtained.

3. Results and Discussion

3.1. Phytochemical Testing of Extracts

Using the method described by Trease and Evans, we characterized the various compounds contained in the ethanolic and acetonc extracts.

All the results are shown in Table 1.

Table-1. Characterization of the chemical groups contained in the extracts

Chemical groups	Leaves		Barks	
	Ethanolic extracts	Acetonc extracts	Ethanolic extracts	Acetonc extracts
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Saponins	-	-	-	-
Reducing compounds	+	+	+	+

Positive reaction: + Negative reaction: -

This table shows that the phytochemical tests carried out on the various ethanolic and acetonc extracts reveal the presence of several families of compounds in both the leaves and the barks. Alkaloids, flavonoids, tannins, terpenoids and reducing compounds were found in both the leaves and the bark. A total absence of saponins was noted in the various extracts. This result could be linked to the method used or the conditions under which the extraction was carried out.

3.2. Evaluation of the Antioxidant Activity of Mucilage Contained in Leaves

Figures 1 and 2 show the variation in the inhibition power of organic leaf extracts from the three sites studied. We obtained affine lines with correlation coefficients close to unity ($r^2 > 0.99$), indicate the good precision of the measures done.

Figure-1. Variation in inhibition power as a function of the concentration of ethanolic extracts of leaves from the various sites: (a) Boof; (b) Ngoyé; (c) Tanime and ascorbic acid (d)

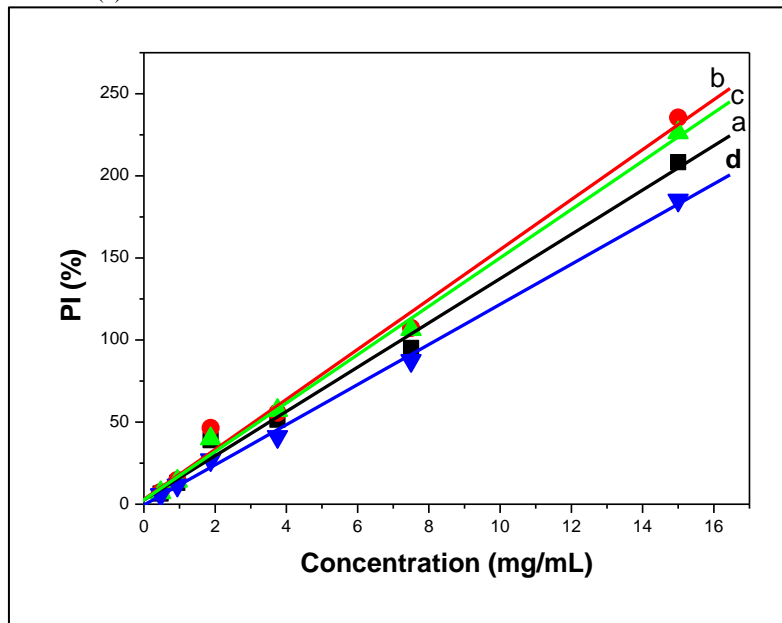
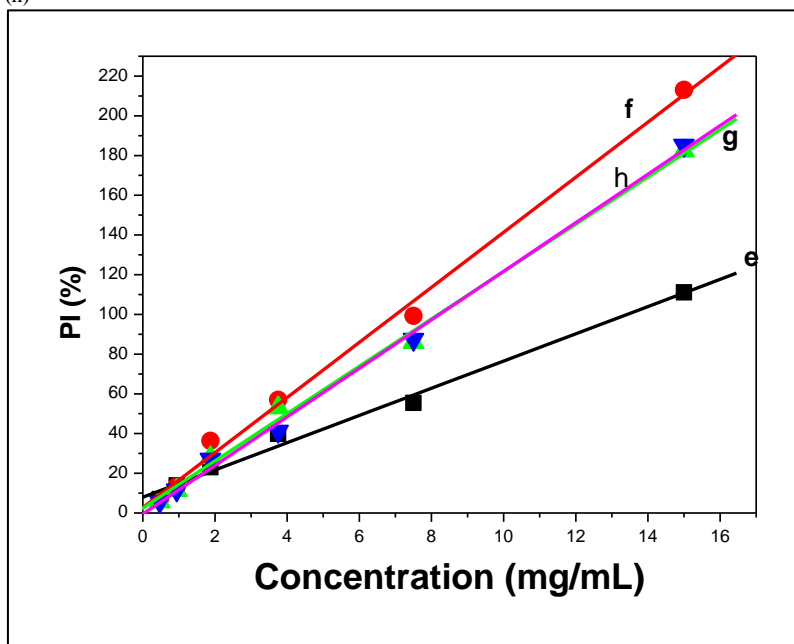


Figure-2. Variation in inhibition power as a function of the concentration of acetone extracts from leaves at various sites: (e) Boof; (f) Ngoyé; (g) Tanime and ascorbic acid (h)



The antioxidant activity of the different extracts is determined from the percentage inhibition curves. This activity is given in the form of IC₅₀ or 50% inhibition concentration.

All the results are shown in Table 2.

Table-2. Antioxidant activity (IC₅₀ mg/mL) of organic leaf extracts and ascorbic acid

Samples	IC ₅₀ (mg/mL)			r ²		
	Boof	Ngoyé	Tanime	Boof	Ngoyé	Tanime
Ethanol extracts from leaves	3.98 ^B	3.58 ^N	3.62 ^T	0.9960 ^B	0.9950 ^N	0.9977 ^T
Acetonic extracts from leaves	5.32 ^B	3.83 ^N	4.39 ^T	0.9950 ^B	0.9980 ^N	0.9980 ^T
Ascorbic acid	4.12			0.9987		

B : Boof site; N : Ngoyé site ; T : Tanime site

This table shows that the IC₅₀ value depends on the site studied and the solvent. We also note that the ethanolic and acetonic extracts of the leaves have a good capacity to trap the DPPH⁻ radical. In fact, the values found are close

to that of ascorbic acid (a bench mark reducing compound), which is 4.12 mg/mL, with the exception of the acetone extract from the Boof site ($IC_{50} = 5.32$ mg/mL). The relatively higher value obtained in this site could be due to a low recovery of anti-radical substances by acetone due to its low polarity compared to ethanol. As a result, the reduction of the DPPH- radical becomes less important and the IC_{50} value becomes high. We also noted a decrease in the IC_{50} value when switching from acetone extracts to ethanol extracts, indicating a greater capacity of ethanol extracts to reduce the DPPH- radical. This reduction capacity is determined by a decrease in absorbance induced by anti-radical substances [24]. Thus, ethanolic extracts of leaves may contain a greater quantity of anti-radical substances. This shows that ethanol solubilizes these substances to a greater extent than acetone. These compounds, which are thought to be highly antioxidant because they are able to release a proton from the hydroxyl group to neutralize the DPPH radical, are polar in nature. Ethanol, which is a polar solvent, will extract more polar compounds. The relatively lower value obtained in these ethanolic extracts indicates greater antioxidant activity than in the case of acetone extracts. In fact, the lower the IC_{50} , the higher the antioxidant activity of a compound [13].

3.3. Evaluation of the Antioxidant Activity of Mucilage Contained in Bark

For each plant extract, we first determined the percentage inhibition at a given concentration. Then, by plotting the variation in the percentage inhibition as a function of the concentration of each plant extract, we obtained affine lines (Figures 3 and 4) with a correlation coefficient of between 0.9978 and 0.9998.

Figure-3. Variation in inhibition potency as a function of concentration of ethanolic extracts of bark from various sites: (a') Boof; (b') Ngoyé; (c') Tanime and ascorbic acid (d')

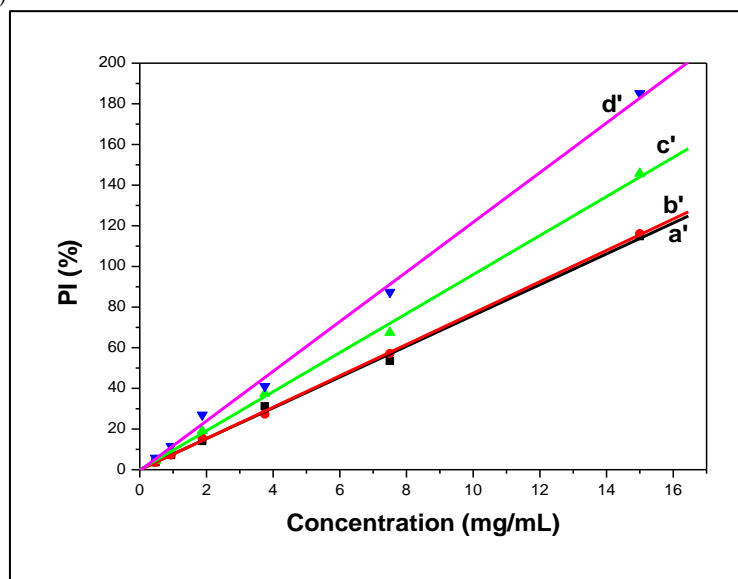
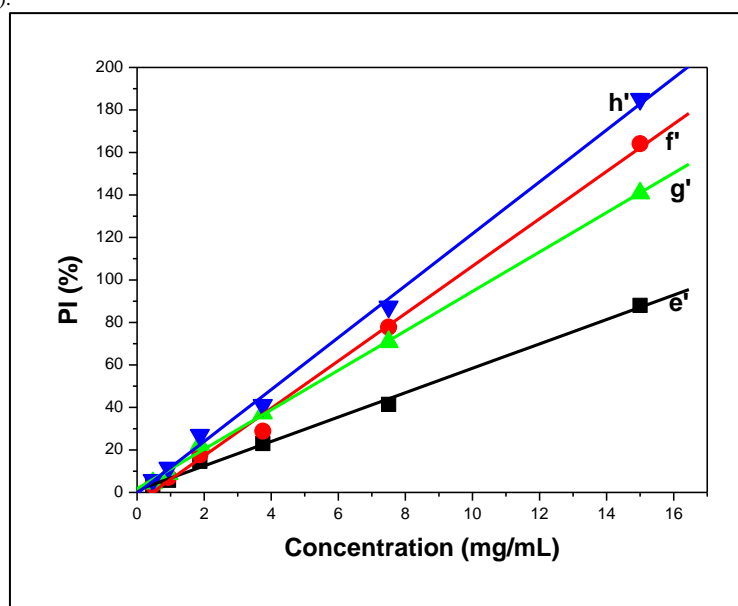


Figure-4. Variation in inhibition power as a function of concentration of acetone extracts of bark from various sites: (e') Boof; (f') Ngoyé; (g') Tanime and ascorbic acid (h').



From these lines, we determined the corresponding IC_{50} values. The results are shown in Table 3.

Table-3. Antioxidant activity (IC₅₀ mg/mL) of organic bark extracts and ascorbic acid

Samples	IC ₅₀ (mg/mL)			r ²		
Ethanol extracts from bark	6.58 ^B	6.49 ^N	8.12 ^T	0.9990 ^B	0.9998 ^N	0.9991 ^T
Acetonic extracts from bark	9.11 ^B	5.51 ^N	5.66 ^T	0.9983 ^B	0.9978 ^N	0.9993 ^T
Ascorbic acid	4.12			0.9987		

B : Boof site; N : Ngoyé site; T : Tanime site

As in the previous case, this table shows that the IC₅₀ value varies according to the harvesting site and the solvent used (the environmental factors at the site that can influence the nature of the chemical compounds are the mineral composition of the soil, but also the humidity, etc.). As in the previous case, this table shows that the IC₅₀ value varies according to the site and the solvent used. In fact, the lowest values for the 2 solvents were obtained at the Ngohé site (5.51 for acetonic extracts and 6.49 for ethanolic extracts). These low values obtained at this site could be due to the greater presence of free radical scavengers in these organic extracts.

On the other hand, at the Boof and Tanime sites, where IC₅₀ values are relatively higher, this increase could be due to the lower presence of free radical scavengers. In fact, the IC₅₀ parameter is inversely proportional to antioxidant activity, and therefore to free radical scavengers. The presence of these anti-radical substances in these extracts could be due to solvent and site factors. Indeed, for the Boof site, we noted an increase in the IC₅₀ value of the acetone extracts compared to the ethanol extracts and the opposite phenomenon for the Tanime site. However, the IC₅₀ values of the various extracts from the three sites studied remained higher than that of ascorbic acid (IC₅₀ = 4.12 mg/mL). These results therefore show that organic extracts from bark have a lower antioxidant power than ascorbic acid, the reference compound. This result could be due to the low presence of anti-radical substances in baobab bark.

3.4. Comparison of the Antioxidant Activities of Mucilage Contained in Baobab Bark and Leaves

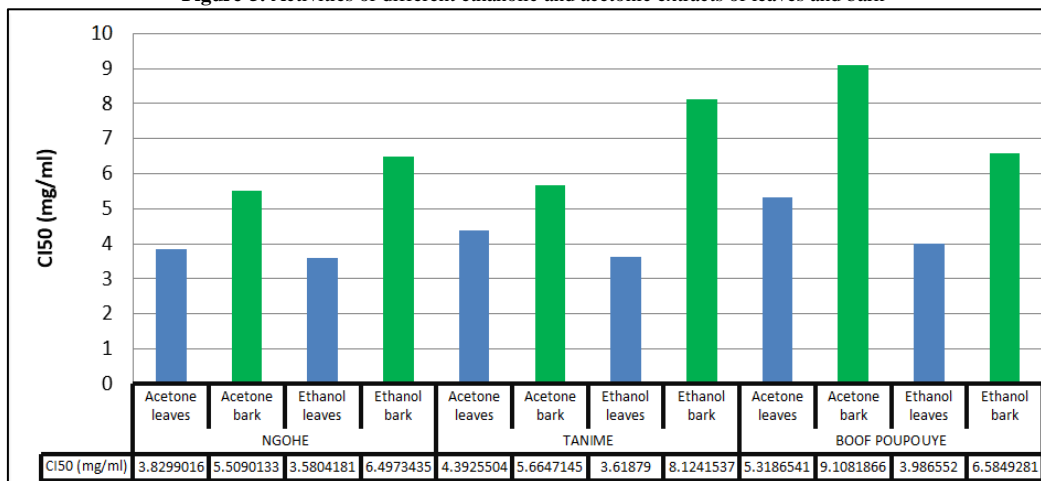
To compare the antioxidant activity of the mucilage contained in baobab leaves and bark from the various sites studied; we have plotted the IC₅₀ histogram in Figure 5 below. This histogram shows IC₅₀ values for leaf extracts that are generally lower than those for ascorbic acid (IC₅₀ = 4.12 mg/mL). The values found vary between 3.58 and 3.98 mg/mL for ethanolic leaf extracts and between 3.83 and 5.32 mg/mL for acetonic leaf extracts. However, for organic extracts of bark, the values obtained are relatively higher than those for ascorbic acid. They ranged from 6.49 to 8.12 mg/mL for ethanolic extracts and from 5.51 to 9.11 mg/mL for acetonic extracts. These experimental results revealed a higher antioxidant activity for leaf organic extracts compared to bark organic extracts. Leaf extracts, particularly ethanolic extracts, are therefore good free radical scavengers and have the most powerful anti-free radical potential. This high antioxidant capacity noted for these leaf extracts could be linked to the quantity, but above all the quality, of the compounds present in the leaves. The reducing power of these extracts is probably due to the presence of hydroxyl groups in the phenolic compounds, which can act as electron donors [21]. Previous work has also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [25, 26].

Studies have shown that the presence of phenolic compounds (flavonoids, tannins) is probably responsible for the antioxidant activity of extracts, as antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins have been shown to reduce and decolorize DPPH due to their ability to release hydrogen [27, 28].

Thus, the compounds responsible for the antioxidant activity of these extracts could be phenolic in nature. This is in good agreement with work carried out on extracts of *S. Montana* L. subsp. *Kitai* be harvested in the Siberian region, a species rich in phenolic compounds which are responsible for numerous biological activities, in particular antioxidant and antimicrobial activity [29]. It is important to note that levels of total polyphenols and flavonoids are correlated with total antioxidant activity. Indeed, in this sense and generally speaking, antioxidant power is strongly dependent on the concentration of phenolic compounds [30]. These data are corroborated by the work of Trabelsi et al, who showed a significant and positive correlation between phenolic compound content and antioxidant activity [31]. However, in the case of our results relating to activity against the DPPH- radical, this relationship is not always obvious, since it may not be significant in some cases. Indeed, Djeridane, *et al.* [32] believe that the existence of a relationship between the different phenolic compounds can be a determining factor in the antioxidant capacity of a given plant. It has been reported that polyphenols are efficient hydrogen atom donors to the DPPH- radical due to their ideal chemical structures [33]. Thus, the high activity noted in leaf extracts could be due to the presence of antioxidant substances of different natures that act in synergy.

This result therefore shows that the plant organ factor has a significant influence on the antioxidant activity of baobab mucilage.

Figure-5. Activities of different ethanolic and acetic extracts of leaves and bark



To confirm the previous result, we carried out a statistical analysis to check whether the site, species or solvent factors had an influence on the result of the analysis, and therefore on the antioxidant activity of the mucilage.

4. Statistical Analysis

We used Google Collaborator to test the hypotheses.

Research hypothesis: ANOVA test

H0: the result does not vary according to the sampling site

H1: results vary depending on the sampling site

Instruction: Si

P_value > 0.05, we accept H0

P_value < 0.05, we accept H1

Sites and Results

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.	
Resultats * Sites	Between Groups	(Combined)	3,922	2	1,961	,556	0,592
	Within Groups		31,741	9	3,527		
	Total		35,663	11			

P_value : 0,592 > 0,05

- Wetherefore accept H0, i.e. that the sampling site has no influence on the result.

Solvents and Results

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.	
Resultats * Solvants	Between Groups	(Combined)	,171	1	,171	,048	0,831
	Within Groups		35,492	10	3,549		
	Total		35,663	11			

P_value: 0,831 >= 0,05

- Solvents and Results are independent we accept H0, i.e. the solvent used has no influence on the result.

Species and results

ANOVA Table

		Sum of Squares	df	Mean Square	F	Sig.	
Resultats * Species	Between Groups	(Combined)	23,412	1	23,412	19,111	0,001
	Within Groups		12,250	10	1,225		
	Total		35,663	11			

P_value : 0,001

Species and Results are dependent on hypothesis H1, i.e. that the species used has an influence on the result. The statistical analysis therefore showed that only the plant species had an influence on antioxidant activity, which confirms the previous result.

5. Conclusion

A study of the antioxidant activity of extracts from baobab leaves and bark at three sites using the DPPH free radical scavenging method showed that leaf extracts have good antioxidant activity compared with bark extracts, whatever the solvent and site. The most active extracts were found at the Ngohé site, but solvent activity varied according to site and species. These leaf and bark extracts contain bioactive substances and could therefore be an alternative to certain synthetic additives for the prevention and treatment of pathologies. Statistical analysis shows that the antioxidant activity of mucilage depends solely on the plant species (leaf or bark) and not on the site or solvent and further research is needed to purify, isolate and identify the molecules responsible for this antioxidant activity.

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