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Characterization Of A Saharan Medicinal Plant: Adansonia Digitata (Bombacaceae): A Qualitative And Quantitative Study

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Abstract

In recent decades, there has been a growing interest in the study of medicinal plants and their traditional use in different parts of the world. Adansonia Digitata; known as the African baobab, is a medicinal plant of the Bombaceae family, widely used in traditional Saharan medicine and as a food condiment. The decoction of the pulp of the Baobab fruit is used as an antidiarrheal. The leaves are recommended to treat the aftermath of indigestion and to soothe stomach aches. As part of an effort to make the most of natural resources, organic extracts from different edible parts of this plant (leaves, fruit and seeds) were prepared by successive maceration in dichloromethane and methanol. The qualitative characterization, carried out by thin-layer chromatography (TLC) was able to reveal the presence of different phenolic molecules with diversified retention factors (Rf). Quantitative estimation of its phenolic compounds, namely total polyphenols, flavonoids and condensed tannins, was carried out by colorimetric methods (ultra violet spectrophotometry) using Folin-Ciocalteu reagent, aluminum chloride and vanillin respectively. The results obtained effectively confirmed the richness of the extracts in these compounds. The evaluation of the antioxidant power, carried out using the DPPH free radical scavenging method, indicated that the various methanolic extracts have a very good antioxidant activity. The quantitative determination of ascorbic acid in different parts of the plant under study was carried out by high performance liquid chromatography (HPLC) and has revealed the presence of this vitamin solely and in a very abounding manner in the fruit pulp with a concentration of 1206.246 mg / l.

Keywords: Adansonia Digitata, Phenolic Compounds, Thin-layer Chromatography, ultra violet spectrophotometry, Antioxidant Activity, DPPH, ascorbic acid, HPLC.

Introduction

Secondary metabolites are part of various chemical groups such as alkaloids, terpenoids, phenolic compounds (J.J. Macheix and al., 2005). Their role is now widely recognized in different aspects of the plants' life as well as for human use. Phenolic compounds can protect from certain human diseases due to their possible interaction with many enzymes and their antioxidant properties (J.J. Macheix and al., 2005).

The African baobab (Adansonia Digitata L.) is one of the main woody species used in food production in various African countries. Leaves and fruits are widely consumed (B.A. Bationo and al. 2009). Ethnobotanical Studies confirmed a high content of antioxidant vitamins different parts of the fruit and leaves in Adansonia Digitata (S. Vertuani and al., 2002).

Given the growing importance of health promotion, and benefits associated with the use of traditional preparations rich in antioxidants, we undertook this study to determine and quantify the antioxidant capacity of the different edible parts of Adansonia Digitata; namely the pulp fruit, leaves, and seeds. To do so; the study focused on an analytical method of separation; TLC, and a quantitative method of colorimetry using Folin-Ciocalteu reagent for total polyphenols, aluminum trichloride for flavonoids and vanillin for condensed tannins. The assessment of antioxidant potency was carried out using the DPPH free radical method and the quantitative determination of ascorbic acid was carried out by high performance liquid chromatography (HPLC).

Until all the active components of this plant are clearly established, this study is a first step in elucidating the therapeutic and nutritional potential of Adansonia Digitata plant products.

Materials and Methods

Plant Material

Adansonia Digitata or African Baobab (vernacular name) is a massive tree belonging to the Plantae kingdom, sub-kingdom of Tracheobionta, phylum of Spermatophyta, sub-branch of Magnoliophyta, class of Magnoliopsida or Dicotyledone subclass of Dilleniidae, order of Malvales, family of Bombacaceae (D. Malgras, 1992), or of Malvaceae (T. Kyndt and al., 2009), Adansonia genus and of digitate species (E. Coulibaly, 2009).

This secular tree, imposing by its size, is the most massive of the known woody species. Unlike other species of the genus Adansonia which are strictly endemic to Madagascar or Australia, A. digitata occurs in most subhumid to semi-arid regions in southern Sahara. Remarkable for its particular shape and large size, A. digitata is very characteristic of savannah landscapes in the Sahelian zones (A.G. Diop and al., 2005).

Despite the physical absence of this tree on Moroccan soil; its products remain widely used in traditional medicine in the region of Laâyoune (O. El Yahyaoui and al., 2015). The pulp of the baobab fruit and its leaves are imported by the Mauritanian population. Our samples are made up of powders; pulp, leaves and seeds of A. digitata, sold in the markets of the city of Laâyoune.

Thin-Layer Chromatography

- Characterization of Tannin

The method used is described by the work of SY GY and al. (2008), with some slight adaptations due to the nature of the samples. 2g of each plant drug were decocted in 15ml of acetone for 1 hour at a temperature of 70°C, then filtered and evaporated to dryness. 0.1mg of the resulting dried material was added to 1ml of methanol. 10µl of the methanolic solutions were deposited using a propette on a TLC plate (silica gel on an aluminum DC-Fertigfolium support ALUGRAM® SIL G/UV254), 1cm from the lower edge, on the baseline, at different locations. The plate was then dried in an oven for 10 seconds and then introduced into a developing tank containing the migration solvent; Ethyl acetate/Methanol/Water (40:8:5). After migration, the plate was removed from the cell and dried in the oven at 100°C for 5 min. The chemical constituents were revealed by spraying the specific reagent; Ferric Chloride/Acetic Acid/Water (2:2:96). When necessary, a UV lamp was used to identify the nature

of the constituents at 365nm. The spots were located and their migration distances were measured to calculate the corresponding Rf.

- Characterization of Flavonoids

1g of dry and ground plant material was extracted with 20ml of 80% methanol. After stirring for 15 min and sonication for 15 min, the extracts were filtered and subjected to TLC, the migration solvent being Ethyl Acetate/Methanol/Ammonia 50% (9:1:1). Revelation is at 365 nm after spraying with Neu's reagent (2-aminoethyl diphenylborate) at 1% in pure methanol (N. Dohou and al. 2004).

- Characterization of Coumarins

2 g of the various plant drugs were macerated in 10 ml of chloroform. After heating for a few minutes, followed by filtration of the mixture, the chloroform extracts were subjected to TLC, the eluent being a Toluene/Ethyl Acetate mixture (93:10). Visualization of the chromatogram, after migration, is done at 365 nm in the absence and presence of ammonia (H. Alilou and al., 2014).

- Characterization of Anthraquinones

Methanolic extracts were made by macerating 1g of each plant drug in 10 ml of pure methanol. After stirring for 10 min and sonication for 10 min, the macerates were filtered and subjected to TLC. The mobile phase consists of the mixture of ethyl acetate/Methanol/water (81:11:8). Revelation is carried out by spraying a 10% solution of potassium hydroxide in ethanol (V.O. Embeya, 2011).

Extraction

The extraction method applied is that of successive exhaustion, which consists of extracting the chemical substances from a plant drug using solvents of increasing polarity. It is a solid-liquid cold extraction, using two solvents; dichloromethane and methanol.

Table 1: Polarity Index and Boiling Temperature of Extraction Solvents

Solvents	Polarity	Boiling Temperature
Dichloromethane CH_2Cl_2	3.1	40°C
Methanol: CH_3-OH	5.1	65°C

In practice, 15g of each drug was macerated in 100 ml of dichloromethane for 24 hours at room temperature and in the dark. The filtrate obtained was then evaporated to dryness using a rotary evaporator and at boiling temperature (Table 1). The resulting extracts, E-DCM, were adjusted to 2 ml as a final volume.

The dry pomace was macerated in 100 ml of methanol for 24 hours at room temperature and in the dark. Apart from the boiling temperature used (Table 1), the same dry evaporation process was carried out in order to obtain the methanolic extracts (E-MeOH) from the pulp of the fruit, seeds, and leaves to which the final volume was adjusted to 2 ml. The yield of the extracts was calculated according to the following formula:

$$Y\ (\%) = \frac{Mext}{Mech} \times 100$$ (H. Falleh and al, 2008)

Where Y is the yeild in %; Mext is the mass of the extract after evaporation of the solvent in mg; Mech is the dry mass of the plant sample in mg.

The extracts were stored in opaque glass jars in the refrigerator until used for the determination of total polyphenols, total flavonoids, and condensed tannins, as well as for the evaluation of antioxidant potency.

Determination of Total Polyphenols

The determination of the total polyphenols of the different parts of A. digitata was carried out according to the method of Folin-Ciocalteu (A. Mansouri and al., 2005). Gallic acid was used as standard.
A 0.1 mg/ml gallic acid stock solution, Folin-Ciocalteu reagent diluted 1:10 with distilled water, and sodium carbonate (7.5%) have been previously prepared. Preparations and incubation are carried out at room temperature.
A range of 9 gallic acid concentrations from 0.025 to 0.6 mg/ml was prepared from a 0.6 mg/ml solution. The calibration curve is made according to the following table.

Table 2: Gallic Acid Calibration Range

Tube n°	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Final Gallic Acid Concentration in mg/ml	0.025	0.5	0.075	0.1	0.2	0.3	0.4	0.5	0.6

300 µl of the gallic acid solution at different concentrations were introduced into the tubes of a first set. A second set of 300 µl of each test sample was placed in tubes. 1500 µl of the reagent and then 1200 µl of the sodium carbonate solution were added to each tube. All preparations were shaken and incubated in the dark for 1 hour. The absorbance reading was taken at 760 nm (A. Mansouri and al., 2005).
The blank was therefore represented by 300 µl of methanol added to 1.5 ml of Folin-Ciocalteu reagent and 1.2 ml of 7.5% sodium carbonate. The values obtained were used to deduce the concentrations of total polyphenols from the calibration curve. The results were expressed in milligrams equivalent of gallic acid per gram of dry weight of the powdered plant using the following formula:

$$C = \frac{(c \times V)}{m}$$

C: Total phenol content (mg gallic acid/g dry matter)
c: Gallic acid concentration established from the calibration curve (mg/ml)
V: Volume of E-MeOH or E-DCM
m: Weight of the dry matter (g)

Dosage of Total Flavonoids

The method used for estimating the total flavonoid content of A. digitata is that described by Bahorun (1997). The reagent used for this was prepared by adding 2 g of Aluminum Chloride (AlCl3) to 100 ml of absolute methanol. A range of 9 concentrations of quercetin from 2.5 to 40 µg/ml was prepared from a 40 µg/ml stock solution (400 µg of quercetin dissolved in 10 ml of methanol).
Aliquots of 0.2 to 3.0 ml of the quercetin stock solution were introduced into a set of test tubes. The final volume in each tube was made up to 3 ml by the addition of absolute methanol. Then 1 ml was

taken from each tube and transferred to another, to which 1 ml of the 2% aluminum chloride methanolic solution was added. After 10 min incubation at room temperature and in the dark, the absorbance was read at 430 nm. The optical densities thus obtained are used to establish a calibration curve representing the concentration of quercetin (µg/ml) as a function of absorbance. (Table 3)

Table 3: Quercetin Calibration Range

N° of tubes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Final Concentration of Quercetin (in µg/ml)	2.5	5	10	15	20	25	30	35	40
Optical Density (430nm)	0.0	0.15	0.33	0.67	0.95	1.36	1.6	2.0	2.38

In order to analyze the extracts, two sets of test tubes were prepared. 1 ml of each extract was introduced into one tube from each of the two sets. 1 ml of the 2% aluminum chloride methanolic solution was added to each of the tubes of the first series and 1 ml of absolute methanol was added to each of the tubes of the second set as a blank value. After 10 minutes, the absorbance was read at 430 nm. The absorbances of the extracts from the tubes of the 2nd series have been subtracted from those of the 1st sets to avoid possible pigment interference. The flavonoid concentration was determined with reference to the calibration curve obtained using quercetin as the standard.

Dosage of Condensed Tannins

The determination of condensed tannins was carried out by the vanillin method described by Hagerman (2002). The reagent for the analysis was prepared by equally mixing the 1% vanillin solution (in methanol) and the 8% methanolic HCl solution. The Standard used is catechin at 0.3 mg/ml. A range of 6 concentrations of catechin from 0 to 0.3 mg/ml was prepared from the stock solution.

Aliquots of 0.2 to 1.0 ml of the catechin stock solution were introduced into a series of test tubes. The final volume in each tube was made up to 1 ml by adding absolute methanol. 5 ml of the analytical reagent were subsequently added at 1 min intervals to each tube of the sets and placed in a water bath set at 30°C for 20 min. The absorbance is read at 500 nm (Table 4).

Table 4: Catechin Calibration Range

N° of tubes	**1**	**2**	**3**	**4**	**5**	**6**
Catechin Concentration (mg/ml)	0	0.06	0.12	0.18	0.24	0.3
Absorbance at 500 nm	0	0.02	0.04	0.06	0.08	0.1

In order to analyze the extracts; two sets of test tubes have been prepared. A first set consists of tubes containing 1 ml of each extract and 5 ml of the analytical reagent added at 1 min intervals in each of the tubes. The second set consists of 1 ml of each extract added to 5 ml of the 4% HCl methanolic solution at 1 min intervals. All the tubes were then placed in a water bath at 30°C for 20 min. Absorbance readings at 500 nm were taken at a one-minute interval. The absorbance of the tubes of the second set (the whites) is subtracted from that of the tubes corresponding to the first set (the white is considerable for fabrics containing a large quantity of pigments). The values obtained are used to deduce the concentrations of the condensed tannin extracts from the calibration curve.

The Antioxidant Potency of DPPH

DPPH radical scanning activity was measured according to the protocol described by Lopes-Lutz and al. (2008). The methanolic solution of DPPH was prepared by dissolving 5.5 mg of DPPH in 100 ml of methanol followed by sonication for 3 min. Test tubes were filled with 2.5 ml of each extract and 1 ml of the methanolic solution of DPPH (55 µg/ml). After stirring through a vortex, the tubes are placed in the dark at room temperature for 30 min. The optical density is read by measuring the absorbance at 517 nm. The results are expressed as anti-radical activity where the free radical inhibition is calculated as a percentage (I %) using the following formula:

$$\textbf{Inhibition } \% = [1 - \left(\frac{\textbf{Abs e}}{\textbf{Abs c}} \right)] \times 100$$

Abs c: Absorbance of the blank
Abs s: Absorbance of the test sample

For each extract, the EC_{50} (Efficient concentration), representing the concentration of the substrate producing the 50% loss of DPPH activity (Samarth and al. 2008), was determined graphically from the curve of percent inhibition versus different concentrations of the extracts tested (Torres and al. 2006). Results can also be expressed in antiradical potency (ARP) (Brand- williams and al. 1995).

Determination of ascorbic acid

The total ascorbic acid content is determined by HPLC using UV detection at 265nm. The conditions for the chromatographic assay are as follows: (NF V 05-10516 JANVIER 1974)

- RP 18 column with a particle size of 5µm, a diameter of 4.0mm and a length of 250mm.
- Composition of the mobile phase:

• Solution A: Dissolve 13.6 g of potassium dihydrogen phosphate in 900ml of water and filter with a 0.45 µm pore size membrane filter.

• Solution B: Dissolve 1.82 g of N-Cetyli-N, N, N-Trimethylammonium bromide in 100ml of methanol. Mix and filter with a 0.45 µm pore size membrane filter.

- Mix 900ml of solution A with 100ml of solution B. If necessary, degas the solution before use.
- Flow rate: 1 ml / min.
- Volume injected: 20 µl.
- Detection wavelength: 265 nm.

Vitamin C content is calculated in mg / l according to the following formula, the result is expressed without decimal.

$$C = 2.5 \times Ed \times Cs \times \frac{Ae}{As}$$

With:

- C: the vitamin C content;
- Ae: the peak area for L-ascorbic acid, obtained with the sample solution;
- As: the peak area for L-ascorbic acid, obtained with the calibration solution;
- Cs: The L-ascorbic acid concentration of the calibration solution (in mg / l);
- Fd: the dilution factor of the sample;
- 2.5: the dilution factor of the reduction step.

Results and Discussion

Thin-Layer Chromatography

The results of the thin-layer chromatography are summarized in Table 5. The TLC showed the presence of different phenolic compounds in all organs of Adansonia digitata represented by bands with various Rfs and colors.

Table 5: TLC results, colors and Rfs of the bands referring to the different organs of A. digitata

Secondary Metabolites	Migration Solvents	Reagents of Revelation	Bands					
			Pulp		Seed		Leaves	
			Spots	Rf	Spots	Rf		
flavonoid	Ethyl acetate/ Methanol/ Ammonia	Neu reagent	Blue Red Red	0.062 0.837 0.987	-	-	Orange Orange Red Red	0.062 0.137 0.775 0.987
Tannin	Ethyl acetate/ Methanol/ Water	Ferric chloride/ Acetic acid/ Water	Red	0.084	-	-	Red Red	0.813 0.987
Anthraquinone	Ethyl acetate/Methanol/ Water	Potassium hydroxide	-	-	-	-	Red spots under fluorescence	0.937 0.962 0.975
Coumarin	Ethyl acetate/ Toluene	Ammonia NH3	-	-	Blue spots under fluorescence	0.114 0.178 0.658 0.734	Blue spots under fluorescence	0.114 0.178 0.658 0.734 0.886 0.975

The leaves represent the richest sample with regard to our results, as they are full of all the compounds we were looking for; namely flavonoids, tannins, coumarins and anthraquinones. This is consistent with other scientific work indicating that the leaves of A. digitata contain many phenolic compounds including flavonoids and tannins (G.K. Oloyede and al., 2010). On the other hand, the pulp is rich in flavonoids and tannins but does not show positive results for coumarins and anthraquinones. These findings are confirmed by the work of IKED Koko and al (2011) with regard to flavonoids. The seeds present different blue spots with diversified Rf which refer to the presence of coumarins. We believe that the different compounds found in the studied organs of A. digitata could justify the use of the latter by traditional practitioners.

Extraction Yields

The extraction yield obtained for each of the plant drugs of Adansonia digitata has been calculated and presented in the following table.

Table 6: Methanolic and Dichloromethane Extraction yield from A. digitata Organs

Organs of Adansonia digitata	Solvent Used	Yield (%)
Pulp	Dichloromethane	0.31
Leaves		1.11
Seeds		17.29
Pulp	Methanol	17.18
Leaves		12.93
Seeds		6.67

Comparative yield results show that methanol is a better extraction solvent for plant drugs from pulp (17.18%) and leaves (12.93%) compared to dichloromethane. The seeds show the opposite result; namely, 6.67% for methanol and 17.29% for dichloromethane. The solvent plays a major role in the extraction process since solubility is the most important parameter. Indeed, the solubility of phenolic compounds is affected by the polarity of the solvent used (S. Mahmoudi and al., 2013). Successive phases of extractions followed by filtration with variation of the solvent were carried out, resulting in fractional dissolution. A low-solvent liquid, dichloromethane, was used initially, followed by an increase in dissolution capacity by the use of a relatively more active solvent, methanol, which resulted in a significantly higher yield of methanolic extraction. Indeed, the most commonly used extraction solvents are alcohols (methanol, ethanol) for very polar compounds such as phenolic acids. Less polar solvents (dichloromethane (DCM), chloroform, hexane, benzene) are used to remove apolar compounds (waxes, oils, sterols, chlorophyll ...) which justifies the high yield observed for seeds that according to previous work are rich in sterols and fatty acids (S. Mahmoudi and al, 2013).

Total Polyphenol Content

The evaluation of the total polyphenol content is carried out by the Folin-Ciocalteu method. Gallic acid was used to produce the calibration curve (Annex 1). The levels of total polyphenols measured for the three parts of A. Digitata (Pulp, Seed and Leaf) ranged from 11.23 to 1194.14 mg EAG/100g DM and from 6.58 to 66.67 mg EAG/100g DM for E-MeOH and E-DCM respectively (Table 7).

Table 7: Total Polyphenol Content of E-MeOH and E-DCM Extracts from the Pulp, Seeds and Leaves of A. digitata Expressed in mg EAG/100g of Dry Plant Material.

Organs	Total polyphenol content in mg EAG/100 g of dry plant material	
	E-MeOH	E-DCM
Fruit Pulp	280.03	22.88
Seeds	11.23	6.58
Leaves	1194.14	66.67

The change of solvent has a direct impact on the metabolite composition of the resulting extract. Indeed; the total polyphenol contents for methanolic extracts are much higher than those observed for dichloromethane extracts. Based on these results, it appears that the phenolic compounds extracted from A. digitata are much more soluble in the polar organic solvents than the apolar ones. Our result is consistent with the work of Lamien-Meda and al. (2008), who report that Adansonia Digitata fruit pulp extracted by methanol is very rich in total polyphenols. They show that this content in the leaves of the same plant is 3753mg EAG/100g versus 1194.14mg EAG/100g for our work. This discrepancy in phenolic compound levels is likely the result of the distribution of secondary metabolites, whose synthesis changes during plant development. The polyphenol content is influenced qualitatively and quantitatively by several climatic and environmental factors; geographical area, drought, soil, stresses and diseases, etc. (N.S. Ebrahimi and al., 2008). Indeed; climatic conditions (high temperature, sun exposure, drought, salinity) stimulate the biosynthesis of secondary metabolites such as polyphenols (Falleh and al. 2008). The gene pool, harvest period and stage of development of the plant may also affect this content (G. Miliauskas and al., 2004).

Total Flavonoid Content

Spectrophotometric determinations were made from the methanolic and dichloromethane extracts of the three parts of A. digitata to determine the total flavonoid content. A calibration curve (Annex 2) is made using quercetin. The results are reported in mg quercetin equivalent/100g of dry plant material (Table 8).

Table 8: Content of Total Flavonoids in Extracts of the Pulp, Seeds and Leaves of A.digitata expressed in mg EQ/100g of Dry Plant Material.

Organs	Flavonoid Content in mg EQ/100 g of Dry Plant Material	
Fruit Pulp	219.19	1.51
Seeds	58.47	3.31
Leaves	824.96	9.11

According to the above table, it is noted that in the case of flavonoids, methanolic extracts have superior contents than dichloromethane extracts. There is also a significant difference in the total flavonoid content depending on the part of the plant; the leaves give the highest content with a value of 824.96 mg EQ/100g DM for the methanolic extract and 9.11 mg EQ/100g DM for the

dichloromethane extract. However, our result in terms of the pulp of the fruit is much better than that found by some authors who have worked on the same plant. It is 11.17 mg EQ/100g MS in the work of Compaoré and al. (2010).

Furthermore; the work carried out by Omale and al. (2008) confirm that the total flavonoid content in the leaves of A. digitata is very important, i.e. 1550 mg EQ/100g MS. It has been shown that flavonoid levels are high when the plant's living environment is not adequate, in which case the plant promotes the synthesis of secondary metabolites in order to adapt and survive.

Dosage of Condensed Tannins

The determination of condensed tannins was carried out by the vanillin method. Catechin was used to carry out the calibration range (Annex 3). Analysis of the condensed tannin levels reported in Table 9 indicates that methanol maceration is much more effective than dichloromethane maceration. The extraction of condensed tannins depends on their chemical nature, the solvent used and the operating conditions (U.D. Chanvan and al. 2001).

Table 9: Content of Condensed Tannins in the E-MeOH and E-DCM Extracts of the Pulp, Seeds and Leaves of A. digitata expressed in mg EC / 100g of Dry Plant Materia.

Organs	Condensed Tannin Content in mg EC/100 g Dry Plant Material	
	E-MeOH	E-DCM
Fruit Pulp	329.124	-
Seeds	86.381	-
Leaves	2650.34	170.15

Methanol showed a better result compared to dichloromethane. Thus, there is a total absence of condensed tannins in the dichloromethane extracts of the fruit pulp and seed. The variation in condensed tannin content depending on the part of the plant is highly significant. Consistent with the results observed for flavonoids, leaves gave the highest levels (2650.34 mg EC/100g DM) compared to values of 329.124 and 86.381 mg EC/100g DM for pulp and seeds respectively. The TLC showed the same result by clearly indicating that the leaves are rich in tannins, followed by the pulp. Previous work, carried out on the same species, reports the richness of this plant in tannins but with results inferior to those found by our work. (Compaoré and al. 2010). Tannins have curative properties against gastric diseases (indigestion, diarrhea, ulcers), venereal diseases, dermatoses and high blood pressure (Sérémé and al. 2008); hence the importance of A. Digitata in the traditional African pharmacopoeia used in the preparation of many remedies, particularly for digestive and inflammatory problems.

The antioxidant Power of DPPH

Figure 1 reports the results of the antioxidant power of methanolic extracts of A. Digitata depending on different concentrations by the DPPH free radical.

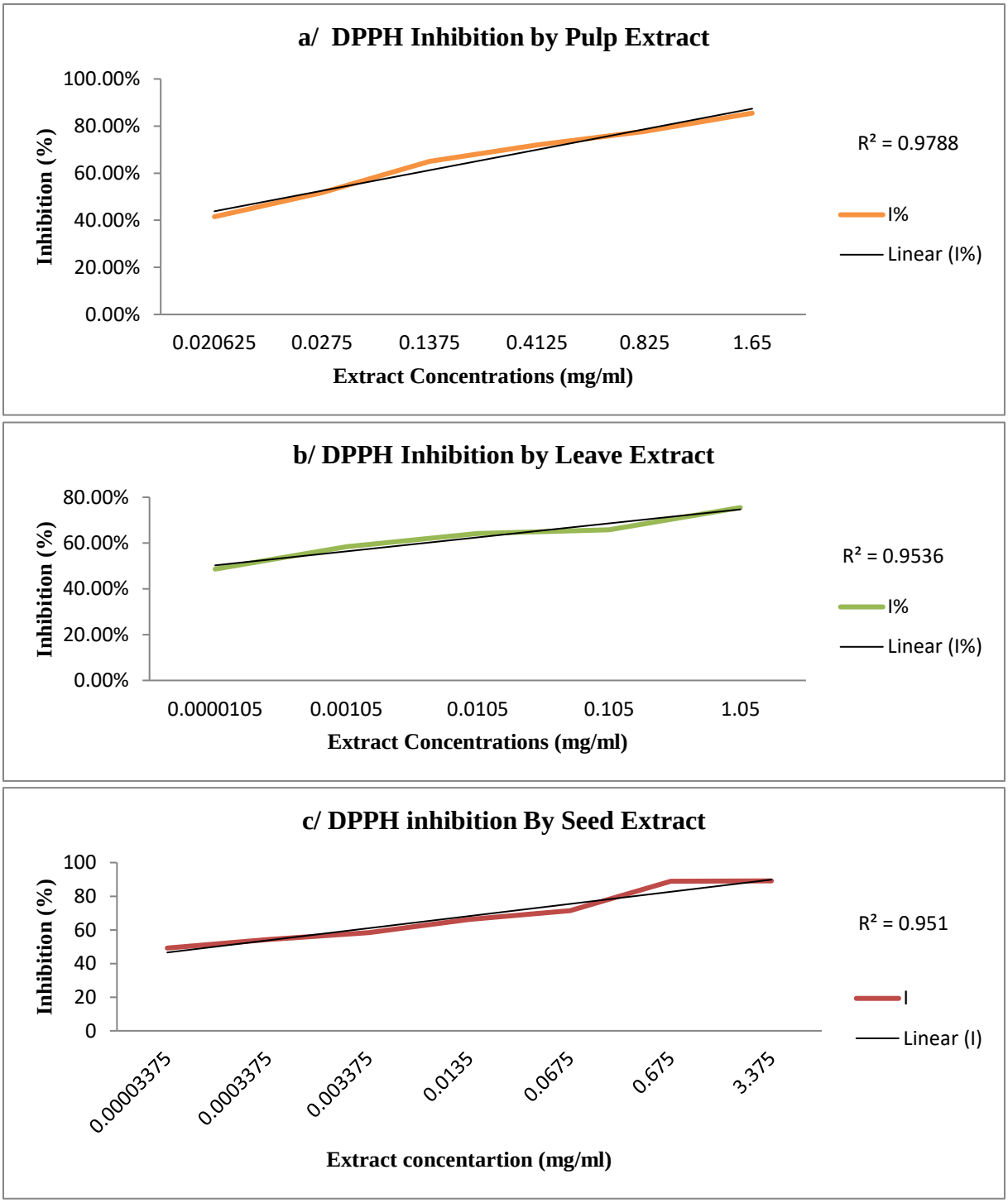

Figure 1: Percentage inhibition of the free radical DPPH depending on the E-MeOH concentrations of the different parts of A. Digitata (a/extract from the pulp; b/extract from the leaves; c/extract from the seeds)

According to the obtained curves shown in Figure 2, all the methanolic extracts of A. Digitata tested produced a significant inhibition of the DPPH free radical with satisfactory correlation coefficients indicating that the two variables (DPPH inhibition and extract concentration) are highly correlated, with remarkable differences in the concentration of the extracts used.

The calculation of the IC_{50} values allowed a better illustration of the results by providing the possible concentration of the substrate producing the 50% loss of DPPH activity; determined on based on the trend curve of the percentages of inhibition in accordance with different concentrations of the extracts tested. The results are shown in the following table. The values of anti-radical power (APR); inversely proportional to the IC_{50} are reported in the same Table 10.

Table 10: IC_{50} and ARP Values of the Different Methanolic Extracts of A. Digitata

Tested Parts of the Plant	IC_{50} (mg/ml)	ARP
Fruit Pulp	0.171	5.848
Seeds	5.383	0.186
Leaves	0.096	10.417

The IC_{50} is inversely related to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the concentration of the free radical DPPH by 50%. Lower values of IC_{50} indicate the effectiveness of the extract and thus a stronger antioxidant power. The methanolic extract from the leaves has a lower IC_{50} than the extracts from the pulp of the fruit and seeds. The antioxidant activity presented by the leaves is better than that of the pulp and seeds. This is also illustrated by the values of antiradical power (APR). The DPPH radical is generally one of the most widely used compounds for rapid and direct assessment of antioxidant activity because of its stability in radical form and simplicity of analysis (Bozin and al. 2006).
The comparative study (Table 11) shows that the IC_{50} values of the methanolic extracts, corresponding to the different parts of Adansonia Digitata, are significantly lower than those of the methanolic extracts of the fruit of Citrus Aurantium (orange) or those of Olea europea var. sylvestris (olive); this shows a rather high and interesting antioxidant power of the studied plant.

Table 11: Comparison of the IC_{50} Values of the different Organs of A. Digitata and Other Species.

Species	Organ	Extract	IC_{50} (µg/ml)	Reference
A. Digitata	Fruit Pulp	MeOH	8.15	Dzoyem and al. (2014)
	Seeds	MeOH	94	Gahane and Kogje (2013)
	Leaves	MeOH	13,5	Ayele and al. (2013)
Citrus Aurantium (orange)	pulp	MeOH	1710.2	Park and al. (2014)
	peel	MeOH	1402.9	
Olea Europea var. sylvestris (olive)	Leaves	MeOH	290	Arab and al. (2013)

In the same context; Vertuani and al. (2002) showed that fruit pulp has high antioxidant activity compared to orange, strawberry, apple and kiwi fruit pulp. These high contents led Nhukarume and al. (2008) to suggest the use of baobab fruit pulp as a botanical antioxidant dietary supplement.

Determination of ascorbic acid

HPLC analysis of ascorbic acid in the different parts of the plant studied revealed the presence of this vitamin solely in the fruit pulp with a concentration of 1206.246 mg/l. (fig.2)

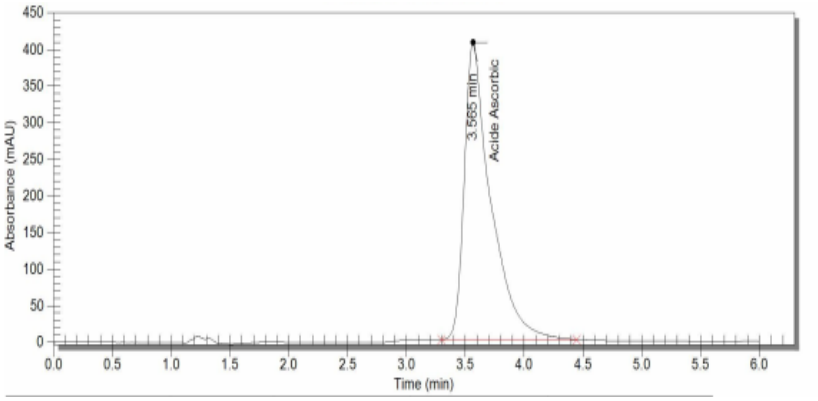

Figure 2: Chromatogram of Adansonia Digitata fruit pulp

Moreover, the chromatograms of the leaves and seeds of this same plant showed an absence of vitamin C expressed by the absence of peaks detection.

Vitamin C content recorded in the pulp of Adansonia Digitata is 120.624 mg/100g, which is in line with the studies conducted by Mady CISSE and al. (2009) ((125 to 312) mg 100 g–1). The work of Kouamé Brice Arnaud Pamba and his colleagues have revealed a content of 240 mg/100g. On the other hand, Gebauer and al. (2002) showed in a study conducted in Sudan on the pulp of the African baobab, values of 30 mg/100g. This variation in levels can result from pedoclimatic conditions, the stage of maturity of the fruits at harvest, and the conditions of preservation and storage of the pulp (A.G. Diop and al. 2006). The value observed for our sample places baobab pulp among the richest fruits (A.R. Proteggente and al. 2002) in vitamin C (orange, 4.6 mg/100g; kiwi, 5.2 to 12 mg/100g; strawberry, 6.1 mg/100g or even apple, 0.6 mg/100g) after camu-camu (Myrciaria dubia (HBK) Mc. Vaugh), acerole (Malpighia glabra L.), ditax (Detarium senegalense Gmel.) or blackcurrant (Ribes nigrum L.) (C.F. Bourgeois 2002, R. Rodrigues and al. 2001).

Conclusion

The antioxidant properties of plants are linked to their therapeutic and protective effects against many diseases such as Alzheimer's, Parkinson's, cancers, cardiovascular disorders, as well as bacterial and viral infections and inflammations (G.K. Oloyede 2010). In this study, Adansonia Digitata proved to be an excellent medicinal plant with a high antioxidant power. Indeed; the qualitative and quantitative study of the different parts of the plant reveals the presence of interesting quantities of phenolic compounds such as flavonoids, tannins, coumarins and anthraquinones, and high content of ascorbic acid, which could be at the origin of its use in traditional medicine and give it much sought-after therapeutic, nutritional and cosmetic properties.

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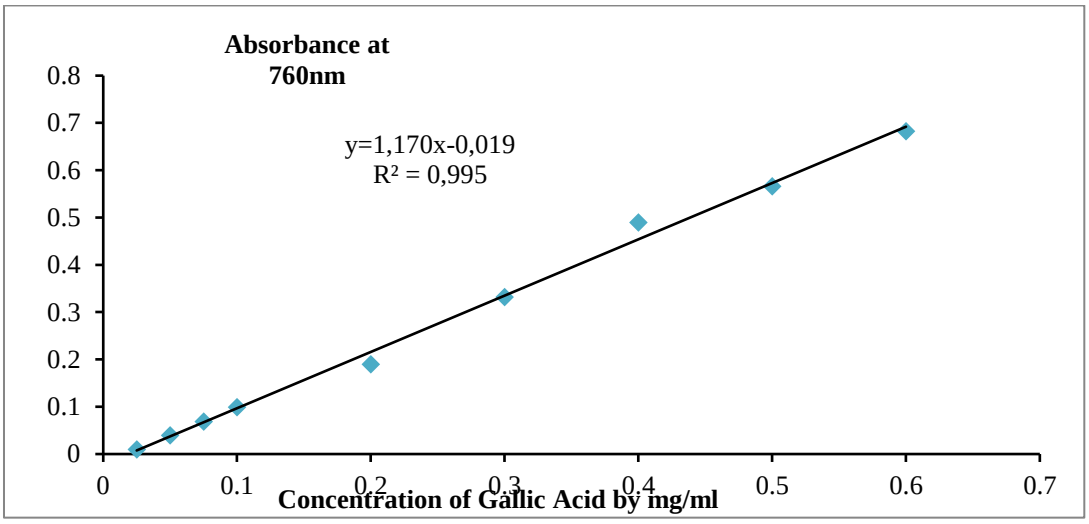
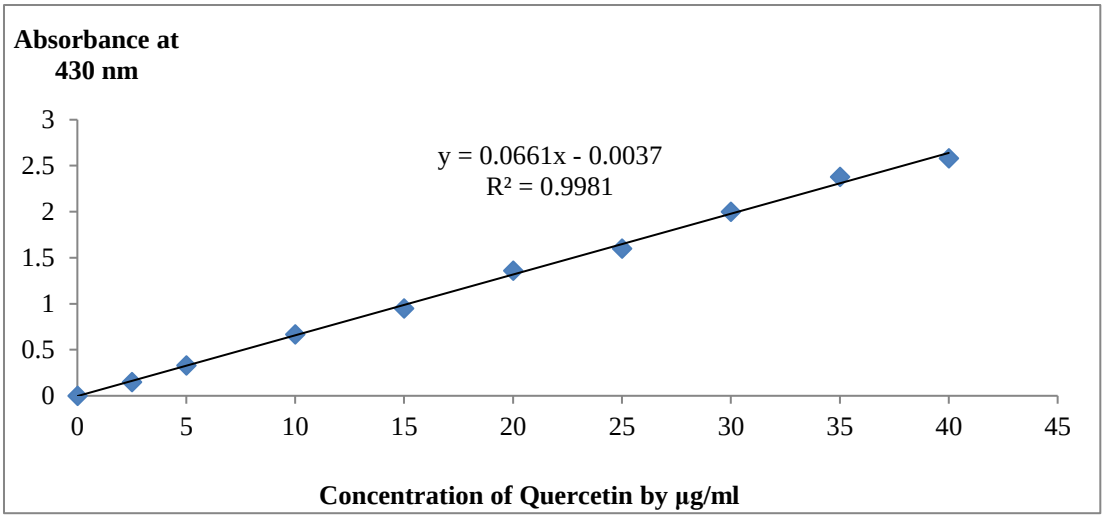
Annex 1: Calibration Curve of Gallic Acid for the Determination of Total Phenols



Annex 2: Calibration Curve of Quercetin for the Determination of Flavonoids



Annex 3: Catechin Calibration Curve for the Determination of Condensed Tannins