

# Determination of polyphenol content and evaluation of antioxidant activity of extracts from different parts of the plant *Diospyros Mespiliformis Hochst Ex. DC*

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**Abstract:** The *Diospyros Mespiliformis Hochst Ex. DC* is a traditional medicinal plant widely used for the treatment of several diseases such as fever, headache, pneumonia, leprosy, syphilis, diabetes, skin infections, bronchial diseases, tuberculosis, etc. Owing to these multiple therapeutic virtues, we have to evaluate the content polyphenolic compounds to which protective virtues, such as antioxidative property, are attributed. The extracts of the leaves, the barks, and the roots of the plant *Diospyros Mespiliformis* are studied. Considering the encouraging results of the assays, the evaluation of the antioxidant activities of ethanolic, aqueous and hydro-ethanolic extracts was carried out by means of the DPPH reduction test. The results of the antioxidant activity evaluation are very satisfactory with a 50% equivalent concentration (IC50) of 0.05 µg/mL for roots and 50% equivalent concentration (IC50) values lower than 0.01 µg/mL for barks and leaves.

**Keywords:** *Diospyros Mespiliformis*, DPPH, IC50, Polyphenol, Flavonoid.

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## I. Introduction

The *Diospyros Mespiliformis Hochst Ex. DC* is one of the giant trees of the Senegalese savannah. It is a tree of variable size with alternating green leaves and a robust trunk with black bark that blooms between January and March and gives a succulent yellow fruit. It is a very familiar tree in the savannah and is usually found on the banks of rivers and termite mounds, so characteristic of heavy and well drained soils [1-5]. It is also found in dry forests (steppe; savannah) sometimes humid and dense. It is listed in the flora of some countries of sub-Saharan Africa, the Gulf of Guinea, Eastern and Southern Africa. Its presence has also been reported in the Near East, Latin America, Australia and part of North America. It is used as medicine in traditional medicine in the fight against certain bronchial affections, various types of evils, infections and psychic diseases [6-8]. These ethno-pharmacological data show the particular interest of phytochemists. The studies carried out until now on the plant have provided very little information on its composition in secondary metabolites. Most of them have been only qualitative, giving information on the different classes of metabolites found by means of chemical screening tests. These present the plant as containing terpenes, saponins, sterols, flavonoids, anthocyanins, quinones and alkaloids [9-11]. In order to deepen our knowledge of the plant, we have further investigated the polyphenol content of the different parts of the plant (leaves, bark, roots) followed by antioxidant activity tests.

## II. Materials and methods

The raw material used for the research was harvested in Ndiémane. A village located on the small coast of Senegal between Mbour (14°25'22.23" N; 16°57'55.35" W) and Joal-Fadiouth (14°09'08" ; 16°49'37" W). The samples collected consisted of fruits, leaves, trunk bark and roots of the plant *Diospyros Mespiliformis Hochst Ex. DC*. They were cleaned with distilled water before being dried under shelter at room temperature. The dried material was crushed and store in glass jars in our laboratory.

### Chemical screening

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They were developed, with the aim of determining in a qualitative way the various families of secondary metabolites present in the extracts of plant. Their evidences were carried out while being inspired by the chemical screening methods described in the literature [12-22].

### **Dosages**

#### ***The total phenolic content of the hydroalcoholic extracts of the various parts of the plant***

The dosage of phenolic compounds allows to see in a quantitative way the richness in these compounds of the studied plant parts. It was carried out according to the procedure described below used by Alara et al. [22]. The total phenol content was calculated in mg of gallic acid equivalent (or ascorbic) (EAG) /g of plant extract.

#### ***Procedure***

To 100  $\mu$ L of each solution (extract, gallic acid or ascorbic acid), 500  $\mu$ L of Folin-Ciocalteu reagent diluted 10 times in water is added. After 2 minutes, 2 mL of 20%  $\text{Na}_2\text{CO}_3$  (20g/100mL) was added to make the medium alkaline to trigger the redox reaction. Then the solutions were kept in the dark for 30min at room temperature. Then, the absorbance was measured at the wavelength of 750 to 760 nm (Ultrospec 7000 UV-vis dual beam spectrophotometer (GE Healthcare, Chicago, IL, USA) by subtracting that of the blank (same solution but without the extract). All analyses were performed in triplicate. Total phenol content was calculated as mg gallic (or ascorbic) acid equivalent (GAE)/g plant extract [22] according to the following equation.

$$T = (C \times V) / M$$

T: total phenolic content in mg gallic acid equivalent (GAE)/g plant extract,

C: concentration of gallic acid determined from the equation of the calibration curve in mg/mL ( $y = ax + b$ , where  $y =$  gallic acid absorbance and  $x = C$ ),

V: volume of the extract solution in mL,

M: mass of the extract in g.

#### ***Total Flavonoid content of the hydroalcoholic extracts of the different parts of the plant***

#### ***Procedure***

250  $\mu$ L of each extract or catechin (1 mg/mL) is mixed with 1 mL of distilled water and then 75  $\mu$ L of 15% sodium nitrite ( $\text{NaNO}_2$ ) solution is added. The first incubation for 6 minutes at room temperature, 75  $\mu$ L of 10% aluminum chloride ( $\text{AlCl}_3, 6\text{H}_2\text{O}$ ) is added. A second incubation for 6 minutes at room temperature was performed and then 1 mL of 1M sodium hydroxide ( $\text{NaOH}$ ) was added. The total volume was made up to 2.5 mL with distilled water (100  $\mu$ L), shaken and then incubated for 15 minutes.

After measuring the absorbance at 510 nm (Ultrospec 7000 UV-vis dual beam spectrophotometer (GE Healthcare, Chicago, IL, USA) of the blank, a standard range was performed under the same operating conditions using catechin at different final concentrations (0.6; 0.5; 0.3; 0.2; 0.1 mg/mL).

### **Antioxidant activity test with DPPH**

#### ***Procedure***

The determination of antioxidant activity of the extracts of the leaves of the plant *Diospyros Mespiliformis Hochst Ex. DC* by DPPH assay was performed as follows. The DPPH solution was prepared and stored in a dark place to avoid oxidation of the solution. Then in a series of test tubes containing 0.1 mL of extract at different concentrations are added 4 mL of the DPPH solution. The solutions are tested at the following concentrations 0.00001; 0.0001; 0.001; 0.01; 0.1; 0.2; 0.3; 0.4 mg/mL and the absorbance of DDPH is measured using (Ultrospec 7000 UV-vis dual beam spectrophotometer (GE Healthcare, Chicago, IL, USA) at 750 to 760 nm. The results are first expressed as percent inhibition (PI) of antioxidant activity.

The IC<sub>50</sub> is obtained from the right-hand equation of the graph representing percent inhibition (%PI) versus concentration (mg/mL). Percent inhibition is calculated according to the following formula: Equation 1

$$\% PI = \frac{A_0 - A_1}{A_0} \times 100$$

% PI = Percentage of Inhibition

A<sub>0</sub> = absorbance of DPPH.

A<sub>1</sub> = absorbance after addition of the extract at a given concentration after the 30 minutes of incubation.

## **III. Results**

### **Results of chemical screening tests**

The families of secondary metabolites found in the ethanolic extract are terpene, polyphenolic and alkaloidal in nature and for the aqueous extract the polyphenols and alkaloids were more represented. These results characterize the richness of these extracts and allow to push the study towards the determination of the content and antioxidant activities of some subfamilies of these secondary metabolites, namely polyphenols and flavonoids by assays.

**Table 1:** Results of extract screening tests

Test Extracts Raw	Alkaloids	Terpene	Saponin	Polyphenols	Flavonoids	Tannins	True Tannins	Condensed Tannins	Coumarin	Antraquinone	Quinone	Leuco anthocyanins	Anthocyanes
Ethanol	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++
Water	++	+++	++	+++	+++	+++	+++	---	---	---	---	+++	---

+++ : very strongly present; ++ : strongly present; --- : absent

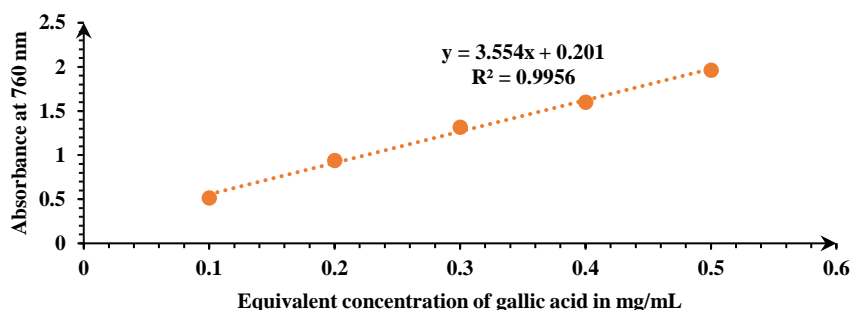
**Results of phenolic and flavonoid assays**

*Determination of the content of phenolic compounds*

*Calibration: gallic acid*

**Table 2:** Absorbance of gallic acid at various concentrations at wavelength 760 nm

Concentration mg/ml	Absorbance at 760nm
0.5	1.964
0.4	1.599
0.3	1.316
0.2	0.941
0.1	0.516

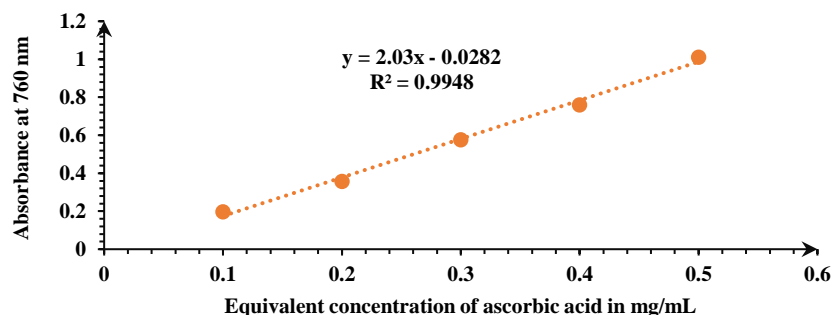


**Figure 1:** Calibration curve of gallic acid

*Calibration: ascorbic acid*

**Table 3:** Absorbance of ascorbic acid at various concentrations at wavelength 760 nm

Concentration mg/ml	Absorbance at 760nm
0.5	1.012
0.4	0.76
0.3	0.576
0.2	0.358
0.1	0.198

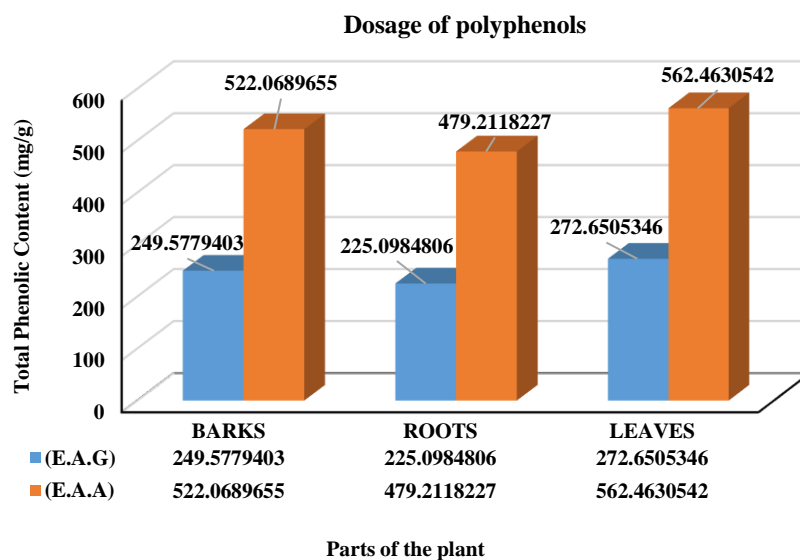


**Table 4:** Absorbance at wavelength 760 nm

Parts of the plant	Absorbance 1	Absorbance 2	Absorbance 3
Barks	1.097	1.063	1.104
Roots	0.86	1.071	1.072
Leaves	1.276	1.171	1.063

**Table 5:** Average contents of E. A. G and E. A. A

Parts of the plant	Average E. A. G content	Average E. A. A content	Standard deviation
Barks	249.5779403	522.0689655	6.170993866
Roots	225.0984806	479.2118227	34.35863973
Leaves	272.6505346	562.4630542	29.96722596



**Figure 3:** Average contents of E. A. G and E. A. A

**Determination of the flavonoid content**

**Calibration: Catechin**

**Table 6:** Absorbance of catechin at various concentrations at wavelength 510 nm

Concentration mg/mL	Absorbance at 510 nm
0.6	1.699

0.5	1.385
0.3	0.854
0.2	0.566
0.1	0.339

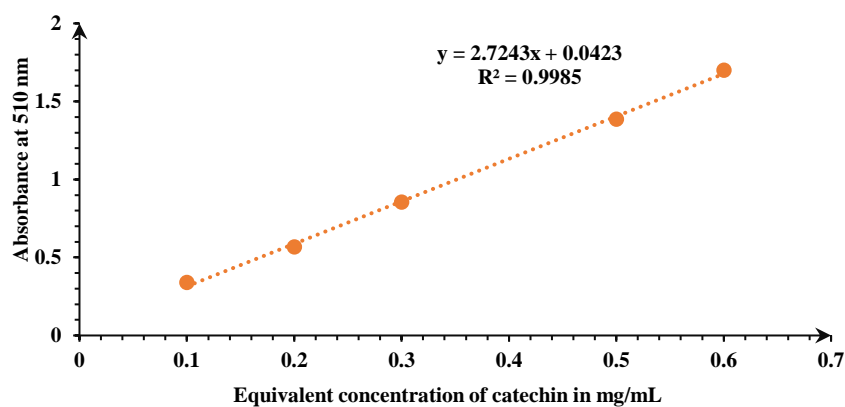


Figure 4: Calibration curve for catechin

Table 7: Concentration of (E. C), absorbance and average flavonoid content

Parts of the plant	Concentration of E. C (mg/mL)	Absorbance at 510 nm	E. C Content
Barks	0.20104247	0.59	50.26061741
Roots	0.213155673	0.623	53.28891825
Leaves	0.165069926	0.492	41.26748155

### Dosage of flavanoids

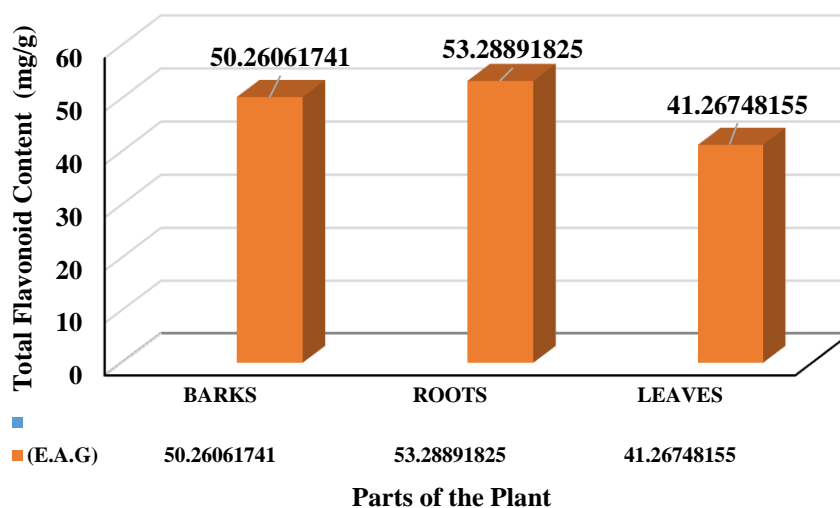


Figure 5: Average (E.C) content

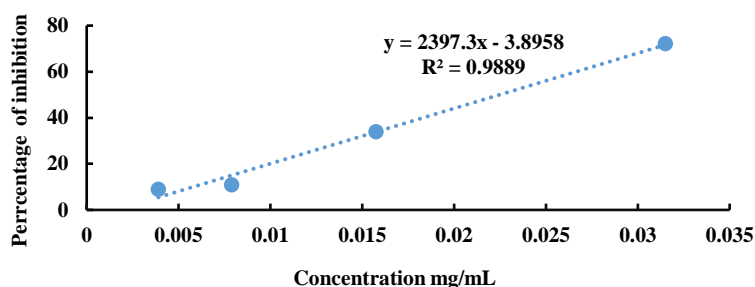
**Antioxidant activity test with DPPH**

*Calibration: Ascorbic acid*

**Table 8:** Percentage of inhibition according to the concentration of ascorbic acid

Concentration mg/ml	Averages in %PI	Standard deviation
0.0039	8.9	0
0.0079	10.963333	0.089566859
0.01575	33.936667	0.089566859
0.0315	72.18	0

**acide ascorbique**



**Figure 6:** Percentage of inhibition of ascorbic acid

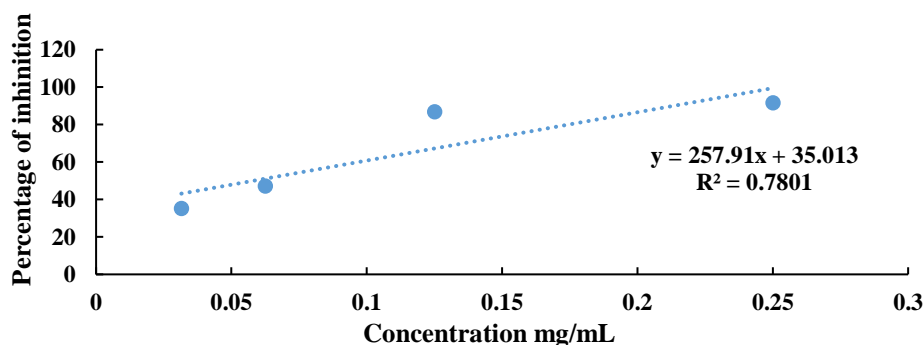
The IC50 of ascorbic acid is 0.022 mg/mL

*Ethanollic extract*

**Table 9:** Percentage of inhibition according to the concentration of the ethanollic extract

Concentration mg/ml	Averages in %PI	Standard deviation
0.0315	35.21	0.10392305
0.0625	47.27	0
0.125	86.9	0
0.25	91.63	0.31176915

**Ethanollic extract**



**Figure 7:** Percentage of inhibition of the ethanollic extract

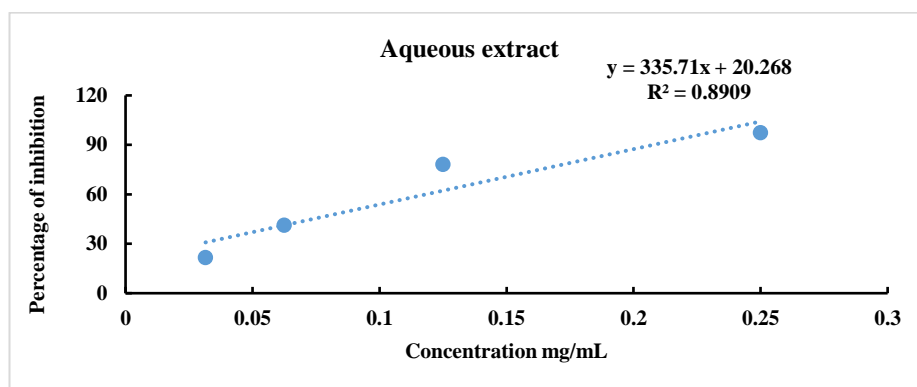
The IC50 of the ethanollic extract is 0.058 mg/Ml

*Aqueous extract*

**Table 10:** Percentage of inhibition according to the concentration of the aqueous extract

Concentration mg/ml	Averages in %PI	Standard deviation
0.0315	21.69333333	0.10392305

0.0625	41.24	0
0.125	78.24	0
0.25	97.34666667	0.31176915



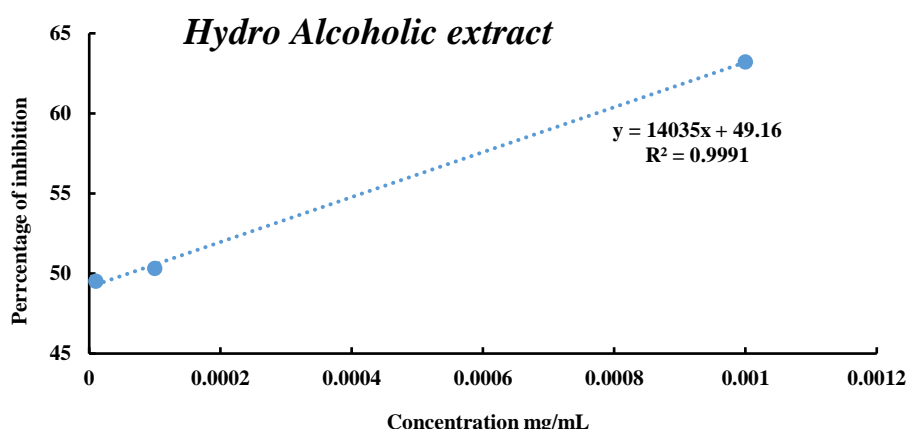
**Figure 8:** Percentage of inhibition of the aqueous extract

The IC<sub>50</sub> of the aqueous extract is 0.088 mg/mL

**Hydro alcoholic extracts**

**Table 11:** Results of the inhibitory percentage of the different extracts

Concentration of the extract mg/mL	%PI		
	Leaves	Barks	Roots
0.00001	54.058314	55.49724	49.52229
0.0001	56.711321	56.17948	50.31847
0.001	58.707644	58.6985	63.21656
0.01	64.696612	63.73655	86.70382
0.1	95.220484	95.91837	96.63372
0.2	95.561878	95.58824	97.06609
0.3	95.874822	95.34814	97.12786
0.4	96.102418	95.01801	97.34404



**Figure 9:** Percentage of inhibition of hydro alcoholic extract of roots

The IC<sub>50</sub> of the hydro-alcoholic extract of the root is 5.98 10<sup>-5</sup> mg/mL (0.0598 µg/mL) and that of the leaves and barks are lower 0.01 µg/mL.

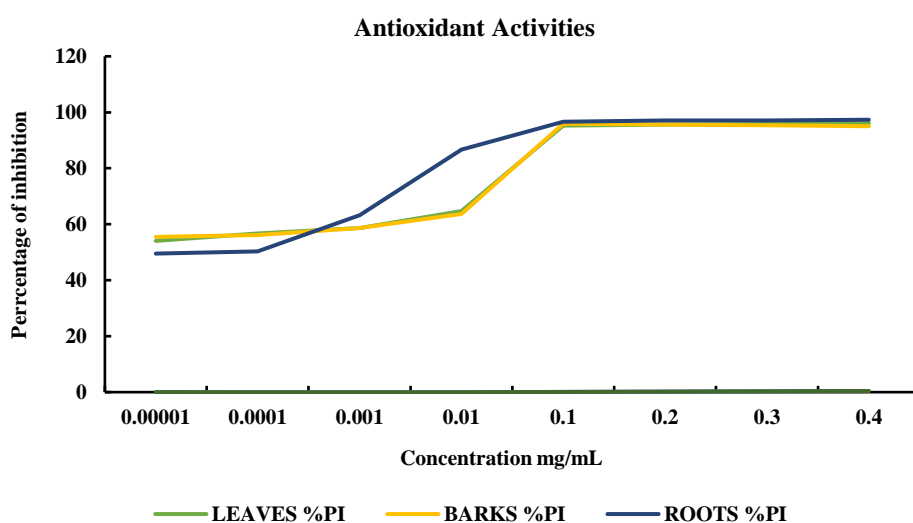


Figure 10: Capabilities of antioxidant activity of hydro ethanolic extracts

#### IV. Discussion

The results of chemical screening carried out on the ethanolic and aqueous extracts show the presence of several secondary metabolites of which one can quote the terpenes, the polyphenolics and the alkaloids and for the aqueous extract the polyphenols and alkaloids. These results characterize the richness of these extracts and allowed us to push the study towards the determination of the content of certain subfamilies of these secondary metabolites, namely polyphenols and flavonoids by assays and evaluation of antioxidant activities.

The results of the assay show that the content of phenolic compounds in the hydroalcoholic extract of leaves is higher, followed by the hydroalcoholic extract of barks and roots.

The studies that have been carried out on *Diospyros Mespiliformis Hochst Ex. DC* have only been focused on the confirmation of the allegations on ethno-pharmacological data of the plant and would have left an important part of information as for the nature, the belonging and even the quantification of the compounds responsible for the declared medical effects. Thus, despite the number of studies carried out on the plant, most of which were conducted in Nigeria, only two studies for the moment, to our knowledge, have been able to provide information on the quantification of certain families of secondary metabolites and those on certain parts of the plant, the leaves on a study of the plant carried out in Benin for one and the fruits for the other carried out in Zimbabwe[23-24]. The values of the content of polyphenolic compounds found in the different parts of the plant were recorded and the leaves held the highest values with  $272.6505346 \pm 29.96722596$  mg (E.A.G) /g (E.P) and  $562.4630542 \pm 29.96722596$  mg (E. A.A) /g(E.P) followed by the trunk with  $249.5779403 \pm 6.170993866$  mg (E.A.G) /g (E.P) and  $522.0689655 \pm 6.170993866$  mg (E.A.A) /g (E.P.) followed by roots with  $225.0984806 \pm 34.35863973$  mg(E.A.G)/g(E.P) and  $479.2118227 \pm 34.35863973$  mg(E.A.A)/g(E.P).

The flavonoid content in the different parts of the plant was calculated on equivalence support in mg of catechin per gram of crude extract. The highest value was obtained with the roots with  $53.28891825$  mg (E.C) /g (E.P) followed by the barks with  $50.26061741$  mg (E.C) /g (E.P) then the leaves with  $41.26748155$  mg (E.C) /g (E.P). The distribution of flavonoids in the parts of the plant is governed by a number of genetic and environmental factors which can induce to the massive production of compounds of a certain class of flavonoid as a barrier for the reinforcement of the tissue, as a dressing, or as a filter to prevent microbial invasion during the absorption of mineral elements from the soil by the roots. For the trunk these compounds will be mainly for a preventive answer to manage possible attacks of aggressors.

The value of the concentration at 50% inhibition for the ethanolic extract is higher than for the aqueous extract. This difference is to be looked in the content of phenolic compounds of the two extracts, the phenolic compounds being soluble in the two solvents because of their polarity. The criterion of differentiation on the content would then be related to the extractions. These suggest that the majority soluble compounds in polar solvents would be carried away during the first extraction and then the second one coming only for the exhaustion of the remaining fraction. Although this hypothesis seems to be more accepted. The amphiphilic character of ethanol could also play an additive role in the extraction in the case of aglycones and heterosides presence in the plant. In this case the total polyphenol content of alcoholic extract increases greatly than of the aqueous extract polyphenol content.



The antioxidant activity of the hydroethanolic extract was studied by means of the DPPH<sup>•</sup> reduction test. It was monitored by UV absorbance of the amount of DPPH<sup>•</sup> present in the solution titrated by a known concentration of plant extract. The absorbance data were then tabulated and expressed as percent inhibitor. The results, expressed as inhibitory percentage, were obtained by means of the formula of inhibitory percentage (IP) presented above. The results of the extracts of the different parts of the plant show that the inhibitory percentage increases as the concentration of the plant extract increases. Although this increasing trend can be divided into three levels of variation with a first slight increase between 0.00001 and 0.0001 mg/mL and then a jump in the activity value between 0.001 and 0.2 mg/mL and then approximately constant with % PI values of 97 (root), 96 (bark) and 95 (leaf). However, from a concentration around 0.001mg/mL, the inhibitory activity value of the root extracts is slightly higher than that of the other parts. The activity values of the bark and leaf extracts give similar results with respect to DPPH. These values seem to be in agreement with the phenolic content of the extracts of the different parts studied above although a global overview of the data allows to see the evidence if we rely on the fact that in relation to phenolic compounds in the fight against free radicals, the role of flavonoids is highly considered. The value of the concentration equivalent for 50% inhibition of 0.00005 mg/mL for the roots is less than 0.000001 mg/mL for the leaves and barks (Table 11). These values compared to the value of 0.09 mg/mL of the results of Adamu and al. [24].in this work are much lower than the latter.

## V. Conclusion

The evaluation of the phenolic composition content of the different parts of the plant and the antioxidant activity of the aqueous, ethanolic and hydroalcoholic extracts of the plant *Diospyros Mespiliformis Hochst Ex. DC* show a good correlation between the total phenolic content and the antioxidant capacity of the extracts although the latter seems to be more in agreement with the content of flavonoids in these extracts. The phenolic content values obtained during the tests are still lower than those of a study carried out by MAHURO et al. [25]. On the other hand, the antioxidant activity is largely superior to those found by ADAMU et al.[24] in their studies. This partly demonstrates the reputation of the plant in traditional medicine. The low concentration of the daughter solutions (diluted raw extracts) for the study of antioxidant activity having produced important antioxidant activities, translates the richness of the plant in biologically active compounds. The high content of these compounds in these extracts can be attributed to the nature of the solvent extraction, itself associated with the combined extractive effect of the two solvents ethanol and water on the plant matrix. The comparative study of the total phenolic content and the antioxidant effect of the plant extracts of these different solvents taken separately seems to confirm this statement.

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