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The Study of the Antioxidant Activity of Oil Extracts from the Seeds of Two Sorts of Red Onion in Morocco

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Abstract :*This work aims at study the antioxidant activity of oils extracted using hexane and ethanol from the seeds of two types of red onion in Morocco, using the method of trapping the free radical DPPH° (2,2-diphenyl-1 picrylhydrazyl). This study was carried out by comparing it to a natural antioxidant ascorbic acid. Both ethanol extracted from the sample of Doukkala (EED) and sample Tetouan (EET) respectively show an effective concentration IC50 33.63 g / ml and 34.20µg / ml compared to the IC50 ascorbic acid, which is 10.79 g / ml with a reaction time reaches equilibrium TEC50 in 5 minutes, while the effective concentrations of EHD and EHT extracts have as IC50 values 54.40µg / ml and 57.11µg / ml. These results were confirmed by calculating the percentage of the anti-radical efficiency which shows that the EED and EET extracts possess anti-radical efficiency 11% higher than the EHD and EHT extracts (5%) are six times lower than that of ascorbic acid (69%).*

Keywords: *red onion, antioxidant activity, ascorbic acid, DPPH°, scavenging activity, effective concentration*

I. Introduction

The toxicological potential risk posed some synthetic antioxidants used in food and cosmetics [1, 2], is becoming increasingly worrying. This risk has led research work on the use of extracts of medicinal plants traditionally known for their health benefit for a long time [3]. These works have implemented techniques to evaluate the antioxidant power of many plant extracts [4-6]. Indeed vegetable oils contain a sufficient amount of beneficial antioxidant for Man [7] can prevent many types of cancer , inflammatory and cardiovascular disease [8] that qualify them to be used as additives in food industry [6, 9], pharmaceutical and cosmetics to replace synthetic antioxidants causing allergic effects and cancer [10].

To participate in the evaluation of new oils extracted from plants of the Moroccan flora [11], we studied the antioxidant capacity of the oil extracted from the seeds of two types of red onion (red onion of Doukkala and oingon red of Amposta) [12] in two regions of Morocco by using the method of trapping the free radical DPPH (2,2- diphenyl - 1 picrylhydrazyl) [13-16].

II. Materials And Methods

2.1. Plant Material

The red onion seeds of Doukkala were collected in June 2012 in the Chaouia region in the west of Morocco and the seeds of red onion Amposta of Téouan region located in northern Morocco were harvested in July 2013. The seeds are then cleaned, dried and finely ground.

2.2. Preparation Of Oily Extracts

100g of powder are placed in a ground glass flask with 300 ml hexane or ethanol. The mixture is then heated to reflux for 20 minutes and then left to stir at room temperature for 12 hours. After filtration, the solvent was removed under reduced pressure. Oil red onion seeds of Doukkala are obtained with a yield of 17% and oil seeds of red onion Amposta is obtained with a yield of 16%.

2.3. Evaluation Of The Antioxidant Activity Of Oil Extracts

The antioxidant activity was evaluated by measuring the DPPH° radical scavenging power (2,2-diphenyl - 1 picrylhydrazyl). The antioxidant power of oils tested was estimated by comparison to a natural antioxidant ascorbic acid. For each concentration, the test is performed three times.

2.3.1. Calibration Curve Of DPPH° Solution

Before starting the tests of antioxidant activity, stability and linearity range of solutions DPPH° radical must be evaluated. We have prepared six solutions of DPPH° at increasing concentrations ranging from 0 µmol, 5, 10, 15, 30, 50 and 60 µmol in solution in methanol. The results obtained are shown graphically in Fig.1. For each concentration of the solution DPPH° on measured its absorbance at 0 min and 60 min. The resulting graph has the correlation coefficient $R^2 = 0.983$.

The activity of the scanning radical DPPH° was measured according to the protocol described by Lopes- Lutz and al. [17] where each of 100µl of methanol solutions of the oily extracts tested at different concentrations after having mixed with 1300 µl of a methanol solution of DPPH° at 0.004 %. After an incubation period of 30 minutes at laboratory temperature, the absorbance is read at 517nm. The inhibition of free radical DPPH° through was also performed ascorbic acid. The kinetics of the reaction and parameters for the calculation of the antioxidant activity for ascorbic acid and oily extracts are then determined.

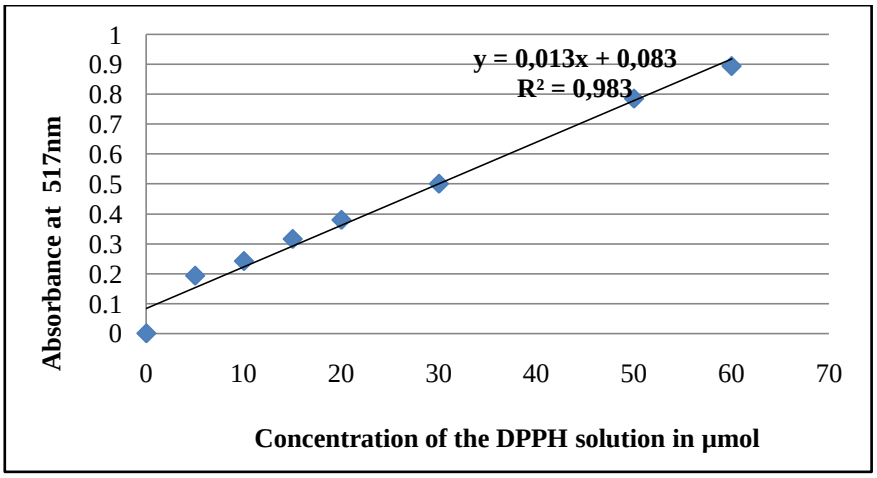


FIGURE 1: Calibration curve of the DPPH solution

2.3.2. Determining the Percentage Of Inhibition

Inhibition of free radicals in percentages (I %) is calculated using the following formula:

I% = [(Abs negative control – Abs sample) / Abs negative control] × 100 (1),

With Abs sample: Sample absorbance,

Abs negative control: absorbance of the negative control.

The reaction kinetics of oil extracts and ascorbic acid with DPPH was entered for each concentration.

To get the effective concentration IC50, (defined as the concentration of antioxidant required to reducing the concentration of initial DPPH° 50%) for each extract, we traced the curves of the oil concentration and ascorbic acid function of DPPH° inhibitions percentages obtained.

2.3.3. Determination Of Balance Time TEC50

The parameter TEC50 was defined as the time reaches to equilibrium with an antioxidant concentration equal to IC50.

2.3.4. Determination Of The Anti-Radical Efficiency EA

Both IC50 and TEC50 factors can be combined to obtain the parameter of anti-radical efficiency:

EA = 1 / IC50 TEC50 × (2).

III. Resultants And Discussion

The analysis of the chemical composition that we have already made, has made it possible to identify the various major constituents of oils red onion seeds of Doukkala region [18] and the red onion of Amposta in the region of Tetouan. Other polyphenols with antioxidant molecules are present in these extracts could be estimated by anti-radical reaction with DPPH°.

3.1. Antioxidant Activity

3.1.1. Reaction kinetics The reduction kinetics of free radical DPPH˙ for each concentration of ascorbic acid is shown by the following Fig.2:

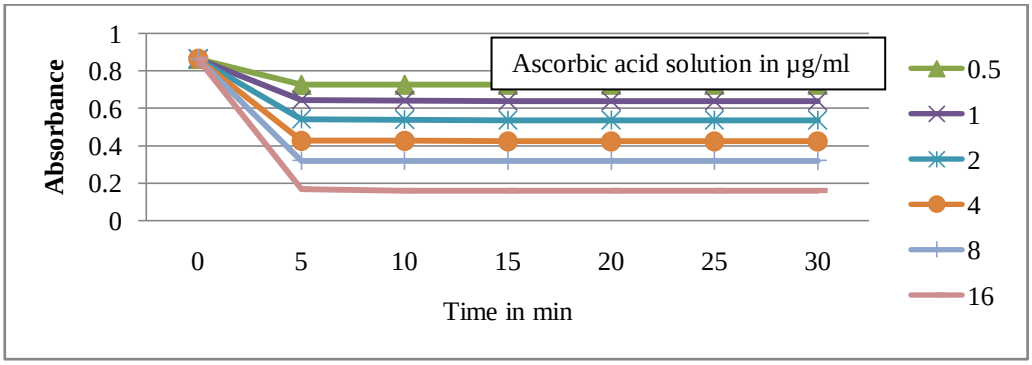


FIGURE 2: reduction kinetics of DPPH° with ascorbic acid

The free radical DPPH° reduction kinetics obtained by reaction with each concentration of the hexane extract oil red onion seeds of Doukkala (EHD) is presented by the following Fig.3:

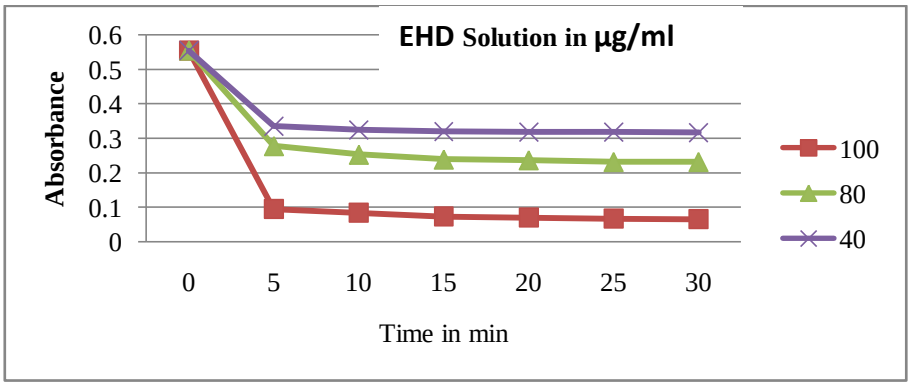


FIGURE 3: reduction kinetics of DPPH° with EHD

The free radical reduction kinetics DPPH° obtained by reaction with each concentration of oil hexane extract of red onion seeds Amposta Tetouan (EHT) is presented by the following Fig4:

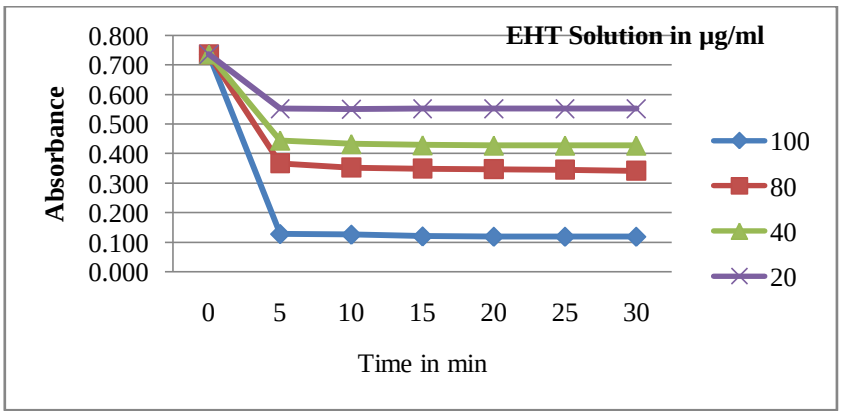


FIGURE 4: reduction kinetics of DPPH° with EHT

The reduction kinetics of free radical DPPH° obtained by reaction with each concentration of the ethanol extract of red onion seeds of Doukkala (EED) is presented by the following Fig.5:

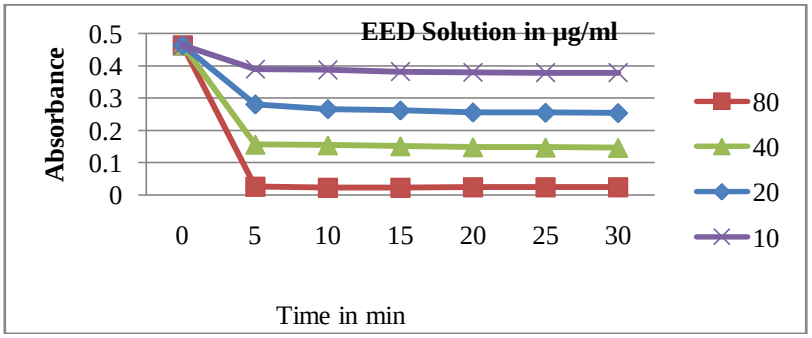


FIGURE 5: reduction kinetics of DPPH° with EED

The kinetics of reduction of free radical DPPH° obtained by reaction with each oil concentration of the ethanol extract of red onion seeds of Amposta Tetouan (EET) is presented by the following Fig.6:

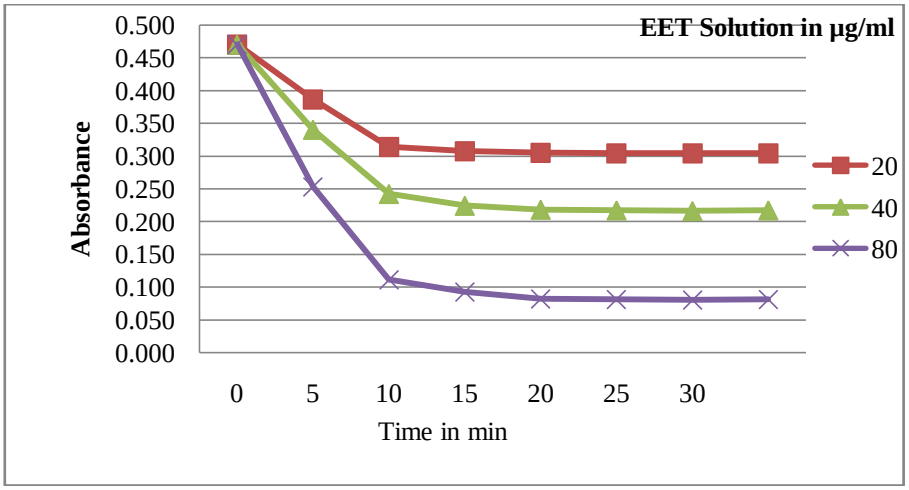


FIGURE 6: reduction kinetics of DPPH° with EET.

We note that for all the samples examined (ascorbic acid and extracts), the curves obtained show two areas:

- the first zone high kinetic trapping of the radical observed after the first five minutes for the ascorbic acid and the ethanol extract after the first 10 minutes, while for the hexane extracts this area extends to 15 min.
- the second zone trapping low kinetic area of DPPH radical or area tend toward equilibrium observed after 5 minutes to ascorbic acid, and the hexane extract of the sample of Tetouan, while this zone is observed after 15 minutes for the rest of the extracts. Thedecrease in the concentration of free radical DPPH° is also reflected in the decrease in absorbance during reaction time to exhaustion of the hydrogen donor antioxidant capacity.

3.1.2. Determining The Percentage Of Inhibition

Measuring the absorbance (or optical density) of different antiradical reactions was carried out by UV spectrophotometry at 517 nm. The percentages of inhibition are calculated using the absorbance values obtained using the equation (1). From the PI values obtained have been plotted curves representing the variation of percentage inhibition against concentration of ascorbic acid. In the same principle PI variation curves as a function of the concentrations of other extracts are shown in Fig.7 and Fig.8.

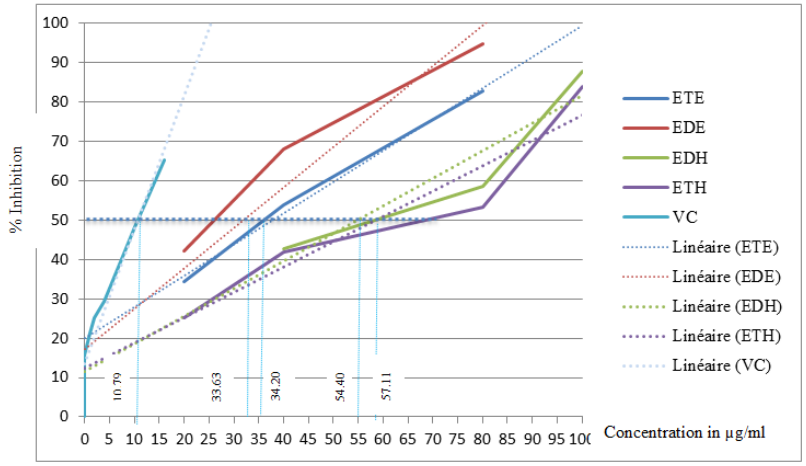


FIGURE 7: % Inhibition of DPPH° against concentration in µg/ml of the extracts (EHD, EET, EED and EED)

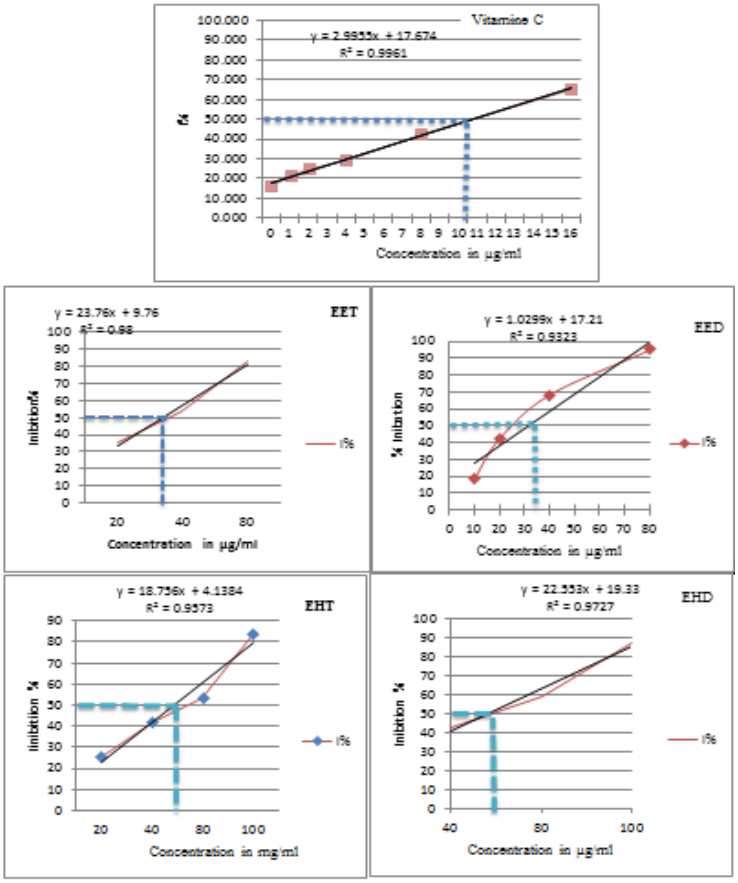


FIGURE 8: Calculate the IC50 of ascorbic acid and extracts EED, EET, EHD and EHT

The curves obtained show that the percentage of inhibition of the free radical increases as the concentration increases. For revealed Percent inhibition of free radical DPPH° in a concentration of 16 µg / ml of ascorbic acid is 65.22%, while for a concentration of 80µg / ml of the EED and extract the EET extract the percent inhibition of free radical DPPH° est 94.83% and 82.77% respectively. For a concentration of 100 mcg / ml of the hexane extracts EHD and EET gives a percentage inhibition of the free radical DPPH˙ approximately 87,81 and 83%, 85%. From the percentages of inhibition of various concentrations of the oily ethanol extracts sand hexane achieved compared to percent inhibition of ascorbic acid is observed that the oily ethanol extract EED has the best ability to inhibit free radical DPPH°.

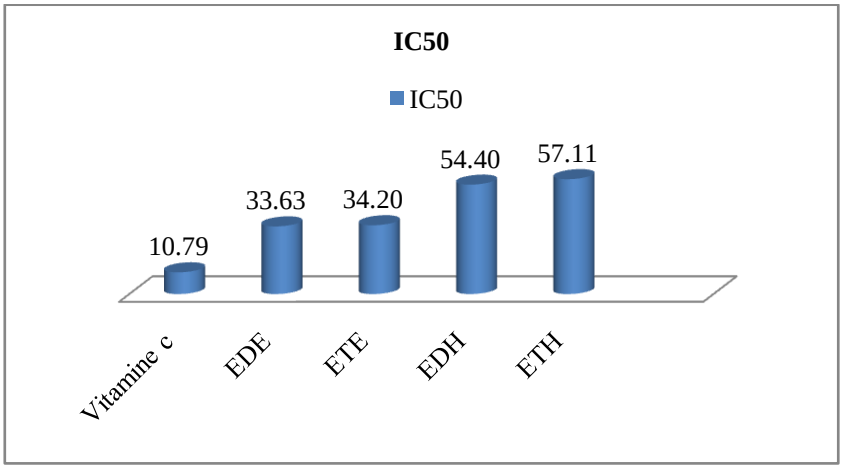
TABLE 1: % inhibition of free radical DPPH° according to extracts concentrations

Sample	Concentration in µg/ml	% inhibition
Ascorbic acid	16	65.22
EED	80	94.83
EET	80	82.77
EHD	100	87.81
EHT	100	83.85

3.1.3. Determination of IC50

The IC50 parameter is inversely related to the antioxidant capacity of a compound, because it expresses the amount of antioxidant required to reduce the concentration of free radical of 50%. Overthe IC50 value is low is more antioxidant activity of a chemical compound is high. IC50 values are extrapolated from the following graph 1for a PI of 50% of radical DPPH°.

The IC50 values determined graphically in µg /ml expresses the effective concentration of the sample to reduce by 50% the amount of free radical DPPH°. The results of various extracts are summarized in Table 2. It is noted that both EED and EET extracts could reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH°) of purple coloration diphenylpicrylhydrazine yellow-colored staining with IC50 de 33.63µg / ml showing antioxidant activity three times lower than that of ascorbic acid, while the extract EHD and EHT extract have as respective IC50 concentration 54.40 µg / ml and 57.11 µg / ml, which shows that the hexane extract it are less effective compared the ethanol extracts which may be due to the presence of more polar antioxidants extract with ethanol graph.2:

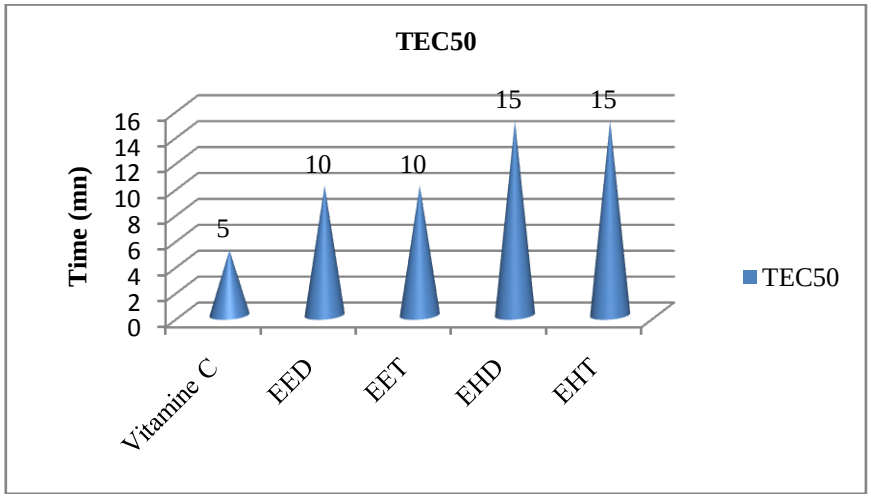


GRAPH 1: inhibitory concentration IC50.

It has been shown that antioxidant molecules such as ascorbic acid, tocopherols, flavonoids and tannins and reduce fade DPPH due to their ability to give hydrogen, which shows that these extracts can contain these substances with antioxidant capacity.

3.1.4. Determining TEC50

We chose the equilibrium state as measurement period where it appears that the reaction between the free radical DPPH and molecules with antioxidant activity of the oily extract reaches the equilibrium phase. The time required to reach steady state depends on the reactivity antioxidants and concentrations used. The results obtained are summarized in Table 2.



GRAPH 2: parameters TEC50

The TEC50 extracts EED and EET corresponding to the reaction of 50% of radical with these extracts is twice less than that of ascorbic acid and the TEC50 of EHD and EHT extracts are also three times less. To characterize the effectiveness of these antioxidants, one calculates the efficiency parameter antiradical EA.

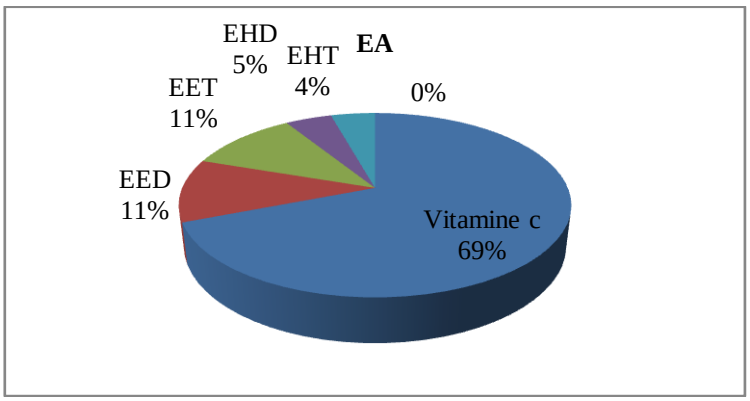
3.2. Parameter Anti-Radical Efficiency:

The anti-radical efficiency combines the two IC50 TEC50 according to parameters and equation (2) to calculate easily in order to characterize the behavior of a substance or mixture as an antioxidant. The calculation parameters of the antioxidant activity of different extracts are summarized in Table 2, which allows us to classify these in relation to the antioxidant activity of ascorbic acid Table 2,

Table 2: The calculation parameters of antioxidant activity

	IC 50 (µg/ml)	TEC50 (mn)	EA (ml/µg.mn)	% EA	ranking
Ascorbic acid	10,79	5	0,01854	69	strong
EED	33,63	10	0,00297	11	medium
EET	34,20	10	0,00292	11	medium
EHD	54,40	15	0,00123	5	Toolow
EHT	57,11	15	0,00117	4	Toolow

From these results, it seems that EED extract has antioxidant activity more effective than the rest of the extracts. The following graph determines the percentage of effective anti-radical EA:



GRAPH 3 : The percentage of anti-radical effectiveness EA

IV. Conclusion

The study of the antioxidant activity of oil extracts from the seeds of two sorts of red onion in Morocco trapping method according to the free radical DPPH° showed that these extracts have moderate antioxidant activity comparable to that of ascorbic acid. The percentage of anti-radical efficiency obtained for each sample shows that both ethanol extracts exhibit anti-radical efficiency of 11% 6 times lower than the 69% ascorbic acid. While both hexane extracts have an EA% to about 5% 14 times lower than that of ascorbic acid 69%, allowing

us to conclude that the oil of EED and EET extracts has an average antioxidant power capable inhibiting the action of free radicals.

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