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Activity Study Antioxidant Extracts Of Flowers and Grains of Chamaerops Humilis L. Western Morocco

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Abstract : *This study aims to evaluate the phytochemical content and antioxidant activity of the extracts of flowers and grains, with two solvents hexane and ethanol, harvested in the region of Chaouia-Ouardigha west of Morocco. However, total phenols are measured by the spectrophotometric method and the antioxidant activity was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH •) as a free radical scavenger, taking as witness antioxidant ascorbic acid. The results revealed a wealth of total phenols ethanol extracts of EEG grains and ethanol extracts of flowers by EEF content respectively 533,14 mg /g and 524,06 mg /g compared to other hexane extracts of EHG grains and flowers EHF . While the extracted EEG reveals a percentage of inhibition of approximately 84.05% to the effective concentration IC50 2,38µg / ml with a reaction time in TEC50 balance of 25 min and a percentage of the antiradical activity of 25%, which shows lower than that of vitamin C (68%). The antioxidant activity of the other three extracts remains significantly lower than that of the EEG extract.*

Keywords*: chamaerops humilis L., total phenols, antioxidant activity, IC50, TEC50, anti-radical.*

I. Introduction

The use of synthetic molecules with antioxidant power, is currently challenged involved because of their potential toxicological risk [1]. Now, new plant sources having a natural antioxidant power are sought [2]. Indeed, polyphenols are natural compounds widely used in plants having a remarkable antioxidant power and are increasingly used due to their beneficial health effects [3]. The role of natural antioxidants attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [4, 5]; they are also used as additives in food, pharmaceutical and cosmetic [6].

Scientific research has been developed for the extraction, identification and quantification of these compounds from medicinal plants [7, 8].

The chamaerops humilis L. is a medicinal plant that grows wild in the region of Chaouia-Ouardigha (Morocco) in the wild, in height between 50 cm to 1m. It enjoys great popular support in Morocco as a remedy against diabetes, digestive diseases disorders, spasm, toning and gastrointestinal disorders. [9]

Several studies have been made on the chamaerops humilis L.. The authors suggest that the presence of flavonoids, tannins, phenolic acids and antioxidant activity and methanol extract of leaves corrosion [10, 11]. However, the study of the antioxidant activity of the extracts of the flower, and grain are little studied. Our work is on the quantitative analysis of total phenols content of flower extracts, and grains of the chamaerops humilis L. and the evaluation of the antioxidant activity [12, 13] of these extracts using free radical DDPH•.

II. Materials And Methods

2.1. Preparation of plant material

The flowers are harvested during the month of March 2014 and the grains are harvested during the month of July 2014 from the Chaouia- Ouardigha region located west of Morocco. The flowers are dried, crushed and stored in glass jars away from light and moisture, and the seeds are separated from the pulp, dried, peeled, finely ground and then packaged to protect from light and moisture.

2.2. Preparation of extracts

200 g of the powders obtained from flowers and seeds are extracted with 600 ml of ethanol or hexane using the Soxhlet for 6 hours. Ethanol or hexane was then removed under reduced pressure by rotavapor obtained oils that are yellow and viscous.

2.3. Moisture determination

The humidity is the amount of water contained in the plant material. The moisture content of plant parts flowers and grains was determined by the method of drying in an oven at 105 ° C ± 5 ° C.

The humidity is expressed as a percentage and calculated by the following formula :

$$H (\%) = (M1 - M2) / M1 \times 100$$

H% = humidity percentage.

M1 = sample weight in grams after harvest (fresh plant).
M2 = weight of sample in grams after drying (dried plant).

2.4. Determination of extraction performance

According to the AFNOR standard (1986), the yield of extract is defined as the ratio between the mass of extract obtained after extraction (M ') and the mass of the plant material used (M). The yield is expressed in percentage and is given by the following formula :

RE (%) = (M '/ M) x 100

RE : extract yield;
M : mass of extract obtained after extraction;
M : mass of dry plant matter used in grams and is 100 g

2.5. Content of total polyphenols

Polyphenols are determined by the Folin-Ciocalteu [14]. This method originally described by Slinkard and Singleton [15], allows knowing the total polyphenols content of a given sample. 0.5 mL of the oily extract and 1 ml of a sodium carbonate solution (20 g / l) are added to 1 ml of 10% (v / v) of Folin-Ciocalteu reagent with gallic acid as standard. After reaction for 2 h at room temperature, the absorbance was measured at 760 nm. The tests were performed three times to ensure reproducibility of results. The total phenolic content was expressed as mg gallic acid equivalent per gram of sample.

2.6. Radical activity by the free radical DPPH•

The test by the free radical DPPH• is performed following the method described by Burits and Bucar (2000) [16]. 100 µl of the solution of each extract at different concentrations ranging from 1.25 µg /ml to 150µg /ml of the ethanolic extract grains EEG and the hexane extract grains EHG, and for the ethanolic extract flowers EEF and the hexane extract flowers EHF and for vitamin C concentrations ranging from 0,5-16µg /ml. These extracts are mixed with 1300 µl of a methanolic solution of the free radical DPPH• 0.004%. After an incubation period of 30 minutes at laboratory temperature, the absorbance is read at 517nm. The inhibition of free radical DPPH• by Vitamin C was also analyzed at the same concentration. Determining the kinetics of the reaction of the oily extracts and vitamin C as standard antioxidant and the parameters of the antioxidant activity.

2.6.1. Determining the percentage of inhibition

According Sharififar and al, (2007) [17], inhibition of free radical DPPH• Percentage (I%) is calculated as follows :

I% = (A white - A sample) x100 / A white

With: White A: Absorbance of the blank and sample A: Sample Absorbance.
All assays were performed three times.

Reaction kinetics extracts and vitamin C with DPPH• was included in each concentration examined. The concentrations of the extracts and of vitamin C according to the percentages of DPPH• inhibited, have been traced to the end of the reaction to obtain the IC50 index. This parameter is defined as the concentration of antioxidant required to reduce the concentration of DPPH• Initial 50% (Sharififar et al., 2007) [17].

2.6.2. Determination of time to balance TEC50

The TEC50 parameter is defined as the time reaches to equilibrium with an antioxidant concentration equal to IC50. This time is calculated graphically (Sharififar et al., 2007) [17].

2.6.3. Determination of the anti-radical efficiency EA

Both IC50 and TE50 factors are combined to obtain the anti-radical efficiency parameter according to the following equation :

EA = 1 / IC50x TEC50

III. Results And Discussion

3.1. Moisture determination

To ensure proper conservation, the water content must be less than or equal to 10 % (Paris and Moyse , 1965) [18] The results of this analysis revealed a moisture content less than 10% for grain (4.98%), which gives a better long-term preservation by against a higher moisture content to 10% for the flowers 18%.

3.2. Determination of extraction performance

The calculation yields relative to the total weight of the powder flowers and grains of the plant chamaerops L show that the two parties had given the masses dry extract greater than 1 g / 100 g as the extract of grains by the two solvents (ethanol and hexane) which gave the highest yield. And grain yield by hexane

(6%) and twice higher than that of the flowers (3%). The ethanol extract of both parties are at least equal 8.82% for flowers and 9% for grains. The appearance and the color extracts are shown in Table 1 below.

TABLE1: Aspects, colors and yields extracts of flowers and grains

The studied parts of Chamaerops humilis L	Hexane extract of flowers (EHF)	Ethanol extract of flowers (EEF)	Ethanol extract of grains (EEG)	hexane extract of grains (EHG)
Aspect	Viscous oil	Viscous oil	Viscous oil	Clear oil
Color	Dark yellow	Dark yellow	Dark Brown	yellow
yields (%)	3	8,82	9	6

The best yield was about 9% obtained for the extraction of flowers and grains with ethanol.

3.3. Determination of polyphenols

Quantitative analysis of total phenols extracts are determined by spectrophotometric according to the procedure of Folin -Ciocalteu from the equations of linear regression of the calibration curve of gallic acid ($y = 0.0513 + 0,0033x$, $R^2 = 0.9919$) (Figure 2) and expressed in mg gallic acid equivalent (table 2).

The results show that the highest value was obtained for the extracted EEG followed by EEF extract and lowest for the EHF extract. However, the content of total polyphenols found in the extracted EEG, EE, EHG, EHF respectively 533,14mg/g, 524,06 mg/g, 21.62 and 8.3 mg/ g. It is found that ethanol extracts EEF, EEG and contain a higher amount of polyphenols that hexane extracts EHF and EHG

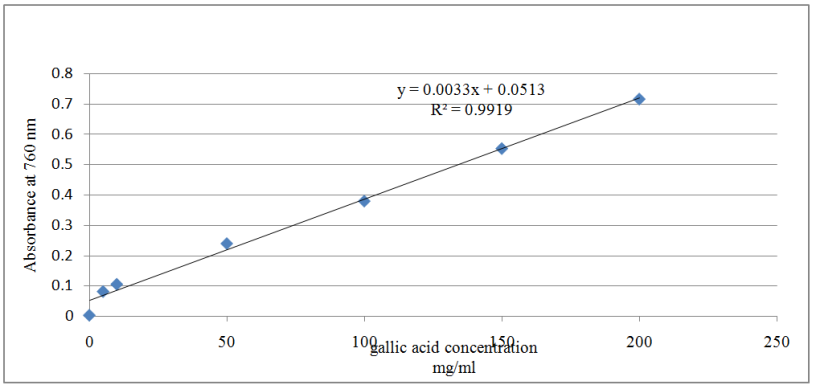


FIGURE 1: Gallic acid calibration curve for the determination of total phenols oily extracts EHF, EHG, EEF and EEG.

TABLE 2: Results of the determination of total polyphenols extracts oily EHF , EHG , EEF and EEG.

Oily extracts	Content of total polyphenols (a)
EHF	8,3
EEF	524,06
EHG	21,62
EEG	533,14

(a) mg gallic acid equivalent per g of extract (EAG mg / g of extract)

3.4. Study of antioxidant activity

3.4.1. Reaction kinetics

A different concentrations of the samples analyzed Vitamin C, extracts of flowers and grains of chamaerops L, the DPPH• reduction kinetics is followed over time until equilibrium results in the presence of a bearing. The results are shown in Figure 2.

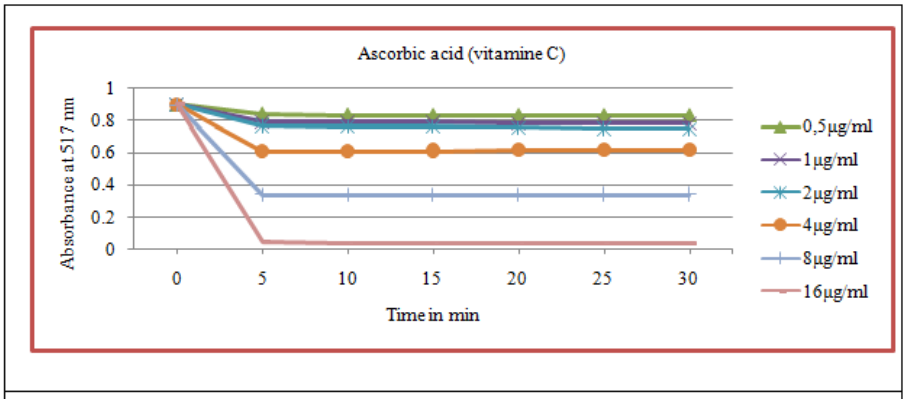
We note that for the compounds examined ascorbic acid and extracted four, the reaction is biphasic which results in a rapid decrease in absorbance within the first minute followed by a slower step until the stage of the balanced. There are two areas :

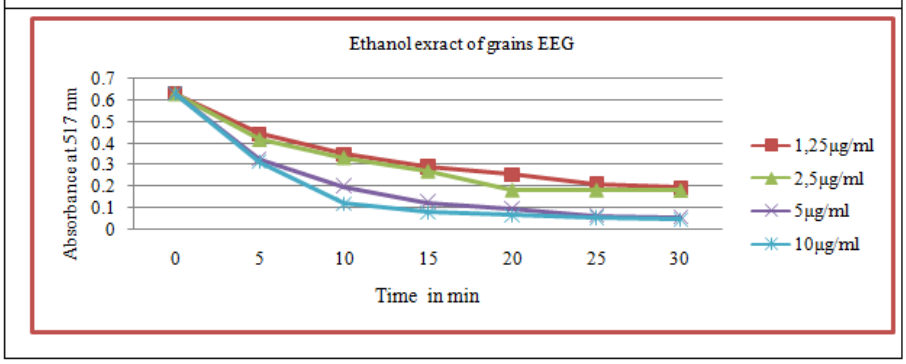
¬ The first zone characterized by a high radical trapping kinetics observed after the first five minutes for ascorbic acid and after 20 minutes for other extracts of flowers and seeds.

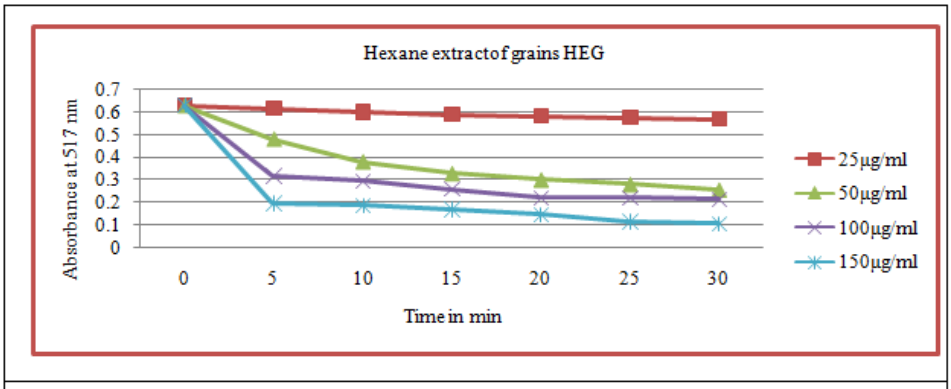
¬ The second zone marked by low DPPH• radical trapping kinetics zone or tendency toward equilibrium observed after five minutes for all concentrations of ascorbic acid, except this zone is observed after 20 minutes for extracts grain and flowers.

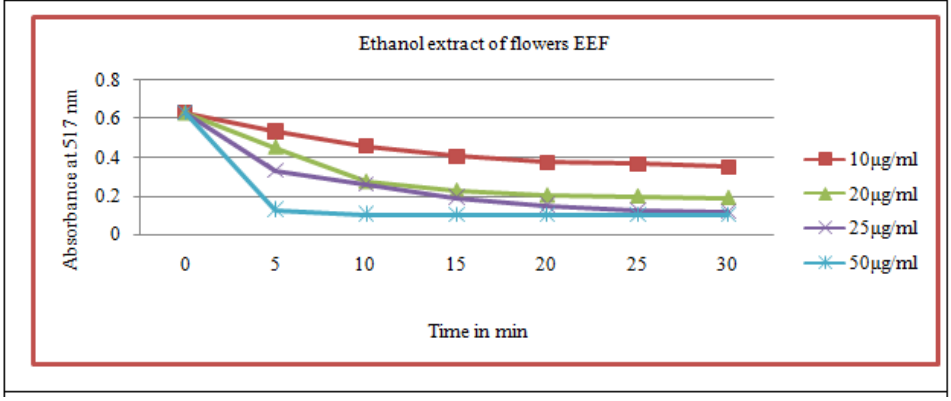
The reaction between the free radical DPPH• and ascorbic hydrogen donors acid reached equilibrium after a very short time compared to extracts chamaerops humilis L. The antioxidant activity is dependent on the

mobility of the hydrogen atom of the hydroxyl group of phenolic compounds of the previews. In the presence of a free radical DPPH•, hydrogen H is transferred to the latter to convert it into a stable molecule DPPH•, this causes a decrease in the concentration of free radical and also the absorbance over time reaction until the completion of the antioxidant hydrogen donor.









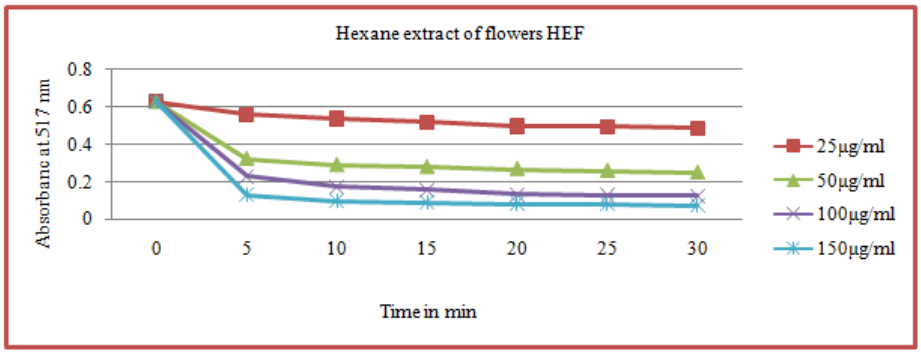


FIGURE 3: Reduction Kinetics of free radical DPPH• Flower extracts and Chamaerops humilis L. grains and vitamin C

3.4.2. Percentage inhibition

Figure 4 below shows the measurement results of the percentage of inhibition at the free radical DPPH• depending on the concentration of the tested extracts.

They show that the percentage of inhibition of the free radical increases with increase in the concentration either ascorbic acid or extracts of flowers and grain chamaerops L.

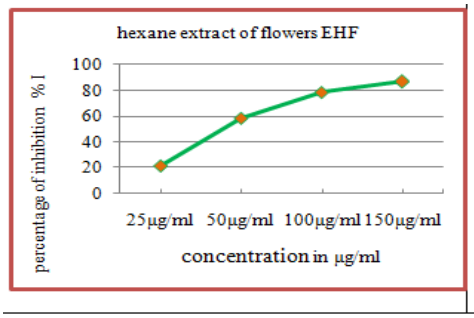
From the results summarized in Table 3, it is noted that the percent inhibition of ascorbic acid is below that of the extract EEG and superior to the rest of EHF extracts, and EHG EEF of chamaerops L.

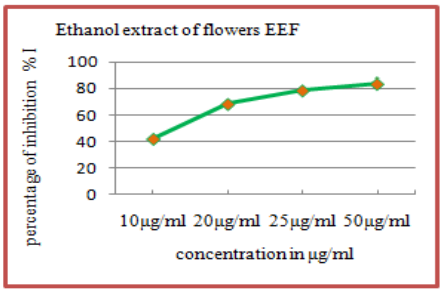
To a concentration of 10 µ / ml was extracted EEG showed a percent inhibition of free radical DPPH• in 84.05%, while for a concentration of 16 µg/ml of vitamin C. The percentage inhibition of the free radical DPPH• is of 95.68%, which corresponds to a 100% inhibition at a concentration of 11.68 µg / ml of the extract EEG. Whereas a concentration of 50 µg / ml of EFE, the percentage inhibition of the free radical DPPH• is 83.65%. As against the percentages of inhibition of EHG and EHF extracts are respectively 80.05% and 87.19% for a much higher concentration of 50 µg /ml.

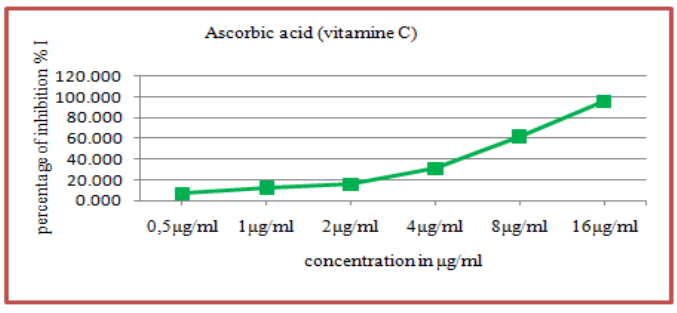
The oily extract EEG shows a percentage of inhibition of free radical DPPH • more important than the other extracts. We can conclude that it contains the largest amount of antioxidants.

TABLE 3: % inhibition of free radical DPPH according to excerpts concentrations

Extracts chamaerops humilis L. and vitamin C	Concentration in µg / ml	% of inhibition
vitamin c	16	95,68
EEF	50	83,65
EHF	150	87,19
EHG	150	80,05
EEG	10	84,05







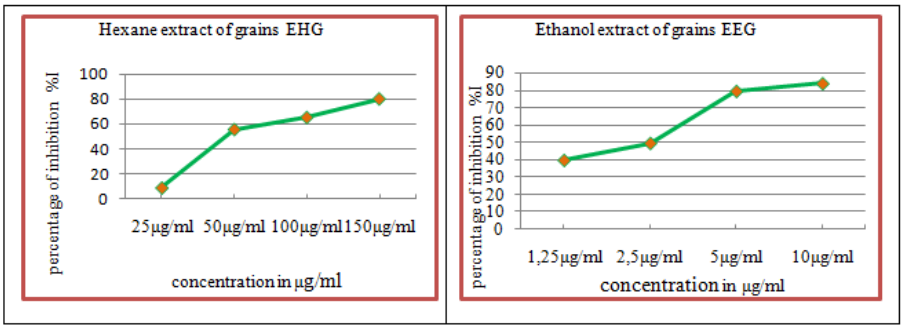


FIGURE 4: Percentage inhibition of extracts Oily Flower and Chamaerops humilis grains and vitamin C.

3.4.3. Determination of IC50

The IC50 is inversely proportional to the antioxidant capacity of a compound, because it expresses the amount of antioxidant required to reduce the concentration of the free radical of 50%. It is found that the lower the value of IC50, the smaller the antioxidant activity of a compound is high.

5 shows the calculation of the IC50 of ascorbic acid and EHF extracts, EEF , EEG, EHG of chamaerops L. Figure 6 :

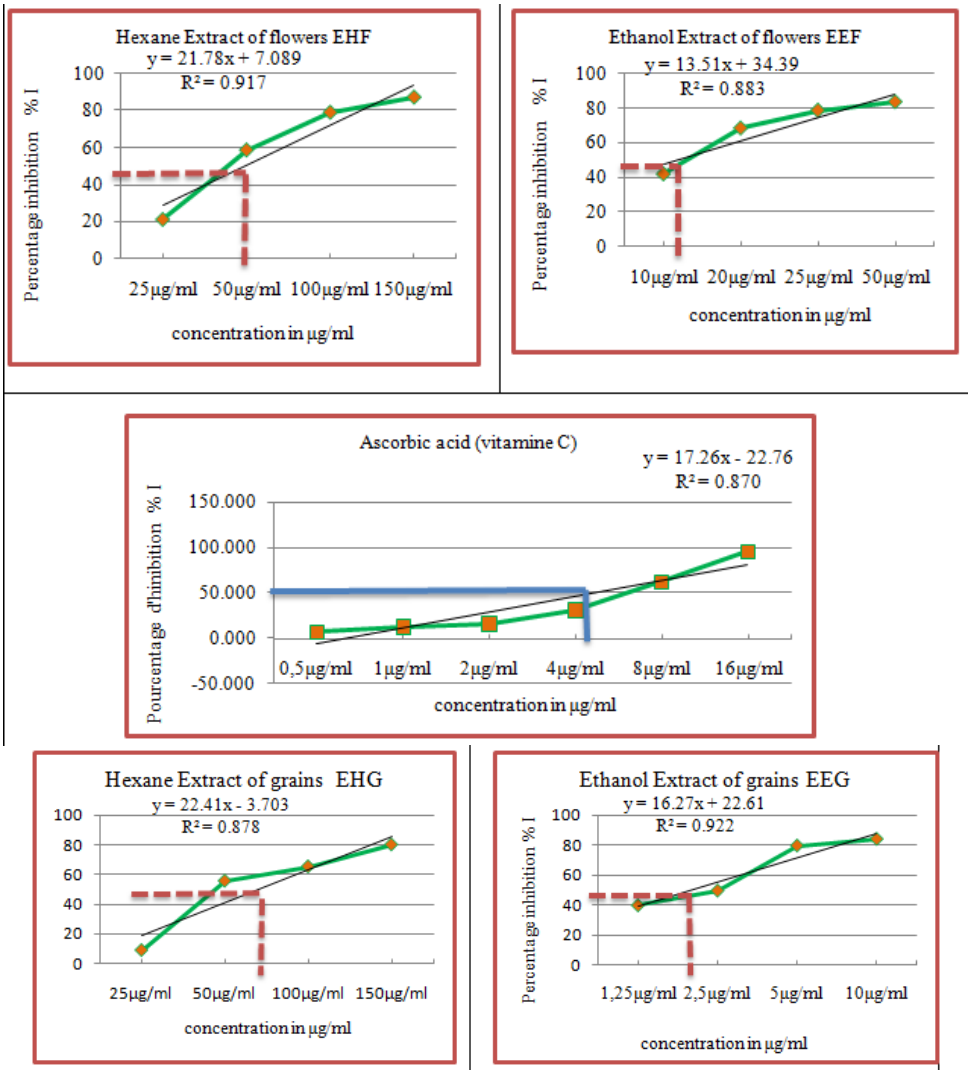


FIGURE 5: The IC50 of oil extracts of flowers and grains of Chamaerops humilis and vitamin C

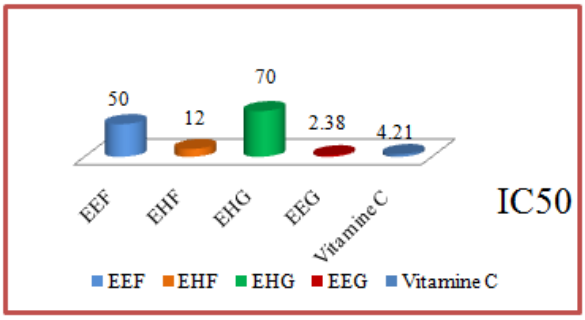


FIGURE 6 : IC50 values of oily extracts of flowers and grains of Chamaerops humilis and vitamin C

The oil extracts EHF EEF, EHG and EEG chamaerops humilis make the free radical (2.2 diphenyl- 1 - picrylhydrazyl) stable in transforming the picrylhydrazine diphenyl- yellow - colored .
The results obtained show that the extract has an antioxidant activity EEG two times higher than that of ascorbic acid with 2,3µg / ml as the IC50, while the EHF extract has a IC50 three times smaller than that of the vitamin C remains more important than the IC50 of EEF extracts (50µg / ml) and EHG (70µg / ml) of the plant studied.

3.4.4. Determining TEC50

Figure 7 shows the values of TEC50 oil extracts EHF EEF EEG EHG of chamaerops and vitamin C. The equilibrium state is chosen as a measurement period in which it appears that the reaction does not progress further. The time to steady state depends on the reactivity of the antioxidants at concentrations employed with free radicals.

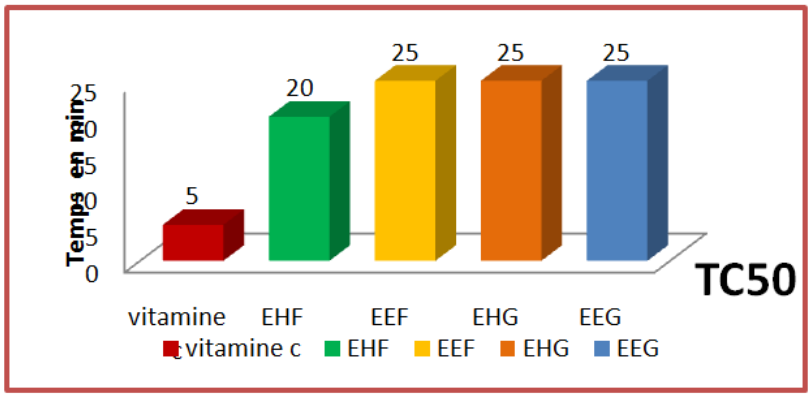


FIGURE 7: the TEC50 values of oily extracts of chamaerops and vitamin C

It is found that vitamin C reacts more quickly with the DPPH •. The TEC50 for the studied extracts from 20 minutes to extract the EHF and EHF for 25 min and extracted EEG, whereas vitamin C needs only 5 minutes to reduce the concentration of free radical of 50%.

3.4.5. Antiradical efficacy endpoint

To easily characterize the behavior of a compound as an antioxidant, IC50 and TEC50 two parameters are combined to calculate the percentage of antioxidant efficiency (% EA). The calculation result is summarized in Table 4

TABLE 4: EA values of oily extracts of flowers and Chamaerops humilis grains and vitamin C

	IC 50 (µg/ml)	TEC50 (mn)	EA (ml/µg.mn)	% EA	classement
vitamin C	4,21	5	0,04751	68	Strong
EEF	12	25	0,00334	5	Low
EHF	50	20	0,001	1	too weak
EHG	70	25	0,00057	1	too weak
EEG	2,38	25	0,01681	25	intermediate

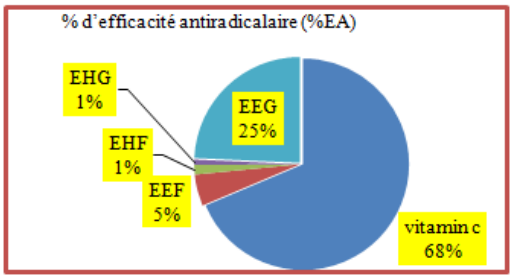


FIGURE 8: the percentages of antiradicalaire efficiency (% AE) of the oily extracts chamaerops and vitamin C

The anti-radical activity of oil extracts of flowers and grains of chamaerops L. was evaluated by the DPPH• radical reduction method. These extracts have a low average scavenging activity compared to that of vitamin C. The EEG extract showed scavenging activity (EA = 25%) than that of the EHF extracts, EEF and EHG but about three times lower than vitamin C.

IV. Conclusion

The study of antioxidant activity by the free radical DPPH• and evaluation of total phenols according to Folin-Ciocalteu procedure for the oil extracts of Chamaerops humilis L allowed us to determine firstly the total content total phenols of each extract and shows that both oil extract EEG, EEF and respectively contain as content 533.14 mg / g and 524.06 mg / g while the total phenolic content of the other two extracts there from is too low 21, 62 mg / g to EHF and 8.3 mg / g for EHG, and on the other hand, the evaluation of the antioxidant activity indicating that the EEG extract has an IC50 of 2.38µg / ml, a TEC50 25min and a % EA 25% .this last therefore reveals interesting antioxidant activity with respect to other extracts which remains relatively lower than that of vitamin C.

This antioxidant activity is due to the presence of molecules capable of trapping free radicals due to their antioxidant power. This extract EEG could play a conservative role in the food or cosmetics.

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