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Study of Antioxidant Activity of Essential Oils Extracted from Moroccan Medicinal and Aromatic Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EI, HS, LA and EM designed the study, authors EI and HS, wrote the protocol and wrote the first draft of the manuscript. Authors LS, LA, EM and EI managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Morocco contains a wide wealth of aromatic and medicinal plants; they constitute natural components, its valorization request a perfect knowledge of the properties to put in value. The present work has been the objective of the valorization of seven plants by studying their chemical compositions and their powers antioxidants by the method of 2.2-diphenyl-1-picrylhydrazyle (DPPH). This study classify the efficiency of oils studied in accordance with the values of IC_{50} obtained, the oregano and the eucalyptus have been identified the most effective with IC_{50} values are respectively 2.47±1.06 and 4.005±0.38 mg/mL, on the other hand the spearmint has shown no effectiveness antiradical whose IC_{50} was not achieved even by increasing its concentration up to 4000 mg/mL.

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Keywords: Essential oil; antioxidant activity; IC_{50}; antiradicular efficiency.

1. INTRODUCTION

Basic radicular phenomena are important to the functioning of the body. The alteration of cellular components and tissue structures occurs when the intensity of these phenomena abnormally increases and exceeds the amount of antioxidants available. The consequence of this imbalance will lead to aggression called (oxidative stress) [1]. All fabrics and all their components can be affected: lipids, proteins, carbohydrates and DNA [2,3]. All these changes increase the risk of more than thirty processes of different diseases [4]. Among them, we include, Alzheimer's disease [5,6], Parkinson's disease [7], Creutzfeldt Jacob, meningitis-cephalad and cancer [8], cardiovascular diseases and cardiac impairment [9], the edema and premature aging of the skin [10].

Synthetic antioxidants, given their efficiency, their low cost and availability, are widely used as additives in foods in order for prevent rancidity. Despite the power of their antioxidant activity, the excess of these synthetic products causes toxicity, responsible of mutagenicites and even present a danger to human health [11,12].

This raises the search for new cures natural antioxidants, suddenly several teams of researchers have been involved in the search for new antioxidants in order for contend oxidative stress and its associated pathologies.

Antioxidant properties of aromatic and medicinal plants are known since ancient times. Its exploitation is mainly through the extraction of their essential oils. These latter are high value added products, used in pharmaceutical, cosmetics and agri-food industries. This study proposes to evaluate and compare the antioxidant activity of essential oils obtained from: *Mentha pulegium*, *Mentha viridis*, *Rosmarinus officinalis*, *Lippia citriodora*, *Cedrus atlantica, Eucalyptus camaldulensis*, *and Origanum campactum*.

2. MATERIALS AND METHODS

2.1 Plant Materials

The essential oils of *Mentha pulegium*, *Mentha viridis*, *Rosmarinus officinalis*, *Lippia citriodora, Eucalyptus camaldulensis* and *Origanum campactum* are collected from different regions of Morocco and extracted by steam distillation from the leaves. Cedar oil is extracted from the branches of the tree. The steam distillation is carried out using Clevenger apparatus during 3 hour average. The chemical composition of essential oils has been identified by gas chromatography coupled to mass spectrometry.

2.2 Evaluation of the Antioxidant Activity

The antiradicular activity of essential oils is measured by using the test in 2.2-diphenyl-1-picrylhydrazyle (DPPH). It is reduced to the form of hydrazine (no radicular) by accepting a hydrogen atom.

2.2.1 Calibration curve of the DPPH solution

Before beginning the tests of the antioxidant activity, the stability and linearity interval of solutions of DPPH must be evaluated and the results are presented graphically. Six solutions of the DPPH (0, 5, 10, 15, 30 and 60 µm) of methanol were tested.

It is observed that there is no significant difference in the absorbance between 0 and 60 min for the concentrations tested and a very good linearity of the absorbance in terms of the concentration.

At the beginning we carried out pretests to define the concentrations that should be used. The effect of each oil on the DPPH is measured by the procedure described by Lopes-Lutz [13]. 100 µL of the methanolic solution of each essential oil and acid ascorbic were introduced in test tubes with 1300 µL of the methanolic solution of DPPH (0.004 %). After agitation by vortex, the reading of the absorbance is made against a white prepared for each concentration to 517 nm after every 5 min during 30 min of incubation in the dark and to the ambient temperature.

2.2.2 Determination of inhibition percentage

The positive control is represented by a solution of standard antioxidant; the ascorbic acid which the absorbance was measured in the same conditions as the samples and for each concentration the test is repeated 3 times. The results were expressed as percentage of inhibition (I %).

$$I\% = [(Abs_{control} - Abs_{test}) / Abs_{control}] \times 100$$

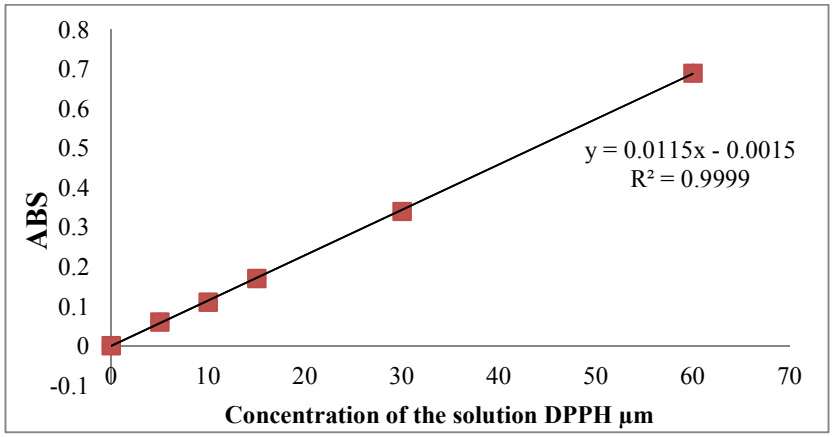

Fig. 1. Calibration curve for DPPH solution

The concentrations of each essential oil tested are plotted graphically according to the percentages of calculated inhibition. It is used to determine the values of IC50 by linear regression. This parameter is defined as the concentration of antioxidant required to decrease the initial DPPH concentration to 50%.

2.3 Determination of the Equilibrium Time TEC_{50}

The parameter TEC_{50} is defined as the time reaches at equilibrium with a concentration of antioxidant equal to IC_{50}. This time is determined graphically.

2.4 Determination of Antiradicular Efficiency EA

The two factors IC_{50} and TEC_{50} can be combined in order to obtain the efficiency parameter antiradicular.

$$EA = 1 / (IC_{50}) \times (TEC_{50})$$

3. RESULTS AND DISCUSSION

3.1 Analysis of the Chemical Compositions of the Chemical Composition of Species

The chromatographic analyzes of the essential oils have highlighted the predominance of monoterpenes. Rosemary contains 63.89% of eucalyptol and 11.96% of camphor. *Origanum compactum* contains 80% of carvacrol and 13% of monoterpenes hydrocarbons (α-pinene and γ-Terpinene). The pennyroyal contains essentially pulegone (99.47%). Table 1 represents the main components of the species tested.

Table 1. The major chemical components of essential oils

Botanical name of the plant	Family	Main components of essential oils
Rosmarinus officinalis	Lamiaceae	1.8-Cineole (63.89%), camphor (11.96%), alpha-pinene (5.91%), camphene (5.36%)
Directed by P. mentha pulegium	Lamiaceae	Pulegone (99.47%), Cymen-8-ol para (0.21%)
Origanum compactum	Lamiaceae	Carvacrol (80.60%), P-cymene (8.90%), γ - Terpinene (4.60%)
Directed by P. mentha viridis	Lamiaceae	Carvone (68.43%), limonene (29.64%)
Eucalyptus camaldulensis	Myrtaceae	1.8 -Cineol (34.22%), cedrol (16.13%), myrtenal (11.34%), Cymen (para) (10.56%), limonene (4.51%)
Lippia citriodora	Verbenaceae	Limonene (36.4%), geranial (12.7%), neral (8.1%)
Cedrus atlantica	Pinaceae	α-pinene (36.45%), 1-tetradecene (15.56%), menthyl acetate (13.24%), caryophyllene (10.69%)

3.2 The Kinetics Study of the Antiradicular Reaction

The antioxidant activity of the various essential oils against DPPH radicular was evaluated spectrophotometrically by following its reduction which is accompanied by its passage from the purple color to the color yellow measured at 517 nm. The following figures show the kinetics of DPPH reduction in terms of time for each concentration of ascorbic acid and essential oils.

Recalling that the extracts are added to the DPPH radical in methanol and the absorbance at 517 nm (absorption maximum of the DPPH) is raised to different time t (min). The hydrogen transfer reaction of the antioxidant to the DPPH is monitored by visible spectroscopy by recording the decrease of the absorption band of the DPPH to 517 nm.

The results shown in Fig. 2 represent two phases of the reaction of EO and DPPH:

- A rapid decrease in absorbance value is explained by the antiradicular reaction of essential oils. This one is drawn after the first five minutes for the ascorbic acid and the essential oils of *Rosmarinus officinalis*, *Mentha pulegium*, *Origanum campactum*, and for *Eucalyptus camaldulensis*. After 10 minutes for both oils of *Lippia citriodora* and *Cedrus atlantica*.
- A slow decrease explained by a low kinetic trapping of the radical followed by a plateau, it is observed after the first five minutes with ascorbic acid and the oil of rosemary especially in the low concentrations (1 and 5 µg/ml), the same goes for the oils of pennyroyal, oregano (in high concentrations 50 and 40 µg/ml). The eucalyptus and cedar oils this balance is only reached after 10 min and 15 min for the verbena.

The antiradical activity of *Mentha viridis* oil has proved to be weak and very slow compared to the antioxidants tested.

Antiradical reaction is carried out by transferring a hydrogen atom or an electron from an antioxidant toward the DPPH radical giving the not radicular form stable DPPH-H. The yellow color obtained at the end of reaction means the antioxidants hydrogen is exhausted.

3.3 Determination of the Percentage of Inhibition

The different optical densities were used to calculate the percentages of inhibition for each essential oil using the formula previously noted. The results obtained after this calculation are presented graphically in terms of the concentrations tested of each essential oil and ascorbic acid, which means the existence of a proportional relationship between the percentage reduction of free radical and the concentration of the extract in the solution. The following figures represent the results of percentage of the radicular inhibition in terms of different concentrations of the ascorbic acid and essential oils.

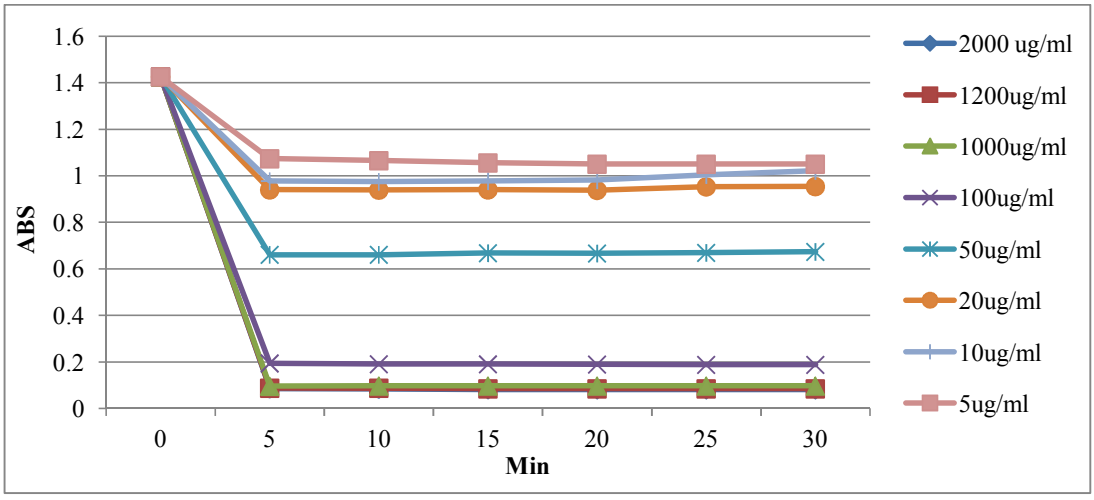

Fig. 2. Kinetics of DPPH reduction obtained with the Ascorbic acid

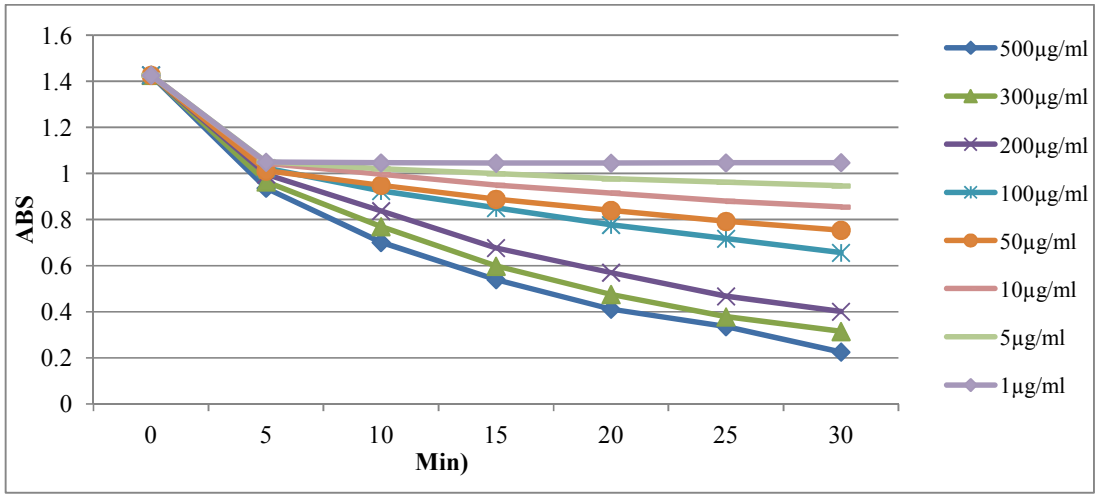

Fig. 3. Kinetics of DPPH reduction obtained with the essential oil of *Rosmarinus officinalis*

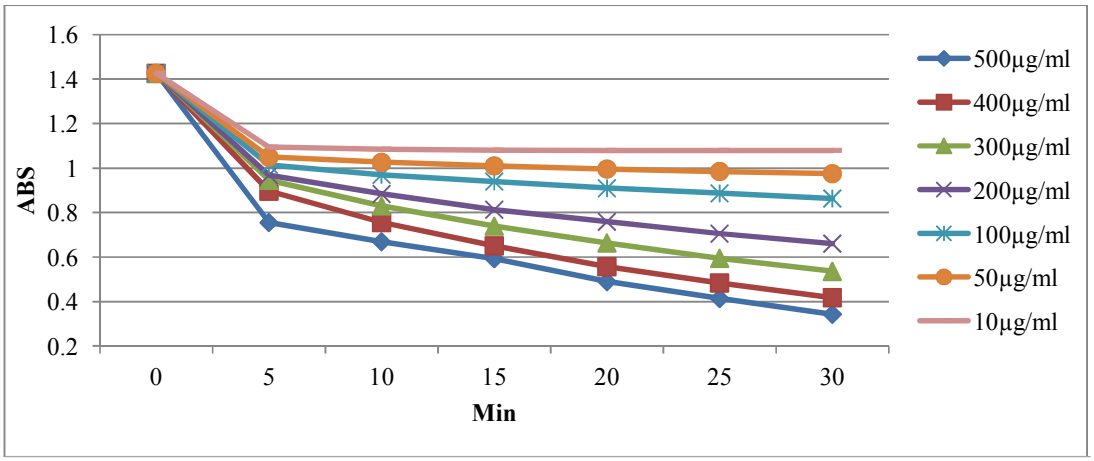

Fig. 4. Kinetics of DPPH reduction obtained with the essential oil of directed by *P. mentha pulegium*

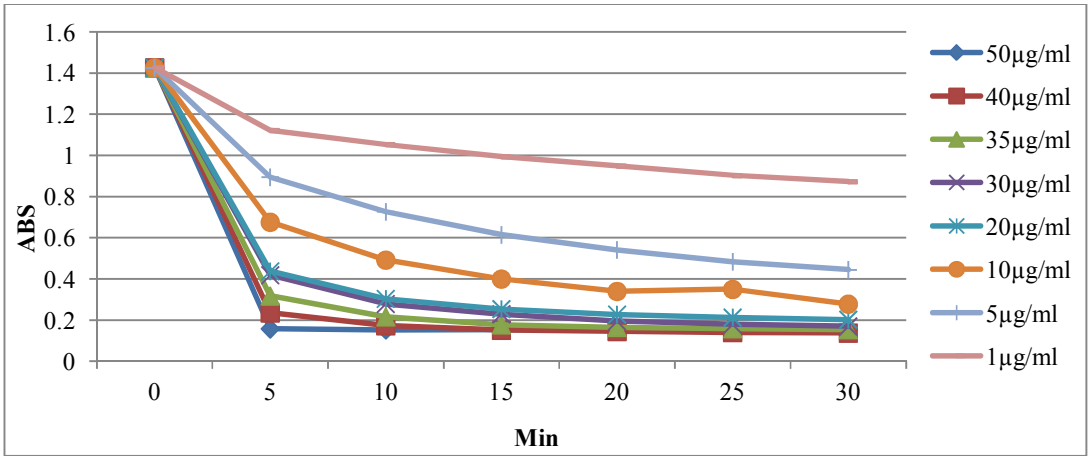

Fig. 5. Kinetics of DPPH reduction obtained with the essential oil of *Origanum campactum*

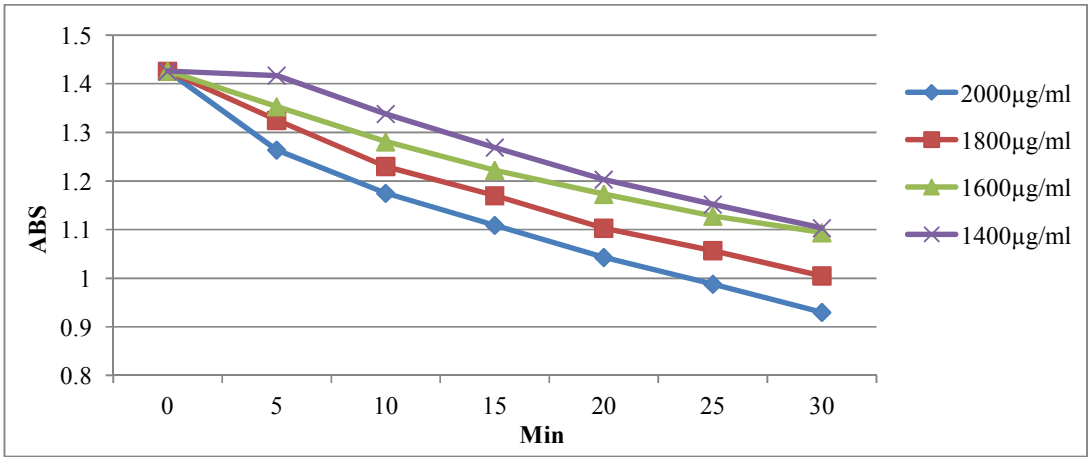

Fig. 6. Kinetics of DPPH reduction obtained with the essential oil of Directed by *P. mentha viridis*

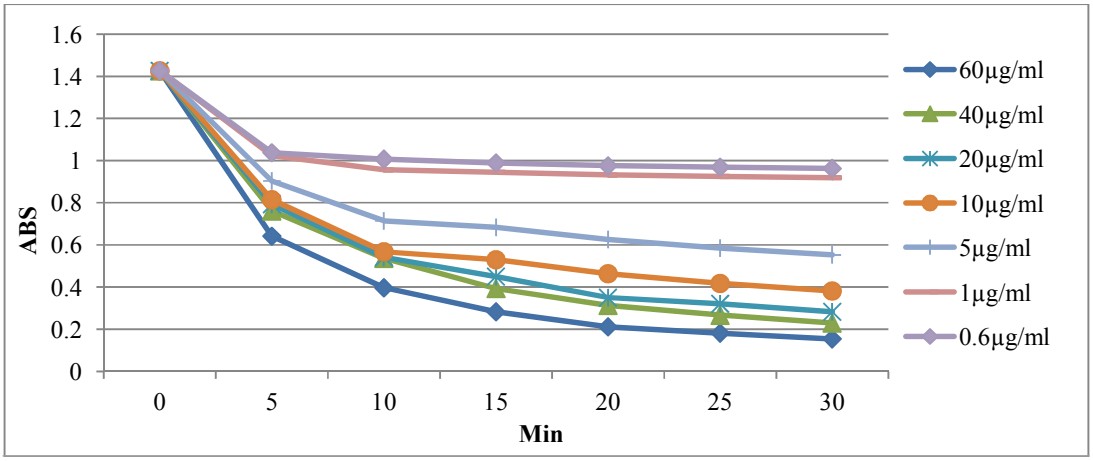

Fig. 7. Kinetics of DPPH reduction obtained with the essential oil of *Eucalyptus camaldulensis*

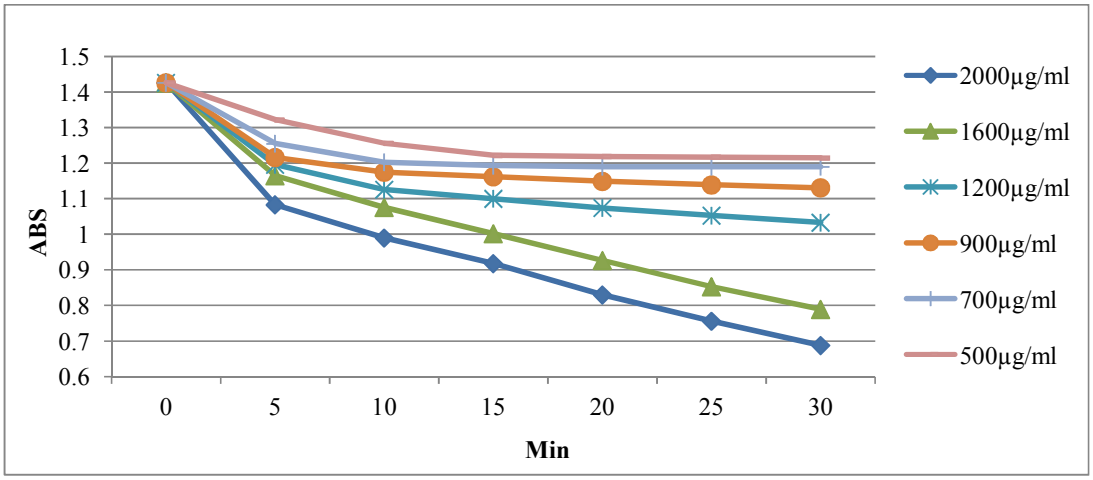

Fig. 8. Kinetics of DPPH reduction obtained with the essential oil of *Lippia citriodora*

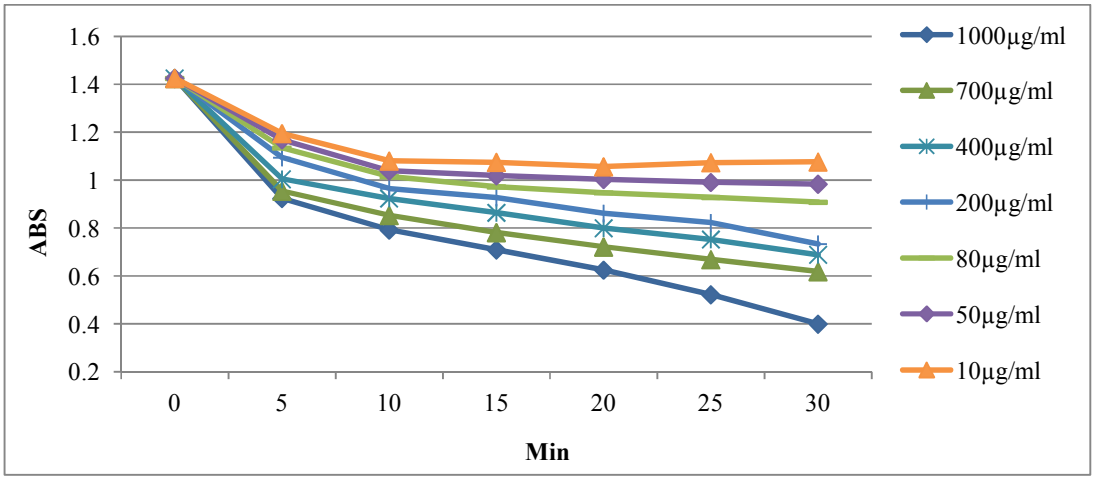

Fig. 9. Kinetics of DPPH reduction obtained with the essential oil of *Cedrus atlantica*

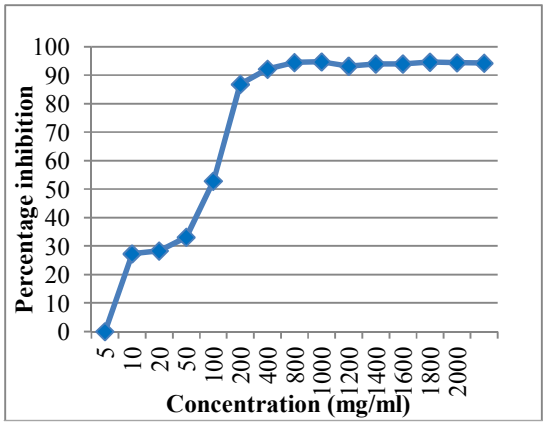

Fig. 10. Percentage of DPPH inhibition in terms of the concentrations of ascorbic acid

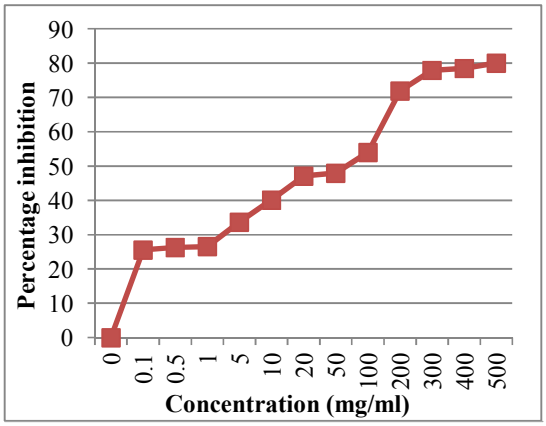

Fig. 11. Percentage of DPPH inhibition in terms of the concentrations of the essential oil of *Rosmarinus officinalis*

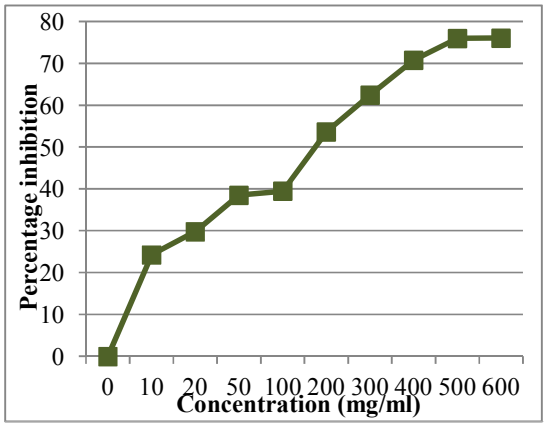

Fig. 12. Percentage of DPPH inhibition in terms of the concentrations of *Mentha pulegium*

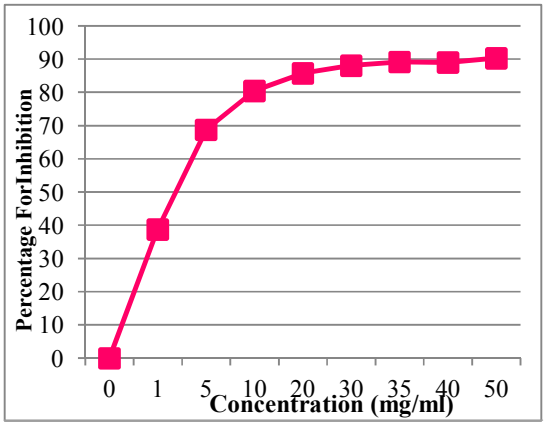

Fig. 13. Percentage of DPPH inhibition in terms of the concentrations *of Origanum campactum*

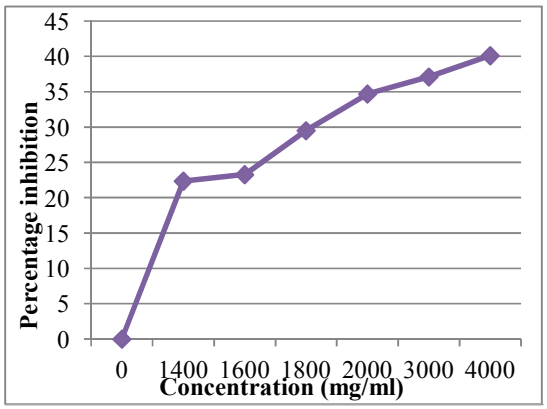

Fig. 14. Percentage of DPPH inhibition in terms of the concentrations of the concentrations of *Directed by P. Mentha viridis*

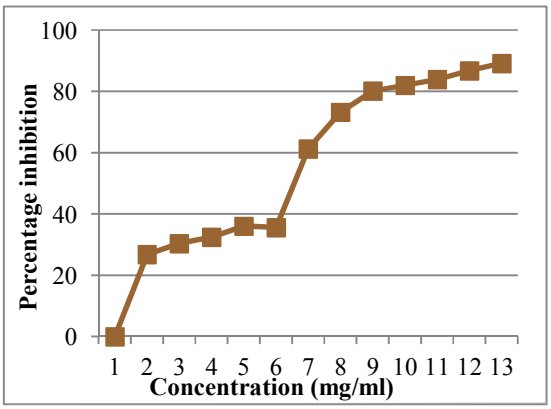

Fig. 15. Percentage of DPPH inhibition in terms of the concentrations of the concentrations of *Eucalyptus camaldulensis*

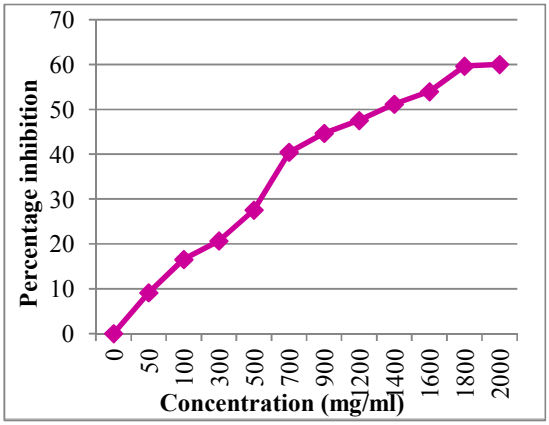

Fig. 16. Percentage of DPPH inhibition in terms of the concentrations of the concentrations of *Lippia citriodora*

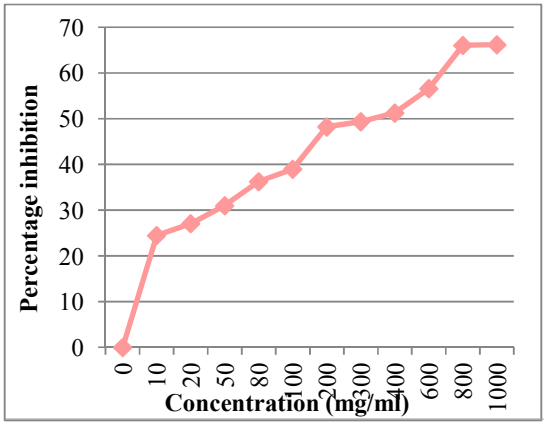

Fig. 17. Percentage of DPPH inhibition in terms of the concentrations of the concentrations of *Cedrus Atlantica*

Generally the results obtained confirm that the inhibition percentage increases proportionally in terms of ascorbic acid and essential oils concentration tested. The previous figures show two categories of plants:

Plants with strong radicular inhibition: *Origanum campactum* presented a high inhibition reached 90.32±3.98%, at low concentration 50 mg/ml followed by *Eucalyptus camaldulensis* at a concentration of 60 mg/ml with a percentage of 89.13±4.65 percent. The essential oils of *Rosmarinus officinalis* and *Mentha pulegium* reduced respectively 80.01±2.34%; 76.06±1.43% of DPPH radicals at concentrations of 500 and 600 mg/ml.

Plants with low radicular inhibition: *Cedrus Atlantica* (1000 mg/ml) and *Lippia citriodora* (2000 mg/ml) were recorded in low percentage of radicular inhibition which does not exceed respectively 66.19±4.32% and 60.03±2.88%. The percentage inhibition of the spearmint was not significant; it is 40.76±3.69%, despite the increase of its concentration up to 4000 mg/ml.

3.4 Determination of IC_{50}

The antioxidant capacity of different EO was determined from the IC_{50}. It is the antioxidant concentration necessary to reduce 50% of the radical DPPH. The IC_{50} and the antioxidant activity of the oils tested are inversely proportional [14].

The values of IC50 were calculated for each extract from the logarithmic equation of the plotted curves and diagrammed in Fig. 11.

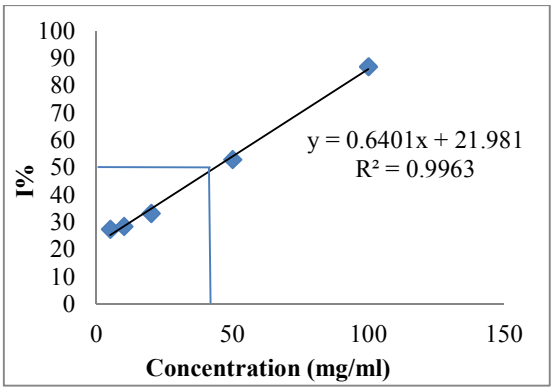

Fig. 18. Calculation of IC_{50} of the ascorbic acid

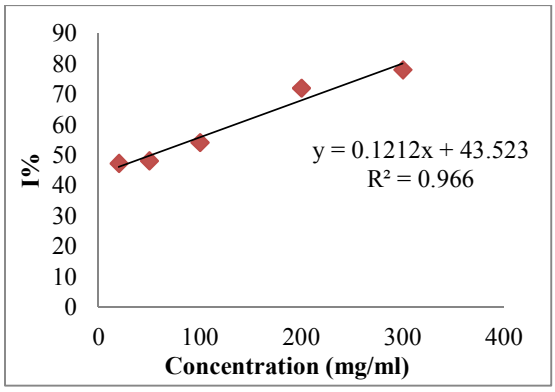

Fig. 19. Calculation of IC_{50} of the essential oil of *Rosmarinus officinalis*

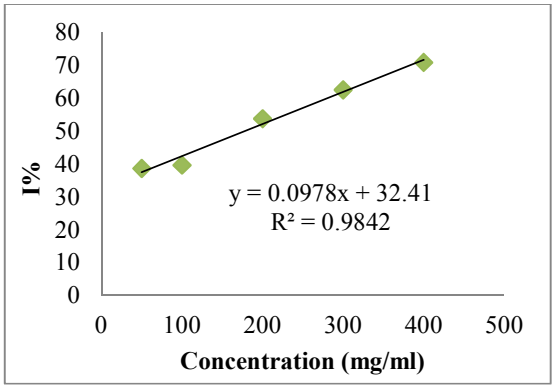

Fig. 20. Calculation of IC_{50} of the essential oil of *Directed by P. Mentha pulegium*

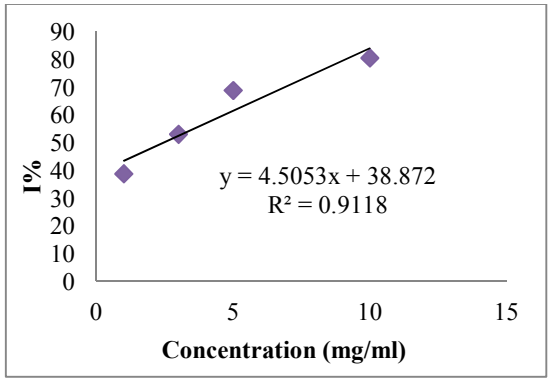

Fig. 21. Calculation of IC_{50} of the essential oil of *Origanum campactum*

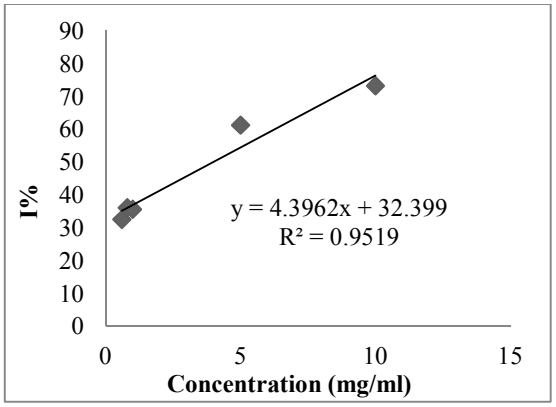

Fig. 22. Calculation of IC_{50} of the essential oil of *Eucalyptus camaldulensis*

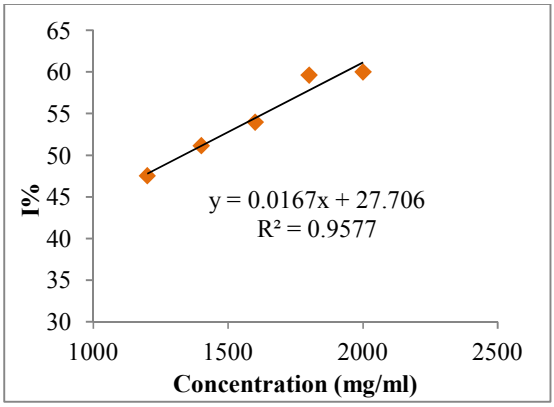

Fig. 23. Calculation of IC_{50} of the essential oil of *Lippia citriodora*

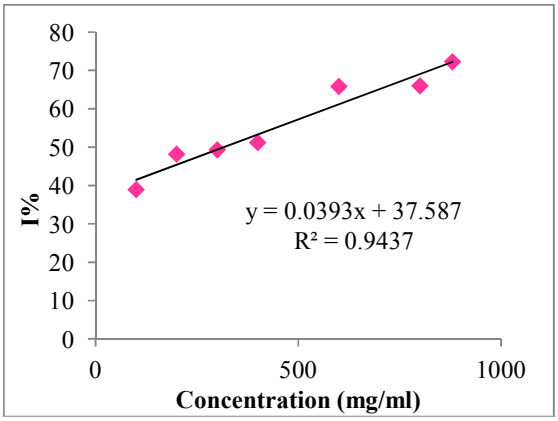

Fig. 24. Calculation of IC_{50} of the essential oil of *Cedrus atlantica*

The values of IC_{50} calculated are presented in the Table 2

Table 2. Result of antioxidant test expressing 50% effective concentration in mg/ml

Oil /Standard	IC_{50}±Ecart type
Ascorbic acid	43.78±2.11
Rosmarinus officinalis	53.55±1.65
Mentha pulegium	179.85±2.47
Origanum campactum	2.47±1.06
Eucalyptus camaldulensis	4.005±0,380
Lippia citriodora	1335.63±1.22
Cedrus atlantica	315.85±0.97

According to the results recorded *Origanum campactum* and *Eucalyptus camaldulensis* have a high antioxidant power compared to other extracts tested and even to ascorbic acid, their IC_{50} is respectively of 2.47±1.06 mg/ml and 4.005±0.38 mg/ml. Many studies have experimentally determined the capacity of natural extracts to trap free radicals, the terpeneoides, flavonoids, alkaloids and tannins are considered as potential antioxidant substances [15], and after the chemical analysis of the extracts tested which has highlighted the predominance of oxygenated monoterpenes and hydrocarbons, the latter are probably responsible for the antioxidant activity of these oils.

Rosmarinus officinalis has proved relatively less efficient than the ascorbic acid whose value is in the order of 53.55±1.65 and 43.78±2.11 mg/ml for the ascorbic acid. The pennyroyal, the cedar of atlas and the verbena lemongrass are almost ineffective, their IC_{50} between 179.85±2.47 and 1335.63±1.22 mg/ml.

The tests conducted on the spearmint shown that this essential oil is ineffective to reduce the free radicals of the DPPH because by increasing the concentration which reaches 4g/ml the IC_{50} is not completed.

3.5 Determination of TC_{50}

It was considered the state of equilibrium as measure of time when it appears that the reaction does not progress further. The time of equilibrium depends on the reactivity antioxidants and concentrations employed.

It is found that ascorbic acid reacts in a faster way with DPPH°. The TC50 recorded are: 11±2 min for the oregano, 12±1.66 min for eucalyptus. Other oils react after 20 minutes as the pennyroyal with TC50 24± 0.96 min, rosemary with 25± 0.98 min. Cedar and verbena reduce the DPPH° after 28 minutes.

3.6 Determination of the Antiradicular Efficiency

The antiradicular efficiency parameter combines two previous settings; IC50 and TC50, the calculation of EA allows to classify the antioxidants tested according to the classification proposed by Sanchez-Moreno, antiradicular activity is low for EAR<1.10^{-3}, intermediate between 1.10^{-3} and 5.10^{-3}, high between 5.10^{-3} and 10.10^{-3}, and very high for EAR> 10.10^{-3}[16]. The parameters for calculating the antioxidant activity are summarized in Table 3.

Table 3. The parameters for calculating the antioxidant activity

	IC_{50} (mg/ml)	TC50 (mn)	EA (ml/mg. mn)	Classification
Ascorbic acid	43.78±2.11	4±0.22	0.09±0.005	Low
Rosmarinus officinalis	53.55±1.65	25±0.98	0.46±0.06	Low
Mentha pulegium	180±2.47	24±0.96	0.13±0.04	Low
Origanum campactum	2.47±1.06	11±2	4.45±0.68	Through
Eucalyptus camaldulensis	4.005±0.38	12±1.66	2.99±1.25	Through
Lippia citriodora	1335.63±1.22	28±2.26	0.02±0.01	Low
Cedrus atlantica	315.85±0.97	28±1.36	0.08±0,005	Low

It appears from these results that the essential oil of oregano which essentially contains a phenol monoterpenoid has very high efficiency compared with the antioxidant of reference ascorbic acid with EA equal to 4.45±0,68 ml/mg.min, even for the eucalyptus oil which its EA is in the range of 2.99±1.25 ml/mg.min. The two essential oils of pennyroyal and rosemary were less effective than the first two oils and more effective than the ascorbic acid with EA respectively of 0.13±0.04 ml/mg.min and 0.46±0.06ml/mg.min. Cedar and verbena presented a very low efficiency.

4. CONCLUSION

This work was conducted the study of the antioxidant activity of a series of essential oils extracted from Moroccan aromatic and medicinal plants.

The study of the antioxidant activity according to the method of trapping free radical DPPH showed that the essential oils of *Origanum campactum* and for *Eucalyptus camaldulensis* are the best hydrogen or electron donors among other essential oils studied. They show a higher efficiency than that of ascorbic acid. However, it would be difficult to believe that the antioxidant activity of these oils is limited only to some of its major constituents; it could also be due to some minor constituents or to a synergistic effect of several constituents. On the other side a more or less moderate efficiency was proven among other essential oils. This study helps to orient the food, cosmetic and pharmaceutical industries toward alternatives to some synthetic additive.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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