Chemical Composition and Antioxidant Activity of the Ceratonia Siliqua L. Seeds Oil

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Abstract

In this work, we are interested in the antioxidant activity of *Ceratonia Siliqua* L. seeds oil. After extraction of seeds oil by Soxhlet, we characterized by GC-MS and evaluated the antioxidant activity by DPPH$^{\bullet}$ and ABTS$^{+\bullet}$. The obtained results show that the seeds oil of *Ceratonia Siliqua* L. contains various organic compounds, the majority of which are fatty acids. Furthermore, the determination of IC_{50} by the DPPH$^{\bullet}$ and ABTS$^{+\bullet}$ methods show that cyclohexane has good IC_{50} compared to hexane.

1. Introduction

The human body naturally produces free radicals by normal metabolism. Due to their high reactivity, these free radicals disrupt the function of the body (Bouyahya et al., 2018; Christodoulou et al., 2018) and its can seriously damage human cells or even stop the function of enzymes or the oxidation of lipids, proteins, amino acids … etc.

To maintain the work of our bodies normal, a balance between free radicals and antioxidant substances is necessary to prevent oxidative stress (El Omari et al., 2018; Jain et al., 2020; Romaniuk et al., 2018). In addition, this latter is considered a cause of several illnesses such as cancer, inflammation … etc.

Previous studies had indicated that natural substances are suitable to protect the human bodies against chronic diseases **(Barati et al., 2020; Rasol et al., 2018**).

Recently, due to the suspicion of many synthetic antioxidants, researchers have focused on plant extracts, which are sources of natural antioxidants. This latter has shown good activity against free radicals (**Du et al., 2019; Liolios et al., 2009; Sy et al., 2018**).

The *Ceratonia Siliqua* L. is known as one of the traditional and most favorable medicinal plants. Its leaf extracts are widely reported in the literature as an excellent antioxidant, anti-diabetic, anti-inflammatory (Tongpoothorn et al., 2012). In addition, its seed oil shows a good anticorrosion activity of iron in acidic solution (Abbout et al., 2018).

The existence of unsaturation and heteroatoms in plant extracts is an indicator of good antioxidant activity. Fatty acids such as omega-6 (linoleic acid) and omega-3 (alpha-linolenic acid) have these properties such as unsaturation and the carboxyl function (oxygen atoms) (Ciocarlan et al., 2018).

The novelty of this work is to investigate the antioxidant activity of the *Ceratonia Siliqua* L. seeds oil and determine the chemical composition of the seeds oil extracted by two solvents. In order to achieve this goal, we are using two methods (DPPH$^{\bullet}$ and ABTS$^{+\bullet}$).

2. Experimental

2.1 Plant and analytical materials

The seeds of *Ceratonia Siliqua* L. were collected from the north of Morocco. Then, the dried seeds were ground using a mix equipped with ring sieve owning trapezoid holes sized 0.5 mm. The powder was reserved in the dark before extraction. The two extraction solvents are cyclohexane and hexane have purity of 99%.

Free radicals used in the study of antioxidant activity, namely 2, 2-diphenyl-picrylhydrazyle ($DPPH^{\bullet}$) and 2, 20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$) with high purity (99.9%).

2.2 Process of extraction

The extraction process was carried out using a Soxhlet extractor for 6 hours with two solvents, cyclohexane (HC) and hexane (HH). At the end of the extraction, the solvent was evaporated using a rotary evaporator and the oil was maintained at 4 ° C to prevent degradation of the compounds for further analysis (Lama-Muñoz et al., 2020).

2.3 GC-MS analysis of the *Ceratonia Siliqua* L. oil

The analysis of the extracted oil was carried out using gas chromatography coupled to mass spectrometry (GC-MS) type Brukur 456-GC operating in the electron impact mode. The capillary column used is an Rxi-5 Sil MS (30×0.25mm ID ×0.25µm). Initially, the column temperature is maintained at 333°K for 5 min, with an increase of 10°C/min to 573 ° K where it remains 10 minutes.

The identification of the structures of the compounds was confirmed by comparing the obtained mass spectra with those of the pure compounds with reference to the library of mass spectra of the apparatus (NIST 2014).

2.4 DPPH free radical scavenging activity

The seeds oil of the *Ceratonia Siliqua* L. was evaluated for their antioxidant activity using the $DPPH^{\bullet}$ method described by Sanchez-Moreno (Hinojosa-Nogueira et al., 2020; Lo Scalzo, 2008). The different concentrations of the solution were prepared by diluting the oil in ethanol. The ascorbic acid was taken as the standard. The $DPPH^{\bullet}$ solution was prepared by dissolving 6 mg of DPPH in 200 ml of the ethanol. In test tubes, 0.1 ml of each solution is added to 2 ml of $DPPH^{\bullet}$ solution freshly prepared. A blank is prepared in parallel only with the ethanol. Then all solutions are stored in a dark place for 30 minutes. The absorbance is measured at 517 nm. Three optical density measurements are determined for each concentration.

The evaluation of the antioxidant activity is expressed as a percentage of inhibition of the $DPPH^{\bullet}$ radical according to the following formula:

$$\% \, Inhibition = \frac{A_{Control} - A_{oil}}{A_{Control}} \times 100$$

$A_{Control}$ and A_{oil} are Absorbance of the DPPH solution without and with oil, respectively.

2.5 ABTS free radical scavenging activity

The seeds oil of the *Ceratonia Siliqua* L. was evaluated for their antioxidant activity using $ABTS^{\bullet+}$ (Rao et al., 2018). Initially, 7 mM of the radical ABTS solution and 2.45 mM of $K_2S_2O_8$ solution was separately prepared using distilled water to dissolve them. Then, the working solution was prepared by mixing the equal quantities of the two stock solutions and allowed to rest for 12–16 h in a dark place at 298K. Further, a fresh working solution was prepared by mixing 1 ml of $ABTS^{\bullet+}$ solution diluting in 60 ml of methanol to obtain a constant absorbance of 1.1±0.02 at the wavelength of 734 nm using a UV–vis Spectrophotometer (Hitachi U-1800, Japan). Thereafter, 150 ml of the oil was mixed with 2.85 ml of $ABTS^{\bullet+}$ solution, then, the absorbance was taken at 734 nm after incubating in a dark place for 2 h. Methanol was used as the blank and the free radical scavenging activity of the oil was calculated using the following formula.

$$\%\ Inhibition = \frac{A_{Control} - A_{oil}}{A_{Control}} \times 100$$

$A_{Control}$ and A_{oil} are the absorbance of the $ABTS^{\bullet+}$ solution without and with oil, respectively.

3. Results And Discussion

3.1 Extraction and GC-MS characterization of *Ceratonia Siliqua* L. seeds oil

Ceratonia Siliqua L. seeds oil was extracted using hexane and cyclohexane as solvents and a Soxhlet extractor as the extraction technique. For both solvents, the oil extract has yellow and yellowish green colors for hexane and cyclohexane, respectively. The characterization by GC-MS analysis shows that the cyclohexane extracts more compounds compared to hexane. The following figure (figure 1) represents the GC-MS spectra for each solvent.

Table 1 shows that the hexane extracts 16 compounds, however, the cyclohexane extracts 18 compounds. In addition, GC-MS analysis showed a difference in the percentage of the extract compounds for each solvent. Which indicates that the nature of the extraction solvent affects the chemical composition of the oil. The extraction technique can also influence the nature and the number of compounds extracted from the seeds (Chen et al., 2021).

The naming and the percentage of compounds detected by GC-MS are presented in Table 1. The obtained result indicates that the most important compounds in the seeds oil of *Ceratonia Siliqua* L. are the fatty acids such as palmitic acid (11.65%), linoleic acid (7.99%), oleic acid (3.63%), stearic acid (2.39%)… .. etc. Also, this oil are rich with the others organic compounds such as 2,4-Di-Tert-butylphenol (4.33%), ar-Tumerone (4.91 %), enzoic acid,1-methylethyl ester (13.50 %), 9-Octadecene,1-methoxy,(E) (15,04 %).

Table 1 : GC-MS chemical composition of Ceratonia Siliqua L seeds oil of the fatty acids with their pharmacological activities

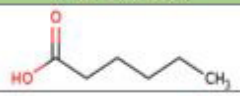
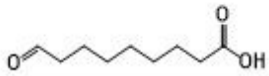
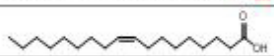
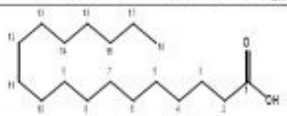
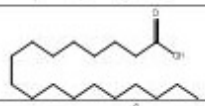
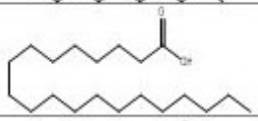
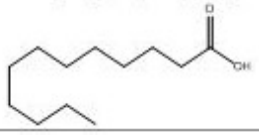
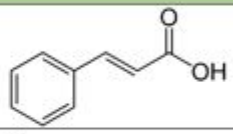
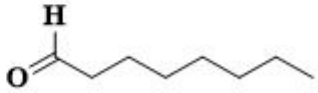
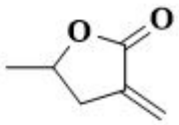
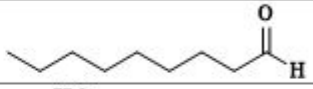
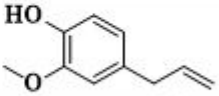
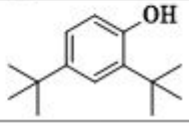
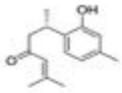
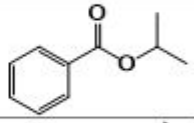
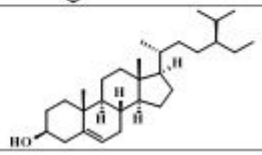
N°	Fatty acids	Structure	HH (%)	HC (%)	Therapeutic activity	Ref
1	Hexanoic acid		3.50	1.64	Antioxidant Hypo-cholesterolemic	[14]
2	Nonanoic acid		4.31	2.91	Antimicrobial Antioxidant	[15]
3	9-Oxononanoic acid		5.78	9.84		
4	Myristic acid		---	7.38	Antifungal Antimicrobial Antioxidant	[16]
5	Palmitic acid		14.79	18.13		
6	Oleic acid		17.48	15.54		
7	Stearic acid		4.73	5.93		
8	Linoleic acid		14.13	13.42		
9	Arachidic acid		---	2.23		
10	Lauric acid		3.27	2.03		

Table 2 : GC-MS chemical composition of Ceratonia Siliqua L seeds oil non fatty acids with their pharmacological activities

N°	Organic compound	Structure	H (%) *	C (%) **	Therapeutic activity	Ref
1	Cinnamic acid		2.55	4.25	Antimicrobial Antioxidant	[17]
2	2-Octenal		1.04	1.01		
3	Dihydro-3-methylene-5-methyl-2-furanone		1.56	2.05		
4	Nonanal		3.15	3.88		
5	Eugenol		2.30	8.13	Antimicrobial Antioxidant	[18]
6	2,4-Di-Tert-butylphenol		3.70	4.39	Agent complexing Antioxidant	[19]
7	Ar-Tumerone		5.48	----	Anticancer	[20]
8	Benzoic acid,1-methylethyl ester		----	2.24	Hypo-cholesterolemic	[21]
9	Beta-sitosterol		4.85	2.17		

Overall, we can conclude that the *Ceratonia Siliqua* L. seeds oil is rich in organic compounds such as esters, alkanes, saturated and unsaturated fatty acids, glycerides and other related organic compounds. In addition, these compounds contained heteroatoms, as we mentioned that these heteroatoms improve the resistance against the corrosion due to adsorption ability on the metallic surface.

On the other hand, another extract from seeds shows an excellent behavior against corrosion as chloroform extracts (Abbout et al., 2021).

3.2 DPPH° radical scavenging activity by the *Ceratonia Siliqua* L. oil

The antioxidant activity of the oil of *Ceratonia Siliqua* L. extracted by cyclohexane and hexane was determined by the DPPH• method represented in the figure 2; this activity was compared to that of ascorbic acid retained as a reference substance.

The obtained results are presented in figure 2, it indicates that the optical density decreases with the increase in the oil concentration.

In order to determine the IC_{50}, we plotted the effectiveness of the inhibition as a function of the oil concentration (Figure 3).

The IC_{50} values issued from figure 3 are represented in table 2.

Table 3 : IC_{50} parameter calculated by $DPPH^{\cdot}$ radical scavenging activity method.

Compounds	IC_{50} parameter (µg/ml)
HH	74 ± 3
HC	68 ± 5
Acid ascorbic	78 ± 4

The analysis of figure 3 shows that the IC_{50} value is equal to 68 ± 5 µg/ml and 74±3 µg/ml for the oil extract with cyclohexane and hexane respectively. These values are lower compared to 78 ± 4 µg / ml of the ascorbic acid and 172.6 µg/ml of the Fenugreek oil (Akbari et al., 2019) and 1850 µg/ml extract of aloe Vera (Benzidia et al., 2018).

This low value of IC_{50} obtained from oil used indicates an excellent antioxidant activity against free radicals. It can be attributed to the existence of groups of heteroatoms with good hydrogen donating capacities such as fatty acid esters (Akbari et al., 2019b; Peng et al., 2016).

This good antioxidant potential may be due to hydrogen-donating abilities of the plant extracts. The capacity of free radical scavenging can be designated by the grade of discoloration in the presence and absence of extracts.

3.3 ABTS radical scavenging activity of the oil

The antioxidant activity of oil extracts by cyclohexane and hexane is determined by the free radical ABTS method. The obtained result was presented in figure 4.

The figure 4 indicates the curves of the inhibition efficiency in function of the oil concentration. For both solvents, this latter increases with the increasing of the oil concentration and then it stabilizes after a 0.3 mg/ml of oil concentration. The efficiency reached the maximum for a concentration of 1mg/ml and it was in order of 97% and 96% for cyclohexane and hexane respectively.

We have also determined the IC_{50} for the free radical $ABTS^{\bullet+}$ method**.** From the figure 4, the IC_{50} of antioxidant activity are 15 ± 1.05 µg/ml and 19 ± 1.05 µg/ml for oil extracts by cyclohexane and hexane respectively. These values are lower than ascorbic acid and 161.3±2.1µg/ml for the Fenugreek oil (Akbari et al., 2019) and 1850 µg/ml for the extract of aloe Vera (Benzidia et al., 2018), which show an IC_{50} of 47 ± 1.1 µg/ml that the values of IC_{50}. The obtained results can be explicated by the presence of

the oil constituents having an excellent antioxidant capacity, such as polyphenol, fatty acids, alkaloids … Etc.

4. Conclusion

In this work, the *Ceratonia Siliqua* L seeds oil were extracted by cyclohexane and hexane using Soxhlet extractor as a technique of extraction. Concern, the characterization of this oil using GC-MS analysis. Finally, the antioxidant activity of *Ceratonia Siliqua* L. was determined by DPPH and ABTS method for oil. From these results, we can conclude that:

The oil extract by the solvents cited above has a yellowish and yellowish green color for hexane and cyclohexane, respectively.

- The *Ceratonia Siliqua* L seeds oil is rich in organic compounds such as esters, alkanes, saturated and unsaturated fatty acids, glycerides and other related compounds such as phenols, flavonoids and alkaloids.
- The obtained results show that the type of extraction solvent affects the chemical composition of the oil.
- The IC50 of antioxidant activity of the oil is:
- 68±5µg/ml and 74±5µg/ml for oil extracted by cyclohexane and hexane respectively for the DPPH method. These values were higher than ascorbic acid (78±4µg/ml).
- 15.00±1.05µg/ml and 19.00±1.05µg/ml for oil extracted by cyclohexane and hexane respectively for ABTS method.

The result of the *Ceratonia Siliqua* L seeds oil shows an excellent efficiency compared to the acid ascorbic.

Generally, based on this study results, it could be recommended that *Ceratonia Siliqua* L seeds oil possibly will be used as an excellent antioxidant that meets the standards used in the food industry and as corrosion inhibitor.

Declarations

Declaration of Interest Statement

We confirm that this work is not submitted in any journal and no conflict between authors. Thank you for your consideration.

Authors' contributions

Conception and design of study: **Said ABBOUT,**

Acquisition of data: **Said ABBOUT, Malak REHIOUI,**

Analysis and/or interpretation of data: **Said ABBOUT, Driss Chebabe; Hamid Erramli, Najat HAJJAJI**

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Figures

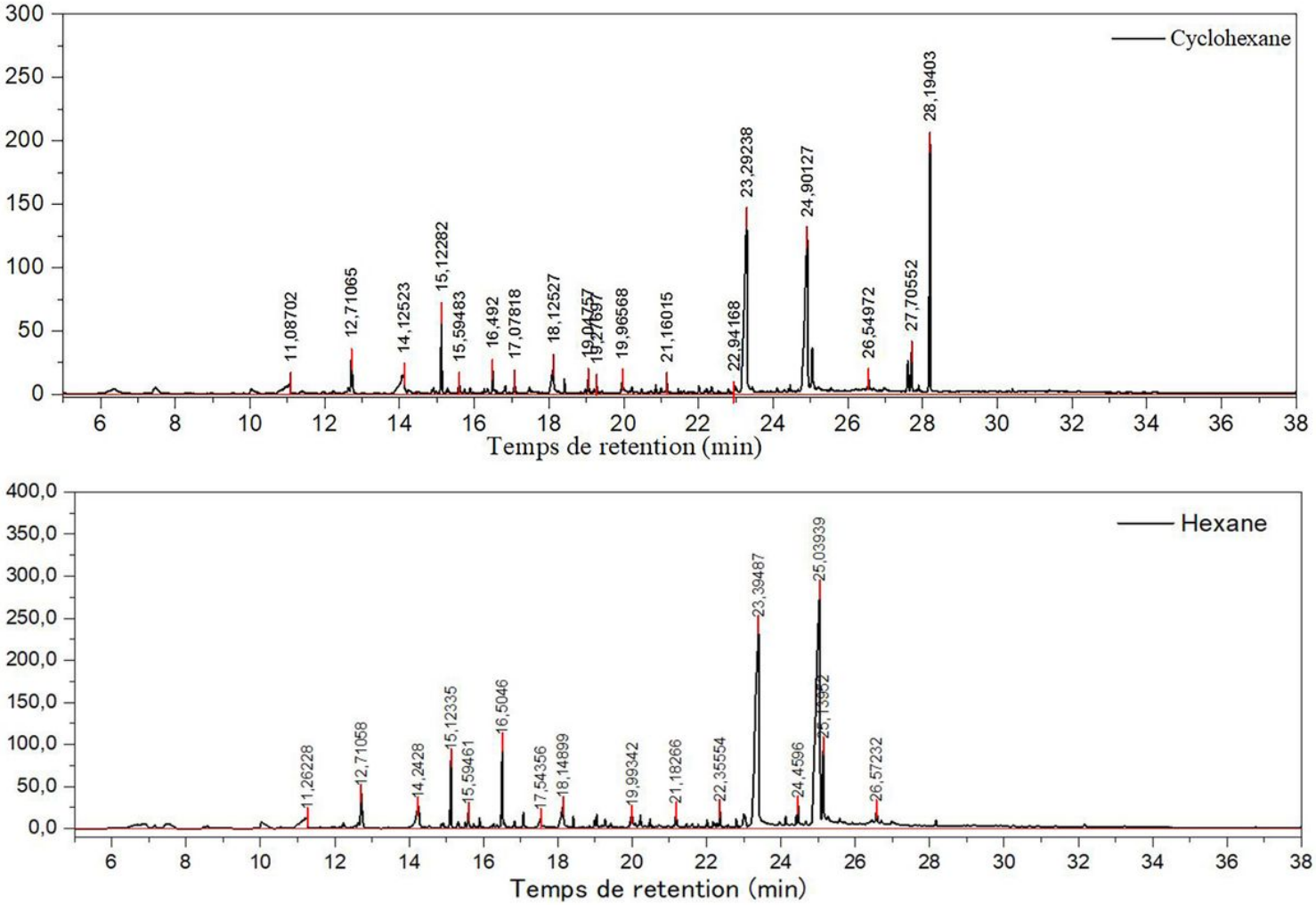

Figure 1

Chromatogram of the Ceratonia Siliqua L. oil

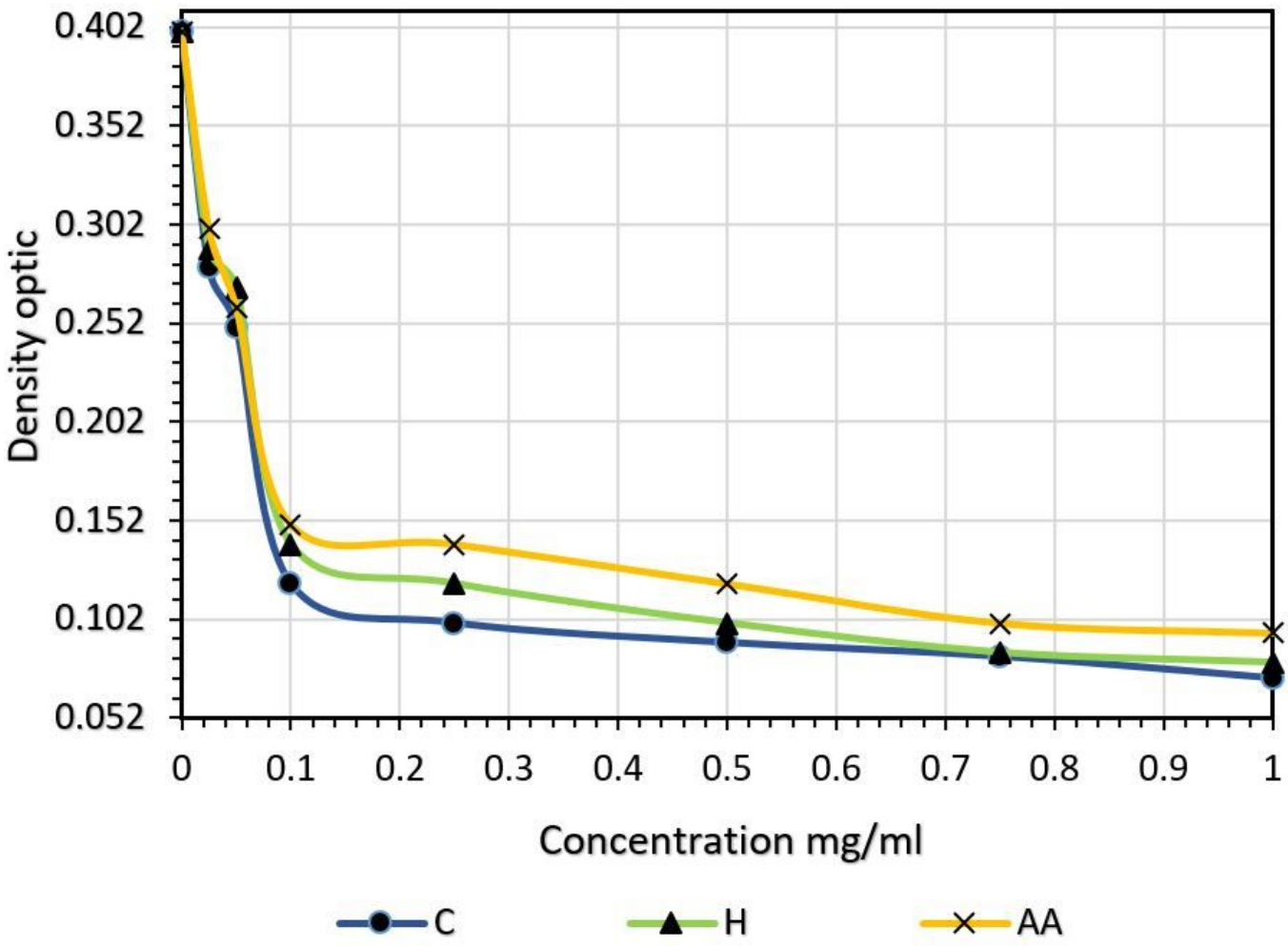

Figure 2

Variation of the density optic in function of the oil concentrations

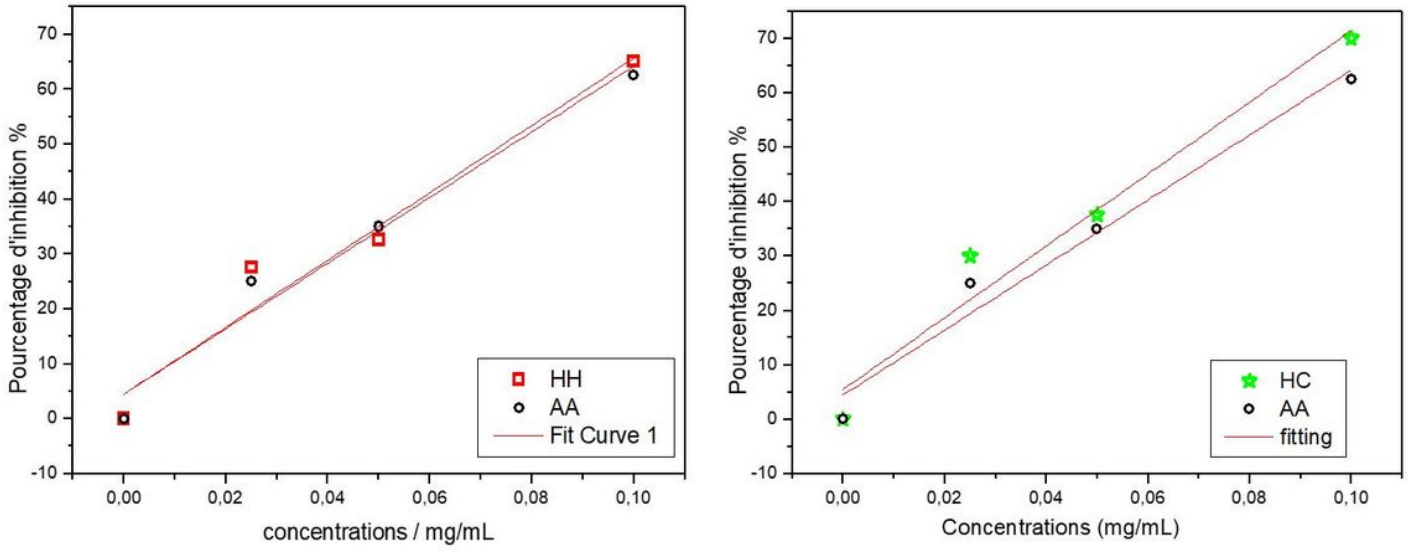

Figure 3

variation of the inhibition efficiency in function of the oil concentration

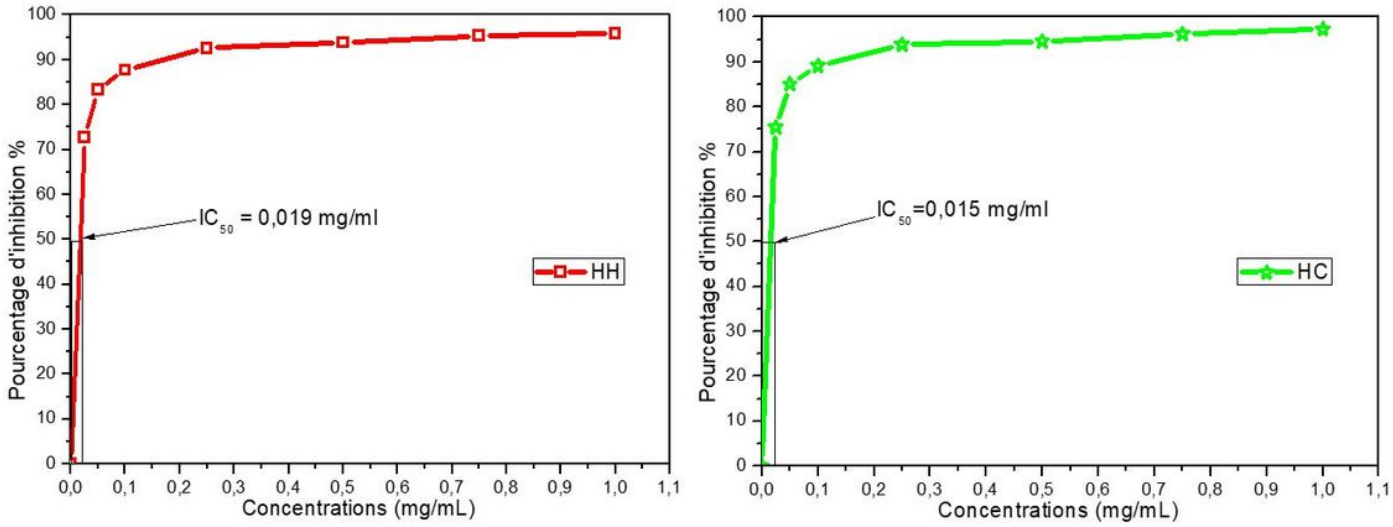

Figure 4

Variation of the inhibition efficiency in function of the oil concentration.