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Antioxidant and antibacterial activities of the essential oils of *Ceratonia siliqua*

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Abstract. The present study investigates the antioxidant and antibacterial activities of the essential oils obtained by hydrodistillation of the pulp and the seeds of *Ceratonia siliqua*. Dried pods (pulp or seeds) were subject to the hydrodistillation for 3 h using a Clevenger–type apparatus. The two essential oils obtained were characterized by its physicochemical indications. Theses oils were used for the determination of the antioxidant activity by free radical scavenging activity, reducing power and liver lipid peroxidation methods and for the evaluation of the antibacterial properties against referenced tested bacteria. The oil of the pulp presents a high scavenging power of free DPPH radical 89.0 ± 1.2 % at concentration 1000 µg/mL and IC50 7.8 µg/mL compared to the seeds 79.0 ± 1.6 %, IC50 31.25 µg/mL. A significant activity for reducing iron and protection against lipid peroxidation induced by Fe^{+2} were obtained by the oil of the pulp. The essential oils of carob showed a good antibacterial activity against tested referenced pathogenic bacteria *Staphylococcus aureus* ATCC 25923, *Pseudomonas aerugenosa* ATCC 25853 and *Escerichia coli* ATCC 25922.
Keyword: Antibacterial, Antioxidant, Carob, Essential oils, Pulp, Seeds.

Introduction

Carob (*Ceratonia siliqua* L.), belonging to the subfamily Caesalpinaceae of the leguminoseae family is a typical tree which has been widely grown in the Mediterranean region and has an economic and environmental importance in Algeria [SEGHIR *et al.*, 2016].

This specie is used for reforestation of arid and degraded areas and also as ornamental purposes [MULET *et al.*, 2016].

The seeds and the pulp have some interesting properties and are often used in pharmacological and food industry [MARKIS and KEFALAS, 2004].

The pod has long been used as a feed for livestock and human consumption including sweets and biscuits [DAKIA et *al.*, 2007]. Pulp of carob is a good source of polyphenols, mainly tannins 16–20 %, and protein (2.7–7.6 %) but it is poor in lipid (0.4–0.8 %) [OWEN *et al.*, 2003; HADDARAH *et al.*, 2013].

The seeds and the pulp are valorized in different applications.

The purpose of the present study was to investigate the antioxidant power by different methods and the antibacterial activity of the essential oils obtained from the pulp and the seeds of *Ceratonia siliqua*.

Material and methods

Vegetable material. The mature carob pods used in current experiments were collected in the month of June 2016, from the region of Mascara (North–West Algeria). The voucher specimen was identified by botanist at SNV faculty, University of Mascara. The collected pods were washed with tap water to remove all impurities and then with distilled water. The samples were dried in darkness at room temperature and chopped into small particles.

Extraction and physicochemical characterization of the essential oils. The sample (pulp or seeds) was submitted to the hydrodistillation for 3h using a Clevenger–type apparatus (ST15

OSA, Staffordshire, UK). Physicochemical characteristics provide a base line for suitability of oils [PARTHIBAN *et al.*, 2011]. The physicochemical indications of the essential oils were determined according to the AOAC method [AOAC, 1995]. The yield of extraction is the ratio between the weight of the essential oil obtained and the weight of the sample to be treated.

Relative density was determined by the ratio between the mass of a certain volume of the essential oil and the mass of the same volume of distilled water taken at the same temperature. Miscibility in ethanol was made by complete solubilization of 1 mL of essential oil in 85% dilute ethanol at 20 ºC. When a limpid solution is achieved we register directly the volume of alcohol added.

Angle of rotation was determined by polarimeter KARL KOLB equipped with cell of 1 dcm length. Refractive Index by using a conventional refractometer ATAGOPOLYSTAT 5A and acid number by titration of 1 g essential oil, 5 mL of 96 % ethanol and about 5 drops of phenolphthalein by alcoholic solution of 0.1N KOH until the solution turns pink. The acid number was calculated by the following formula:

$$\text{Acid number} = \frac{\text{V (KOH)}.\,\text{Concentration (KOH)}.\,56.1}{\text{Mass of the test}}$$

For Ester value determination, 1.5 g of essential oil was introduced into a glass flask. It was added through burette 25 mL of ethanol solution of KOH 0.5 mol/L.

The condenser was adapted and was placed the ball on the heating mantle and allowed to heat for one h. After cooled, 20 mL of distilled water and 5 drops of 0.2 % phenolphthalein were added to it. Finally, as the excess of KOH solution with hydrochloric acid 0.5 mol/L alongside the operation cited, blank was made under the same conditions and with the same reagents. For the determination of Iodine value, 0.2 g of oil was weighed into a conical flask. 10 mL of carbon tetrachloride and 20 mL of the Wij's solution were added to the flask and the solution was kept in dark for 30 min at room temperature. 15 mL of 10 % potassium iodide solution with 100 mL of distilled water were added to the flask.

The resulting solution was titrated against 0.1 M sodium thiosulphate ($Na_2S_2O_3$), using starch as indicator till the end point where the blue-black coloration becomes colorless. A blank titration was carried out at the same time starting with 10 mL carbon tetrachloride. Iodine value was then calculated by the following formula:

$$\text{Iodine number} = \frac{(\text{B} - \text{S}).\,\text{N}.\,12.69}{\text{Weight of the sample}}$$

Where B: 0.1N sodium thiosulfate required (mL) by blank, S: 0.1N sodium thiosulfate required (mL) by sample, N: Normality of sodium thiosulfate solution.

The physicochemical indications of the essential oils of *Ceratonia siliqua* were presented in table 1.

Table 1.

Physicochemical characteristics of the essential oils.

Characteristics	Pulp	Seeds
Yield %	2.32 ± 0.18	1.2 ± 0.2
pH	4.3 ± 0.5	5.2 ± 0.3
Density (g/mL)	0.833 ± 0.03	0.910 ± 0.04
Rotators power (°)	+ 0.05 ± 0.01	+2 ± 0.01
Indication of refraction	1.426 ± 0.002	1.422 ± 0.003
Miscibility in ethanol (v/v)	12	15
Indication of iodine (g iodine/100g EO)	2.04 ± 0.01	2.01± 0.02
Indication of acid (mg KOH/g EO)	3.82 ± 0.1	2.2 ± 0.2
Indication of ester (mg KOH/g EO)	42.61 ± 0.4	33.22 ± 0.2
Indication of saponification (mg KOH/g EO)	21.34 ± 0.2	37.2 ± 0.1

Values are means triplicate determination ± standard deviation of mean.

For the determination of the Saponification value, 2 g of each essential oil were weighted into a clean dried conical flask and 25 mL of alcoholic potassium hydroxide was added. A reflux condenser was attached to the flask and heated for an h with periodic shaking. The appearance of clear solution indicated the

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completion of saponification. Then 1 mL of 1 % phenolphthalein indicator was added and the hot excess alkali was titrated with 0.5 M HCl until it reached the end point where it turned colour less. A blank titration was carried out at the same time and under the same condition. The Saponification value was calculated by the following formula:

$$\text{Saponification value} = \frac{(b - a).8.05}{m}$$

Where b: 0.5 N HCl required (mL) by the blank, a: 0.5 N HCl required (mL) by the sample. The essential oils extracted were stored at 4 ºC in an opaque glass bottle sealed to protect it from air and light (main agents of degradation).

Evaluation of the antioxidant activity. No single method was adequate to investigate the total antioxidant activity of a sample, due to the variability of the essential oils composition and the conditions of the test used. Antioxidant activity assays can be classified into two methods depending on the following two chemical reactions: methods based on hydrogen–electron transfer and methods based on single–electron transfer. The antioxidant capacity of the essential oil was estimated by different antioxidant methods, including 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing power and liver lipid peroxidation assay.

DPPH free radical scavenging activity. The ability of the carob essential oil to scavenge DPPH free radicals was evaluated by the protocol as described by Kirby and Schmidt [KIRBY and SCHMIDT, 1997] with some modifications. The essential oils were prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99 and 0.97 µg/mL. A volume of 50 µL of these solutions was mixed with 1950 µL of methanol solution of DPPH (6.10^{-5} M) as free radical source. The mixtures were stirred vigorously for 30 seconds and then incubated for 30 min in the dark at room temperature. Scavenging activity was recorded by monitoring the decrease in absorbance at 517 nm against a blank consisting of methanol solution of DPPH [SHIMADA *et al.*, 1992, LACHKAR *et al.*, 2016]. Ascorbic acid and catechin were used for comparison. Lower absorbance of the reaction mixture indicated higher free radical scavenging capacity. DPPH free radical scavenging activity was calculated as:

$$\%\ inhibition = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) * 100$$

Control sample contained all the reagents except the essential oil. Percentage of inhibition was evaluated using the equation as described previously, whilst IC50 (concentration of substrate that inhibits 50 % of the DPPH radicals present in the reaction medium) values were estimated from the % of inhibition versus concentration plot, using a non–linear regression algorithm. Tests were carried out in triplicate.

Ferric–reducing activity. The ferric reducing power of all essential oils was evaluated by the protocol described by Yildirim and collab. [YILDIRIM *et al.* 2001]. 1 mL of each oil at various concentrations was added to 2.5 mL of 0.2 M phosphate buffer pH 6.6 and 2.5 mL of potassium ferricyanide solution $K_3Fe(CN)_6$, 1 %.

After incubation for 20 min at 50 ºC, 2.5 mL of trichloroacetic acid 10 % was added and the mixture was centrifuged for 10 min at 3000 rpm. An aliquot of 2.5 mL of the supernatant from each sample mixture was added in test tube to 2.5 mL of distilled water and 0.5 mL of ferric chloride solution 0.1 % prepared freshly in distilled water. After 20 min of reaction time at 35 ºC, the absorbance was recorded at 700 nm against a blank that contains all reagents except the essential oil and ferric chloride. The control was achieved by various concentrations of ascorbic acid and catechin. Higher absorbance of the reaction mixture indicated higher reducing power. Tests were carried out in triplicate.

Liver lipid peroxidation method. Lipid peroxidation levels in the liver tissues were evaluated using the thiobarbituric acid reactive substances (TBARS) assay as described by Tatiya and Saluja [TATIYA and SALUJA, 2010]. The solution contained 0.5 mL of homogenate 10 %, 1 mL of 0.15 M KCl and 0.5 mL of

various dilutions of each essential oil. The liver lipid peroxidation was initiated by adding of 100 µL of ferric chloride 1 mM.

After incubation for 30 min at 37 ºC, the reaction mixture was stopped by addition of 2 mL of iced HCl 0.25 N containing 15 % TCA trichloroacetic acid (tissue homogenate was deprotenized by TCA), 0.38 % thiobarbituric acid and 0.2 mL of BHT, 0.05 %. The mixture was heated at 80 ºC for 60 min, cooled and centrifuged for 15 min at 6900 rpm. The absorbance of the supernatant was measured by spectrophotometer (Cary 50, Varian, Palo Alto, CA, USA) at 532 nm against a blank containing all reagents with the exception of liver homogenate and essential oils. Identical experiments were carried out to determine the normal (without essential oil and $FeCl_3$) and the level of lipid peroxidation in the tissues (with $FeCl_3$ and without essential oil).

For preparation of the homogenate, the liver was quickly removed after dissection of the rats, rinsed with physiological saline and homogenized at 4 ºC in a solution of 0.15 M KCl by 10 %. The homogenate was centrifuged for 15 min at 800 rpm to remove cellular debris; the supernatant was recovered to examine the *in vitro* lipid anti peroxidation [SINGH *et al.*, 2007]. The percentage effect anti lipid peroxidation (% ALP) was determined by the following formula:

$$\%\ \mathrm{ALP} = \left(\frac{\text{Absorbance of FeCl3} - \text{Absorbance of sample}}{\text{Absorbance of FeCl3} - \text{Absorbance of normal}}\right) * 100$$

Evaluation of the antibacterial activity

Tested bacteria and inoculums. The essential oils of the pulp and the seeds of carob were tested against referenced pathogenic bacteria that are now involved in nosocomial infectious, some of which have acquired resistance to antibiotics. Gram negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aerugenosa* (ATCC 25853) and Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) were kindly provided by the Laboratory of Medical Analysis located in Hospital of Mascara City, situated in Western of Algeria. All the microbial strains were stored in nutrient agar slope at 4 ºC then these were sub cultured at 37 ± 1 ºC for 24 h before use. The essential oils were dissolved in DMSO (dimethyl sulfoxide) and sterilized by filtration through filter paper 0.45 mm. The first test was carried out to determine the effect of various concentrations of DMSO on the bacterial growth. The protocol of inoculums preparation described by Atwal [ATWAL 2003] was prepared from a culture of 18–24 h of the tested bacteria on agar medium equivalent to 0.5 McFarland (10^8 CFU/mL). The inoculums thus prepared was then diluted 1/100 in sterile saline to a final concentration of 10^6 CFU/mL. The tests were conducted on agar and liquid medium Muller Hinton [OKIGBO *et al.*, 2009].

Antibiotic selected are: Amoxicillin (AMX, 25 µg), Tetracycline (TE, 30 µg), Erythromycin (E, 15UI), Spiromycine (SP, 100 µg), Gentamicin (G, 10UI) and Ampicillin (AM, 10 µg). We placed on the agar Muller Hinton different disks of selected antibiotics, and then the boxes were left dried for 30 min at room temperature to get a good spread of antibiotics. The plates were incubated for 18–24 h at 37 ± 2 ºC. The measure of the diameter of the zones of inhibition allows classifying bacteria in three categories: sensitive, intermediate or resistant.

Agar diffusion method. Antibacterial activities of the extracted essential oils were determined by the disk diffusion method using Mueller–Hinton agar [OKIGBO *et al.*, 2009]. The sterile disks were separately impregnated by the selected essential oil for a few h. In sterile *Petri* dishes, culture mediums Muller Hinton was poured, leave for 15 minutes to solidify, where 1 mL of inoculums was deposited beforehand prepared and inoculated with a rake. Then the disks containing the test products were transferred to the inoculated box.

After incubation for 24 h at 37 ± 1 ºC, the results were determined by the measure of the diameter of the zone of inhibition in mm including disk diameter of 6 mm [LINO and DEOGRACIOUS, 2006]. The antibacterial activity was considered positive from a diameter greater than 6 mm according to the antibiogram

committee of the French Society of Microbiology [RIOS *et al.*, 1988].

Determination of the MIC and MBC. The minimal inhibition concentration and minimal bactericide concentration of the tested oils were evaluated by agar dilution method [AKOMO *et al.*, 2009]. 100 µL of each oil were added to the first well, then serial dilutions were performed, then 100 µL of the tested bacterial suspension of 18 h in the broth Muller Hinton (10^6 CFU/mL) were added to each well. The plates were shaken and incubated for 24 h at 37 ºC.

The lowest concentration required to completely inhibit the growth of the tested bacteria was designated as the MIC and expressed in µg/mL. The MBC was defined as the lowest concentration that kills 99.99 % of the burden of the initial inoculums. For the evaluation of the MBC, 100 µL of each well that showed no change in culture were inoculated on nutrient agar and incubated for 48 h at 37 ºC and the lowest concentration showing no growth after incubation was designed as the MBC.

Results and discussion

Evaluation of the antioxidant activity

DPPH free radical scavenging activity. As shown in figure 1, the essential oil of the pulp showed a high scavenging activity of free DPPH 89.0 ± 1.2 % at concentration 1000 µg/mL compared to the oil of the seeds 79.0 ± 1.6 % at some concentration. These results are colses to thoses found with ascorbic acid and catechin (97.53 ± 4.72 %, 94.87 ± 1.78 %).

The antioxidant activity of different essential oils studied directly depends on the concentration used.

The mechanism of the reaction between the antioxidant and the DPPH radical depends on the structural conformation of the antioxidant [KOURI *et al.*, 2007; FABRI *et al.*, 2009].

The scavenger effect of active compounds on free radicals depend on the presence of free OH groups, in particular 3–OH, with a configuration 3',4'–rthodihydroxy [HEIM *et al.*, 2002].

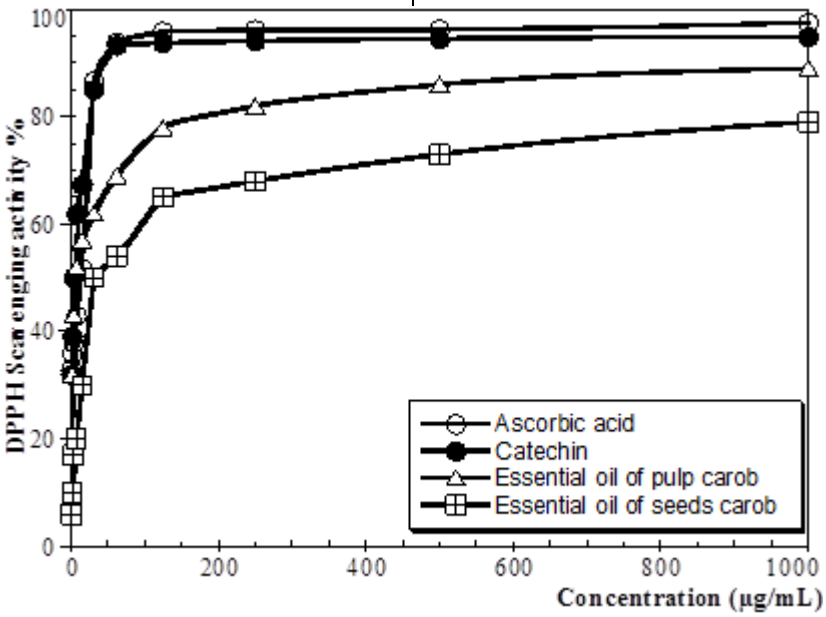


Figure 1. Free radical–scavenging capacities of the positive controls (ascorbic acid, catechin) and the essential oils of the pulp and the seeds of carob measured by DPPH assay.

The obtained herein were found to be in agreement with the work of several authors who reported that the efficiency of an antioxidant component to reduce DPPH essentially depends on its hydrogen donating ability, which is directly related to the presence of phenolic compounds [HAZZIT *et al.*, 2009], the abundance of monoterpenes hydrocarbons [RUBERTO and BARATTA, 2000], and oxygenated

monoterpenes [TEPE *et al.*, 2004]. Our results are high to those cited by Torun and collab. [TORUN *et al.* 2013].

The IC50 parameter commonly employed to measure the antioxidant capacity is necessary for each essential oils to reduce 50 % of free DPPH concentration in a defined period of time, a low IC50 value corresponds to a higher antioxidant activity. In term of IC50, the essential oil of pulp present IC50 of 7.8 µg/mL, followed by catechin 14.26 µg/mL, then ascorbic acid 17.21 µg/mL and finally essential oil of seeds 31.25 µg/mL.

Reducing power assay. The reducing power method is often used to determine the ability of the natural antioxidant to donate an electron or hydrogen [SHIMADA *et al.*, 1992].

The evaluation of the ferric reducing/antioxidant was based on the reduction of Fe^{+3}/ferricyanide complex to the ferrous form in the presence of antioxidants in the tested samples.

The Fe^{+2} were then monitored by the measuring of the formation of Perl's Prussian blue at 700 nm. In fact, it is widely accepted that higher absorbance at 700 nm is correlated to power reducing [OZTURK *et al.*, 2007].

The reducing activity of the essential oils from the pulp and the seeds of carob increased in a concentration–dependent manner (Figure 2).

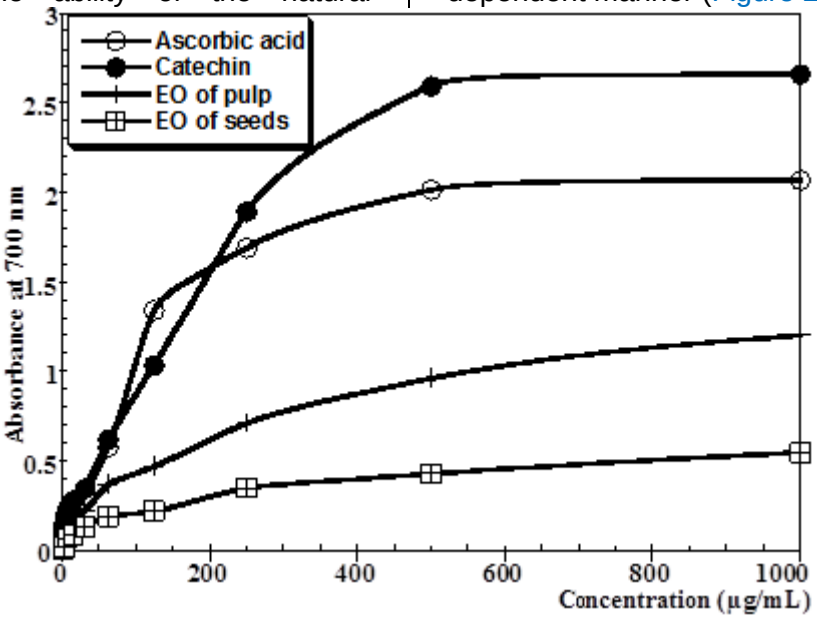


Figure 2. Antioxidant capacities of the positive controls (ascorbic acid, catechin) and the essential oils of the pulp and the seeds of carob using ferric reducing power method.

For the pulp essential oil, we note that a significant activity for reducing iron, values observed by optical density exceeding 1 ± 0.2, while ascorbic acid and catechin provide respectively an OD of 2.069 ± 0.03 and 2.66 ± 0.016 in the same concentration 1000 µg/mL.

We can classify power reduction of iron as follows: Catechin, ascorbic acid, pulp and seeds essential oils. Shimada and collab. noted that the reductive power may be directed to the presence of the phenolic compounds, such as isothymol and carvacrol, due to the hydroxyl substitutions in the aromatic ring, which possesses potent hydrogen–bonding abilities [SHIMADA *et al.*, 1992 BUTNARIU, 2012, BUTU, *et al.*, 2014b].

Liver Lipid peroxidation. The liver lipid peroxidation is related to the loss of membrane fluidity and increase with membrane permeability, causing a decrease in physiological performance [BALU *et al.*, 2005]. The inhibitory effect of the essential oils of the pulp and the seeds on Fe^{2+} induced lipid peroxidation in rats homogenates was shown in figure 3.

The chemical structure of iron, and its ability to drive one electron reactions, makes iron a key factor in the formation of free radicals [FRAGA and OTEIZA, 2002]. The oil of the pulp protects against liver lipid

peroxidation induced by Fe^{+2}, considerably increase percentage of anti–lipid peroxidation in a dose–dependent manner and was close to those of catechin and ascorbic acid with a percentage more than 60 % at 1000 µg/mL.

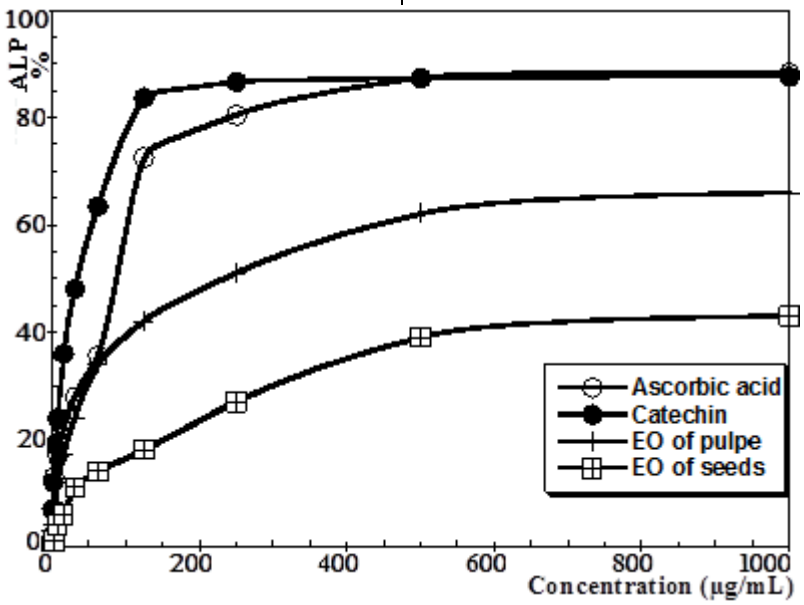
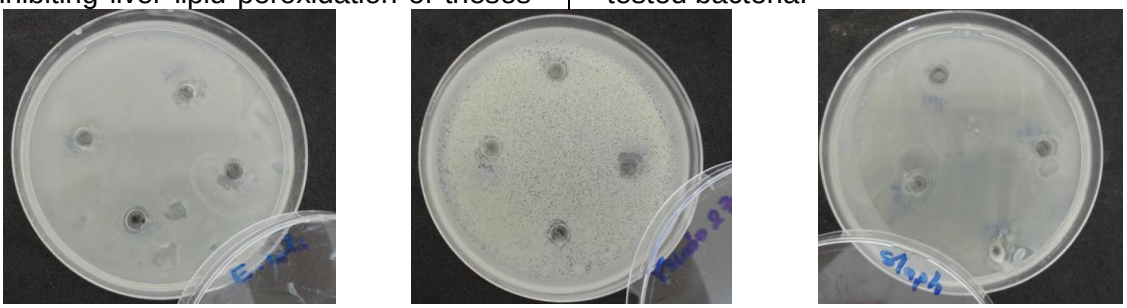


Figure 3. Antioxidant capacities of the positive controls (ascorbic acid, catechin) and the essential oils of the pulp and the seeds from carob using liver lipid peroxidation assay.

This oil was effective in inhibiting the liver lipid peroxidation induced in rat's homogenate by the system Fe^{+2} ascorbate [BUTNARIU, *et al.*, 2005, BUTU, *et al.*, 2015]. The generation of malondialdehyde (MDA) and related substances which react with the thiobabiturique acid are inhibited by pulp oil [TATIYA and SALUJA, 2010,]. This indicates significant activity of inhibiting liver lipid peroxidation of theses oils. The preventive effects by theses oils could be related to the presence of antioxidants compounds [OBOH *et al.*, 2007].

Determination of the antimicrobial activity. The first test with DMSO at various concentrations (12.5 %, 25 %, 50 % and 100 %) was performed in order to select the concentration which has no effect on the normal growth of the tested bacteria.



E. coli (ATCC 25922) *P. aeruginosa* (ATCC 25853) *S. aureus* (ATCC 25923)

Figure 4. The effect of the different concentrations of DMSO (100 %, 50 %, 25 %, and 12.5 %) on the growth of three tested bacteria

As shown in figure 4, the DMSO at different concentrations has no effect on the growth of tested strains; these results are confirmed by several authors [OKWU, 2005; DOHERTY *et al.*, 2010, BUTNARIU, *et al.*, 2016].

According to the obtained results, it appears that the among the most active antibiotics on Gram negative bacteria, first spiramycin include any which acts on the two strains followed by gentamycin, then tetracycline, and erytromycine. as against, amoxicillin, generally specific antibiotic of Gram–negative bacteria were inactive on

all strains; ampicillin was also inactive [BUTNARIU, *et al.*, 2015a, CAUNII, *et al.*, 2015].

For Gram–positive bacteria, *Staphylococci* were sensitive to the most antibiotics except Ampicillin. All tested bacteria were resistant to the antibiotics.

As shown in table 2, the oils of the pulp showed some good activity against all bacterial tests, the diameter of the zones of inhibition exceed 16 mm.

The highest activity was noted against *P. aeruginosa* with an average value of 26.44 ± 0.6 mm.

Table 2.

Results of antibiogram and antibacterial activity of the pulp and seeds essential oils.

Tested bacteria	*E. coli*	*P. aeruginosa*	*S. aureus*
Antibiotics			
Ampicillin	0	0	0
Amoxicillin	1 ± 0.1	0	15 ± 0.3
Tetracycline	4 ± 0.2	3 ± 0.2	5 ± 0.3
Gentamicin	9 ± 0.1	8 ± 0.3	16 ± 0.3
Erythromycin	4 ± 0.1	5 ± 0.2	6 ± 0.1
Spiromycine	15 ± 0.3	18 ± 0.5	7 ± 0.1
Essential oils			
Pulp of carob	20.22 ± 1.02	26.44 ± 0.6	16.08 ± 0.9
MIC (µg/mL)	34.23 ± 1.1	134.31 ± 2.3	124.31 ± 2.6
MBC (µg/mL)	68.53 ± 1.6	269.31 ± 2.7	229.31 ± 3.3
Seeds of carob	13.31 ± 1.3	17.52 ± 0.7	12.11 ± 1.07
MIC (µg/mL)	113.53 ± 3.1	175.31 ± 2.3	221.31 ± 1.6
MBC (µg/mL)	227.23 ± 2.7	375.31 ± 2.6	443.31 ± 2.3

These results are in agreement with the work of Meziou–Chebouti and collab. [MEZIOU–CHEBOUTI *et al.*, 2015] who showed a very good activity of carob leaves extract against tested strains. In a second step, the antibacterial activity by microdilution assay was determined [BUTNARIU and GIUCHICI, 2011, IANCULOV, *et al.*, 2004, BUTNARIU, 2014].

These antibacterial capacities were not related to the presence of particular compounds but only in the antagonistic or synergistic effects of each substances of the natural product [CHENG *et al.*, 2001, BUTNARIU, *et al.*, 2012, [PETRACHE, *et al.*, 2014].

Alligiannis and collab. described a classification of plant material on the basis of MIC results as follows: Strong inhibition: MIC less than 500 µg/mL and moderate Inhibition: MIC ranges from 600 to 1500 µg/mL [ALLIGIANNIS *et al.*, 2001, BUTNARIU and CORADINI, 2012, BUTU, *et al.*, 2014a].

Pulp essential oil has a good activity compared to the seeds oils with MIC of 34.23 ± 1.1 µg/mL against *E. coli*, 124.31 ± 2.6 µg/mL against *Staphylococcus aureus* and 134.31 ± 2.3 µg/mL against *P. aeruginosa*. The essential oil of the seeds also showed good antibacterial activity with MIC and MBC very low.

Conclusions

This work was assessed out to evaluate the antioxidant capacity and antibacterial activity of the essential oils obtained from the pulp and the seeds of *Ceratonia siliqua*. The oil of the pulp present highest yield 2.32 ± 0.18 % compared to the seeds 1.2 ± 0.2 %.

The oils of the pulp and the seeds have acidic an pH respectively 4.3 ± 0.5 and 5.2 ± 0.3, high indication of acid (3.82 ± 0.1, 2.2 ± 0.2 mg of KOH/g of EO), indication of ester (42.61 ± 0.4, 33.22 ± 0.2 mg KOH/g EO), indication of iodine (2.04 ± 0.01, 2.01± 0.02 g iodine/100g EO), indication of saponification (21.34 ± 0.2, 37.2 ± 0.1 mg KOH/g EO), relative density (0.833 ± 0.03, 0.910 ± 0.04 g/mL), fairly high refractive index (1.426 ± 0.002, 1.422 ± 0.003), rotator power (+ 0.05 ± 0.01, +2 ± 0.01) and miscibility in ethanol of 12 for pulp and 15 (v/v) for seeds. The antioxidant capacity of two essential oils directly depends on the concentrations.

The oil of the pulp presents a high scavenging power of free DPPH radical 89.0 ± 1.2 % at concentration 1000 µg/mL and IC50 7.8 µg/mL compared to the

seeds 79.0 ± 1.6 %, IC50 31.25 µg/mL. A significant activity for reducing iron and protection against Lipid Peroxidation induced by Fe^{+2} were obtained by the oil of the pulp. For the evaluation of the antibacterial activity, the essential oils of carob obtained from the pulp and the seeds showed a good activity against all bacterial tests.

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