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ORIGINAL RESEARCH PAPER

EXTRACTION OF *NIGELLA SATIVA* SEEDS AT A PILOT SCALE AND THEIR EFFECTS ON THE FORMATION OF *PSEUDOMONAS AERUGINOSA* BIOFILMS

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Abstract: The extraction of vegetable oils from aromatic and medicinal plants has been the subject of research in several recent studies. Because of their richness in secondary metabolisms which are part of the manufacture of drugs, food supplements and cosmetic products. We are interested in discontinuous solid-liquid extraction of seeds of *Nigella sativa at* a pilot scale. Biological and physicochemical analysis was carried out on the extracts of these seeds, in order to valuate them. The results have shown that the extracts of *Nigella sativa* seeds are very rich in fatty acids and polyphenols in addition to the presence of other nutrients such as vitamins, amino acids and free quinones which have shown potential in phytotherapy. The extracts of the plant prevents the formation of *Pseudomonas Aeruginosa biofilms*. This study has given very promising results with regard to the efficiency of the extracts on free radicals and bacterial strains.

Keywords: *antioxidant, biological analysis, Nigella sativa, phytochemical analysis, solid-liquid extraction, vegetable oil*

INTRODUCTION

Plants, there are those who feed, those who kill and those who care. The plant world intersects with scientific research until today. Medicinal plants have developed into a real pharmacy. These plants have become essential in human life and resulted in a medicine that continues to reveal its pure virtues. Among these plants, the seeds of *Nigella sativa* have not ceased to show their therapeutic virtues and consecration [1 – 6].

A large amount of free radicals can be generated in the human body during the metabolic process.

These free radicals can damage DNA and lead to the oxidation of lipids and proteins in cells, resulting in oxidative stress and homeostatic imbalance, even some chronic and degenerative diseases and some cancers. Currently, many studies are devoted to the exploration and use of natural antioxidants to eliminate excessive free radicals in the human body, thereby achieving prevention and treatment of disease. On the one hand, all these studies can constitute a theoretical basis for the development of medicines and healthy foods; on the other hand, to propose new development ideas for the food industry, in particular for the food additive industry [7, 8]. Antioxidants are molecules with various origins able to neutralize activated forms and toxic oxygen species (singlet oxygen, superoxide anion, hydrogen peroxide, peroxyl radicals, and hydroxyl radical) and to slow the degradation of materials or organic compounds due to oxidation effects.

This work is a part of the development of *Nigella sativa* seeds. In order to study the development of the solid-liquid extraction of these seeds at a pilot scale, we have carried out the extraction of their oils, the determination of the total polyphenols and the total flavonoïds. The study of their antioxidant, antimicrobial, non-stick, anti-biofilm activities and physico-chemical analyzes were also performed.

MATERIALS AND METHODS

In the fields of natural substance chemistry, analytical chemistry and therapeutic chemistry, the extraction of organic molecules is a crucial phase.

MP1035 pilot description

Principle of operation

Solid-liquid extraction is a semi-continuous process, coupling distillation with a Soxhlet type cartridge containing the solid product impregnated with an active principle (solute) to be extracted by dissolution in a hot solvent (Figure 1). The solvent can also be fed in a single "pass" for infusion and the extract obtained is withdrawn manually [9].

Filling of products

The boiler must be filled with the working solvent. In the present study we used two types of solvents:

- *n*-hexane to extract the fixed oil contained in *Nigella* seeds.
- *phytochemical analysis* mixture of 80 : 20 (v / v) ethanol-water solvent was used for the extraction of the crude extract.

When the boiler is full or sufficiently charged, we:
- close the front filler cap of the boiler with its seal, open the flange of the extraction cartridge cover;
- unscrew the fittings of the upper solvent supply tube from the extraction cartridge by hand;
- push the upper solvent supply tube from the extraction cartridge towards the upper solvent supply valve so as to completely disengage the head from the extraction cartridge,
- fill with solid study product (dried and crushed *Nigella* seeds), the bag thus prepared;
- position the bag containing the solid product to be studied with its metal support in the extraction cartridge;
- position the upper solvent supply tube from the extraction cartridge to the latter so as to supply the solvent in the centre of the cartridge;
- hand tighten the fittings of the upper solvent supply tube of the extraction cartridge;
- position the cover of the extraction cartridge again in its place;
- close the flange of the extraction cartridge cover**.**

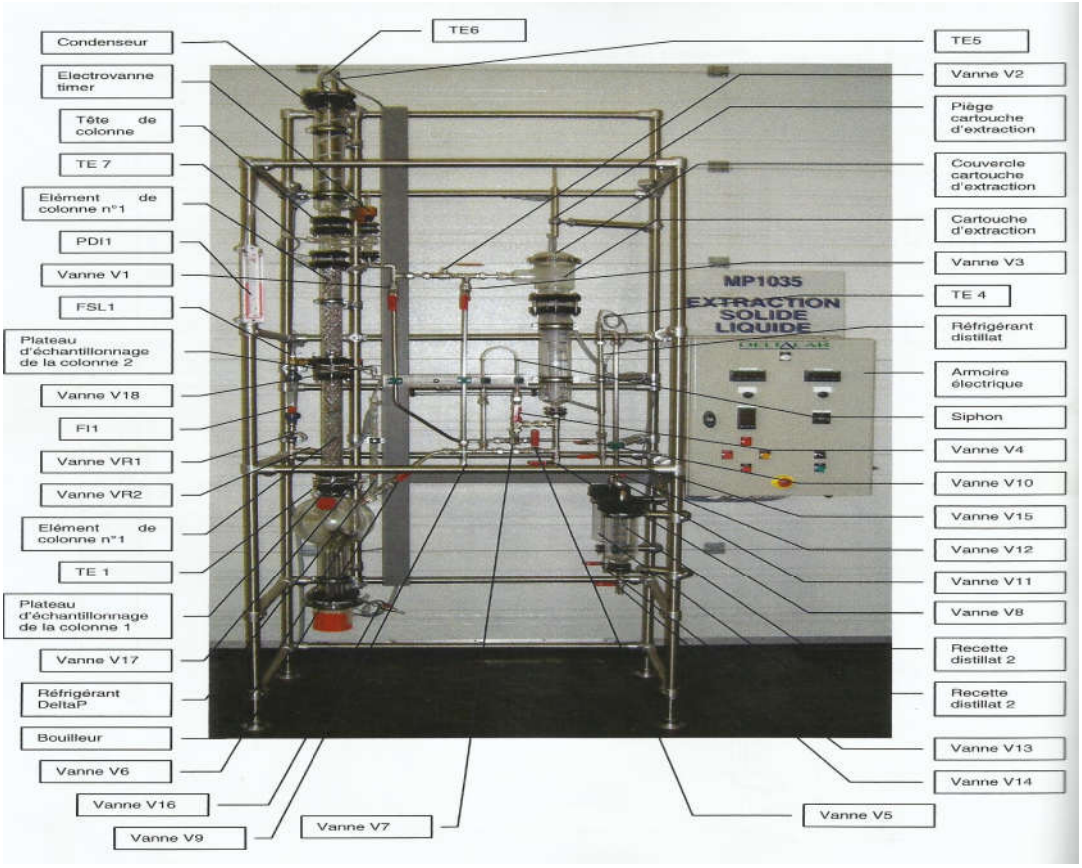

Figure 1. *Solid-liquid extraction pilot and distillation MP1035*

Solid-liquid extraction

• Start the pilot;
• Open valves V2, V4 and V6 successively;
• Switch on the boiler heating by the "on" button located on the electrical cabinet;
• The heating power of the boiler is adjusted to the value desired by the corresponding regulator;
• Temperatures are increasing; the value of the column pressure drop is indicated by the U-shaped pressure gauge in mm of water column;
• When the solvent vapors reach the column head, they are condensed, then flow back into the column;
• Start the distillate sampling timer with the "on / off" button located on the electrical cabinet;
• The condensed solvent filled the extraction cartridge to the upper level of the overflow;
• When the solution contained in the extraction cartridge overflows, it returns to the boiler;
• The condensed solvent refills the extraction cartridge for a new filling-siphoning cycle.

Plant material

This study was carried out on the *Nigella sativa* seeds. The harvest of the seeds of *nigella* was made in September 2019 in the nursery of Timsadart, region of Ain Ghoraba – Tlemcen, Algeria.
The plant material (seeds) was dried in the shade at 20 °C. To obtain better extraction, it is advisable to use dry plant material in order to eliminate any risk of degradation of the antioxidant compounds under the action of the enzymes of fresh plant material.
The material thus dried was finely ground using an electric grinder (Retsch KM 100), then sieved through three sieves (1 mm, 400 µm, 200 µm) in order to be able to recover the powder with a particle size between (200 and 400 µm), compatible with the pores of the pilot cartridge.
The pilot is then ready for an extraction.

Oil extraction

Fixed oil extraction

A mass of 200 g of the seeds of *Nigella* prepared previously undergoes an extraction with an apolar solvent (*n*-hexane 95 % supplied by Biochem) for a ratio 1 / 10 (w / v) for 4 hours (25 cycles) to ensure exhaustion any apolar substance contained in the seeds. The maximum of solvent was recovered by distillation on the same pilot. The quantity of hexane remaining in the boiler was recovered and then evaporated using a rotavapor (BÜCHI R200). The final product was red to brown in color, fluid in shape and spicy in smell.

Crude extract extraction

At the end of the extraction with the polar solvent, the seeds were left to dry overnight before being reused for a second extraction with a polar solvent (ethanol-water) 80 / 20

(v / v) in order to extract the polar and moderately polar compounds; the protocol indicates to do the extraction for a longer time than the first, about 6 hours (37 cycles). At the end of the process and after distillation, the alcoholic extract in turn was put on a rotary evaporator to remove all traces of solvent. The product thus obtained has a dark brown and pasty shape and a pronounced odor.

Essential oil extraction

When the distilled water is brought to the boil, the water vapors enter the seeds and break the plant cells releasing the volatile molecules. The vapors are then cooled and collected in a bottle. Diethyl ether solubilizes the essential oil and is immiscible with water; we opted for a liquid-liquid extraction. By decantation we manage to separate the two phases, the organic phase containing the essential oil was recovered, the solvent was then evaporated and then the essential oil was recovered.

Extraction yield

The yield of the extracts was compared to the dry matter, by calculating for each extract the difference between the mass of initial seeds and that of product, on the initial mass. The yield is calculated according to the following relationship (Equation 1):

$$Y(\%) = \frac{mass_{initial} - mass_{product}}{mass_{initial}} x100 \quad (1)$$

Determination of total polyphenols

The most suitable protocol for the evaluation of the phenolic compounds contained in the extracts obtained was based on the reducing power of these compounds, on the mixture of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic ($H_3PW_{12}O_{40}$) acid of the Folin-Ciocalteu reagent. A blue complex was formed; the reading was carried out on a UV-Visible spectrophotometer of type "SPECORD 210/plus" at a wavelength of 750 nm [10].

Protocol

2 mL of freshly prepared 2 % sodium carbonate solution were mixed with 100 µL of the extract (to be diluted if it is too concentrated). The mixture was stirred with a vortex for 5 minutes, 100 µL of Folin-Ciocalteu reagent (supplied by Prolabo) at 1 N were then added to the mixture, the whole was left to stand for 30 minutes at room temperature before reading at 750 nm against a blank containing all the reagents except the extract to be analyzed.

The absorbance read at 750 nm corresponds to the amount of polyphenols present in the extracts.

A standard Gallic acid curve was performed in parallel under the same conditions as a control, the results obtained were expressed in mg equivalent of Gallic acid per gram of crude extract (GAE / g E) [11].

Determination of total flavonoids

The aluminum trichloride method was used to quantify flavonoids (Figure 2), the appearance of the yellow color due to the fixation of Al^{3+} ions on the oxygen atoms of carbons 4 and 5 of flavonoids is shown in the structure below, and the reading is carried out on a UV-Visible device at 415 nm.

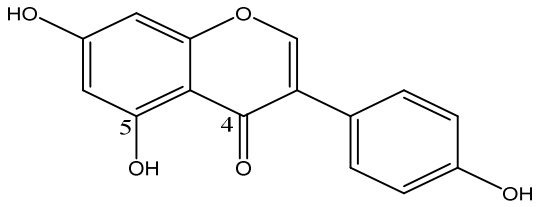

Figure 2. *Structure of flavonoids*

Protocol

The quantification of flavonoids was carried out by the colorimetric method [12].

500 µL of the extract were added to 2 mL of distilled water followed by 150 µL of a 15 % sodium nitrite ($NaNO_2$) solution. After 6 minutes, 150 µL of 10 % aluminium chloride ($AlCl_3 \cdot 6H_2O$) were added to the mixture; the whole was left for 6 minutes without stirring, then 2 mL of 4 % sodium hydroxide were added to the tubes and the final volume was immediately completed with 200 µL of distilled water.

The reading was performed at 415 nm after 15 minutes; the white was prepared by replacing the extract with methanol.

The calibration curve was carried out in parallel under the same working conditions using quercetin as a positive control.

The results were expressed in mg quercetin equivalent per gram of crude extract (ERQ / g E) [12].

Biological activities

Study of antioxidant activity

Iron reduction: FRAP METHOD (Ferric reducing antioxidant power)

The reducing power of an extract is mainly linked to its antioxidant power. The reducing activity of our extracts was summed up by the ability to reduce the Fe (III) present in the potassium ferricyanide complex $K_3[Fe(CN)_6]$ to Fe (II). The absorbance was proportional to the reducing power of the tested extracts. Reading was carried out at 700 nm on a UV-Visible device [13].

Protocol

1 mL of the sample at different concentrations was put in a mixture of 2.5 mL of phosphate buffer solution (*p*H = 6.6) and 2.5 mL of a potassium ferricyanide solution $K_3[Fe(CN)_6]$ to 1 %. The tubes were left in the oven at 50 °C. After 20 minutes of incubation, the tubes were cooled to room temperature; to stop the reaction 2.5 mL of 10 % trichloroacetic acid were added, and then centrifuged at 3000 rpm for 10 minutes.

A volume of 2.5 mL was removed from the supernatant to add it to 2.5 mL of distilled water and 500 µL of a solution of $FeCl_3 \cdot 6H_2O$ (0.1 %). The mixture was analyzed by UV-Visible spectrometry, against a blank at 700 nm [14].
Ascorbic acid was used as a positive control while keeping the same operating conditions.
The results were represented on a diagram which indicates the absorbance for each concentration of the extracts and the ascorbic acid.

Trapping of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl)
2,2-diphenyl-1-picrylhydrazyl (DPPH) is a radical widely used in antioxidant activity tests, this is due to its stability as a radical and also as a molecule by accepting an electron or a hydrogen radical. In our work the antioxidant effect is translated by the possibility of giving a hydrogen radical, and this is observed by the initial color change from purple to yellow; the reducing capacity on the DPPH was read at its maximum absorbance located at 515 nm.
DPPH was dissolved in methanol before being used, then stored in an opaque flask away from any light source to avoid its oxidation. The maximum absorbance of DPPH was checked before use, it must be between (0.40 and 1.0) [15].

Protocol
1 mL of each extract at different concentrations was prepared in methanol, and was added to an equal volume of the freshly prepared DPPH solution. The tubes were vortexed and kept in the dark for 30 minutes. A blank was prepared containing the extract without DPPH, the absorbance of the samples was read at the same maximum absorbance of DPPH alone (515 nm).
For the standard, reference was made to ascorbic acid as in the handling of FRAP.
- The percentage of anti-free radical activity was calculated by the Equation 2:

$$I(\%) = \frac{A_C - A_T}{A_C} x100 \quad (2)$$

where:
A_C - absorbance of negative control (Abs.)
A_T - absorbance of the test (Abs.)

The concentration of extracts necessary to trap 50 % of free radicals was calculated from the linear regression equation obtained by plotting the percentages of inhibition calculated according to different concentrations of prepared samples. This concentration is known by the term (Efficient concentration 50 - EC50 or inhibitory concentration 50 - IC50) [16]. The lower value of IC50, the greater the antioxidant effect.

Antimicrobial activity
To assess the antimicrobial activity of *Nigella sativa* oil, two techniques were performed on a strain of *pseudomonas aeruginosa*.
Pse*udomonas aeruginosa* is a robust gram-negative bacterium, very resistant to many antiseptics, often used in hospitals, responsible for nosocomial infections. The mortality rate reaches 50 % in vulnerable patients. It is renowned for the ease of contagion and its

speed of adaptation to drug attacks; further tests have shown that it is a biofilm trainer [17].
The different extracts from *Nigella sativa* seeds have a broad spectrum of inhibition against different strains of gram-positive and gram-negative bacteria [18].

Non-stick activity
Urinary catheter pieces of 1 cm^2 were prepared, sterilized and placed in tubes containing 1 mL of the bacterial suspension at an OD of 0.1 and 1 mL of *Nigella sativa* oil at concentrations of 50, 40 and 30 % diluted in DMSO, were incubated at 37 °C for 24 hours. The pieces were collected, washed thoroughly with sterile distilled water and then placed in 1 mL of physiological water. Sonication was carried out 3 times using the Wise Clean WUC-D06H ultrasound model for 5 minutes, interspersed with a 20 seconds vortex passage. A series of dilutions was made for each sample, and then inoculated on nutrient agar. The colonies were counted after 24 hours of incubation at 37 °C [19].

Anti-biofilm activity
After the formation of the biofilm on pieces of 1 cm^2 of urinary catheter, the recovered pieces were rinsed with sterile distilled water and placed in tubes containing *Nigella* oil at concentrations of 20, 30 and 50 % diluted in DMSO, then incubated at 37 °C for 24 hours.
After incubation, the pieces were rinsed and then treated by successive passage on the ultrasound and vortex to detach the bacteria adhered to the probe. A series of dilutions were made for each sample, followed by seeding on nutrient agar. The colonies were counted after 24 hours of incubation at 37 °C [20].

Physicochemical analysis

Density
The relative density of *Nigella* oil was carried out by a hydrometer, it is in the form of pycnometers of 1 mL of volume and mass known with precision.
The hydrometer was then filled with *Nigella* oil, and then closed by its cap; the excess oil which overflows on the walls was wiped off before weighing. As before, the weighing was repeated until a stable value was reached.

Refractive index
The refractive index was read from a light prism refractometer witch was directed to a light source and calibrated with distilled water.
If the reading was taken at a slightly different temperature at 20 °C, an empirical formula Equation 3 makes it possible to evaluate the refractive index at 20 °C exactly.

$$n^{20} = n^{T} + 0.0004(T - 20) \quad (3)$$

Acid index
In order to determine whether a fatty acid was not deteriorated, the concentration of free acid it contains was calculated. It was determined by the amount of potash (in mg)

needed to neutralize all free acids; a deteriorated fatty acid was linked to the transformation of triglycerides into glycerol.
This index was determined by a dosing in return, the free acids react with the known amount of potassium added; the excess potassium was then measured by an HCl solution of precisely known concentration, the acid index was then calculated as follows (Equation 4):

$$I_A = \frac{(V_T - V_E)C_{HCl}M_{KOH}}{m} \quad (4)$$

where:
I_A - acid index [mg KOH/g]
V_T - volume of hydrochloric acid solution used for the control [mL]
V_E - volume of hydrochloric acid solution used the sample solution [mL]
C_{HCl} - given hydrochloric acid concentration,[$mol \cdot L^{-1}$]
M_{KOH} - molar mass of KOH [$g \cdot mol^{-1}$]
m - mass of oil exactly weighed [mg]

Saponification index
The saponification index corresponds to the mass in mg of KOH necessary to neutralize the esterified fatty acids contained in 1 g of fat. If a fatty substance is brought to a boil in the presence of potash, the esters saponify. It is a total and slow reaction; it takes forty to sixty minutes to gently boil. The KOH reacts with the fatty acids released to form soap; the excess is then dosed with a hydrochloric acid (HCl) solution [21] (Equation 5).

$$I_S = \frac{(V_T - V_E)C_{HCl}M_{KOH}}{m} \quad (5)$$

where:
I_S - saponification index [mg KOH/g]
V_T - volume of hydrochloric acid solution used for the control [mL]
V_E - volume of hydrochloric acid solution used the sample solution [mL]
C_{HCl} - given hydrochloric acid concentration [$mol \cdot L^{-1}$]
M_{KOH} - molar mass of KOH [$g \cdot mol^{-1}$]
m - mass of oil weighed [mg]

Infrared spectrometry

The infrared spectra of the samples tested and the standards were established via an FTIR device by Agilent technology CARY 630 FTIR, the interval is between (400 and 4000 cm^{-1}). It is widely used in the identification of functional groups of secondary plant metabolisms.
The technique was used on three different samples (black seed powder, fixed oil and raw extract). Gallic acid, quercetin and ascorbic acid serve as witnesses for the interpretation of our spectra.

RESULTS AND DISCUSSION

This work involves the study of the extraction of crude extract, fixed oil and essential oil from the *Nigella sativa* seeds using a solid-liquid pilot extractor.

Extraction yield

The three extracts recovered after extraction were filtered and stored for the determination of dry weight. The yield was calculated relative to 100 g of crushed and dried seeds. The results were presented as a percentage in Figure 3.
According to the diagram, the extraction with the two solvents gave good yields (greater than 10 %) compared to the hydro distillation which has a clearly low yield (1.55 %).
The extraction with the non-polar solvent (*n*-hexane) represented by the fixed oil in the diagram, has given the best yield (26.43 %), this is due to the amount of non-polar compounds contained in the seeds such as lipids and mainly proteins.
Similarly, the crude extract from the extraction by polar solvent (water-ethanol) has given a yield of 13.38 %, represented by the secondary compounds such as polyphenols and alkaloids.
The volatile compounds (thymoquinone and thymohydroquinone) present in small quantity in the seed justify the low yield.
These results thus obtained by the pilot MP 1035 remain more important as for the work carried out on the traditional Soxhlet assemblies, and this shows the efficiency of the extraction on pilot.

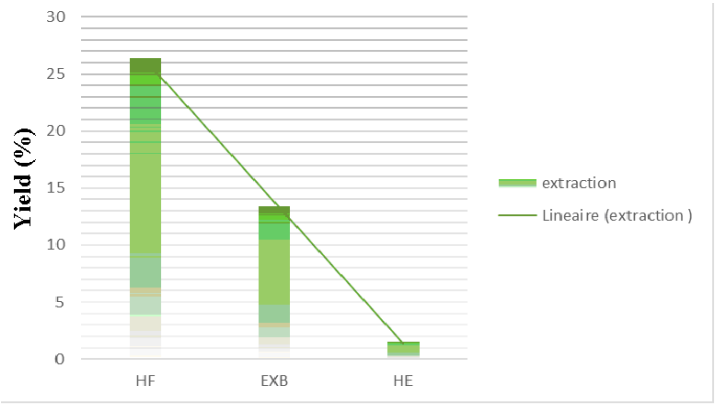

Figure 3. *Yield of Nigella sativa extracts*

Determination of total polyphenols

The Folin-Ciocalteu colorimetric method was used for the determination of total polyphenols. The polyphenol content was determined from the positive control calibration curve performed (Gallic acid); it was expressed in mg equivalent of gallic acid per gram of extract (mg EAG / g E) (Figure 4a).
The results obtained are shown in the histogram (Figure 5a).
The raw extract of *Nigella* seeds showed a high level of polyphenols (20.89 mg EAG / g E), while the fixed oil has a remarkably low value (6.81 mg EAG / g E).

Determination of total flavonoids

The dosage of flavonoids was carried out by the method of aluminum trichloride [20]. The flavonoid content in our extracts was expressed in mg equivalent of quercetin per g of the extract (mg EQ / g E) from the calibration curve performed (Figure 4b).
The results obtained are shown in Figure 5b.
The crude extract from the seeds of *Nigella sativa* has a flavonoid content of 3.16 mg EQ/g E, unlike the fixed oil which has an indefinite value for a solubilization stress in the solvents described in the protocol.

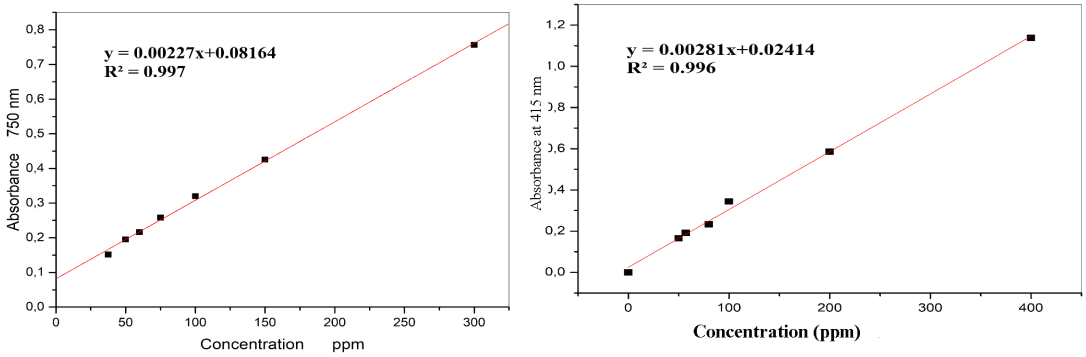

Figure 4. *Calibration curve* a) *Gallic acid; b) Quercetin*

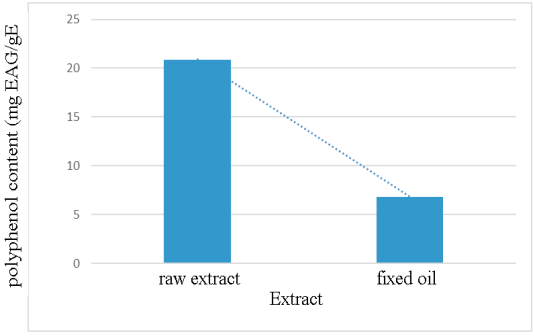

Figure 5a. *Polyphenol content of extracts*

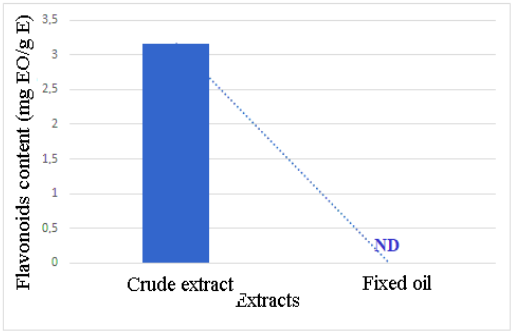

Figure 5b. *Flavonoids content of extracts*

Study of antioxidant activity

Trapping of the DPPH radical
The reduction in the DPPH radical by the antioxidant present in the extracts tested was expressed by the reduction in the absorbance of this radical followed on a UV-Visible spectrophotometer.
The curves plotted in Figures 6a and 6b represent the percentage of inhibition of the extracts as a function of the concentration; the standard curve (in ascorbic acid) was presented in Figure 6c.

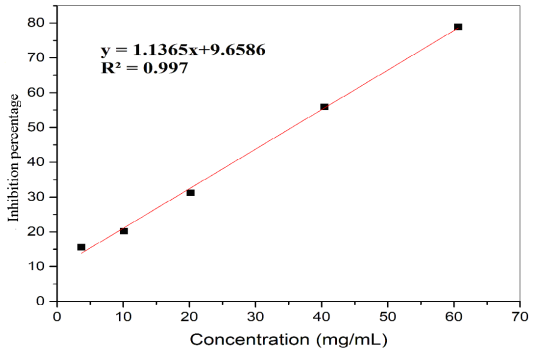

Figure 6a. *Inhibition percentage as a function of the different concentrations of the fixed oil*

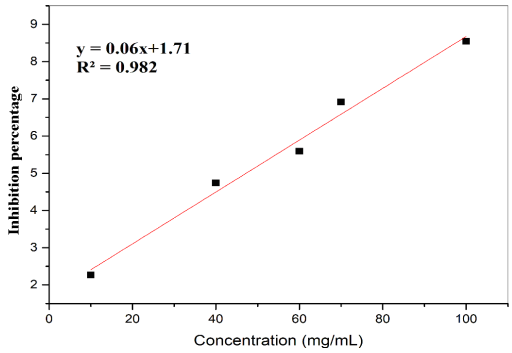

Figure 6b. *Inhibition percentage as a function of the different concentrations of the crude extract*

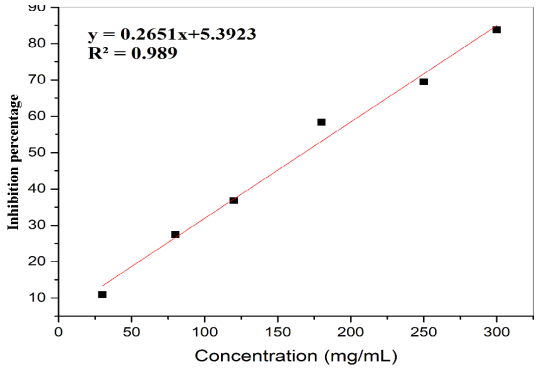

Figure 6c*. Inhibition percentage based on different concentrations of ascorbic acid*

The antioxidant activity of the different extracts was measured by the IC50, the parameter which corresponds to the concentration necessary to reduce 50 % of the DDPH radical; a low IC50 value implies a higher antioxidant activity.
The histograms below represent a comparison between the IC50 of our extracts and that of ascorbic acid.
The results presented (Figure 7) show an appreciable difference between the antioxidant effect of the standard and the raw extract of *Nigella* seeds.
Nigella sativa's fixed oil IC50 remains undetermined due to the unavailability of a suitable solvent to dissolve the oil.
According to Figure 7, the crude extract exhibited remarkably significant activity compared to ascorbic acid (the standard antioxidant).

FRAP reduction test

Figure 8 represents the histograms of the absorbance as a function of three different concentrations of the crude extract and the ascorbic acid; the increase in absorbance indicates an increase in reducing power.
According to our results, the increase in iron reduction is proportional to the concentrations used.

From sight, it is observed that the slope of the trend curve of ascorbic acid is greater than that of the crude extract which presents a slight increase in the reducing power of the three solutions tested.
The reducing power of our extracts due to the richness of these extracts in polyphenolic substances tends to release their electron, or proton from the hydroxide group [21].

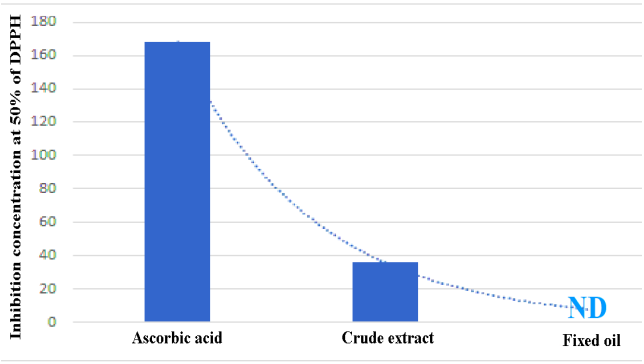

Figure 7*. The IC50 of the different extracts and ascorbic acid*

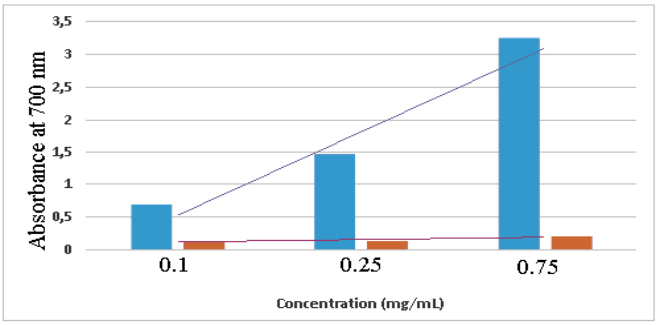

Figure 8. *Reducing power of crude extract (brown) and ascorbic acid (blue)*

Antimicrobial activity

Antiadhesive activity

Once the evaluation by the Transmission Control Protocol method was completed, our choice was based on the most formative strain of biofilm.
After incubating the probe pieces with the bacterial suspension and the various concentrations of HF for 24 h at 37 °C, a series of dilutions were made at each concentration (10, 20, 30 and 50 %).
According to the results obtained from the counting of the boxes inoculated on cetrimide agar, the black cumin oil prevented the installation of the biofilm on the pieces of probe at the various concentrations (Figures 9 and 10).

Anti-biofilm activity

After incubation with the bacterial suspension of *P. aeruginosa* for 24 h at 37 °C, the pieces of probes were rinsed with distilled water then put in tubes containing various

concentrations of HF (10, 20, 30 and 50 %). A series of dilutions were made for each sample.
The results obtained after enumeration on cetrimide agar, showed the elimination of the biofilm formed by the oil, at a concentration of 10, 20, 30 and 50 % (Figure 11), which shows the strong bacterial activity of *Nigella sativa* against *P. aeruginosa*.

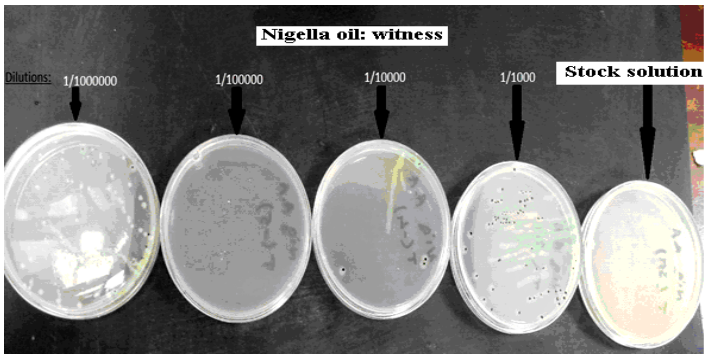

Figure 9. *Witness count of Nigella sativa*

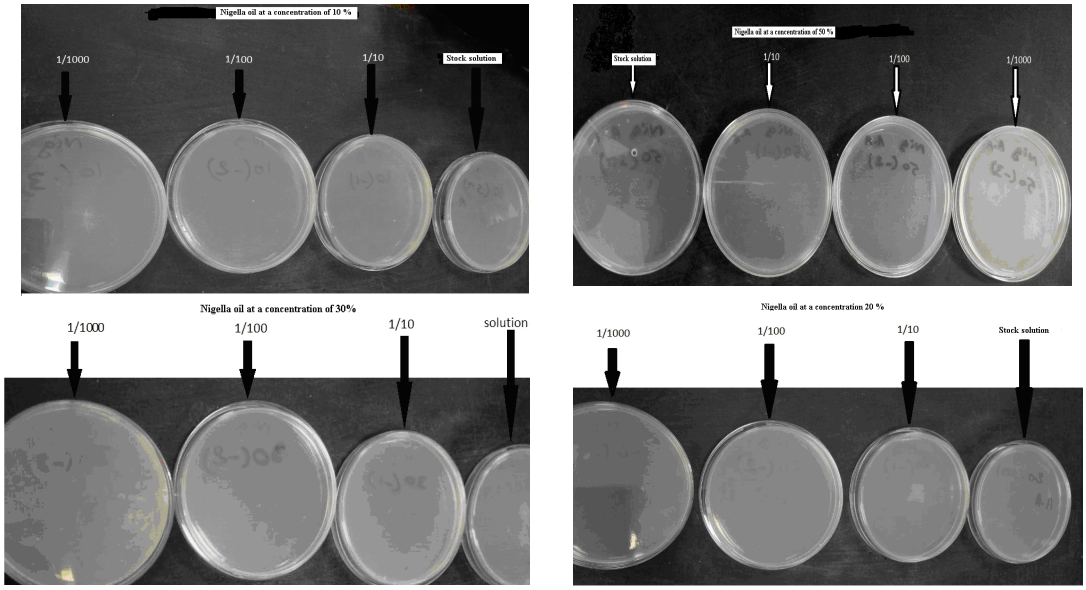

Figure 10. *Non-stick activity of N. sativa on P. aeruginosa at 10, 20, 30 and 50 % respectively*

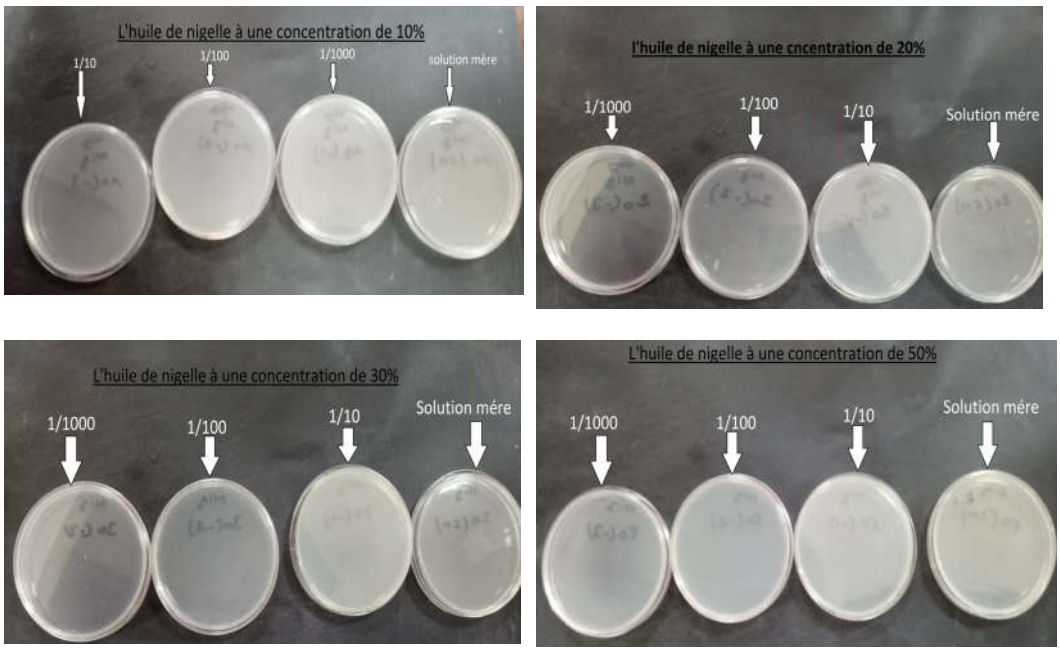

Figure 11. *Anti-biofilm activity of N. sativa on P. aeruginosa at 10, 20, 30 and 50 % respectively*

Spectroscopy infrared

The spectra obtained were presented in Figures 12 and 13.

The tables 1 and 3 indicate the characteristic bands of the main functional groupings.

The same groupings characterizing the standards were observed in the samples tested, the interest of the infrared technique was reduced so to speak to an analytical use in the identification of our samples by comparison with reference products.

Inductive and mesomeric effects can cause frequency shifts in the absorption bands.

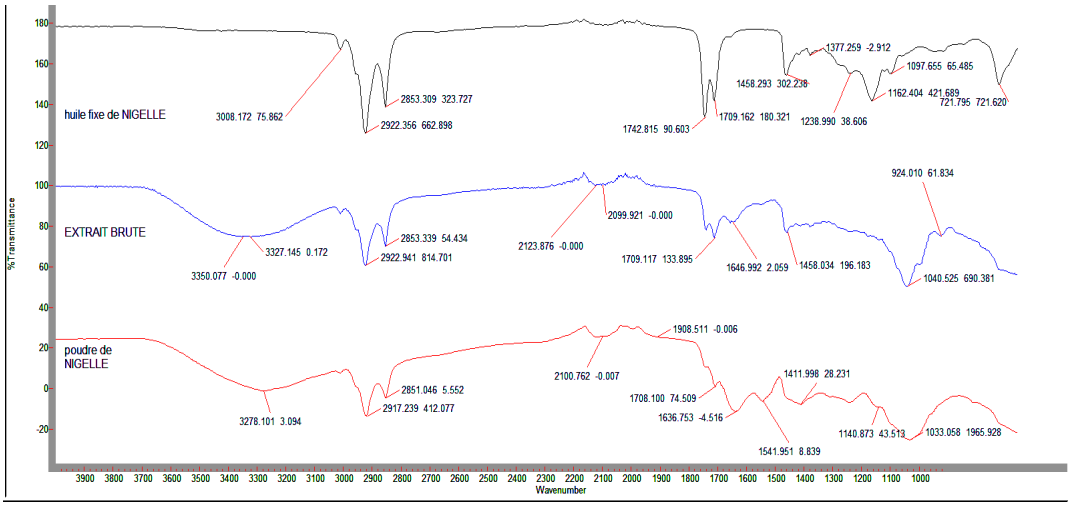

Figure 12. *Superimposition of the 3 spectra of the samples tested*

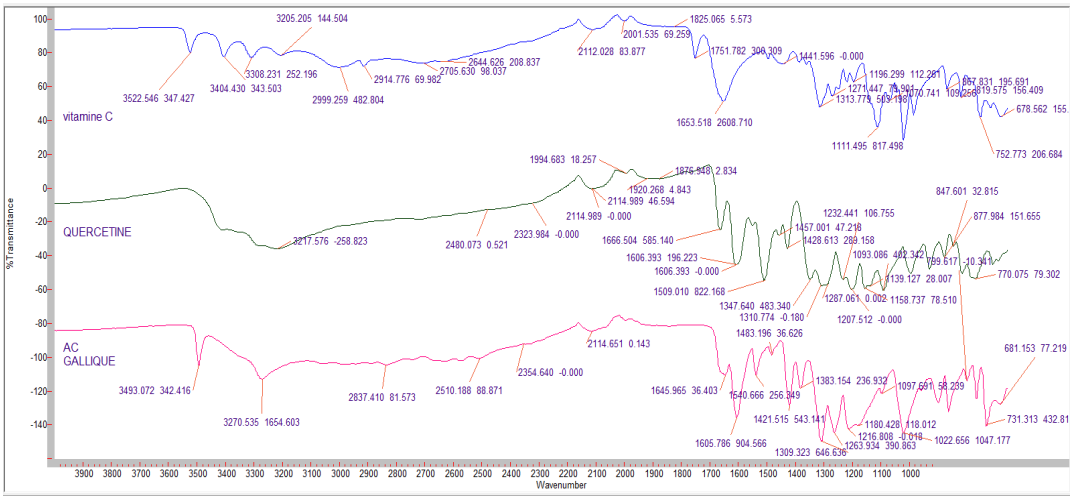

Figure 13. *Superimposition of the 3 spectra of the standards used*

Table 1*. Characteristic bands of the samples*

Functional grouping	Fixed oil [cm^{-1}]	Crude extract [cm^{-1}]	*Nigella* powder [cm^{-1}]
C=O	1709	1646	1636
C-H	2853-2922	2853-2922	2851-2917
O-H	-	3350	3278

Table 2*. Characteristic bands of standards*

Functional grouping	Quercetin cm^{-1}]	Gallic acid [cm^{-1}]	Ascorbic acid [cm^{-1}]
C-H	770	731	754
C=C	1606	1605	1455
C=O	1666	1645	1656
O-H	3217	3270	3311

Physicochemical parameters

The density index was considered to be a physical criterion which corresponds to a density ratio of a substance compared to the density of water. The density of the *Nigella* oil studied in this work reveals a value of 0.9584; this value is higher than that found in previous works [22].

The refractive index was an important parameter for checking the purity of the oil, the reading (1.4725) was similar to the data reported [23].

The degree of alteration of oil was determined through its acid number; it is a chemical parameter of freshness and purity of oil. The result given by our oil (7 mg KOH / g) falls within the range characterizing good oil, less than 10 mg KOH / g depending on the distributing company.

The saponification index corresponds to the quantity in mg of KOH necessary to saponify 1 g of fat. The value of 187.11 mg KOH/g was given by the *Nigella* oil analyzed, the latter is lower than that found in the literature [24]. The saponification index provides information on the length of the carbon chain of the fatty acids which constitute the triglycerides (majority fraction of a fatty substance).

The determination of the saponification index of *Nigella* oil gave a value equal to 187.11 mg KOH / g for *N. sativa* and 175.88 mg KOH /g for olive oil.
It is noted that the value of the saponification index of *Nigella sativa* is greater than olive oil, which shows that *Nigella sativa* contains less long chains of fatty acids.
We can deduce that *Nigella sativa* oil is pure oil rich in long-chains and unsaturated fatty acids.
These indices were grouped in the Table 3 below reporting a comparison between the values found in our study, in the literature and of commercial oil.

Table 3. *Physicochemical parameters of fixed oils*

Physicochemical indices	***Nigella* oil (present study)**	***Nigella* oil (literature) [21]**	***Nigella* oil (commercial) [22]**
Density at 20 °C	0.9584	0.913	0.900 to 0.925
Refractive index at 20 °C	1.4725	1.684	1.470 to 1.476
Acid index [mg KOH/g]	7	2.11	< 10
Saponification index [mg KOH/g]	187.11	223.57	185.205

CONCLUSION

At the end of this experimental study, we obtained very convincing and encouraging results. Indeed, the results of this study have shown that the extracts of *Nigella sativa* seeds are very rich in fatty acids and polyphenols in addition to the presence of other nutrients such as vitamins, amino acids and free quinones which have shown potential in phytotherapy.
On the other hand, a fixed oil supplementation of *Nigella sativa* has shown a beneficial effect against the reproduction of a bacterial strain of gram negative type *Pseudomonas aeruginosa*, black cumin oil prevents the formation of biofilms on hospital probes, as well as elimination of the biofilm on infected probes.
Furthermore, we have found that solid-liquid extraction on the MP1035 pilot gives a significantly better yield than the other conventional techniques used.
MP1035 pilot has two techniques (extraction and distillation), allows the recovery of the solvents used at the end of the process; which is beneficial in term of time and cost.

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