Phytochemical Screening, Antimicrobial Activity Study and Evaluation of in Vivo Anti-inflammatory Activity of Essential Oils of *Opuntiaficus-Indica* Seeds

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Abstract:

The *Opuntiaficus-indica* commonly called "prickly pear" cactus or Indian fig. The yield rates of extraction of essential oils from seeds by the steam stripping method and followed by liquid-liquid extraction by cyclohexane from the aqueous phase gave low seed yields equal to 0.29%.nIn the present study the phytochemical screening reveals Gallic Flavones Genincathecole Sterols and triterpenes. GC-MS has allowed to identify twenty nine compounds. On the other hand, the in vivo anti-inflammatory study in Wistar rats showed that our essential oil has a very important effect that persists and reaches an inhibition rate of 89.6%.The essential oil showed a very good antimicrobial activity against the tested bacteria.

Keywords:*Opuntiaficus-indica*, screening phytochimique, essential oïl, anti-inflammatoirein vivo, antimicrobienne, rats Wistar.

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I-Introduction

Opuntiaficus-indica, is a fruit of the genus Opuntia affiliated to the family Cactaceae native to Mexico, well adapted to the climate of the Mediterranean basin **(Butera and al., 2002)**, it was introduced in North Africa around the 16th century **(Araba et *al*., 2000; El Mannoubi et *al*., 2008).**This arborescent plant is well adapted to arid and semi-arid climates such as Algeria. This shrub was marginalized **(Maataoui and al, 2006).** However, this plant is attracting more and more interest, in recent decades different parts of this plant have been studied (snowshoes, fruits, flowers) but little work has been devoted to the seeds, generally neglected while they can be valued as by-products, it is in this context that our research work which aims to identify new natural active ingredients, and the evaluation of antimicrobial and anti-inflammatory activities in vivo of the essential oil of the seeds of *opuntiaficus-indica*in Wistar rats.

II-Materials

II.1-Plant and animal materials

-Prickly pear fruits (o*puntiaficus-indica*): the harvest was made during the month of April 2021 in the region of Telaghwilaya of SidiBel Abbes.

-The grains: we recovered the grains after the crushing fruits of prickly pear by crushing robot, we dried the grains in the sun .

-Wistar rats from the breeding of the laboratory of pharmacology and toxicology, Institutpasteur in Algiers (Algeria).

-Nine bacterial strains Gram+ and Gram- and a yeast

II.2-Products used

-Carragenin

II.3-Equipment

II.3.1-Gas chromatography coupled to mass spectrometry (GC/MS) :

GC. The analysis of essential oils was performed using a Shimadzu GC - 2010 chromatograph, equipped with a flame ionization detector and a fused silica capillary column of 25 m × 0.25 µm, type SE 30. The temperature range is 55 to 250 °C. The carrier gas is Helium, with a flow rate of 0.77 ml/min and a pressure of 24.4 kPa.

The GC/MS coupling was performed on a Shimadzu GCMS - QP 2010, operating under helium pressure with a flow rate of 0.6 ml/min, the ionization potential is set at 70 eV. The injection and detection temperatures are respectively 200 and 250 °C.

II.3.2-UV visible spectrophotometer:

The determination of sugars was performed using a UV visible spectrophotometer of the brand SHIMADZU model UV mini 1240.

II.3.3-Microplate reader:

The follow-up of the microbial activity was carried out thanks to the microplate reader of mark TECAN SPECTRA which has the same principle as the visible spectrophotometer.

II.4-Methods

II.4.1-The extraction of essential oils

II.4.1.1-Hydro distillation:

Essential oils are volatile components; distillation is widely the most used method to obtain them from aromatic plants.

II.4.1.2-Extraction by liquid-liquid chromatography:

The volatile principles dissolve only very partially in water and the essence can be separated by decanting the distillate after cooling. The liquid obtained is put in the refrigerator for 24 hours. To separate the essential oil from water, we add NaCl before proceeding to a liquid-liquid chromatograph in a separating funnel with an apolar organic solvent in our case we used cyclohexane, once the organic phase recovered we add Na_2SO_4 to remove traces of water and filter

II.4.1.3-Purification of essential oils:

The organic phase is introduced into the steam rota under vacuum to separate the solvent from the essential oils. The temperature is set at 40°C because it is the lowest possible temperature under vacuum that allows the evaporation of cyclohexane.

II.4.2-Physical properties of essential oils:

a-Density

The relative density at 20 ° C of an essential oil is the ratio of the mass of a sample of essential oil at 20 ° C mass (m1), to a mass (m2) of distilled water at 20 ° C of the same volume.

We determine the mass relative to the empty pycnometer (m0), then filling the pycnometer with distilled water (m1), priming the pycnometer with the sample (m2) using a precision balance at a temperature of 20°C. It is given by the formula.

$d20 = (m2 - m0) / (m1 - m0)$

In this formula, d20 represents the relative density at 20 °C of the essential oil. The values m0, m1, and m2 represent the masses as described in the procedure:

m0: Mass of the empty pycnometer

m1: Mass of the pycnometer filled with distilled water at 20 °C

m2: Mass of the pycnometer filled with the sample of essential oil at 20 °C

By subtracting the mass of the empty pycnometer from the mass of the pycnometer with the sample (m2 - m0) and dividing it by the difference between the mass of the pycnometer with distilled water and the mass of the empty pycnometer (m1 - m0), we can determine the relative density of the essential oil at 20 °C.

b-Refractive index

The refractive index of a substance is the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a monochromatic light ray (D line of sodium) that passes through the substance at a constant temperature of 20°C.

The refractive index nd20 is determined by direct reading on a conventional Abbe refractometer.

After cleaning the surfaces of the prism and calibrating the apparatus, 2 drops of essential oil are placed in the middle of the prism using a pipette.

Two areas are observed in the eyepiece, one dark and one light, separated by a more or less iridescent band; the intersection of the reticle on the separation line is brought by the right button, then the iridescence is removed. The value is indicated by the reading scale.

II.4.3-Chromatographic study by TLC:

Thin layer chromatography (TLC) involves physical-chemical phenomena, based on adsorption power. The speed of progression of each of the constituents of the mixture entrained by the solvent is not the same. Consequently, spots are formed which are revealed by means of UV or colored reagents.

We used two migration systems on a 20x20 plate of silica gel 60F254 from MERCK

System1 :Butanol / Acetic acid / Water (4/1/5) System2 : Cyclohexane / Acetone (2/1)

System 2 was chosen because it gave the best separation. The revelation of the spots is done with UV and Vanillin 1%.

II.4. 4-Study and evaluation of antimicrobial activity

The microbial kinetics technique in microplate

This method consists of two steps: The revival and calibration of the microbial strains and the plating of the microplate

II.4. 4-Revivification and calibration of the strains:

First of all, it is necessary to defrost the preserved microbial strains and leave them at room temperature

II.4. 4-For bacterial strains:

A young bacterial culture of 18 to 24 hours is prepared for each strain on nutrient agar incubated at 37°C.After incubation, a bacterial suspension is prepared in a nutrient broth, which reaches an optical density equal to 70% at a wavelength of 620nm, measured with a molecular absorption spectrophotometer.This optical density (70%) corresponds to a microbial load that equals 2 x108 germs/ml.For the strain of Candida albicans which represent the fungal flora in this work, we proceed the same steps but instead of nutrient agar, we put the agar sabouraud and instead of nutrient broth we put liquid Sabouraud.

II.4. 4-Plating of the microplate

The kinetics of microbial growth is carried out in duplicate, in microplate, in each well is introduced 50µl of nutritive broth and 50µl of the studied extract. From the first well, we take 50µl of the mixture and we put it in the second well and from the third to the fourth.... And so on until the exhaustion of the wells, in order to realize a series of dilution to ½. Lastly, 50µl of the microbial suspension is added and followedby spectrophotometer of mark TECAN and read at 0h, 2h, 18h, 24h, 48h and 72 hours with a wavelength equal 620nm.

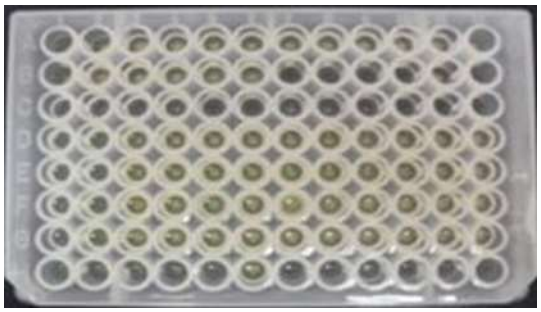


Fig 01 : Microplate 96 wells.

II.5-Determination of anti-inflammatory activity

II.5. 1-Description (Carrageenan-induced paw edema)

Rats are of both sexes, equal in number, 02 months to 03 months old, weighing (160-220) grams, and grouped into three (03) groups in batches of (05). The females are not pregnant and the animals are healthy; the temperature of the animal house is between 20 and 25 °C, with a humidity of 75% and a photoperiod of 12 /24 hours. The rats are housed in transparent plastic

cages, the dimensions and structure meet the guidelines of good practice, the rats are fed a standard diet of sticks and free access to drinking water.

Rats were fasted 16 hours before experimentation. In vivo studies were performed in accordance with current guidelines for laboratory animal care and ethical guidelines for the investigation of experimental pain in conscious animals

The anti-inflammatory activity was performed on the rat model of plantar edema (male and female Wistar, 160-220 gr) by injecting a 2% suspension (0.2 ml) of carrageenan into the right paw; this is a technique inspired by that described by **(Winter and al.,1962)** and (**Adeyemi and al.,2002)** .Chemical inflammation was induced by injecting 0.2 ml of 2% carrageenan in isotonic saline under the plantar fascia of the rat's right hind paw. The studied essential oil of *opuntiaficus-indica* was administered orally (gavage); 30 min before carrageenan injection.

Rats were fasted 16 hours before treatment and divided into three groups of five rats each:

Group 1: Control (untreated) Rats (n=05): received a 0.9% isotonic saline solution (10 ml/kg body weight) by gavage 30 min before an injection of 0.2 ml of 2% carrageenan, subcutaneously in the plantar fascia of the right hind leg of the rat

Group 02: Reference (indomethacin at a dose of 12 mg/kg). In this group, the rats received indomethacin at a dose of 12 mg/kg by gavage 30 min before an injection of 0.2 ml of 2% carrageenan under the skin in the plantar fascia of the right hind leg of the rat **(Mokhort and Riabukha, 1971).**

Group 03: This group of rats received by gavage, the essential oil of our plant species in a dose (500 mg/kg), and in the same way as for the two other groups a subcutaneous injection of carrageenan was administered, 30 minutes before.

The evaluation of edema was followed by recording the diameter in millimeters of the inflamed paw, one hour, 2, 3, 4, 5 and 6 hours after the injection of carrageenan using a digital caliper within 300mm to iso 9001:2000.

For each group, the mean diameters obtained at these different readings (vt) were compared with those obtained before any treatment (v0), thus allowing calculation of the percentages of edema (percentage of inflammation), according to the formula: (vt - v0)/v0 ×100. While the percentage of edema inhibition was calculated from the formula:

III-Results and discussion

III.1-Determination of yields (essential oils):

The yield rates of extraction of essential oils from seeds by the steam stripping method and followed by liquid-liquid extraction by cyclohexane from the aqueous phase gave low seed yields equal to 0.29%.

III.2-Physical properties of essential oils:

Table 01: Physical properties of essential oils.

	Density (d20)	Refractive index (nd20)
Essential oilsof grains	0.709	1.511

III.3-Chromatographic study by TLC:

The results of the separation of the constituents of the essential oil in a Cyclohexane / Acetone system (2/1) as well as their detection with the help of the different developers are reported respectively on the tables:

Table 02: Retention factor and color of essential oils of the seeds.

Spots	Rf	UV	Vanilline1%
1	0.130	254	Greenish
2	0.351	/	Pink
3	0.450	254	Purple
4	0.565	254	Whitish brown
5	0.648	/	green

III.4-Photochemical study :

III.4.1-The results obtained are expressed in the following table:

Table 03: Characterization of the phenolic compounds of the studied plants.

Plants Phenolic compounds		grains
Tannins	Gallic	+
	Catechetical	-
Flavonoids	Anthocyanins	/

	Flavones	+
	Genin	+
	cathécole	+
Saponosides		-
Sterols and triterpenes		+

III. 6-GC/MS chromatographic analysis of essential oils:

The identification of the products was done, by comparing their retention times and mass spectra with those of reference compounds from the literature stored in the database of the apparatus. Indeed, 100% of the chemical composition of the essential oil of the seeds was determined.

III. 6.1-Chemical composition of the essential oils of the seeds :

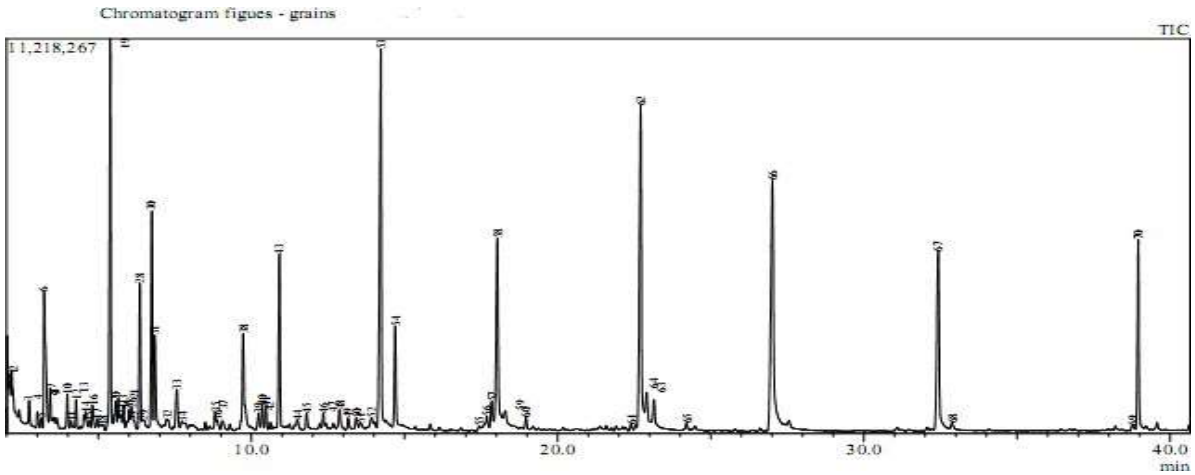


Fig 02: Chromatogram of essential oils in the seeds.

The chemical composition of the essential oil of the seeds consists of hydrocarbons and aldehydes.

III. 7-Study and evaluation of antimicrobial activity:

The results obtained by the microplate method, ninety-six (96) fruits seem to be indicative of priming, according to the spectra because they show a clear effect on the following strains:

III. 7.1-The activity of the essential oils of the seeds:

The antimicrobial activity of the essential oils of the*Opuntiaficus-indica*seeds on different strains is represented in the following graphs:

III. 7.1.1-Wild *Enterococcus faccalis*

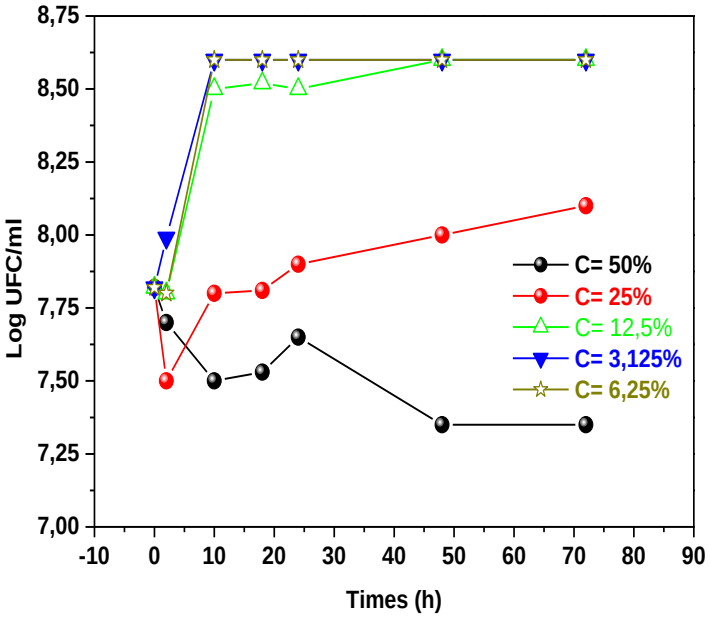


Fig 03 :The influence of essential oils of the seedson the growth of *Efaccalis S* strain.

The control underwent normal growth by representing the different phases of growth (latent, exponential, stationary and decline).

We note that the concentrations 3.125%, 6.25% and 12.5 have no effect on the growth of the strain, but there is a concentration between 12.5 and 25% which plays a bacteriostatic role.

III. 7.1. 2-*Escherichia coli* ATCC 25922

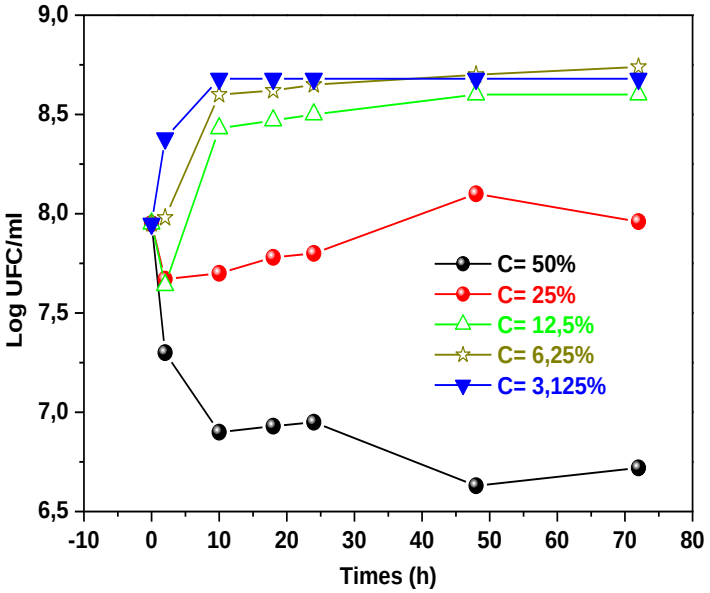


Fig 04 *:* The influence of essential oils of the seedson the growth of *E coli strain* ATCC 25922.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).It is noted that the concentrations 3.125%, 6.25% and

12.5 have no effect on the growth of the strain, but there is a concentration between 12.5 and 25% which plays a bacteriostatic role.

III. 7.1. 3-*Bacillus spizizenii* ATCC6633

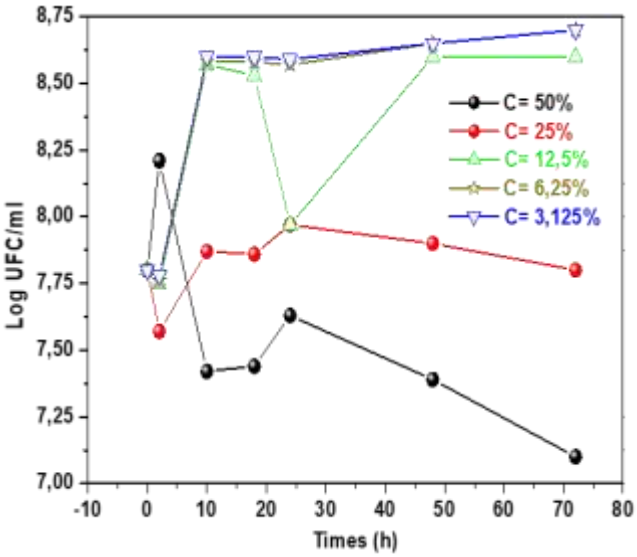


Fig 05: The influence of essential oils of the seedson the growth of the*Bacillusspizizenii* ATCC 6633 strain.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).

The concentrations 3.125%, 6.25% and 12.5% have no effect on the growth but have a bacteriostatic effect at 25%.

III. 7.1. 4-*Pseudomonas aeruginosa* ATCC 10145

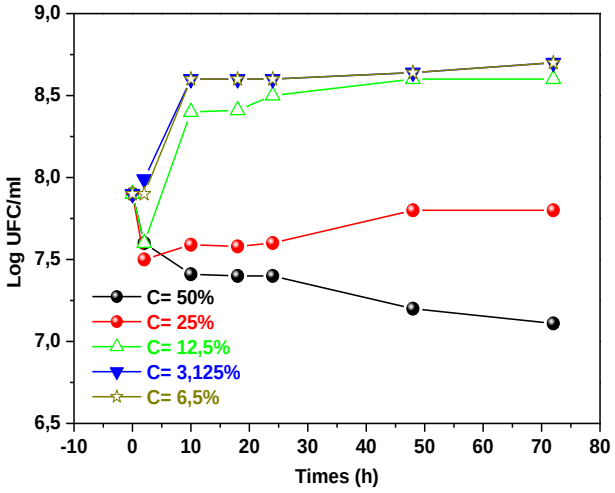


Fig 06: The influence of essential oils of the seedson the growth of*Pseudomonasaeruginosa*ATCC 10145.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).The concentrations 3.125%, 6.25% and 12.5% have no effect on the growth but there is a concentration between 12.5%, 25% to a bacteriostatic effect.

III. 7.1. 5-*Salmonella hemdelberg*sauvage

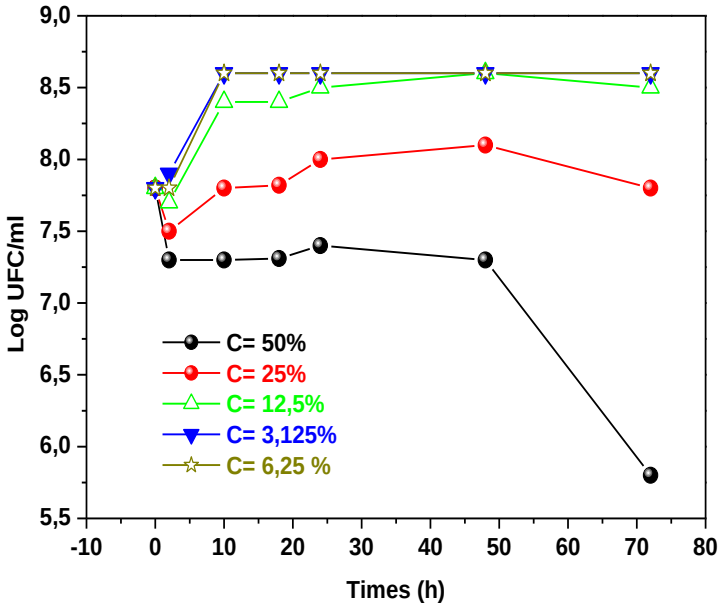


Fig 07: The influence of essential oils of the seedson the growth of the *Salmonellahemdelberg* S strain.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).We note that 3.125%, 6.25% and 12.5 have no effect on the growth of the strain, but the 25% concentration played a bacteriostatic role.

III. 7.1. 6-*Enterobacter cloaeae* ATCC13047

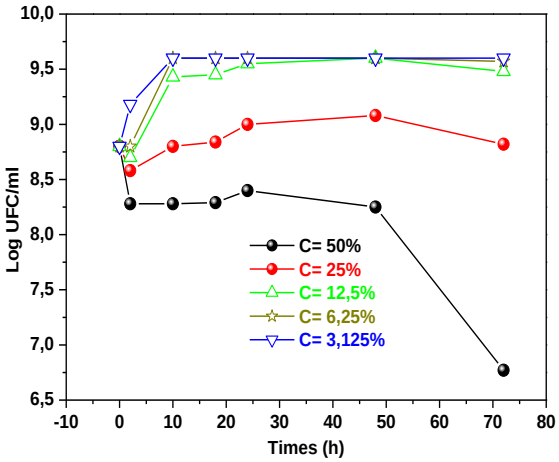


Fig 08: The influence of essential oils of the seedson the growth of*Enterobactercloaeae* ATCC 13047.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).The concentrations 3.125%, 6.25% and 12.5% have no effect on the growth but there is a concentration between 12.5%, 25% to a bacteriostatic effect

III. 7.1. 7-*Staphylococus aureus* ATCC6538

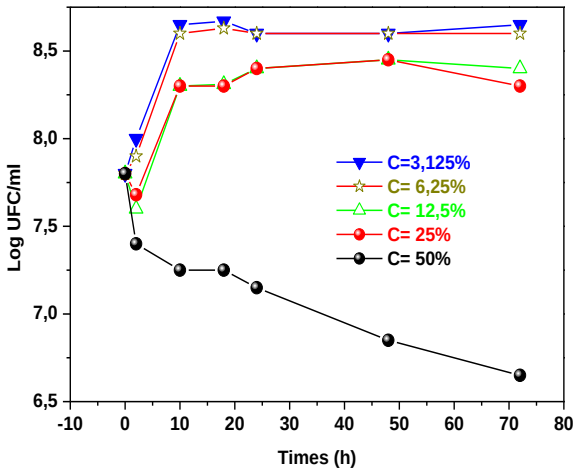


Fig 09: The influence of essential oils of the seedson the growth of *Staphylococusaureus* ATCC 6538.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).

The concentrations 3.125%, 6.25%, 12.5% and 25% have no effect on the growth but there is a concentration between 25%, 50% to a bacteriostatic effect.

III. 7.1. 8-*Pseudomonas aeruginosa*ATCC 27853

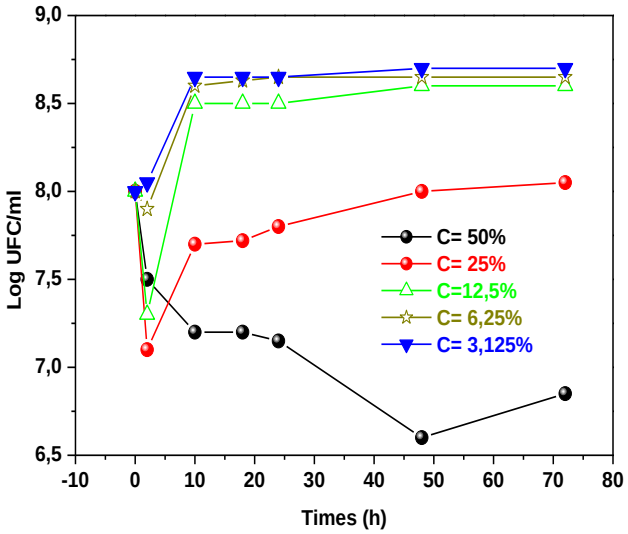


Fig 10: The influence of essential oils of the seedson the growth of *Pseudomonasaeruginosa* ATCC 27853.

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline).The concentrations 3.125%, 6.25% and 12.5% have no effect on the growth but there is a concentration between 12.5%, 25% to a bacteriostatic effect.

III. 7.1. 9-*Klebsiella pneunomiae*sauvage

The control underwent normal growth representing the different phases of growth (latent, exponential, stationary and decline). The concentrations 3.125%, 6.25% and 12.5% have no effect on the growth but there is a concentration between 12.5%, 25% to a bacteriostatic effect

Injection of 0.2 mL of 2% carrageenan in isotonic saline under the plantar fascia of the rat's right hind paw induced edema that gradually increased with time or even (Table 04).

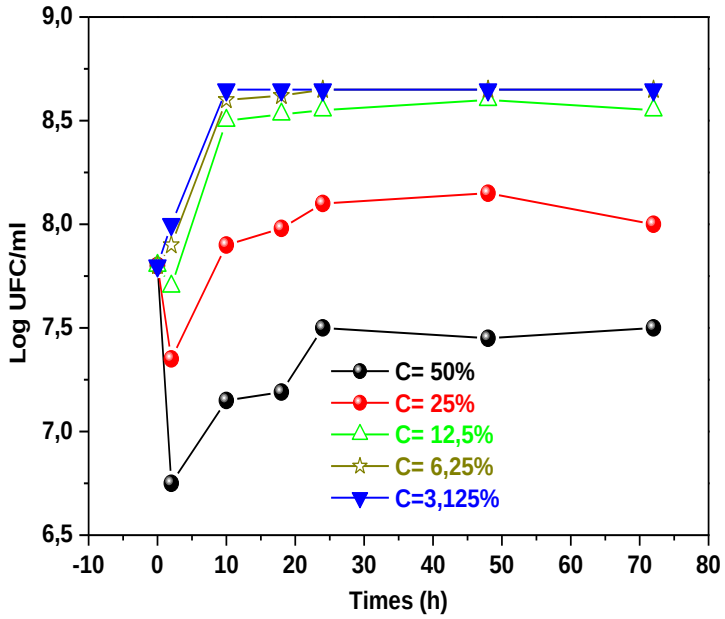


Fig 11: The influence of essential oils of the seedson the growth of *Klebsiellapneunomiae* S strain.

Table 04: Effect of essential oils of the seeds of *opuntiaficus-indica*graveolensandindometacin on plantar edema in (mm) induced by carrageenan in rats.

Traitements	Avant	1h	2h	3h	4h	5h	6h
Indométacine	3.12±0.10	5.05±0.40	5.09±0.49	5.73±0.46	5.46±0.65	4.89±0.69	4.31±0.6
essential oils of the seeds	2.81±0.20	4.69±0.31	4.29±0.25	4.08±0.22	3.63±0.22	3.61±0.23	3.32±0.15
NaCl	3.12±0.07	5.95±0.11	6.22±0.11	6.88±0.20	7.29±0.38	7.71±0.42	8.12±0.22

We compare the results obtained in the three batches: the indomethacin group (reference group), the second group (control group) that is received saline and the third group that is

gavaged by our essential oils of the seeds. progression of inflammation in the three groups is represented by the (Fig 12); while the percentage of inhibition for the three groups (Fig 13).

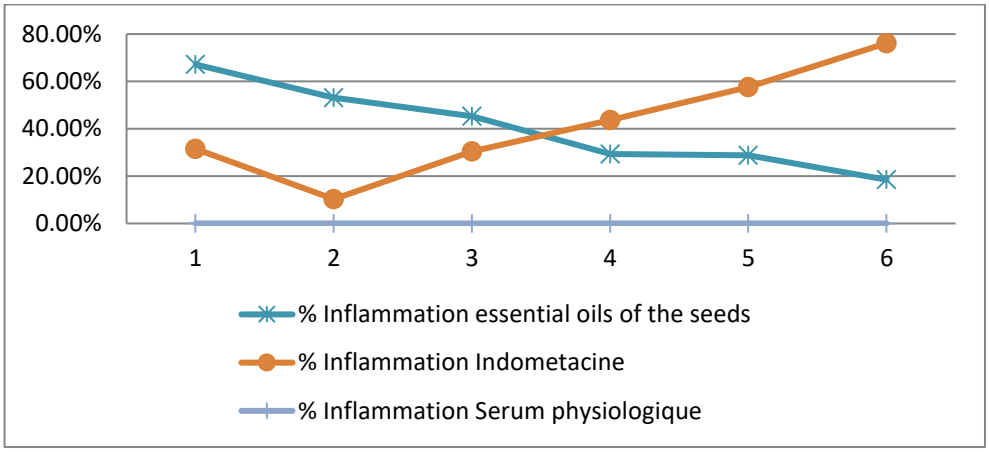
We observe for the control group that is received saline that the inflammation caused by carrageenan increases with time and it reaches its maximum value 98.7%.After one and a half to two hours of injection of carrageenan this and attributed to the release of histamine and serotonin and continue until exceeding 100% after six hours, in the group of indomethacin at a dose of (12mg/kg bw), we notice a very significant reduction ($p<0.05$) of the thickness of the rat's paw after the second hour of the injection of carrageenan with an inhibition rate of 30.42% to reach the maximum value in the fifth and sixth hours with an inhibition rate of 57.42% and 76.13%.

On the other hand, for the lot treated with essential oils of the seeds of *opuntiaficus - indica*significantly ($p<0.05$) prevents plantar edema in rats from the second hour of treatment with an inhibition rate that is (66.22%). Our essential oils of the seeds has a very important effect that persists and reaches an inhibition rate of 89.6% after six hours of injection of carrageenan and is significantly different from the control lot.

So we notice that our essential oils of the seeds of *opuntiaficus-indica* has a quite important anti-inflammatory effect 89.6% compared to the synthesized drug indomethacin during the second phase of the inflammation.

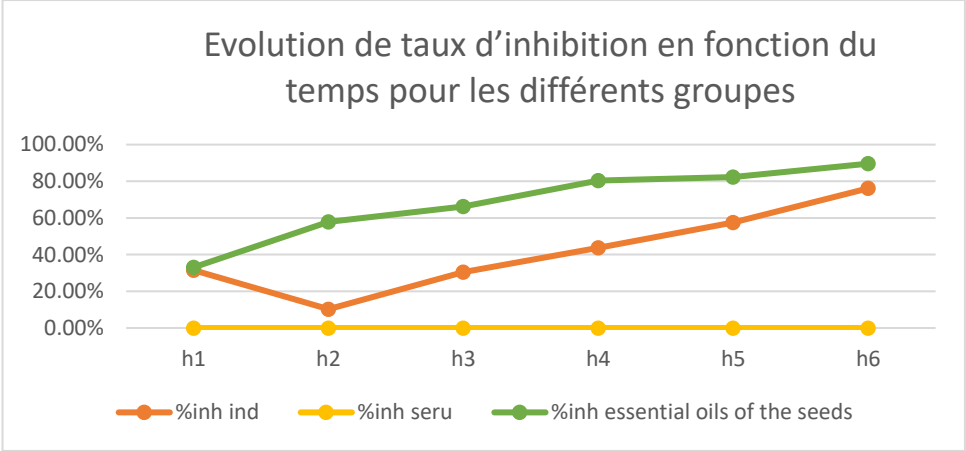
On the other hand we notice a strong decrease in the percentage of inflammation during the second phase, that is to say from the second hour for the dose administered by essential oils of the seeds this can be explained by an inhibition of cycloogenase that could mean that essential oils of the seeds of *opuntiaficus-indica* inhibits the cycloogenase 2 (COX2) and prostaglandin-like, which is responsible for the synthesis of prostaglandins and prostaglandin-like responsible for the appearance of edema, which explains the disappearance of inflammation at the sixth hour of the administration of essential oils, which is reflected in the decrease in the volume of the inflamed paw and the absence of swelling. These results indicate that the effect of essential oils of the seeds of opuntiaficus-indica is dose dependent. The evaluation of the percentage of inhibition shows that this essential oil has an important anti-inflammatory activity and this is due to the richness of essential oils of the seeds of opuntiaficus-indica in bioactive compounds; studies are proven that saponins have a very important anti-inflammatory power (**Della Loggia, 1994**) and (**Navarro, 2001**).

The results of this study, which complement the data in the literature, confirm that opuntiaficus-indica is an interesting plant that could be used in a reasoned manner in the treatment of inflammation. Indeed, the significant results obtained during this study have shown essential oils of the seeds of opuntiaficus-indica at a dose of 500 mg/Kg have anti-inflammatory properties more important than that of indomethacin.



Groupe 1 : témoin traité avec le sérum physiologique (10 ml/kg PC) ; **Groupe 02:**référence ont l'Indométacine à la dose de (12 mg/kg) ; **Groupe 03 :**essentialoils of the seedsof*opuntia ficus indica*

Fig 12 : Evolution de l'inflammation (œdème plantaire) en fonction du temps pour les différents groupes.



Group 01: control treated with saline (10 ml/kg PC); **Group 02:** reference received Indometacin at the dose of (12 mg/kg); **Groups (03):** essential oils of the seeds of *opuntiaficusindica.*

Fig 13: Evolution of inhibition rate versus time for the different groups.

IV- Conclusion

Within the framework of a valorization of these resources, the plant *opuntiaficus indica*was the subject of a phytochemical study of essential oils of the seeds and an evaluation of its antibacterial and anti-inflammatory potential in vivo.The phytochemical screening reveals that essential oils of the seeds are rich in various secondary metabolites that participate in the adaptation of this plant to their environments as well as their plant to their environments as well as their biological properties and therapeutic properties.The results of the evaluation of

antibacterial activity are very promoting, since essential oils of the seeds revealed an activity on the tested strains (clinical and reference).The orientation of research to deepen the aspects seen in this work, also the exploration of other facets of the biological properties of this medicinal plant could be realized.

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