



Formulation and development of an antiacne patch containing black seed and clove extracts by using chitosan-gelatin as polymers matrix

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Abstract

Acne is an inflammatory condition caused by bacterial colonization of hair follicles on the face, neck, chest and back. To address this issue, there are typically two well-known treatment options: topical treatments and systemic treatments, which are used when acne is challenging to manage with topical medications. For overcoming the resistance of acne-causing bacteria to antibiotics and local cutaneous irritation, this work aims to characterize another solution that is less hazardous to the skin, health and more effective, this alternative is presented as a patch based on the ethanol extracts of two therapeutic plants (*Nigella sativa*, *Syzygium aromaticum*) as well as the chitosan that serves as a polymer of the patch and the gelatin is the essential matrix for the release of the extracts. The results showed that this patch has a strong inhibitory effectiveness against three tested bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*).

Keywords:

Acne, Patch, Chitosan, Gelatin, *Nigella sativa*, *Syzygium aromaticum*.

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1. Introduction

Acne can affect how people are seen by others, as the face is often the first thing noticed [1][2]. This disease is a prolonged inflammatory infection of the pilosebaceous unit caused by an increase in androgen-induced sebum production, altered keratinization, inflammation and bacterial colonization of the hair follicles on the face, neck, chest and back. Early colonization by *P.aeruginosa* acnes and family history can play an important role in the disease. Antibiotics have always been used to treat acne, both topically and orally. The most commonly recommended topical antibiotics for treating mild to moderate acne are erythromycin and clindamycin, while cyclines are the broad-spectrum antibiotics often used for moderate to severe cases [3][4]. However, there is growing interest in alternative treatments, as medicinal plants could potentially replace conventional topical acne therapies [5]. For instance, *Nigella sativa* is a valuable ingredient in skin care due to its antibacterial, antioxidant, and anti-inflammatory properties. It not only fights the bacteria responsible for acne but also soothes skin irritations and reduces redness associated with outbreaks. Therefore, it has all the qualities needed to combat acne and excess sebum. Additionally, it may help fade scars and marks from acne or eczema. Similarly, *Syzygium aromaticum* extract is recognized for its antibacterial and antiseptic properties, further supporting the effectiveness of natural remedies in acne treatment... Scientific studies have shown that clove extract has significant inhibitory activity against certain strains of bacteria, including those involved in acne, such as *Propionibacterium acnes*. These antibacterial properties help to eliminate bacteria present on the skin, thereby contributing to the reduction of acne imperfections [6]. Chitosan, derived from chitin, a polysaccharide found in shellfish in crustaceans, has been extensively studied for its antibacterial and anti-inflammatory properties. It provides protection against the bacteria responsible for acne and helps reduce skin inflammation. In addition, chitosan promotes wound healing, which can be beneficial for acne-prone scars. Gelatin, a protein substance derived from animal collagen, is known for its beneficial properties on the skin. It strengthens the skin barrier, improves elasticity and helps reduce the appearance of acne scars. Gelatin can also help maintain skin hydration, thus promoting healthy and balanced skin. In the search for effective solutions against acne, many products and treatments have been developed over years. Among these, anti-acne patches have proven to be a promising option. These patches are designed to target specifically the areas affected by acne and offer localized treatment. This work aims to evaluate the biological activities of our plant extracts, to extract our polymer from the crab and finally to create and characterize a powerful patch from these ingredients.

2. Material and Methods

2.1. Raw material

The plant material is represented by two species, one belongs to Ranunculaceae family: *N. sativa* grains that were purchased from an herborist, from Biskra. The other to Myrtaceae family: *S. aromaticum* grains, were purchased from an herbalist from Iben Ziyad Constantine.

2.2. Extraction process

In our study, we performed a maceration extraction of two plants; we placed 100g and 50g of *N. sativa* and *S. aromaticum* respectively, in 200 mL or 100mL of solvent (ethanol) for 48 hours.

2.3. Filtration and evaporation

First, a simple filtration was effectuated with filter paper by using a conical tap to remove solid particles from plants. The main purpose of evaporation by rotary evaporator is to quickly remove a volatile solvent. Their principle is based on the lowering of the boiling point with the decrease of pressure.

Extract yield is the ratio between the mass of the extract (M) and the initial mass (I) of a plant to be treated. The formula for calculating the yield is: $\text{Yield (\%)} = M/I * 100$.

2.4. Chitosan extraction from crab shells

2.4.1. Preparation of chitin

The carapaces of the invasive blue crabs were cleaned with tap water and then dried with absorbent paper. The next step is to grind the carapaces with a high-speed grinder mill to obtain a powder.

- Demineralization according to Haidra (2018): 40 g of dry powder from crushed shells was dispersed in 250 mL of HCl solution (1M). All was mixed for 2 hours at room temperature. The resulting product is then recovered, filtered and washed several times until a neutral pH of 7. The recovered product was then dried in 80°C bowl for 24 hours [7].
- Deproteinization according to Haidra (2018): the demineralized product was placed in an alkaline solution of NaOH (2N) for 2 hours at room temperature. The treated product was filtered from the alkaline solution, washed with distilled water and eventually dried in a 60°C oven for at least 48 hours until a stable weight was obtained. This product is raw chitin, which is used in the subsequent deacetylation reaction to prepare chitosan [7].
- Discoloration according to Tolaimate et al., 2003 and Hafsa et al., 2016: the chitin obtained was slightly colored, so the pigments responsible for this color are removed using an oxidizing treatment [H₂O₂ (0.1M), HCl (1mM) in a volume ratio of 9:1, and mixed with acetone 1:5(p/v) for ten min, the mixture is washed and dried at 37°C for a 24 hours [8] [9].
- Chitosan desacetylation according to Haidra (2018): In order to obtain the chitosan sample, the raw chitin was treated with an alkaline solution of NaOH (10%) for 24 hours. After treatment, the product was filtered and then washed with distilled water to a neutral pH before drying at 80°C in a vault [7]:

$$DDA = 100 - \frac{(A_{1320} / A_{1420}) - 0.3822}{0.03233}$$

A₁₃₂₀: absorbance at 1320 cm⁻¹ corresponding to amide III.

A₁₄₂₀: absorbance at 1420 cm⁻¹ corresponding to the symmetrical deformation of CH₃ and CH₂.

2.4.2. Preparation of chitosan/gelatin solution loaded with *N. Sativa*/*S. aromaticum* extract

To prepare a solution of 1% p/p, chitosan powder was dissolved in acetic acid at 0.5% v/v. A gelatin solution (25% p/p) was prepared by dissolving gelatin powder in deionized water at 40°C. The solution was then loaded with 5mL of 1% p/p chitosan solution, 1mL of 25% p/p gelatin solution, 2ml of *N. sativa* extract at a concentration of 100mg/mL, and 2mL of *S. aromaticum* extracted at a 50 mg/mL concentration.

2.5. Preparation of the patch

The patch was formulated according to Kuo et al., 2021. A mixture of 10 mL of gelatin/chitosan solution containing two plant extracts was poured into 85 mm cup and left to dry at room temperature for 48 hours. Subsequently, we reticulated with glutaraldehyde at a concentration of 10% v/v for 1 hour at room temperature. The obtained patch was then cut into a circular shape with a diameter of 8mm using a steel mold [5].

Drug Release Test was effectuated for each weighed patch, 3mL of distilled water was added in a screw tube, and then the samples were incubated for 30min, 1, 3, 24 and 48 hours at 37°C. Each sample was taken and weighed.

2.6. In vitro biological tests

2.6.1. Evaluation of antimicrobial activity

- Microbial strains

Three bacterial strains were used in this study: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These strains were provided to us by the Microbiology Laboratory of the CRSP of Constantine. In addition, the fungal strains used in this experimental study are represented by: *Candida albicans*, *Aspergillus niger*.

- Evaluation of antibacterial activity

The disc diffusion method was used to evaluate the in vitro antibacterial activity of two plant extracts.

- The re-isolation of bacterial strains: the seed in an enrichment medium which is the nutrient agar. Incubate at 37 °C for 24 hours in the stove.
- Preparation of bacterial suspension: In a tube containing 9 mL of sterile physiological water a few colonies are placed, in order to prepare a suspension for each bacterium. Additionally, the cell density was adjusted either by dilution in physiological water or by adding colonies until an absorbance range between 0.08 and 0.1 at a wavelength of 625nm was obtained.

• Seeding: The medium (MH) was poured into the sterile petri dishes. These dishes were inoculated with the bacterial suspension.

100 mg and 50 mg for each extract weighed in Eppendorf tubes and mixed with 1mL of DMSO. Then, sterile discs (Watman No. 3 paper with a diameter of 6mm) are placed on the agar surface. Through a micropipette, 10 μ L of the extract was taken and put on the disc. The dishes were placed in the refrigerator for 2 hours at 4°C to allow the active substances to diffuse in the medium, and then incubated in the stove for 24 hours at 37°C. The antibacterial activity of the patch has also been determined [10].

- Evaluation of antifungal activity

The filamentous fungi are ripped onto the Sabouraud medium and incubated at 28°C for a week. From young and pure crops, fungal cell suspensions of *Candida albicans* and *Aspergillus niger* are prepared in 10mL sterile physiological water. The suspension is diluted to DO equal to 0.20 (equivalent to approximately 10⁸ cells/ml) at 650nm. On sterile petri containing Sabouraud agar, these suspensions were seeding. On sterile discs that are placed on the agar surface, 10 μ L of plant extract were deposited, then, the dishes were placed in the refrigerator for 2 hours at 4°C to allow the active substances to diffuse in the medium. The incubation of the dishes was carried out for 3 days at 28°C for the strain *A. niger*, while *C. albicans* was incubated for 24 hours at 37°C [10].

2.6.2. Evaluation of anti-inflammatory activity

The principle of in vitro anti-inflammatory activity is determined by the method of Kandikattu et al., (2013). First, the Tris-HCl buffer was prepared (0.05% / pH= 6). Different concentrations of the extracts are prepared (0.8mg/mL, 0.6mg/mL, 0.4mg/mL, 0.2mg/mL) from the stock solution (1mg/mL). Different concentrations of sodium diclofenac (injectable form) (1mg/mL, 0.8mg/mL, 0.6mg/mL, 0.4mg/mL, 0.2mg/mL) are prepared in water distilled from the mother solution as regards the standard. For the whites were made a white extract and a white BSA (which was prepared from the dissolution of 0.2g of BSA in 100ml of Tris-HCl buffer. Then 1ml of BSA was added to the different concentrations of the extracts and the drug. The control tubes contain 1ml distilled water plus 1mL BSA. Finally, a first incubation is carried out at 37°C for 15 min, then a second incubatory at 72°C in a water bath for 5 min [11].

2.6.3. Evaluation of antioxidant activity

The antioxidant capacity of two extracts was determined using DPPH (2,2-Diphenyl Picryl-Hydrazyl) as a relatively stable free radical [12]. In this test, purple DPPH was reduced to a yellow compound, diphenylpicrylhydrazine, whose intensity of color is inversely proportional to the reducing power of the antioxidants present in the medium. The reaction is carried out by means of a microplate reader with 96 wells of 200 μ L volume for each well. The DPPH solution is prepared from the dissolution of 4mg DPPH in a volume of 100mL of methanol, then the absorption is measured in the spectrophotometer at a wavelength of 517nm. The DPPH solution is stored at -20°C.

3. Results and discussion

3.1. Determination of the ethanol extracts yield

The yield of the ethanol extracts of *S. aromaticum* and *N. sativa* from the powder of the grains of *N. sativa* and the flower buds of *S. aromaticum* is mentioned in Table (01).

Table.1. Determination of the ethanol extracts yield

Plant	Sample mass (g)	Mass extract (g)	Yield (%)
<i>S. aromaticum</i>	100	2.7	2.7
<i>N. sativa</i>	100	6.5	6.5

3.2. Determination of antimicrobial activity

For the *N. sativa* extract results, according to Figure (1) below: For *E. coli* (ATCC25922), a low activity was found for the *N. sativa* extract with a D=7.5mm for the C1 concentration=100 mg/mL, so this strain is resistant to this extract. The *P. aeruginosa* strain (ATCC27853) has a sensitivity for a single concentration (C1=100mg/mL) of the ethanol extract of *N. sativa* with a diameter of D=8.5 mm, therefore is a sensitive strain for this extract. Moreover,

for the *S. aureus* (ATCC25923), a sensitivity was found for this extract for the two concentrations with a diameter of D=09mm for the first concentration (C1) and D=8.5mm for the second concentration (C2), then this strain is also sensitive for the ethanol extract of *N. sativa*. Our results agree with the results of Harzallah (2012) and his team who proved that *N. sativa* has a very low inhibition power on *E. coli* translated by a D = 07mm [13]. Several studies have been carried out on the antibacterial effect of this plant. TQ is the majority constituent, it has been found to have antibacterial power against different types of bacteria, in particular gram-positive bacteria such as *S. aureus* [14]. The ability to modify bacterial resistance to antibiotics is one of the antimicrobial characteristics of TQ [15]. According to these results, we conclude that the species of *N. sativa* has an antibacterial power and that the responsible for these properties is: Thymoquinone, thymohydroquinone and p-cymene [16].

For the extract of *S. aromaticum* using the technique of diffusion of discs, we were able to identify the antibacterial power of the ethanol extract of *S. aromaticum* vis-à-vis the bacterial strains tested. According to Ponce et al., (2003), it can be concluded that all the strains studied are sensitive to this extract [17]. According to Figure (1), under this, *E. coli* (ATCC25922), a high activity was found for this extract with a D=15 mm for the concentration of C=100mg/mL, so the strain is very sensitive to this concentration. In addition, even with C=50mg/mL, it can be considered that this strain is sensitive (D=10mm). On the other hand, in the *P. aeruginosa* strain (ATCC27853), an antibacterial effect was found for the two concentrations of the ethanol extract of *S. aromaticum* with a diameter of D=10 mm for C1 and D=13 mm for C2 Figure (1) and table (2), so is a sensitive strain for the ethanol extract of cloves. Sensitivity was also found in the *S. aureus* strain (ATCC25923) for this extract for both concentrations with a D=09 mm for the first concentration and D=11 mm for the second concentration, then we can say that this strain is sensitive for the ethanol extract of *S. aromaticum*. Several components present in plant extracts have antibacterial properties, in particular phenols. According to the study by Rhayour (2016), it can be explained that the extract of *S. aromaticum* has an antibacterial effect thanks to a main compound which is eugenol which belongs to the phenol family [18]. As we know, the unsaturated fatty acids in the bacterial cell are responsible for the membrane fluidity, so the presence of extracts or essential oils induces disturbances at the level of the outer envelope of the cell which translates to structural modifications of the membrane. Eugenol can increase the content of saturated fatty acids in C16 and C18 and decrease the content of unsaturated fatty acids in C18 [19]. This action is not limited to the genes themselves, but can affect the enzymes responsible for their biosynthesis. Some of the studies have shown that the inhibition of enzymes involved in anabolism and the conversion of AG such as desaturase and cis-trans isomerase can be achieved by different mechanisms (competitive, incompetent, non-competitive inhibition) [19]. The fixation of eugenol on the bacterial membrane causes changes in structure, permeability and bacteria lysis. These results may be related to origin of the plants studied, the concentration of the extracts, antibacterial capacity of the compounds of interest in our extracts, extraction method, extracts storage conditions and functioning of certain enzymes.

Table 2. Diameters of inhibition microbial growth of *N. sativa* and *S. aromaticum* extracts against several microbes.

Microbes	Strains	ATCC	NS extract		SA extract		DMSO
			C1	C2	C1	C2	
Bacteria	<i>E.coli</i>	25922	7.5	-	15	10	-
	<i>S.aureus</i>	25923	9	8.5	11	9	-
	<i>P.aeruginosa</i>	27853	8.5	6.5	13	10	-
Yeast	<i>C.albicans</i>	10231	-	-	-	-	-
Fungi	<i>A.niger</i>	16404	-	-	9	-	-

ATCC: American Type Culture collection, NS: *N. sativa*, SA: *S. aromaticum*, C1: 100 mg/mL, C2: 50 mg/mL, DMSO: negative control, (/): No activity, Data are expressed in mm. According to their diameter of inhibition (m), strains are represented as: resistant (-): D<8mm, sensible (+): 9mm≤D≤14mm, highly sensible (++) : 15mm≤D≤19mm, very highly sensible (+++) : D≥20mm.

The antifungal activity was tested on two fungal strains (*A. niger*, *C. albicans*). Concerning the extract of *S. aromaticum* showed a low efficacy against the strain *C. albicans*, on the other hand the extract of *N. sativa* does not express any antifungal power with this strain, which is in agreement with the results of (Tanis et al., 2009) and those of (Abu-Al-Basal 2009) [20][21]. For the *A. niger* strain, an activity was found with the extract of *S. aromaticum* (D=09 mm, for C=100 mg/mL), then this strain is sensitive to this extract. With the extract of *N. sativa* we can consider this strain as a resistant strain because of the lack of antifungal power. According to (Harzallah et al., 2012),

C. albicans has a slight sensitivity to *N. sativa* oil [13]. So, we can conclude that the strong activities are thanks to the oil and not the extract. Numerous studies have confirmed that the essential oil of *S. aromaticum* and with the help of eugenol has antifungal power against the species of *A. niger* and other fungal species (*Fusarium oxysporum*, *Fusarium culmorum*, *Penicillium sp*, *Aspergillus* and *Trichoderma*) indeed, this is similar to the work of many researchers such as Hmiri and their colleagues [22]. The chemical composition of the extracts and oil is varied according to the extraction method. Khosavi (2011) used the extraction by maceration of *N. sativa* (our method used during our experiment), the results indicate the absence of certain compounds, from these results we can say the TQ is present in low content, and for this reason we did not obtain a strong inhibitory activity [23].

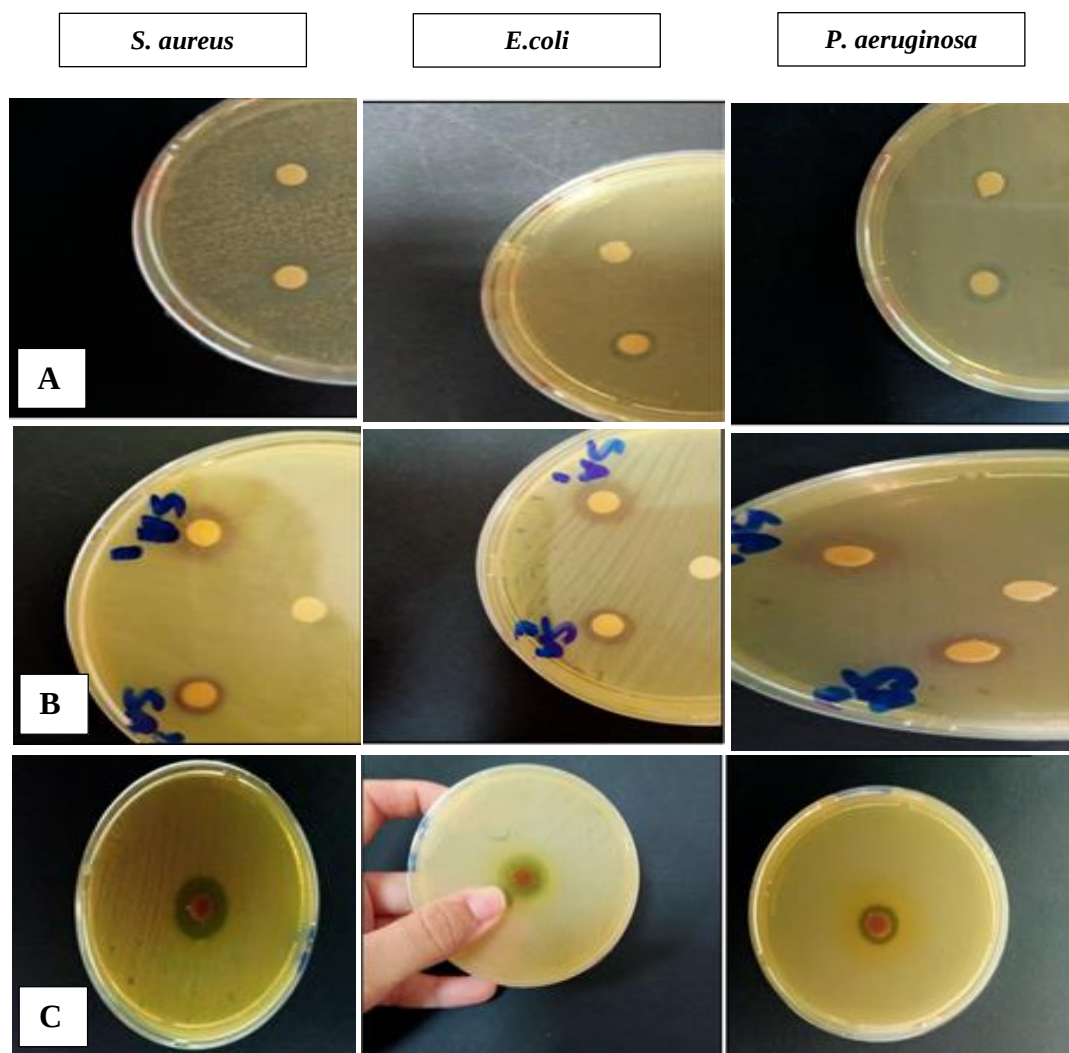


Figure.1. Pictures of bacterial strains developed on agar plates treated by *N. sativa* (A) and *S. aromaticum* (B) ethanolic extracts and the formulated patch (C): *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

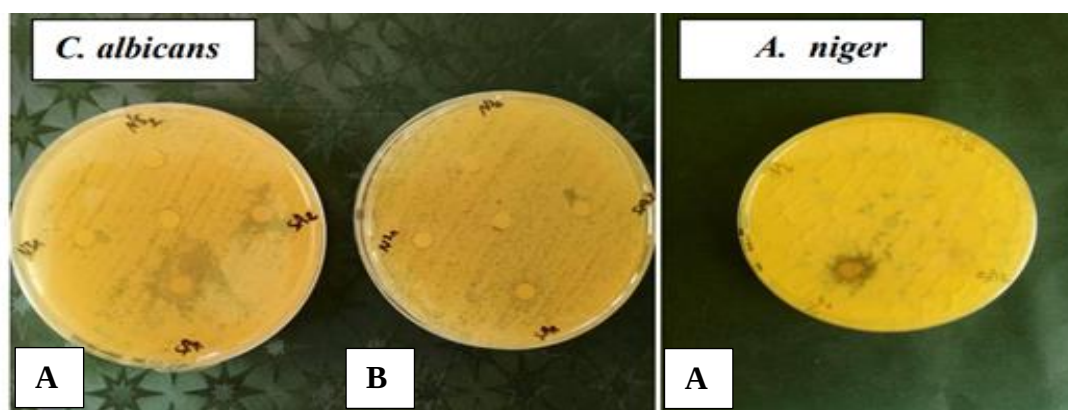


Figure.2. Pictures of fungal strains developed on agar plates treated by *N. sativa* (A) and *S. aromaticum* (B) ethanolic extracts: *Candida albicans* and *Aspergillus niger*.

3.3. Determination of anti-inflammatory activity

The IC_{50} of our extracts of *S. aromaticum*, *N. sativa* and standard have been determined in Figure (3). The results obtained indicate that the extract of *S. aromaticum* (clove) and *N. sativa* (*Nigella* seed) have demonstrated anti-inflammatory effects. The IC_{50} , which represent the concentration necessary to inhibit 50% of the inflammation, were measured at 196.67 μ g/mL for *S. aromaticum* and 185.27 μ g/mL for *N. sativa*. Compared to the standard used, diclofenac, which has shown anti-inflammatory power with an IC_{50} of 132.4 μ g/mL, the extracts of *S. aromaticum* and *N. sativa* seem to have a slightly less powerful anti-inflammatory activity. However, it is important to note that these extracts have demonstrated significant activity in inhibiting inflammation. Numerous studies report that TQ is the active ingredient responsible for the anti-inflammatory power of *N. sativa* extract. For *S. aromaticum*, several studies show that eugenol is the main compound responsible for antioxidant capacity. We have not found similar works on the species of *S. aromaticum* and *N. sativa* having studied the anti-inflammatory potential of an ethanol extract. Most of the studies have been carried out *in vivo*.

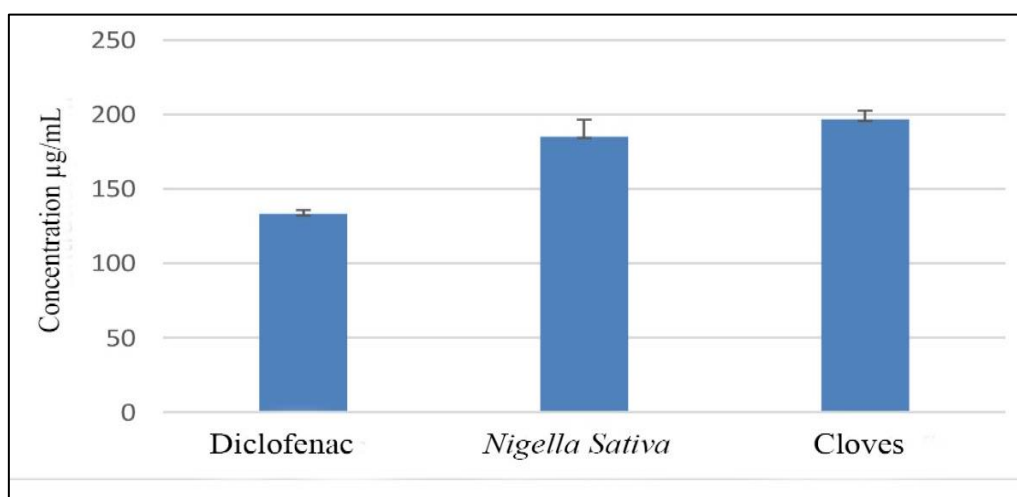


Figure. 3. *In-vitro* anti-inflammatory effect of *N. sativa* and *S. aromaticum* (cloves) extracts (IC_{50} inhibitory concentrations). *N. sativa* extract, cloves: *S. aromaticum* extract, Diclofenac: positive control.

3.4. Determination of antioxidant activity

3.4.1. DPPH test

The antioxidant power of the extracts with respect to the free radical DPPH was experimented with using a spectrophotometer at 517 nm. According to the Figure (4) : Thus, the percentages of inhibition of the DPPH radical of the *S. aromaticum* extract as a function of the concentration are of the order of 85.98% to 7.92%.

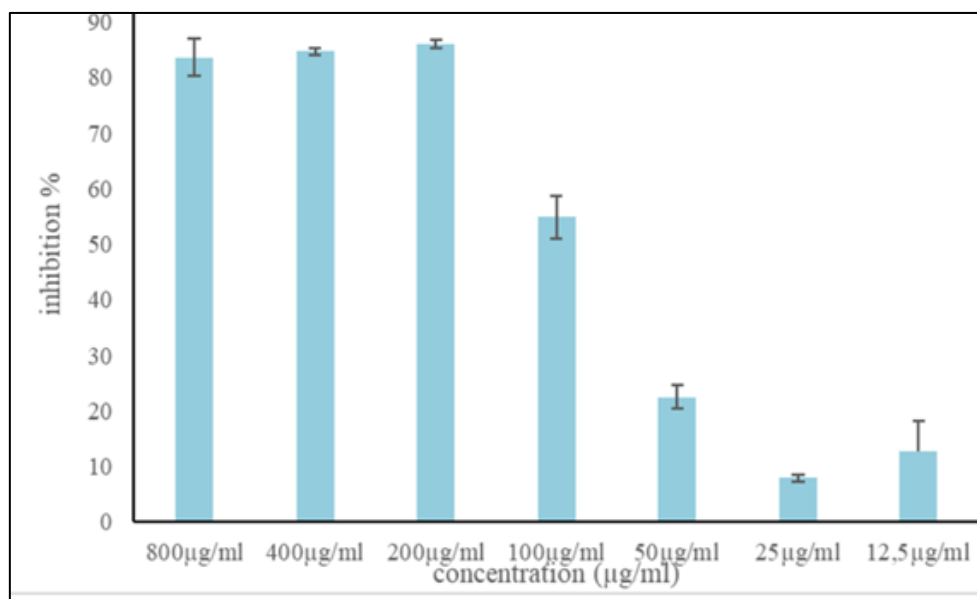


Figure.4. Percentage inhibition of DPPH free radical effect of *N. sativa* and *S. aromaticum* extracts.

The antioxidant power of the extracts with respect to the free radical DPPH was experimented with using a spectrophotometer at 517 nm. According to the Figure (5), the percentages of inhibition of the DPPH radical of the *S. aromaticum* extract as a function of the concentration are of the order of 85.98% to 7.92%. Thus, the percentage of inhibition of the DPPH radical increases with the concentration of ethanol extracts.

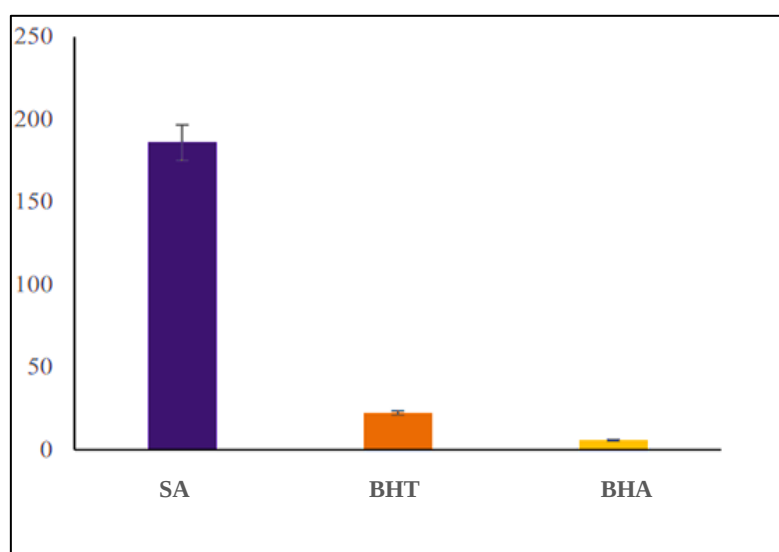


Figure.5. Histogram of the IC₅₀ values of the antioxidant activity (DPPH) of *S. aromaticum* extract (SA) in comparison with positive controls (BHT and BHA).

3.4.2. Phenanthroline test

The anti-radical power of the extracts with respect to the phenanthroline radical was experimented with using a spectrophotometer at 510 nm. According to the results shown in Figures (6, 7) we find that :

* The extract of *S. aromaticum* with an $A_{0.5}=123.69\mu\text{g/mL}$, while the two standards used for this test BHA and ascorbic acid with an $A_{0.5}=8.41\mu\text{g/mL}$ and $A_{0.5}=9.01\mu\text{g/mL}$, respectively. The comparison of these results shows that the antioxidant activity of our *S. aromaticum* extract is lower than the activity of the standards (because the IC_{50} of the extract is 14.70 times higher than that of BHA and 13.72 times that of ascorbic acid).

* Eugenol is a compound with anti-radical potential because of its chemical structure and its reactive properties. Several studies have shown its ability to neutralize free radicals. Among these studies, the published study by Chaieb et al. (2007), which showed that eugenol has a significant antioxidant power [14].

• We have not found similar works on the species of *S. aromaticum* having studied the phenanthroline potential of an ethanol extract.

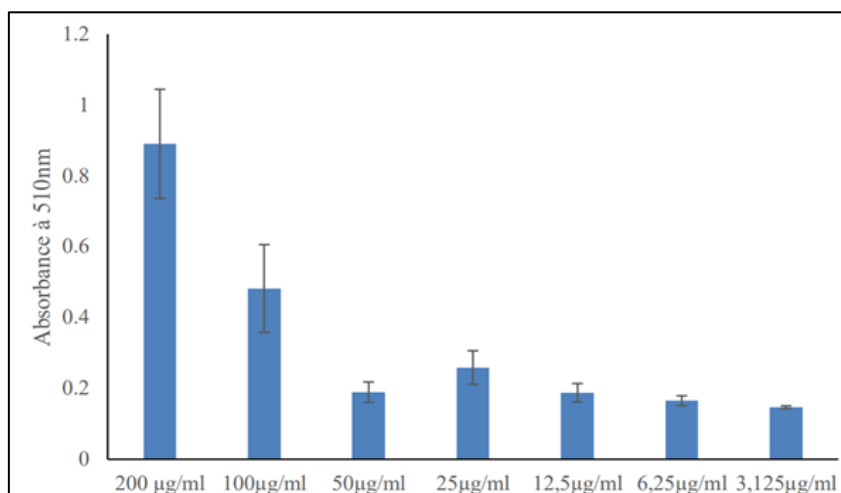


Figure.6. Histogram showing the reducing effect of phenanthroline by the ethanolic extract of *S. aromaticum* (SA).

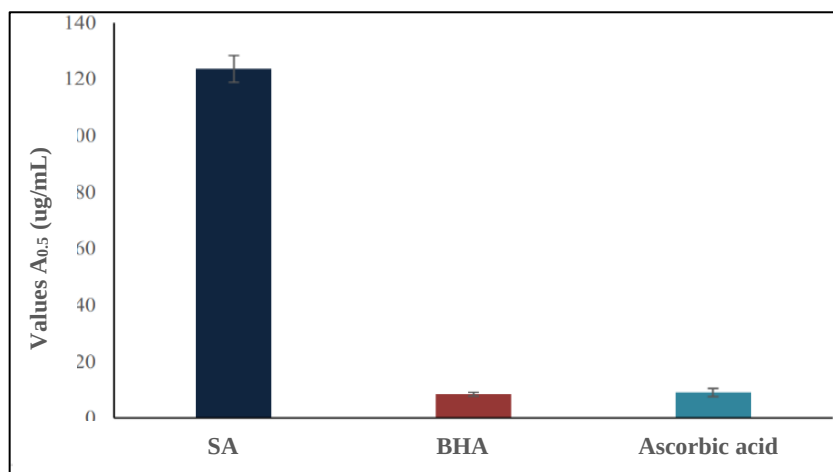


Figure.7. Histogram of the IC_{50} values of the antioxidant activity (phenanthroline).

For the extract of *N. sativa*, the results of our study on the antioxidant activity of *Nigella sativa* showed a non-significant activity. In the tests carried out, the tested *Nigella* extracts did not show significant antioxidant effects, unlike the initial studies based on the well-known antioxidant properties of the plant. The methodology used in our study followed standardized protocols to evaluate antioxidant activity. We used an ethanol-based extraction and measured the antioxidant activity using two well-established tests, the DPPH test and the Phenanthroline test. However, our results differ from some previous studies that have reported significant antioxidant activity of *Nigella*

sativa. These differences can be attributed to a variety of factors, the natural variation of the plants, the growing conditions, the extraction methods or the antioxidant tests carried out.

4. Results of the patch

4.1. Characterization of chitosan

Analysis by FTIR (Fourier transform infrared spectroscopy) of chitosan revealed a DDA (degree of deacetylation) of 79.54%. The degree of deacetylation is a measure of the amount of acetyl groups present in the structure of chitosan. It represents the proportion of free amine groups (NH_2) relative to the total number of amine and acetyl groups. A chitosan with a DDA of 79.54% indicates that nearly 79.54% of the acetyl groups present in the structure of the chitosan have been deacetylated, leaving free amine groups.

4.2. Characterization of patch

After 48 hours of drying in the open air, a gelled patch is obtained, Figure (8) shows the result

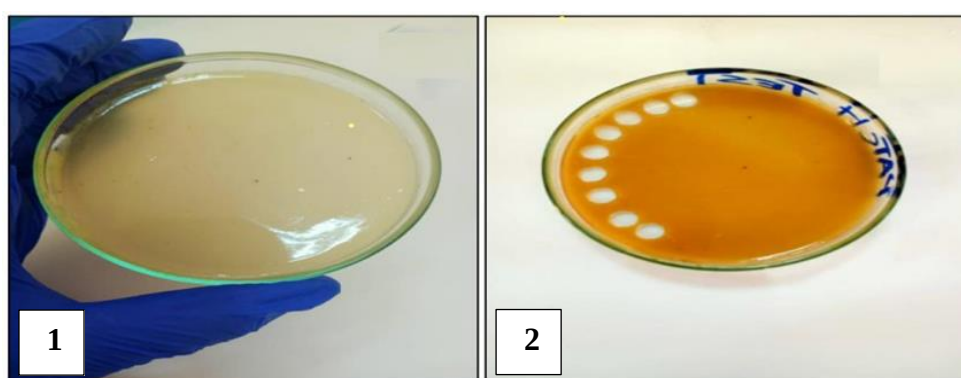


Figure.8. Solution of the obtained patch (1: before crosslinking with glutaraldehyde; 2: after crosslinking with glutaraldehyde).

4.3. Antibacterial activity of the patch

Looking for the antibacterial activity of patch, as we have already mentioned that the extract of *S. aromaticum* had a good antibacterial power on the three strains tested, and even the extract of *N. sativa* had an antibacterial activity on these strains. In addition, our polymer (chitosan) also has a certain antibacterial effect. The results are mentioned in Table (3) and Figure (9). A high activity was found against the three strains, according to the classification of (ponce et al, 2003), *E. coli* and *S. aureus* are very sensitive strains ($D=18\text{mm}$ and $D=15\text{mm}$ respectively) compared to the *P. aeruginosa* strain is sensitive ($D=13\text{mm}$) [17].

To analyze the results of the patch antibiogram, it is necessary to emphasize the synergistic effect which designates the interaction between at least two or more things (*S. aromaticum* extract, *N. sativa* extract and chitosan: each of these compounds has antibacterial power), whose combined effects are greater than the sum of their own effects. We have not found similar works that have studied the antibacterial effect of the ethanol extracts of *N. sativa* and *S. aromaticum* combined with chitosan.

Table.3. Diameters of the patch inhibition zones.

Strains	Inhibition zones (mm)
<i>E.coli</i>	15
<i>S.aureus</i>	18
<i>P.aeruginosa</i>	13

Conclusion

The increased antibiotic resistance and local cutaneous irritation should be given more attention. Therefore, developing novelty treatment patches with solid antibacterial effects and fewer side effects is desiderating. However, this first work in Algeria highlighted the formulation of an anti-acne patch based on plants extracts and chitosan with different yields. The evaluation of the biological activities showed that *S. aromaticum* extract was effective, in particular as antibacterial, antifungal, antioxidant and anti-inflammatory agent. On the other hand, *N. sativa* extract seems to be an antibacterial and anti-inflammatory agent. The extraction of chitosan from the blue crab was carried out successfully, and the polymer obtained had a perfect degree of deacetylation. This indicates the quality and purity of the chitosan used in the formulation of the patch. Regarding the results of the patch, it has been shown to be robust against three tested bacteria, namely *E. coli*, *P. aeruginosa* and *S. aureus*. The drug release test showed that the patch has almost constant release kinetics. It can be used for 24 hours. However, this study constitutes a preliminary step in the development of the anti-acne patch based on plant extracts and chitosan. Additional studies are needed to evaluate the effectiveness of the patch against *P. acnes*, to conduct clinical studies with patients with acne, to compare with other treatments available on the market and finally to optimize the formulation of the patch. This could include adjustments to the concentration of plant extracts, chitosan and gelatin in order to improve the therapeutic effectiveness of the patch.

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