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Evaluation of the antibacterial and anti-inflammatory activities of two plants of the Apiaceae family: BuniumCrassifoliumBatt and OenantheFistulosa L

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Abstract:
Current research primarily focuses on exploring natural molecules with anti-inflammatory and antibacterial properties. In this context, we have become interested in two medicinal plants, Oenanthefistulosa L. and BuniumcrassifoliumBatt., from the North-eastern Algeria region. The objective of this study is to evaluate the antibacterial activity of extracts: n-butanol, ethyl acetate, precipitated n-butanol, and dichloromethane from Oenanthefistulosa and the 100% methanol extract from Buniumcrassifolium. This is done using the agar diffusion method with discs impregnated with different concentrations of extracts on three types of bacteria: one Grampositive (Staphylococcus aureus) and two Gram-negative (Escherichia coli and Pseudomonas aeruginosa). The anti-inflammatory activity, based on the ability of these two extracts (ethyl acetate and precipitated n-butanol) from Oenanthefistulosa to reduce thermal denaturation of proteins, was also evaluated. The ethyl acetate extract of Oenanthefistulosa shows more effective antibacterial activity compared to the other extracts, although it exhibits moderate efficacy. The precipitated nbutanol and dichloromethane (CH_2Cl_2) extracts show notable but limited antibacterial activities. The methanol extract from Buniumcrassifolium shows moderate activity against all strains at the initial concentration. Both extracts exhibit a significant ability to reduce the rate of protein denaturation, with a maximum inhibition rate recorded at 96.41% at a dose of 250 µg/ml for the precipitated nbutanol extract, while the ethyl acetate extract achieved a rate of 84.35% at the same dose. The results obtained contribute to proving the efficacy of Oenanthefistulosa and Buniumcrassifolium in inhibiting the growth of certain pathogenic bacteria. The tests also showed that the plant Oenanthefistulosa has anti-inflammatory properties that make it interesting for the treatment of inflammation.
Keywords: Oenanthefistulosa, Buniumcrassifolium, antibacterial activity, anti-inflammatory activity.

1. Introduction:

Since ancient times, man has been drawing on the impressive reserve of plants that nature abounds. The use of these plants and their extracts as treatments is a very ancient practice. However, herbal

medicine is nowadays enjoying considerable success in many regions of Africa, Asia and Europe (Najja et al., 2010). Plants, vital elements of biological diversity, are mainly used for human well-being. After having long fought traditional medicine, doctors and health organizations are more interested in the values and effectiveness of plant treatments. Many scientific studies have been undertaken to study the botanical and therapeutic aspects of these plants and to integrate their medicinal properties into a modern health system (Mpondo et al., 2015). They contain chemical components that are divided into large groups. Proteins, carbohydrates, lipids and nucleic acids on the one hand, pigments, tannins, polymers, hormones and essential oils on the other hand. The first are the constituents of primary metabolism. They exist permanently within the plant. The others come from secondary metabolism and are not always present in all plants (Bouameret al., 2005). A large number of aromatic and medicinal plants, spice plants and others have very interesting biological properties, which find application in various fields, namely in medicine, pharmacy, cosmetics and agriculture thanks to the active ingredients they contain: flavonoids, heterosides, alkaloids, saponins, quinone, vitamins, and essential oils (PhytoChem., 2007). These active ingredients are at the origin of several biological activities such as anti-inflammatory, antimicrobial, antiseptic, diuretic and antioxidant activity (haddouchi et al., 2014).

Algeria is a country rich in medicinal and aromatic plants due to the diversity of its climate and the nature of its soil and constitutes a real phylogenetic reservoir, with approximately 4000 species and subspecies of vascular plants. Despite this, medicinal plants in Algeria are not yet fully known (Hamel et al., 2018). Secondary metabolites are the subject of numerous researches based on in vivo and in vitro cultures of plant tissues. This is particularly the case of plant polyphenols which are widely used in therapy as vasculoprotectors, anti-inflammatories, enzyme inhibitors, antioxidants and antiradicals (Bahorun, 1997).

Faced with the emergence of forms of resistance of several bacteria to certain antibiotics,as well as the emergence of many diseases of inflammatory origin, the search for new active molecules with a broad spectrum of action has become a necessity. For this the evaluation of phytotherapeutic properties such as antibacterial and anti-inflammatory activity remains a very useful task and one of the most interesting avenues to explore, particularly for medicinal plants of rare or unknown use (Chaouche et al., 2021).

The Apiaceae family, rich in secondary metabolites, has economic and medicinal interests, comprising coumarins, flavonoids, acetylenic compounds and sesquiterpene lactones, as well as a great richness in essential oil. This family of plants is well known for having a significant amount of essential oil in almost all of its anatomical organs. To date, 760 constituents of essential oils have been isolated from Apiaceae (Chaker El calamouni., 2010).

2. Materials and methods:

The aim of our work is to evaluate the antibacterial and anti-inflammatory activity of extracts of Buniumcrassifolium and Oenanthefistulosa. This research aims to explore the therapeutic properties of natural compounds present in plants with a view to developing new drugs or alternative therapies to treat bacterial infections and inflammatory processes. 1. Plant material Different extracts of two plants, namely Buniumcrassifolium and Oenanthefustulosa, were used to evaluate the antibacterial and anti-inflammatory activities. These extracts were provided to us directly by the laboratory of Genetics, Biochemistry and Plant Biotechnology.

The extracts tested A total of five extracts of the plants studied were the subject of our studies (Table 1).

Table 1: The different extracts of the plants studied.

	extracts
01	N-butanol precipitated from Oenanthefistulos

02	Oenanthefistulosaethylacetate
03	DichloromethanefromOenanthefistulosa
04	N-butanol from Oenanthefistulosa
05	Methanol 100% from the aerial part of Buniumcrassifolium

2.1. Antibacterial activity

Bacterial strains used

To demonstrate the antibacterial activity of the different extracts of the two plants studied,three ATCC type reference bacterial strains were used (two Gram bacteria and one Gram+) (Table 2).

Table 2 :Bacterialstrainstested.

Bactérie	Gram	Origine
Staphylococcus aureus	+	ATCC 6538
Escherichia coli	-	ATCC 25922
Pseudomonas aeruginosa	-	ATCC 27853

Diffusion test (disc method)

The diffusion test is a method used in microbiology to assess the antibacterial efficacy of asubstance. The basic principle is based on the diffusion of antibacterial agents of different concentrations in an agar medium. After a certain time of contact between the antibacterial compounds and the bacterial strain, the effect of the antibacterial product appeared as an inhibition zone around the disc. Depending on the inhibition diameter, the strain is considered either sensitive, very sensitive, extremely sensitive or resistant (Biondi et al.,1993).

Revivification of strains

Antibacterial tests must be carried out on young cultures aged 18 to 24 hours in the exponential growth phase. Reactivation of the strains was carried out by seeding each bacterial species in streaks on the surface of the nutrient solution previously poured and solidified in the Petri dishes. Incubation in an incubator for 18 to 24 hours at a temperature of 37 °C is carried out. (Moroh et al., 2008). (figure 01).

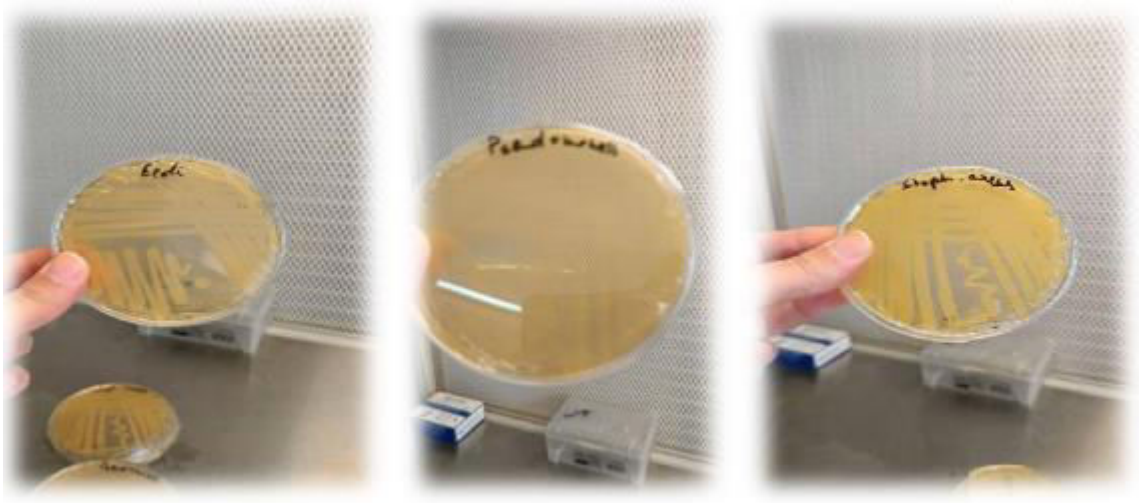

Fig 01: the three bacterial strains tested (E.coli, Pseudo, staph).

Preparing the discs

A sheet of Wattman No. 3 paper is cut into 6 mm diameter discs. Then they are placed in a glass tube, and sterilized in an autoclave and stored until use.

Sterilization of equipment

The working material was first sterilized (test tubes, 6 mm Wattman paper disks, Muller hinton agar, Eppendorf, micropipette tips) in an autoclave at 121 °C for 15 minutes as well as by the UV rays of the hood and the benzene burner.

Culture medium

The culture medium used is Mueller-Hinton, which is the medium most used for sensitivity tests to antibacterial agents (Francois et al., 2007).

The agar is boiled until completely dissolved in a water bath, the culture medium is then poured into the Petri dishes, then left to cool (Figure 02).

Fig 02: Poured Muller-Hinton culture medium.

Preparation of dilutions of plant extracts

The extracts of the plants B. crassifolium and O. fistulosawere dissolved in dimethyl sulfoxide (DMSO), a solvent that has no effect on bacteria, as has been demonstrated in previous studies.
The different concebtrations were prepared by performing successive dilutions (SM, 1/2, 1/4, 1/8), knowing that the concentration of the mother solution of each extract is 100 mg/ml (figure 03).

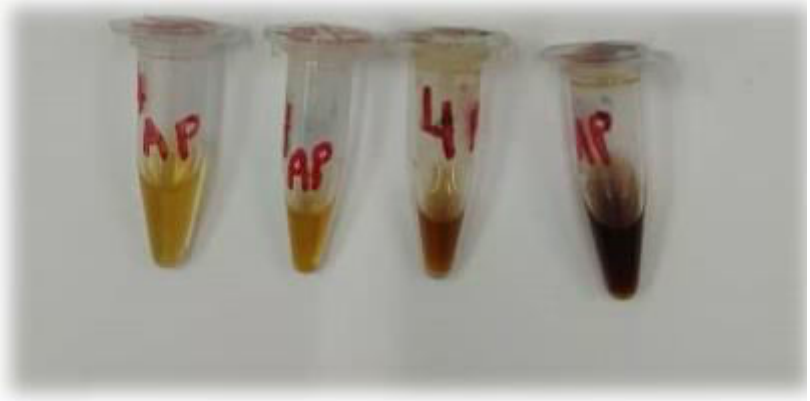

Fig 03 : preparation of dilutions.

Préparation de la suspension bactérienne :

Bacterial suspensions of the strains to be tested are prepared from 24-hour pure cultures on isolation medium, by scraping a few well-isolated colonies of each bacterial species with a platinum solution (Fig 04), which are then introduced into a tube containing phthisiological water (0.9 percent NaCl). Homogenization of the bacterial suspension using a vortex for a few seconds is carried out. The bacterial suspension should be cloudy with a density of 0.5 Mcfarland (Mohhamdi, 2006).

Figure 04: Preparation of bacterial suspension.

Inoculation of the agar

The Muller Hinton (MH), melted and sterilized at 120ºC, in an autoclave is poured into Petri dishes near the Bunsen burner (the agars are dried before use).
Dip a sterile swab in the bacterial suspension. Squeeze it out by pressing it firmly (by rotating it) on the inner wall of the tube, in order to discharge it as much as possible. Rub the swab on the entire dry agar surface, from top to bottom, in tight streaks (Figure 5).
The operation is repeated twice, rotating the dish 60° each time without forgetting to rotate the swab on itself. Finish the inoculation by passing the swab on the periphery of the agar. The Petri dishes are finally placed at 4°C for 1 hour before placing the discs.

Figure 05: preparation of bacterial suspension.

Depositing the disks

Near the Bunsen burner and using sterile forceps, the disks containing 10 µl of extract are placed on the already inoculated agar. Leaving a space between each disk, a disk impregnated with DMSO is used as a control (Figure 6).

The Petri dishes are then hermetically sealed and incubated in the incubator at 37°C for 24 hours.

Figure 06: Depositing the discs.

Reading

The data from the antibiogram tests were analyzed by measuring the diameters of the inhibition zones around the discs after 24 hours of incubation. The results are expressed as a function of the diameter of the inhibition zone (Ponce et al., 2003).

• For non-sensitive (-) or resistant strains: the diameter is less than 8 mm.
• For sensitive (+) strains: the diameter is between 9 and 14 mm.
• For highly sensitive (++) strains: the diameter is between 15 and 19 mm.
• For extremely sensitive (+++) strains: the diameter is greater than 20 mm.

2.2. In-vitro anti-inflammatory activity

Principle

The in vitro anti-inflammatory effect of the different extracts of Oenanthefustulosa L. is determined using the Bovine Serum Albumin (BSA) denaturation inhibition test according to the protocol of Kandikattu (2013).

Briefly, a range of concentrations of each extract ranging from 0 to 10 mg/ml is carried out. 1 ml of each dilution is added to 1 ml of the 0.2% BSA solution prepared in TrisHCl (0.05 M at pH 6.6). The

mixture is then incubated at 37 C ° for 15 min and then at 72 ° C for 5 min. At the end of the incubation, and after vortexing, the mixture is cooled rapidly, then the turbidity is measured at 660 nm using a spectrophotometer (Figures 7 and 8).
For each concentration of extract, a blank consisting of 1 ml of extract and 1 ml of Tris-HCl (0.05 M at pH 6.6) is prepared. The purpose of this blank is to subtract the absorbance of the extract and Tris-HCl from the results obtained.
In this test, diclofenacwas used as a reference anti-inflammatory. The evaluation of its anti-inflammatory activity was carried out under the same operating conditions as those applied to the samples. The percentage of inhibition of bovine serum albumin (BSA) denaturation was determined using the following formula :
% inhibition = [(A control – A test) / A control] x 100
A control: absorbance of blank
A test: absorbance of the sample.

Figure 07: The mixture before incubation at 72°C.

Figure 08: spectrophotometer.

3. Results and discussions :

3.1. Evaluation of antibacterial activity

Antibacterial activity of concentrated and diluted extracts :

The study of antibacterial activity was carried out by the solid medium diffusion technique. This technique allows us to qualitatively estimate the antibacterial effect of the extracts by measuring the diameters of the inhibition zone, in millimeters (mm) of the disks containing a volume of plant extract at a predefined concentration (figure 6).
The results are presented in figures (09,10,11,12,13 and 14).

Figure 09: Antibacterial activity of the extracts on the strains tested.

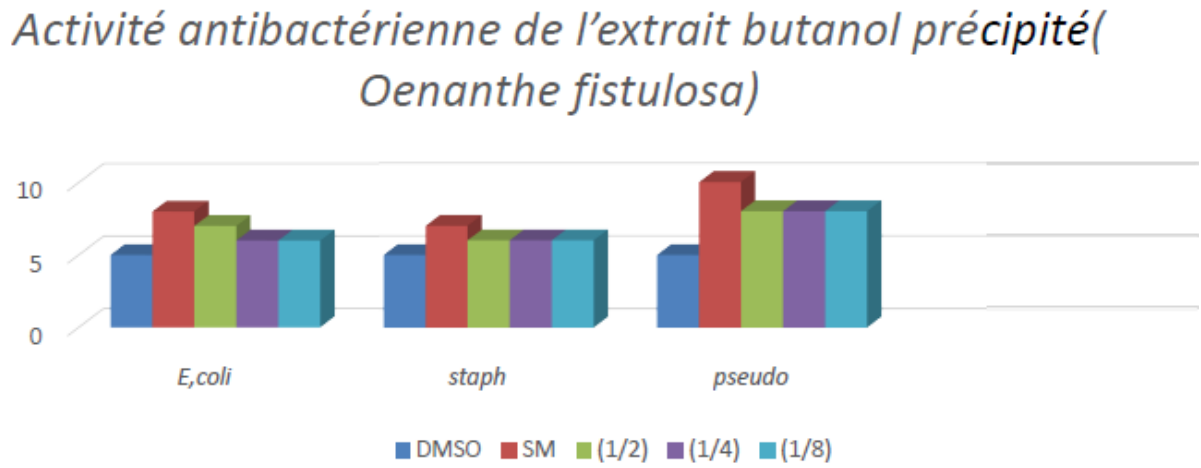

Figure 10: Antibacterial activity of precipitated butanol extract (Oenanthefistulosa).

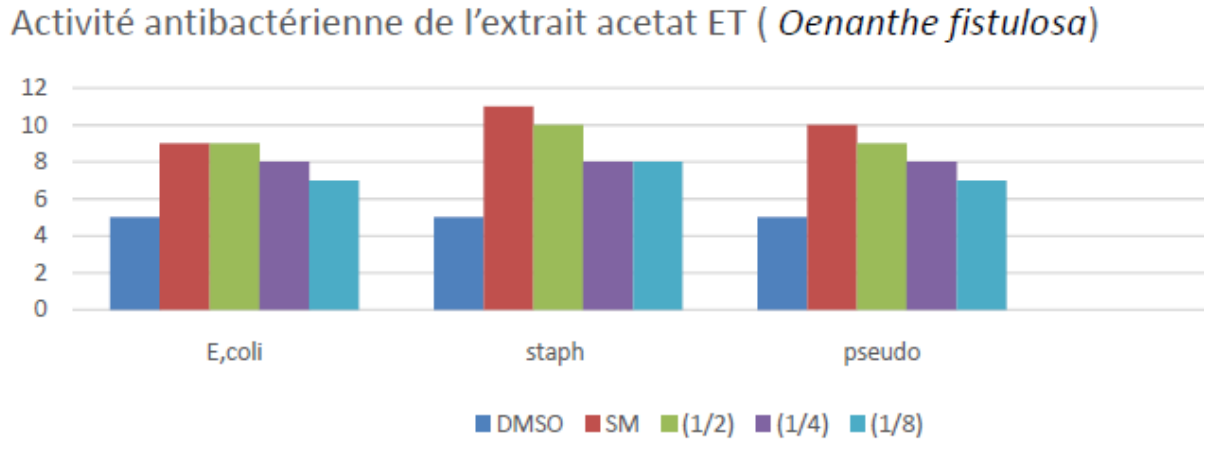

Figure 11: Antibacterial activity of ethyl acetate extract (Oenanthefistulosa).

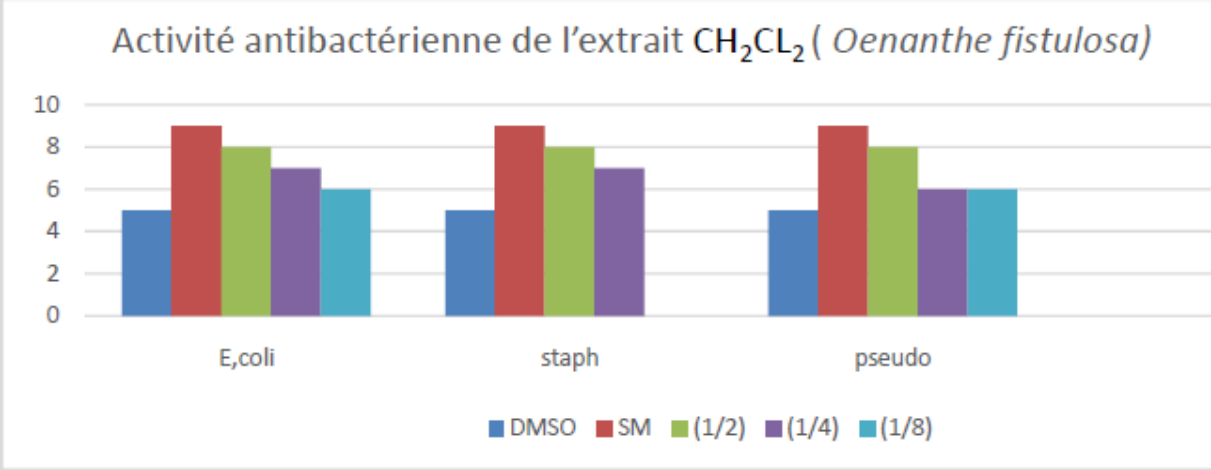

Figure 12: Antibacterial activity of CH_2Cl_2 extract (Oenanthefistulosa).

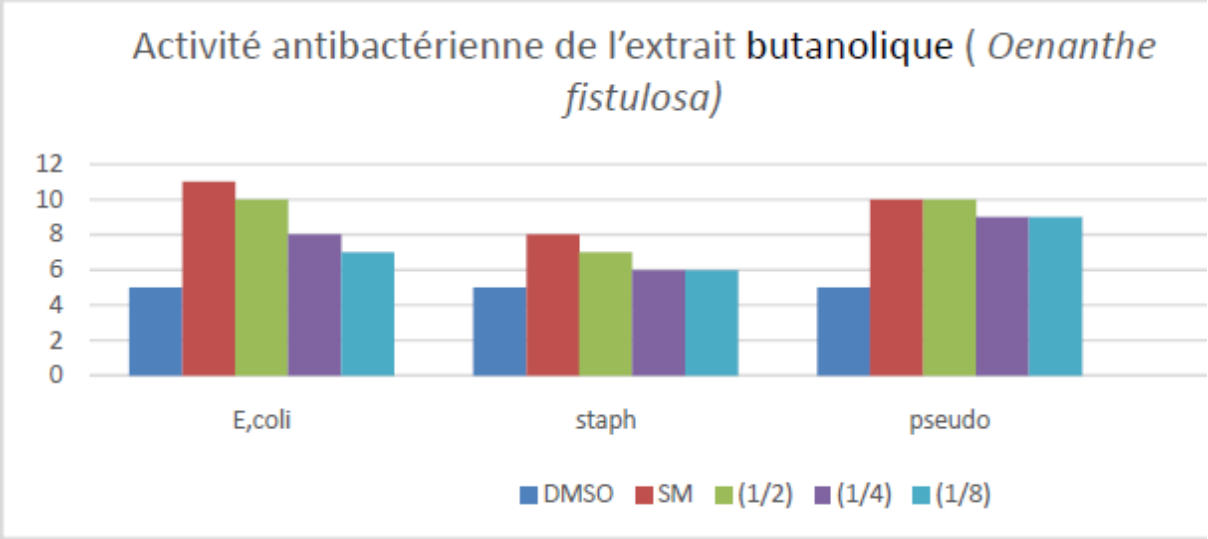

Figure 13: Antibacterial activity of butanolic extract (Oenanthefistulosa).

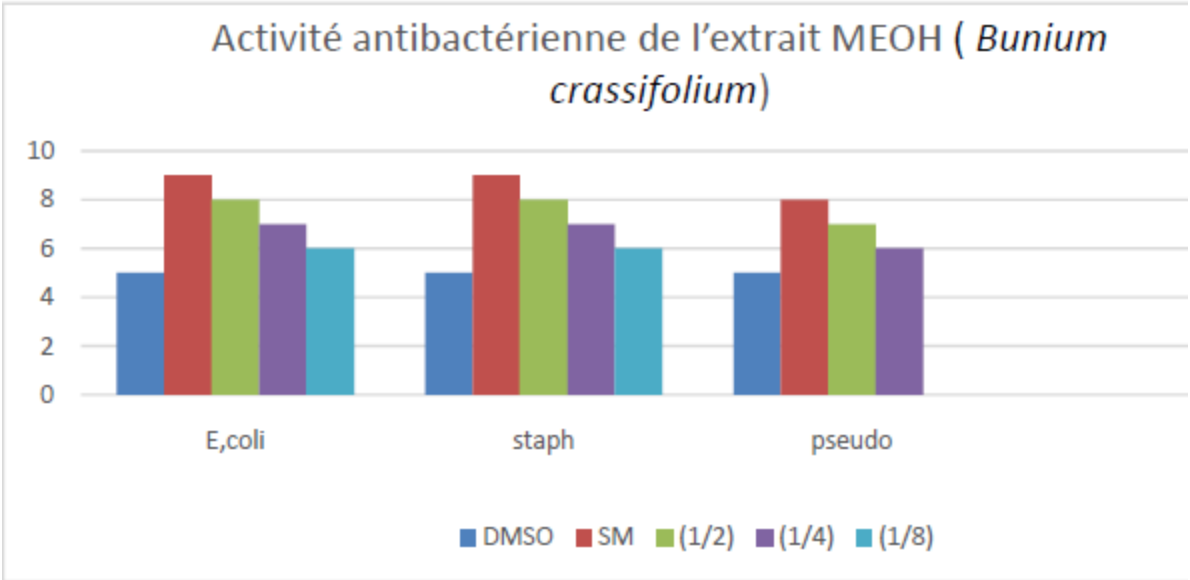

Figure 14: Antibacterial activity of Methanolic extract (Buniumcrassifolium).

From the results expressed in the figures:

It is observed that Staphylococcus aureus is the most sensitive strain to the extract of Oenanthefistulosa. L (ethyl acetate) with concentrations SM, 1/2 whose inhibition diameter values reached (11, 10) mm, respectively. Pseudomonas aeruginosa shows sensitivity to the same extract with concentration SM and 1/2 whose inhibition diameter is (11,10) mm. Escherichia coli also shows sensitivity with concentration SM and 1/2 whose inhibition diameter is (9,9) mm. For the n-butanolic extract (Oenanthefistulosa), the most sensitive strain is Escherichia coliwhose inhibition diameters are (11 and 10) mm with concentrations SM and 1/2. Pseudomonas aeruginosa also shows sensitivity with the concentration SM and 1/2 whose inhibition diameter is (9.9) mm. The same extract does not exert any inhibitory effect on Staphylococcus aureus.

The ethyl acetate extract shows an average efficacy against the three strains. However, the n-butanol extract is effective against Staphylococcus aureus and Escherichia coli.

According to the previous results, it can be deduced that the ethyl acetate extract shows a more effective antibacterial activity than the n-butanol extract.

The precipitated n-butanol extract has an antibacterial activity with the initial concentration (SM) against the bacterial strain Pseudomonas aeruginosa and Escherichia coli whose inhibition diameter values reached are (10 and 8) mm, respectively. The other dilutions do not have any antibacterial activity (diameter less than 8 mm). While the dichloromethane CH_2Cl_2 extract of Oenanthefistulosahas antibacterial activity against the three bacterial strains with the initial concentrations (SM) (a diameter of 9 mm).

The dichloromethane CH_2Cl_2 extract has an average efficacy against the three strains. However, the precipitated n-butanol extract is effective against Pseudomonas aeruginosa and Escherichia coli.

According to the previous results, it can be deduced that the dichloromethane CH_2Cl_2 extract has a more effective antibacterial activity than the precipitated n-butanol extract.

The MeOH extract of the species Buniumcrassifolium has an antibacterial activity against the strains (Escherichia coli, Staphylococcus aureus, pseudomonas aeruginosa), with the initial concentration (SM) whose inhibition diameter values reached are (9, 9, 8) mm, respectively. The other dilutions have no antibacterial activity (diameter less than 8 mm).

Therefore, the dichloromethane CH_2Cl_2 extract has an average efficacy against the three strains tested.

Comparing our results to those of (Chaibeddra, 2020) who tested the ethyl acetate and n-butanolic extract of the plant OenanthevirgataPoiret, where he recorded that the ethyl acetate extract has antibacterial activity against three bacteria, while the butanolic extract has antibacterial activity against only one bacteria. The ethyl acetate extract shows good inhibition against Pseudomonas aeruginosa and Staphylococcus aureus, and no effect was shown against E.coli (the diameter of the inhibition zone: Pseudomonas aeruginosa = 20 mm, Staphylococcus aureus = 16 mm). On the other hand, n-Butanol shows moderate inhibition against Pseudomonas aeruginosa (the diameter of the inhibition zone: Pseudomonas aeruginosa =11mm).

We find that our Oenanthefistulosa extract has less antibacterial activity compared to OenanthevirgataPoiret extracts.

In another study carried out by Karouche et al., (2020) on the methanolic extract of the plant Buniummauritanicum, the latter reacted positively on the two reference strains: Staphylococcus aureus and Pseudomonas aeruginosa, the latter have inhibition zones with diameters that vary between 8 and 12mm. The results we obtained are rather comparable to those of this study.

The results obtained indicate that the presence of antibacterial activity in the extracts of the plant Buniumcrassifolium and Oenanthefistulosa is due to the presence of different chemical components such as alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids.

The appearance of an inhibition zone around the paper disk impregnated with the crude extracts studied indicates a bacteriostatic action. The diameter of the inhibition zone varies from one bacterium to another and from one extract to another. In accordance with the information reported in the literature, we have established that an extract has a bacteriostatic action if its inhibition diameter is greater than 8 mm (Marjorie, 1999).

2. Evaluation of anti-inflammatory activity

To evaluate the anti-inflammatory effect of two Oenanthefistulosa extracts, we performed an in vitro test of inhibition of thermal denaturation of BSA. The results obtained (Figure 15) were then compared to those obtained under the same conditions for the commercial anti-inflammatory diclofenac sodium.

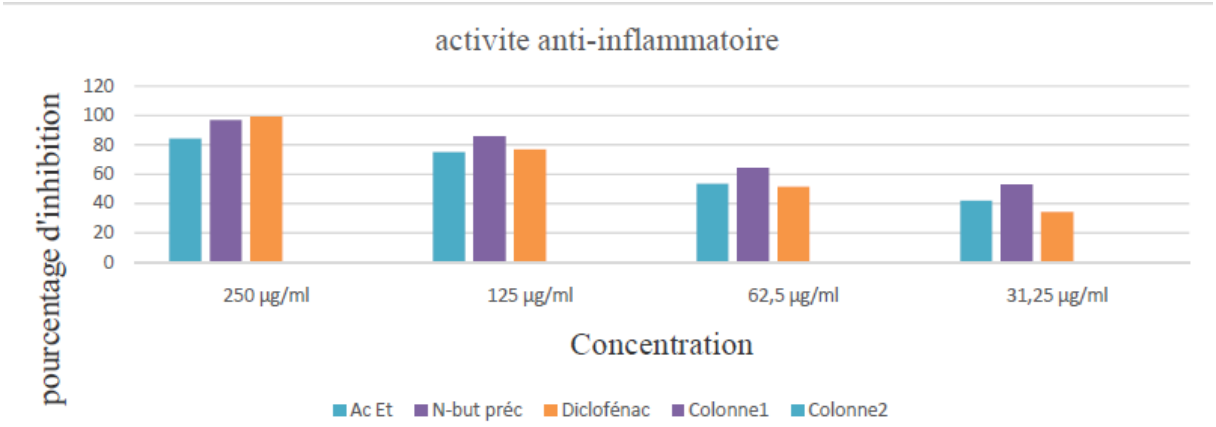

Figure 15: Percentage of inhibition of protein denaturation of the two extracts compared to the standard.

The ability of different extracts to inhibit BSA denaturation was estimated. The results obtained show that both extracts have an inhibitory effect on BSA denaturation. The evaluation of the percentage of inhibition shows that the extract of Ethyl acetate and N-butanol precipitated from Oenanthefistulosa has an in vitro anti-inflammatory activity at the dose of 125, 62.5, 31.25µg/ml higher compared to the standard solution of diclofenac in the same concentrations. On the other hand, in the concentration of 250 µg/ml, diclofenac presents a maximum inhibition of protein denaturation at a percentage of 99.23%, and the percentage of inhibition for the extract of n-butanol precipitated at the same concentration remains relatively close to 96.41%, while the percentage of inhibition for the extract of ethyl acetate is 84.35%.

The percentage of inhibition of denaturation by the precipitated n-butanol extract is higher compared to the ethyl acetate extract and close to that of the reference anti-inflammatory (diclofenac).

The comparison between the percentages of inhibition of protein denaturation of the OenanthevirgataPoiret ethyl acetate extract where it recorded a protection of the order of (81.58%) in the study of (Chaibeddra, 2020) and the n-butanol extract of OenanthevirgataPoiret was ineffective. It is found that our Oenanthefistulosa extract presents a relatively superior protection.

Protein denaturation is defined as a process due to external factors such as heat, strong acid or base, organic solvent or concentrated inorganic salt, which means that the tertiary structure and secondary structure of the protein are disoriented (Dharmadeva et al, 2018). Protein denaturation is one of the well-known events that cause inflammatory and arthritic diseases. In some arthritic diseases, the production of autoantigenscan be caused by the protein degradation that occurs in the body (Chandra et al, 2012).

From our results, we find that both extracts (Ethyl acetate and N-butanol precipitate) of Oenanthefistulosa were able to control protein denaturation and thus inhibit the production of autoantigens. The inhibitory activity of BSA denaturation can be attributed to the presence of

different bioactive compounds such as flavonoids in the extracts. It can be concluded that the crude extracts possess an anti-inflammatory effect.

4. Conclusion and perspectives:

Medicinal plants remain and will remain for a long time a reliable source of active ingredients for their therapeutic properties.

The present study was conducted to evaluate the antibacterial and anti-inflammatory activity of the extracts, ethyl acetate, n-butanolic, precipitated n-butanol, dichloromethane and methanol of two plants belonging to the Apiaceae family. These extracts were tested against three pathogenic bacteria: Escherichia coli, Staphylococusaureus, Pseudomonas aeruginosa.

The evaluation of the antibacterial activity of these extracts with a view to developing new natural antibiotics at different concentrations against the bacterial strains tested revealed that the extracts of the plants studied have a more or less significant inhibitory effect.

This study highlights the potential of Oenanthefistulosa and Buniumcrassifolium extracts as sources of natural antibacterial agents. Their ability to inhibit the growth of common pathogenic bacteria suggests that they could be further explored for the development of novel antibacterial treatments. However, further studies are needed to understand the specific mechanisms of action of these extracts and to evaluate their in vivo efficacy.

Evaluation of the anti-inflammatory activity of crude ethyl acetate and precipitated n-butanol extracts of Oenanthefistulosa shows that they have promising potential as natural anti-inflammatory agents. Their inhibitory effects on protein denaturation suggest that they could be further explored for the development of treatments against inflammatory and arthritic diseases. However, further studies are needed to identify the specific active compounds and understand their exact mechanisms of action.

All of these results obtained constitute only a first step in the search for biologically active substances of natural origin, which is why it would be important to further research on these two interesting plants and the resulting perspectives are as follows:

• It would be important to isolate and identify the bioactive compounds responsible for the antibacterial and anti-inflammatory activity observed in the extracts of Oenanthefistulosa and Buniumcrassifolium.

• In vivo studies would be needed to evaluate the efficacy of the extracts in animal models against inflammatory and infectious diseases.

• Research could also be conducted on the synergy between the extracts and other antimicrobial or anti-inflammatory agents, such as antibiotics or conventional anti-inflammatories.

• Extensive studies on the safety and toxicity of the extracts, particularly in the long term and at higher doses.

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