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Evaluation of the antibacterial activity of essential oils of *Rosmarinus officinalis* L and *Rosmarinus eriocalyx* from the region of Sidi Bel Abbes (Algeria).

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ABSTRACT

Antibiotic resistance is currently a major public health problem facing hospitals around the world, another concern and that of the increased risks of additive synthetic food, added to foods that objective is to preserve foodstuffs. All these parameters are behind the urgent need for a natural antimicrobial agent. Rosmarinus officinalis L. is used in traditional medicine as an antispasmodic, ingredients in beauty products and so good in the preservation of food products. In this context, the objective of this study is to evaluate the antimicrobial activity of essential oils extracted from two species of the same genus of rosemary of wild origin "Rosmarinus eriocalyx" and "Rosmarinus officinalis" of a home garden in the Sidi Ali Benyoub region, against three pathogenic bacteria (Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa). Our work is divided into two parts, the first consists of extracting a volume of essential oil from 100g of each plant species by the hydrodistillation method which is the most effective and the most useful one, the second part is based on the evaluation of the effect of these oil extracts on the growth of the three pathogenic bacteria "Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa" taken from a biological sample "the urine" in the presence of a urinary tract infection at the level of the Central Laboratory, bacteriology department of the SBA center, which is highlighted by the aromatogram tests and the monitoring of bacterial growth in the absence and in the presence of essential oils. The diffusion method on the agar medium made it possible to determine the diameters of the zones of inhibition. The results show that the quantitative analysis of the essential oils of Rosmarinus eriocalyx and Rosmarinus officinalis L gave good yields of 1% and 0.63%. Regarding the antibacterial activity of both oils against pathogenic bacteria, the results show us that Rosmarinus eriocalyx (wild) has a higher activity compared to "Rosmarinus officinalis L". We can see that these oils have antibacterial activity only against Pseudomonas aeruginosa but the other two bacteria "Staphylococcus aureus and Escherichia coli" can be resistant for these oil extracts.

***Keywords*:** Antibacterial activity, aromatogram, essential oils, pathogenic bacteria, *Rosmarinus eriocalyx*, *Rosmarinus officinalis* L, yield

INTRODUCTION

Through the years, interest of using plant compounds has been growing faster in worldwide due to their benefits on health (Nandi and Ghosh, 2016). Many researchers have reported essential oil (EO) as potential alternative antimicrobials (Solórzano-Santos and Miranda-Novales, 2012). Rosemary *Rosmarinus officinalis L.* is a spontaneous aromatic plant widely distributed in Algeria, belonging to the Labiatae family (Lamiaceae) commonly called by the local population "Eklil". Rosemary likes limestone soils and adapts very well to arid and rocky regions. It is easily recognized, all year round availability. Its leaves, the flowering tops, which we will have taken care to dry or the essential oil which are used in

herbal medicine. Rosemary has been the subject of recent research in the pharmaceutical and food industries. It has anti-inflammatory and antispasmodic properties (Gianmario *et al.*, 2007) and action on the nervous system (Gonzalez *et al.*, 2007; Suzana *et al.*, 2007). Rosemary has excellent antioxidant and antimicrobial properties (Thoresen and Hildebrand, 2003). Recent research has shown that it has a variety of pharmacological activities, such, as cancer chemoprevention, anti-diabetic, hepatoprotective. Thus, rosemary is considered one of the most effective herb for treating headaches, poor circulation (Rocha *et al.*, 2015). Rosemary, like all aromatic and medicinal plants, contains chemical compounds with antibacterial properties. The use of these plant-based molecules can have many advantages over current synthetic products. Whose essential oils have antibacterial activity against some pathogens responsible for several human diseases such as Escherichia coli, staphylococcus aureus, pseudomonas aeroginosa, etc. (Bennadja *et al*., 2013). With this in mind, our choice fell on these two plants answered in Algeria (*Rosmarinus officinalis* L and *Rosmarinus eriocalyx*), widely used in culinary flavoring. In our region of Sidi-bel-abbés *Rosmarinus officinalis* L and *Rosmarinus eriocalyx* are important and more used as a condiment and/or food uses, found in the kitchen; it is also a bee plant, medicinal plants traditionally used against inflammation, bacterial and viral infections, and antispasmodics.

The aim of this work is to evaluate the antibacterial activity of the essential oil of *Rosmarinus officinalis* L, *Rosmarinus eriocalyx* from the ecotype of the region of Sidi Ali Benyoub and Mazaourou of Sidi-Bel-Abbés (Algeria) against pathogenic bacteria such as *staphylococcus aureus, pseudomonas aeroginosa, Escherichia coli.*

MATERIALS AND METHODS

Place of internship

Our experimental study is programmed at the level of the laboratory of Physiology and Nutrition and General Microbiology within the Department of Biology at the Faculty of Natural and Life Sciences of the University of Djilali Liabes of Sidi-Bel-Abbés (SBA).

Plant material

This work is focused on the two plants of "*Rosmarinus officinalis L*" and "*Rosmarinus eriocalyx"*, the choice of these plants is linked on the one hand to the importance of this species "Rosemary" as a medicinal and aromatic plant and among the most used throughout the world and on the other hand to the strong use of their extract of essential oils in traditional medicine. The species of "*Rosmarinus eriocalyx"* was provided at the level of the forest of ''Sidi Ali Benyoub" which is located in Sidi-Bel-Abbés province in the north-west of Algeria, it is located at 34°56'44 "N, 0°43'10"W. Latitude: 34.9456, Longitude: -0.719433 between Tabia and Mezaourou.), and for the second plant *'Rosmarinus officinalis L'* was provided at a small home garden at ''MEZAOUROU'' which is located in the province of Sidi-Bel-Abbés in the north-west of Algeria Latitude: 34.8173, Longitude:-0.623319, 34° 492 23 North, 0° 372 243 West). Both plants were dried to extract these oils

Extraction of essential oils (EO_s)

The extraction of EO from the dry leaves of the two plants *Rosmarinus officinalis L* and *Rosmarinus eriocalyx* is carried out using the hydrodistillation method at the level of the laboratory of Physiology and Nutrition of the department of biology, of the Faculty of Sciences of the Nature and Life at SBA.

Extraction process

This is the simplest and the oldest method of steam distillation. The process consists of immersing (50g) of the vegetable raw material (Rosemary) in a flask filled (100ml) with distilled water on a heat source. Everything is then brought to boil. The heat allows the plant cells to burst and then release odorous molecules. These aromatic molecules form an azeotropic mixture with water vapor. The vapors are condensed in a cooler and the essential oils separate from the water by density difference (the same steps for both species). The duration of a hydrodistillation can vary considerably, up to several hours depending on the equipment used and the plant material to be treated. The duration of the distillation influences not only the yield but also the composition of the extract.

EO analysis

Quantitative analysis

Determination of extraction yield

According to the standard (AFNOR, 1982), the yield of essential oil (Y_{EO}) is defined as the ratio

between the mass of essential oil obtained after extraction (m) and the mass of the plant material used (m_0). The yield is expressed as a percentage, it is expressed by the following formula. $Y_{EO}(\%)=m/m_0 X 100$ (Chama *et al.*, 2020).

Y_{EO}: Essential oil yield in %

m : Mass of essential oil in grams.

m_0: Mass of fresh plant material used in grams

Qualitative analysis

Organoleptic control

It consists of determining the organoleptic characteristics of the EOs obtained: smell, color, appearance, and flavor.

Study of Physico-chemical properties

In order to determine the quality of the EOs, we determined a certain number of physico-chemical characteristics.

Determination of relative density

The density of an oil is the ratio of the mass of a certain volume of oil at 20°C (Chama *et al.*, 2020).

$$D\frac{20}{20}=\frac{m}{m_0}$$

Where: m: the mass in grams of the oil, m_0: the mass in grams of distilled water

Determination of the refractive index

The refractive index is used for the identification and as a criterion of purity of EOs and liquid compounds, and to check the quality of the extraction.

pH

The pH or "hydrogen potential" measures the chemical activity of hydrogen H+ ions in solution. pH measures the acidity or basicity of a solution. This method describes the ionic acidity of the product to be analyzed; its principle consists in introducing the electrode of the pH meter into the product after adjusting the calibration temperature. The reading is done directly on the pH meter.

Research method and identification of the three pathogenic bacteria

The tested bacterial strains of *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa* are brought from a biological sample (urine) of a patient suffering from a urinary tract infection; this experiment of isolation of the strains of interest is carried out at the central laboratory level CHU Abdelkader Hassani SBA in the department of bacteriology. Once, the samples are subjected to a direct examination including microscopic observations in the fresh state and a Gram stain. The identification of the strains was carried out at the level of the General Microbiology laboratory of the Faculties of Nature and Life Sciences, Department of Biology, University Djilali Liabés SBA.

Sampling: The collection of urine is carried out during the morning in a sterile pot, throwing away the first jet and then collecting the other urine.

Storage: The urine is stored at 4°C to prevent the proliferation of germs.

Sowing Seeding is carried out using a sterile platinum loop in streaks in three Petri dishes, each dish containing a selective culture medium for the isolation of the strains of interest. The media used are: Chapman for *Staphylococcus aureus*, Mac Conkey for *Escherichia coli*, Hektoen for *Pseudomonas aeruginosa*

Incubation: the inoculated Petri dishes are incubated at 37° C. for 18-24 hours.

Identification of isolated strains

Identification is based on the determination of morphological (macroscopic and microscopic) and biochemical characters

Macroscopic observation makes it possible to distinguish the shape, color, and morphology of bacterial colonies.

Microscopic observation makes it possible to study the morphological aspect of the cells of a microbial species. It includes the examination:

Fresh Microscopic

Observation in the fresh state consists of depositing a drop of bacterial suspension between slide and sterile cover slip and observed at magnification (X40). This method makes it possible to observe: The morphology of bacteria, Mobility, Grouping mode.

In the colored state Microscopic observation in the stained state is carried out on a bacterial smear, fixed and stained by the Gram method, this method makes it possible to observe: Color: Gram-positive or Gram-negative

Biochemical tests

The study of biochemical bacteria is essentially based on the search for catalase, oxidase to confirm that it is a bacterium of interest.

Oxidase test

Principle

The purpose of this test is to determine the presence of an active cytochrome oxidase using a determined substrate: N-dimethyl-para phenylene diamine, which will react in its presence with a color change (formation of a purple-colored compound).

Technical

The search for oxidase consists of placing a disk of it on a sterile slide and soaking it with a drop of distilled water. Then, pick up a colony using a Pasteur pipette and place it on the disc. A rapid color change to purple indicates a positive reaction.

Catalase test

Principle

In the presence of molecular oxygen, certain metabolic reactions lead to the formation of hydrogen peroxide. Catalase is an enzyme that breaks down hydrogen peroxide H_2O_2 into water H_2O and oxygen O_2.

Technical

It consists of depositing a drop of hydrogen peroxide H_2O_2 on a slide. Using a sterile Pasteur pipette, pick up a few colonies of the strain to be tested and place them in the drop of hydrogen peroxide. The appearance of many gas bubbles indicates a positive reaction.

Conservation

The conservation was carried out in the short term (about a week); the boxes are maintained at more than 4°C in anaerobic atmosphere.

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Aromatogram technique on solid medium

Principle

The aromatogram is based on a technique used in medical bacteriology and which is carried out in vitro, called antibiogram or method by diffusion in agar medium or even disc method. This method has the advantage of being very flexible in the choice of essential oils tested, of applying to a very large number of bacterial species, and of having been widely evaluated for more than 50 years of worldwide use (Wilkinson, 2006). It is the technique we used to initially assess the antibacterial activity of EO (Chama *et al.*, 2020).

The antibacterial activity is evaluated by the aromatogram method, which makes it possible to determine the sensitivity of the different bacterial species to the essential oils used.

Technical

This method consists of depositing sterile cellulose discs 6mm in diameter on the surface of Petri dishes containing agar which is already solidified and seeded with the microorganisms of interest, these discs will be soaked in a quantity of essential oil to test; Petri dishes were incubated at 37°C for 24 hours. After incubation, the reading of the results is done by measuring the diameter in millimeters of the clear zone around the discs; this zone is called the zone of inhibition (formation of a translucent halo). The larger the diameter of the zone of inhibition, the more sensitive the strain is to the substance tested, the smaller it is, the more resistant the microbial strain.

Revivification and transplanting of stumps

Revivication of microbial strains are carried out by the streak method. The latter is then revived from storage boxes of selective media for each bacterium on nutrient agar. The cultures are incubated in an oven at 37°C for 24 hours.

Preparation of the inoculum

It was prepared from a young 24-hour culture. For this, bacterial suspensions were made by taking 3 to 5 well-isolated colonies, which were deposited in 10 ml of distilled water. Then, we ensured a good agitation. Then incubated at 37°C for 24 hours. After incubation, centrifugation of this bacterial suspension was performed.

Preparation of culture media

The culture medium used for the aromatogram test is Mueller Hinton (MH) medium, the process consists of pouring this agar into sterile petri dishes with a diameter of 90 mm to a thickness of 4 mm

and then allowing them to cool and solidify at room temperature in the sterile area.

Preparation of different EO concentrations

The preparation of EO concentrations consists of making a stock solution of tween 80 for preparing the different dilutions of essential oil. The process consists of diluting 2.5 ml of pure Tween 80 in 90 ml of distilled water, this solution is sterilized at 120°C for 15 minutes. In a volume of 9ml of this stock solution, we added 1ml of essential oil, in order to obtain a well-homogenized solution, we stirred the contents with a vortex, and then we carried out the successive dilutions of 10^{-1} to 10^{-6}.

Preparation of EO discs

The discs are prepared from Whatman paper 6mm in diameter, then they are put in a test tube, sterilized in an autoclave at 120°C for 20 minutes, then soaked in the extracts of essential oils of *Rosmarinus officinalis* and *Rosmarinus eriocalyx* at room temperature.

Seeding

The seeding of the bacterial suspension is carried out by the calibration technique using a Pasteur rake pipette on the Mueller Hinton medium prepared beforehand. In order to better appreciate the reading of the inhibition zones, two tests are carried out for each oil.

Disc repositories

Using sterile forceps; Whatman paper discs previously immersed in essential oils were placed on the surface of the inoculated Petri dishes.

Incubation

In order to inoculate the bacterial strains and deposit the discs, the boxes are incubated at 37°C for 24 to 48 hours.

Expression of results

The reading of the results is done after the incubation time by measuring the diameter of the zone of inhibition around each disc using a caliper or a ruler graduated in millimeters. The larger the diameter of this zone, the more the strain is sensitive to the essential oil (Djenane *et al*., 2012). To interpret the results, we used the scale of estimation of antimicrobial activity, which is given in the following table (Table 1).

Table 1: Reading of antimicrobial activity results (Djenane *et al*., 2012)

Diameter (∅) (mm)	Strain sensitivity
∅ < 7	**Not susceptible or resistance (-)**
8< ∅ <14	**Sensitive (+)**
15< ∅ <19	**Very sensitive(++)**
∅ >20	**Extremely sensitive(+++)**

RESULTS AND DISCUSSION

EO analysis

Rosmarinus officinalis L

Y_{EO}: Essential oil yield in %

m: Mass of essential oil in grams 0.63g.

m_0: Mass of fresh plant material used in grams 100g.

$$Y=\frac{0.63}{100}\times 100 \text{ , } Y = 0.63$$

Rosmarinus eriocalyx

YEO: Essential oil yield in %

m: Mass of essential oil in grams 1g.

m_0: Mass of fresh plant material used in grams.

$$Y=\frac{1}{100}\times 100 \text{ , } Y = 1$$

The yield obtained during the extraction is 0.63% for *Rosmarinus officinalis* and 1% for *Rosmarinus eriocalyx*, the yield may vary from one harvest to another this may be due to the various factors that come into play, among them, we cite the nature of the soil, the harvest period, the drying time, the extraction method, but this value remains within the standards between 0.5-2 according to (AFNOR, 1982).

The results obtained on the yield of essential oils extracted from Rosemary of the two populations studied in the region of Sidi Ali Beyoub and Mezaourou of the wilaya of Sidi-Bel- Abbés

comply with Afnor standards (0.5-2%). However, they show a significant difference between the sample from sidi ali benyoub (1%) of the *Rosmarinus eriocalyx* species and the sample from Mezaourou (0.63%) of the *Rosmarinus officinalis* species, his difference is due to the different factors such as; soil type, harvest time, drying time, etc. Regarding *Rosmarinus eriocalyx* is obtained from the mountain of Sidi Ali Benyoub (soil type) which is an upper region in the month of April, and the drying time is 12 days. Moreover, *Rosmarinus officinalis* is obtained from a garden in Mezaourou which is a lower region in the month of April and the drying time is 7 days; these conditions are influenced on the yield results.

Few are the works on Rosmarinus essential oil, and comparing to the yield of essential oil, our recorded value is close to that mentioned by Bendif *et al.* (2017) which was 0.73% for the oil extracted from leaves 0,6% and 0.82%, respectively. On the other hand, it is higher than that found in Annaba by (Ouibrahim, 2014) which was estimated at 0.36% and that obtained in Morocco (0.54%) (Derwich *et al.*, 2011). Low compared to that reported by Bendif *et al*. (2018) but this is obtained after supercritical CO_2extraction, which was 3.7%. The yield of essential oil from plants is often minimal, typically 1% or less (Carson and Hammer, 2011). The difference in yield is quite normal because it varies according to several parameters including climate, plant nutrition, stress (Croteau, 1986) and the collection period (Akrout *et al*., 2003).

Qualitative analysis

Organoleptic control

The essential oil of *Rosmarinus officinalis* and *Rosmarinus eriocalyx* are extracted by a simple hydrodistillation technique, this extraction method is standardized for the extraction of essential oils (Marie *et al*., 2004). The organoleptic parameters of this essential oil are in agreement with those listed in the standards (AFNOR, 1999). It is in liquid form with a light yellow color and it has a camphor odor, these results with the AFNOR standard are presented in the table (Table 2)

Table 2: Organoleptic characteristics of essential oils of *Rosmarinus officinalis L* and *Rosmarinus eriocalyx*.

	Appearance	Color	Odor	Flavor
AFNOR (fresh form)	Mobile, clear liquid	Almost colorless to pale yellow	Characteristic fresh, more or less camphor depending on the origin	Bitter and very slightly spicy
Essential Oil	Liquid	Pale yellow	Camphor	Spicy and fragrant

Study of Physico-chemical properties

Determination of relative density

The density of an oil is the ratio of the mass of a certain volume of oil to20°C, it varies between 0.900 and 0.905 (AFNOR, 1994). Our result showed that the essential oil density of *Rosmarinus eriocalyx* is 0.895g/cm.

The determination of the refractive index of *Rosmarinus eriocalyx* oil at 20°C is 1.464

pH

The hydrogen potential "pH" of *Rosmarinus eriocalyx* is 6.28

Identification of isolated strains

After the incubation of the dishes for the three strains, we identified:

Macroscopic identification

Staphylococcus aureus: fairly large colonies of about 1mm in diameter, round, regular, domed, smooth and shiny of the Smooth type. They are also cream, pigmented with a yellow color.

Escherichia coli: they are rounded, smooth colonies of a red or colorless color with regular edges 2 to 3mm in diameter.

Pseudomonas aeruginosa: these colonies are smooth, of average size of 1 to 2mm, with a domed central part and an irregular edge, shiny and mucous or even runny.

Microscopic identification

In the fresh state

Table 3: microscopic characteristics in the fresh state of the isolated strains

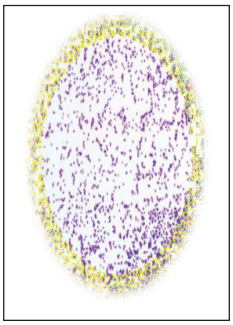
This table represents the microscopic characteristics of the different bacterial strains isolated

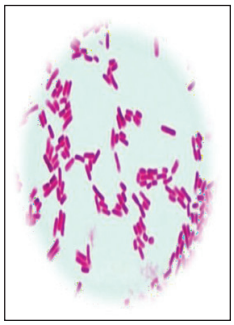
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
Form	Shell	Rod bacillus	Bacillus
Mobility	Motionless	Mobile	Very mobile
Mode of grouping	Motionless Bunch	Mono/diplobacillus	Isolated of grapes or diplobacillus

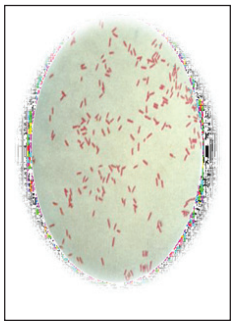
Table 4: Gram type of strains isolated

This table represents the gram type of each bacterial strain

Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
Gram typeGram-positive	Gram-negative	Gram-negative







Biochemical identification

Oxidase test

Staphylococcus aureus: absence of color change on the oxidase disc: oxidase negative

Escherichia coli: the absence of color on the oxidase disc: oxidase negative

Pseudomonas aeruginosa: presence of dark color on the disc: positive oxidase

Catalase test

Staphylococcus aureus: the appearance of gas bubbles on the drop of H_2O_2: positive catalase

Escherichia coli: presence of gas bubbles in the H_2O_2: positive catalase

Pseudomonas aeroginosa: the appearance of gas bubbles: positive catalase

Table 5: Catalase and Oxidase Biochemical test results

This table represents the biochemical characteristics

	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
Oxidase test	-	-	+
Catalase test	+	+	+

Results of the antibacterial activity of essential oils against pathogenic bacteria "aromatogram"

These results showed us the zones of inhibition around the discs soaked in essential oils for the purpose of evaluating the activity of these oils vis-à-vis the strains tested.

Rosmarinus eriocalyx

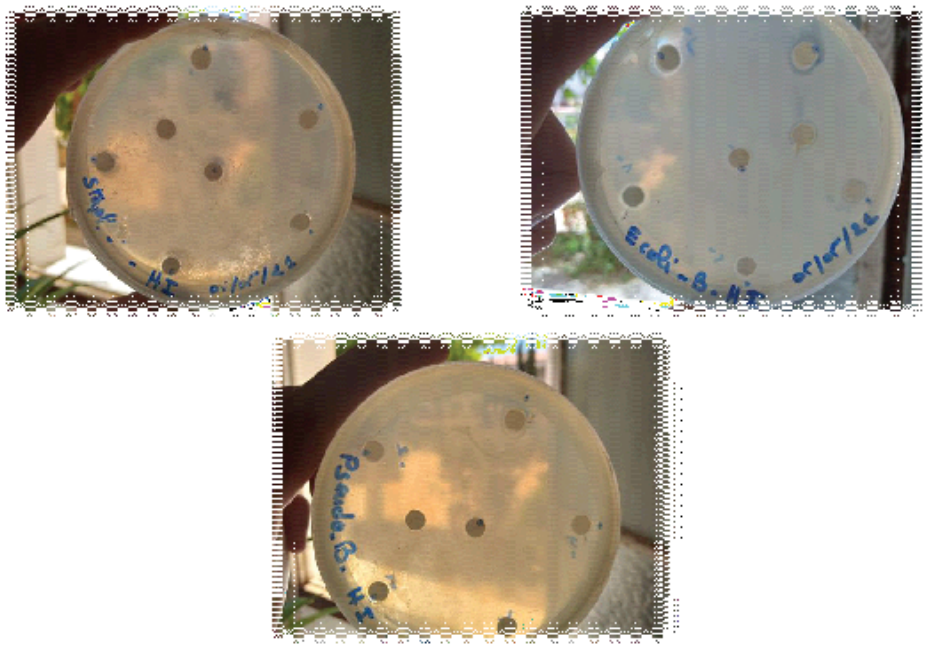


Fig. 1: Results of the effect of essential oil of *Rosmarinus eriocalyx* against pathogenic strains inoculated on MH medium at 37°C for 24 to 48 hours.

Rosmarinus officinalis

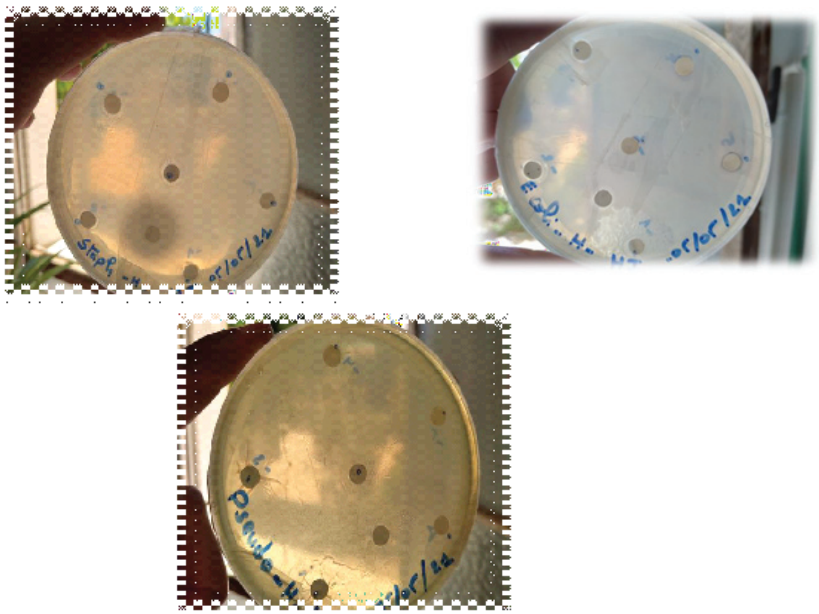


Fig. 2: Results of the effect of essential oil of *Rosmarinus officinalis* against pathogenic strains inoculated on MH medium at 37°C for 24 to 48 hours.

The results obtained from the aromatogram showed that *Staphylococcus aureus* and *Escherichia coli* were the most resistant bacteria to extracts of *Rosmarinus officinalis L* and *Rosmarinus eriocalyx* from both sources. Knowing that, *E.coli* has a strong resistance against the extract of *Rosmarinus eriocalyx* with a diameter of 2mm compared to the other extract of *Rosmarinus officinalis* with a diameter of 6 mm, on the other hand *Staphylococcus aureus* has a weak resistance to the extract of *Rosmarinus eriocalyx* with a diameter of 6mm compared to the other essential oil of *Rosmarinus officinalis* which represents a diameter of 3mm. On the other hand, the species of *Pseudomonas aeruginosa* marked a significant zone of inhibition of 19mm; therefore it has a high sensitivity compared to the essential oil of *Rosmarinus eriocalyx* on the other hand it has a lesser sensitivity vis-à-vis the extract of *Rosmarinus officinalis* by a diameter of 13 mm.

Previous studies on the essential oil of *Rosmarinus officinalis* reveal antimicrobial activity and indicate a similarity with the results obtained in the present work (Boutabia *et al*., 2016) compared to the strains studied; they noticed that bacteria (*Pseudomonas aeruginosa, E.coli*) represent inhibition diameters of (20, 2 mm) respectively while the results are not similar by the work presented by Bertella, (2019) they found that the *Staphylococcus aureus* bacteria is very sensitive vis-à-vis the two extracts tested by a diameter of 16.5 mm. Benbelaid *et al*. (2016) found that *Rosmarinus tournefortii* essential oil exhibited significant antibacterial activity against *Staphylococcus aureus* with an inhibition zone of 40 mm, so the results reported by Benbelaïd *et al.* (2016) and Bendeddouche *et al*. (2011) found that there is no antimicrobial activity of the essential oil of *Rosmarinus tournefortii* on *Pseudomonas aeruginosa* with an inhibition zone which was zero. While the results exhibited by Baratta *et al.*(1998) showed that rosemary essential oils destroyed *Pseudomonas aeruginosa*. Our results on the antibacterial activity of rosemary essential oils are consistent with those obtained by Lograda, (2014). The diameters of the zones of inhibition obtained do not exceed 20 mm, they are close to our results and reflect the modest or even weak antibacterial potential of the EO of *R. officinalis*. On the other hand, Djeddi and his colleagues (2007) reported the strong antibacterial activity of the essential oil of *R. officinalis* from the National Park of El Hamma (Algiers). On the other hand, according to Caillet and Lacroix, (2007*) S. aureus* and *E. coli* were moderately sensitive. Several studies have highlighted the high sensitivity of Gram (+) bacteria compared to Gram (-) bacteria (Falleh *et al*., 2008; Hayouni *et al*., 2007; Turkmen *et al*., 2007; Shan *et al*., 2007; Koné *et al*., 2004), This can be attributed to the difference in the outer layers of Gram (-) and Gram (+) bacteria. Gram (-) bacteria, apart from the cell membrane, have an additional layer the outer membrane, which consists of phospholipids, proteins and lipopolysaccharides, this membrane is impermeable to most molecules. Nevertheless, the presence of porins in this layer will allow the free diffusion of molecules with a molecular mass below 600 Da. However, inhibition of the growth of Gram (-) bacteria has been reported, particularly in combination with factors that may disturb cell integrity and/or membrane permeability, such as low pH values and increased NaCl concentrations (Georgantelis *et al*., 2007). The hypersensitivity of the *Staphylococcus aureus* strain ATCC can be explained by the probability of sensitivity of Gram (+) bacteria to external environmental changes, such as temperature, pH and natural extracts due to the absence of the outer membrane (Balentine *et al.*, 2006). Some studies reveal no selective antimicrobial activity against Gram (+) or Gram (–) bacteria (Guesmi and Boudabous, 2006). The resistance of the *Sterptocoque* sp strain can be attributed to the ability of the antibacterial agent to diffuse uniformly in the agar (Hayouni *et al*., 2007). The zone of inhibition increases considerably with the concentration of the extracts, which was also observed by Dordevic and his collaborators, (2007). Several classes of polyphenols such as phenolic acids, flavonoids and tannins serve as a defense mechanism in plants. against pathogenic micro-organisms, insects and herbivores (Falleh *et al*., 2008). Polyphenols, such as tannins and flavonoids like epigallocatechin, catechin, myricetin, quercetin, (Shan *et al*., 2007) and luteolin (Askun *et al*., 2009) are important antibacterial substances. Generally, all plants of the *Lamiaceae* family known for their phenolic compounds, have been

proven active against a variety of microorganisms (Gortzi *et al*., 2007). The antimicrobial activity of rosemary has also been attributed to rosmarinic acid, chlorogenic acid and caffeic acid (Tsai *et al*., 2007), carnosic acid and a few essential oil compounds, mainly borneol and camphor (Ramirez *et al*., 2007). The differences found can be attributed to several factors such as inherent factors (variety, ambient conditions, ecological factors, seasonal variations), extraction methods (Moreira *et al*., 2005; Sagdic and Ozcan 2003; Celiktas *et al*., 2007a, Turkmen *et al*., 2007). The antimicrobial action of HES takes place in three phases: Phase 1- The destruction of the bacterial wall by EO, causing an increase in permeability and then the loss of cell constituents. Phase 2- Acidification of the interior of the cell, blocking the production of cellular energy, and synthesis of structural components. Phase 3- Destruction of the genetic material, leading to the death of the bacteria. Several studies have shown that $Gram^-$ bacteria are supposed to be more resistant than $Gram^+$, this is due to structural differences in their outer membranes (Inouye *et al*., 2001; Lopez *et al*., 2005; Bozin *et al*., 2006), the penetration of active compounds present in HES is therefore different (Kheyer *et al*., 2014). In $Gram^-$ bacteria, the outer membrane constitutes an effective permeability barrier, rich in lipopolysaccharide whose negative surface charges prevent the diffusion of hydrophobic molecules (Nikaido, 2003), however, some low molecular weight phenolic compounds can adhere to these bacteria by attachment to membrane proteins and lipopolysaccharides using their functional groups and sneak up to the more vulnerable inner membrane (Dorman *et al*., 2000). In other words, hydrophobic compounds are capable of disrupting the plasma membrane and the outer membrane of $Gram^-$ bacteria by inducing its permeability and cell death (Wang *et al*., 2012).

CONCLUSION

In this work, we undertook a study on the antimicrobial activity of essential oils of *Rosmarinus officinalis* from a garden in Mezaourou and *Rosmarinus eriocalyx* is harvested at the level of a drill in Sidi Ali Benyoub of Sidi-Bel-Abbés. It can be seen that these two essential oils have a weak antibacterial activity against pathogenic strains (*Staphylococcus aureus* and *Escherichia coli*), on the other hand Pseudomonas aeruginosa was sensitive to the essential oil of *Rosmarinus eriocalyx* and *Rosmarinus officinalis*. This is why we can conclude that Rosemary has an antibacterial activity against pathogenic bacteria especially *Pseudomonas aeruginosa*, the results vary according to the type of plant material, its region and the dilutions of which they can present another effect, against these tested bacteria . This leads us to say that the essential oil of Rosemary could constitute a good basis of treatment for various problems due to the bacterial strain of *Pseudomonas aeruginosa*. All of these results obtained constitute only a first step in the search for biologically active substances of natural origin. A chemical analysis is desirable to obtain a more in-depth view of the qualitative and quantitative composition of these oils studied in order to highlight the therapeutic effect of this medicinal plant *Rosmarinus officinalis L* Finally, the results obtained on the antimicrobial activity made it possible to justify the traditional use of plant extracts from two plants chosen at the level of our region.

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