GSC Biological and Pharmaceutical Sciences, 2020, 12(01), 181-188

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(Research Article)

Evaluation of the antibacterial activity of three essential oils extracted from plants used in traditional medicine in Algeria (*Salvia officinalis* L, *Melissa officinalis* L and *Origanum vulgare* L)

Derradji Leila [1,*], Saidi Ouided [1] and Hadef Youcef [2]

[1] *Laboratory of Pharmacognosy, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar-Annaba University, Algeria.*
[2] *Laboratory of Development and Control of Pharmaceutical Preparations, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar-Annaba University, Algeria.*

Publication history**:** Received on 14 June 2020; revised on 06 July 2020; accepted on 08 July 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.12.1.0183

Abstract

This study consists in highlighting the antibacterial activity of three essential oils extracted from aromatic plants used in traditional medicine in Algeria which are: *Salvia officinalis* L, *Melissa officinalis* L and *Origanum vulgare* L, in order to demonstrate the therapeutic value, we evaluated their antibacterial activity using the antibiogram method. We previously extracted the essential oil from the flowering stems and dried leaves. This is done using a standard device. To mount the antibiogram, we used pathogenic germs resistant to many antibiotics: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* carbapénemase positif (KPC+), *Klebsiella pneumoniae* carbapénemase négatif (KPC-), *Enterobacter cloacea, Serratia marcescens*, *Acinétobacter baumanii, Pseudomonas aeruginosa* ATCC 27853, *Citrobacter freundi.* The results that we have obtained indicate that the three raw and diluted essential oils exhibit significant antibacterial activity on pathogenic strains which are all very sensitive to raw and diluted essential oils. The three essential oils studied are precious natural bioactive substances that can be used to fight against infectious diseases.

Keywords: Essential oils; Antibacterial activity; Pathogenic germs; Multidrug-resistant; Antibiogram

1. Introduction

Phytotherapy continue to be a popular healthcare choice with the general public not only for health maintenance and wellbeing, minor ailments (e.g. coughs and colds), chronic conditions (e.g. back pain) and serious chronic diseases (e.g. asthma, cancer, depression, diabetes) [1]. *Salvia officinalis* is used for its tonic, choleretic and antiperspirant properties. *Melissa officinalis* is used as stomachics and antispasmodics. *Origanum vulgare* is used as a stomachic and antispasmodic [2]. For our study we have selected these spontaneous species used in traditional medicine in Algeria. These three species have essential oils rich in citral (*Melissa officinalis*), carvacrol (*Salvia officinalis*) and thyone (*Origanum vulgare*) [3]. The extraction of essential oils is carried out according to a standardized process, by the hydrodistillation method [4]. The purpose of our study is to assess the antimicrobial activity of the three essential oils against multidrug-resistant pathogens. The pathogenic strains we have chosen come from the microbiology laboratory at the University Hospital Center (C.H.U) in Annaba (a city in eastern Algeria). These strains are made up of: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* carbapénemase positif (KPC+), *Klebsiella pneumoniae* carbapénemase négatif (KPC −), *Enterobacter cloacea, Serratia marcescens*, *Acinétobacter baumanii, Pseudomonas aeruginosa* ATCC 27853 and *Citrobacter freundi.* The method of studying the antibacterial

* Corresponding author: Derradji Leila

activity that we have recommended is that of the antibiogram. This method makes it possible to evaluate the sensitivity of a bacterium with regard to antibiotics [5]. The results of the aromatogram show us that these three raw and diluted essential oils exhibit significant antibacterial activity on the pathogenic strains studied.

2. Material and methods

2.1. Vegetal material and extraction of essential oil

The flowering stems of *Salvia officinalis*, *Melissa officinalis* and *Origanum vulgare* were harvested in the Annaba region (northeastern Algeria). The extraction of the essential oils is carried out by the method of steam distillation, which uses a Likens Nickerson type extraction apparatus, from the selected flowering stems and dried. 100 grams of dried flower stems are introduced into a balloon distilled water impregnated until quarters.

2.2. Selection of bacterial strains

Our choice fell on non-demanding bacterial strains often isolated in hospital environments and often incriminated in infections in humans. These bacterial strains are collected in hospital, obtained at the central laboratory of Microbiology C.H.U Annaba. These strains are resistant and sensitive: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* carbapénemase positif (KPC+), *Klebsiella pneumoniae carbapénemase négatif* (KPC −), *Enterobacter cloacea*, *Serratia marcescens*, *Acinétobacter baumanii*, *Pseudomonas aeruginosa* ATCC 27853 and *Citrobacter freundi*.

2.3. Reactivation of bacterial strains

The bacterial strains are reactivated by subculture from the preservation medium on a solid and nonselective culture medium (nutrient agar) previously melted, poured into petri dishes 4 cm thick (these cans are cooled and dried before to be sown) for 24 hours of incubation in the oven. The petri dishes are then removed and the raw and young cultures previously prepared will be used for the preparation of bacterial suspensions**.**

2.4. Preparation of bacterial suspensions

The method of preparation of the inoculum is that recommended by the SFM (French Society of Microbiology) which consists of preparing, from an 18 to 24 hour culture of the bacterium studied on the agar medium. A suspension in saline solution (0.9% NaCl) equivalent to the standard Mac Farland 0.5 (~106 CFU / ml can be obtained by measuring the optical density (OD) ranging from 0.08 to 0.1 read at 625 nm.

2.5. Environment of culture

Mueller-Hinton agar prepared as follows: We introduced 35 g of agarose powder into one liter of water which was heated until completely dissolved. Then we sterilized the solution obtained by autoclaving at 121 ° C. for 15 minutes. We then poured the solution into Petri dishes 4 cm thick and cooled to 45˚C.

2.6. Seeding proper

The petri dishes are seeded from the bacterial suspensions using the sterile swabs. The swab is impregnated with the bacterial suspension, wrung out against the inner wall of the test tube. Seeding is by tight streaks, from top to bottom. The operation is repeated two to three times, turning the box 60˚ each time. The swab should be passed on the periphery of the agar plate.

2.7. Choice of solvent for diluting essential oils

The aprotic organic solvent (DMSO) is chosen for its innocuity against bacteria (lacking antibacterial activity). In the presence of the drug, it does not give interference (since the desired activity is that of the drug).

2.8. Preparation of the dilutions of the essential oils

A range of five dilutions has been prepared and the raw essential oil.

Going from ½, ¼, ⅛, $^{1}/_{16}$ from the raw essential oil by adding 0.5 ml of DMSO in the five tubes, 1ml of pure oil in one tube then take 0.5 ml of the tube of the Raw oil and pour it into the second tube and each time we take 0.5 ml of the previous tube and add to the next tube to the last tube where we throw the last 0, 5 ml so each tube contains 0.5 ml.

2.9. Disk distribution

Sterile discs of blotting paper 6 millimeters in diameter are grasped with sterile forceps and impregnated with a small amount of our essential oil previously prepared at different concentrations. The discs are applied to previously seeded culture media. A maximum of 6 disks is arranged per box of 8.5 centimeters in diameter, with a gap of 24 millimeters between the disks.

2.10. Reading

After 24 hours of incubation, the dishes are removed and the results are read by measuring the diameter, in millimeters, of the possible inhibition area around the discs.

3. Results and discussion

By comparing the results of the antibiogram with the critical values, we were able to classify the bacteria in the following categories: sensitive, intermediate and resistant. We measured in millimeters the diameter of the inhibition halo or clear area surrounding the discs. This clear area represents the destruction of pathogenic germs on which we have deposited our essential oils. This allows us to assess the degree of antibacterial activity of the three essential oils (Table 1).

Table 1 Sensitivity and degree of antibacterial activity according to the inhibition diameter

Inhibition diameter	**< to 8mm**	**From> 8 to 14 mm**	**From> 14 mm to 20 mm**	**≥ to 20 mm**
Sensitivity of the germ	resistant	Limited sensitivity	Average sensitivity	Very sensitive
Degrees of activity	-	+	++	+++

The antibiogram obtained from the essential oil of *Salvia officinalis* shows us that the majority of the germs tested are sensitive to the essential oil but their sensitivity differs from one germ to another but other germs are resistant. We give the case of *Staphylococcus aureus* ATCC 43300 and *Escherichia coli* ATCC 25922 which are very sensitive to raw oil and oil diluted to ½. We also give the case of *Enterobacter cloacea* which is moderately sensitive to raw oil; but it is resistant to diluted oil. *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae*, (KPC+) and *Klebsiella pneumoniae* (KPC −) (Table 2).

Table 2 Result of the antibiogram of the essential oil of *Salvia officinalis* L

Microbial strains	***Salvia officinalis* L**				
	Degrees of dilution				
	Raw E. o	**1/2**	**1/4**	**1/8**	**1/16**
Enterococcus faecalis ATCC 29212	**+**	**-**	**-**	**-**	**-**
Staphylococcus aureus ATCC 43300	**+++**	**+++**	**-**	**-**	**-**
Escherichia coli ATCC 25922	**+++**	**++**	**+**	**+**	**-**
Pseudomonas aeruginosa ATCC 27853	**+**	**-**	**-**	**-**	**-**
Klebsiella pneumoniae (KPC+)	**+**	**+**	**-**	**-**	**-**
Klebsiella pneumoniae (KPC −)	**+**	**-**	**-**	**-**	**-**
Enterobacter cloacea	**++**	**-**	**-**	**-**	**-**
Serratia marcescens	**-**	**-**	**-**	**-**	**-**
Acinétobacter baumanii	**-**	**-**	**-**	**-**	**-**
Citrobacter freundii	**-**	**-**	**-**	**-**	**-**

Strains sensitive to raw essential oil and diluted to ½ of *Melissa officinalis* are *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* (KPC+), *Klebsiella pneumoniae* (KPC −) and *Citrobacter freundii.* Strains resistant to raw and diluted oil are *Serratia marcescens* and *Acinétobacter baumanii*. All the strains tested are not very sensitive to resistant from the ¼ dilution (Table 3).

Table 3 Result of the antibiogram of the essential oil of Melissa officinalis L

Microbial strains	***Melissa officinalis* L**				
	Degrees of dilution				
	Raw E.o	**1/2**	**1/4**	**1/8**	**1/16**
Enterococcus faecalis ATCC 29212	+	-	-	-	-
Staphylococcus aureus ATCC 43300	+++	++	-	-	-
Escherichia coli ATCC 25922	+++	++	+	+	-
Pseudomonas aeruginosa ATCC 27853	+	+	-	-	-
Klebsiella pneumoniae (KPC+)	++	-	-	-	-
Klebsiella pneumoniae (KPC −)	++	-	-	-	-
Enterobacter cloacea	+	-	-	-	-
Serratia marcescens	-	-	-	-	-
Acinétobacter baumanii	-	-	-	-	-
Citrobacter freundii	+++	+++	++	++	+

The majority of the strains tested are very sensitive to raw essential oil from *Origanum vulgare* and moderately sensitive to diluted oil. However, strains like *Serratia marcescens, Acinétobacter baumanii* and Pseudomonas aeruginosa ATCC 27853 are resistant to raw and diluted oil (Table 4).

Table 4 Result of the antibiogram of the essential oil of *Origanum vulgare* L

Microbial strains	***Origanum vulgare* L**				
	Degrees of dilution				
	Raw E.o	**1/2**	**1/4**	**1/8**	**1/16**
Enterococcus faecalis ATCC 29212	+++	+++	+++	++	++
Staphylococcus aureus ATCC 43300	+++	+++	+++	+++	++
Escherichia coli ATCC 25922	+++	+++	++	++	+
Pseudomonas aeruginosa ATCC 27853	+	-	-	-	-
Klebsiella pneumoniae (KPC+)	+++	+++	+++	++	++
Klebsiella pneumoniae (KPC −)	+++	+++	++	++	-
Enterobacter cloacea	+++	+++	+++	+++	++
Serratia marcescens	-	-	-	-	-
Acinétobacter baumanii	-	-	-	-	-
Citrobacter freundii	+++	+++	+++	+++	++

The three raw essential oils are very active against *Staphylococcus aureus* ATCC 43300 and *Escherichia coli* ATCC 25922. The areas of inhibition observed around these germs reach a maximum of 38 mm in diameter. The raw essential oil of *Origanum vulgare* is very active against *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* (KPC+), *Klebsiella*

pneumoniae (KPC −), *Serratia marcescens, Acenitobacter baumanii*, *Pseudomonas aeruginosa* and *Citrobacter freundii.* The areas of inhibition observed around these germs are large with diameters ranging from 35 mm to 44.8 mm. But the raw essential oils of *Salvia officinalis* and *Melissa officinalis* are weakly active against these germs since the areas of inhibition observed around these strains are insignificant with diameters ranging from 8.5 mm to 10.5 mm (Figure 1).

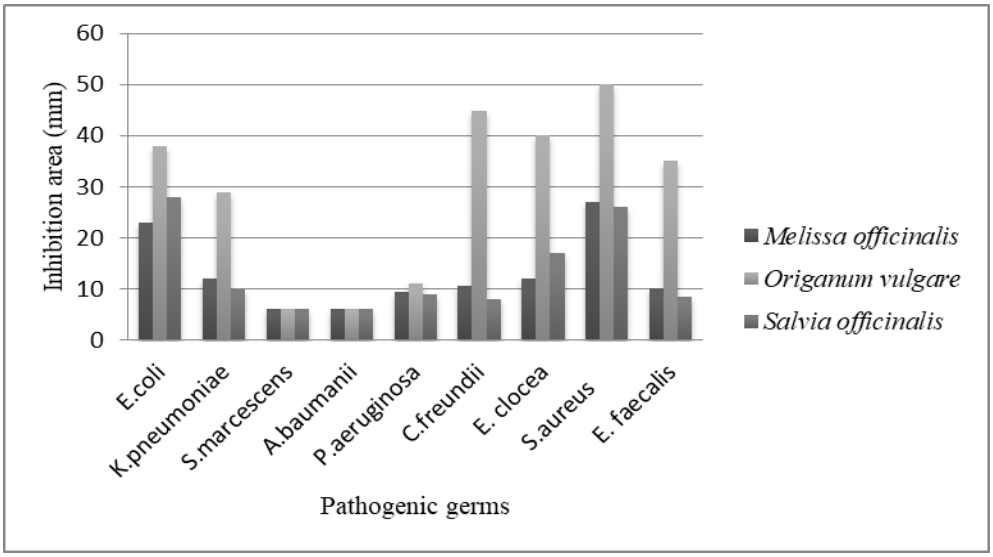

Figure 1 Antibacterial activity of raw three essential oil

The essential oil diluted to ½ of *Origanum vulgare* is very active against most of the germs tested. The area of inhibition has a diameter which arrives at 38 mm. It is weakly active against *Serratia marcescens Acinétobacter baumanii* and *Citrobacter freundii.* The essential oils of *Salvia officinalis* and *Melissa officinalis* diluted to ½, lose their activity on most strains. But remain weakly active towards *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 43300 (Figure 2).

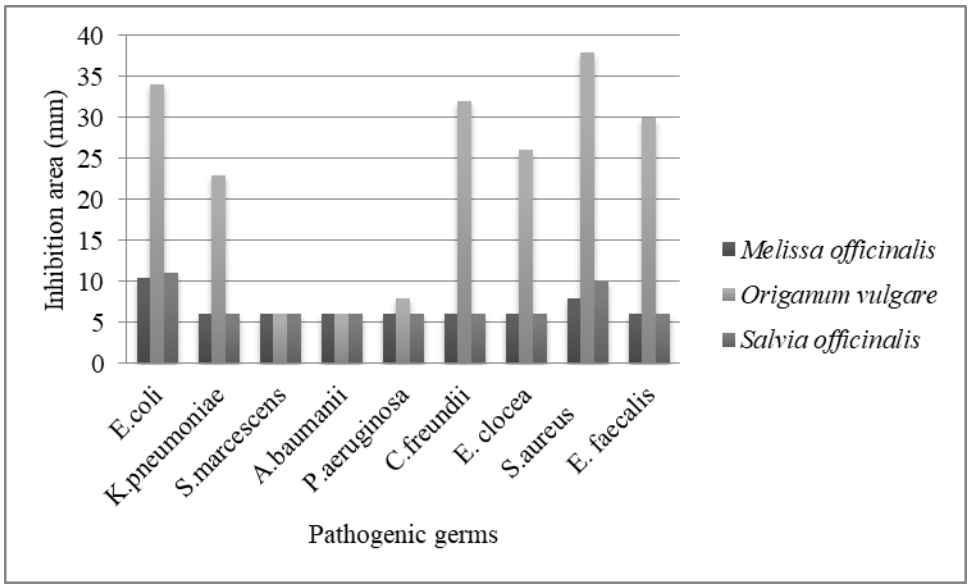

Figure 2 Antibacterial activity of the three essential oils after dilution with ½

The essential oil of *Origanum vulgare* diluted to ¼ is very active against most of the strains tested. The inhibition area arrives at a diameter of 30 mm. On the other hand, it is inactive against *Serratia marcescens*, *Acinétobacter baumanii* and *Pseudomonas aeruginosa* ATCC 27853. The essential oils of *Salvia officinalis* and *Melissa officinalis* diluted to ¼ are inactive against all the strains tested (Figure 3).

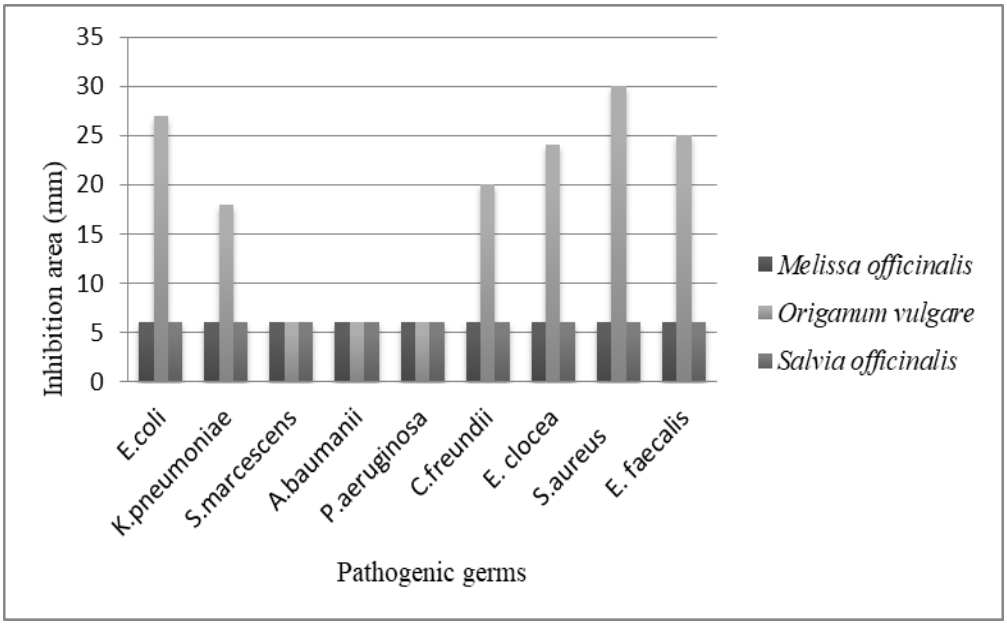

Figure 3 Antibacterial activity of the three essential oils after dilution with ¼

The essential oil of *Origanum vulgare* diluted in ⅛ is very active against *Escherichia coli* ATCC 25922 with a area of inhibition of 29 mm. However, it is not very active to inactive against other germs. The essential oils of *Salvia officinalis* and *Melissa officinalis* diluted in ⅛ are inactive against all the strains tested (Figure 4).

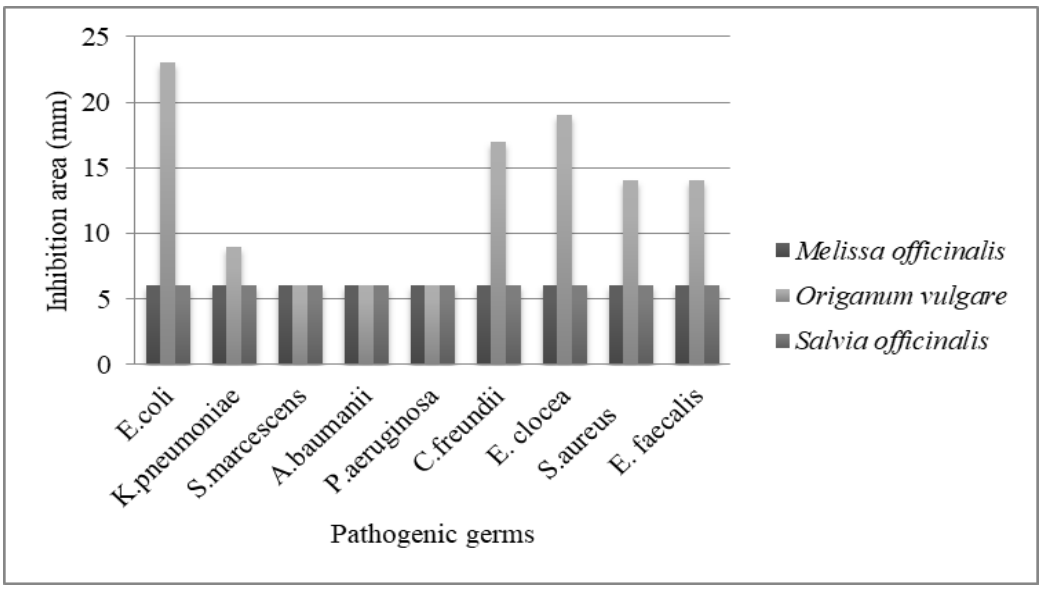

Figure 4 Antibacterial activity of the three essential oils after dilution with 1/8

The essential oil of *Origanum vulgare* diluted to $^{1}/_{16}$ is weakly active against *Escherichia coli* ATCC 25922 and *Enterobacter cloacea* with a maximum inhibition area of 14 mm diameter. On the other hand, it is inactive against other germs, as is the case for the essential oils of *Salvia officinalis* and *Melissa officinalis* (Figure 5).

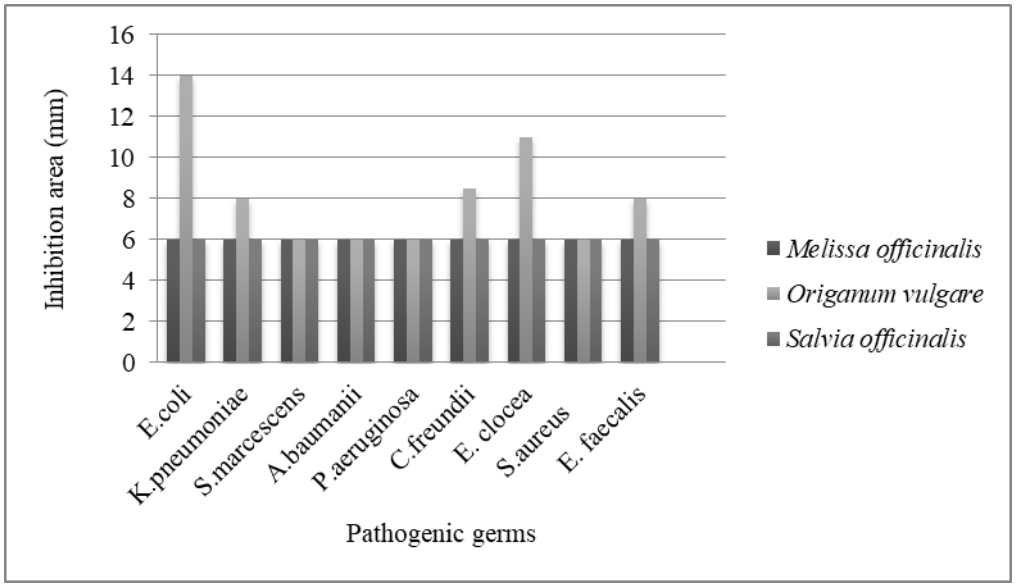

Figure 5 Antibacterial activity of the three essential oils after dilution with 1/1**6**

Marino and Bersani [6] obtained a similar result for the essential oil of Salvia officinalis which showed inhibition against the bacteria tested at low concentrations, the most sensitive was Escherichia coli and that Salvia officinalis has limited activity against these germs ». « Mimica-Dukic and Bozin [7] obtained a different result for the essential oil of *Melissa officinalis* which has an important activity against *Pseudomonas aeruginosa* and *Escherichia coli* which is particularly sensitive to this essential oil.

4. Conclusion

We have demonstrated that three essential oils obtained from aromatic plants have significant antibacterial activity on multi-resistant pathogenic germs. These tested germs are very sensitive to these three raw essential oils but their sensitivity is variable when these oils are diluted.

Compliance with ethical standards

Acknowledgments

We thank the personal manager of the microbiology laboratory for allowing us to perform the antibiogram tests and we thank the staff for helping us in handling the tests performed.*Disclosure of conflict of interest*

Declaration of conflict of interest

All authors declare that we participated in the design, execution and analysis of the document and that I approve the final version. In addition, there is no conflict of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

References

[1] Barnes J, Anderson LA and Phillipson JD. (2007). Herbal medicines. Third edition, organisation of the royal pharmaceutical society, Grande Bretagne, 9.

[2] Teuscher E, Anton R and Lobstein A. (2005). Les plantes aromatiques, épices, aromates, condiments et huiles essentielles. Lavoisier, éditions médicales internationales, Paris, 301-445.

[3] Bruneton J. (1993). Pharmacognosie, Phytochimie, Plantes médicinales, quatrième édition. Lavoisier, 580.

[4] Paris R and Hurabielle M. (1981). Abrégé de matière médicale (pharmacognosie), Généralités-monographies. Second edition, Masson, Paris, 229-231.

[5] Delarras C. (2014). Pratique en microbiologie de laboratoire : Recherche de bactéries et de levures-moisissures. Paris: Lavoisier, Paris.

[6] Marino M, Bersani C and Comi, G. (2001). Impendance me asurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. International Journal of Food Microbiology, 187-195.

[7] Mimica-Dukic N, Bozin B, Sokovic M and Simin N. (2004). Antimicrobial and Antioxidant Activities of Melissa officinalis L (Lamiaceae) Essential oil. Journal of agriculture, food chemistry, 2485 –2489.

How to cite this article

Derradji L, Saidi O and Hadef Y. (2020). Evaluation of the antibacterial activity of three essential oils extracted from plants used in traditional medicine in Algeria (*Salvia officinalis* L, *Melissa officinalis* L and *Origanum vulgare* L). GSC Biological and Pharmaceutical Sciences, 12(1), 181-188.