ejpmr, 2017,4(4), 613-616

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF THE BACTERIOSTATIC EFFECT OF THE ESSENTIAL OIL OF *THYMUS HIRTUS* OF THE ALGERIAN SOUTH ON RESISTANT PATHOGENIC GERMS

Leila Derradji*[1] and Youssef Hadef[2]

[1]Laboratory of Pharmacognosy, Faculty of Medicine, Badji Mokhtar-Annaba University, Algeria.
[2]Laboratory of Development and control of the pharmaceutical preparations hospital workers, Faculty of Medicine, Badji Mokhtar-Annaba University, Algeria.

***Corresponding Author: Dr. Leila Derradji**

Laboratory of Pharmacognosy, Faculty of Medicine, Badji Mokhtar-Annaba University, Algeria.

Article Received on 17/02/2017 **Article Revised on 11/03/2017** **Article Accepted on 31/03/2017**

ABSTRACT

The flowery stalks dried by hirtus Thymus are collected in the region of El-Oued (the South of Algeria). The species is spontaneous, used in the traditional medicine in Algeria. To make the evaluation of the bacteriostatic effect of the essential oil, we used the method of the aromatogramme. The selected bacterial strains are collected in a hospital environment (central laboratory of Microbiology C.H.U Annaba)*: Klebsiella pneumoniae, Proteus vulgaris, Esherichia coli, Pseudomonas aeroginosa* and *Serratia marcescenc*. The results of the aromatogramme show us that the essential oil burned is active on the selected origins which sensitive differs according to the degrees of its dilutions. Indeed important zone of inhibition are observed with diameters affecting 36 mm. The species *Thymus hirtus* the region of the South of Algeria establishes a source of bioactive natural substances. We have to exploit this source because she could be a solution to the treatment of the infectious pathologies.

KEYWORDS: essential oil, sensibility, bacteriostatic germs, resistance, aromatogram.

INTRODUCTION

The herbal medicine contributes to the good balance of our body by stimulating the natural defenses.[1] Plants contribute to cure health problems[2]. Most of the aromatic botanical species possessing therapeutic virtues for the active molecules which they possess.[3] The kind Thymus, presents recognized therapeutic interests[4,5]. For our study we selected a spontaneous species used in the traditional medicine in Algeria: hirtus thymus of the Algerian South. Just after the harvest, we proceeded to the process of dehydration, realized by methods suited for the inhibition of the enzymatic reactions susceptible to destroy active ingredients[6]. Our study has for objective of evaluation the bacteriostatic effect of the essential oil of this species. We used the method of distribution of records, aromatogram. The selected bacterial strains are collected in a hospital environment (central laboratory of Microbiology C.H.U Annaba): *Esherichia coli, Staphylococcus aureus, Pseudomonas aerogenosa, Acinetobacter baumannii, Klebsiella pneumonia, Proteus vulgaris, Serratia marscecence*. The extraction of the essential oil is realized by training in the steam or hydro- distillation[7]. The results of the aromatogramme show us that the essential oil burned is active on the selected origins which sensitive differs according to the degrees of its dilutions. Indeed important zones of inhibition are observed with diameters affecting 38 mm. The species *Thymus hirtus* the region of the South of Algeria establishes a source of bioactive natural substances. We have to exploit this source because she could be a solution to the treatment of the infectious pathologies.

1. MATERIAL AND METHOD

1. 1. Vegetal material and extraction of the essential oil

The harvest of the flowery stalks of Thymus hirtus was made in the region of El-Oued (the South of Algeria). These samples are dried in the shade. The preservation is made in Kraft paper bags shielded from the light and from the humidity. The extraction of essential oil is made by the method of hydro distillation, by training in the vapor which uses a device of extraction of type Likens Nickerson, from the selected and dried flowery stalks. 100 grams of dried flowery stalks are introduced into a ball(balloon) of the distilled water filled until quarters. The set (flowery stalks and distilled water) is carried in the hanging boiling two at three o'clock. Vapors loaded with essential oil cross a cooler, then part by difference of density. The obtained essential oil is put in hermetic coloured glass opaque flasks and preserved in the refrigerator shielded from the heat and from the light to avoid its change.

1.4. Evaluation de l'activité antibactérienne de l'huile essentielle

It is determined by the aromatogramme in solid environment using the distribution of disks agar. It consists in putting depositing steril disks of blotting paper soaked(filled) with essential oil of drug on the surface of agar boxes (solid environment of MULLER-HINTON) carrier of the studied bacterium.

1.4.1. Selection of bacterial strains

- **Choice of bacterial strains**

Our choice concerned to not demanding bacterial strains frequently isolated in circles hospitable and often incriminated in infections in man. These bacterial strains are collected in a hospital environment, obtained in the central laboratory of Microbiology C.H.U Annaba[11]. These stumps are resistant and sensitive: *Esherichia coli, Staphylococcus aureus, Pseudomonas aerogenosa, Acinetobacter baumanii, Klebsiella pneumonia, Serratia marscecence*.

- **Reactivation of bacterial strains**

Bacterial strains are reactivated by transplanting from the environment of preservation on a not selective and solid environment of culture agar nourishing) beforehand melted, poured into boxes of molded on 4cm of thickness (these boxes are cooled and dried before being sowed) for 24heures of incubation in the steam room. The boxes of molded are then removed and the young cultures beforehand prepared are going to be of use to the preparation of the bacterial suspensions.

- **Preparation of the bacterial suspensions**

The method of preparation of the inoculum is the one recommended by the SFM (Frenchman company of Microbiology) which consists in preparing, from a culture from 18 to 24 hours of the bacterium studied on the environment agar. A suspension in salt solution (0,9 % Na Cl) amounts to the standard MACFARLAND 0,5 (106 UFC/ml can be obtained by the measure of the optical density (D.O) going from 0,08 to 0,1 read in 625nm.

1.4. 2. Sowing

- **Environment of culture**

We introduced 35 g of powder of agar into a liter of water which we warmed until its complete dissolution. Then we proceeded to a sterilization of the obtained solution to the autoclave in 121°C during 15 minutes. We poured, then, the solution into boxes of Molded on 4cm of thickness and cooled in 45°C. Agar is dried before employment. The boxes of molded are sowed from the bacterial suspensions by means of the sterile swabs. The swab is soaked with the bacterial suspension, pressed against the internal wall of the test tube.

- The sowing is made by one streak squeezed of high below
- The operation is repeated two-three times, by turning the box of 60 ° every time
- The swab must have passed on the periphery of the agar.

1.4. 3. Preparation of the dilutions of essential oil

- **Choice of the solvent of dilution**

The aprotic solvent (DMSO) is chosen as its Harmlessness towards bacteria (devoid of antibacterial activity) .En presence of the drug, he does not give interference (because sought activity is the one some essential oil).

- **Preparation of the dilutions of the essential oil**

Going of ½, ¼, 1/16th, 1/32nd from the raw essential oil (tab 1), by addition of 0,5ml of DMSO in five tubes, 1ml some oil pure in a tube may take 0,5ml of the tube of the raw oil and pay him into the second tube and every time we take 0,5 ml of the previous tube and thus add them to the tube following up to the last tube where we throw the last ones 0,5 ml every tube contains 0,5ml.

1.4. 4. Distribution of disks

The sterile disks of blotting paper 6 millimeters in diameter, are seized in the sterile crowbar and soaked with a small quantity of our the essential oil beforehand prepared for various concentrations.
Disks are applied to circles of previously sowed cultures.
A maximum of 6 disks is arranged by box 8,5 centimeters in diameter, with a space of 24 millimeters between disks..

1.4. 5.Reading

After 24 hours of incubation, boxes are removed and the reading of the results(profits) is made by the measure of the diameter, in millimeter, of the zone of possible inhibition around records(disks) by means of a metallic caliper, outside of the closed boxes.

Table1: proceeded to the various dilutions

Degree of dilution	He	DMSO
½	0,5ml Hes brute	0,5ml
¼	0,5ml Hes au 1⁄2	0,5ml
⅛	0,5ml Hes au 1⁄4	0,5ml
1⁄16	0,5ml Hes au 1⁄8	0,5ml
1⁄32	0,5ml Hes au 1⁄16	0,5ml

2. RESULTS AND DISCUSSION

2.1. Yield of essential oil

Having extracted the essential oil of the plant by training in the steam, the extract is got back in a smoked glass, hermetically closed to avoid any oxidation and change of the essential oil. Stalks decorated with flowers after extraction by the method of hydrodistillation give a yield of 11cm on extract, what corresponds to 02 ml of essential oil for a taking of 100 gr.

2. 2. The aromatogram

By comparing the results of the aromatogram with the critical values, we were able to classify bacteria in the sensitive, intermediate or resistant categories. We measured in millimeter the diameter of the zone of inhibition surrounding disks or clear zone which shows the destruction of pathogenic germs what allows us to estimate the degree of the bactericidal activity of the essential oil (tab. 2).

Table 2: Sensibility and degree of bacteriostatic activity according to the diameter of inhibition

Diameter of inhibition	Inferior in 8mm	From 8 to 14 mm	From 14mm to 20mm	Superior or equal to 20 mm
Sensibilité du germe	résistante	Sensibilité limité	Sensibilité moyenne	Très sensible
Degrés d'activité	-	+	++	+++

- ***Proteus vulgaris***
- The zone of inhibition of the raw oil and the dilutions 1/2,1/4 is of 30mm of diameter and respectively de12mm, 10mm, 10mm for the dilutions 1/8,1/16,1/32 (figure 1)..
- ***Klebsiella pneumoniae***
- The zone of inhibition of the raw essential oil and the dilution 1/2 is 26 mm in diameter. The dilutions 1/4, 1/8, 1/16 give a zone of 20 mm inhibition diameter and of 13mm for the dilution 1/32 (figure 2).
- ***Escherichia coli***
- The raw oil and the dilution 1/2 give a zone of inhibition of 30mm diameter. The dilution 1/4 gives a zone of inhibition of 20mm. Diameter. For the dilutions 1/8,1/16, 1/32, the diameter of the zone is respectively of 12mm, 11mm, 10mm.
- ***Staphylococcus aureus***
- The raw oil and all the dilutions give a zone of inhibition of 30mm diameter
- ***Pseudomonas aeroginosa***
- The raw oil and the dilutions 1/2,1/4 give a zone of inhibition respectively of 11mm,
- 9mm, 8mm. The zone of inhibition for the dilutions 1/8, 1/16,1/32 presents one
- Diameter of 6mm.
- ***Acinetobacter baumanii***
- The raw oil and the dilutions 1/2, 1/4, 1/8 give a zone of inhibition of 30mm of diameter. The zone of inhibition of the dilutions 1/16, 1/32 is of 15mm, 10mm diameter (figure 3).
- ***Serratia marcescenc***
- The zone of inhibition of the raw oil and the dilution 1/2 is of 18mm, 12mm of diameter and that of the dilutions 1/4, 1/8,1/16,1/132 is of 10mm of diameter (figure 4).

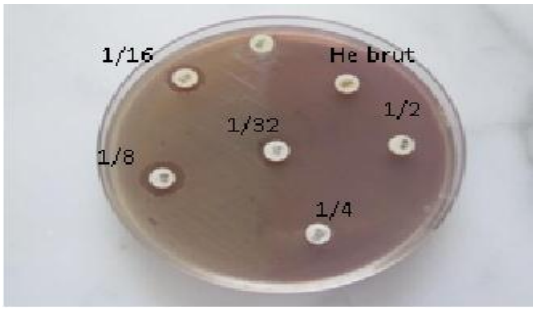

Figure 1: ***Proteus vulgaris***

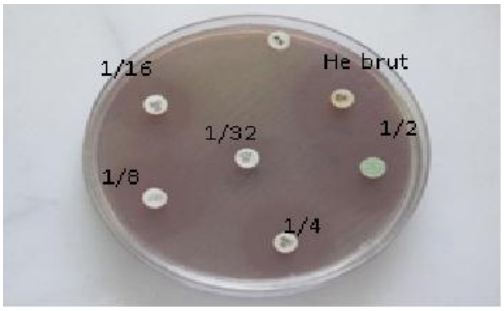

Figure 2: ***Klebsiella pneumonia***

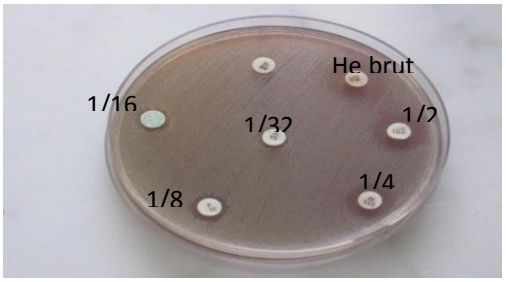

Figure 3: ***Acinetobacter baumanii***

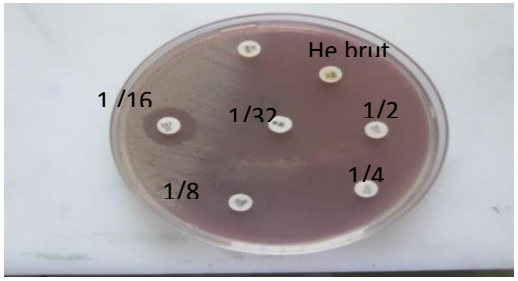

Figure 4: ***Serratia marcescenc***

The zones of inhibitions appearing with the raw oil are the dilutions on tested origins vary 6 in 30mm. The classification in touch with the specter of antibacterial activity (picture 1), indicates the degrees of sensibility of each of the tested bacterial strains, so:

- *Klebsiella pneumoniae:* Is very sensitive for the raw oil and all the dilutions 1/2,1/4,1/8,1/16 and presents a sensibility limited for the dilution 1/32.
- *Proteus vulgaris*: is very sensitive for the raw oil and the dilutions 1/2,1/4,1/8,1/16 and present a sensibility limited for the dilution 1/32.
- *Pseudomonas aeroginosa:* Has a sensibility limited for the raw oil and the dilutions 1/2,1/4 and it is resistant for the dilutions 1/8,1/16,1/32.
- *Serratia marcescenc*: a sensibility limited for all the dilutions is of an average sensibility for the raw oil and.
- *Acinetobacter baumanii:* Is very sensitive for the raw(raw) oil and all the dilutions
- *Staphylococcus aureus*: is very sensitive for the raw(raw) oil and all the dilutions
- *Esherichia coli*: is very sensitive for the raw(raw) oil and the dilutions 1/2,1/4,1/8 and present an average sensibility for the dilution 1/16 and a sensibility limited for 1/32.

CONCLUSION

Stalks and flowery leading experts of *Thymus hirtus* of the Algerian South are used in the Algerian traditional medicine. This aromatic supplied species a rich essential oil there carvacrol, polyphenols the bacteriostatic activity of which for certain pathogenic germs is proved. We tested the raw essential oil and diluted with this spontaneous species on pathogenic stumps. the sensibility of these germs, results of our study, show that the raw essential oil of this species as well as its dilutions present one activate bacteriostatic that it is useful to exploit(run) to fight against the resistant germs in antibiotics.

RÉFÉRENCES

1. Debelmas A. M., delaveau. M. Guide des plantes dangereuses. Ed: Maloine. Paris. 1983.
2. Barnes J., Anderson A., phillipson L., David J. Herbal medicine. Ed: pharmaceutical press. Grande Bretagne. 2002.
3. Bremness L. Les plantes aromatiques et médicinales. Ed: Larousse Bordas. Paris. 1996.
4. Ryrie C. Jardin de tradition, Ed : Nathan. 2000.
5. Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales ; Technique et documentation. Ed : LAVOISIER. 1993.
6. Mahmoudi Y. La thérapeutique par les plantes communes en Algerie. Ed : Palais. 1987.
7. Purchon N. La Bible de l'aromathérapie. Ed MARABOUT. 2001.
8. Paris .M. Harabelle. M. Abrégé de matière médicale ; tome I. Ed : Masson. 1981.
9. Iserin P. Encyclopédie des plantes médicinales. 2000.
10. Ozenda P. Les végétaux. Organisation et diversité biologique, Ed Dunod. 2000.
11. Laboratoire de microbiologie, Faculté de Médecine d'Annaba (avril2011).