

Antibacterial Activity of the Extracts Obtained from *Rosmarinus officinalis*, growin in wild in Bechar region, south west of Algeria

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Abstract – The aim of this work is to determine the antibacterial activity of *Rosmarinus officinalis* L against *Escherichia coli* and *Klebsiella pneumoniae* and to identify the specific plant fraction responsible for the antimicrobial activity. The plant was extracted with ethanol to yield the crude extract which was further sub fractionated by different solvents to obtain the petroleum ether, the dichloromethane, the ethyl acetate, and the aqueous fractions. The Minimum Inhibitory Concentrations (MIC) was determined using broth microdilution. The MICs ranged between 20 and 90 µg/µl. The majority of these microorganisms were inhibited by 90 µg/µl of the crude extracts. The petroleum ether fraction of *Rosmarinus officinalis* L significantly inhibited 100% of the tested strains. Ethyl acetate extracts of the studied plant exhibited relatively low MICs and could be therefore described as strong antibacterial.

Keywords: *Rosmarinus officinalis* L , Antibacterial activity , *Escherichia coli* a, *Klebsiella pneumoniae*, Bechar.

1. Introduction

Disease causing bacteria have always been considered a major cause of morbidity and mortality in humans. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased.. Essential oils and extracts from aromatic plants have long been used for a wide variety of

medicinal and domestic purposes (Makhloufi *et al.*, 2012). Antimicrobial properties of essential oils obtained from aerial parts and seeds of aromatic plants such as *Matricaria pubescens* (Desf.), *Rosmarinus officinalis* L and *Artimisia herba alba*, *Cotula cinerea* against food-related microorganisms as well as their applications in food system have been investigated and reviewed (Atik bekkara *et al.*, 2007; Chanthaphon *et al.*,

2008;Makhloufi *et al* ., 2011; Mebarki ,2018). Many studies have investigated the antimicrobial activity of different plant species in various geographical regions in search for new antibiotics. The use of plant derivatives as antimicrobials has not been extensively addressed until recently since most antibiotics were derived from bacterial or fungal origin. With the increase in resistance and the realization that the effective life span of any antibiotic is limited, new sources especially plant sources are currently being heavily investigated. Thousands of phytochemicals with antimicrobial activity have already been identified but they should be subjected to animal and human studies to study their toxicity and their effectiveness in whole organism systems. Several phytochemicals are already being studied in humans (Abdel-Massih *et al.*, 2010)

The region of Bechar is located in the south-west of Algeria. It is located at an altitude of 769 m and belongs to the pre-Saharan climatic zone with two main seasons (summer and winter). With a strong sunshine, exceeding 3500h / year, and an intense direct solar radiation which can reach 800 W / m² on a horizontal plane. In summer the temperature easily exceeds 50 ° C in the shade, and the relative humidity remains low around 27%. Moreover, in

winter the outside temperature can drop to -5 ° C at night with rare and irregular rainfall of the order of 60 mm / year (Akermi *et al.*, 2017). The aim of this work is to study the antimicrobial activity of *Rosmarinus officinalis*, of Antar Mountain in the region of Bechar against bacteria which they have high level of acquired resistance to some antibiotics (*Escherichia coli* and *Klebsiella pneumoniae* and to identify the specific fraction/s responsible for the antibacterial activity.

2. MATERIALS AND METHODS

2.1. Bacterial Strains

Five strains of *Escherichia coli* and four strains of *Klebsiella pneumoniae* were isolated at Torabi Boudjemaa Hospital in Bechar Town, between March 2015 and May 2015. These isolates exhibited different profiles of resistance.

2.2. Selected Plants

The leaves of *Rosmarinus officinalis* L (**Fig 1**) were collected directly from nature in Antar Mountain which is located in the north of Bechar .



Figure 1: *Rosmarinus officinalis* L. Antar Mountain, région de Béchar, Mars 2015

2.3. Antibacterial Activity

Dry extracts were weighed and dissolved in sterile distilled water. The solutions were filtered through 0.22 μ m sterile filter membranes and stored at 4°C for further use. The concentration of the original solution of the plant extract/fraction corresponds to the concentration obtained after resuspension of the dried plant extracts. This was used as the stock solution and the most concentrated one from which the Minimum Inhibitory Concentration MIC series were prepared (Abdel massih *et al.*, 2010).

2.4. Preparation of Crude Extract

Fresh plants were dried in the shade at room temperature and ground in a coffee bean grinder. The dried plant material was weighed and then soaked in 80% ethanol for 7 days with continuous shaking in a shaker at room temperature. At day seven, the plant material was filtered and the

filtrate collected. This was repeated and the filtrates were combined and concentrated in a rotary evaporator to obtain the crude extract.

2.5. Fractionation Method

The crude extract of the plant was further partitioned by extraction with different solvents in a 1 : 1 (v/v) ratio in order to subfractionate the plant components according to their polarity: petroleum ether (fraction 2), dichloromethane (fraction 3), and ethyl acetate (fraction 4). Extractions were repeated three times and fractions were combined. The remaining aqueous layer was collected as fraction number 5. Fractions 1 and 5 were dried using a freeze dryer, but fractions 2, 3, and 4 were dried under the hood to dryness due to the inconvenience of introducing vapor solvent into the freeze dryer. Controls were prepared for each fraction by drying the same amount of solvent and following the same subfractionation method without plant extract (solvent control) (Abdel massih *et al.*, 2010).

2.6. Study of Antibacterial Activity of the Plant Extracts

The plant powders were weighed and dissolved in sterile distilled water. The solutions were filtered through 0.22 μ m sterile filter membranes and stored at 4°C for further use. The concentration of the

original solution of the plant extract/fraction corresponds to the concentration obtained after resuspension of the dried plant extracts. This was used as the stock solution and the most concentrated one from which the Minimum Inhibitory Concentration MIC series were prepared.

2.7. Determination of the MIC

We tested antibacterial activity of rosemary extracts against two different species: *Escherichia coli* and *Klebsiella pneumoniae*. The Microdilution Broth Method was used for the determination of the MIC of plant extracts. Broths (100 µl) were dispensed in each well of a sterile microdilution tray. An appropriate volume of plant extract suspension was added to the first tube in each series (after removing the same volume of broth) in order to achieve the desired concentration after the addition of the bacterial inoculum (Abdel massih *et al.*,2010).

Cultures were grown in BHI broth (Brain Heart Infusion broth) at 37 °C for 20-24 h with shaking. A standardized bacterial inoculum was prepared and adjusted till final concentration of 10^7 CFU/. The wells were inoculated with 100 µl of this inoculum resulting). A routine bacterial count was performed in triplicates to verify the bacterial concentration. Positive and

negative control wells were used. The negative control well consisted of 200 µl of Mueller Hinton Broth (MHB); the positive well consisted of 200 µl MHB with a bacterial suspension but without a plant extract. The tray was incubated at 37°C for 18–24 hours after which the MIC was recorded as the highest dilution of each plant extract that still retained an inhibitory effect resulting in no visible growth or in other terms absence of turbidity observed with the naked eye. (Rožman and Jeršek,2007; Daoud *et al.*,2011)

2.8. Antibiotic resistance of test strains

Antibiotic sensitivity of test strains was determined by the standard Disc diffusion method of Iqbal and Arina (2001) against a number of antibiotics. The potency of antibiotics per disc is as follows. Ampicillin, Amoxicillin and Gentamicin, was used to check the potency of the discs.

3. Results and discussion

3.1. Resistance Phenotypes of the Tested Strains

For antibiotic resistance, the growth inhibition zones measured by disc diffusion method are presented in Table 1. The patterns of resistance of the tested strains against some antibiotics show that we can divide the tested bacteria into two categories: Sensitive and Resistant.

Table 1: Phenotypic profiles of susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* strains

Strains	Ampicillin,	Amoxicillin	Gentamicin
<i>E.coli</i> 1	R	R	S
<i>E.coli</i> 2	R	R	S
<i>E.coli</i> 3	R	R	S
<i>E.coli</i> 4	R	R	S
<i>E.coli</i> 5	R	R	S
<i>K. pneumoniae</i> 1	R	R	S
<i>K.pneumoniae</i> 2	R	R	S
<i>K.pneumoniae</i> 3	R	R	S
<i>K.pneumoniae</i> 4	R	R	S

S: Sensitive and R: Resistant .

All the tested strains are resistant to Ampicillin and amoxicillin , and they are sensitive to Gentamicin. Our results are correlated with Daoud et al.,(2011). Due to the high level of resistance to the tested antibiotics , the choice of effective and safe antibiotic treatment is becoming limited. Alternative agents or extracts obtained from natural medicinal plants need to be introduced or combined with antibiotics for therapeutical use.

3.2. Inhibitory and Bactericidal Activities of the Plant Fractions

3.2.1. Antibacterial Activity of *Rosmarinus officinalis* L

The antibacterial activity of rosemary extracts against *Escherichia coli* and *Klebsiella pneumonia* strains was assessed by evaluating the presence of MIC values. Results (Table 2), showed that the rosemary extracts have great potential of antibacterial activity against all strains tested.

Table 2: MICs of the different fractions of *Rosmarinus officinalis* on *Escherichia coli* and *Klebsiella pneumonia*.

Strains	Crude ($\mu\text{g}/\mu\text{l}$)	Ethyl acetate ($\mu\text{g}/\mu\text{l}$)	Aqueous ($\mu\text{g}/\mu\text{l}$)
<i>E.coli</i> 1	90	25	85
<i>E.coli</i> 2	70	20	80
<i>E.coli</i> 3	80	20	80
<i>E.coli</i> 4	90	25	85
<i>E.coli</i> 5	80	25	80
<i>K. pneumoniae</i> 1	50	25	60
<i>K.pneumoniae</i> 2	60	30	75
<i>K.pneumoniae</i> 3	55	30	65
<i>K.pneumoniae</i> 4	60	25	70

Rosmarinus officinalis exerted both inhibitory and bactericidal effects on *Escherichia coli* and *Klebsiella pneumoniae*. This effect was observed with the crude extract, the ethyl acetate, and the aqueous fractions. The MIC values for were in the range of 50-90 $\mu\text{g}/\mu\text{l}$ for crude extract, for the Ethyl acetate extract The MICs ranged from 20 to 30 $\mu\text{g}/\mu\text{l}$,in the

other hand the MICs ranged from 60 to 85 µg/µl with Aqueous extract.

Dichloromethane fraction did not show any inhibitory effect within the tested concentrations. The MICs for Dichloromethan and Petroleum ether extracts are not detected.

The solvents' controls that were systematically run for all solvents did not exert any antibacterial activity. Bacterial growth was observed for the positive controls while no growth was observed for the negative controls.

Antibacterial results indicated that the crude extract of *Rosmarinus officinalis*, the ethyl acetate, and the aqueous fractions extracts showed a significant inhibitory activity, according to Weckesser et al. (2007), The extracts exhibited an effect against the Gram-negative bacteria (*E. coli* and *P. aeruginosa*). However, this effect was less efficient than that presented against the Gram-positive bacteria, since a higher MIC value was obtained with the Gram-negative bacteria .The same results were reported by Panizzi et al. (1993). In the study of Gislène et al.,(2000) it was shown that *E. coli* and *Klebsiella pneumonia* were resistant to hydro-alcoholic fraction.

4. Conclusion

The results of this study show that the preparation of the extracts can be used as an alternative of antibiotics.

5. References

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