The evaluation of antibacterial and antiproliferative activities of *Rosmarinus officinalis* essential oil from Morocco

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Abstract: Essential oils of plants contain many bioactive molecules and have recently a considerable place of application in pharmaceutical, cosmetic and food industry. The aim of this work is to study antibacterial and antiproliferative activities of *Rosmarinus officinalis* essential oil.

Essential oil was extracted from dried plant by hydrodistillation, and then analyzed by gas chromatography-mass spectrometry (GC/MS). The antibacterial activity of the essential oil was tested by micro-atmosphere technique and agar diffusion against seven strains bacteria.

The antiproliferative activity was determined by viability test against P3X63Ag8 myeloma cell in presence of various amount of essential oil. The morphological alterations were searched after staining of treated cells by le May-Grünwald-Giemsa

Our results showed that the major component detected in *R. officinalis* essential oil is 1.8-cineole (42%). This essential oil revealed an important antibacterial activity against resistant bacteria. The viability test showed a cytotoxic effect of essential oil on P3X63Ag8 myeloma cell. The morphological of the treated tumor cells analysis revealed a cellular lysis with membrane rupture, a hypertrophy and chromatin condensation.

In conclusion, the *R. officinalis* essential oil could have an antibacterial activity against resistant bacteria and antiproliferative activity against myeloma cells.

Keywords: antibacterial activity, antiproliferative activity, essential oil, *Rosmarinus officinalis*.

Introduction

Rosmarinus officinalis L. (rosemary) is a wild plant belonging to the family Lamiaceae that is abundant in arid and dry regions, especially on low hills and mountains (Fechtal et al. 2001). The natural geographical area of rosemary is practically limited to the Mediterranean basin but it is cultivated around the world. In Morocco, rosemary can be found on the left bank of the Moulouya and in the Rifain Atlas Mountains. In the Middle Atlas, it is very abundant in the Lahmer mountain, the Fegouss pass and the Bou Iblane massif. In the Great Atlas, it is common from mountain Ayachi, Titahoune and Tizin Teleghmet. Moreover, it is particularly abundant in the regions of Midelt and in the forest of Debdou and rare in the western part (Fechtal et al. 2001). It is also found in the Central and Central-Western Rif.

Rosemary is used as a spice in Mediterranean cuisine and a natural preservative in the food industry but generally it is used as ornamental and medicinal plant to relieve headaches, improve memory and concentration, strengthen convalescents, combat the effects of stress and fatigue and treat epilepsy (Takaki et al. 2008; Raskovic et al. 2014; Eddouks et al. 2017, Capatina et al. 2020). It is also a pulmonary antiseptic, antidiarrhoeal, diuretic and sudorific (Al-Sereiti et al. 1999; Haloui et al. 2000; Slamenova et al. 2002). Internally, rosemary is used in the treatment of hepatic disorders, biliary and gastrointestinal lithiasis (Bahri et al. 2020). Rosemary also acts externally in rheumatic and peripheral blood circulation disorders and in accelerating wound healing (Al-Hader et al. 1994; Erenmemisoglu et al. 1997). *In vitro*, rosemary has been shown to be effective against a large number of bacteria and fungi that parasitize the digestive system, respiratory tract and various organs (Mangena and Muyima, 1999; Daferera et al. 2000; Mahgoub et al. 2019). In addition, it has an antispasmodic effect on the smooth muscles of the trachea and intestines, which increases its effectiveness in the treatment of respiratory and digestive infections (Al-Sereiti et al. 1999). The aim of this study is to evaluate the antibacterial and antiproliferative activities of *R. officinalis* essential oil.

Materials and Methods

1. Chemical study of *R. officinalis* essential oil

1.1 Plant material

R. officinalis was collected from spontaneous stands in Errachidia in southern Morocco (31° 55′ 55″ north, 4° 25′ 28″ west) during the period April – May 2019.

The plant was identified by Prof. Alaoui-Ismaili, at the Institut Agronomique and Veterinary of Rabat (Morocco).

1.2 Extraction by hydrodistillation

The freshly harvested plant (200 g) was dried under shade at 25 °C in a ventilated room for one week and was grounded with a blender. The essential oil was extracted by hydrodistillation in a modified Clevenger-type apparatus (Clevenger 1928) under optimal operating conditions; a quantity of 100g of rosemary powder was added to 800 ml of distilled water (2 liter) (Fadil et al. 2015). The set was placed in a balloon heater attached to a refrigerator to ensure condensation of essential oil for 3hours. At the end the distillation, two phase were observed, an organic phase (essential oil) and aqueous phase (aromatic water), more dense than oil. The essential oil was collected, dried under anhydrous sodium sulphate and stored in sealed vials in the dark at 4°C, until used.

The yield of essential oil were expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation (Ban et al. 2016) :

Yield (%) = Amount of extracted oil (g) / Amount of dry vegetal matter mass (g) x 100

1.3. Analysis by gas chromatography (GC)

The chemical composition of the essential oil of *R. officinalis* was determined by gas chromatography-mass spectrometry (GC/MS).

Chromatographic analyses were performed on an electronically pressure regulated gas chromatograph (Chromapack Cp 9001 type) coupled to a Polaris Q MS ion trap mass spectrometer. The gas chromatography was equipped with a flame ionisation detector (FID) fed by a Hydrogen/Air mixture whose respective pressures are 0.4 bar and 2.2 bar and a fused silica capillary column (30 m long and 0.25 mm internal diameter). The temperature of the column is automatically increased by 4 °C/min between 50 °C and 230 °C in two steps,

one at 50 °C for 5 min and the other at 230 °C for 10 min. The injector and the detector keep constant temperatures of 235 °C and 240 °C respectively. One microliter of the sample was injected as a 10% (V/V) solution in hexane. The mass spectrometer was operated under the following conditions: the fragmentation temperature was carried out by electronic impact under a field of 70 eV. The temperature of the ionization source is equal to 200.

Component identification was carried out by comparing spectral and chromatographic data with literature data (Adams 2005).

2. Antibacterial activity

2.1. Bacterial strains

The different germs used for this study are pathogenic hospital germs, taken at random from the bacteria most commonly encountered in the different samples supplied from Bacteriology Laboratory of the Pasteur Institute of Morocco (table 1). In parallel, reference strains were also used for this study. The strains were kept in Kliglller-Hanja medium at 4 °C.

Colonies of each germ were emulsified in a tube containing 5 ml of physiological water. Various dilutions were made to obtain a suspension with an opacity equivalent to that of the 0.5 standard of the Mac Farland scale which corresponds to approximately 10^8 bacteria/ml.

Table 1: The bacterial strains used.

Bacterial strains	Famille	Origin
Escherichia coli	Enterobacteriaceae	Urine
Escherichia coli CIP54127	Enterobacteriaceae	Pasteur Institut of Paris
Proteus vulgaris	Enterobacteriaceae	Vaginal sampling
Proteus vulgaris CIP5860T	Enterobacteriaceae	Pasteur Institut of Paris
Klebsiella pneumoniae CIP8291T	Enterobacteriaceae	Pasteur Institut of Paris
Salmonella enteritidis	Enterobacteriaceae	Coproculture
Staphylococcus aureus	Enterobacteriaceae	Vaginal sampling

2.2 Study of the sensitivity of bacterial strains to antibiotics

The dishes containing Muller-Hinton medium were swabbed and dried at 37 °C for 15 min. The individual discs of selected antibiotics (Table 2) were deposited using a fine flaming forceps. Then the dishes were placed at 4 °C for 30 min and incubated at 37 °C for 18 h.

Table 2: List of antibiotics used

Disc load (µg)	Antibiotic	Resistance	Sensitivity
10	Amikacin	≤14	≥ 19
25	Amoxicillin	≤ 14	≥ 21
20/10	Amoxicillin + clavunic acid	≤ 14	≥ 21
75	Ticarcillin	≤ 18	≥ 22
75	Pipéracillin	≤ 12	≥ 18
30	Tetracyclin	≤ 17	≥ 19
30	Aztréonam	≤ 15	≥ 17
10	Gentamicin	≤12	≥ 16
5	Péfloxacin	≤ 16	≥ 22
5	Norfloxacin	≤ 19	≥ 22
5	Ciprofloxacin	≤ 19	≥ 22

The inhibition diameter is in mm

The antibiotics used and their standard inhibition diameters of resistance and sensitivity.

2.3. Study of antibacterial activity

2.3.1. Micro-atmosphere technique

The micro-atmosphere method was used in this study. The microorganisms used were inoculated in radial streaks in a sterile Petri dish containing a CLED medium. On the inside of

the lid of the Petri dish, a filter paper 2 cm in diameter was placed and soaked with a given quantity of essential oil ranging from 0 to 100 µl. The Petri dishes were then incubated in the reverse position (lid down). Evaporation of the volatile substances that saturate the atmosphere of the Petri dish and exert their effects on the seeded germs takes place. Reading the results allows the minimum inhibitory quantity to be detected. The tests were carried out in duplicate and repeated three times. The efficacy of the essential oil was determined by the scoring systems adopted by Benjilali et al (1984) (Table 3).

Table 3: Rating system for the inhibitory efficacy (Benjilali et al., 1984).

Minimum inhibitory quantity (MIQ) (mm)	Efficiency rating
MIQ ≤ 5	5
5 < MIQ ≤ 10	4
10 < MIQ ≤ 20	3
20 < MIQ ≤50	2
50 < MIQ≤ 100	1
100 < MIQ	0

The inhibition efficiency of Rosmarinus officinalis essential oil achieved by the micro-atmosphere technique was determined by the scoring systems adopted by Benjilali et al (1984).

2.3.2. Direct solid-state contact technique

The previously prepared bacterial suspension was diluted 1:100 to obtain a suspension of approximately 10^6 bacteria/ml. A Petri dish containing Muller-Hinton medium was swabbed. The dish was then dried in the oven for 15 min at 37 °C. Cellulose discs of 6mm diameter were deposited on the surface of the medium. A volume of 10µl of the essential oil studied was deposited on the cellulose discs using a micropipette. The boxes were then deposited for 30 min at 4 °C. After incubation at 37 °C for 24 hours, the inhibition zones were measured. The tests were carried out in duplicate and repeated three times.

3. Study of the antiproliferative activity of rosemary essential oil

3.1. Cell line

The cells used come from myeloma line P3X63Ag8 of the Balb/c mouse donated by the French Blood Establishment of Bordeaux (France). The cells were cultured in RPMI 1640 vials containing 5% supplemented fetal calf serum, penicillin at 100 U/ml, streptomycin at 100 mg/l, glucose at 4 g/l, sodium pyruvate at 200 mM and glutamine at 200mM. The cells were incubated at 37 °C in a 5% CO_2 humid atmosphere.

3.2. Cell viability test

Cell suspensions of 5 x 10^4 cells / ml were distributed in a 24-well plate.

The essential oil was diluted 1:10 in absolute ethanol and then dissimilar dilutions were made in the culture medium (RPMI). Ethanol showed no toxicity on cells at 1% (Jung-Woo et al. 2004). A 10µl volume of each dilution was added to each well. The cells were incubated at 37 °C in a 5% CO_2 humid atmosphere for 48 hours. A control was made under the same conditions by adding 10µl of ethanol. The assays were performed in triplicate.

A 1/3 dilution of the cell suspension was carried out in a buffered solution of Trypan blue. The counting of the living cells was carried out under an inverted stand microscope on the whole Malassez cell whose final volume was 1 µl (European Collection of Authenticated Cell Culture).

The concentration of live cells in each well was calculated according to the following formula:

Number of cells/ml = N x D x 1000

(N = number of living cells per µl, D = dilution factor).

The percent cytotoxicity was calculated according to the following formula:

C% = (N - Ne) / N control cells x100

(N = number of cells in the control well, Ne = number of cells in the test well).

Results

1. Chemical study

1.1 Extraction yields

The essential oil of *R. officinalis* is lightly colored, with a strong and slightly mentholated odor. The yield in essential oil is 0.85% ± 0.053

1.2 Chemical composition of the essential oil

The analysis of the chemical composition by gas chromatography of the essential oil of *R. officinalis* has identified 15 components that represent 95.75% of the totality of this essential oil (table 4). 1,8-cineole is the majority component of this essential oil, with a percentage of 42%. Monoterpenic hydrocarbons present 27.77% and alcohol present 7.66% of this essential oil.

Table 4 : Chemical composition of the essential oil of *Rosmarinus officinalis*.

	Compound	Retention time (R_t)	Percentage %
1	α-pinene	7.55	11.92
2	Camphene	8.09	4.55
3	β-pinene	9.22	7.71
4	β-myrcene	9.94	1.41
5	p-cymene	11.12	0.93
6	1,8-cineole	11.49	42
7	γ-terpinene	12.66	0.86
8	terpinolene	13.85	0.39
9	Linalool	14.39	0.88
10	Camphre	15.98	13.99
11	Bornèol	16.81	3.57
12	1-terpinen-4-ol	17.35	0.81
13	α-terpineol	17.86	2.40
14	acetate-bornyl β-	21.44	0.73
15	caryophyllene	26.07	3.60

2. Antibacterial activity

2.1. Sensitivity of bacterial strains to antibiotics

The antibiogram revealed that *S. aureus* is sensitive to all the antibiotics used. However, *E. coli*, *Proteus vulgaris* and *S. enteritidis* presented resistance to some antibiotics. *E. coli* is resistant to amoxicillin (AMX), AMX + clavulic acid and furan. Whereas, *P. vulgaris* is resistant only to amoxicillin and AMX + clavulanic acid and *S. enteritidis* is resistant to cefalotin.

2.2. Micro-atmosphere technique

The minimum inhibitory quantities of the essential oil studied are shown in Table 5. All the strains used presented sensitivity to the essential oil studied. These strains were inhibited at minimal quantities which varied from 40 µl and 90 µl of *R. officinalis* essential oil. The *S. aureus* strain was the most sensitive of the bacterial strains used. This bacterium was inhibited at 40 µl of *R. officinalis* essential oil. The bacterial strain *K. pneumonia* was more resistant, it was inhibited at an amount equal to 90 µl of *R. officinalis* essential oil.

Table 5: Minimal inhibitory quantities of *Rosmarinus officinalis* essential oil on seven bacterial strains using the micro-atmosphere technique.

Bacterial strains	Minimum Inhibitory Quantities (µl)
Escherichia coli CIP54127	65
Proteus vulgaris CIP5860T	40
klebsiella pneumonia CIP8291T	90
Proteus vulgaris	40
Salmonella enteritidis	60
Escherichia coli	70
Staphylococcus aureus	40

The bacterial strains *P. vulgaris* and *P. vulgaris* CIP5860T were inhibited at comparable amounts of essential oil, as were *E. coli* and *E. coli* CIP54127T. The degrees of effectiveness of the essential oil studied are reported in Table 6.

Table 6: Degrees of effectiveness of *Rosmarinus officinalis* essential oil according to the minimum inhibitory quantities.

Bacterial strains	Effectiveness degrees
Echerichia coli CIP54127	1
Echerichia coli	1
Proteus vulgaris CIP5860T	2
Proteus vulgaris	2
k. pneumoniae CIP8291T	1
Salmonella enteritidis	1
Staphylococcus aureus	2

2.3 Direct contact technique

The diameters of inhibition of the essential oil of *R. officinalis* varied between 20 mm and 35 mm. The *S. aureus* strain is the most sensitive to the inhibitory action of the essential oil of *R. officinalis* with inhibition diameters of 35 mm (Table 7).

Table 7: Diameter of inhibition of *Rosmarinus officinalis* essential oil on seven bacterial strains obtained by the direct contact technique.

Bacterial strains	Diameter of inhibitions (mm)
Escherichia coli CIP54127	32
Proteus vulgaris CIP5860T	22
klebsiella pneumoniae CIP8291T	29
Proteus vulgaris	31
Salmonella enteritidis	20
Escherichia coli	31
Staphylococcus aureus	35

3. Antiproliferative activity

The cell viability test showed that the essential oil of *R. officinalis* exerts a dose-dependent cytotoxic effect on P3X63Ag8 myeloma cells (Figure 1).

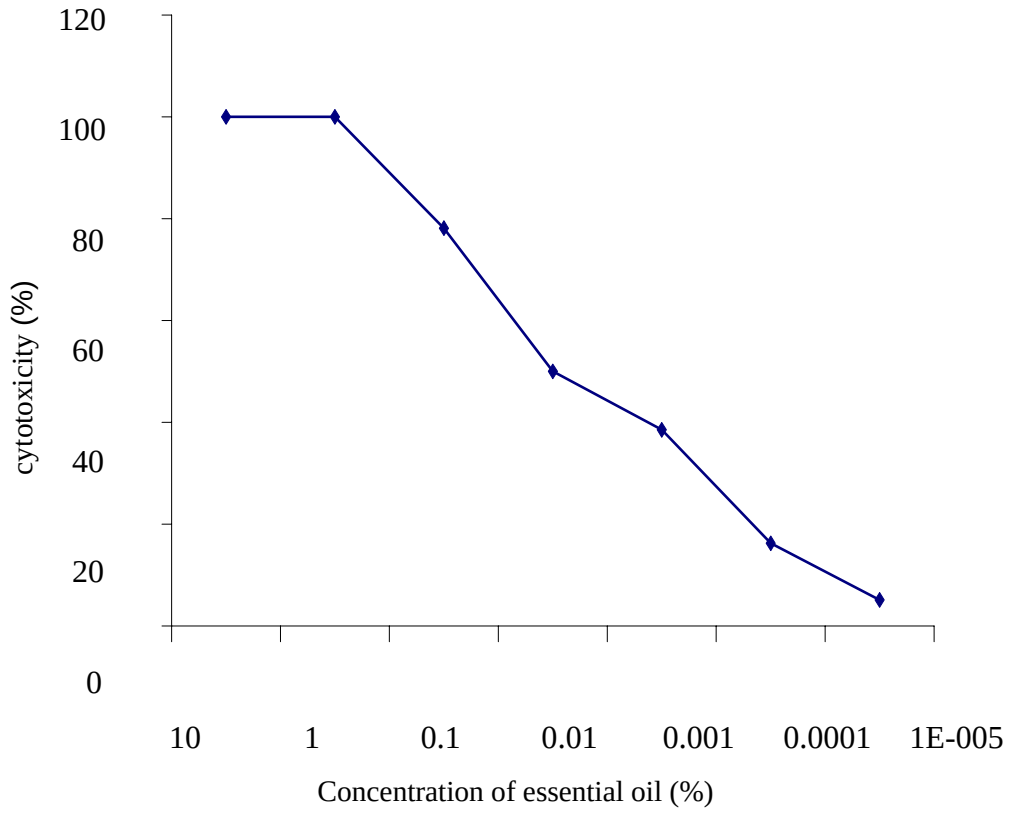

Figure 1: Percentage cytotoxicity of *Rosmarinus officinalis* essential oil on P3X63Ag8 myeloma cancer cells, measured after 48 hours.

The 50% inhibitory concentration (IC_{50}) after 48h incubation is 100 µg/ml (0.01%).

Total cell death was observed at the 1% concentration of *R. officinalis* essential oil. Microscopic examination of the cells showed hypertrophy of the treated cells. Lysed cells and damaged membranes were also noted.

Discussion

Essential oils are complex products that contain many biologically active molecules. These

oils occupy a considerable place in the pharmaceutical market and in the cosmetics and food industry (Silva et al. 2003; Benkeblia 2004; Hongratanaworakit 2009).

The comparison of our results with literature data shows that the yield of our rosemary (0.82%) is lower than those found by other Moroccan researchers (1.6 to 2.41%) (Fadil et al. 2015; El Kamli et al. 2017, Elyemni et al. 2019), but is close to that found by several researchers (Djeddi et al. 2007; Tomi et al. 2016). Indeed, there are several factors that influence the yield of quality and quantity of essential oils of this plant such as the extraction method, the period and the region of harvest.

The percentages of the identified products present approximately 95.75% of the total essential oil of *R. officinalis*. 1,8-cineole is the major component of this essential oil, with a content of 42%. These results are consistent with those previously reported for other Moroccan and Mediterranean rosemary samples (Tunisia, Turkey, Greece, Serbia, Italy and France), where 1,8-cineol represents the major component with more than 40% (Chalchat et al. 1993; El Amrani et al. 2000; Yesil Celiktas et al. 2007; Zaouali et al. 2010). While in some samples of rosemary essential oil also Moroccan, Algerian, Spanish, Italian and Bulgarian, the level of this product is lower, approximately between 12% and 32% (Tomei et al. 1995; De Mastro et al. 2004; Djeddi et al. 2007, Elyemni et al. 2019). Next to cineole, monoterpenic hydrocarbons are the most dominant class. α-pinene (11.92%) and β-pinene (7.71%) are quantitatively the main constituents of this class. These results are consistent with those of Aberchane et al. (2001). Monoterpene alcohols represent 7.66% of our *R. officinalis* essential oil; these alcohols are dominated by borneol (3.57%) and γ-terpineol (2.4%). Similar alcohol content in the essential oil of Moroccan oriental rosemary has also been reported by Fechtal et al. (2001). These variations in yield and chemical composition could be explained by several factors, namely, geographical origin, environmental, climatic and agronomic conditions, the type of plant material subjected to extraction, the time of harvest, the maturity of the leaves and their degree of drying, as well as the oil extraction technique (Fechtal et al. 2001, Jamshidi et al. 2009; Moghtader and Afzali 2009; Okoh et al. 2010).

To get an idea of the extent of the field of action of the rosemary essential oil studied, we chose six bacterial strains of the Enterobacteriaceae family and one bacterial strain of the

Micrococcaceae family (*S. aureus*). These bacteria are frequently encountered in many infections in Morocco and pose a clinical and therapeutic problem. The study of the sensitivity of *S. aureus*, *E. coli*, *P. vulgaris* and *S. enteritidis* to antibiotics has shown that *S. aureus* is sensitive to all antibiotics, while the other strains have been found to be resistant to some antibiotics. We chose the micro-atmosphere technique because it aims to highlight the antibacterial activity of the volatile phase of essential oils. By this technique, *S. aureus* proved to be more sensitive than the other strains, while *K. pneumonia* is the most resistant of the strains studied; it is inhibited only at high quantities of essential oil. Kozlowska et al. (2015) tested the antibacterial activity of several essential oils on the same bacterial strains. They showed that *P. vulgaris* and *S. aureus* are the most sensitive of the strains studied. This antimicrobial activity is probably due to the major components of the oil. Indeed, the studies of methanolic (Shan et al. 2007~~32~~, Abolfazl and Parisa 2020) and ethanolic (Weerakkody et al. 2010) extracts of *R. officnalis* showed significant inhibitory properties against *S. aureus*. However, Jiang et al. (2011) showed that the antimicrobial activities of rosemary essential oil were superior to those of α-pinene and 1,8-cineole. Thus, it is rather difficult to attribute the antimicrobial effect of this oil to one or more active ingredients, as extracts always contain a mixture of different chemical compounds. In addition to these major components, minor components can also contribute significantly to the antimicrobial activity of the extracts. Thus, the antimicrobial activity of rosemary essential oil is the synergistic effect of its components.

The micro-atmosphere technique prevents any direct contact between the germs and the essential oil and allows only the antibacterial activity of the volatile fraction of the essential oils to be evaluated. Non-volatile products may be responsible for a certain activity that would escape experimentation. For this reason, we used the technique of direct contact where the essential oil acts directly on the micro-organisms studied. As already observed by the micro- atmosphere technique, *S. aureus* remains the most sensitive strain of the bacteria studied. These results are in agreement with those of Vardar-Unlu et al. (2003) who found that *S. aureus* is more sensitive (37 mm, inhibition diameter) than *E. coli* (23 mm id) and *K. pneumonia* (22 mm id) to the essential oil of *Thymus pectonatus*. The antibacterial activity of essential oils would be due to the molecular interaction of the functional groups of its

components with the wall of the bacteria which causes deep lesions which increase the rate of cellular mortality (Pattanaik et al. 1997).

No significant difference in sensitivity for our essential oil has been observed between resistant bacteria from hospital and reference bacteria used.

This would indicate that the essential oil of *R. officinalis* would act in the same way on sensitive bacteria as on resistant bacteria. Similar results have been reported by other researchers (Mondello et al. 2003; Nostro et al. 2004, Zhong et al. 2021). Thus this essential oil could be used as a natural replacement treatment for infections caused by antibiotic resistant bacteria and become a potential antimicrobial in the food and pharmaceutical industries.

The cell viability test was performed to evaluate the acute cytotoxicity of *R. officinalis* essential oil on P3X63Ag6 myeloma cells. It is also used to determine the dose-response relationship. This test showed that the essential oil tested presented a very high cytotoxicity against P3X63Ag6 myeloma cells. This cytotoxicity is proportional to the concentration of the essential oil for the chosen dilution interval.

The cytotoxicity of rosemary oil has also been reported in literature. Indeed, an IC_{50} greater than 250µg/ml has been reported when the oil has been exposed to cancer cells of the ovaries and liver (Satyal et al. 2017), while a low IC_{50} of 14.15 µg/ml was calculated after exposure to cancer cells (Lorenzo-Leal et al. 2019). Our IC_{50} (100 µg/ml) is intermediate between the two results. Similarly, these variations can be attributed to the composition of the essential oil used.

Microscopic examination of the cells after treatment with the essential oil revealed hypertrophy and lysis of the myeloma cells treated with membrane bursting. These effects could be due to the direct action of the constituents of the essential oils with the constituents of the cell membranes. These components would induce deep lesions that must have caused the culture medium to penetrate into the cell and lead to hypertrophy resulting in membrane rupture. This confirms the explanation of the mechanism of action of essential oils on bacteria. This activity would be due to the presence of monoterpenes in this essential oil. Many monoterpenes, such as geraniol and 1,8-cineole, are the main constituents of essential oils and present an antiproliferative activity (Moteki et al. 2002; Hibasami et al. 2003).

Conclusion

From these results, we can conclude that *R. officinalis* essential oil has antibacterial activity against resistant bacteria and can therefore be an effective alternative to bacterial control. Moreover, it presents an antiproliferative effect on P3X63Ag6 myeloma tumor cells by acting on their membrane and therefore could be used as an antiproliferative agent. Although clinical trials on patients after the use of *R. officinalis* extract are recommended for confirmation of these data.

References

- Aberchane M, Fechtal M, Chaouche A, Bouayoune, T. (2001). Influence de la durée et de la technique d'extraction sur le rendement et la qualité des huiles essentielles du cèdre de l'Atlas (*Cedrus atlantica Manetti*). Ann Rech For Maroc. 34:110-118.

- Abolfazl JS, Parisa HN (2020). Antimicrobial effects of Rosmarinus officinalis methanolic extract on Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa in laboratory conditions. Journal of Medicinal and Chemical Sciences. 3: 103-108. 10.26655/jmchemsci.2020.2.2

- Adams RP (2005). Identification of essential oil components by gas chromatography / mass spectroscopy. American Society for Mass Spectrometry. 16(11): 1902-1903. org/10.1016/j.jasms.2005.07.008

- Al-Hader, AA, Hasan ZA, Aqel MB (1994). Hyperglycemic and insulin release inhibitory effects of Rosmarinus officinalis. J of ethnopharmacology. 43(3):217-221.

- Al-Sereiti MR, Abu-Amer KM, Sen P (1999). Pharmacology of rosemary (Rosmarinus officinalis linn) and its therapeutic potentials. Indian J. Exp. Biol. 37(2):124-130.

- Bahri S, Ben Ali R, Abdennabi R, Ben Said D, Mlika M, Ben Fradj MK, El May MV, Jameleddin SBK (2020). Comparison of the Protective Effect of Salvia officinalis and Rosmarinus officinalis Infusions against hepatic damage Induced by hypotermic-ischemia in Wistar Rats. Nutr cancer. 72(2):283-292. 10.1080/01635581.2019.1631359

- Ban L, Narasimhamoorthy B, Zhao L, Greaves A, Schroeder D (2016). Antioxidant activities from differentrosemary clonal line. Food chemistry. 201: 259-263. 10.1016/j.foodchem.2016.01.082

- Benjilali B, Tantaoui Elaraki A, Ayadi M, Ihlal M (1984). Method to study antimicrobial effects of essential oils: application to theantifungal activity of six Moroccan essences. J Food Prot. 47:748-753.

- Benkeblia N (2004). Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). LWT-Food Science and Technology. 37: 263-268. org/10.1016/j.lwt.2003.09.001

- Capatina L, Boiangiu RS, Dumitru G, Napoli EM, Ruberto G, Hritcu L, Todirascu-Ciornea E (2020). Rosmarinus officinalis Essential Oil Improves Scopolamine-Induced Neurobehavioral Changes via Restoration of Cholinergic Function and Brain Antioxidant Status in Zebrafish (Danio rerio). Antioxudants. 9(1):62. 10.3390/antiox9010062

- Chalchat JC, Gary P, Michet A, Benjilali B, Chabart JL (1999). Essential oils of rosemary (Rosmarinus officinalis L.). The chemical composition of oils of various origins (Morocco, Spain, France). J Ess oil research. 5(6):613-618. org/10.1080/10412905.1993.9698293

- Clevenger JF (1928). Apparatus for the dermination of volatil oil. J Am Pharm Assoc 17: 345-346. org/10.1002/jps.3080170407

- Daferera DJ, Ziogas BN, Polissiou MG (2000). GC-MS analysis of essential oils from some greek aromatic plants and their fungitoxicity on Penicillium digitatum. J Agric Food Chem. 48(6):2576-2581. 10.1021/jf990835x

- De Mastro G, Ruta C, Mincione A, Poiana M (2004). Bio-morphological and chemical characterization of rosemary (Rosmarinus officinalis L.) biotypes. Acta horticulturae. 629:471-480. 10.17660/ActaHortic.2004.629.61

- Djeddi S, Bouchenah N, Settar I, Skaltsa H. (2007). Composition and antimicrobial activity of the essential oil of Rosmarinus officinalis from Algeria. Chemistry of Natural Compounds. 43(4):487-490. 10.1007/s10600-007-0172-4

- Eddouks M, Ajebli M, Hebi M (2017). Ethnopharmacological survey of medicinal plants used in Daraa-Tafilalet Region (Province of Errachidia), Morocco. J Ethnopharmacol. 198:516-530. 10.1016/j.jep.2016.12.017

- El Amrani, A, Zrira S, Benjilali B, Berrada M (2000). A study of Moroccan rosemary oils. J Ess Oil research. 12(4):487-495. org/10.1080/10412905.2000.9699572

- El Kamli T, Errachidi F, Eloutassi N, Majid H, Chabir R, Bour A (2017). Comparaison

quantitative et qualitative des huiles essentielles de rosmarinus officinalis obtenues par différentes méthodes. European Scientific Journal July 13(21): 171-182. 10.19044/esj.2017.v13n21p172

- Elyemni M, Louaste B, Nechad I, Elkamli T, Bouia A, Taleb M, Chaouch M, Elouatassi N (2019). Extraction of essential oils of Rosmarinus officinalis L. by Two Different Methods: Hydrodistillation and Microwave Assisted Hydrodistillation. org/10.1155/2019/3659432

- Erenmemisoglu A, Saraymen R, Ustun S (1997). Effect of a Rosmarinus officinalis leave extract on plasma glucose levels in normoglycaemic and diabetic mice. Pharmazie. 52(8):645-646. PMID: 9292920

- Fadil M, Farah A, Ihssane B., Haloui T, Rachiq S (2015). Optimization of parameters influencing the hydrodistillation of Rosmarinus officinalis L. by response surface methodology. M J Mater Environ Sci. 6(8): 2346-2357.

- Fechtal M, Ismaili R, Zine El Abidine A (2001). Effet de la transplantation sur la qualité et le rendement en huiles essentielles du romarin (Rosmarinus officinalis). Ann Rech For Maroc. 34:94-102.

- Haloui M, Louedec L, Michel JB, Lyoussi B (2000). Experimental diuretic effects of Rosmarinus officinalis and Centaurium erythraea. J of ethnopharmacology. 71(3):465-472. 10.1016/s0378-8741(00)00184-7

- Hibasami H, Yamada Y, Moteki H, Katsuzaki H, Imai, K., Yoshioka, K., Komiya, T. (2003). Sesquiterpenes (costunolide and zaluzanin D) isolated from laurel (Laurus nobilis L.) induce cell death and morphological change indicative of apoptotic chromatin condensation in leukemia HL-60 cells. Int J Mol Med.12(2):147-151. PMID: 12851709

- Hongratanaworakit T (2009). Simultaneous aromatherapy massage with rosemary oil on humans. Sci Pharm. 77:375-387. 10.3797/scipharm.0903-12

- Jamshidi R, Afzali Z, Afzali, D. (2009). Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with othercountries. American-Eurasian Journal of Agriculture and Environment Science. 5(1):78–81.

- Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, Zu YG, Liu XL (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environmental

Toxicology and Pharmacology. 32(1):63-68.

- Kozłowska M, Laudy AE, Przybył J, Ziarno M, Majewska E (2015). Chemical composition and antimicrobial activity of some medicinal plants from Lamiaceae family. Acta Pol Pharm. 72(4):757-767. PMID: 26647633

- Jung-Woo L, Jung-Gul S, Eun-Hee K, In Been Y, Ji Yeon K, Hong-Gu J, Hee Jong W (2004). Immunompdulatory and antitumor effects in vivo by the cytoplasmic fraction of Lactobacillus casei and Bifidobacterium longum. J Vet Sci 5 (1): 41-48.

- Lorenzo-Leal, AC, Palou E, López-Malo A (2019). Antimicrobial, cytotoxic and anti-inflammatory activities Pimenta dioica and Rosmarinus officinalis Essential Oils. BioMed Res Int. Volume 2019, Article ID 1639726, 8 pages. org/10.1155/2019/1639726

- Mahgoub SAM, El-Hack MEA, Saadeldin IM, Hussein MA, Swelum AA, Alagawany M (2019). Impact of Rosmarinus officinalis cold-pressed oil on health, growth performance intestinal bacterial populations, and immunocompetence of Japanese quail. Poult Sci. 98(5):2139-2149. org/10.3382/ps/pey568

- Mangena T, Muyima NY (1999). Comparative evaluation of the antimicrobial activities of essential oils of Artemisia afra, Pteronia incana and Rosmarinus officinalis on selected bacteria and yeast strains. Lett Appl Microbiol 1999. 28(4):291-296. 10.1046/j.1365-2672.1999.00525.x

- Moghtader M, Afzali D (2009). Study of the antimicrobial proprieties of the oil of Rosemary. American-Eurasian Journal of Agriculture and Environment Science. 5 (3):393-397.

- Mondello F, De Bernadis F, Girolamo A, Salvatore G, Cassone A (2003). In vitro and in vivo activity of tea tree oil against azole-susceptible and resistant human pathogenic yeasts. J of Antimicrobial Chemotherapy. 51:1223-1229. 10.1093/jac/dkg202

- Moteki H, Hibasami H, Yamada, Y., Katsuzaki H, Imai K, Komiya, T (2002). Specific induction of apoptosis by 1,8-cineole in two human leukaemia cell lines, but not in a human stomach cancer cell line. Oncol. Rep. 9:757-760. PMID: 12066204

- Nostro A, Blanco AR, Cannatelli MA, Euea V, Flamini G, Morelli I (2004). Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiology Letters. 230(2):191-195.

- Okoh OO, Sadimenko AP, Afolayan AJ (2010). Comparative evaluation of theantibacterial activities of the essential oils ofRosmarinus officinalisL. Obtainedby hydrodistillation and solvent free microwave extraction methods. Food chemistry.120 (1):308-312. doi.org/10.1016/j.foodchem.2009.09.084

- Pattanaik S, Subramanyam VR, Kole C (1997). Antibacterial and antifungal activity of the essential oils in vitro. Microbios. 86(349):237-246. PMID: 9218354

- Raskovica M, Milanovic I, Pavlovic N, Ćebovic T, Vukmirovic, S., Mikov M. (2014). Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med ; 14 :225-234. 10.1186/1472-6882-14-225

- Satyal P, Jones T, Lopez E, McFeeters R, Awadh Ali N, Mansi I, Al-kaf A, Setzer W (2017). Chemotypic characterization and biological activity of Rosmarinus officinalis. Foods 2017; 6(3):20. 10.3390/foods6030020

- Shan B, Cai YZ, Brooks JD, Corke H (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol. 117(1):112-119. org/10.1016/j.ijfoodmicro.2007.03.003

- European Collection of Authenticated Cell Culture. Fundamental techniques in cell culture. Laboratory Handbook 3RD edition Sigam-Aldrich.

- Slamenova D, Kuboskova K, Horvathova E, Robichova S (2002). Rosmary-stimulated reduction of DNA strand breaks and FPG-sensitive sites in mammalian cells treated with H2O2 or visible light-excited Methylene Blue. Cancer Letters.177: 145-153. 10.1016/s0304-3835(01)00784-4

- Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, Matos FJA (2003). Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. Journal of Ethanopharmacology. 89:277-283. 10.1016/j.jep.2003.09.007

- Takaki I, Bersani-Amado LE, Vendruscolo A, Sartoretto SM, Diniz SP, Cuman RK (2008). Anti-inflammatory and antinociceptive effects of Rosmarinus officinalis L. essential oil in experimental animal mode. J of Medicinal Food. 11(4):741–746. 10.1089/jmf.2007.0524

- Tomei PE, Cioni PL, Flamini G, Stefani A (1995). Evaluation of chemical composition of essentiel oils of same Lamiaceae from Serrania de Ronda (Andalucia, Spain). J Essent Oil

Res. 7(3):279-282.

- Tomi K, Kitao M, Konishi N, Murakami H, Matsumura Y, Hayashi T (2016). Enantioselective GC–MS analysis of volatile components from rosemary (Rosmarinus officinalis L.) essential oils and hydrosols. Organic Chemistry. 80(5):840-847. org/10.1080/09168451.2016.1146066

- Vardar-Unlu G, Candan F, Sokmen A, Daferera D, Polissiou M, Sokmen M, Donnez E Tepe B (2003). Antimicrobial and antioxida,t activity of the essential oil and methanol extracts of thymus pectinatus Fisch. Et Mey. Var. pectinatus (Lamiaceae). J Agric Food Chem. 51:63-67. 10.1021/jf025753e

- Weerakkody NS, Caffin N, Turner MS, Dykes GA (2010). In vitro antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. Food Control. 21(10):1408-1414. doi.org/10.1016/j.foodcont.2010.04.014

- Yesil Celiktas O, Hames Kocabas E, Bedir E, Vardar Sukan F, Ozek T, Baser KH (2007). Antim- icrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chemistry. 100(2):553- 559.

- Zaouali Y, Bouzaine T, Boussaid M (2010). Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem Toxicol. 48: 3144-3152. 10.1016/j.fct.2010.08.010

- Zhong X, Hao X, Wang X, Gan M, Zhou N, Li J, Lin P, Liu J, Shang X (2021). Chemical characterization of the polar antibacterial fraction of the ethanol extract from Rosmarinus officinalis. Journal Food Chemistry. 15(344): 128674. 10.1016/j.foodchem.2020.1286.