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Seasonal Evaluation Of The Antibacterial Activity Of Rosemary Essential Oil (Rosmarinus Officinalis L.) In A Semi-Arid Region (Djelfa)

TELIBI Arbia[1], MOUAS Yamina[2], AISSAOUI Regadia [3]
[1]University of Blida1, Faculty of Natural and Life Sciences – Department of Biotechnology, Research Laboratory in Biotechnology of Plant Production, BP 270, route Soumaa, Blida, Algeria.
telibiarbia@gmail.com
[2]University of Blida1, Faculty of Natural and Life Sciences – Department of Biotechnology, Research Laboratory in Biotechnology of Plant Production, BP 270, route Soumaa, Blida, Algeria
aminaagro@outolook.com
[3]Laboratory of Exploration and Valorization of Steppic Ecosystems, Faculty of Natural and Life Sciences, University Ziane Achour of Djelfa, BP 3117 Djelfa – Algeria. Tele Laboratory of Organic Chemistry and Natural Materials, Faculty of Exact and Computer Sciences, Ziane Achour University in Djelfa
Corresponding author:
r.aissaoui@univ-djelfa.dz
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Abstract
The objective of this study was to evaluate the yield and antibacterial activity of essential oils from rosemary leaves (Rosmarinus officinalis L.) collected in the semi-arid region of Djelfa, Algeria, against three bacterial strains over different seasons. Essential oils were extracted by hydrodistillation. The antibacterial activity was tested using the disk diffusion method against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Oil yields were 0.7% in autumn, 0.2% in winter, 1.04% in spring, and 1.90% in summer. All tested bacterial strains were sensitive to rosemary essential oil in most seasons, but with variability depending on the strain and season. The findings highlight seasonal variation as a determining factor for both yield and antibacterial efficacy of rosemary essential oil.
Keywords: *Rosmarinus officinalis L.; Essential oil; Antibacterial activity; Seasonality variation; Semi-arid; Djelfa.*

INTRODUCTION

Aromatic and medicinal plants represent a valuable resource due to the progressive discovery of the applications of their bioactive molecules in healthcare, as well as their uses in various economic sectors such as cosmetics, pharmaceuticals, and the perfume industry. According to several authors, natural compounds offer a vast diversity of chemical structures and exhibit a wide range of biological activities **[1].** The antimicrobial properties of aromatic and medicinal plants have been recognized since antiquity; however, it was not until the early 20th century that scientific interest in these properties increased significantly **[2].**

Rosemary (Rosmarinus officinalis L.) is an aromatic herb belonging to the Lamiaceae family, valued for its antioxidant, antimicrobial, antispasmodic, emmenagogue, and antitumor properties. It is extensively used in pharmaceutical products and traditional medicine **[3].**

This species is among the most widely used medicinal plants globally and in Algeria. Its essential oil extracts are also widely utilized **[4].**

Current research predominantly focuses on the study of antioxidant and antimicrobial molecules such as vitamins, carotenoids, and polyphenols. Accordingly, this study aims to evaluate the yield and antibacterial activity of essential oils from Rosmarinus officinalis L. harvested across four different seasons, tested against three bacterial strains: Escherichia coli (Gram-negative), Pseudomonas aeruginosa (Gram-negative), and Staphylococcus aureus (Gram-positive).

2. MATERIALS AND METHODS

2.1. Study Area

The "Senalba Chergui" forest represents a small part of a forest and pastoral complex located in the Ouled Naîl mountains (Saharan Atlas). It extends over the mountainous hills over an area of 19,000 hectares,

northwest of the city of Djelfa (Fig. 1), this forest constitutes the last bulwark against the advance of the desert. The vegetation is dominated by three species: Aleppo pine, holm oak and juniper [5]. According to the ombrothermic diagram of precipitation and temperature recorded over the period 2004–2014, the climate of the region is characterized by a long dry season and a short rainy season, with very high average temperatures, this type of climate belongs to the category of semi-arid climates **[6].**

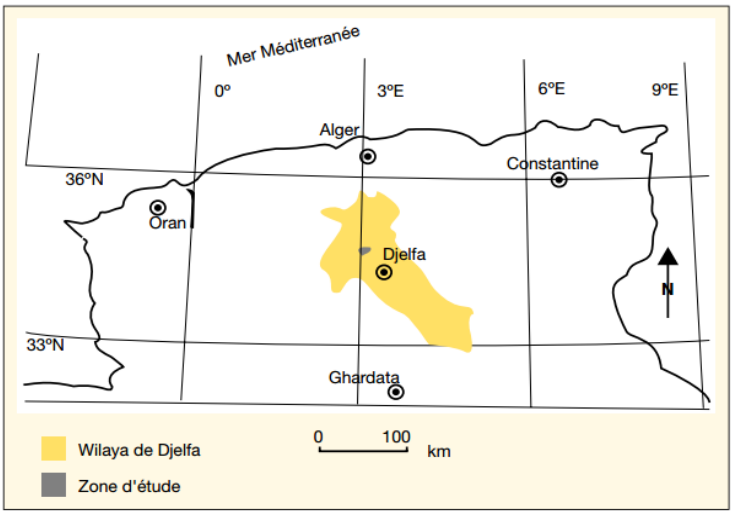

Figure 1: Location of the study area (Bencherif, 2010)

2.2. Plant Material and Extraction

Plant material

Leaves of Rosmarinus officinalis were randomly collected from healthy plants during September (autumn), December (winter), April (spring), and July (summer). Plant material was shade-dried at room temperature, until its weight stabilized.

Extraction of essential oil

100 g of dried leaves are poured into a flask containing distilled water, the whole is brought to a boil. The oil-laden vapors, passing through a condenser, condense and are collected in an Erleen Mayer flask. Sodium chloride is added until the distillate is saturated, then stirred to dissolve, a thin layer of essential oil appears on the surface. To recover it, the distillate is poured into a separating funnel and 100 ml of Dichloromethane are introduced. After stirring and decanting (liquid-liquid extraction), two phases appear: a lower organic phase and an upper aqueous phase. The organic phase is recovered (Dichloromethane + essential oil), then anhydrous magnesium sulfate is added to remove traces of water. After filtration, a solution is obtained. The solvent is then evaporated using a rotary evaporator. The essential oil recovered after evaporation is quantified (weighed).

Determination of extraction yield

According to AFNOR standards, the essential oil yield (EOY) is defined as the ratio between the mass of essential oil obtained after extraction (M') and the mass of the plant material used (M). The yield is expressed as a percentage by the following formula: EOY (%) = (M'/M) ×100 **[7].**

2.3. Antibacterial Activity Testing

The antibacterial efficacy was determined by the disc diffusion method on Mueller-Hinton agar. Bacterial inocula (adjusted to 0.5 McFarland standard) included Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 27853), and Pseudomonas aeruginosa (ATCC 25923). Sterile 6-mm discs were impregnated with the essential oil and placed on inoculated agar. After incubation at 37°C for 24 hours, the diameter of inhibition zones was measured and used to classify sensitivity.

Antibacterial test

Seasonal assessment of antibacterial activity of the essential oil of our species study is carried out by the disc diffusion method This method has the advantage of being very flexible in the choice of essential oils tested. Sterile Mueller Hinton agar is poured into Petri dishes at a rate of 15 ml per dish and then left to cool. The bacterial inoculum, adjusted to 0.5 McFarland, is rubbed over the entire surface of the Mueller Hinton from top to bottom in tight streaks [8]. Wattman paper discs No. 4, 6 mm in diameter, are sterilized in aluminum foil in an autoclave at 121 °C for 15 minutes, three discs per box, are removed using sterilized forceps, then soaked with rosemary essential oil (crude) until the disc is completely

impregnated, the discs thus treated are placed on the surface of the inoculated agar diffused, the boxes are incubated in an oven at 37°C for 24 hours, the absence of microbial growth is reflected by a translucent halo around the disc, identical to sterile agar, the diameter of which is measured using a caliper, the sensitivity to oil was classified by the diameter of the inhibition halos: not sensitive (-) for diameters less than 8 mm; sensitive (+) for diameters from 8 to 14 mm; very sensitive (++) for diameters of 15 to 19mm and extremely sensitive (+++) for diameters over 20mm **[9].**

Statistical analysis

For each bacterial strain the tests were repeated ten (10) times for each essential oil of the season (S1, S2, S3, S4) and discs impregnated in DMSO were also used as negative controls. 50 tests in total. The results obtained were processed by analysis of variance (MANOVA) at a probability level of 5%, and by software Excel.

3. RESULTS AND DISCUSSION

3.1. Essential Oil Yield and Characterization

The yields of essential oil varied with the season: autumn (0.7%), winter (0.2%), spring (1.04%), and summer (1.90%) **Fig. 2**. Organoleptic properties are summarized in **Table 1**.

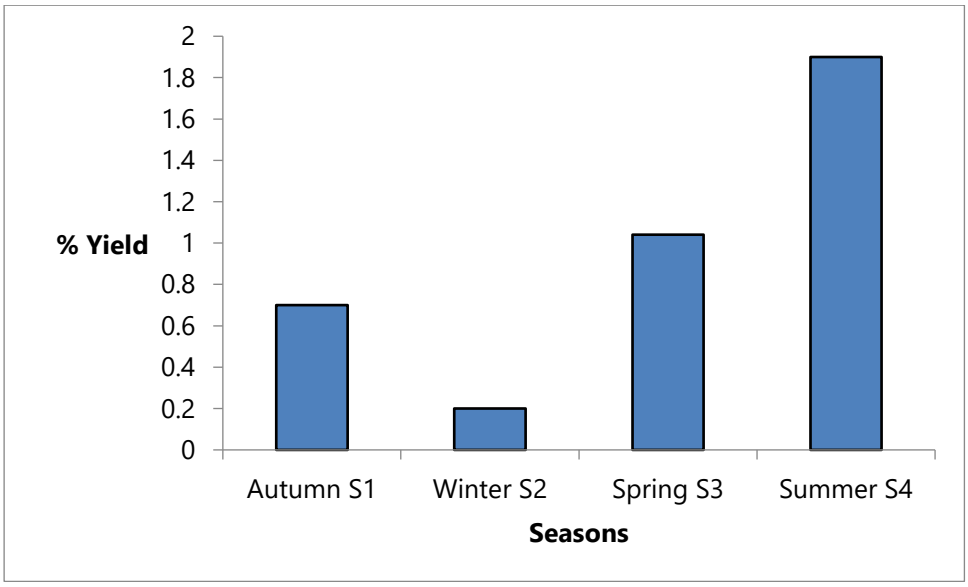

Figure 2: Yields of rosemary essential oil depending on the season

Table 1: Organoleptic characteristics of rosemary essential oil according to the 4 seasons.

Characteristics of rosemary essential oil			Season
Smell	Color	Appearance	
More camphor	Dark yellow	Mobile liquid	S1
Less camphorated	Pale yellow	Mobile liquid	S2
More camphor	yellow	Mobile liquid	S3
less camphorated	Pale yellow	Mobile liquid	S4

3.2. Antibacterial Activity Against Escherichia coli

The Rosemary essential oils from seasons S1, S3 and S4 exhibit significant antibacterial activity against Escherichia coli, with respective inhibition zones of 13.33 mm, 11.33 mm and 9 mm. In contrast, the essential oil from season S2 exhibits no antibacterial activity against Escherichia coli. Thus, our results indicate that the Escherichia coli bacterial strain is sensitive to the essential oil of our study species **Fig. 3**.

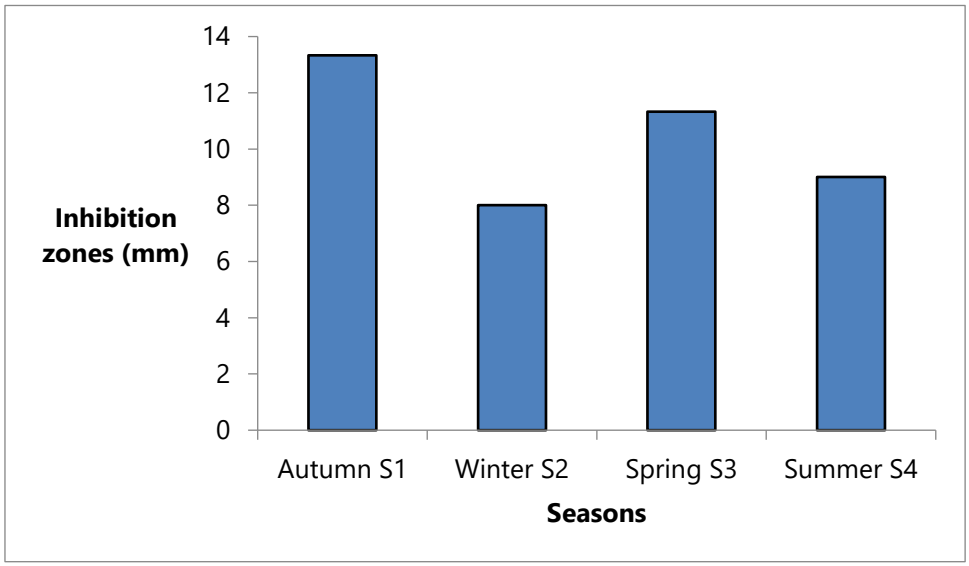

Figure 3: Antibacterial power of the essential oil of four-season rosemary (S1, S2, S3 and S4) against Escherichia coli

3.3. Antibacterial Activity Against Staphylococcus aureus

The bacterial strain Staphylococcus aureus was highly sensitive to rosemary essential oil S4, with an average inhibition of 17.66 mm. Rosemary essential oils S1 and S3 inhibited the growth of Staphylococcus aureus with inhibition zones of 12.33 mm and 14.33 mm, respectively. In contrast, the tested bacterial strain was highly resistant to S2 essential oil, with an average inhibition of 7.66 mm **Fig. 4**

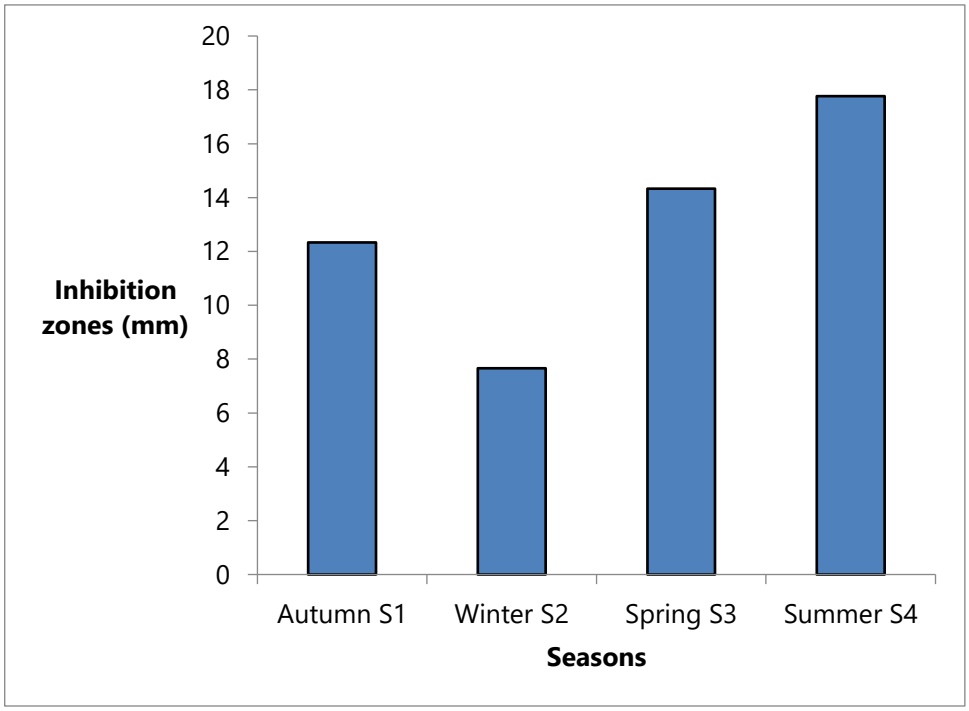

Figure 4: Antibacterial power of the essential oil of four-season rosemary (S1, S2, S3, S4) against Staphylococcus aureus

3.4. Antibacterial Activity Against Pseudomonas aeruginosa

The essential oil of our study species from season S1 exhibited antibacterial potency against the bacterial strain Pseudomonas aeruginosa, with an average inhibition of 10.30 mm. In contrast, the essential oils from seasons S2, S3, and S4 did not inhibit the growth of the same bacterial strain, with respective inhibition diameters of 8.30 mm, 7.23 mm, and 7 mm **Fig. 5.**

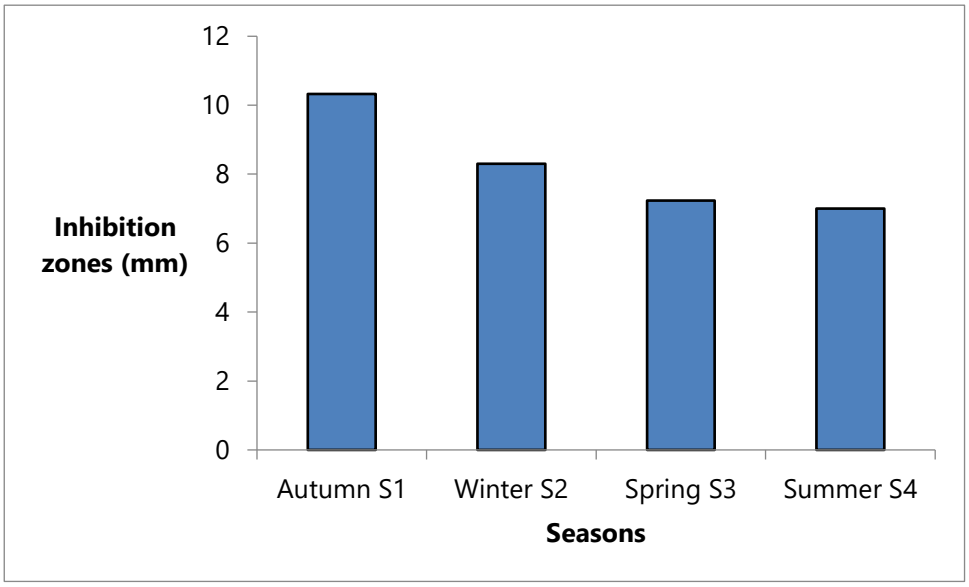

Figure 5: Antibacterial power of the essential oil of four-season rosemary (S1, S2, S3, S4) against Pseudomonas aeruginosa

DISCUSSION

Extraction of the essential oil

The results obtained from the extraction of the essential oil of Rosmarinus officinalis L. during the four seasons show the existence of a notable difference between the yields. The summer yield is the highest, estimated at 1.9%, followed by the spring yield (1.04%), while the lowest yields are recorded in autumn (0.7%) and winter (0.2%), these variations are explained by several factors: ecological conditions, especially climatic (temperature and humidity), the plant species itself, the organ used, the stage of development, the harvest period, the conditions of conservation of the plant material and the extraction method **[10], [11].**

The organoleptic parameters of rosemary essential oil are in accordance with those listed in AFNOR standards **[7]**, with a mobile liquid appearance, a yellow color and more or less camphor.

Seasonal evaluation of the antibacterial activity of rosemary essential oil

The disk diffusion method allowed us to highlight the antibacterial power of the essential oil against the three bacterial strains tested, according to the classification of **[10].** All strains are sensitive to the essential oil of autumn (S1) and resistant to that of winter (S2). E. coli (Gram -) and Staphylococcus aureus (Gram +) strains are sensitive to essential oils of seasons S3 and S4, while Pseudomonas aeruginosa (Gram -) is resistant to essential oils of the same seasons. The sensitivity of a microorganism to an essential oil depends on its properties as well as the type of microorganism **[12].**

The hypersensitivity of Staphylococcus aureus ATCC strain can be explained by the likelihood of sensitivity of Gram (+) bacteria to external environmental changes, such as temperature, pH and natural extracts due to the absence of the outer membrane **[13].**

The Gram-negative bacterium Pseudomonas aeruginosa is the most resistant. Several studies have confirmed the high resistance of Gram (−) bacteria compared to Gram (+) ones. This observation can be attributed, on the one hand, to the action of certain volatile compounds in the essential oil studied, and on the other hand, to the presence of a lipopolysaccharide (LPS) layer in Gram (−) bacteria, which could function as an effective barrier against any incoming biomolecule [14]. The bacterial strain Escherichia coli is, on the other hand, sensitive, although it is also Gram-negative. It is postulated that the different components of essential oils show varying degrees of activity against Gram (−) and Gram (+) bacteria **[15].** On the other hand, the activity of essential oils and extracts of Oregano and Laurel plants shows an inhibitory power on Pseudomonas aeruginosa **[16].**

The composition of essential oils of the same species varies according to geographical location, climatic conditions, harvest period, part of the plant used, etc. Consequently, their antimicrobial properties also vary **[17].**

4. CONCLUSION

In the context of expanding knowledge on the antibacterial properties of medicinal and aromatic plants in semi-arid areas, we studied the effect of seasonal variation on the yield and antibacterial activity of the essential oil from the leaves of Rosmarinus officinalis L., known by the vernacular name "klileldjabel". It is a medicinal plant belonging to the Lamiaceae family, widely used in traditional medicine in the Arab world. The extraction of the essential oil was carried out by hydrodistillation. The yields obtained were 0.7%, 0.2%, 1.04% and 1.90% for autumn, winter, spring and summer, respectively. Concerning the antibacterial activity, the aromatogram method made it possible to highlight the inhibitory power of rosemary essential oils, harvested during the four seasons, against the three bacterial strains tested (E. coli Gram –, Pseudomonas aeruginosa Gram –, Staphylococcus aureus Gram +). The three strains were found to be sensitive to the autumn essential oil, and resistant to the winter one. The E. coli (Gram –) and Staphylococcus aureus (Gram +) strains showed sensitivity to the spring and summer essential oil, while Pseudomonas aeruginosa (Gram –) remained resistant. Finally, our results indicate that the essential oil of Rosmarinus officinalis L. presents an interesting antibacterial activity. However, these results obtained in vitro constitute only a first step in the search for natural substances with biological activity. Further tests will be necessary to confirm the observed performances. New perspectives could be explored, in particular by studying in more depth the antibacterial activity of essential oils, whether used alone, by their main components, or in combination, in order to highlight possible synergies. It would also be relevant to continue this work on other pathogenic bacteria, in order to validate or not the effectiveness of Rosmarinusofficinalis essential oil.

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REFERENCES

[1] Bérubé-Gagnon J. (2006). Isolation and identification of antibiotic compounds from Picea mariana bark. MSc Thesis, Department of Renewable Resources, Quebec.

[2] Yano Y., Satomi M.,&Oikawa H. (2006). Antimicrobial effect of spices and herbs on Vibrio parahaemolyticus. International Journal of Food Microbiology, 111:6-11.

[3] Atikbekkara F., Bousmaha L., Talebbendiab S.A., Boti J.B.,&Casanova J. (2007).

Chemical composition of the essential oil of Rosmarinus officinalis L. growing wild and cultivated in the Tlemcen region. Biology&Health, 7:6-11.

[4] Hostettmann K. (1997). Learn all about the power of plants. ED Favre SA, Lausanne, Switzerland.

[5] Bencherif K. (2010). Drought research. Department of Forestry, Faculty of Science, 21(3):179-186.

[6] Bouafia L.&Boubekeur H. (2014). Physiological and biochemical responses of rosemary to abiotic stress in the Djelfa region. MSc Thesis, Biology APV, University of Djelfa, p.16.

[7] AFNOR Standard. (2000). Collection of standards: essential oils. Volume 2. Paris: AFNOR.

Bendahou M., Benyoucef M., Benkada D., Soussa Elisa M.B.D., Galvao E.L., Marque M.M.O., **[8]** Muselli A., Desjobert J.M., Bernardini A.F.,&Costa J. (2007). Influence of the extraction process on essential oil of Origanum glandulosum. Journal of Applied Sciences, 7(8):1152-1157.

[9] Ponce A.G., Fritz R., Delvalle C.,&Roura S.I. (2003). Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensmittel-Wissenschaft und Technologie, 36:679-684.

[10] Granger M.M.R., Passet J., & Arbousset G. (1973). The essence of Rosmarinus officinalis L.: Influence of the method of processing the plant material. Parfums Cosmétiques Savons France, 3(3):133-137.

[11] Rosua J.L. & Granados A.G. (1987). Analysis of essential oils of species of the genus rosemary and their interest as a taxonomic character. Medicinal Plants and Phytotherapy, XXI (2):138-143.

[12] Kalemba D.&Kunicka A. (2003). Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 10:813-829.

[13] Balentine C.W., Crandall P.G., O'Bryan C.A., Duong D.Q.,&Pohlman F.W. (2006). The pre- and post-grinding application of rosemary and its effects on lipid oxidation and colour during storage of ground beef. Meat Science, 73:413-421.

[14] Inouye S., Yamaguchi H.,&Takizawa T. (2001). Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens using a modified dilution assay method. Journal of Infection and Chemotherapy, 7(4):251-254.

[15] Dorman H.J.&Deans S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2):308-316.

[16] Ohno T., Kita M., Yamaoka Y., Imamura S., Yamamoto S.M., Kodama T., Kashima K.,&Imanishi J. (2003). Antimicrobial activity of essential oil against Helicobacter pylori. Helicobacter, 8(3):207-215.

[17] Stéphane C.&Monique L. (2007). Essential oils: their antimicrobial properties and their potential applications in food. RESALA, Laboratory in Applied Food Sciences, INRS-Institut Armand-Frappier, p.7.