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Physicochemical Characterization and Antimicrobial Activity of Essential Oil from *Rosmarinus officinalis L,* Growing in the Wild West Saharan Atlas of Algeria

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Abstract - The antimicrobial activity of *Rosmarinus officinalis L* essential oil has been recognized for a long time. In the present study, the antimicrobial properties of the essential oils obtained from the leaves of *R. officinalis L*, have been investigated against six strains of bacteria and six fungi strains.

R. officinalis L, provided a yield of 1.5% of essential oil. The Physicochemical characterization shows that refractive index is about (1.467). Specific density at 20 °C (0.949), optical rotation (+11.7), colour and odour were also determined.
R. Officinalis L. essential oil exhibited stronger antimicrobial activity, with a maximum inhibition zone of 28 mm against *E. Faecalis*. In addition, the essential oil has a remarkable antifungal activity. *P. Escpansum* is the most sensitive strain with an inhibition diameter of 24 mm. All fungal strains were inhibited at a low concentration of 1/600.

Keywords: Essential oil, *Rosmarinus officinalis L*, antimicrobial activity, physicochemical characterization.

1.Introduction

Essential oils and extracts from aromatic plants have long been used for a wide variety of medicinal and domestic purposes. Antimicrobial properties of essential oils obtained from aerial parts and seeds of some aromatic plants such as *Matricaria pubescens* (Desf.), *Rosmarinus officinalis* L and *Artimisia herba alba* have been investigated and reviewed against food-related microorganisms as well as their applications in food systems (Gachkar et *al.*,2007 ; Atik bekkara et *al.*,2007;

Chanthaphon *et al.*, 2008 ; Makhloufi *et al.*, 2012 ; Makhloufi *et al.*,2015).

Rosemary has been the subject of recent research in the pharmaceutical and food industries. It has excellent antioxidant and antimicrobial properties, due to certain compounds (carnosol, carnosic acid, ursolic acid, betulinic acid, and the rosmaridiphenol rosmanol) (Thoresen *et al.*,2003; Boutabia *et al.*, 2016).

R. officinalis L. is a shrub belonging to the Lamiaceae family, in the Mediterranean area, including Algeria, which lies on the arid slopes and hills (Dellile,2007; Makhloufi,2012). In the west Saharan Atlas of Algeria, it is quite frequent in the mountains of Mourghad, Mekthern, Bouaboud, and Founassa, etc.

2. Materials and Methods

R. officinalis L. specimens were collected from Morghad Mountain in the region of Founassa (32°30'43.047" N de latitude, 0°50'9.669" O), Saharan Atlas, West of Ain Sefra (Figure 1) during December 2016, and January 2017. These biomasses were dried for fifteen days in the dark at an ambient laboratory temperature (20-28 °C).

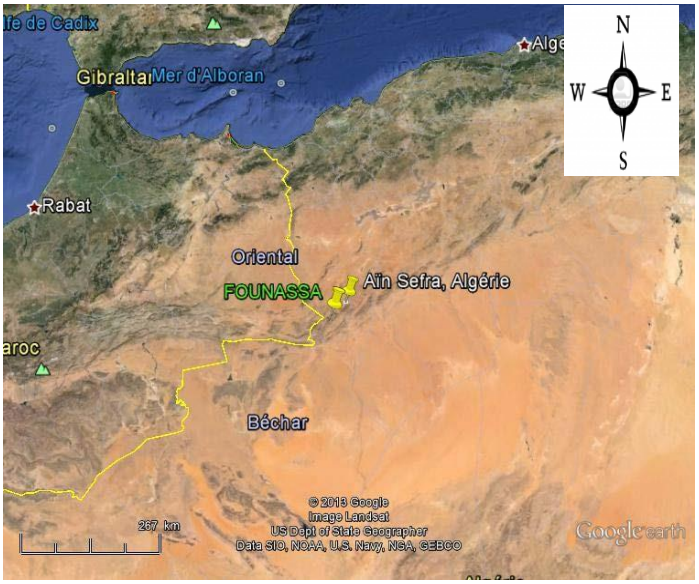

Figure 1: Geographical Situation of the Study Area (Founassa) (Bouyahiaoui, 2017)

2.1. Distillation of the Essential Oil

The dried aerial parts were ground before the operation, and then, 100 g of ground *R. officinalis L* were submitted to hydrodistillation for 3h using a Clevenger apparatus (Amarti et al., 2008; Makhloufi *et al.*, 2011: Akermi *et al.*,2017). The distilled essential oils were dried over anhydrous sodium sulphate, filtered and stored at +4 °C until it was used (Chanthaphon *et al.*, 2008;Ayoughi *et al.*, 2011).

2.2. Physical and Chemical characterization of essential oil *R. officinalis L.*

Methods conform to A.F.N.O.R.(1986) have determined the specific density at 20°C ; Optical Rotation (OR) was measured with a polarimeter. The refractive index (RI) was measured with a refractometer at

20ºC. The methods used for the determination of the acid index and ester are also used following the AFNOR standards.

2.3 Antimicrobial activity

2.3.1 Microbial strains

The antimicrobial activity was evaluated by paper disc diffusion and dilution methods against six selected fungi, namely: *Aspergillus niger*, *A.flavus(1)* , *Penicillium purpurogenum(Isolated from dates)*, *P.Jensinii*, *P.escpansum* and , *A. flavus (2) isolated from Wheat, and* six selected Gram-positive and Gram-negative species: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC27853, *Bacillus cereus* ATCC 11778, *Enterococcus feacalis* ATCC 29212 and *Listeria monocytogenes* ATCC19115.

2.3.2 Disc diffusion method

The agar disc diffusion method was employed for the determination of antifungal activities of the essential oil in question. Briefly, the fungal cultures were grown on PDA. The 7-day old culture of mycelial mat was washed, and then suspended in normal saline solution. The colony forming units (CFU/ml) of suspension of the test fungus was determined and test inoculum was adjusted to 10^6 CFU/ml. These conidia were used for antifungal essay tests. Inocula (0.1ml) were applied on the surface of the PDA plate and spread using a sterile glass spreader (Bansod & Rai, 2008). The qualitative antibacterial was performed using culture growth at 37 °C for 18 h and adjusted to approximately 10^8 colony forming unit per milliliter (CFU/ml). The culture medium used for the bacteria was Mueller Hinton Agar (MHA) (Gachkar et al., 2007). Five hundred microliters of the inoculums were spread over plates containing MHA and a Whatman paper disc (6 mm in diameter) were impregnated with 10 µl of the undiluted oil and were placed on the inoculated plates. The plates were left for 30 min at room temperature, and incubated at 37 °C for 24 h (Shunying *et al*., 2005; Bekhchi *et al*., 2008; bourkhiss *et al*., 2007). The diameters of the inhibition zones were measured in millimeters. Control assay discs were also used; all tests were performed in triplicate (Yesil *et al*., 2007).

2.3.3 Dilution method (MIC)

Antimicrobial tests were performed according to the method reported by Remmal et al. (1993), and Farah et al. (2001). The essential oil is emulsified with an agar solution of 0.2% in order to disperse the compounds and improve their contact with the tested germs, before being diluted to one tenth in the agar solution. Quantities of this dilution were added to

test tubes containing Mueller Hinton agar for bacteria, and PDA for fungi. The final concentrations of essential oil are from 1 / 100, to1 / 1000 (v / v). In parallel, Control assay containing only the culture medium and agar solution at 0.2% were also used. The MIC is the lowest concentration of essential oil giving no visible growth to the naked eye (Makhloufi *et al.*,2011).

3. Results and discussion

The essential oil, with pale yellow colors, was obtained by hydrodistillation from *R. officinalis L* with the yield of 1.5 % (v/w) on dry weight basis.

This yield is comparable to that quoted by Hilan *et al.* (2006) (1.52%) and is higher than that quoted by Bekkara Atik *et al.* (2007) (0.8%), Biljana *et al.* (2007) (1.18 %). In contrast, this performance was revealed lower than that given by the same species from different regions such as Sardinia whose performance can reach 1.75% (Angioni *et al.*, 2004).

Table 01: Physico-chemical index of essential oil of *R. officinalis L.*

Refractive index	Optical Rotation	**Specific density at 20 °C**	**Ester index**	**Acid index**
1.467	+11.70	0.949	18.50	0,550

As can be seen from Table 1, the major differences were found in density and Refractive index. The main properties of *R. officinalis* of Campestre da Serra (Rio Grande do Sul State) : The physico-chemical parameters averaged 0.8887 g/cm3 for specific gravity, 1.4689 for refractive index, and +11.82° for optical rotation, and there were no significant variations in either the chemical or physico-chemical data in the different years (Atti-Santos,2005), for Morocco the density is about 0.898 and the Refractive index equals 1.468. (EL Kamli *et al.*, 2017)

The Physico-chemical properties of *R. officinalis* essential oil and yield and chemical composition depend on environmental factors which include cultivation and habitat climate conditions (temperature, humidity, radiation, wind, soil properties, geographical location, and harvest time and methods), and postharvest techniques **.**

The Physico-Chemical Characteristics Value of British Pharmacopoeia (2008) are: Relative density 0.895 to 0.920, Refractive index 1.464 to 1.473, Optical

rotation -5 to 8 , the Appearance between Colourless to pale yellow liquid .

3.1. Antimicrobial activity

3.1.1. Disc diffusion assay

The growth inhibition zones measured by disc diffusion method are presented in Fig. 2and 3. According to these results, the essential oil of *R. officinalis L.* have great antimicrobial activity against most of the investigated strains. The diameters of growth inhibition zone ranged from 16 to 28 mm (including the diameter of the disc- 6 mm) with the highest inhibition zone values observed against *E. faecalis* (28mm)for Bacteria and *P.escpansum* (24mm) for fungi.

As For the direct contact method, the results differ depending on the seeds used (Table 2 and 3). in vitro, *R. officinalis L.* essential oil proved to have a good inhibitory activity against the tested germs. However, the microorganisms studied did not show the same sensitivity against the essential oil. Rosemary essential oil inhibited both fungal agents tested at a concentration of 1/600 v/v.

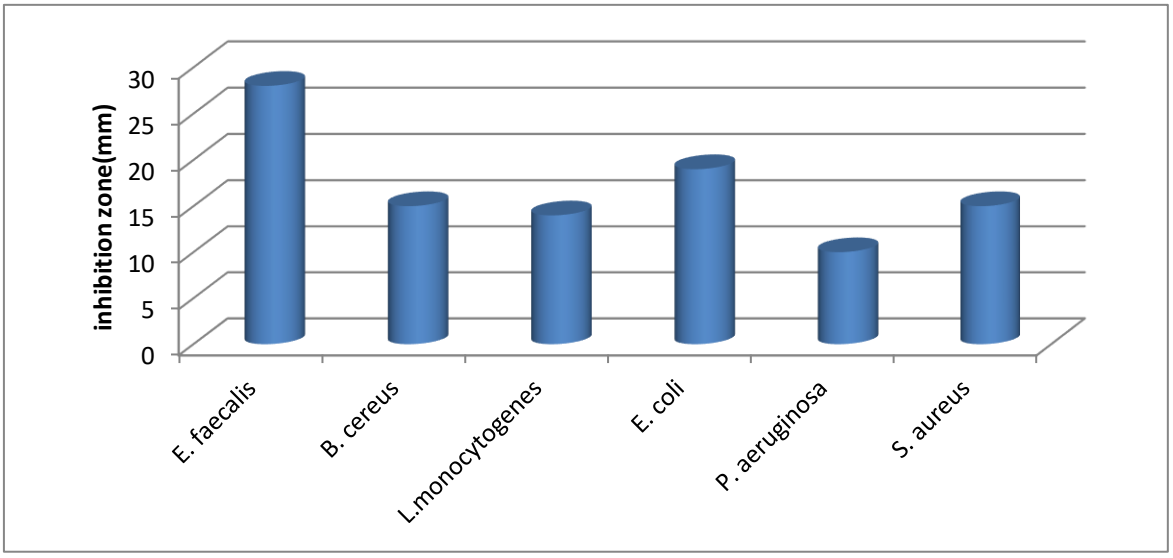

Figure 02 : Antibacterial activity of essential oil (EO) by disc diffusion assay

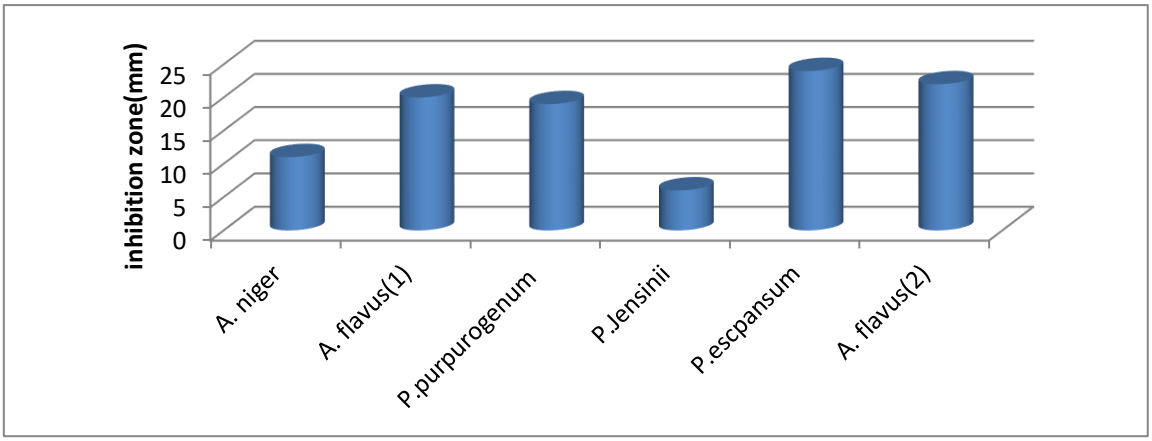

Figure 03: Antifungal activity of essential oil (EO) by disc diffusion assay

Table 02: MIC of essential oil (EO)(v/v) on bacteria strains

Concentrations / Bacteria	1/10	1/25	1/50	1/75	1/100	1/125	1/150	1/175
P.aeruginosa	-	-	-	+	+	+	+	+
S.aureus	-	-	+	+	+	+	+	+
B.cereus	-	-	+	+	+	+	+	+
L.monocytogenes	-	-	-	+	+	+	+	+
E.faecalis	-	-	-	-	-	+	+	+
E.coli	-	-	+	+	+	+	+	+

Table 03: MIC of essential oil (EO)(v/v) on fungal strains

Concentrations / Fungi	1/500	1/550	1/600	1/750	1/800	1/850	1/900	1/950
A.niger	-	-	-	+	+	+	+	+
A.flavus(1)	-	-	-	+	+	+	+	+
P.purpurogenum	-	-	-	-	+	+	+	+
P.jensinii	-	-	-	-	+	+	+	+
A.flavus(2)	-	-	-	+	+	+	+	+
P.escpansum	-	-	-	-	+	+	+	+

(-):Inhibition (+): Growth

The data indicated that Gram-positive bacteria were more sensitive to essential oils than to Gram-negative bacteria due to their outer membrane barriers (Burt, 2004). Among Gram-positive bacteria, *E. faecalis* was the most sensitive to essential oil from rosemary (MIC 1/100 v/v) whereas *S.aureus* was the most resistant (MIC 1/25v/v). Several researchers reported that *R. officinalis L* essential oil inhibited some Gram-positive and negative bacteria (*S.aureus, L.monocytogenes, E.coli….)* (Mathlouthi *et al.*, 2009; Rozman and Jeršek, 2009). Moreno *et al*. (2006) reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol.

4. Conclusion

In conclusion, Hydro distillated-essential oil from *R. officinalis L*. has a good inhibitory activity. The strong antifungal activity of *R. officinalis L.* against filamentous fungi strains is an indication of the broad spectrum of the oil antifungal potential. This Activity could be a natural alternative to synthetic antimicrobial and preservatives to enhance the safety and the shelf life of food.

5. Acknowledgments

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6. References

-A.F.N.O.R. (Association française de normalisation). (1986). Recueil des normes françaises « Les huiles essentielles » », Ed A.F.N.O.R., Tour Europe, 2ème Ed. Paris.

- Akermi M. M, Moussaoui A, Makhloufi A, Dalil H (2017). Phytochemistry, antimicrobial activities of the essential oils of the branches of Juniperus phoenicea in Bechar (Algeria). Applied Biology in Saharan Areas Vol. 1, N. 2, p. 1-7, December 2017

-Amarti F, Satrani B, Aafi A, Ghanmi M, Farah A, Aberchane M ,El Ajjouri M, El Antry S , Chaouch A (2008). Composition chimique et activité antimicrobienne des huiles essentielles de Thymus capitatus et de Thymus bleicherianus du Maroc.J Phytothérapie.(6) :342–347 .

- A. Angioni, A. Barra, E. Cereti, D. Barile, J.D. Coïsson, M. Arlorio and P. Cabras. (2004). Chemical composition, plant genetic differences, anti- microbial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis L*. J. Agricult. Food Chem., 52,11, 3530-3535

-Atik bekkara, F., Bousmaha, L., Taleb bendiab, S.A., Boti, J.B., Casanova, J., (2007). Composition chimique de l'huile essentielle de *Rosmarinus officinalis L poussant* à l'état spontané et cultivé de la région de Tlemcen. *J Biologie & Santé* vol. 7, n° 1,p. 6-11.

- Atti-Santos A; Rossato M; Gabriel Pauletti F; Duarte Rota L; Rech J; Pansera M; Agostini F; Atti Serafini L; Moyna P.(2005). Physico-chemical evaluation of *Rosmarinus officinalis L.* essential oils. Braz. Arch. biol. Technol . vol.48 no.6 Curitiba Nov. 2005

-Ayoughi F,Barzegar M,Sahari MA, Naghdibadi H (2011). Chemical Compositions of Essential Oils of *Artemisia dracunculus* L. and Endemic *Matricaria chamomilla* L. and an Evaluation of their Antioxidative Effects. *J. Agr. Sci. Tech.* 13:79-88

- Bansod S and Rai M (2008). Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic *Aspergillus fumigatus* and *A. niger. World Journal of Medical Sciences,* 3(2): 81-88.

-Bekhechi C,Atik-Bekkara F,Abdelouahid JD (2008) . Composition et activité antibactérienne des huiles essentielles d'Origanum glandulosum d'Algérie : J Phytothérapie. 6 :153-159.

-Bourkhiss M, Hnach M,Bourkhiss B,Ouhssine M ,Chaouch A (2007). Composition chimique et propriétés antimicrobiennes de l'huile essentielle extraite des feuilles de Tetraclinis articulata (Vahl) du Maroc .J Afrique Science, 03(2) :232 – 242.

-British Pharmacopoeia Commission. British Pharmacopoeia (2008); London: Stationery Office.

-Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods: a review. Int. J. Food Microbiol. 94: 223-253.

-Chanthaphon S,Chanthachum S, Hongpattarakere T (2008). Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. Songklanakarin J. Sci. Technol. 30 (Suppl.1): 125-131

-Delille, L. , (2007).les plantes médicinales d'Algérie. Édition BERTI. Alger,122

-Farah A, Satrani B, Fechtal M, Chaouch A, Talbi M (2001) . Composition chimique et activités antibactérienne et antifongique des huiles essentielles d'*Eucalyptus camaldulensis* et son hybride naturel (clone 583) . *Acta Bot. Gall.* 148(3) : 183-190.

-Gachkar L , Yadegari D , Bagher Rezaei M, Taghizadeh M, Alipoor Astaneh S, Rasooli I (2007). Chemical and biological characteristics of Cuminum cyminum and *Rosmarinus officinalis* essential oils.Food Chemistry ,102 , 898–904

-Hilan C, Sfeir R, Jawish D et Aitour S (2006). Huiles essentielles de certaines Plantes médicinales libanaises de la Famille des *lamiaceae : Lebanese Science Journal*,7(2).

-Kaloustian J,Chevalier J, Mikail C,Martino M, Abou L, Vergnes MF (2008) . Étude de six huiles essentielles : composition chimique et activité antibactérienne . J Phytothérapie. 6 :160-164 .

- Makhloufi A, Moussaoui A, H.A. Lazouni, N. Hasnat and D.E. Abdelouahid ,(2011). Antifungal activity of Rosmarinus officinalis L. and its impact on the conservation of a local variety of dates.Medicinal Plants, 3(2) June 2011.

-Makhloufi A,(2012) Etude de l'activité biologique de deux plantes médicinales de la région de Bechar (Matricaria pubescens (Desf.) et Rosmarinus officinalis L) et leur impact sur la conservation des dattes et du beurre cru. Thèse de doctorat en science en biologie ,Université de Tlemcen.

-Makhloufi, A. , Moussaoui ,A., Lazouni ,H.A, (2012). Antibacterial activities of essential oil and crude extracts *from Matricaria pubescens* (Desf.) growing wild in Bechar, South west of Algeria. Journal of Medicinal Plants Research Vol. 6(16), pp. 3124-3128.

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