AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

Phytochemistry and Antibacterial Activity of Essential Oils of Two Species of Rosmarinus the High Atlas Morocco

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ARTICLE INFO

Article history:
Received 8 August 2014
Received in revised form
12 September 2014
Accepted 25 September 2014
Available online 2 November 2014

Keywords:
Rosmarinus officinalis, Rosmarinus Eriocalyx, essential oils, Phytochemistry,Antibacterial Activity, GC-MC,chemical composition.

ABSTRACT

Background: Rosemary is a well known aromatic and medicinal plant whose consumption serves to remedy number of disorders. Its Essential oil (EO) constitutes an important ingredient for well-being feeling improvement through beauty products such as soaps, perfumes, deodorants. In Morocco, two species of rosemary which have never been studied are mentioned. They are *Rosmarinus officinalis*. L and *Rosmarinus Eriocalyx*. **Objective:** This study was designed to valorize rosemary species: *Rosmarinus officinalis* and *Rosmarinus eriocalyx* harvested in Eastern Moroccan High Atlas. It reveals chemical composition (non-volatile and volatile) and antibacterial activity of their essential oils. **Results:** The species are rich in flavonoids, tannins, sterols, triterpenes, saponins, free anthraquinones, mucilages, cardiac glycosides and catechols. But tests for alkaloids, reducing compounds, carotenoids, holosids,tetrahydrocannabinols and coumarins were negative. Average yields of essential oils from aerial part of *Rosmarinus officinalis* and *Rosmarinus eriocalyx* extracted by hydrodistillation are respectively 2.37% and 2.79%. Analytical studies of essential oils by GC / MS showed that both species have almost the same profil: α-pinene, β-pinene, camphene, camphor, borneol were found as predominant compounds and 1,8-cineole as chemotype with 43% for *Rosmarinus officinalis* and 45% for *Rosmarinus eriocalyx*. Results of microbiological tests showed antibacterial effects against the tested pathogens. Minimal Inhibitory Concentrations vary from 1.04 mg/ml to 16.7 mg/ml. **Conclusion:** This study on the species *Rosmarinus officinalis* and *Rosmarinus Eriocalyx* proved that 1, 8-cineole is the chemotypes. The phytochemical screening of the species has highlighted that both plants are rich in secondary metabolites. Preliminary results of antibacterial study showed in vitro efficiency of the species.

To Cite This Article: Kamal Fadili, Safae Ayane, Oussama Hadic, Fatima Elhilali, Youssef Khabbal, Touria Zair., Phytochemistry and antibacterial activity of essential oils of two species of Rosmarinus the high Atlas Morocco. ***Aust. J. Basic & Appl. Sci.***, *8(16): 287-295, 2014*

INTRODUCTION

Rosemary (*Rosmarinus officinalis*),which grows wild around Mediterranean area, is a species from Lamiaceae family. It grows as a shrub or herbaceous plant with about 0.8 to 2m height (Atik bekkara *et al*, 2007).This plant likes dry and arid regions, hills and low mountains, calcareous, shale, clay and rocky substrates (El Amrani *et al*, 1997).

Its use since ancient times in traditional medicine is justified by its antiseptic (Bult *et al*, 1985), antirheumatic (Makino *et al*, 2000), anti-inflammatory, antispasmodic (Juhas *et al*, 2009; .Beninca *et al*, 2011), antimicrobial and anti-hepatotoxic properties (E. Stefanovits-Banyai *et al* 2003). Its appreciation as a spice for seasoning and food preservation (Arnold *et al*, 1997) is supported by a very high antioxidant activity (Wang *et al*, 2008).

This antioxidant activity of *Rosmarinus officinalis* is due to its phenolic compounds including: carnosic acid, carnosol, rosmarinic acid and hydroxycinnamic acid ester (R. Inatani *et al*, 1983, Vassiliki G *et al* 2013).

Rosemary uptake improves memory and it is sometimes used as an antidepressant. It is also useful against cough and digestive disorders such as diarrhea, spasms and flatulence. Thanks to diuretic and antispasmodics properties (Bedrossain, 1999), aerial parts of rosemary are orally used to relieve renal colic and dysmenorrhoea (Gonzalez-Trujano *et al*, 2007).

Recent research shows that rosemary extracts possess strong anticancer properties (Vassiliki G *et al* 2013).

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Essential oil (EO) of rosemary widely used for centuries in beauty products manufacturing such as soaps, perfumes, deodorants (Arnold *et al*, 1997) has been the subject of many studies (Lawrence B.M.1997).Based on the main compounds identified in essential oils (1,8 cineole, camphor, borneol, ...) different chemotypes were identified and classified according to geographical and climatic regions. (El Amrani, 1999).

In Morocco, two species of rosemary are mentioned: *Rosmarinus officinalis*. L and *Rosmarinus Eriocalyx*. They are found in eastern, Atlas Rif, great Atlas, Midelt area, Debdou forest regions and rarely in the western region (Yakoubi, 1999). Moroccan oriental High Atlas region with its desert climate is characterized by an abundance of *Rosmarinus officinalis* L and *Rosmarinus eriocalyx* species which have never been studied. The aim of the present work is to give chemical and biological knowledge about these species: *Rosmarinus officinalis* L and *Rosmarinus eriocalyx,* through a study of their chemical composition (non-volatile and volatile compounds) and an evaluation of antibacterial activity of their essential oils. Bacterial species used in this work cause skin infections (*Staphylococcus aureus*), urinary tract infections (*Escherichia coli*) and nosocomial infections (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). In addition, emergence of multidrug resistance in these bacteria species causes treatment failure and constitutes a major public health problem.

MATERIALS AND METHODS

❖ ***Materials:***

- ***Plant material:***

Rosmarinus officinalis was collected in Mzyzle rural commune at 15Km from Rich in Eastern Moroccan High Atlas and *Rosmarinus Eriocalyx* was harvested in the region of Tizi Ntalghemt at 20 km south from Midelt. The samples were collected in March 2012 at the time of flowering and were dried in the shade for about ten days. Species identification was carried out at the Scientific Institute of Rabat in the laboratory of Floristic.

- ***Microbiological material:***

We chose four bacterial strains common in human pathology, belonging to Gram positive and Gram negative classes. The strains were isolated from neonatal unit at the University Hospital of Fez. Bacterial strains used were: *Pseudomonas aeruginosa* (Gram negative), *Escherichia coli* (Gram negative), *Klebsiella pneumoniae* (Gram negative) and *Staphylococcus aureus* (Gram positive). These bacterial species are responsible for skin infections (*Staphylococcus aureus*), urinary and digestive tract infections (*Escherichia coli*), and nosocomial infections (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*).

❖ ***Phytochemical Study:***

- ***Extraction of essential oils:***

Essential oils extraction was performed by hydrodistillation in a Clevenger-type apparatus for three hours. They were then dried with anhydrous sodium sulphate and stored in a refrigerator at 4°C until use. Yield of essential oil is evaluated after three extractions of dry plant material.

- ***Analysis and identification of essential oils constituents:***

Chromatographic analysis of Rosmarinus EOs samples was performed on a gas chromatograph Thermo Electron type (Trace GC Ultra) coupled with a mass spectrometer Thermo Electron Trace MS system type (Thermo Electron: Trace GC Ultra , Polaris Q MS), fragmentation is performed with 70 eV-intensity electron impact. The chromatograph is equipped with a DB-5 column (5% phenyl-methyl-siloxan) (30m x 0.25 mm x 0.25 microns film thickness) , a flame ionization detector (FID) supplied with H2/Air gas mixture. Column temperature progressively increases at a rate of 4 ° C / min from 50 to 200 ° C for 5 min. The injection mode is split. EOs' chemical compounds of both *Rosmarinus* species were identified based on comparison of their Kovats indices (IK) with those of known compounds in the literature (Kovats 1965, Adams, 2007). This step was supplemented by a comparison of indices and mass spectra with different references (Adams, 2007; National Institute of Standards and Technology, 2014). Kovats indices compare the retention time of an unknown product with the retention time of a linear alkane of the same carbon number. They are determined by injecting a mixture of alkane (C_7-C_{40} standard) under the same operating conditions.

- ***Phytochemical tests:***

Various chemical tests were performed with a phytochemical screening. It is a qualitative test based on color reactions and / or precipitation.

Characterization tests were made according to the protocol of Dohou *et al* (2003); JUDITH (2005); Diallo (2005); Bekro *et al* (2007); Bruneton, (2009) and N'Guessan *et al* (2009).

The extracts have been obtained by extraction with the following solvents: petroleum ether, methanol, ethanol, chloroform and distilled water.

The phytochemical screening is also based on the use of several reagents. Alkaloids search need Dragendorff reagent. Characterization of catechic tannins is done by isoamyl alcohol and hydrochloric acid, gallic tannins by Stiasny reagent, sodium acetate and ferric chloride. To detect sterols and triterpenes, we used acetic anhydride and concentrated sulfuric acid. Alcohol, diluted hydrochloric acid; magnesium chips and isoamyl alcohol were used to search flavonoids. Chloroform, diluted hydrochloric acid and ammonia were allowed to search quinone substances.

- ***Antibacterial activity:***
- ***Evaluation of antibacterial activity by disc-diffusion method:***

A bacterial suspension of 10^8 CFU / ml, made in isotonic sterile solution was inoculated by flooding on 90 mm-diameter Petri dishes containing Mueller Hinton agar medium. After drying, 6 mm- diameter sterile Whatman paper discs impregnated with 2µl of EO were placed on agar (a disc per box). Three repetitions were made. Inhibition diameters were read after 18 to 24 hours of incubation at 37 °C. A positive control was conducted with antibiotics: Nalidixic Acid 30µg (NA30), tetracycline30µg (TE30), amikacin30µg (AK30) and gentamicin 10µg (GM10).

The antibacterial activity is determined by measuring diameters of inhibition zones (mm) around the discs.

Sensitivity to oil was graded according to the diameter of inhibition zones **Ponce and al (2003)**: non sensitive (-) for diameters less than 8mm; sensitive (+) for diameters from 8 to 14mm; very sensitive (+ +) for diameters from 15 to 19mm and extremely sensitive (+ + +) for diameters over 20mm.

- ***Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in liquid medium:***

This method consists in preparing a standardized bacterial inoculum in sterile saline from a 18 to 24 hours-bacterial culture. Emulsion of tested essential oils was prepared with a dimethyl sulfoxide solution (40% DMSO). In test tubes containing 4 ml of Mueller Hinton broth and 40 µl of bacterial inocula (108UFC/ml), essential oils were aseptically added with different volumes. The final concentrations range C1 to C9 obtained was as follow: 0.26; 0.52; 1.04; 4.17; 8.35; 16.7 and 33.4 mg/ml and final bacterial inocula was 10^6CFU/ ml.

Three repetitions were made. Test tubes used as negative controls do not contain the extract and those who have served as positive controls contain Amoxicillin (4µg/ml). After incubation for 18-24 hours at 37 ° C, the MIC was determined.

MBC was evaluated after plating de 100 µl aliquot from each tube where no visible bacterial growth was observed. Petri dishes were then incubated at 37°C for 18 to 24 hours. MBC / MIC ratio allowed us to determine the bactericidal or bacteriostatic power of essential oils. If this ratio is greater than four, the essential oil has is bacteriostatic, otherwise it is bactericidal (Canillac N. and Mourey A., 2001).

RESULTS AND DISCUSSION

- ***Yield of essential oils:***

Yields of essential oil obtained from the aerial parts (leaves and flowers) are 2.37% for *Rosmarinus officinalis* and 2.79% for *Rosmarinus eriocalyx*.

Thus, our species are essential oil-rich compared to species from other countries such as *Rosmarinus officinalis* from Algeria (0. 8%) (Atik bekkara *et al,* 2007). The species from Tunisia yielded 1.17% for *typicus* breed and 2. 7% for the *troglodytorum* breed (Zaouali *et al*,2010).Results also showed that Eriocalyx species is richer in EO than officinalis species.

- ***Chemical composition of essential oils:***

Chemical composition of *Rosmarinus officinalis* and *Rosmarinus Eriocalyx* essential oils are described in Table 1.

Chemical analysis allowed us to identify 29 compounds, representing 99.16% for *Rosmarinus officinalis*' essential oil with major compounds: 1,8-cineole (43, 77%); Camphor (18.74%); α-pinene (9.49%); Borneol (5.88%); β-pinene (5.18%). For *Rosmarinus eriocalyx'* essential oil, 23 compounds were identified representing 99.33% with the following major compounds: 1,8-cineole (45.01%); Camphor (18.74%); α-pinene (11.29%); β-pinene (6.08%), borneol (5.14%) (Table1).

We found that both essential oils have 1,8-cineole as major component, so both species are 1,8-cineole chemotype. Both EOs are qualitatively similar, differences appear in a quantitative point of view. We note the absence of some compounds (Isobornylformate, Isoborneol, thujanol <iso-3> 0.1-terpineol, Delta-3-carene, Terpinolene) in *R.eriocalyx* EO while they are in low percentages in *R.officinalis*.

We also note that oxygenated monoterpenes fraction is the most abundant with 74.01% and 73.27% for *R. officinalis* and *R.eriocalyx* respectively.

Table 1: Chemical composition of *Rosmarinus officinalis* and *Rosmarinus Eriocalyx* essential oils.

N°	Constituents	Kovàts Index KI	Percentages %	
			R.officinalis	*R.eriocalyx*
1	Tricycline	926	0.17	0.12
2	α –thujene	930	0.34	0.10
3	α-pinene	939	9.49	11.29
4	Camphene	954	4.46	4.19
5	β- pinene	979	5.18	6.08
6	Myrcene	990	0.46	0.05
7	α –Phellandrène	1002	0.14	0.09
8	Delta-3-carene	1011	0.03	-
9	α - terpinene	1017	0.45	0.25
10	p-cymene	1024	1.15	1.08
11	1,8-Cineole	1031	43.77	45.01
12	γ-terpinene	1059	0.69	0.25
13	Cis-Sabinene hydrate	1070	0.20	0.10
14	Terpinolene	1088	0.24	-
15	Trans-Sabinene hydrate	1098	0.01	0.08
16	Linalol	1096	0.61	0.51
17	Sabina ketone<dehydro>	1022	0.04	0.06
18	1-terpineol	1133	0.05	-
19	Thujanol<iso-3>	1138	0.04	-
20	Camphor	1146	18.74	18.43
21	Isoborneol	1160	0.03	-
22	Borneol	1169	5.88	5.14
23	Terpinen-4-ol	1177	0.99	0.81
24	α-terpineol	1188	3.69	3.19
25	Isobornylformate	1239	0.02	-
26	Isobornylacetate	1285	1.62	0.88
27	E-caryophyllene	1419	0.46	1.06
28	Cubebol	1515	0.02	0.11
29	Caryophyllene oxide	1583	0.19	0.45
Monoterpenes %			22,84	23,56
Sesquiterpenes %			0,46	1,06
oxygenated Monoterpenes %			74,01	73,27
oxygenated Sesquiterpenes %			0,21	0,56
Esters %			1,64	0,88
Total			99,16	99,33

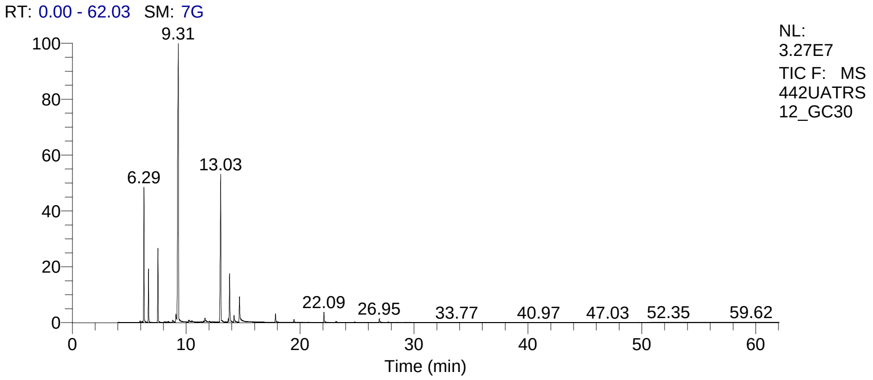


Fig. 1: *Rosmarinus eriocalyx' essential oil chromatogram*

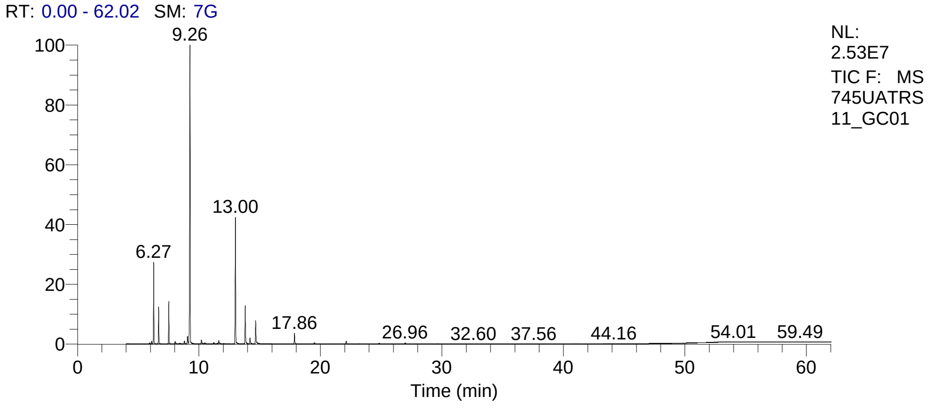


Fig. 2: *Rosmarinus officinalis' essential oil chromatogram*

Our results confirm those of El Amrani (1999) who found that Eastern Moroccan rosemary has 1,8-cineole chemotype with regional differences in 1,8-cineole'content that varies from 41% to 63 %.

Comparing our results with those of other researchers, we found that Rosemary's OE from Bibans area (Algeria) is characterized by 1,8-cineole (52.4%) followed by camphor (12.6 %) (Boutekedjiret C, 1998) whereas the sample from Bourj Bou Arreridj (Algeria) contains only 7.5% of 1, 8-cineole, camphor (12.1%), borneol (10.1%) and α-terpineol (9.5%) (Benhabiles N.E.H, 2001). Sample from El-Harrach's region (Algeria) contains α-pinene (43.6%), camphor (8, 6%), verbenone (7.65%), and limonene (5.53%) (Bousbia N, 2011).

*Rosmarinus officinalis'*EO from Tunisia is mainly composed of 1.8 cineole (40%), camphor (17.9%) and α-pinene (10.3%) (Zaouali *et al*, 2010).

In addition, rosemary harvested in Portugal is rich in myrcene (25%), 1,8-cineole, and camphor (Serrano *et al.*, 2002) while Rosemary from North East of Spain presents an EO containing camphor and α-pinene as main constituents (Guillen Maria D, 1995). Furthermore, essential oil of Lebanese rosemary is characterized by 1,8-Cineole (20%) and α-pinene (18.8 – 38.5%) (Diab *et al*, 2002).

The major compounds of Rosmarinus officinalis' essential oil from Eastern Cape Province in South Africa are : verbenone (17.43%), camphor (16.57%), 1,8 cineole (11.91%), α-pinene (11.47%), borneol (5.74%) and camphene (5.70%) (Okoh.O. *et al*, 2010).

Many factors affect yield and chemical composition of essential oils such as drying, harvest period, harvest region, extraction technique, and the age of the plant (Aberchane M, 2001; Bourkhiss M, 2009).

- ***Phytochemical screening:***

Phytochemical study led to the same results for both species. These results are shown in table 2.

Table 2: Results of phytochemical screening of *Rosmarinus* extracts

Chemical group	Results	
	R.Officinalis	*R.Eriocalyx*
Alkaloids	-	-
Flavonoids	+	+
Leucoanthocyanes	-	-
Catechols	+	+
Gallic tannins	+	+
Catechic tannins	-	-
Saponins	+ (Foam index = 230)	+ (Foam index = 200)
Sterols and triterpenes	+	+
Free anthraquinones	+	+
Combined anthraquinones	+	+
Reducing compounds	-	-
Mucilages	+	+
Cardiotonic heterosids	+	+
Carotenoids	-	-
Oses and holosides	-	-
Tetrahydrocannabinols	-	-

Presence (+); absence (-)

Results of phytochemical characterisation proved that both species are rich in flavonoids, tannins, sterols, triterpenes, saponosids, free anthraquinones, mucilages, cardiotonic heterosids and catechols. However alkaloids, reducing compounds, carotenoids , oses , holosids , Tetrahydrocannabinols and coumarins were not observed.

Rosemary wealth in sterol explains its diuretic effect. On the other hand, anthracenosids have irritant and laxative effects on the large intestine that explain the stomachic effect of rosemary. Saponins have analgesic, antiinflammatory and anti-oedematous properties (Roux *et al*. 2007) which justify their use in traditional treatments for venous insufficiency.

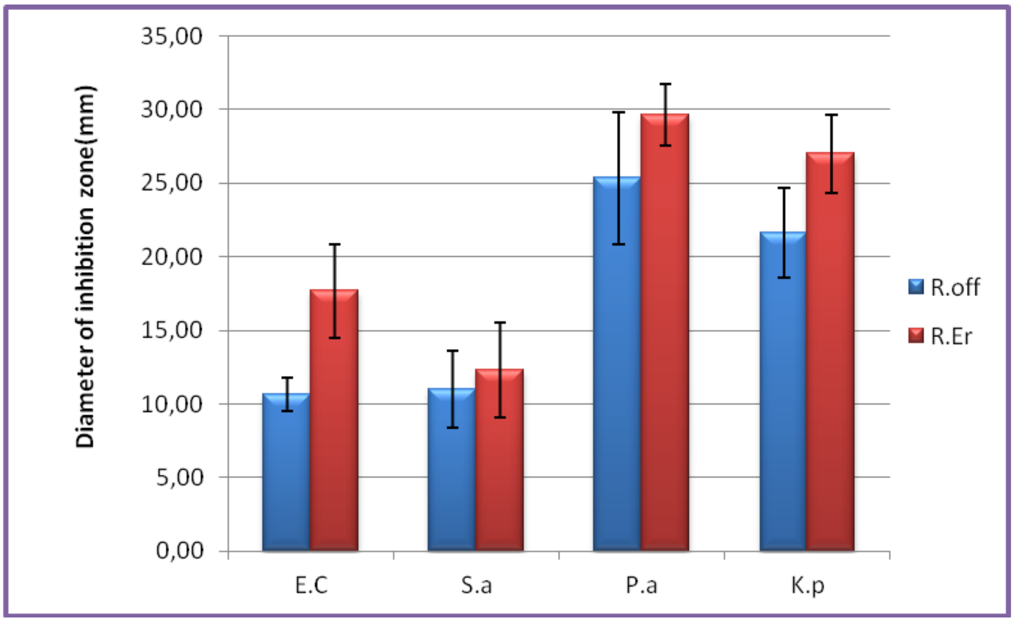
- ***Antibacterial activity:***

Aromatogramm performed through disc-diffusion method:

Results of antibacterial test of *R.officinalis* and *R.eriocalyx* Eos are mentioned in figure 3.

The above results obtained for antimicrobial activity using the disc-diffusion method (aromatogramm) show that antibacterial activity of tested EO depend on the target bacterium.

For disc- diffusion method, an essential oil is considered inactive (-) for diameters less than 8mm; active (+) for diameters between 8 and 14mm; very active (+ +) for diameters of 15-19 mm and extremely active (+ + +) for diameters over 20 mm Ponce *et al*, (2003).We note that essential oil of *Rosmarinus eriocalyx* shows a higher activity compared to *Rosmarinus officinalis*. Diameters of inhibition are higher for *R.eriocalyx* EO (*E.coli*: 17.66 mm, *S. aureus*: 12.33 mm, *P. aeruginosa* and *K. pneumoniae* 29.67 mm and 27 mm). We note that *R.eriocalyx* EO is active on all tested bacterial strains, and this activity remains higher than antibacterial effect such as nalidixic acid (3 mm) tested on *E. coli*, tetracycline (0 mm) tested *K. pneumoniae* and amikacin (0mm) tested *P.aeruoginosa* but remains low compared to gentamicin (32 mm). These antibiotics were used as reference controls recommended by MFC 2010 and EUCAST 2013.



E.c: Escherichia coli *P.a: Pseudomonas aeruginosa*
S.a: Staphylococcus aureus *K.p* : *Klebsiella pneumoniae*

Fig. 3: Means diameters of inhibition zones against the test bacterial strains: *Escherichia coli*, Pseudomonas *aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*

R. officinalis EO, was also active on all tested bacterial strains (*E. coli*: 10.67mm, *S.aureus*: 11 mm, *P.aeruoginosa*: 25.33 mm and *K. pneumoniae*: 21.67 mm). We note that inhibition diameters are higher than those induced by antibiotics (Nalidixic Acid / *E.coli*: 3 mm) (Tetracycline / *K. pneumoniae*: 0 mm) and (Amikacin / *P.aeruoginosa*: 0 mm). But inhibition diameter induced by *R. officinalis* essential oil against *S.aureus* is lower compared to that of gentamicin (32 mm).

R. officinalis and *R.eriocalyx* EOs are extremely active on *P. aeruginosa* and *K. pneumoniae* strains. This result is very important especially for *P.aeruoginosa* which is known for its high resistance to all antibiotics.

These results have some similarities with those of Taoufik Ouassil since he found that R.officinalis is active against the four species of bacteria (*E.coli* :< 14mm, *S.aureus* : <14mm, *P.aeruoginosa* :14mm, *K.pneumoniae* :14mm). Diameters of inhibition concerning *E. coli* and *S. aureus* are similar to ours but a great difference can be observed concerning *P.aeruginosas* and *K.pneumoniae* (Taoufik Ouassil, 2010).

Many researchers have highlighted sensitivity of Gram (+) bacteria compared to Gram (-) while testing natural extract (Bari M.A, 2010; Derwich E, 2010; Falleh, H, 2008) but in our case it seems that *Rosmarinus'* essential oils are more active against Gram (-) bacteria.

Table 3: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal concentration (MBC) of *Rosmarinus officinalis* and *Rosmarinus eriocalyx* EOs against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae.*

Bacteria	*Rosmarinus Officinalis*		*Rosmarinus Eriocalyx*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Escherichia coli	16,7	33,4	1,04	2,08
Staphylococcus aureus	8,35	33,4	8,35	16 ,7
Pseudomonas aeruginosa	16,7	33,4	16,7	33,4
Klebsiella pneumoniae	2,08	4,17	1,04	2,08

For *R.Eriocalyx* essential oil, a strong inhibitory activity is observed against *E. coli* and *K. pneumoniae* with 1.04 mg/ml as MIC for both bacterial strains. *S. aureus* and *P.aeruoginosa* have respectively 8, 35 mg/ml and 16, 7 mg/ml as MIC. The bactericidal effect of *R.Eriocalyx* EO was found at 2.08 mg/ml against *E.coli* and *K.pneumniae*. Minimal Bactericidal Concentrations against *S.aureus* and *P. aeruginosa* were 16, 7 mg/ml and 33.4 mg/ml respectively (Table 3).

R. officinalis essential oil expressed a strong inhibitory activity against *K. pneumoniae* with a MIC of 2.08 mg/ml,and *S. Aureus* with a MIC of 8,35 mg/ml .*E. coli* and *P. aeruginosa* were inhibited with 16,7 mg/ml (Table 3). *R. officinalis* EO has also a bactericidal power. Minimal bactericidal concentrations were 4.17 mg/ml for *K. pneumoniae* and 33.4 mg/ml for *E. coli*, *S. aureus* and *P. aeruoginosa*.According to our results, the MBC / MIC ratios are lower than 4 for all strains, so both essential oils have a bactericidal power against the tested strains.

Taoufik Ouassil (2010), found that *Rosmarinus officinalis*'EO has a great antibacterial activity against strains *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* since he found 2.5 µg / ml and 0.625µg/ml as MIC respectively.

In Turkey (Izmir), Yesil Celiktas.O,*et al*.(2007)worked on *Rosmarinus officinalis* and found the following MIC: *E.coli* (20mg/ml), *S.aureus* (10mg/ml), *P.aeruginosa* (10mg/ml), *K. pneumoniae* (20mg/ml).

Okoh.O *et al*, (2010) found that South African sample of *Rosmarinus officinalis* (oriental region of the Cape) exhibited the following MIC: E. *coli* (7,5mg/ml), *S.aureus* (3,75mg/ml) and *K. pneumoniae* (0, 94mg/ml).

These results show that the antimicrobial activity of essential oils to be linked to the nature of its major constituents.

Conclusion:

In this work, we have studied chemical composition and antibacterial activity of essential oils of two *Rosmarinus* species. Qualitative and quantitative analyses of their essential oils revealed 29 and 23 compounds for *Rosmarinus officinalis* and *Rosmarinus Eriocalyx* respectively. Both species are 1, 8-cineole chemotypes.

The phytochemical screening of the species has highlighted that both plants contain flavonoids, tannins, sterols and triterpenes, saponins, free anthraquinones, mucilages, cardiac glycosides and catechols. Preliminary results of antibacterial study showed in vitro efficiency of *Rosmarinus officinalis* and *Rosmarinus eriocalyx* on all tested bacteria with minimum inhibitory concentrations ranging from 1,04 mg/ml to 16,7 mg/ml. The results presented here may contribute to the knowledge of antimicrobial potential of these species. Other studies on extracts activities of these species are needed to compare them with essential oils activity.

ACKNOWLEDGMENTS

We are grateful to Mr M. Ibn Tattou, Professor at the Scientific Institute of Rabat, for the species identification. We also thank Ms Soro K. Aminata for help in writing step.

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