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RESEARCH ARTICLE

STUDY OF THE INDIVIDUALITY EFFECT ON THE ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF THE *ROSMARINUS OFFICINALIS VAR. PROSTRATUS* EXTRACTS OBTAINED BY THE ULTRASOUNDS

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ABSTRACT

This work aimed to compare the yields and to evaluate the *in vitro* antibacterial activity and phytochemical constituents of hexane, ethyl acetate, dichloromethane and methanol extracts of seven individuals of *Rosmarinus Officinalis Var. Prostratus* obtained by the ultrasounds. The statistical study shows that there is an individual effect on the yield and on the antibacterial activity of these extracts. The Phytochemical screening revealed that the flavonoïdes and tannins are present in important quantities in the ethyl acetate extract, especially in individuals 4 and 7.The results of the *in vitro* antibacterial activity show that the extracts have significant inhibitory effect against *M. smegmatis*, *Bacillus* and *E.coli* especially in individuals 4 and 7. These results suggest that the flavonoids and tannins can be responsible for the differentiation of the antimicrobial property of *Rosmarinus Officinalis Var. Prostratus*. The quantitative study (CMI) confirmed the results of the qualitative study and showed that the ethyl acetate extract of individuals 4 and 7 present the highest antibacterial effect.

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INTRODUCTION

The emergence of new diseases affecting and weakening of the immune system in a more sustained manner, and the emergence of microbial strains, which are more resistant and virulent, requires the development of new antibacterial agents, more than indispensable. These substances must be both available and less toxic compared to antibiotics obtained via chemical means. Many researchers around the world are trying to find new active substances from the aromatic plants extracts as sources of useful and novel antimicrobial agents that would overcome these disavantages : in Morocco (Sqalli *et al*., 2009), Australia (Prasad and Seenayya, 2000), Italy Tampieri *et al*., 2005), Turkey (Dulger *et al*., 2006), Iran (Bonjar, 2004) and others. Morocco is recognized for a generous diversification in medicinal and aromatic plants (MAP) having therapeutic efficiency. Still, the exploitation of these MAP in Morocco is not rational because of intensive harvests in natural habitats that can only deplete spontaneous MAP. The logical result is a threat to the survival and the sustainability of the stands as well as risk of loss of the genetic capital. Domestication and cultivation of MAP are proving to be relevant solution to counteract this damaging phenomenon. Improved farming techniques are a promising way to increase the performance and efficiency of cultivated MAP but which are not identical to spontaneous MAP that are more efficient. Considering the quantity and / or quality of a natural substance in plant is controlled by three main factors: genotype, environment and its interaction. It is in this context that the present work is inscribed, which aims to study the effect of individuality on *Rosemary* var. prostratus" extracts. Rosmarinus (rosemary) is

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one of the natural plants of Morocco which is known for its medicinal and aromatic properties since antiquity, but the relatively recent enhancement of interest in 'green' consumerism has led to a renewal of scientific interest in these substances in the pharmaceutical and food industries. Besides, it has been shown to exhibit antibacterial properties (Celiktas *et al*., 2007), anti-inflammatory (Gianmario *et al*., 2007) antiulcer-genic (Patricia *et al*., 2000), antispasmodic (Al- Seret *et al*., 1999) and antioxidant properties (Malo *et al*., 2011).

Our work identifies three components, namely a qualitative study including the evaluation of the antibacterial effect of seven individual extracts of *Rosemary var. prostratus*, a quantitative study that has allowed the determination of the CMI of these elements and a phytochemical study.

MATERIALS AND METHODS

Plant material

Samples of the aerial part (stems, leaves and flowers) of seven individuals (I1 to I7) of Rosmarinus officinalis var. prostratus were collected from the experimental garden of the National Institute of Medicinal and Aromatic Plants of Taounate (NIMAP) in February 2012, when the plants were in full bloom. It should be noted that no agronomic treatment was imposed during the cultivation of these species. The Botanical identification and the authenticated voucher specimens were deposited in the Herbarium of the National Institute of Medicinal and Aromatic Plants (code: FA/RP/INPMA/003), University of Sidi Mohamed Ben Abdellah, Fez, Morocco.

Bacterial Strains

The extracts were tested for antibacterial activity against the following bacterial strains:

M.smegmatis (MC$_2$155):	Nonpathogenic atypical mycobacterium, it presents a susceptibility to antituberculous agents similar to that of *M. tuberculosis*. (Mitscher and Baker, 1998).
*Escherichia coli (*ATCC 25922*)*	Pathogen Gram-negative bacteria known for its strong antibiotic resistance and its toxic and invasive power in human beings. It causes intestinal diseases which vary in severity from benign to serious or even life threatening forms.
*Bacillus subtilis (*ATCC 23857*)*	Non-pathogenic Gram-positive bacterium for human beings, but may contaminate food and may exceptionally cause food poisoning. It is regarded as an excellent model for the pathogenic bacteria study, such as Staphylococcus aureus, Streptococcus pneumoniae, and Bacillus anthracis.

These strains belong to the bacteria culture collection of the Microbial Biotechnology Laboratory at the Faculty of Sciences and Techniques (FST) in Fez, Morocco.

Ultrasonic Extraction

Extraction was carried out in an ultrasonic bath (Elma–Transsonic TI-H-15) (Power: 100 W). This is a technique used to isolate compounds of plant origin without degrading them. This extraction method assisted by ultrasound was chosen for its various advantages in terms of energy saving, solvent and time (Biesaga, 2011; Schinor *et al*., 2004; Jennan *et al*., 2014). Flasks containing 50 g of a dry powdered plant were subjected to successive solvent extraction (hexane, dichloromethane, ethyl acetate and methanol) in the ultrasonic bath. Sonication was performed with ultrasound frequency 35 KHz for 45 min (Two repetitions were performed). After filtration, each mixture was evaporated under vacuum to obtain crude extracts.

Antibacterial Activity

Disc diffusion method

To highlight the antibacterial and antimycobacterial activity of the extracts, the disk diffusion method (Bayoub *et al*., 2010; Bauer *et al*., 1966) was used because of its simplicity and efficiency to test bacteria sensitivity (Rožman and Jeršek, 2009). For this purpose, sterile Whatman paper discs of 6 mm diameter were placed in the center plate of 90 mm in diameter containing 30 ml of LB medium previously inoculated with 100µl liquid cultures of bacterial test strains. The discs were then impregnated with 10µl of extracts. The control corresponds to a disc impregnated with 10µl of pure solvent. After incubation, the inhibition zones formed around the discs were measured. Three replicates were performed for each strain.

The MIC Determination

The Minimal Inhibiting Concentration (MIC) was performed using the macro broth dilution method (Rastogi *et al*., 1991). The MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after a time of incubation. Its determination was made by observation of the disorder induced by the growth of the bacteria in each tube.

The MIC of the ethyl acetate extracts of the different individuals was determined. This technique consists in adding variable volumes of the plants extract in tubes containing media culture LB liquid inoculated by 250 µl of liquid cultures of the stocks to test, in order to obtain final concentrations in ethyl Acetate residue from 0.94 to 9.43 Mg /ml (Concentration in soluble residue). The pilot tube corresponds to a tube deprived of the extract of plant. The study was resumed three times.

The MIC was defined as the lowest concentration of plant extracts that caused growth inhibition after the period of incubation. The total volume of the tubes is 6 ml, and the percentage in DMSO in all the tubes is lower to 2%, concentration which does not inhibit the growth of the tested bacteria used (Molina-Salinas and al., 2006).

Test statistics

The results of statistical analysis are obtained by analyzing variance using the software 'Lumiére'.

Phytochemical Screening

In order to identify the molecules responsible for the bacterial activity of Rosmarinus var. prostratus, we conducted a qualitative test to highlight the polyphenols, flavonoids, tannins and alkaloids in extracts of different individuals. The principal chemical constituents were characterized in the extracts by

colorimetric essays. Classic reactions used to detect the various chemical entities were the yaniding reaction for flavonoids (Karumi *et al*, 2004), the ferric chloride for tannins (Kablan *et al*., 2008), the Folin ciocalteu reagent for polyphenols (Singleton *et al*., 1999) and the Dragendorff reagent (Bekro *et al*., 2007) for alkaloids. The experimental protocols are:

- Detection of flavonoids: the test consists of adding to a minimum volume of extract a few drops of hydrochloric acid (2N) and 0.5 g of magnesium, which allowed reacting for 3 min. The appearance of a pink-orange, pink purple or red color indicates the presence of flavonoids.
- Detection of tannins: The reaction is to introduce 5 ml of extract in a test tube to which was added 1 ml of aqueous solution of 2% ferric chloride. The presence of tannins is characterized by the formation of a greenish color or blackish blue, up to precipitate.
- Identification of polyphenols: to l00 µl of the extract added 500 µl of Folin-Ciocalteu reagent (diluted ten times) and 400 µl of a solution of Na_2CO_3 (75 mg / ml distilled water), allowed incubate 2 h at room temperature. The presence of polyphenols leads to the appearance of a dark blue color.
- Identification of alkaloids: alkaloids have been identified in extracts with Dragendorff reagent (precipitation reagent): 0.1 g of the extract is taken up in 6 ml at 60% ethanol, followed by adding 2 drops of Dragendorff reagent. The appearance of an orange or brown-red precipitate - red indicates a positive test.

For these tests, a white is prepared in the same conditions but without extract. The tests are repeated three times.

RESULTS AND DISCUSSION

The Yield Extract

The extracts of hexanic, Ethyl Acetate, dichlorométhane and methanolic alcohol were concentrated under vacuum with the rotavapor. After filtration and evaporation under vacuum of solvent, the total extracts so obtained are weighed in order to estimate their yield, which is calculated according to the following formula:

Rdt= (EB/MS) x 100

Rdt= Yield; EB= Raw extract obtained after the extraction; MS = Dry matter from which the extraction was realized.

Seven *Rosmarinus var.prostratus* individuals supplied yields varying between 6.7 % and 9.8 % for hexanic extract, between 7.9 % and 11.35 % for ethyl acetate extract, between 3.5 % and 6.8 % for the dichloromethane extract and between 5.8 % and 7.8 % for the méthanolique extract. These obtained results show that the best yield is obtained with ethyl acetate extract for which the percentage varies from 7.9% up to 11.6%. This can be attributed to the fact that the great majority of the compounds of this plant are soluble in the ethyl acetate. With a percentage 9.8 %, 11.35 %, 5.2 % and 7.8 % for hexanic extract, ethyl acetate extract, dichloromethane extract and méthanolique extract the individual n°7 presents the highest content.

Table 1. Yield extracts of *Rosemary var. Prostratus* obtained by the ultrasound from seven individuals

Individuals of Rosemary prostratus	Yields (%)			
	Hexane	Ethyl Acetate	Dichloromethane	Methanol
I1	8.8± 0.02	8.8± 0.03	4.1± 0.01	6.6± 0.01
I2	7.5± 0.03	8.7± 0.02	4.8± 0.03	5.8± 0.02
I3	7.8± 0.03	7.9± 0.03	6.8± 0.01	6.4± 0.03
I4	9.3± 0.01	11.6± 0.01	4.4± 0.03	6.8± 0.01
I5	6.7± 0.03	10.8± 0.01	3.5± 0.02	7.2± 0.02
I6	9.2± 0.01	11.4± 0.03	4.5± 0.03	6.4± 0.03
I7	9.8± 0.01	11.35± 0.01	5.2± 0.03	7.8± 0.02

Table 2. Inhibition halos produced by hexane, ethyl Acetate, dichloromethane and méthanolique extracts of *R. officinalis var. prostratus*

	Solvent	Strains		I1	I2	I3	I4	I5	I6	I7
Diameter of inhibition (mm)*	Hexane	E. coli	Test	6.5±0.5	7.66±0.57	7.66±0.57	8.5±0.5	8.33±0.57	7.33±0.57	8.66±0.5
			Témoin	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0
		Bacillus	Test	7.66 ±0.57	8.33 ±0.57	6 .66 ±1.13	10.66 ±0.57	7.16 ±0.28	9.66 ±0.57	9.83 ±1.04
			Témoin	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0
		Smeg	Test	7 ± 0.57	14.33 ± 0.57	15.33 ± 0.57	29.66±0.57	15 .3± 0. 57	16 ± 1	24.66 ± 1.15
			Témoin	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0	6.00 ± 0
	Ethyl Acetate	E. coli	Test	9±1.0	11.33±0.57	15.66 ±0.57	18.66±0.57	16±1	14 .33 ±0.57	17.33 ±0.57
			Témoin	6.33 ± 0.33	6.33 ± 0.33	6.33 ± 0.33	6.33 ± 0.33	6.33 ± 0.33	6.33 ± 0.33	6.33 ± 0.33
		Bacillus	Test	10.66±0.57	17.33 ±0.57	15 .66 ±1.15	24.33 ±1.52	15.66±0.57	18.33 ±1.15	28 ±1
			Témoin	7.2 ± 0.22	7.2 ± 0.22	7.2 ± 0.22	7.2 ± 0.22	7.2 ± 0.22	7.2 ± 0.22	7.2 ± 0.22
		Smeg	Test	9.66 ± 0.66	14.33 ± 0.33	15.33 ± 0.33	29.66 ± 0.33	15.33 ± 0.33	16 ± 0.9	24.66±1.33
			Témoin	6.33± 0.22	6.33± 0.22	6.33± 0.22	6.33± 0.22	6.33± 0.22	6.33± 0.22	6.33± 0.22
	Dichlorometh ane	E. coli	Test	9.33±0.33	8.33±0.57	11.33 ±0.57	12.33±0.57	9.33±0.57	9.66 ±0.57	14.33 ± 0.57
			Témoin	7,5±0,00	7,5±0,00	7,5±0,00	7,5±0,00	7,5±0,00	7,5±0,00	7,5±0,00
		Bacillus	Test	11.33±0.57	10.66 ±0.33	12.33 ±0.33	16±0.57	11 ±0.8	14.33 ±0.33	19.33 ±0.33
			Témoin	8.33 ± 0.57	8.33 ± 0.57	8.33 ± 0.57	8.33 ± 0.57	8.33 ± 0.57	8.33 ± 0.57	8.33 ± 0.57
		Smeg	Test	12.66±0.57	11.66±0.57	14.66 ±0.33	17.66±0.54	14±0	13.33 ±1.57	17.66 ± 0.57
			Témoin	8,5±0,33	8,5±0,33	8,5±0,33	8,5±0,33	8,5±0,33	8,5±0,33	8,5±0,33
	Methanol	E. coli	Test	6.66±0.57	8.33±0.57	9.33 ±0.57	9.83±0.25	7.12±0.28	8.33±0.57	10.33 ±0.57
			Témoin	7.3 ± 0.33	7.3 ± 0.33	7.3 ± 0.33	7.3 ± 0.33	7.3 ± 0.33	7.3 ± 0.33	7.3 ± 0.33
		Bacillus	Test	9.46 ±0.56	11.33 ±0.22	9 .35 ±0.54	16.35 ±0.33	12.43 ±0.22	12.6 ±0.54	18.2 ±0.33
			Témoin	8.3 ± 0.33	8.3 ± 0.33	8.3 ± 0.33	8.3 ± 0.33	8.3 ± 0.33	8.3 ± 0.33	8.3 ± 0.33
		Smeg	Test	9.16 ± 0.28	12.33 ± 0.57	13.66 ± 0.57	13.66 ± 1.15	12.33 ± 0.57	10.33 ± 0.57	13.66±0.57
			Témoin	8.33± 0.22	8.33± 0.22	8.33± 0.22	8.33± 0.22	8.33± 0.22	8.33± 0.22	8.33± 0.22

Generally, the achieved results show that the yield varies according to individuals and extracts. Consequently, the yield is affected by individuality and solvent type. These yields are close to those quoted in the literature. Indeed, (Balouiri *et al*., 2014) obtained a yield of 8.7 for Rosmarinus extract by using ultrasounds. Indeed, a study led by (Fadil *et al*., 2015) showed that there is an individuality effect on the yield.

Antibacterial activity using disc method

In vitro antimicrobial activity of the extract is estimated by the diameter of inhibition and varies according to individual plant and bacteria strains. The method disc results (± standard deviation) are presented in Table 2 and Figure 1.

inhibition diameter for the 7 individuals of *Rosemary officinalis var. prostratus* tested on the three strains (*Escherichia coli*, *Bacillus subtilis*, *and smegmatis*). The results of this testing are set out in Table 4. The ANOVA table decomposes the variance to two components: the inter-groups component and the intra-groups component. Since the probability value for the F test was less than 0.05, there is a statistically significant difference between the means of the inhibition diameters between the seven individuals at the 95.0% confidence level for the three tested strains. This shows the effect of individuality on the antibacterial activity. The results of antibacterial activity showed that the seven individual extracts of *Rosmarinus officinalis var. Prostratus* revealed an

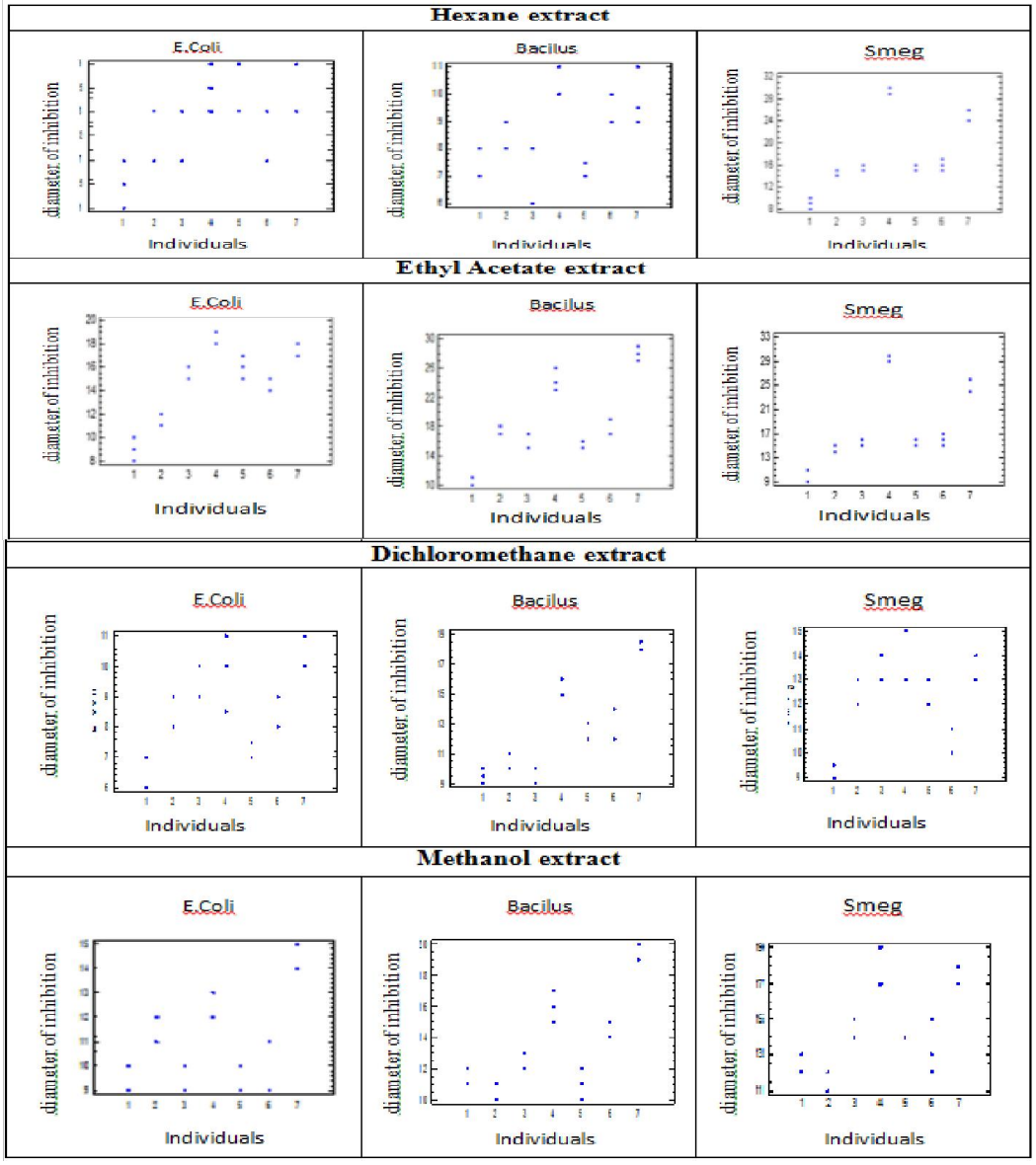

Figure 1. Antibacterial activity of extracts estimated by diameter of inhibition of the seven individuals of *Rosmarinus officinalis var. prostratus*

Analysis of variance

Analysis of variance (ANOVA) was used with a factor in order to check if there are significant differences between the average

inhibiting effect on all the tested strains, except the hexane extract. This activity differs from one individual to another and from one extract to another. In fact, these findings demonstrate

that the hexane was not effective in the extraction of bioactive compounds (the diameter of the inhibition halo is significantly similar to that of the control), whereas, the other three solvents (dichloromethane, ethyl acetate and methanol) allowed the extraction of antibacterial substances resulting in the formation of the halos inhibition around the discs.

The highest antibacterial activity was observed for the ethyl acetate extracts of *R. officinalis* var. prostratus followed by dichloromethane extracts, methanolic extract and this for the three used strains (Table 2; Figure 1). Barrows *et al*. (2007) tested the effect of different extracts (hexane, ethyl acetate and methanol) of Evodia elleryana and showed that the extract of

Table 4. Variance Analysis of ANOVA with a factorial combination of treatment

	Strains	Variance analysis				
		Source of Variation	Sum of Squares	Ddl	Average Square	F	Probabilities
Hexane extract	*E. coli*	Inter- groups	10.9762	6	1.82937	5.91	0
		Intra-groups	4.33333	14	0.309524		
	B. subtilis	Inter- groups	40.9762	6	6.82937	12.47	0.0001
		Intra-groups	7.66667	14	0.547619		
	M. smegmatis	Inter- groups	873.905	6	145.651	219.62	0
		Intra-groups	9.3333	14	0.666671		
Ethyl Acetate extract	*E. coli*	Inter- groups	207.619	6	34.6032	66.06	0
		Intra-groups	7.33333	14	0.5238		
	B. subtilis	Inter- groups	609.143	6	101.524	101.52	0
		Intra-groups	14	14	1		
	M. smegmatis	Inter- groups	57.5	6	9.583	197.071	0
		Intra-groups	6.16667	14	0.44		
Dichlorometha ne extract	*E. coli*	*Inter- groups*	32.9762	6	5.49603	11.54	0.0001
		Intra-groups	6.66667	14	0.47619		
	B. subtilis	*Inter- groups*	187.452	6	31.2421	72.90	0
		Intra-groups	6.0	14	0.428571		
	M. smegmatis	*Inter- groups*	57.5	6	9.583	21.76	0
		Intra-groups	6 .16667	14	0.444571		
Methanol extract	*E. coli*	*Inter- groups*	65.9048	*6*	10.9841	23.07	0
		Intra-groups	6.66667	*14*	0.47619		
	B. subtilis	*Inter- groups*	183.81	*6*	30.6349	58.48	0
		Intra-groups	*3.833*	*14*	0.279		
	M. smegmatis	*Inter- groups*	99.2381	*6*	16.5397	23.16	0
		Intra-groups	10.0 14	*14*	0.714286		

Table 5. MinimumInhibitory Concentration (MIC) of the Ethyl Acetate extract of seven individuals of *Rosmarinus officinalis* var. *prostratus*

	MIC (mg/ml)						
Strains	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7
E coli	8.01	6.6	5.18	3.77	5.18	5.18	3.77
B.subtilis	6.6	5.18	5.18	3.77	6.6	6.6	2.35
Smeg	5.18	5.18	5.18	2.35	5.18	5.18	3.77

Table 6. Phytochimic characterization of extracts *Rosmarinus officinalis var. prostratus*

Individuals	Extracts	Chemical groups			
		Polyphénols	Flavonoïdes	Tannins	Alkaloids
I1	hexanic extact	-	-	-	-
	ethyl Acetate extract	++	+++	++	-
	Dichloromethane extract	++	++	+	-
	Methanolic extract	++	++	+	-
I2	hexanic extract	-	-	-	-
	ethyl Acetate extract	++	+++	++	-
	Dichloromethane extract	++	++	+	-
	Methanolic extract	++	++	+	-
I3	hexanic extract	-	-	-	-
	ethyl Acetate extract	++	+++	++	-
	Dichloromethane extract	++	++	+	-
	Methanolic extract	++	++	+	-
I4	hexanic extract	-	-	-	-
	ethyl Acetate extract	+++	+++	+++	-
	Dichloromethane extract	++	++	+	-
	Methanolic extract	++	++	+	-
I5	hexanic extract	-	-	-	-
	ethyl Acetate extract	+++	+++	++	-
	Dichloromethane extract	++	++	+	-
	Methanolic Extract	++	++	+	-
I6	hexanic extract	-	-	-	-
	ethyl Acetate extract	+++	+++	++	-
	Dichloromethane extract	++	++	+	-
	Methanolic extract	++	++	+	-
I7	hexanic	-	-	-	-
	ethyl Acetate extract	+++	+++	+++	-
	Dichloromethane extract	++	++	+	-
	Methanolic extract	++	++	+	-

Compounds presence: (+++) = important; (++) = average ;(+) = weak; (-) = absent

the ethyl acetate from this plant has the most inhibitory effect compared to the other extracts. These results show that the molecules responsible for the activity are more extractable with the ethyl acetate than the other solvents and these active molecules of this plant are of medium polarity since this solvent allows the extraction of average polar compounds (Derbel *et al*., 2010). Several previous studies reported that the extracts obtained by the ethyl acetate at several plants, including *Rosmarinus*, have an effect against pathogenic bacteria including *M. smegmatis* (Balouiri *et al*., 2014; Barrows *et al*., 2007; Lu *et al*., 2012). Statistical analysis of the results showed that the ethyl acetate extract of the individuals 4 and 7 recorded respectively the highest significant activity with an average of the inhibition zone of 28.5 and 23.7 for M. smegmatis, 24.33 and 18.33 for Bacillus, and 18.66 and 17.33 for E. coli.

Minimal inhibitory concentration

The CMI is the weakest substance concentration for which there is no visible growth after the incubation time. Its determination was made by observing disorder induced by the bacteria growth in each tube. Results are represented in table 5. Ethyl acetate extracts of the various individuals of *Rosmarinus Officinalis Var. Prostratus*, appeared active towards all the bacterial stocks tested by the disc method but with different degrees which was translated by the difference of the CMI. These extracts inhibit the growth of bacteria with CMI values which vary between (3,77 and 8,01 mg of résidus/ml) for *B.subtilis* and between (2.35 and 6.6 mg/ml) *M.smegmatis*. Indeed, all the microbial strains were inhibited at concentration 5.18mg / ml except for the individual 1 where stocks are inhibited only from 6.6mg/ml. The most sensitive microorganism in all extracts is M.smegmatis whose growth was stopped in the weak concentration 5.18mg / ml. Results showed that CMI of individuals 4 and 7 are weakest compared to the other individuals and this for M.smegmatis, E.coli and B.subtilis; whereas individual 1 presented the largest CMI. This shows that individual extracts of 4 and 7 present interesting antibacterial characteristics on tested micro-organisms. These results confirm those obtained by disc method.

Phytochemical Screening

Revelation tests of polyphenols, flavonoïdes, tannins and alkaloids were carried out, the results of this photochemical screening carried out for 7 studied Rosmarinus individuals are gathered in (table 6). The results are negative for alkaloids but positive for polyphenols, flavonoids and tannins (table 6). According to Gonzalez-Trujano and al., (2007), the phytochemical screening of *Rosmarinus* extract indicates the presence of flavonoids and tannins. The phytochemical study of the various individuals shows a little difference of the chemical composition, we can give up this difference to factors influencing the plants composition as genetic factors (Beekkara *et al*., 2007), the growth of its organs (Al-Ramamneh, 2009), the stage of development of plant organs (ontogeny of sheets (leaves), flowers and fruits) (Ozguven., Ettansi., 1998). We notice that flavonoids and tannins are present in great quantities in ethyl acetate extract of individual 7. We have already noticed that the ethyl acetate extract presents the best antibacterial effect. Thus, this activity could be due to the presence of flavonoids and tannins in these extracts. Indeed, according (Elâgoun, on 2003), the ethyl acetate is used for the flavonoids extraction. Flavonoids play very important roles in plants, like protecting them against the hydric stress and generating their tolerance of the heavy metals present in soils. Besides, flavonoids possess several pharmacological effects (El-Sakka and al., 2010) ; they increase the solidity of capillaries (Vierling.,2004), they protect vegetable food of the oxidation, they are antioxidants renowned for their anti-radicular action (Benhayoun *et al*.,2007), they possess antiviral activities, spasmolitiques, anti-inflammatory, cytotoxique activities (Bernard and al.,1974; Nobuyuki and al., 1994; Das and al.,1994).

Several studies were led concerning the biological activity of flavonoids of other plants, and showed that these molecules are endowed with an antibacterial effect (Hernandez *et al*., 2000; Schinor *et al*., 2007), and of an antimycobactérial effect (Kuete *et al*., 2010; Koysomboon *et al*., 2006; Kuete*et al*., 2008; Okunade *et al*., 2004; Copp, 2003; Newton *et al* ., 2000). Hence, the importance to connect the presence of such metabolites with the *Rosmarinus* antibacterial activity. We also note the presence of tannins. These molecules are endowed with antibacterial activities as several works show such as: (Kolodzize and al.1990; Mahamat on 1999; Bassene on 1995). According Iserin (2001), tannins can stop bleedings and fight against infections.

Conclusion

This investigation focused to identify and compare yields, antibacterial activities of hexane extracts, ethyl acetate, dichloromethane and methanol and phytochemical screening of seven individuals of *Rosemary officinalis var. prostratus*. The statistical study shows that there is an individuality effect on the yield, on the antibacterial activity of *Rosmarinus Officinalis Var. Prostratus* extracts obtained by the ultrasounds at the level of confidence 95.0 %. The phytochimical study shows that the flavonoïdes and tannins are present in the ethyl acetate extract and especially in individuals 4 and 7 with important quantity . The results of the antibacterial activity show that the extracts present, in vitro, a significant inhibitory activity against the three strains tested especially in individuals 4 and 7. The flavonoids and tannins can be responsible for the differentiation of the antimicrobial activity. The quantitative study (CMI) confirmed the results of the qualitative study and showed that the ethyl acetate extracts in individuals 4 and 7 present the highest antibacterial effects. All results have shown that extracts of ethyl acetate of individual 4 and 7 gave the best yield, best activities against strains tested, especially M. smegmatis, screening phytochemical showed that flavonoids and tannins are present in large amounts in these extracts of *Rosemary var .prostratus.* These two individuals (4 and 7) can be selected for a future vegetative multiplication in order to have clones that are able to ensure a standard production. The latter will ensure source extracts of this very important activity against the Mycobacterium tuberculosis and against the other stocks tested.

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