International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 3, 2011

Research Article

IN VITRO ANTIBACTERIAL ACTIVITY AND GC/MS ANALYSIS OF THE ESSENTIAL OIL EXTRACT OF LEAVES OF *ROSMARINUS OFFICINALIS* GROWN IN MOROCCO

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Received: 23 Feb 2011, Revised and Accepted: 01 May 2011

ABSTRACT

The chemical composition and antibacterial activity of essential oils obtained from *Rosmarinus officinalis* (family *Lamiaceae*) were determined. Their chemical composition was determined by hydro-distillation, analysed by GC/MS and GC-FID. To evaluate the antibacterial activities of these aromatic extracts; their in vitro antibacterial activity was determined by disk diffusion testing and minimum inhibitory concentration (MIC). *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,, Kellebsiella pneuomonae, Salmonella typhi, Staphylococcus intermedius, Bacillus subtilis, Streptococcus mutans, Micrococcus luteus,* and *Proteus mirabilis* were used as test bacterial strains. The analyses for leaves resulted in the identification of forty seven compounds, representing 65.61% of the total oil and the yields were 0.54%. The major component was α-pinene (18.25%); other predominant components were camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%). The bacterial strains tested were inhibited at minimum inhibitory concentration (MIC) values in the range of 4 at 48.2μg/mL. The presence of monoterpenes as the major constituents of the essential oil extracted from leaves could be responsible for the potential antibacterial activity observed in this study.

Keywords: Antibacterial activity, *Rosmarinus officinalis,* Essential oils, GC/MS, α-pinene

INTRODUCTION

Rosmarinus officinalis, a member of the family *Lamiaceae* is a flowering plant that grows in Mediterranean countries, southern Europe and in the littoral region through minor Asia areas wildly. Essential oils are also called volatile oils, and are generally aromatic oils obtained by the steam or hydro-distillation of plants. Different parts of plants have been used to obtain essential oils. These include the flowers, leaves, seeds, roots, steams, bark, and wood though secretionary parts. Multiple studies have been reported on the chemical composition of the essential oils of *Rosmarinus officinalis* belonging to different regions in the world [1-3]. The essential oil of *Rosmarinus officinalis* has been the object of several studies antioxidant activity [4-8], antibacterial [9-13], Toxicity insecticidal [14, 15], Anti-Inflammatory and Antinociceptive [16], antifungal [17, 18] and only, in recent years have these oils been commercialised as pest control products [19]. In our previous studies on the toxicity of *Rosmarinus officinalis*, essential oil and blends of its major constituents against *Tetranychus urticae Koch* (Acari: Tetranychidae) on two different host plants [20]. In traditional medicine, Rosemary is used to treat different diseases including: depression, insomniac and arthritic pains [21]. Moreover volatile compounds obtained from plants, have known antimicrobial, antifungal and insecticidal activities [22-24]. Essential oils have many therapeutic and they aid the distribution of drugs and antiseptics [25]. Their most important characteristics are their anti- infection, antibacterial, antifungal, allelopatic, and antoxidative effects [26, 22, 23]. Although the antimicrobial properties of essential oils and their components have been reviewed in the past [27, 28], the mechanism of action has not been studied in great detail. Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell [29, 11]. The objective of this study was to determine the chemical composition and antibacterial activity of the essential oil isolated from leaf of *Rosmarinus officinalis* from Morocoo.

MATERIALS AND METHODS

Chemicals and standards

All solvent were of analytical grade, unless otherwise specified. Hexane solution, anhydrous sodium sulfate and series of alkanes (C_4-C_{28}) standards were obtained from Faculty of Sciences, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

Plant material

The leaves of *Rosmarinus officinalis* were collected during April 2010 in Atlas median region (Taferdoust) from Morocco, 15 km in the south east of Boulmane city (latitude: 25° 31 '11" longitude: 5° 22' 21"; altitude: 2100 m); the climate was semi-desertic with strong continental influence with an annual average temperature of 20°C. Specimens were then dried in the open air for sixteen days. The plant was identified by Dr. Elhoussine Derwich and was then isolated from the other specimen and was deposited in Regional Center of Interface, University Sidi Mohamed Ben Abdellah.

The amount of oil obtained from each plant material was calculated as:

Oil (% v/w) = observed volume of oil (ml)/ weight of sample (g) x 100

Extraction of essential oils

The essential oils were extracted by hydro-distillation using an apparatus of Clevenger in Faculty of Sciences of Fez (Morocco). The extraction took 2.5 hours for mixing 200g of plants in 1400 ml of distilled water. The yellowish oil (0.5 ml) for leaves was dissolved in hexane and then dried over anhydrous sodium sulfate. After determining the yield and after filtration, the solvent was eliminated by pressure distillation reduced in rotary evaporator at 35°C and pure oil stored at 4°C in obscurity till the beginning of analysis.

Gas chromatography analysis (GC-FID and GC/MS)

The essential oils from leaves of *Rosmarinus officinalis* were analysed by gas chromatography (GCFID) and gas chromatography–mass spectrometry (GC–MS) using a CP-SIL- 5 CB column in Unity of GC/-MS and GC, Regional Center of Interface, Sidi Mohamed Ben Adellah University, Fez, Morocco.

The GC (TRACE GC-ULTRA, S/N 20062969, Thermo-Fischer) analysis equipped with flame ionisation detector (GC-FID), Varian capillary column Test Report CP 7770 (CP-SIL- 5 CB; 50m length, 0.32mm of Inside diameter, 0.45mm Outside diameter and Film thickness 1.20 μm). Column temperature was initially kept at 40 °C for 2 min, and then gradually increased to 260 °C at 5 °C/min rate and finally held for 10 min at 260 °C. The temperature of the injector was fixed to 260°C and the one of the detector (FID) to 270°C. The debit of gas vector (nitrogen) was fixed to 1ml/min.The volume of injected specimen was 0.5µl of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

The identification of different chemical compounds was realised by gas phase chromatography (TRACE GC-ULTRA, S/N 20062969, Thermo-Fischer) coupled with mass spectrometry (PolarisQ, S/N 210729, Thermo Fischer) (GC/MS). The utilised column was Varian capillary column Test Report CP 7770 (CP-SIL- 5 CB; 50m length, 0.32mm of Inside diameter, 0.45mm Outside diameter and Film thickness 1.20 µm). The column temperature was programmed from 40 to 260°C for 5°C/min. The temperature of the injector was fixed to 250°C and the one of the detector (PolarisQ) to 200°C. Ionisation of the sample components was performed in electron impact mode (EI, 70 eV). The debit of gas vector (Helium) was fixed to 1ml/min. Transfer line temperature was 300°C. The mass range from 40 to 650 amu was scanned at a rate of 2.9scans/s. The volume of injected specimen was of 1µl of diluted oil in hexane solution (10%). The constituents of essential oils were identified in comparison with their retention indices, calculated in relation to the retention time of a series of lineary alkanes (C_4- C_{28}) with those of reference products and in comparison with their retention indices with those of the chemical components gathered by [30] and in comparison with their spectres of mass with those gathered in a library (NIST-MS Search Version 2.0).

Antibacterial activity

Recent studies revealed that *Rosmarinus officinalis* oil is an effective antibacterial agent which can control many food micro-organisms such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli*, *Shigella dysenteria, Bacillus cereus* and *Staphylococcus aureus* [29]. Volatile compounds from plants, especially essential oils have anti-inflammatory [31, 32], antimicrobial, fungicidal and insecticidal activities [33-36]. These products have frequently been reported to be antimicrobial agents [37-41]. The volatile essential oils released from leaves, flowers and fruits into the atmosphere and from roots into the soil defend herbivores and pathogens [42]. The selected essential oils were screened against four: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Kellebsiella pneuomonae, Salmonella typhi, Staphylococcus intermedius, Bacillus subtilis, Streptococcus mutans, Micrococcus luteus,* and *Proteus mirabilis.* The minimal inhibition concentration (MIC) values were evaluated according to published procedures [43-45].The minimal inhibitory concentration (MIC) was determined only with micro-organisms that displayed inhibitory zones. MIC was determined by dilution of the essential oils in dimethyl sulfoxide (DMSO) and pipetting 0.01 mL of each dilution into a filter paper disc. Dilutions of the oils within a concentration range of 4- 48.2µg/mL were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth [46]. A negative control was also included in the test using a filter paper disc saturated with DMSO to check possible activity of this solvent against the bacteria assayed. Diameter of inhibition zone including disc diameter of 6 mm. The experiments were repeated at least twice.

In this study, antibacterial activity of *Rosmarinus officinalis* oil was examined using different bacterial species. In addition, chemical composition of volatile compounds, were also determined.

RESULTS AND DISCUSSION

Phytochemical screening of the essential oil

The retention time and chemical composition of essential oils of *Rosmarinus officinalis* are presented in Figure 1 and Table 1.

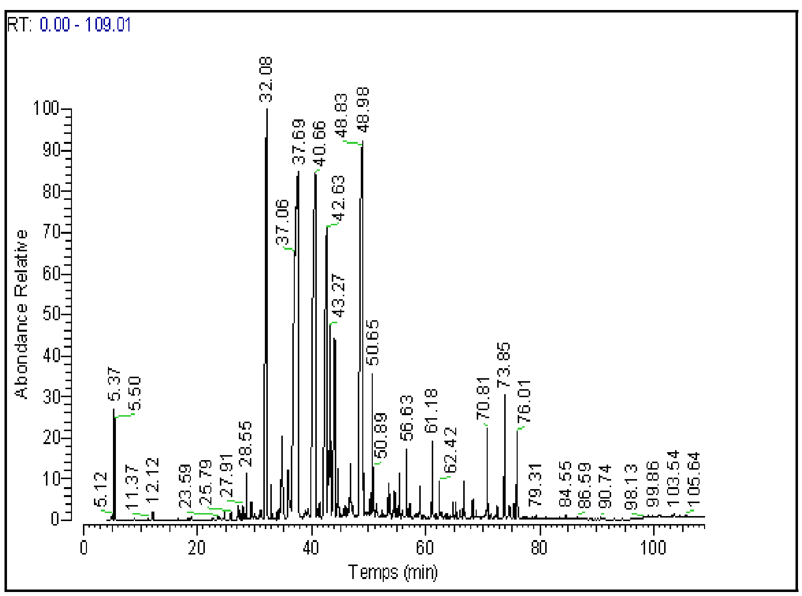

Fig. 1: Chromatogram of *Rosmarinus officinalis*

The constituents of *Rosmarinus officinalis* from Morocco are listed in order of their elution on the CP-SIL- 5 CB column (fig 1). In total, forty seven volatile compounds, representing 65.61% of the total composition, were identified in the leaves oils (Table 1). Monoterpene hydrocarbons were found to be the major group of compounds, the main one being α-pinene (18.25%) followed camphor (6.02%). The most abundant components found in the leaf oil were α-pinene (18.25%), other predominant components were camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%). The essential oils yield of *Rosmarinus officinalis* collected from mountainous region (Tafardoust) (Morocco) was 0.54%. It is relatively low than other plants industrially exploited as a source of essential oils: *Artemisia herba-alba* (0.59%), *Artemisia absinthium* (0.57%) and *Artemisia pontica* (0.31%)[47], *Rosmarinus officinalis* (1.9%) [17], *Mentha piperita* (1.02%)[48], lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%) and Laurel (0.1-0.35%) [49] and *Rosmarinus officinalis* (0.48-1.75%) [50].

Table 1: Chemical composition of essential oils of *Rosmarinus officinalis* from Morocco

Peak	Constituents	*RT (min)	**RI	Air (%)	***Mass range (m/z)
1	verbenol	25.79	944	0.05	(152),109,41,94,81,39,69,55,97,43,57
2	terpinolene	26.35	945	0.12	(136),93,121,91,136,79,77,105,39,41,107
3	1-octen-3-ol	28.02	948	0.03	(128),57,72,29,41,55,27,85,58,39,43
4	β-phellandrene	28.55	964	0.10	(136),93,77,91,136,79,94,41,80,92,39
5	α-pinene	32.08	969	18.25	(136),93,91,39,121,77,92,79,43,41,105
6	α-thujene	33.12	973	0.28	(136),93,41,91,77,79,39,27,69,94,43
7	α-myrcene	34.01	981	0.02	(136),41,93,69,39,27,53,79,77,67,91
8	sabinene	35.01	983	0.35	(136),93,41,91,77,79,39,27,69,94,43
9	α-terpinene	37.06	994	0.45	(136),93,91,136,121,77,92,79,43,41,105
10	camphene	37.69	998	5.02	(136),93,79,91,77,41,121,67,27,107,39
11	3-carene	38.20	1018	0.78	(136),93,91,79,77,92,121,80,136,94,105
12	β-pinene	40.66	1040	4.58	(136),93,91,69,39,77,92,79,53,41,27
13	trans-ocimene	41.25	1042	0.01	(136),93,41,79,91,77,92,39,27,80,53
14	cymen-8-ol	41.32	1120	0.01	(134),119,134,91,120,117,41,77,39,65,115
15	bornyl acetate	42.63	1277	4.35	(196),95,43,93,436,121,41,80,55,108,69
16	limonene	43.27	1005	3.56	(136),68,93,39,67,41,27,53,79,94,92
17	linalool	45.50	1082	0.20	(136),71,41,43,93,55,69,80,39,121,27
18	1.8-cineole	48.83	1059	5.25	(154),43,93,81,71,69,84,68,108,41,55
19	camphor	48.98	1122	6.02	(152),95,41,81,39,55,69,108,67,83,27
20	cis-Verbenol	49.13	1126	0.24	(152),109,41,94,81,39,69,55,97,43,57
21	terpinen-4-ol	50.62	1137	1.98	(154),71,111,93,43,86,41,69,55,68,154
22	borneol	50.65	1138	3.10	(154),95,41,110,93,55,67,139,121,96,69
23	menthofuran	50.89	1155	0.04	(150),108,150,79,109,39,77,41,91,51,53
24	α-copaene	53.71	1221	0.02	(204),161,119,105,93,41,91,92,81,120,204
25	carvone	56.60	1181	1.13	(152),95,67,68,82,81,41,152,69,109,55
26	pulegone	56.61	1220	0.02	(152),152,81,67,109,82,41,137,69,95,55
27	α-terpineol	56.63	1174	2.89	(154),59,93,121,136,81,43,68,95,67,41
28	sabinyl acetate	56.64	1224	0.01	(194), 92,91,81,41,134,55,109,79,43,53
29	linalyl acetate	56.65	1252	0.02	(196),93,43,41,69,80,121,68,55,71,79
30	carvacrole	56.66	1262	0.03	(150),135,150,91,136,77,107,117,115,79,105
31	isobornyl acetate	56.67	1265	0.11	(196),95,43,121,93,136,41,108,110,55,82
32	α -elemene	56.68	1410	0.02	(204),161,119,204,41,105,189,91,121,93,133
33	solanone	57.02	1296	0.06	(194),43,93,136,121,41,79,81,91,77,39
34	isosativene	57.05	1339	0.01	(204),94,91,41,105,79,93,204,119,39,77
35	α-cubebene	58.02	1344	0.01	(204),161,105,119,41,81,91,120,93,55,204
36	geranyl acetate	58.79	1352	0.01	(196),69,43,41,68,93,136,67,121,80,39
37	calarene	60.01	1403	0.01	(204),161,41,105,91,119,93,162,107,189,133
38	cymene	61.18	1045	2.56	(134),119,134,91,120,117,41,77,39,65,115
39	ledene	61.23	1419	0.04	(204),107,105,135,93,161,41,91,81,119,204
40	α -farnesene	61.41	1452	0.58	(204),41,93,69,107,55,79,91,119,77,123
41	α -cubenol	61.55	1458	0.58	(204),41,93,69,107,55,79,91,119,77,123
42	β-caryophyllene	62.02	1494	2.02	(204),93,133,91,41;79,69,105,107,120,77
43	caryophyllene oxide	62.42	1506	0.01	(220),43,41,79,93,91,95,69,55,67,81
44	germacrene-D	64.05	1515	1.02	(204),161,105,91,41,119,79,81,93,77,27
45	cadinene	64.85	1529	0.01	(204),161,189,204,105,91,133,119,95,41,55
46	α -eudesmol	65.50	1542	0.02	(222),59,149,43,41,108,93,79,81,67,164
47	Terpinyl isovalerate	69.02	1567	0.01	(238),136,121,93,85,41,57,60,81,137,68
Total identified constituents (%)				65.61	
Yields (%)				0.54	

*RT: Retention time obtained by chromatogram (Fig1).

**RI: Retention indices were determined by GC-FID on CP-SIL- 5 CB column.

***Mass range (m/z) was determined by mass spectrometry (PlarisQ).

The chemical compositions revealed that this leaves had compositions similar to those of other *Rosmarinus officinalis* essential oils analyzed by [51], which the major constituent in lab sample have been reported as α-pinene (30.3%) and 1.8-cineole (15.2%) and in main combinations in semi-industrial essential oil are α-pinene (30%) and 1.8-cineole (12.2%). Phytochemical studies have reported the occurrence of α-pinene (43.9-46.1%), 1.8-cineole (11.1%) and camphor (24-5.3%) in Rosemary essential oil [52]. Other studies by [53] reported the major constituent in leaves of *Juniperus phoenicea* collected from Atlas median in the region of Boulmane (Morocco) as α-pinene (49.15%). Contrary it's different to the composition of essential oil of leaves of *Lavandula dentate* study in Sardinia which the major component were verbenone (21.8%) [54]. These differences in oil composition are correlated with different regions or countries where the plant is cultivated [55, 56]. Intensive research on the chemical characteristics has been conducted on this species [57, 58]. The leaves essential oil of *Rosmarinus officinalis* has been reported in varying detail[59].

In this study the yield and total oil composition of essential oils of *Rosmarinus officinalis* collected from region of Boulmane (Morocco) where 0.54% and 65.61%. This total oil composition (63.81%) is low than other *Rosmarinus officinalis* study in Iran (99.74%) [13], in Brazil (90.6%) [16] and in Algeria (98.2%) [60] (Table 2). [17], studied the composition and antifungal activity of *Rosmarinus officinalis* L oil from Turkey; they reported that the yields and the total oil obtained were 1.9%% and 99.93% respectively and the composition is characterized by a high content of *p*-cymene (44.02%), linalool

(20.5%), γ-terpinene (16.62%), thymol (1.81%), β-pinene (3.61%), α-pinene (2.83%) and eucalyptol (2.64%). In our previous the essential oils obtained from leaves of *Rosmarinus officinalis* in Spain contained: α-pinene, 1.8-cineole, camphor, verbenone, and borneol [61]. Furthermore, in Egypt [62] studies on the chemistry of the essential oils extracted from the fresh leaves of *Rosmarinus officinalis* collected from Sinai and Giza, the yields were 0.14 and 0.40% respectively. These components represented 82% of the total composition of the oil identified in Sinai. verbenone (12.3%), camphor (11.3%), bornyl acetate (7.6%) and limonene (7.1%) were the major constituents and 86% of the total composition of the oil, were identified in Giza: camphor (14.9%), α-pinene (9.3%), and 1.8-cineole (9.0%) were the main constituents.

The essential oil content shows variations in plants of different geographical origin, periods and also in different part of the tree: [63] reported the rate of constituents in leaves of *Rosmarinus officinalis* plant being collected in three periods (before, after and during blooming) as α-pinene (20.08%, 27.65% and 17.82%), 1.8-cineole (7.32%, 7.55% and 9.99%) and camphor (9.11%, 8.84 and 15.68%). In our previous studies on the chemistry of Iran [64], considerable differences were observed in the essential oil composition between *Cuminum cyminum* and *Rosmarinus officinalis*: α-pinene (29.1% and 14.9%), 1.8-cineole (17.9% and 7.43%) and linalool (10.4% and 14.9%) respectively [65].

Furthermore, studies on the chemistry of *Juniperus phoenicea* [66], considerable differences were observed in the essential oil composition between leaves and berries: α-pinene [38.22% and 39.30%], [α-cedrol (31.23%) and sabinene (24.29%)] respectively.

Antibacterial activity

For thousands of years, there has been target interest in biologically active constituents, isolated from plant species for the elimination of pathogenic micro-organisms, because of the resistance that micro-organisms have built against antibiotics [67] or because they are ecologically safe compounds [68].

Table 2: Comparisons of the total oil and major compounds of leaves of essential oils of *Rosmarinus officinalis* analyzed in other countries

Extraits	**Iran**[13]		**Brazil**[16]		**Turkey**[17]		**Algerie** [60]		**Egypt**[62]		**Morocco**	
	TO (%)	MC (%)	TO (%)	MC (%)	TO (%)	MC (%)	TO (%)	MC (%)	TO (%)	MC (%)	TO (%)	MC (%)
Rosmarinus officinalis	99.74	α-pinene (15.52)	90.6	myrcene (24.6)	99.93	cymene (44.02)	98.2	1.8-cineole (29.5)	82-86	verbenone (12.3) - camphor (14.9)	63.61	α-pinene (18.25)

- TO: Total oil

- MC: Major compounds

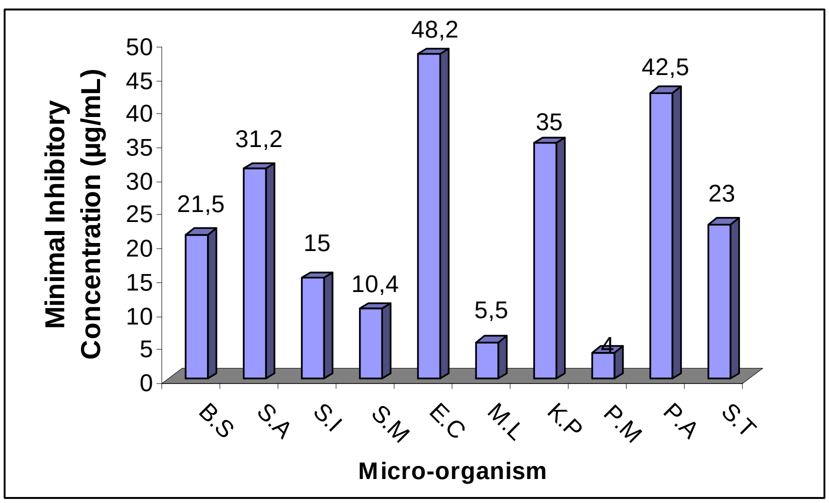

Fig. 2: Minimal Inhibitory Concentration (µg/mL) evaluation of the essential oil of *Rosmarinus officinalis (B.S: Bacillus subtilis, S.A: Staphylococcus aureus, S.I: Staphylococcus intermedius, S.M: Streptococcus mutans, E.C: Escherichia coli, M.L: Micrococcus luteus, K.P: Kellebsiella pneuomonae, P.M: Proteus mirabilis, P.A: Pseudomonas aeruginosa* and *S.T: Salmonella typhi).*

In recent years due to an upsurge in antibiotic-resistant infections, the search for new prototype drugs to combat infections is an absolute necessity and in this regard plant essential oils may offer great potential and hope.

Results obtained in the antibacterial study of the essential oils are shown on Figure 2 and 3. With the agar disc diffusion assay, oils were found to be active *Proteus mirabilis, Micrococcus luteus, Pseudomonas aeruginosa, Kellebsiella pneuomonae, Staphylococcus aureus*, *Streptococcus mutans, Bacillus subtilis* and *Salmonella typhi* at a minimal inhibitory concentration (MIC) of 4, 5.5, 10.4, 21.5 and 23µg/mL respectively. Against *Kellebsiella pneuomonae, Staphylococcus intermedius, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli;* the oil from the leaves was found to be more active; the oils showed MIC values of 35, 15, 31.2, 42.5 and 48.2µg/mL respectively (fig 2). The data indicated that *Proteus mirabilis, Micrococcus luteus* and *Streptococcus mutans* were the most sensitive strain tested to the oil of *Rosmarinus officinalis* with the strongest inhibition zone 21, 19.5 and 16mm respectively. *Bacillus subtilis, Salmonella typhi, Kellebsiella pneuomonae* and *Staphylococcus intermedius*, were found to be more sensitive among bacteria with inhibition zone of 13, 12, 10.5 and 10mm respectively. Modest activities were observed against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli;* with inhibition zones of 9.5, 8 and 7.5mm (fig 3). The antibacterial activity revealed that this leaves had relatively similar to those of other *Rosmarinus* *officinalis* essential oils analyzed by [20] which the oil is an effective antibacterial agent which can control many food micro-organisms such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli*, *Shigella dysenteria, Bacillus cereus* and *Staphylococcus aureus* [29]. The major components of this oil, α-pinene, have been known to exhibit antimicrobial activity against the bacterial strains (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *pseudomonas aeroginosa, Shigella flexniri, Kellebsiella pneuomonae, Salmonella typhi, Serratia marcescens* and *Escherichia. Coli*[13]. Essential oils rich in α-pinene demonstrated potential antibacterial activity [69, 70]. Monoterpenes hydrocarbons, terpinenes, have also shown antimicrobial properties that appear to have strong to moderate antibacterial activity against Gram-positive bacteria [71]. The bridged bicyclic monoterpenes α-pinene and β-pinene showed considerable biological activity [72]. On the other hand, enantiomers of α-pinene, β-pinene, limonene and linalool have a strong antibacterial activity [73]. The antimicrobial activity revealed that this leaves had similar to those of other *Rosmarinus officinalis* essential oils analyzed by[2], which the major component was α-pinene. In other study revealed the 1.8- cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus typhi, Staphylococcus aureus, Staphylococcus intermedius,* and *Bacillus subtilis*) [74]. Intensive research has been conducted on this antibacterial activity [75, 12, 76].

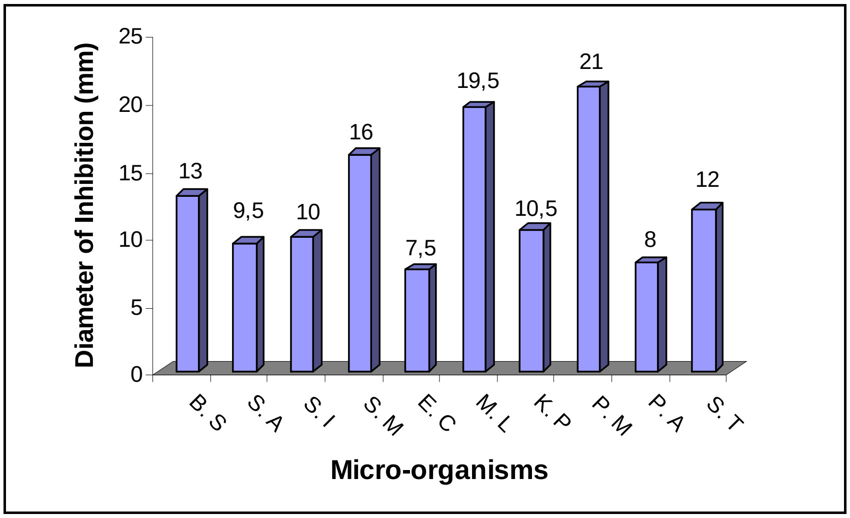

Fig.3: Diameter of Inhibition (mm) evaluation of the essential oil of *Rosmarinus officinalis* (B.S: *Bacillus subtilis*, S.A: *Staphylococcus aureus*, S.I: *Staphylococcus intermedius*, S.M: *Streptococcus mutans*, E.C: *Escherichia coli*, M.L: *Micrococcus luteus*, K.P: *Kellebsiella pneuomonae*, P.M: *Proteus mirabilis*, P.A: *Pseudomonas aeruginosa* and S.T: *Salmonella typhi*).

CONCLUSION

This study has been concerned with determining the chemical composition characteristics and antibacterial activity of essential oils extracted from *Rosmarinus officinalis*, collected in the middle Atlas region of Morocoo. The leaf oil obtained from *Rosmarinus officinalis* grown in Morocco was characterized by GC-MS, GC-FID and forty seven volatile compounds were identified which made up 63.61% of the total essential oil. The essential oil yields of the studies were 0.54%. *Rosmarinus officinalis* biosynthesized essential oils of one chemotype principal α-pinene (18.25%), according to the first major constituent: camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%). The oil was found to have significant antibacterial activity and therefore can be used as a natural antimicrobial agent for the treatment of several infectious diseases caused by these germs, which have developed resistance to antibiotics.

ACKNOWLEDGEMENTS

The authors thank Unity of GC/MS and GC, Regional Center of Interface, University Sidi Mohamed Ben Abdellah, Fez, Morocco for the gas chromatography coupled with mass spectrometry (GC/MS) and gas chromatography with flame ionization detection (GC-FID) analysis.

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