Chemical composition, antioxidant, and antimicrobial activities of rosemary (*Salvia rosmarinus* Spenn.) essential oils from Argentina

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Manuscript received: August 17, 2023

Keywords: ABTS, FRAP, DPPH, Principal Component Analysis, gas chromatography, 1,8-cineole

ABSTRACT

This work aimed to study the chemical variability of rosemary (*Salvia rosmarinus* Spenn.) essential oils from the Argentinean provinces of Mendoza and San Luis, and to compare them with commercially available oils. Additionally, this study aimed to assess the antioxidant and antimicrobial properties of the essential oils being examined. One of the essential oils from Mendoza (M23) and San Luis provinces belonged to myrcene, 1,8-cineole, and camphor chemotype. In contrast, the M24, M25, and M26 samples from Mendoza were found to belong to a chemotype characterized by high levels of 1,8-cineole, camphor, and α-pinene, also observed in both commercial oils. The antioxidant properties of essential oils were evaluated using three standardized methods: ABTS, FRAP, and DPPH. The results revealed that all essential oils exhibited lower levels of antioxidant activity when compared to established standards. The oils were also tested against 15 strains of Gram-positive and Gram-negative bacteria using the disk diffusion method. The oils from Argentina were active against *Staphylococcus aureus, Bacillus cereus, Paenibacillus larvae, Escherichia coli, Pseudomonas savastanoi pv glycinea* B076*, and Pseudomonas syringae pv syringae* 728*,* with inhibition zones ranging from 0.75 to 1.5 cm. In the future, rosemary essential oil could be used to avoid food deterioration and to control pathogenic microorganisms.

INTRODUCTION

Argentina has a rich diversity of native and introduced aromatic plants due to its diverse geography and climate. The country's vast territory includes regions with unique environmental conditions that favor the growth and development of a wide range of aromatic plants. Many are used for various purposes, including culinary, medicinal, and ornamental (Juliani et al., 2007).

Rosemary, *Salvia rosmarinus*, formerly known as *Rosmarinus officinalis* L., is an aromatic shrub native to the Mediterranean, belonging to the *Lamiaceae* family (Tropicos.org, 2023), and probably introduced to Argentina by the first European migration. It is common in gardens and family orchards due to its aromatic properties and because it is used as a spice in food and popular medicine (di Paola, 2006). As an aromatic crop, it is cultivated to supply the food, pharmaceutical, and cosmetic industries. The captivating aspect of this species lies in its attributes for health, encompassing qualities such as being a natural antioxidant, stimulant, tonic, and possessing antiseptic properties (Corbino, 2009). In addition, rosemary has been used as a controlling factor in the proliferation of tumor cells (Wang et al., 2012; Kontogianni et al., 2013), as an antibacterial agent (Wang et al., 2012; Jordán et al., 2013a; Coy Barrera and Acosta, 2013; Harmankaya and Vatansever, 2017; Ruales and Salazar, 2018), and as a food preservative (Lax Vivancos, 2014; Miranda et al., 2015; Castillo Ruilova, 2016).

A limited number of studies have been conducted to study the chemical diversity of Argentinean rosemary varieties. In Buenos Aires, two phenotypes of rosemary, wide (WP) and narrow leaf (NP), were evaluated to assess their chemical composition. The WP phenotype was dominated by α-pinene (31.2%), 1,8-cineole (21.6%), camphor (7%), and camphene (5%). The NP phenotype was dominated by myrcene (31.1%), 1,8-cineole (18.7%), and camphor (15.4%). This oil was characterized by lower levels of α-pinene (4.9%) (Ojeda-Sana, 2013). In Cordoba province, rosemary essential oils contained high levels of camphor (35.7%), verbenone (26.6%), and β-caryophyllene (15.8%) (Olmedo et al., 2015).

The typical composition of rosemary essential oils contained varied levels of α-pinene, 1,8-cineole, and camphor. For rosemary from Spain growing in different bioclimatic areas, Supra-Mediterranean (SM) and Lower Thermo-Mediterranean (LTM), the essential oils showed similar levels of α-pinene (13.0-13.1%), 1,8-cineole (19.6-21.2%), and camphor (17-18.6%), with myrcene remaining low (1.5-3.6%). Both varieties showed a similar aroma profile (Jordan et al., 2013b).

A comprehensive study conducted in the Balkans showed this general chemical profile (Lakusic et al., 2012). These authors identified three main chemotypes. The first chemotype was dominated by high levels of 1,8-cineole (27 - 16.7%) and varying levels of α-pinene (11.9 - 39.4%) and camphor (2.4 - 16.7%), with one of the samples containing high amounts of borneol (21.4%). The camphor type had high levels of camphor (24 - 28.1%), lower and varying levels of α-pinene (8.2 - 13.5%) and 1,8-cineole (10.9 - 18%), with one variety containing 11.6% of borneol. A variety from Lustica (Montenegro) was composed of high levels of camphene (10.2%) and myrcene (11.5%). The intermediate type, represented by two varieties, was dominated by both 1,8-cineole (16.7 and 30.3%) and camphor (17.3 to 28%), and had lower and varying amounts of α-pinene (8.7 and 11.2%) and borneol (16.5 and 12.9%). Rosemary from Serbia showed an unusual composition, dominated by limonene (21.7%) and camphor (21.6%), with lower amounts of α-pinene (13.5%), linalool oxide (10.8). Borneol, camphene, sabinene, 1,8-cineole, and α-terpineol were found in lower quantities (Bozin et al., 2007).

Essential oils (EO) from Morocco have been studied in detail. The major oil constituents were 1,8-cineole (28.6–51.1%), α-pinene (9.9–16.2%), camphor (5.3–16.8%), β- pinene (2.2–8.0%), camphene (2.3–7.7%), myrcene (0–4.5%), α-terpineol (0–3.8%), and limonene (0–3.3%) (Annemer et al., 2022).

Another study of rosemary essential oils from plants collected in Morocco found that essential oils were dominated by α-pinene (14%), 1,8-cineole (23.6%), camphor (18.7%), and borneol (15.5%), with minor amounts of verbenone, camphene, and caryophyllene (Bouyahia et al., 2017). In contrast, other oils from the same region contained high levels of α-pinene (14.1%), 1,8-cineole (48.8%), and camphor (15.3%), with lower amounts of myrcene, β-pinene, camphene, p-cymene, borneol, α-terpineol, and caryophyllene (Bouzid et al., 2023).

Monoterpenes characterized essential oils from Brazilian rosemary as the most commonly found terpenes, including 1,8-cineole, α-pinene, camphene, β–pinene, camphor, borneol, bornyl acetate, p-cymene, beta myrcene, limonene, α-terpinene, verbenone, β-terpineol, linalool, and terpinen-4-ol. Many of these monoterpenes are the most representative of the essential oils, with many of them being chemical markers of this oil (Soussa Borges et al., 2019).

Natural plant products are strongly considered a new and safe alternative for controlling microbial pathogens and for avoiding oxidation of products in food and pharmaceuticals (Bozin et al., 2007; Isman et al., 2011). In some cases, the use of preservatives to avoid food spoilage resulted in products that were not acceptable for human consumption (Bozin et al., 2007). In the case of antibiotics, misuse has led to rapid resistance of microorganisms, resulting in ineffective antibiotic compounds (Kokoskova et al*.,* 2011).

Hence, there exists a necessity to discover novel ecologically conscious substitutes within essential oils. Antioxidant activity is one of the remarkable bioactivities to be further explored for new uses and potential applications, particularly within the food, cosmetic, and pharmaceutical sectors (Veenstra and Johnson, 2021). In rosemary from Palestine, the essential oils showed lower antioxidant activity levels than Trolox (Vitamin E analog). The half-maximum inhibiting concentration (IC_{50}) for the oil was 22.38 ± 0.7 µg/mL and for Trolox was 2.7 ± 0.5 µg/mL (Eid et al., 2022). Similarly, researchers have conducted numerous studies using rosemary oils from various sources. A Chinese rosemary essential oil was more inhibitory against *S. aureus, E. coli, P. aeruginosa,* and *C. albicans* compared to oils from the other five Lamiaceae family members (Luo et al., 2019).

This study aimed to assess the chemical composition of essential oils of rosemary varieties grown in Mendoza and San Luis (Western Argentina) provinces and compare them with commercial types to identify chemotypes within the Argentinean varieties. This work also sought to study rosemary essential oils' antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Essential oils. The essential oils of *Salvia rosmarinus* were extracted by hydrodistillation with Clevenger trap from dried plants of the five varieties of rosemary: four provided by National Institute of Agricultural Technology (Instituto Nacional de Tecnologia Agropecuaria, INTA) in La Consulta, Mendoza, Argentina (M23, M24, M25, and M26); and one (SL) provided by the School of Tourism and Urbanism, National University of San Luis (Facultad de Turismo y Urbanismo, Universidad Nacional de San Luis). Two commercial essential oils were purchased from Auracacia (RA from Morocco) and Sigma Chemical Company (RS from Tunisia). The percentage yield (volume/weight) was determined.

GC-MS analysis of the essential oils. The essential oils were analyzed by Gas Chromatography/Mass Spectrometry using a Shimadzu TQ8040 equipped with an autosampler AOC-6000 and a column. Essential oils were diluted in Tert-butyl ethyl ether (TBME), 5 µL of essential oil/1 mL TBME, and 0.2 mL of this solution was injected in the GC (split 1:100). The oven was programmed as follows: Initial temperature 30 ºC (1 min), rate 1 7.5 ºC/min up to 150 ºC (1 min), rate 2 15 ºC/min up to 220 ºC (1 min). Total time 23.67 min. Oils were run in a Shimadzu capillary column (SH-Rxi-5 sil MS, equivalent to DB5, length 30 m, stationary phase thickness 25 µm, diameter 0.25mm).

Ferric Reducing Antioxidant Power assay (FRAP). The ability of the essential oils (EO) to reduce Fe^{+3} to Fe^{+2} was analyzed using the ferric reducing antioxidant power assay (FRAP), in which an increase in absorbance (blue color) at 593 nm was observed (Benzie and Strain, 1996). An essential oil dilution of each oil was mixed with the FRAP reagent ($FeCl_3$: buffer acetate: TPTZ; 1:10:1), and absorbance (OD) was measured at 593 nm in a spectrophotometer. A control without essential oil was also measured at the same OD. A calibration curve was made using ascorbic acid (AA) (1 mg/mL), and the result values were expressed as mg EO/mg AA. The experiments were carried out in duplicate.

Total radical scavenging capacity ABTS assay. This assay was used to analyze the ability of essential oils to scavenge free radicals. The ABTS reagent was prepared with potassium persulfate and left in the dark until the following day. This solution was diluted in ethanol, OD at 734 nm was measured and adjusted to 0.7, and the solution was kept at 30 ºC. A dilution of each essential oil in ethanol was mixed with the ABTS reagent and the OD was measured. Dilutions of TROLOX (T) were used to build the calibration curve. Results were expressed as mg EO/mg T. The experiments were carried out in duplicate (Bendif et al., 2017).

Free radical scavenging capacity ABTS assay. For this assay, a solution of DPPH (2,2-diphenyl-1-picrylhydrazyl radical) was prepared by solubilizing the reagent in ethanol, and TROLOX was used for the calibration curve. The reagent OD at 517 nm was adjusted below 1. Optical density of essential oil dilutions was measured and compared to TROLOX. The results were expressed as mg EO/mg T. The tests were performed in duplicate (Bendif et al., 2017).

Antimicrobial activity. Microorganisms. The following microorganisms were used to test the antimicrobial activity of rosemary essential oils. Gram-positive strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 9341, *Staphylococcus epidermidis, Bacillus cereus, Paenibacillus larvae* T, and *Paenibacillus larvae* 9. Gram-negative strains: *Escherichia coli, Pseudomonas aeruginosa* PAOI, *Pseudomonas fluorescens, Pseudomonas syringae* Q, *Pseudomonas syringae* pv. *Tomato, Pseudomonas syringae* pv. *Syring*ae 728, and *Pseudomonas savastanoi* pv. *glycinea* B076.

Disk diffusion method. All bacteria except *P. syringae* were incubated for 18-20 h, at 37 ºC in tubes containing Müeller Hinton Broth. *P. syringae* strains were incubated in King B broth (CKB) for 18-20h at 28 ºC. Ten-fold dilutions were made of each inoculum until an OD at 620 nm of 0.04 (10^6 CFU/ml) was reached. Then, 100 µl of each inoculum was spread over plates containing either Mueller-Hinton agar (or King B agar for *P. syringae*). Paper filter discs of 6 mm were impregnated with 10 µl of each essential oil and were placed on the surface of the agar media. The plates were left for 30 minutes at room temperature to allow the oil diffusion into the agar. Plates were then incubated at 37 ºC (or 28 ºC for *P. syringae*) for 24 h. After this time, the inhibition zone around each disc was measured in cm (Oliva et al., 2010).

RESULTS AND DISCUSSIONS

GC-MS analysis of the essential oils

The yields of the essential oils were: M23, 3.08%; M24, 0.51%; M25, 0.50%; and M26, 1.95%; and SL, 1.23%. The rosemary essential oils from the varieties M23, M24, M25, and SL were harvested at the end of August (winter) when plants were in the flowering stage, and M26 was harvested in June.

The essential oils of rosemary varieties from Argentina showed significant variations in composition (Table 1, Figure 1). The major components of all *S. rosmarinus* essential oils that dominated the profile were α-pinene, camphene, myrcene, limonene, 1,8-cineole, and camphor (Figure 1). In contrast, β-pinene, α-terpinene, *para*-cymene, γ-terpinene, linalool, camphor, terpinen-4-ol, α-terpineol, and (E)-β-caryophyllene were found in minor amounts. All these compounds have been previously described as components in the different chemotypes of rosemary essential oils (Annemer et al., 2022; Bouzid et al., 2023). All the oils were characterized by high levels of 1,8cineole and camphor, and varying levels of myrcene and α-pinene (Figure 1).

The major components in all the oils were 1,8-cineole (23.52-51.91%) and camphor (9.5-15.38%); however, myrcene was present as the main component in M23 (32.42%) and SL (22.62%) with low levels of α-pinene (7%) (Table 1; Figure 1). These values coincide with the narrow phenotype (NP) described by Ojeda-Sana (2013) for essential oils from Buenos Aires, Argentina. Another study with two Argentinean rosemary essential oils from Castelar and Sumalao found similar results in the composition (Mizrahi et al., 1991).

In this study, all the varieties from Mendoza, M23 to M26, presented high levels of 1,8-cineole (24.46%, 23.64%, 23.52%, and 29.85%, respectively) (Table 1; Figure 1); however, they did not reach the 50% of the commercial oils from Morocco and Tunisia. They presented similar amounts of camphor compared to the Morocco and Tunisia oils. M24, M25, and M26 oils were dominated by higher levels of α-pinene, 14.35%, 11.87%, and 19.36 %, respectively. These values were similar to commercial oils. These three varieties contained the highest levels of borneol (6.49%, 6.68%, and 3.35%, respectively) compared with the other samples, including the commercial ones from Morocco and Tunisia (Table 1). The Morocco and Tunisia essential oils showed a similar profile showing high levels of 1, 8 cineole (50.91% and 51.91%), α-pinene (14.44% and 10.68%), and camphor (13.36% and 9.5%).

There were slight differences between the M24, M25, and M26 samples (Table 1). Camphene was highest in M26 (6.53 %) and lower in the other two (3.82% and 2.95%). Linalool was high in M24 (3.81%) and M25 (34.38%) and low in M26 (0.44%). Borneol and verbenone showed similar trends, being higher in M24 and M25 (Table 1).

All varieties grown in Mendoza (M23-26) showed the presence of marker components that were not found in the other samples, including thuja 2,4-(10)-diene, verbenone, *cis* and *trans* pinocamphone (Table 1).

M24, M25, and M26 essential oils composition could be compared to that obtained from Morocco, in which the main compounds identified were α-pinene, 1,8-cineole, and camphor. Similar results were obtained in an investigation of the chemical composition of *S. rosmarinus* from Morocco, in which these three terpenes represented the main compounds (Bouzid et al., 2023).

The two commercial essential oils contained very low levels of myrcene (1%), similar to the Moroccan oil described by Annemer et al. (2022) in which the compound was below 3% for all the oils. Similar results of composition were described for three rosemary essential oils from Paraná (Brazil), which contained α-pinene (9.3–20.1%), 1,8-cineole (13.0–49.1%), and camphor (12.1–20.5%) as the main compounds (Trombin de Souza et al., 2022). The most representative compounds for the essential oils of *S. rosmarinus* from Italy were 1,8-cineole, α-pinene, and camphor. They also contained camphene, sabinene, β- pinene, myrcene, limonene, γ–terpinene, terpinolene, borneol, α-terpineol, bornyl acetate, and trans caryophyllene (Tundis et al., 2020). The essential oil of *S. rosmarinus* from Greece was similar to the Italian oil with α-pinene, camphene, β- pinene, *para*-cymene, 1,8-cineole, borneol, α-terpineol, bornyl acetate, and caryophyllene as the main components (Papageorgiou et al., 2008).

In other rosemary species, *Salvia jordanii* J.B.Walker, ex *Rosmarinus eriocalyx* Jord. & Fourr., the main components of the oils were camphor, α-pinene, camphene, 1,8-cineole, β-pinene, limonene, borneol, E-caryophyllene, and α-bisabolol (Bendif et al*.,* 2017). This is a very similar composition to *S. rosmarinus* essential oils described above.

Research studies have demonstrated that the physiological age, the variety, the part of the plant, the origin, the soil, the climate, and many other factors are responsible for the chemical composition of the essential oils, and that the variations are found more frequently in quantity than in quality (Sousa Borges et al*.,* 2019). The major components of essential oils define chemotypes. Five chemotypes have been determined based on the chemical composition of the EO: 1- α-pinene /1,8-cineole, where α-pinene is the primary compound; 2- verbenone/α-pinene /camphor/ 1,8-cineole, where verbenone is the main compound; 3- myrcene/1,8-cineole/camphor, dominated by myrcene; 4- 1,8-cineole/camphor/α-pinene , where 1,8-cineole is the major component; and 5- α-pinene /beta pinene/camphene, where pinenes and camphene are present almost in the same quantity (Satyal et al., 2017). The M23 and SL essential oils were similar to chemotype 3 (myrcene/1,8-cineole/camphor), which has been characteristically found in Argentina; while, M24, M25, M26, and both commercial oils belonged to chemotype 4 (1,8-cineole/camphor/α-pinene).

The principal component analysis (PCA) of the chemical variability of the essential oils (Table 1, Figure 1) separated the different essential oils based on their chemical profile. The first principal component explained the 89.6% of the total variance in the dataset. In comparison, the second principal component explained the 8.6%, with these two principal components explaining most of the total variance (98.2%).

Three main groups were identified, each in a different quadrant. The varieties M23 and SL formed a distinct group that belonged to the chemotype myrcene/1,8-cineole/camphor. This group (M23-SL) was opposite (negatively correlated) to the commercial group (Morocco and Tunisia). The M24-M26 varieties formed the third group, sharing a similar composition belonging to the chemotype 1,8-cineole/camphor/α-pinene, with variety M26 being the most dissimilar. The commercial group represented by Northern Africa varieties, Morocco and Tunisia, was clearly separated from the Mendoza group, suggesting that the Northern Africa group belonged to a different chemotype that is high (> 50%) in 1,8-cineole (Table 1, Figure 2).

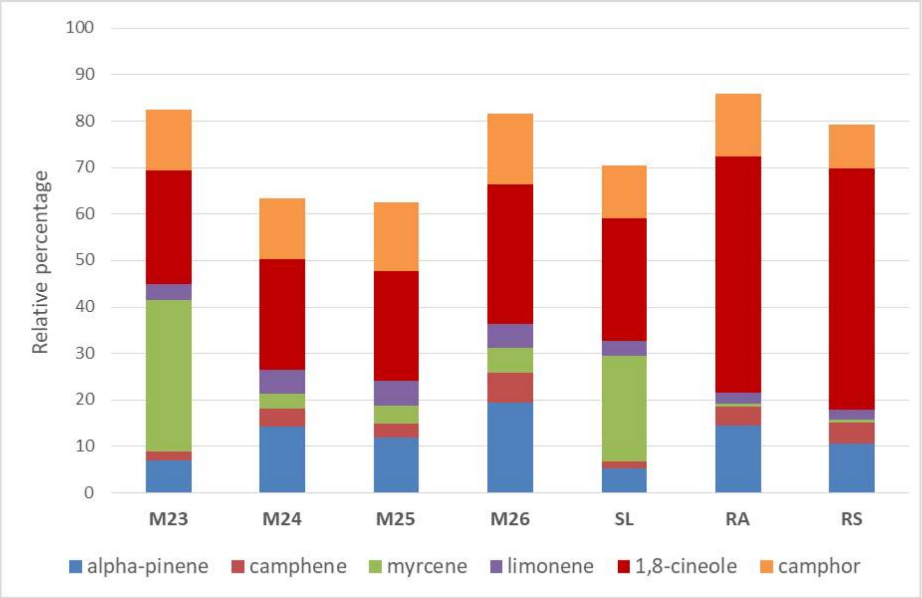

Figure 1. Relative percentages of major components of rosemary (*Salvia rosmarinus*) essential oils from Mendoza (M23-M26), San Luis (SL), and commercial samples from Morocco and Tunisia for comparison.

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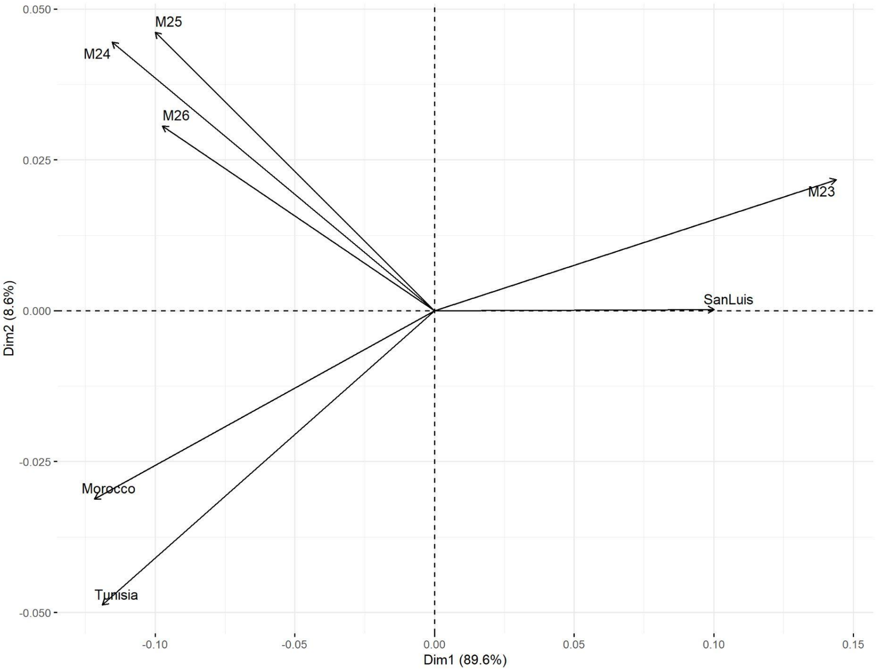

Figure 2. Principal component analysis of the chemical variability of rosemary (*Salvia rosmarinus*) essential oils from Mendoza (M23-M26), San Luis (SL), and commercial samples from Morocco and Tunisia for comparison.

Table 1. Chemical composition of rosemary (*Salvia rosmarinus*) essential oils of the varieties M23, M24, M25, and M26 (INTA La Consulta, Mendoza, Argentina) and SL (San Luis, Argentina) from Argentina and their comparison with essential oils from Morocco and Tunisia.

Peak[1]	RT	RI	Components	M23	M24	M25	M26	SL	Morocco (RA)[2]	Tunisia (RS)[3]
3	6.557	923	unknown					0.8		
4	7.936	923	tricyclene	0.06	0.12	0.07	0.21		0.09	0.1
5	8.013	927	α-thujene	0.19			0.03	0.1	0.08	0.26
6	8.18	934	α-pinene	6.97	14.35	11.87	19.36	5.31	14.44	10.68
7	8.54	951	camphene	2.04	3.82	2.95	6.53	1.49	4.16	4.45
8	8.63	955	thuja 2,4-(10)-diene	0.12	0.41	0.41	0.15			
9	9.01	972	verbenene				0.01			
10	9.05	974	sabinene		0.65	0.52	0.02	0.06		0.09
11	9.16	979	beta pinene	0.54	1.1	0.89	1.07	0.34	4.01	7.98
12	9.28	984	3-octanone		0.1	0.1	0.02			
13	9.39	989	myrcene	32.46	3.14	4.05	5.25	22.62	0.7	0.69
14	9.78	1007	α-phellandrene	0.45	0.55	0.48	1.23	2.28	0.08	
15	9.85	1010	*delta*-3-carene		1.19	1.17	1.02			0.11
16	10.02	1018	α-terpinene	1.16	0.55	0.56	1.2	0.93	0.28	0.18
17	10.18	1026	*para*-cymene	2.52	2.37	2.27	2.59	8.39	1.74	1.46
18	10.3	1031	limonene	3.37	5.3	5.27	5.32	3.24	2.23	2.09
19	10.36	1034	1,8-cineole	24.46	23.64	23.52	29.85	26.48	50.91	51.91
20	10.92	1060	γ-terpinene	1.6	0.49	0.68	0.97	4.55	0.38	0.42
22	11.51	1087	terpinolene	0.4	0.3	0.43	0.68		0.13	0.17
23	11.59	1091	*para* -cymenene		0.26	0.27	0.08			
24	11.76	1099	unknown					0.11		
25	11.77	1099	linalool	1.33	3.81	4.38	0.44	3.74	0.46	0.22
26	11.82	1101	Unknown[4]		0.52	0.41				
28	12.26	1123	chrysanthenone	0.06	2.89	1.8				
29	12.37	1128	α-campholenal		0.13	0.05				
30	12.69	1144	*trans*-pinocarveol		0.3	0.11				
31	12.82	1151	camphor	13.09	13.13	14.91	15.38	11.28	13.36	9.5
32	13.10	1164	*trans*-pinocamphone	0.12	0.75	0.65	0.27			
33	13.22	1170	pinocarvone		0.11	0.11				
34	13.27	1172	δ-terpineol	0.16	0.18	0.18	0.08		0.22	0.15
35	13.33	1175	borneol	0.26	6.49	6.68	3.35		2.21	2.7

[1] Listed peaks of a total of 59 integrated peaks, blank spaces indicate the component was not detected. [2] Commercial rosemary essential oil from Auracacia, and [3] Sigma Chemical Co. [4] Mass spectra: 55(15%), 70(30%), 80(100%), 91(45%), 107(50%), 122(25%), 150 (10%), structure like filifolone.

Table 1*(continuation)*. Chemical composition of rosemary (*Salvia rosmarinus*) essential oils of the varieties M23, M24, M25, and M26 (INTA La Consulta, Mendoza, Argentina) and SL (San Luis, Argentina) from Argentina and their comparison with essential oils from Morocco and Tunisia.

Peak[1] #	RT	RI	Components	M23	M24	M25	M26	SL	Morocco (RA)[2]	Tunisia (RS)[3]
36	13.41	1180	*cis*-pinocamphone	0.25	2.08	1.69	0.54			
37	13.49	1183	terpinen-4-ol	0.95	0.67	0.81	0.5	2.36	0.48	0.55
38	13.58	1187	*para*-cymen-8-ol		0.06	0.06				
39	13.77	1197	α-terpineol	1.65	1.37	1.52	0.81	0.5	1.8	1.28
40	13.92	1204	γ–terpineol		0.39	0.37	0.03			
41	14.03	1210	verbenone	1.42	3.02	4.85	0.17			
43	14.68	1244	*cis*-myrtanol		0.31	0.28	0.01	0.35		
44	14.83	1251	*trans*-myrtanol		0.52	0.51	0.02			
45	15.2	1271	piperitone		0.07					
46	15.52	1287	*iso*-bornyl acetate		1.3	0.42	1.28		0.37	1.41
47	15.55	1289	thymol					3.13		
48	15.73	1297	carvacrol		0.07			0.83		
49	17.3	1381	β-copaene		0.31	0.3				
50	18.18	1430	(*E*)-β-caryophyllene	2.79	1.35	2.34	1.01	0.77	2.33	3.03
51	18.54	1450	(Z)-beta farnesene		0.08	0.06				
52	18.84	1466	α-humulene	0.8	0.17	0.34		0.07	0.18	0.25
53	19.13	1483	α- morphene		0.24	0.23				
54	19.50	1503	β-guaiene		0.09	0.07				
55	19.61	1511	β-bisabolene			0.05				
56	19.73	1521	γ-cadinene		0.17	0.14				
57	19.80	1525	δ-cadinene	0.05	0.32	0.33				0.07
58	19.84	1529	*trans*-calamenene		0.11	0.07				

[1]Listed peaks of a total of 59 integrated peaks, blank spaces indicate the component was not detected. [2]Commercial rosemary essential oil from Auracacia, and [3]Sigma Chemical Co.

Antioxidant capacity

The antioxidant activity of essential oils from Argentina was assessed by three standardized methods, with all essential oils showing some level of antioxidant activity as compared with known standards Trolox (a vitamin E analog) and ascorbic acid. In the ABTS assay, SL essential oil had the highest antioxidant activity, having a Trolox Equivalent Antioxidant Capacity (TEAC) of 1.8 mg of the essential oil (EO) per mg of Trolox, while M24 showed the lowest activity, with a TEAC of 11.1 mg EO per mg of Trolox (Table 2). The FRAP antioxidant assay, a water-based analysis, used ascorbic acid to determine the Ascorbic acid Equivalent Antioxidant Capacity (AEAC). The essential oil M25 showed the highest antioxidant activity (9.2 mg of EO/mg ascorbic acid), while the Moroccan essential oil showed the lowest activity, with an AEAC of 43.6 mg EO/mg ascorbic acid. In the DPPH assay, Trolox was used as a standard to determine TEAC. The M24, M25, and SL samples were the essential oils with the highest antioxidant activity (164.8 to 348.5me EO/mg Trolox), while M23 was the oil that showed the lowest TEAC (706.0 mg EO/mg Trolox) (Table 2).

To determine the antioxidant properties of natural products, it is advisable to use more than one in-vitro test as each has a different mechanism of action. The DPPH test measures the ability of essential oils to act as hydrogen donors; the FRAP assay tests the ability of essential oils to reduce Fe^{+3} (Bozin et al., 2007); and the ABTS assay tests the essential oil's ability to scavenge free radicals (Bendif et al., 2017). Of the three methods, the ABTS assay seemed to be the most sensitive as less oil concentration was needed to observe an antioxidant effect. For the FRAP assay, the highest antioxidant level was equivalent to 9.2 mg per mg of ascorbic acid. Much lower activities were found with the DPPH method, where the highest antioxidant activity was 164.8 times lower than that of a unit of Trolox.

The sample SL, as determined by the ABTS method, had the highest antioxidant activity, with 1.8 mg of oil equivalent to 1 mg of Trolox. The other oils showed lower levels (from 3.6 to 6.6 mg EO per mg of Trolox). The higher antioxidant activity of the SL oil coincides with the presence of thymol (3.13%) and carvacrol (0.83%), which are almost devoid of the rest of the oils. In fact, these two components are important chemical markers (Table 1).

Table 2. Antioxidant activity (ABTS, FRAP, and DPPH) of rosemary (*Salvia rosmarinus*) essential oils and their comparison with essential oils from Morocco.

Rosemary variety	ABTS* mg EO/mg Trolox	FRAP* mg EO/mg AA	DPPH* Mg EO/mg Trolox
Mendoza 23	4.7	35.5	706.0
Mendoza 24	11.1	32.4	164.8
Mendoza 25	4.3	9.2	348.5
Mendoza 26	3.6	15.8	390.5
San Luis	1.8	11.7	258.1
Morocco	6.6	43.6	484.9

*Average of duplicate trials.

The differences observed in these assays could be due to the chemical nature of compounds in the oils, and either synergistic or antagonistic combinations between them (Bozin et al., 2007).

Several authors have attributed the antioxidant ability of essential oil to the amount of total phenolic compounds present (Bouzid et al., 2023; Bouyahya et al., 2017; Leporini et al., 2020). Such composition makes an oil suitable to be considered for food preservation purposes. Extracts and essential oils from rosemary (*S. rosmarinus*) have been extensively studied. The European Union (EU) has classified both extracts and oils of rosemary as nontoxic, with no adverse effects observed (NOAEL). For this reason, rosemary essential oils are of particular interest to the pharmacological and food industries (Veenstra and Johnson, 2021).

Antimicrobial activity

The antimicrobial activity of rosemary essential oils was tested against different bacteria that can be classified either by their cell wall composition as Gram-positive or Gram-negative bacteria, or by the hosts that they infect, such as humans, other animals, or plants. *S. epidermidis* is a skin commensal responsible for opportunistic, nosocomial, and cardiovascular infections. *S. aureus, E. faecalis, B. cereus, E. coli*, and *P. aeruginosa* are responsible for many diseases in humans and animals and can contaminate foods by producing toxins and causing their deterioration (Pires Amaral et al., 2019). *P. larvae* is an insect pathogen and producer of the devastating bee disease Loque Americana, which has led to substantial economic losses (Paletti Rovey et al., 2023). The *P. syringae* complex has been related to disease in many plants, including farm vegetables such as tomatoes and lettuce, and field products such as soybean and wheat (Oliva et al., 2015).

As observed in the disc diffusion method, all the Argentinean essential oils showed activity against *S. aureus*, with inhibition zones 0.9-1 cm (Table 3; Figure 3). The oil from Morocco showed the highest activity, with an inhibition zone of 10.5 cm (Table 3). The rosemary oils from Argentina were inactive against *S. epidermidis*, while the oils from Morocco and Tunisia showed inhibition zones of 0.95 cm. All oils, except San Luis, were active against *M. luteus*, with inhibition zones varying between 0.8 and 1.5 cm. The oil from San Luis was inactive. The oil from Morocco was the only one active against *E. faecalis*, with a zone of inhibition of 0.9 cm. All oils showed similar levels of activity against *B. cereus*, with inhibition zones of 0.85-1.15 cm, and against *E. coli*, with inhibition zones of 0.8-1.2 cm (Figure 3). M25, Morocco, and Tunisia oils were active against *P. larvae* 9 and *P. larvae* T. *P. larvae* T was also sensitive to SL oil. The M24 and Tunisia were the only oils showing activity against a strain of *Klebsiella sp.*, with inhibition zones of 0.9 and 1, respectively. None of the oils were active against three Gram-negative bacteria, *P. aeruginosa* (PAOI), *P. fluorescens*, and *P. syringae* (Q) (Table 3). All essential oils showed activity against *P. savastanoi* pv *glycinea* B076, with zones of inhibition ranging from 0.7 to 1.3 cm, with the oils from Tunisia showing the highest antibacterial activity (1.3 cm). A similar trend was observed for *P. syringae* pv *syringae* 728, with all oils being active and having inhibition zones of 0.75 to 1.2 cm. The oils from Morocco and Tunisia were active against *P. syringae* pv *tomato* DC3000, having inhibition zone of 0.8 cm, while the Argentinean oils were inactive against this strain (Table 3, Figure 3).

Table 3. Antimicrobial activity of rosemary (*Salvia rosmarinus*) essential oils of the varieties M23, M24, M25, and M26 (INTA La Consulta, Mendoza, Argentina) and SL (San Luis, Argentina) and their comparison with essential oils from Morocco and Tunisia.

Microorganism/Essential oil	M24	M25	M26	SL	Morocco (RA)	Tunisia (RS)
S. aureus ATCC (+)	1	1	1	0.95	10.5	0.9
S. epidermidis (+)	NI	NI	NI	NI	0.95	0.95
M. luteus ATCC (+)	1	1.5	0.8	NI	1.25	1.25
E. faecalis ATCC (+)	NI	NI	NI	NI	0.9	NI
B. cereus (+)	1.15	0.85	0.85	0.9	0.9	1.15
P. larvae 9 (+)	ND	1.4	ND	ND	1.1	2
P. larvae T (+)	ND	1.2	ND	1.1	1.2	1.2
Escherichia coli (-)	1	0.85	0.9	0.8	1.2	0.8
Klebsiella sp (-)	0.9	NI	NI	NI	NI	1
P. aeruginosa PAOI (-)	NI	NI	NI	NI	NI	NI
P. fluorescens (-)	NI	NI	NI	NI	NI	NI
P. *savastanoi* pv *glycinea* B076 (-)	1	1	0.8	0.7	0.85	1.3
P. syringae pv *syringae* 728 (-)	0.85	0.95	0.75	0.8	0.95	1.2
P. syringae pv *tomato* DC3000 (-)	NI	NI	NI	NI	0.8	0.8
P. syringae Q (-)	NI	NI	NI	NI	NI	NI

Antimicrobial activity expressed as inhibition zones measured in cm, average of duplicate trials. (+) Gram-positive bacteria; (-) Gram-negative bacteria; ND, not done; NI, no inhibition.

On average, Gram-positive strains showed more sensitivity to the oils than Gram-negative strains, with slightly higher inhibition zones. Similar results were observed with three essential oils of *S. rosmarinus* collected from different regions of Morocco, where *B. subtilis* was more inhibited than *E. coli* (Annemer et al., 2022). Bouyahya et al. (2017) evaluated the chemical composition, antioxidant, and antimicrobial properties of *S. rosmarinus* essential oils from Morocco, reporting that the main components were 1,8-cineole, α-pinene, and camphor, and that there was higher antimicrobial activity against Gram-positive strains than Gram-negative ones.

Another study, conducted by Bendif et al. (2017), found antimicrobial activity against Gram-positive, Gram-negative bacteria, and a yeast strain using rosemary essential oils from Algeria. In this study, the Gram-positive bacteria *S. aureus* and *M. luteus,* and the sporulated bacteria *B. cereus* and *P. larvae*, were inhibited by all the oils tested, Gram-negative bacteria that were inhibited by these oils included *E. coli, P. savastanoi* pv *glycinea* B076, and *P. syringae* pv *syringae* 728. Gram-negative *P. aeruginosa, P. fluorescens*, and *P. syringae* Q were the most resistant strains to the oils, as no inhibition zones were observed in the agar plates.

Similar results showing a lack of rosemary essential oil activity against *P. aeruginosa* were obtained by other researchers (Annemer et al., 2022; Bendif et al., 2017). The resistance of Gram-negative cells could be attributed to the amphipathic lipopolysaccharide external membrane that might act as an external barrier, preventing the entrance of hydrophobic compounds. In addition, the mechanism of action of essential oils on bacterial cells has been reported to affect several targets, including disrupting the cytoplasmic membrane and disturbing metabolic processes including respiration, transport of molecules, and chemical signaling processes such as the quorum sensing system. All these metabolic processes have been observed to be affected by essential oils (Carvalho et al., 2019).

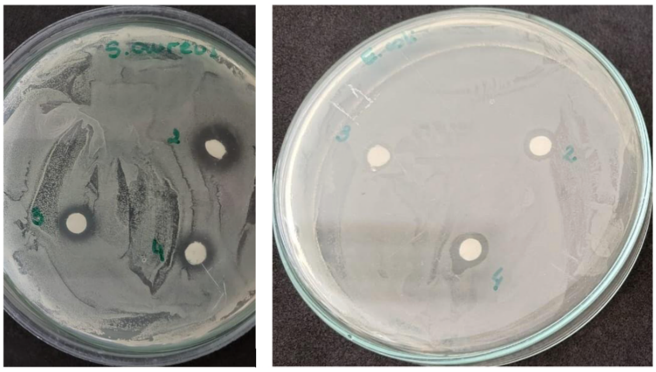

Figure 3. Antimicrobial activity of rosemary (*Salvia rosmarinus*) essential oils from Argentina on *S. aureus* (left) and *E. coli* (right) by the disc diffusion method. Zones of inhibition were measured in cm. Disk number 2, M24; number 3, M25; number 4, M26.

The antimicrobial activity of essential oils has been attributed to their main components and to the synergic or antagonist relationships between these components (Bouyahya et al*.*, 2017; Burt, 2004). The most active oils in this study were the Morocco and Tunisia oils, with 1,8-cineole as their main component, followed by camphor and α-pinene. Other researchers have also tested the antimicrobial activity of individual terpenes, demonstrating that they are active compounds against microorganisms and that this activity depends on the chemical structure. For example, oxygenated groups further improve the antimicrobial effectiveness of essential oil components, in contrast to the lower antimicrobial activities of hydrocarbonated terpenes (Koroch et al., 2007).

Other studies have focused on the antimicrobial activity of 1,8-cineole. The essential oil of *Perovskia abrotanoides*, a medicinal plant from Iran used to treat leishmaniasis, has major components similar to *S. rosmarinus*, including 1,8-cineole, camphor, and α-pinene as primary terpenes. These terpenes are responsible for the inhibitory activity of this essential oil (Mahboubi and Kazempour, 2009). An investigation of the antimicrobial activity of 1,8-cineole and limonene showed that the 1,8-cineole was more active on *S. aureus, B. cereus, E. coli,* and *P. aeruginosa* than on the yeast *Cryptococcus neoformans*. In the same study, the association of both terpenes was tested with the chequerboard technique showing no difference between them (van Vuurem and Viljoen, 2007).

The PCA analysis in this study showed that the antimicrobial activity was related to the chemistry of the essential oils (Figure 4). The essential oils were separated into three different quadrants of the PCA, with this segregation reflecting the geographical origins of the oils, as the essential oils from Argentina, Morocco, and Tunisia formed separate groups. The proximity of oils M24 and M25 in the PCA showed that the antimicrobial activity of these oils was closely related, while the oils M26 and SL were more dissimilar (Table 1, Figure 4).

The essential oils from Morocco and Tunisia were the most effective against all the microorganisms, showing inhibition activity against 11 strains out of 15. The major component of both oils was 1,8-cineole, representing more than 50% of the composition, followed by α-pinene and camphor. The Argentinean essential oils showed lower antimicrobial activity, inhibiting only 7 strains out of the 15. These oils showed a slightly different composition than the Morocco and Tunisia commercial oils. The Argentinean oils showed lower levels of 1,8-cineole (24-26%), and higher levels of α-pinene, myrcene, and camphor (Table 1).

Our results are in accordance with those of Annemer and collaborators (2022), where rosemary essential oils from Morocco produced by farmers' cooperatives showed high levels of 1,8-cineole (46.2-51.1%). In addition, they also observed that the oils containing the highest amount of this component exhibited the highest antimicrobial activity (Annemer et al., 2022).

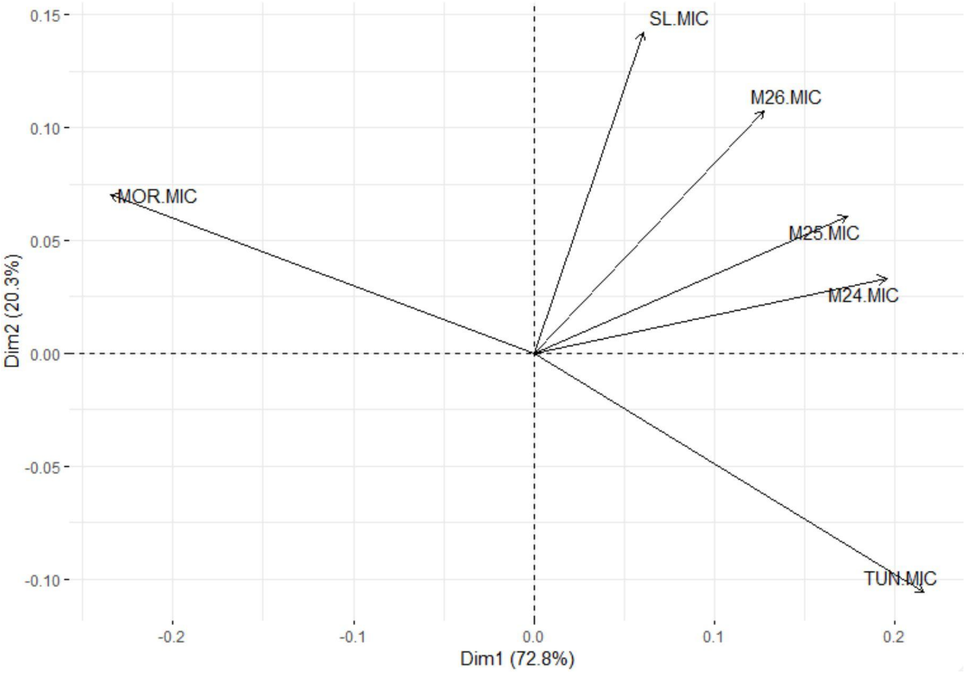

Figure 4. Principal component analysis of the antimicrobial activities of rosemary (*Salvia rosmarinus* essential oils from Mendoza (M24-26), San Luis (SL), and commercial samples from Morocco and Tunisia for comparison.

Contrasting outcomes were observed in a research study involving an Algerian essential oil, wherein the inhibitory effects were less pronounced than those observed with the Morocco and Tunisia oils. One potential rationale for this result could be that the predominant constituent of this oil was camphor rather than 1,8-cineole, as the authors observed that 1,8-cineole showed higher activities than camphor. Nevertheless, despite this oil's limited effectiveness, it exhibited antimicrobial properties against *S. aureus, E. faecalis*, and *E. coli* (Mahboubi and Kazempour, 2009).

CONCLUSION

This study characterized the chemical variability of Argentinean rosemary essential oils. These oils belong to the chemotypes myrcene/1,8-cineole/camphor and 1,8-cineole/camphor/α-pinene. The essential oils showed high (SL) to moderate/low levels (M23-26) of antioxidant activity, thus making them candidates for controlling oxidation. In addition, the Argentinean oils showed varied levels of antimicrobial activities against both Gram-positive and Gram-negative bacterial strains, especially against *B. cereus*, *S. aureus*, and certain strains of *P. syringae*. Further studies are needed to characterize the chemical variability of essential oils of Argentina's rosemary varieties nationally. Such studies, coupled with additional efforts to assess the practical application of these oils as antioxidants and antimicrobial agents, will contribute to finding new uses and applications for rosemary essential oils.

ACKNOWLEDGMENTS

M.M. Oliva and H.R. Juliani are grateful to the Fulbright Program (funded by the U.S. Department and State) and “Consejo National de Investigacion Cientificas y Tecnologicas (CONICET)” for co-financing the Fulbright scholarship for Dr. Oliva. We also thank Shimadzu Instrument Company, the New Jersey Agricultural Experiment Station, the Insitute for Food, Nutrition and Health, and its Center for Agricultural Food Ecosystems (RUCAFE). I.M. Lorello and C. Baglio thank the “Instituto Nacional de Tecnología Agropecuaria (EEA La Consulta y EEA Mendoza, Argentina) and Facultad de Ciencias Agrarias, Universidad

Nacional de Cuyo. A. Posadaz acknowledges the support of the “Universidad Nacional de San Luis (San Luis, Argentina), Facultad de Turismo y Urbanismo”, with funding provided PROICO 2023-CyT-UNSL.

REFERENCES

Annemer, S., Farah, A., Stambouli, H., Assouguem, A., Almutairi, M.H., Sayed, A.A., Peluso, I., Bouayoun, T., Talaat Nouh, N.A., and El Ouali Lalami, A. 2022. Chemometric investigation and antimicrobial activity of *Salvia rosmarinus* Spenn. Essential Oils Molecules 27, 2914.

Bendif, H., Boudjeniba, M., Djamel M. M, Biqiku, L., Bramucci, M., Caprioli, G., Lupidi, G., Quassinti, L., Sagratini, G., Vitali, L.A., Vittori, S., and Maggi, F. 2017. *Rosmarinus eriocalyx*: An alternative to *Rosmarinus officinalis* as a source of antioxidant compounds. Food Chem. 218:78-88.

Benzie, I. F., and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239(1):70-6.

Bouyahya, A., Et-Touys, A., Bakri, Y., Talbaui, A., Fellah, H., Abrini, J, and Dakka, N. 2017. Chemical composition of *Mentha pulegium* and *Rosmarinus officinalis* essential oils and their antileishmanial, antibacterial and antioxidant activities. Microb. Pathog. 111: 41-49.

Bouzid, H. A., Oubannin, S., Ibourki, M., Bijla, L., Hamdouch, A., Harhar, Sakar, E.H., Harhar, H., Majourhat, K., Koubachi, K., and Gharby, S. 2023. Comparative evaluation of chemical composition, antioxidant capacity, and some contaminants in six Moroccan medicinal and aromatic plants. Biocatal. Agric. Biotechnol. 47: 102569.

Bozin, B., Mimica-Dukic, N., Samojlik, I., and Jovin, E. 2007.Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. J. Agric. Food Chem. 55(19):7879-85.

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

Carvalho, M. F. N. N., Leite, S., Costa, JP., Galvão, A., and Leitão., J. H. 2019. Ag(I) camphor complexes: antimicrobial activity by design. J. Inorg. Biochem. 199: 110791.

Castillo Ruilova, V.A. 2016. Efecto del uso del romero (*Rosmarinus officinalis* L.) como aditivo antibacterial en salchichas de pollo tipo frankfurt. Quito. 80p. http://dspace.udla.edu.ec/handle/33000/5276. Accessed July 27, 2023.

Corbino, G. B. 2009. Informe. El valor terapéutico de las plantas aromáticas y medicinales. Instituto Nacional de Tecnología Agropecuaria. http://inta.gob.ar/documentos/el-valor-terapeutico-de-las-plantas-aromaticas-y-medicinales. Accessed July 27, 2023

Coy Barrera, C.A., and Acosta G. E. 2013. Actividad antibacteriana y determinación de la composición química de los aceites esenciales de romero (*Rosmarinus officinalis*), tomillo (*Thymus vulgaris*) y cúrcuma (*Curcuma longa*) de Colombia. Rev. Cuba. Plantas Med. 18(2):237-246.

Di Paola, M. M. 2006. Un modelo de producción de aromáticas. Apuntes Agroecon. 4(5):1-14.

Eid, A. M., Jaradat, N., Issa, L., Abu-Hasan, A., Salah, N., Dalal, M., Mousa, A., and Zarour, A. 2022. Evaluation of anticancer, antimicrobial, and antioxidant activities of rosemary (*Rosmarinus Officinalis*) essential oil and its Nanoemulgel. Eur. J. Integr. Med. 55: 102175.

Harmankaya, S., and Vatansever, L. 2017. The effect of essential oils of rosemary and clove on shelf life chicken meat. Van. Vet. J. 28, 11–19.

Isman, M.B., Miresmailli, S., and Machial, C. 2011. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochem. Rev. 10:197–204.

Jordán, M. J., Lax, V., Rota, M. C., Lorán, S., and Sotomayor, J. A. 2013a. Effect of the phenological stage on the chemical composition, and antimicrobial and antioxidant properties of *Rosmarinus officinalis* L essential oil and its polyphenolic extract. J. Ind. Crops Prod. 48, 144–152.

Jordán, M. J., Lax, V., Rota, M. C., Lorán S, and Sotomayor, J.A. 2013b. Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. Food Control. 30 (2): 463–468.

Juliani, H.R., Biurrun, F.N., Koroch, A.R., De Carli, A., and Zygadlo, J.A. 2007. The production of native and exotic herbs, medicinal and aromatic plants in Argentina. In: Issues in New Crops and New Uses. J. Janick, A. Whipkey (Eds.). *Am. Soc. Hortic. Sci.* (ASHS) Press, Alexandria, Virginia. pp, 316-321.

Kokoskova, B., Pouvova, B., Pavela, R. 2011. Effectiveness of plant essential oils against *Erwinia amylovora, Pseudomonas syringae pv. syringae* and associated saprophytic bacteria on/in host plants. Plant Pathol. J. 93 (1), 133-139.

Kontogianni, V. G., Tomic, G., Nikolic, I., Nerantzaki, A. A., Sayyad, N., Stosic-Grujicic, S., Stojanovic, I., Gerothanassis, I. P., and Tzakos A.G. 2013. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. Food Chem. 136(1): 120–129.

Koroch, A. R., Juliani, H. R., and Zygadlo, J. A. 2007. Bioactivity of essential oils and their components. Chapter 5. In: RG Berger (Ed.) Flavours and Fragrances. Chemistry, Bioprocessing and Sustainability. Springer-Verlag. Berlin. Heidelberg. Germany. pp 87-115.

Lakusic, D. V., Ristic, M. S., Slavkovska, V. N., Sinzar-Sekulic, J., and Luksic, B. S. 2012. Environment-related variations of the composition of the essential oils of rosemary (*Rosmarinus officinalis* L.) in the Balkan peninsula. Chem. Biodivers. 9 (7): 1286-1302.

Lax Vivancos, V. 2014. Estudio de la variabilidad química: propiedades antioxidantes y biosidas de poblaciones espontáneas de *Rosmarinus officinalis* L. en la Región de Murcia. http://hdl.handle.net/10201/41969. Accessed July 27, 2023.

Leporini, M., Bonesi, M., Loizzo, M. R., Passalacqua, N.G., and Tundis, R. 2020. The essential oil of *Salvia rosmarinus* Spenn. From Italy as a source of health-promoting compounds: chemical profile and antioxidant and cholinesterase inhibitory activity. *Plants*. 9, 798.

Luo, W., Dua, Z., Zhenga, Y., Lianga, X., Huanga, G., Zhanga, Q., Liua, X., Zhangb, K., Zhengc, X., Lind, L., and Zhang, L. 2019. Phytochemical composition and bioactivities of essential oils from six Lamiaceae species. Ind. Crops. Prod. 133: 357–364.

Mahboubi, M., and Kazempour, N. 2009. The antimicrobial activity of essential oil from *Perovskia abrotanoides* Karel and its main components. Indian J. Pharm. Sci. *71 (3): 343-347.*

Miranda, J.M., García-Soto B., Quitral, V., Trigo, M., López, M., Barros-Velázquez, and J., Aubourg, S.P.. 2015. Aplicación de hielo incluyendo conservantes naturales para la mejora de la calidad de especies marinas refrigeradas. Inves. Cienc. Cult. Tecn. 7(14): 14-19.

Mizrahi, I., Juarez, MA., and Bandoni, L.A. 1991 The essential oil of *Rosmarinus officinalis* growing in Argentina. J. Essent. Oil Res. 3:1, 11-15.

Ojeda-Sana, A. M., van Baren, C. M., Elechosa, M. A., Juárez, M. A., and Moreno, S. 2013. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control. 31(1). 189-195.

Oliva, M. de las M., Beltramino, E., Gallucci, N., Casero, C., Zygadlo, J., and Demo, M. 2010. Antimicrobial activity of essential oils of *Aloysia triphylla* (L'Her.) Britton from different regions of Argentina. Bol. Latinoam. Caribe Plant Med. Aromat. 9(1):29 – 37.

Oliva, M., Carezzano, E., Giuliano, M., Daghero, J., Zygadlo, J., Bogino, P., Giordano, W., and Demo, M. 2015. Antimicrobial activity of the essential oils of *Thymus vulgaris* and *Origanum vulgare* on phytopatogenic strains isolated from soybean**.** Plant Biol. 17:758-765.

Olmedo, R., Asensio, C., and Grosso, N. 2015. Thermal stability and antioxidant activity of essential oils from aromatic plants farmed in Argentina. Ind. Crops. Prod. 69. 21-28.

Paletti Rovey, M. F., Sotelo, J. P.; Carezzano, M. E.; Huallpa, C., and Oliva, M. M. 2023. Hexanic extract of *Achyrocline satureioides*: antimicrobial activity and *in vitro* inhibitory effect on mechanisms related to the pathogenicity of *Paenibacillus larvae.* Vet. Res. Commun. 47(3):1379-1391

Papageorgiou, V., Gardeli, A., Mallouchos, A., Papaioannou, M., and Komaitis, M. 2008. Variation of the chemical profile and antioxidant behavior of *Rosmarinus officinalis* L. and *Salvia fruticosa* Miller Grown in Greece. J. Agric. Food Chem. 56:7254-64.

Pires Amaral, G., Rigon Mizdal, C., Terra Stefanello, S., Loureiro Mendez, A. S., Puntel, R. L., de Campos, M. M. A.; Antunes Soares, F. A., and Fachinetto, R. 2019. Antibacterial and antioxidant effects of *Rosmarinus officinalis* L. extract and its fractions. J. Tradit. Complement. Med. 9: 383-392.

Ruales, C., and Salazar, C. 2018. Efecto inhibitorio del aceite esencial de *Rosmarinus officinalis* al 100% y extracto etanólico *de Rosmarinus officinalis* al 100% a diferentes tiempos sobre *Porphyromonas gingivalis*. Estudio in vitro. Facultad de Mecánica Automotriz. UIDE. Quito. 92p.

Satyal, P., Jones, T. H., Lopez, E. M., McFeeters, R. L., Awadh Ali, N. A., Mansi, I., Al-kaf, A., and Setzer, W. N. 2017. Chemotypic characterization and biological activity of *Rosmarinus officinalis*. Foods. 6, 20; doi:10.3390/foods6030020.

Sousa Borges, R., Sánchez Ortiz, B. L., Matias Pereira, A. C., Keita, H., and Tavares Carvalho, J. C. 2019. *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J. Ethnopharmacol. 229: 29-45.

Trombin de Souza, M., Trombin de Souza, M., Bernardi, D., Oliveira, D. D. C. , Morais, M. C., de Melo, D. J., Richardi, V. S., Zarbin, P. H. G., and Zawadneak, M. A. C. 2022. Essential oil of *Rosmarinus officinalis* ecotypes and their major compounds: insecticidal and histological assessment against *Drosophila suzukii* and their Impact on a nontarget parasitoid. J. Econ. Entomol. 115(4):955-966.

Tropicos database. 2023. *Salvia rosmarinus* Schleid. www.tropicos.org/name/100254189. Accessed on June 21, 2023.

Tundis, R., Leporini, M., Bonesi, M., Rovito, S., and Passalacqua, N.G. *Salvia officinalis* L. from Italy: A comparative chemical and biological study of its essential oil in the Mediterranean context. 2020. Molecules. 25: 5826.

van Vuuren, S. F., and Viljoen, A. M. 2007. Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour Fragr. J. 22: 540–544.

Veenstra, J. P., and Johnson, J. J. 2021. Rosemary (*Salvia rosmarinus*): Health-promoting benefits and food preservative properties. Int. J. Nutr. 6(4):1-10.

Wang, W., Li, N., Luo, M., Zu, Y., and Efferth, T. 2012. Antibacterial activity and anticancer activity of *Rosmarinus officinalis* l. essential oil compared to that of its main components. Molecules. 17(3): 2704-2713.