Aromatic herb with antibacterial activity in infections with Gram negative bacteria

Ciucă Alina Maria[1], Roman Luminița[2], Hosu Anamaria[3], Chelariu Mihaela[2], Roman Horațiu[4], Ciucă Gheorghe[5], Mihăescu Grigore[2]

[1]Faculty of Medicine Carol Davila, University of Bucharest, [2]Faculty of Biology, University of Bucharest, [3]Faculty of Chemistry and Chemical Engineering, University Babes-Bolyai Cluj-Napoca, [4]Faculty of Geology, University of Bucharest, [5]Faculty of Electronics, University of Bucharest.



Abstract: *Rosmarinus officinalis* is a member of the family Lamiaceae that is native to the Mediterranean region and grows well near the sea. Known in antiquity as a symbol of love and loyalty, *R. officinalis* is known as stimulating the brain and nervous system, tonic, astringent, diaphoretic, stimulant, with antibacerial and antiviral activity, due to the antioxidant capacity against free radicals and peroxides. GC-MS (Gas chromatography-mass spectroscopy) analysis ethanolic extract of *R. officinalis* were obtained more than 300 compounds, alpha-pinene camferol and had the greatest abundance. Ethanolic extract of *R. officinalis* (1v / 4w) was tested on a total of 60 strains of Gram-negative MDR by disc diffusion method and decimal dilutions. Antibacterial activity against all strains had effect, MIC values between 6.25 and 50 µl / ml.

Keywords: *Rosmarinus officinalis* , antibacterial activity



Materials and methods: Microbial inoculum suspension adjusted to 1.5x108 CFU / mL (0.5 McFarland standard), 15-18 h was cloth seeded in Petri dishes with solid Sabouraud medium. Then spot of 10 ml extract was placed in an medium. Plates were incubated at 37 degrees for 24 hours after which a measured area of inhibition by the appearance of clear zones around the spot. MIC testing, we used 96 well plates with BHI medium. I was no turbidity monitored in wells seeded with microbial suspensions **(20 µl)** and plant extract in decimal dilutions. Isolation of active compounds from ethanol extract of *R. officinalis* was done with GC-MS Surveyor LC Pump Plus

Results and discussion: In Figure 1 is shown the antimicrobial activity of ethanol extract from a strain of Gram negative bacteria by the appearance of clear zones around the spot, the size of the inhibition zone is proportional to the concentration of the extract. In figure 1**e** is shown CMI ethanolic extract against a strain of Gram negative bacteria. The last row represents the positive control wells in which no bacterial suspension was seeded and positive control line 10 is not added ethanolic extract.

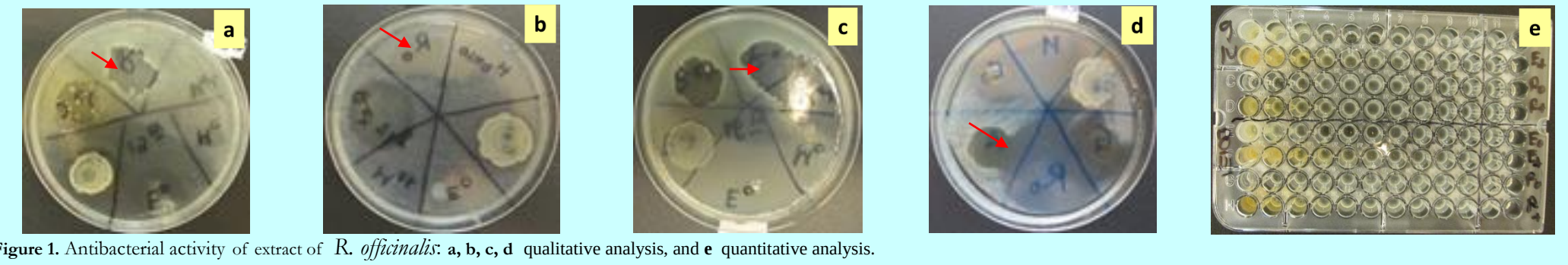


Figure 1. Antibacterial activity of extract of *R. officinalis*: **a, b, c, d** qualitative analysis, and **e** quantitative analysis.

Highest antibacterial activity against strains was for *Pseudomonas aeruginosa* and *Escherichia coli*, MIC was 6.25 µl/ml, against *Klebsiella pneumoniae* and *Proteus mirabilis*, MCI was 125.5 µl/ml and against *Acinetobacter baumannii* 50 µl/ml.

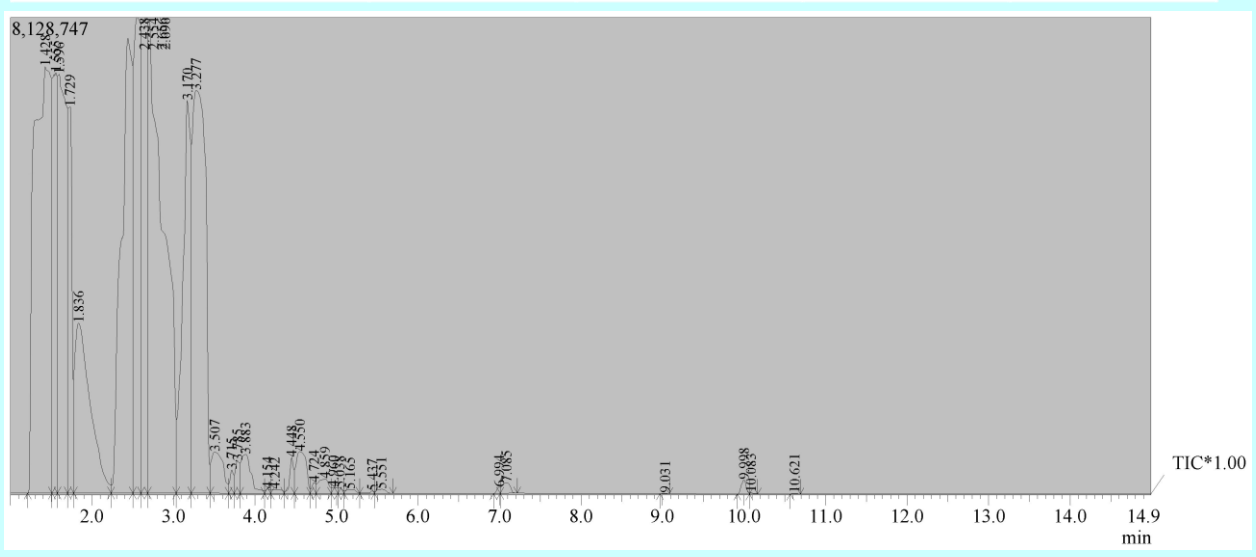
Tabele 1. The minimal inhibitory concentrations (MICs) for the essential oil and ethanolic extracts of *R. officinalis* against Gram negative bacteria.

	MICs ethanolic extract (ml/ml)		MICs essential oil (ml/ml)	
Strain	CMI_{50}	CMI_{90}	CMI_{50}	CMI_{90}
Escherichia coli	31.25	62,5	31.25	62.5
Klebsiella pneumoniae	62,5	125	31.25	125
Proteus mirabilis	31,25	62,5	15.625	31.25
Alcaligenes faecalis	31,25	62,5	15,625	31,25
Acinetobacter baumannii	15,625	125	15.625	62.5
Pseudomonas aeruginosa	31,25	62,5	31.25	62.5



Tabele 2. Active compounds isolated from *R. officinalis* extract.

Peak	R. Time	Area%	Height	Compound
1	1.428	15.81	7255947	Rosmanol
2	1.555	4.29	7155358	Carnosic acid
3	1.596	7.72	7125622	Carnosol
4	1.729	3.33	6572308	α-Amyrin
5	1.836	5.73	37967088	β-Amyrin
6	2.438	11.01	7739074	Luteolin
7	2.554	7.17	8095066	α-pinen
8	2.656	6.08	8094181	β-pinen
9	2.696	15.67	8068706	Eucaliptol
10	3.170	6.90	6687047	Camfor
11	3.277	11.45	6855123	Acetat de bornil
12	3.507	1.05	704751	Caffeic acid
13	3.715	0.19	395057	Chlorogenic acid
14	3.785	0.33	547666	Labiatic acid



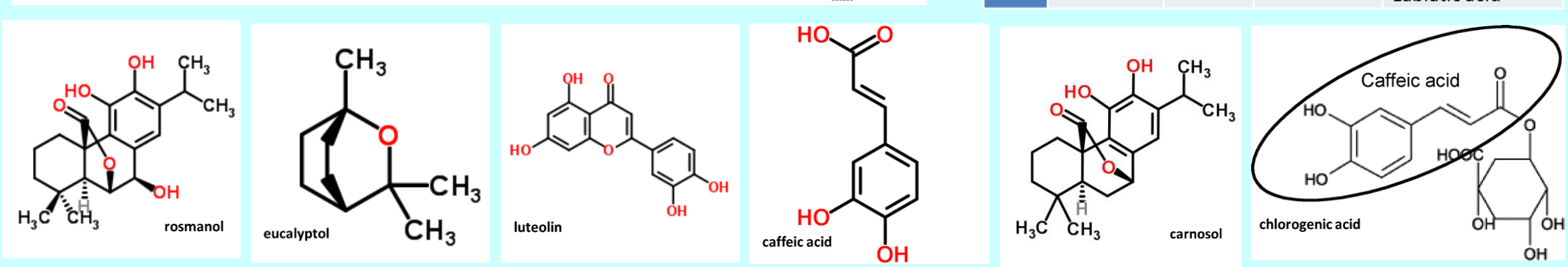
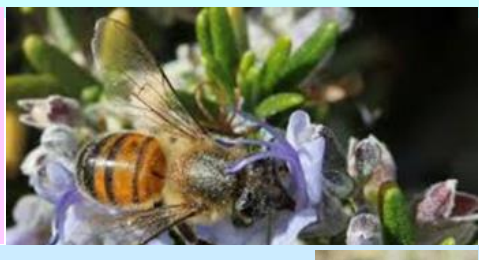


Figure 2. Chromatograme of ethanolic extract of *R. officinalis*. Chromatogram analysis it is observed that the highest concentration had a rosmanol, eucalyptol, luteolin, caffeic acid, carnosol and chlorogenic acid . Although other compounds found in small concentrations were still important for their work.

Conclusion: Gram negative infections are the most common for both people and plants. The antibacterial activity of ethanolic extracts and oil of *R. officinalis* proved effective due to the synergistic action of compounds resulting from the plant secondary metabolism. Literature revealed that most identificași compounds by GC-MS showed antioxidant and antibacterial activity individually. As a general conclusion, as well as the essential oil ethanolic extracts of *R. officinalis* can be used in the treatment of infections caused by Gram negative bacteria, or may be used as a preventive treatment. Mode of action of the metabolism compounds, with multiple targets of action, prevent the development of new virulence factors for bacteria. Compared to synthetic drugs, plant action on the human body does not create side effects such as drug depent or damage to other organs. In the future, the results justify the need for continuing studies at the molecular level in order to clarify the mechanisms of action of essential oils and their fractions of microbial cells and their specific targets for action.



References:

1. CHIFIRIUC MARIA CARMEN. 2011. Comparative analysis of disk diffusion and liquid medium microdillution methods for testing the antibiotic susceptibility patterns of anaerobic bacterial strains isolated from intra-abdominal infections. Biointerface Res Appl Chem. **1**: 209-220.
2. CHIFIRIUC MARIA CARMEN, MIHĂESCU G., LAZĂR VERONICA. 2011. Microbiologie și Virologie Medicala. Ed.Universitatii din Bucuresti



Acknowledgements: I bring gratitude to Professor Coordinator, Mihăescu Gregory, Mrs. Professor Carmen Chifiriuc director of the Research Institute of the Faculty of Biology and Mrs. head of Theodor Burghele laor Hospital Bucharest.