Rosmarinus officinalis L. (ROSEMARY), A LEGENDARY HERB WITH MANY BENEFICIAL EFFECTS ON THE HUMAN BODY

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Abstract. *Rosmarinus officinalis* Linnaeus 1753 comes from the countries of the Mediterranean region, but it has adapted to different climate and soil variations, and modifying its dimensions according to the aforementioned conditions; however, the flowers remain the same. The study of the activity of this plant on the human body (antibacterial activity, antifungal, relaxing effect on the smooth muscles of trachea and intestines, hepatoprotective and antitumorogenic activity) preoccupied and still is a current study for many specialists. In this study, we revealed the importance of this plant against Gram negative bacteria isolated from nosocomial infections. Identifying the compounds of essential oil and ethanol extracts of rosemary leaves was made by GC-MS (Gas chromatography coupled with mass spectrometer impact ionization). The most important compounds identified were rosmanol, eucalyptol, luteolin, caffeic acid and bornyl acetate. The antibacterial activity of ethanol and oil extracts of rosemary was reported against all supported strains, MIC ranging, depending on the species or on its virulence factors strain, between 15.625 and 125 µl / ml. The antibacterial activity of ethanolic extract and oil essential of rosemary was due to the synergistic action of all the compounds resulting from the secondary metabolism of the plant by inhibition of efflux pump, having a direct effect on the bacterial membrane. As a general conclusion, the essential oil and ethanolic extracts of rosemary can be used with success in the prevention and in the treatment of infections caused by Gram negative bacteria.

Keywords: *Rosmarinus officinalis*, antibacterial activity, Gram negative bacteria.

Rezumat. *Rosmarinus officinalis* L. (rozmarinul), o plantă legendară cu multiple efecte benefice asupra organismului uman. *Rosmarinus officinalis* Linnaeus 1753, provine din țările din regiunea mediteraneană, dar s-a adaptat la diferite variații de climă și sol, modificându-și în funcție de acestea dimensiunile, florile rămânând însă aceleași. Studiul activității acestei plante asupra organismului uman (activitatea antibacteriană, antifungică, hepatoprotectoare, relaxare a mușchilor netezi ai traheei și intestinului și antitumorogenetică), a preocupat și încă reprezintă un studiu de actualitate pentru mulți specialiști în domeniu. În acest studiu am relevat importanța acestei plante împotriva unor bacterii Gram negative izolate din infecții nosocomiale. Identificarea compușilor din extractele etanolice și ulei din frunze de rozmarin a fost efectuată prin metoda GC-MS (cromatografia de gaz cuplată cu spectrometru de masă cu ionizare prinimpact). Cei mai importanți compuși identificate au fost rosmanol, eucaliptol, luteolina, acidul cafeic și acetat de bornil. Activitatea antibacteriană a extractelor etanolice și de ulei esențial din rozmarin a fost raportată împotriva tuturor tulpinilor studiate, CMI variind funcție de specia tulpinii sau factorii de virulență ai acesteia, fiind cuprins între 15.625 și 125 µl/ml. Activitatea antibacteriană a extractelor etanolice și uleiului de rozmarin s-a datorat actiunii sinergice a tuturor compușilor rezultați din metabolismul secundar al plantei, prin inhibiția pompelor de eflux, având acțiune directă asupra membranei bacteriene. Ca o concluzie generală, extractele etanolice și uleiul esențial din rozmarin pot fi utilizate cu succes atât în prevenirea cât și în tratamentul infecțiilor cauzate de bacterii Gram negative.

Cuvinte cheie: *Rosmarinus officinalis*, activitate antibacterială, bacterii Gram negative.

INTRODUCTION

Rosmarinus officinalis Linnaeus, commonly known as rosemary, is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers, belonging to the family Lamiaceae (Room, 1988). The name rosemary derives by combining two words, Rose and Mary referring to the flower as a symbol of the Virgin Mary, first recorded in the eighteenth century. It is found in continental Europe as rosemary and Rosa Maria. After mid-nineteenth century, when flower names became common, it may also refer to the herb rosemary, Latin *ros marinus* "dew of the sea". In our popular tradition, rosemary is considered a flower of protection, purification, worn at important events, such as wedding or Christmas carolling. In addition to its properties as a spice, its antibacterial, anti-inflammatory activity, the role of modulator of the nervous system, and hyperglycaemia, gives it well-deserved appreciation. The main compounds obtained from the essential oil of rosemary, identified by GC-MS reported by TSCHIGGERL & BUCARAM, 2010, were: 1,8-cineole, camphor, α-pinene, camphene, borneol, bornyl acetate, myrcene, limonene, α- terpineol and caryophyllene. HUSSAIN et al., 2010, demonstrated in vitro antiproliferative activity of cancer cells, antioxidant and antibacterial activity of the essential oil of rosemary, reporting a total of six major compounds identified by GC: 1,8-cineole (38.5%), camphor (17.1%), α-pinene (12.3%), limonene (6.23%), camphene (6.00%) and linalool (5.70%). By vacuum liquid chromatography (VLC) on silica gel, OLUWATUYI et al., 1994, isolated five compounds from the extract ethanolic of *R. officinalis*: carnosic acid, carnosol, 12-methoxy-trans-carnosic acid, 12-methoxy-trans-carnosic acid and 12-methoxy-cis-carnosic acid to test the antibacterial activity against MDR bacterial with efflux pump. Most of the studies have shown synergistic activityof several compounds resulting from the secondary metabolism of the plant as having activity against MDR bacteria. Gram negative bacteria are responsible for most of antibiotic resistant infectious diseases due to the impermeability of external membrane (PAGÈS et al., 2008; DAVIES & DAVIES, 2010). Penicillin G was the first antibiotic introduced in the clinical therapy but its use is limited because the side chain does not allow passage through the external membrane of enteric Gram negative bacteria (*Escherichia coli*). Similarly, macrolides does not penetrate the outer membrane, although the *E. coli* cell-free

systems, as effective erythromycin inhibit protein synthesis in cell-free systems as a Gram positive bacteria. Permeability of the external membrane is the cause for which vancomycin (inhibitory glycopeptide antibiotic of the formation of peptidoglycan transversal links after binding to the peptides of D-Ala-D-Ala) cannot inhibit the growth of Gram negative bacteria, but is inhibitory against Gram positive bacteria (CHIFIRIUC et al., 2011). The inaccessibility of the target may be due to the impermeability of the external membrane by an activation of efflux pumps (porins), which removes the drug from the interior of the cell, against the concentration gradient (PAGES et al, 2008). Adaptive resistance, a variant of intrinsic resistance signifies temporary increase in bacterial cell capacity to survive in the presence of inhibitors or toxic chemical agents by altering the activity of gene expression. Adaptive resistance may be mediated by efflux. Their activity varies with the concentration of NaCl. Physiological NaCl concentration is associated with the increased level of resistance due to the increased expression of efflux pumps. Adaptive resistance is induced by subinhibitory concentrations of antibiotics during therapy: cells do not die, but become more resilient. Also, biocides used excessive induce the adaptive resistance of bacteria. High resistance to bacteria grown in biofilm is on the one hand due to the increased activity of the efflux pumps, but with increasing thickness of the biofilm antibiotic diffusion rate decreases and the cells adapt to subinhibitory concentrations. Natural production of antibiotics is insignificant, and the trade is practically the only source of antibiotics biosphere. Under pressure from the presence of antibiotics, selection of resistant strains is much faster because it is geared towards survival in a hostile environment, and not to adapt to the environment, characteristic of populations that evolve slowly. Therapeutic exposure to high antibiotic concentrations of pathogenic bacteria creates conditions of severe selection pressure and induces high-level resistance. Antibiotic resistance cannot be removed. The chemical modification of aminoglycosides, macrolides and other classes of antibiotics has led to the semisynthesis of the chemical derivatives resistant to the inactivation mechanisms of bacteria. But chemical modification is limited to no influence of the antimicrobial activity. New semisynthetic compounds had the effect of extending the use of antibiotic classes (meticylin β-lactam), azithromycin (macrolide), amikacin (aminoglycoside), etc. and the resistance genes evolved in response to the new chemical compounds and clinical efficiency diminished progressive (DAVIS, 2010). Recycling antibiotics is a short-term measure, because resistant strains do not disappear, and when reintroduced into the antibiotic therapy resistance genes will be selected soon (CHIFIRIUC et al., 2011). In this context, the effectiveness of antibiotics against bacteria is no longer valid and finding new compounds, such as those obtained in the synthesis of herbs known for their antibacterial properties, represents the only solution.

MATERIALS AND METHODS

1. Analysis of phenotypic resistance to antibiotics of Gram negative strains

Gram-negative strains, which were the subject of this article, were isolated from urogenital infections from the patients hospitalized at Theodor Burghele Hospital, Bucharest. Strains were identified in the unit, automatic identification method VITEK®2 compact. For determining spectrum antibiotic sensitivity of bacterial strains from the collection, it was used the disc-diffusion method. Gram-negative strains were seeded in the cloth using a sterile swab Müller-Hinton medium, a bacterial suspension with a turbidity corresponding to McFarland standard 0.5. On seeded plates, there were applied the disks impregnated with the antibiotic, applied to β-lactam antibiotic discs specific family of bacterial strains tested according to the CLSI 2009. The plates were incubated 16-18 hours at 37° C with the cover down. The reading of the results was conducted by measuring the diameters of the zones of inhibition generated by different antibiotics, using a graduated ruler. The interpretation of results was performed according to standard CLSI 2009.

2. Preparation of the extract and analysis of the compounds

The ethanolic extract was prepared by macerating leaves of *R. officinalis* powder in 95% ethyl alcohol for 24 hours (1: 4 w / v), after which it was introduced into a rotary evaporator for 10-15 minutes and then the supernatant was purified by Watthmann filter paper no. 41. The extract thus obtained was stored in an amber glasscontainer at 4° C. The volatile oil of the leaves of *R. officinalis* was obtained by hydrodistillation in a Clevenger- Neo for 4 h. The volatile oil was dried with Na_2SO_4 and stored in a dark glass bottle at 4°C. Oil samples were diluted in dichloromethane (1/200) for the analysis of chemical compounds. To identify the compounds of the *R. officinalis* oil extract we used a GC-MS (Shimadzu GC-2010 Plus gas chromatograph). A multi-dimensional GC/GCMS system performed separations using two columns that had different chromatographic selectivity. When the components of interest are insufficiently separated on the first column, they can be selectively introduced ("heart-cut") to a second chromatographic column with different selectivity. The first used capillary column was MEGA SE-52 0.25 x 25 m df=0.25 μm. The operating conditions were: split splitless injector (injection mode - split flow dividing ratio 1/100 at 250° C). Oven temperature is up from 50°C - 280°C (3°C/min). Monitoring FID (Flame Ionization Detector) was at 290° C (H_2: 50 mL/min, Air: 400 mL/min, Make-up: 0 mL/min). Switching for the second column was 8 times. Second capillary column was: MEGA DetTBuSililBeta 0.25 x 25 m df=0.25 μm (oven temp: 45 °C (12.00 min) - 180 °C (2 °C/min). The relative concentration of the compounds was calculated using the values of the chromatographic peak areas under the curves, without applying correction factors.

3. Determination of antimicrobial activity of rosemary extract

The quantitative determination of the antimicrobial activity and establishing of the minimum inhibitory concentration (MIC) was made by the method of serial microdilution in a liquid medium BHI (Hearth Infusion Broth) in 96 well plates / Ependorf tubes of 1.5ml (CHIFIRIUC et al., 2011). MIC was established macroscopically as being

the last concentration where no growth of the microbial medium was observed, respectively, the beginning of turbidity medium appearance. Also, for higher precision, a spectrophotometric absorbance read at 620 nm was made. Antimicrobial activity was determined by disc diffusion method adapted to standardized control antimicrobial activity of antibiotics, CLSI, 2009 (Kirby-Bauer). The microbial inoculum represented by a suspension obtained from a culture grown on a solid medium 16-18h adjusted to 0.5 McFarland nephelometric standard cloth seeded on one Mueller-Hinton agar plate. Mueller-Hinton medium plates after inoculation were allowed to dry before applying the plant extract of the stock solution at 10 mL. The plates are incubated for 16-18 hours at 35 ± 2 ° C, with the lid down. Reading the results was done by measuring the diameters of the zones of inhibition comparatively. The influence on the ability adhesion to the inert substrate was quantified after the protocol quantitative analysis of the effect of antimicrobial, evaluating the biomass, after fixation with methanol and staining with crystal violet. The optical density was determined spectrophotometrically and the biological material resuspended at 490 nm (NORUZI et al., 2010).

RESULTS AND DISCUSSION MCI was established macroscopically as being the last concentration

The results of the antibiotic resistance by disk diffusion method are listed in the following table:

Table 1. Antibiotic resistance of Gram-negative strains.

Strain	STX	MPM	ERT	AMC	AMP	CTX	CAZ	CIP	ATM	GM	CXM	NOR	FEP	CL	FOT	IMP	TZP	LEV
E. coli	21	3	1	18	17	0	20	16	3	12	6	7	18	1	2	3	8	6
Klebsiella pn.	15	5	7	17	8	2	21	10	1	13	2	3	17	0	0	2	18	7
JProteus m.	3	0	0	3	0	0	1	1	0	3	0	0	3	0	0	0	1	2
Ps. aeruginosa	0	2	0	3	0	0	3	3	3	2	0	0	2	0	0	2	3	0
Acinetobacter b.	3	1	0	3	0	0	2	2	1	3	0	0	2	0	0	2	2	0
Alcaligenes f.	1	0	0	2	0	0	2	0	2	2	0	0	2	1	0	2	2	2
Total strins	35	67	70	35	53	76	28	46	68	43	70	68	35	76	76	67	44	61

Of the total of 40 strains of *E. coli*, 22 (44%) showed resistance to SXT (Trimethoprim / Sulfamethoxazole), 25 (50%) strains to AMC (ampicillin/clavulanic CAID), 20 (0.40%) to CAZ (ceftazidime), 18 (36%) to FEP (cefepime). A greater resistance to STX had a *Klebsiella pneumoniae* strains- out of 26 strains, 15 (0.57%) showed resistance. Resistance of *K. pneumoniae* strains to CAZ was 58% and to EFP and AMC was 0.47%. Phenotypic analysis spectrum antibiotic resistance as penicillins, cephalosporins and carbapenems, showed the existence of ESBL. β-lactamases are the main factor of resistance of β-lactam antibiotics Gram-negative bacteria. These hydrolyses the β-lactam bond (C - N) of this class of antibiotics. This chemical linkage is essential for β-lactam antibiotic activity, as it has the role of analog peptide bond linking the terminal D-Ala peptide of peptidoglycan monomer. β-lactamases are highly effective enzymes: a single molecule can hydrolyse over 100,000 β-lactam molecules. They constitute a large family of enzymes with unitary structure; the β-lactam ring cleaves and inactivates the antibiotic, but differs in amino acid sequence (MIHĂESCU et. al. 2007). *P. aeruginosa* and *Acinetobacter baumannii* strains had MPM high resistance to carbapenems (meropenem) and IMP (imipenem) by developing additional mechanisms of resistance, biofilm formation, porins with a role in drug efflux. All Gram negative strains, particularly *K. pneumoniae* showed high resistance to CAZ (ceftazidime- generation cephalosporin III). The high resistance to FEP (cephalosporin- antibiotics cefepime generation IV) is due to the phenomenon of adaptation of strains to the antibiotic by developing new resistance mechanisms, efflux pumps whose activity increases due to cellular stress. A high resistance to ERT (ertopenem, a carbapenem with a structure very similar to meropenem but a somewhat less broad spectrum of activity) was registered in case of the strains of *K. pneumoniae* (0.26%).

In vitro action of fosfomycin against ESBL strains had higher efficiency compared to lactam antibiotics. FOT may be a suitable, effective and cheap alternative in the treatment of ESBL strains. FOT is a phosphonic acid, a bactericidal agent with in vitro activity against most pathogens: *Proteus mirabilis, K. pneumoniae, P. aeruginosa, A. baumanni, Alcaligenes faecalis, E. coli*. Fosfomycin tromethamine is well tolerated, with a low incidence of adverse events. Fosfomycin tromethamine achieves high clinical and bacteriological cure rates in patients with acute uncomplicated lower urinary tract infection and is well tolerated (PATELL et. al., 1997). A lower resistance to antibacterial treatment is observed when using CL (colistin). In the last two decades, the paucity of novel antibiotics to treat drug-resistant infections, especially those caused by Gram-negative pathogens, has led to the reconsideration an old antibiotic (fosfomycin and colistin) as a therapeutic option. The polymyxin group of polypeptide antibiotics, discovered in the 1940s, was among the first antibiotics with significant activity against Gram negative bacteria. Colistin is polymyxin E, used clinically. Colistin has no activity against Gram positive bacteria, all cocci, and anaerobes. The rates of colistin resistance have been relatively low, probably because of its infrequent use. The emergence of colistin-resistant *K. pneumoniae* has been described following widespread use of colistin. The most common mechanism of colistin resistance is the modification of LPS and efflux pump/potassium system. Empirical treatment with colistimethate sodium should be considered for patients at high risk for infection by carbapenem-resistant bacteria with severe sepsis (YAHAV et al., 2011).

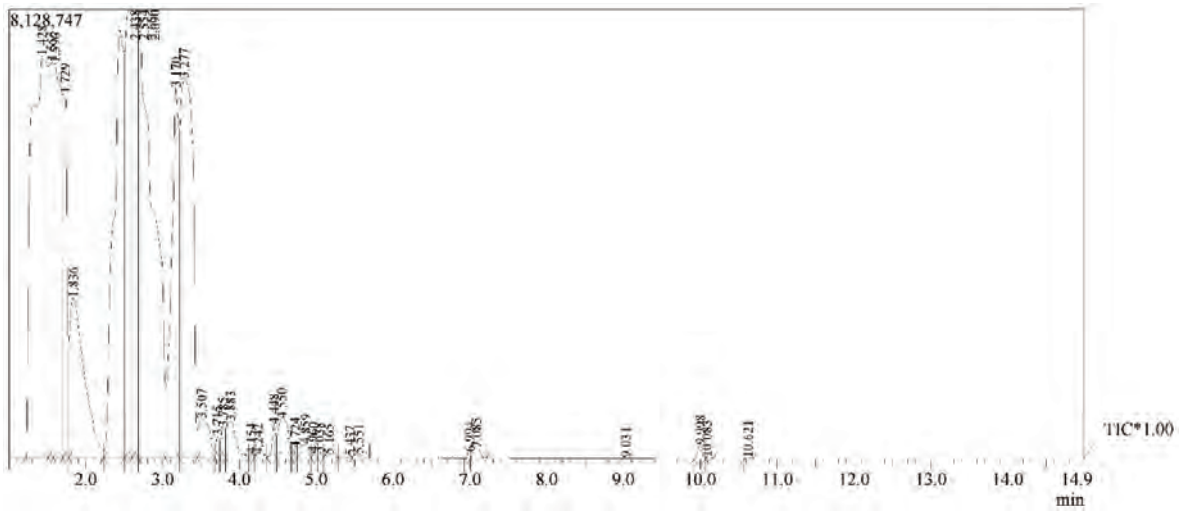
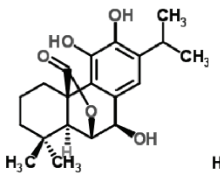


Diagram 1. Chromatogram of the extract of *R. officinalis.* In abscise it is passed retention time and in ordinate, abundance. The peaks represent the concentration of the compound.

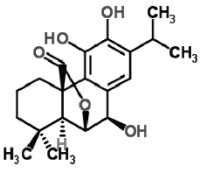
Table 2. The most representative compounds of *R. officinalis* identified by GC-MS depending on the retention time of the eluent.

Peak	Compound	R.T.	Area%	Height
1	Rosmanol	1.428	15.81	7255947
2	Camosic acid	1.555	4.29	7155358
3	Camosol	1.596	7.72	7125622
4	α–Amyrin	1.729	3.33	6572308
5	β–Amyrin	1.836	5.73	37967088
6	Luteolin	2.438	11.01	7739074
7	α–pinene	2.554	7.17	8095066
8	β–pinene	2.656	6.08	8094181
9	Eucalyptol	2.696	15.67	8068706
10	Camphor	3.170	6.90	6687047
11	Bornyl acetate	3.277	11.45	6855123
12	Caffeic acid	3.507	1,05	704751
13	Chlorogenic acid	3.715	0.19	395057
14	Labiatic acid	3.785	0.33	547778

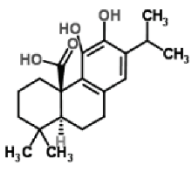
Rosmanol is a terpenoid glycoside and has the molecular formula $C_{20}H_{26}O_5$. ZHANG et al., 2014 refers to a further 5 terpenoid glycoside in addition to the existing, (1S,4S,5S)-5-exo-hydrocamphor 5-O-β-d-glucopyranoside, isorosmanol, rosmanol, 7-methoxyrosmanol, epirosmanol, ursolic acid, micromeric acid, oleanolic acid, niga-ichigoside, glucosyl tormentate and asteryunnanoside B, were obtained from the aerial parts of *R. officinalis* L. The principal antioxidative components of *R. officinalis* leaf extract are the phenolic diterpenes carnosol and carnosic acid. Highly oxidized diterpenes increase in rosemary plants exposed to drought and high light stress (FIUME, 2013). Carnosic acid reached the maximum concentrations in December, decreasing by 50% during the summer months, while rosmarinic acid showed a constant concentration during the year (LUIS & JOHNSON, 2005). The essential oil of rosemary is reported to have antimicrobial activities (FIUME, 2013). Rosmarinic acid and carnosic showed strong activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) absorption radical *in vitro*. Rosmarinic acid scavenging activity was always much higher than other tested compounds such as vanillin acid, naringin and even ascorbic acid, the "classic" antioxidants. These results highlight the importance of a catechol group moiety for hydrogen-donating activity, which is present in rosmarinic, caffeic, and carnosic acid all three showing a similar DPPH scavenging capacity (LUIS & JOHNSON, 2005; AL-SEREITIA et al., 1999). Carnosol and carnosic acid are powerful inhibitors of lipid peroxidation in microsomal and liposomal systems. Carnosic acid reacted with HOCl in such a way as to protect the protein alpha 1-antiproteinase against inactivation (ARUOMA, 1992). Carnosol has a bridging epoxide group straddling the 1,4 positions on one of the cyclohexane rings. The main isolated triterpenes have been α–amyrin and β– amyrin. Amyrin has the chemical formula C30H50O. OLIVERIA et al., 2005 reported the hepatoprotective potential of alpha- and beta-amyrin against toxic liver injury and suggest that the diminution in oxidative stress and toxic metabolite formation as likely mechanisms involved in its hepatoprotection. Epidemiological evidence suggests that flavonoids may play an important role in the decreased risk of chronic diseases associated with a diet rich in plant-derived foods. Flavonoids are also common constituents of plants used in traditional medicine to treat a wide range of diseases (LOPÉZ-LÁZARO, 2009). Luteolin (3′,4′,5,7-tetrahydroxyflavone) is a yellow flavonoid used as a dye. It is found mostly in the leaves of plants, but it is also present in the edible parts of celery, dandelion, thyme oregano citricus and carrot. It is one of the most common flavones and plays a role in the human body as an anti-oxidant, scavenging dangerously reactive free-radicals. It is also an anti-inflammatory, a promoter of carbohydrate metabolism, and a moderator of the immune system. The anti-inflammatory activity may be linked to its anticancer property. Luteolin anticancer property is associated with the induction of apoptosis, and the inhibition of cell proliferation, metastasis and angiogenesis (LIN et al., 2009; LOPÉZ-LÁZARO, 2009).



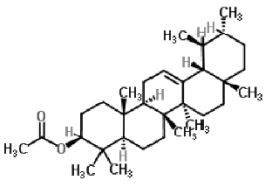
Rosmanol



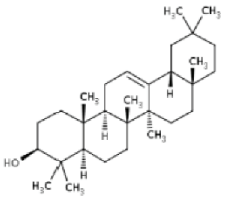
Carnosic acid



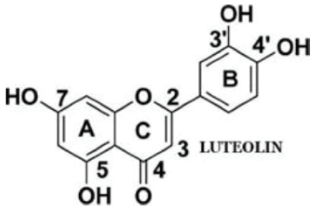
Carnosol



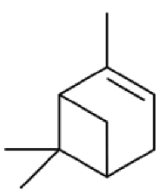
α– amyrin



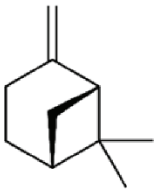
β- amyrin.



3-H =Luteolin, 3-OH=Quercitin

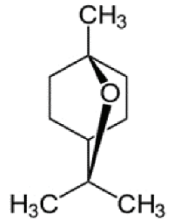


α–pinen



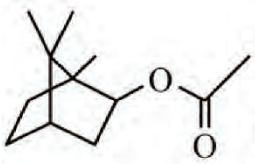
β-pinen

Eucalyptol

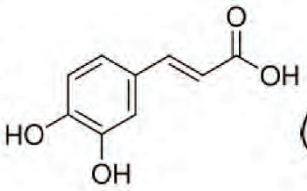


Camphor

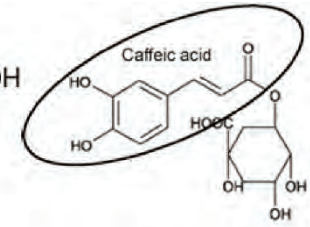
Pinene (C10H16) is a bicyclic monoterpene chemical compound. Pinene has been recognized for centuries for its antifungal activity, insecticidal and aromatic property. Several biological activities are associated with pinenes, including the use as a natural insecticide. The antimicrobial activities of the isomers and enantiomers of pinene were evaluated against bacterial and fungal cells. The agar diffusion test showed that only the positive enantiomers of the a- and β-isomers of pinene were active (RIVAS DA SILVA et al., 2014). α-pinene was chosen as a surrogate for the endocyclic structured monoterpenes. Its carbon double bond is located inside a C6-ring structure. Therefore, the oxidation reactions, especially by ozone, cause primarily ring-opened structured products, such as pinonaldehyde, possessing different saturation vapour pressures, than found for the exocyclic monoterpene reactions with predominantly ring-retaining products, such as nopinone in the case of β-pinene (BONN & MOORTGAT, 2002).



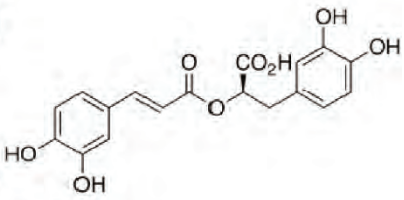
Bornyl acetate



Caffeic acid



Chlorogenic acid



Labiatic acid

Eucalyptol (1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane); the molecular formula $C_{10}H_{18}O$ is a bicyclic monoterpene with a distinctive aroma. Eucalyptol and camphor ($C_{10}H_{16}O$) are found in the majority of aromatic herbs. Camphor and eucalyptol showed the strongest antimicrobial activity (HASOUNA et al., 2013). ASGHAR et al., 2012, reported the antibacterial activity of the essential oil obtained from the aerial parts of *Artemisia aucheri*. The CG-MS analysis identified among major compounds with antimicrobial activity, bornyl acetate and borneol in a concentration of 2.7% and 7.8% respectively. Caffeic acid ((2E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid, 3-(3,4-dihydroxyphenyl)acrylic acid) and molecular formula ($C_9H_8O_4$) is an antioxidant with *in vitro* and *in vivo* activity (OTHOF, 2001). Antibacterial activity of the caffeic acid was reported by ALMEIDA et al., 2006, in a study against virulent strains, *Serratia marcescens* and *Enterobacter cloacae*, using disk diffusion. Phenolics are the most widespread dietary antioxidants, and among these, chlorogenic acid (CGA) accumulates to high levels in some crop plants. Chlorogenic acids (3-(3,4-Dihydroxycinnamoyl) quinic acid) are cinnamic acid derivatives and they are an important intermediate in lignin biosynthesis. Green coffee is a major source of CGA. Antibacterial activity of CGA has been reported by several authors. Chlorogenic acids, trigonelline and caffeine are the compounds with the greatest action against the strains of *Streptococcus mutans* (ANTONIA et al., 2010). *Hyptis atrorubens* Poit (Lamiaceae) is a plant species used as an antimicrobial agent in Guadeloupe. The hydromethanolic extract of the stems showed the best antibacterial activity against bacteria, mostly Gram-positive ones. The bactericidal power of rosmarinic acid was much faster in the time kill study (ABEDINI et al., 2013). Rosmarinic acid (R)-O-(3,4-Dihydroxycinnamoyl)-3-(3,4-dihydroxyphenyl) has the molecular formula $C_{18}H_{16}O_8$.

Qualitative and quantitative evaluation of the antimicrobial activity R. officinalis extract

The qualitative testing by disc diffusion method Kirby-Bauer, adapter (CHIFIRIUC et al., 2011) emphasized that hydroalcoholic extracts and essential oil of *R. officinalis* shoots had antimicrobial activity for all bacterial strains from the collection achieved, quantified by the appearance of a zone of growth inhibition around the spot stock solution deposited on the seeded agar medium (Figure 1).

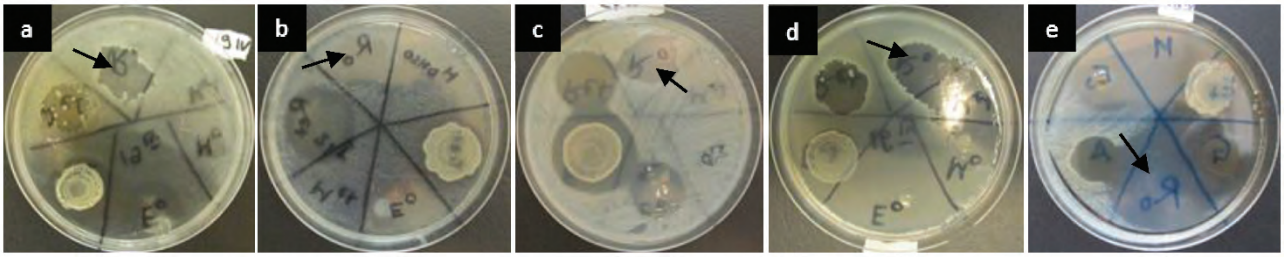


Figure 1. Qualitative Testing of susceptibility to volatile oil and ethanol extract of *R. officinalis* strains studied, (from left to right): *Ps aeruginosa*$_{9IV}$ (a), *E. coli*$_{28II}$ (b), *P. mirabilis* (c), *K. pneumonia* (d), *A. baumannii* (e) (original).

Qualitative evaluation is an estimate, because it is not known the extent of absorption of the compound by the medium. Quantitative determination of antimicrobial activity was performed by the technique of successive binary microdilution in a liquid medium which is a method of determining the minimum inhibitory concentration (MIC). The assay was performed according to the method described by CHIFIRIUC et al., 2011. Out of stock solutions of the compounds to be analysed there are performed micro binary successive dilutions in liquid BHI medium, distributed in 96-well plates. No dilutions were made in the wells treated for positive control (culture medium) and negative control (the control microbial culture). MIC values are between 15.6 and 125 µl / ml for ethanolic extracts and essential oils of *R. officinalis*. The antimicrobial activity of extracts from *R officinalis* could be attributed to compounds resulting from the secudar metabolism of the plant having protective against phytopathogens, most of which are Gram negative bacteria.

Table 3. The minimal inhibitory concentrations (MICs) for the essential oil and ethanolic extracts of *R. officinalis* against Gram negative bacteria.

	MICs ethanolic extract (µl/ml)		MICs essential oil (µl/ml)	
Strain	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{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

The antimicrobial activity of essential oils and plant extracts, evaluated qualitatively and quantitatively, is often first demonstrated for these pharmacological properties, but the specificity and complexity of interactions at the molecular level between cell components and components of plant extracts are not yet understood fully.

It was demonstrated that ethanolic extracts and essential oil of *R. officinalis*, reported by different authors, have antimicrobial effects that are highly differentiated according to the chemical structures of the components present in the extract, the specific mechanisms of the synergistic influence / antagonism between the components or environmental factors or specific microorganisms. Ethanolic extract and essential oil of *R. officinalis* contain a variety of compounds, most with intrinsic antimicrobial activity, others without antimicrobial activity. After determining the MIC, there can be applied a variety of methods to elucidate the mode of action of the compounds resulting from the secondary metabolism of a plant against microbial cells. The establishment of a mechanism of action should emphasized the importance of knowing the molecular interactions between the studied substance and specific targets of the host cell.

CONCLUSIONS

Antimicrobial resistance is not new but the number of resistant organisms are unprecedented. Diseases and pathogens that were once thought to be controlled by antibiotics are returning in new forms of resistance. Antibiotic-resistant strains have first appeared in hospitals, where the use of antibiotics is varied. Resistance to multiple drugs was first detected among enteric bacteria (*E. coli*, 1950s to early 1960s). To several antibiotic resistance bacteria increased especially in developing countries where antimicrobials were readily available without prescription. The severity of and difficulty in treating MDR strains necessitates the use of several, sometimes six to seven different, drugs. Among the Gram negative bacteria, hospital infections caused by *P. aeruginosa* and *A. baumanii* are sometimes resistant to all, or all but one, which seriously challenges the treatment of immunocompromised individuals and can result in death. ESBL (Extended Spectrum β-lactamases), carried among Enterobacteriaceae destroy even the latest generations of penicillin and cephalosporins. Metallo-β-lactamases that inactivate carbapenems are often the 'last resort' in serious infections of Gram negative bacteria (LEVI & MARSHALL, 2004).

In this context, the pharmaceutical industry seems to be no longer effective and the return to medicinal plants seems to be the only opportunity in the fight against MDR bacteria. The use of plants is as old as mankind. Rosemary is a spice with multiple uses in both food and seasoning in many diseases: cancer, diabetes, digestive diseases, respiratory and bacterial

infections. The most important compounds of rosemary are caffeic acid and its derivatives such as rosmarinic acid (labiatic acid) and chlorogenic acid. These compounds have antioxidant effect and activity against Gram negative bacteria. The phenolic compound, rosmarinic acid, obtains one of its phenolic rings from phenylalanine via caffeic acid and the other from tyrosine via dihydroxyphenyl-lactic acid. Relatively large-scale production of rosmarinic acid can be obtained from the cell culture of Coleus blumei Benth when supplied exogenously with phenylalanine and tyrosine (AL-SEREITI et al., 1999). Eucalyptol was also found in high concentration, being one of the major monoterpene constituents. Camphor and eucalyptol showed the strongest antimicrobial activity (HSOUNA et al., 2013). Carnosol and carnosic acid have been for over 90% of the antioxidant properties of rosemary extract. Ethanolic extracts and essential oil of *R. officinalis* have been shown to have a strong antibacterial activity. The studied strains showed antibacterial resistance both intrinsic and acquired to antibiotics. 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 of the identified compounds by GC-MS showed antioxidant and antibacterial activity individually. As a general conclusion, 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. The mode of action of the metabolism compounds, with multiple targets of action, prevents 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 dependence 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.

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