Microbial Pathogenesis 99 (2016) 173–177

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Inhibition of the NorA multi-drug transporter by oxygenated monoterpenes

Mayara Ladeira Coêlho [a], Josie Haydée Lima Ferreira [b], José Pinto de Siqueira Júnior [c], Glenn W. Kaatz [d, e], Humberto Medeiros Barreto [b, *], Ana Amélia de Carvalho Melo Cavalcante [a]

[a] Laboratory of Toxicology Genetic Research, Federal University of Piauí, Teresina, Piauí, Brazil
[b] Laboratory of Research in Microbiology, Federal University of Piauí, Teresina, PI, Brazil
[c] Laboratory of Genetics of Microorganisms, Federal University of Paraiba, João Pessoa, Paraiba, Brazil
[d] The John D. Dingell Department of Veteran's Affairs Medical Center, Detroit, MI, USA
[e] Department of Internal Medicine, Division of Infectious Diseases, Wayne State University, Detroit, MI, USA

ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form
20 August 2016
Accepted 22 August 2016
Available online 24 August 2016

Keywords:
Monoterpene
Fluoroquinolone
NorA
Efflux pump inhibitor
Bacterial resistance

ABSTRACT

The aim of this study was to investigate intrinsic antimicrobial activity of three monoterpenes nerol, dimethyl octanol and estragole, against bacteria and yeast strains, as well as, investigate if these compounds are able to inhibit the NorA efflux pump related to fluoroquinolone resistance in *Staphylococcus aureus*. Minimal inhibitory concentrations (MICs) of the monoterpenes against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* strains were determined by micro-dilution assay. MICs of the norfloxacin against a *S. aureus* strain overexpressing the NorA protein were determined in the absence or in the presence of the monoterpenes at subinhibitory concentrations, aiming to verify the ability of this compounds act as efflux pump inhibitors. The monoterpenes were inactive against *S. aureus* however the nerol was active against *E. coli* and *C. albicans*. The addition of the compounds to growth media at subinhibitory concentrations enhanced the activity of norfloxacin against *S. aureus* SA1199-B. This result shows that bioactives tested, especially the nerol, are able to inhibit NorA efflux pump indicating a potential use as adjuvants of norfloxacin for therapy of infections caused by multi-drug resistant *S. aureus* strains.

1. Introduction

The prevalence of infectious diseases caused by multi-drug resistant microorganisms has increased dramatically worldwide [1,2] despite of the wide range of available antimicrobial agents. Infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* resistant to cephalosporin of third-generation and fluoroquinolones are commonly acquired in hospitals and communities of all countries of the world [3,4]. Fluoroquinolones have been proposed as a possible alternative to vancomycin therapy against methicillin resistance *S. aureus* (MRSA) infections [5], however resistance to these antibacterial agents has become common and widespread [6–8].

Fluoroquinolones are able to binding to the complexes formed between DNA and DNA gyrase or topoisomerase IV, inactivating these bacterial enzymes, leading to a rapid inhibition of DNA replication [9] Bacterial resistance to fluoroquinolones occur due to mutation in one or more genes encoding these target enzymes or by expression of multidrug efflux pumps capable of actively removing fluoroquinolones from bacterial cell [10–12], as NorA efflux protein overexpressed by SA1199-B strain tested in the present study [13,14].

The knowledge about resistance mediated by efflux pumps has motivated the search for efflux pump inhibitor (EPI) compounds which could recover the efficacy of current antibiotics [15]. In this sense, synthetic or natural products from vegetable origin have been investigated for its ability to act how EPI [16–20].

Essential oils from plants are rich in bioactive compounds, such

* Corresponding author. Laboratory of Research in Microbiology, Department of Parasitology and Microbiology, Center of Health Sciences, Federal University of Piauí – UFPI, Campus Petronio Portella, University City, Ininga, 64049-550, Teresina, PI, Brazil.
E-mail address: hmbarreto@ufpi.edu.br (H.M. Barreto).

http://dx.doi.org/10.1016/j.micpath.2016.08.026
0882-4010/

as terpenes and terpenoids, which have showed promising results as potential therapeutic agents [21–23]. Monoterpenes, the main constituents of essential oils [24], have been reported for their antifungal, anti-aflatoxin and antioxidant activities [25]. The aim of the present study was to evaluate if monoterpenes nerol, estragole and dymethil-octanol, are potential intrinsic antimicrobial agents and/or efflux pump inhibitors of the NorA multi-drug transporter of *S. aureus.*

2. Materials and methods

2.1. Strains and drugs

The intrinsic antimicrobial activity of the monoterpenes was tested against Gram-positive (*S. aureus* ATCC 25923, SA1199, SA1199-B and SA10), Gram-negative (*E. coli* ATCC 25922) or yeast (*Candida albicans* NEWP031) strains. The inhibitory effect on NorA activity was performed with *S. aureus* SA1199-B strain which over-express the *norA* gene encoding NorA. NorA can efflux hydrophilic fluoroquinolones and other drugs such as DNA-intercalating dyes [13]. Bacterial strains were maintained on Brain Heart Infusion Agar (BHIA, Himedia, India) slant at 4 °C, and prior to assay the cells were grown overnight at 37 °C in Brain Heart Infusion (BHI, Himedia, India). The yeast strain was maintained on Sabouraud Dextrose Agar (SDA, Himedia, India) slant at 4 °C and prior to assay the cells were grown for 24 h at 37 °C in Sabouraud Dextrose Broth (SDB, Himedia, India).

Oxygenated monoterpenes 3,7-dimethyl-octan-1-ol, (2Z)-3,7-dimethylocta-2,6-dien-1-ol (nerol), and 1-methoxy-4-(prop-2-en-1-yl)benzene (estragole), norfloxacin and ethidium bromide were obtained from Sigma Chemical Corp., St. Louis. Antibiotics and ethidium bromide were dissolved in sterile water.

2.2. Log P estimation

Estimation of the Log *P* was performed using the MarvinSketch 6.2.2 (Chemaxon), by the Phys method. Log *P* has been calculated for the uncharged molecule due to the typical range of pKa for aromatic hydroxyl is 8.0–10.0.

2.3. Evaluation of the intrinsic antimicrobial activity

Stock solutions of nerol, 3,7-Dimethyl-1-octanol and estragole were prepared by dissolving 10000 μg of each monoterpene in 1 mL of dimethyl sulfoxide, thus starting with an initial concentration of 10000 μg/mL. This stock solution was then diluted in sterile distillated water to obtain the test solution (1024 μg/mL). Minimal inhibitory concentrations (MICs) of monoterpenes were determined by micro-dilution assay in BHI broth 10% with bacterial suspensions of 10^5 CFU/mL and monoterpene solutions ranging from 8 to 512 μg/mL. Microtiter plates were incubated at 37 °C for 24 h, then 20 μL of resazurin (0.01% w/v in sterile distilled water) was added to each well to detect bacterial growth by color change from blue to pink. MICs were defined as the lowest concentration at which no bacterial growth was observed.

Antifungal assays were performed by micro-dilution method in SDB double concentrated with yeast suspension of 10^5 CFU/mL and monoterpene solutions ranging from 8 to 512 μg/mL. Microtiter plates were incubated at 37 °C for 24 h. The MIC was defined as the lower concentration of the monoterpene solution able to inhibit the visible growth. Inhibition of the fungal growth was confirmed transferring an aliquot from each well of the MIC test microtiter plate to a Petri dish containing SDA and checking cell viability after incubation at 37° for 24 h.

2.4. Evaluation of the NorA efflux pump inhibition

For evaluation of the monoterpenes as modulators of fluoroquinolone resistance, MICs of the norfloxacin for SA1199-B strain were determined in the presence or absence of each compound at sub-inhibitory concentrations (1/8 MIC, 1/4 MIC or 1/2 MIC). Antibiotic or ethidium bromide (EtBr) concentrations ranged from 0.125 to 128 μg/mL. Microtiter plates were incubated at 37 °C for 24 h and readings were performed with resazurin as previously described.

2.5. Statistical analysis

All experiments were performed in triplicate and results were normalized by calculation of geometric average values. Error deviation and standard deviation of the geometric average were revealed. Differences between treatment with antibiotics alone or associated with monoterpenes were examined using one-way analysis of variance (ANOVA). Differences mentioned above were analyzed by Bonferroni posttest and $p < 0.05$ were considered statistically significant.

3. Results

Chemical structures and Log P values of the oxygenated monoterpenes tested are presented in Table 1. MICs found to every compound against *S. aureus*, *E. coli* and *C. albicans* strains are presented in Table 2. Monoterpenes tested did not present activity against all *S. aureus* strains [26]. On the other hand, the nerol showed a weak inhibitory activity (512 μg/mL) against *E. coli* e *C. albicans* strains.

Addition of oxygenated monoterpenes to the growth medium at sub-inhibitory concentrations caused a decrease in the MIC for norfloxacin against SA1199-B (Figs. 1–3). The nerol and 3,7-dimethyl-octan-1-ol enhanced the antibiotic activity of norfloxacin against SA1199-B in a concentration-dependent manner. On the other hand, a modulatory effect was also verified when antibiotics were replaced by ethidium bromide, a well-known substrate of NorA protein (Fig. 4).

4. Discussion

Essential oils have been proposed as a natural source of compounds with antibacterial activity against multi-drug resistant bacteria, as well as, a natural source of compounds able to inhibit bacterial resistance mechanisms [27–29]. In this work we investigate the intrinsic antibacterial activity of three oxygenated monoterpenes and their potential as EPI of the NorA efflux pump which is related with resistance to hydrophobic fluoroquinolones in *S. aureus* SA1199-B.

In respect to intrinsic antimicrobial activity, only nerol was active against *E. coli* ATCC 25923 and *Candida albicans* NEWP031.

Table 1
Chemical structure and Log P estimation for oxygenated monoterpenes tested.

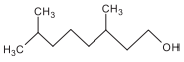
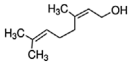
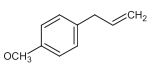
Monoterpene	Structure	Log P
3,7-dimethyl-octanol		3,12
(2*Z*)-3,7-dimethyl-2,6-octadien-1-ol (nerol)		3,02
1-allyl-4-methoxybenzene (estragole)		2,89

Table 2
Minimal inhibitory concentrations (MIC) showed by Nerol, 3,7-Dimethyl-1-octanol and Estragole against *S. aureus* strains (geometrical means of three simultaneous tests).

CEPAS	MIC (μg/mL)		Estragole
	Nerol	Dimethyl octanol	
S. aureus ATCC 25923	≥1024	≥1024	≥1024
S. aureus SA1199	≥1024	≥1024	≥1024
S. aureus SA1199-B	≥1024	≥1024	≥1024
S. aureus SA10	≥1024	≥1024	≥1024
E. coli ATCC 25923	512	2048	2048
C. albicans NEWP031	512	2048	2048

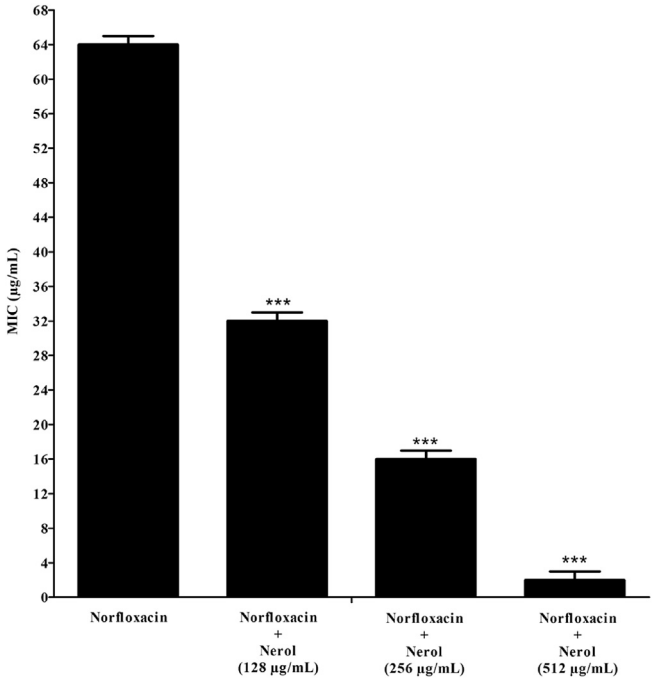


Fig. 1. MIC of the norfloxacin in absence or presence of nerol against SA1199-B. Each result is the geometric mean of three simultaneous experiments. (***) Statistically significant values ($p < 0.001$).

Similar results have been reported by previous studies which showed the effectiveness of nerol as antifungal compound against *Aspergillus* strains [30]. Also was verified that essential oils from *Ocimum basilicum* Linn., *Thymus algeriensis* and *Citrus aurantium* L., which contain nerol in its composition were actives against bacteria [31–35]. Besides, its trans isomer, geraniol, displayed strong activity against *C. albicans, E. coli* and plant pathogenic bacteria, *Agrobacterium tumefaciens, Erwinia carotovora, Corynebacterium fascians, and Pseudomonas solanacearum* [36–38]. It was also reported that the nerol is more effective as an antibacterial than as an antifungal agent [39,40].

Although oxygenated monoterpenes have not presented intrinsic activity against *S. aureus*, when they were placed on the growth medium at sub-inhibitory concentrations they were able to reduce the MIC for norfloxacin in a concentration dependent manner against SA1199-B strain (Figs. 1–3). The oxygenated monoterpenes also were able to improve the activity of the ethidium bromide against SA1199-B (Fig. 3), a substrate of NorA protein. These results indicate that the compounds tested are EPIs for NorA efflux pump. Besides, the monoterpenes of open chain (nerol and 3,7-dimethyl-octan-1-ol) were more effective as NorA inhibitors than the monoterpene 1-methoxy-4-(prop-2-en-1-yl) benzene which presents a benzene ring.

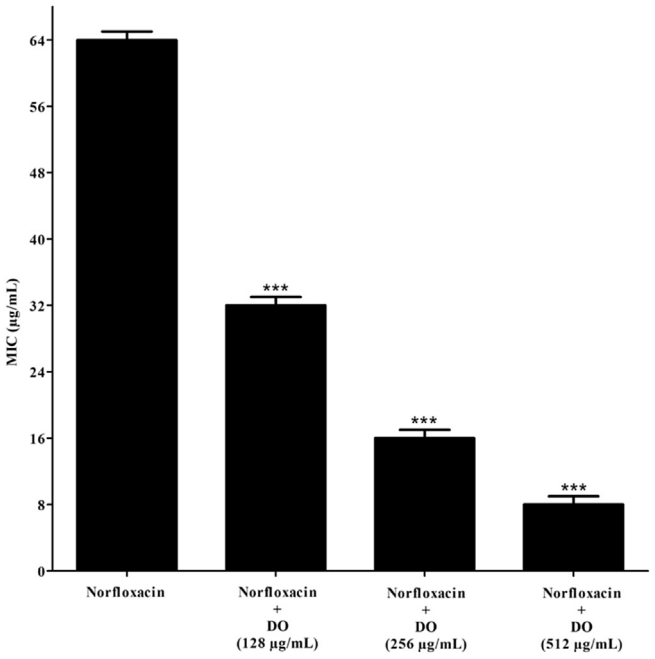


Fig. 2. MIC of the norfloxacin in absence or presence of 3,7-dimethyl-1-octanol (DO) against SA1199-B. Each result is the geometric mean of three simultaneous experiments. (***) Statistically significant values ($p < 0.001$).

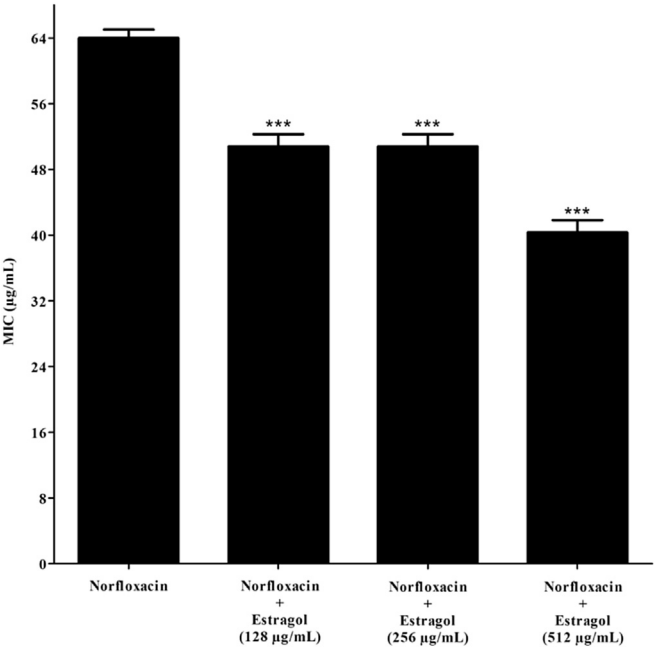


Fig. 3. MIC of the norfloxacin in absence or presence of estragole against SA1199-B. Each result is the geometric mean of three simultaneous experiments. (***) Statistically significant values ($p < 0.001$).

Taking into account the criteria adopted by the Clinical and Laboratory Standards Institute (CLSI, 2013) nerol decreased the MIC of the norfloxacin for SA1199-B, changing its phenotype from resistant to sensible. In the case of 3,7-dimethyl-octan-1-ol, the SA1199-B phenotype was changed from resistant to intermediate.

Lipophilicity studies are relevant to know if a molecule is able to interact with cell membranes [41]. The Log *P* of oxygenated

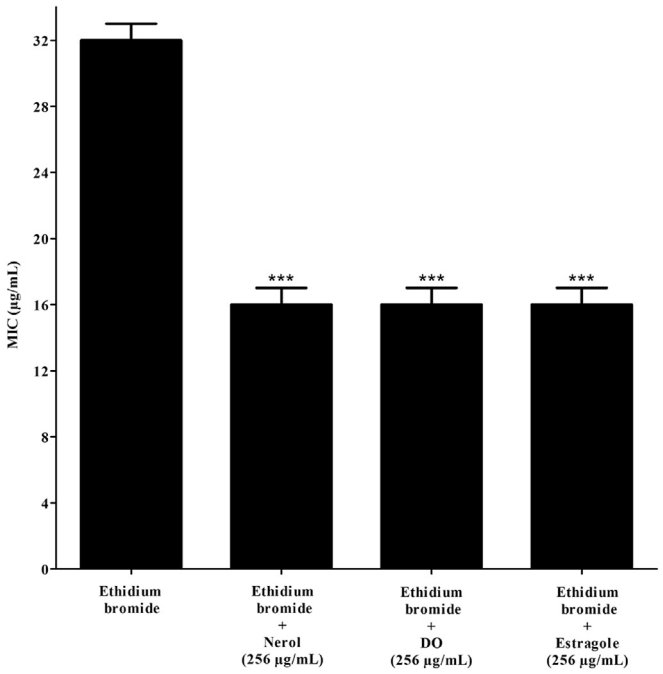


Fig. 4. MIC of the ethidium bromide in absence or presence of nerol, dimethyl-octanol (DO) and estragole against SA1199-B. Each result is the geometric mean of three simultaneous experiments. (***) Statistically significant values ($p < 0.001$).

monoterpenes was calculated and results showed that all of them were found to be very hydrophobic, thus them are able to intercalate in the phospholipid bilayer. Interaction of hydrophobic compounds with plasma membrane can increases its permeability leading to dissipation of the proton-motive force [42,29].

Once NorA activity is dependent of the proton motive force [43], we believe that partition of nerol or 3,7-dimethyl-octan-1-ol molecules into the plasma membrane affecting the proton gradient, could be related with the inhibitory effect verified for NorA [44]. Despite, a damaged membrane by nerol or 3,7-dimethyl-octan-1-ol could become more permeate to norfloxacin molecules, also contributing to increase its intracellular concentration. Additionally, damaged plasma membrane could lead to conformational changes and inhibition of membrane proteins, such as NorA.

5. Conclusion

Oxygenated monoterpenes nerol and 3,7-dimethyl-1-octanol were able to potentiate the antibiotic activity of norfloxacin against SA1199-B, indicating that this essential oil components act as efflux pump inhibitors of NorA. Thus, these compounds are potential components of pharmaceutical forms for use in association with norfloxacin in the antibiotic therapy of infections caused by fluoroquinolone resistant *Staphylococcus aureus*.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgments

The authors acknowledge the Brazilian research agencies FAPEPI and CNPq for the financial support and gs3:Universidade Federal do Piauí.

References

[1] R. Laxminarayan, Antibiotic effectiveness: balancing conservation against innovation, Science 345 (2014) 1299–1301, http://dx.doi.org/10.1126/science.1254163.
[2] N. Brusselaers, D. Vogelaers, S. Blot, The rising problem of antimicrobial resistance in the intensive care unit, Ann. Intensive Care 47 (2011) 1–7, http://dx.doi.org/10.1186/2110-5820-1-47.
[3] K.Y.L. Chua, B.P. Howden, J.-H. Jiang, T. Stinear, A.Y. Peleg, Population genetics and the evolution of virulence in *Staphylococcus aureus*, Infect. Genet. Evol. 21 (2014) 554–562, http://dx.doi.org/10.1016/j.meegid.2013.04.026.
[4] A.C. Uhlemann, M. Otto, F.D. Lowy, F.R. DeLeo, Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*, Infect. Genet. Evol. 21 (2014) 563–574, http://dx.doi.org/10.1016/j.meegid.2013.04.030.
[5] N. Gade, M. Qazi, Fluoroquinolone therapy in Staphylococcus aureus infections: where do we stand? J. Lab. Physicians 5 (2013) 109, http://dx.doi.org/10.4103/0974-2727.119862.
[6] A. García-Fernández, S. Gallina, S. Owczarek, A.M. Dionisi, I. Benedetti, L. Decastelli, Emergence of ciprofloxacin-resistant *Salmonella* enterica serovar typhi in Italy, PLoS one (2015) 10, http://dx.doi.org/10.1371/journal.pone.0132065 e0132065.
[7] L. Vali, A. Dashti, M.M. Jadaon, S. El-Shazly, The emergence of plasmid mediated quinolone resistance qnrA2 in extended spectrum β-lactamase producing *Klebsiella pneumoniae* in the Middle East, DARU J. Pharm. Sci. Eur. J. Pharm. Sci. 23 (2015) 34, http://dx.doi.org/10.1186/s40199-015-0116-7.
[8] V. De Lastours, F. Chau, C. Roy, B. Larroque, B. Fantin, Emergence of quinolone resistance in the microbiota of hospitalized patients treated or not with a fluoroquinolone, J. Antimicrob. Chemother. 69 (2014) 3393–3400, http://dx.doi.org/10.1093/jac/dku283.
[9] K.J. Aldred, R.J. Kerns, N. Osheroff, Mechanism of quinolone action and resistance, Biochemistry 53 (2014) 1565–1574, http://dx.doi.org/10.1021/bi5000564.
[10] L.S. Redgrave, S.B. Sutton, M.A. Webber, L.J.V. Piddock, Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success, Trends Microbiol. (2014) 438–445, http://dx.doi.org/10.1016/j.tim.2014.04.007.
[11] P. Mc Gann, M. Milillo, Y.I. Kwak, R. Quintero, P.E. Waterman, E. Lesho, Rapid and simultaneous detection of the chlorhexidine and mupirocin resistance genes qacA/B and mupA in clinical isolates of methicillin-resistant *Staphylococcus aureus*, Diagn. Microb. Infect. Dis. 77 (2013) 270–272, http://dx.doi.org/10.1016/j.diagmicrobio.2013.06.006.
[12] T.J. Opperman, et al., Efflux-mediated bis-indole resistance in *Staphylococcus aureus* reveals differential substrate specificities for MepA and MepR, Bioorganic Med. Chem. 18 (2010) 2123–2130, http://dx.doi.org/10.1016/j.bmc.2010.02.005.
[13] G.W. Kaatz, S.M. Seo, Inducible NorA-mediated multidrug resistance in *Staphylococcus aureus*, Antimicrob. Agents Chemother. 39 (1995) 2650–2655, http://dx.doi.org/10.1128/AAC.39.12.2650.
[14] G. Kaatz, S. Seo, Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*, Antimicrob. agents 37 (1993) 1086–1094, http://dx.doi.org/10.1128/AAC.37.5.1086.
[15] E.F.F. Matias, et al., Potentiation of antibiotic activity of aminoglycosides by natural products from *Cordia verbenacea* DC, Microb. Pathog. 95 (2016) 111–116, http://dx.doi.org/10.1016/j.phytol.2008.01.002.
[16] F. Fontaine, et al., Boronic species as promising inhibitors of the *Staphylococcus aureus* NorA efflux pump: study of 6-substituted pyridine-3-boronic acid derivatives, Eur. J. Med. Chem. 95 (2015) 185–198, http://dx.doi.org/10.1016/j.ejmech.2015.02.056.
[17] S.T. Nguyen, S.M. Kwasny, X. Ding, S.C. Cardinale, C.T. McCarthy, H.-S. Kim, Structure–activity relationships of a novel pyranopyridine series of Gram-negative bacterial efflux pump inhibitors, Bioorganic Med. Chem. 23 (2015) 2024–2034, http://dx.doi.org/10.1016/j.bmc.2015.03.016.
[18] J.B. Bharate, et al., Discovery of 4-acetyl-3-(4-fluorophenyl)-1-(p-tolyl)-5-methylpyrrole as a dual inhibitor of human P-glycoprotein and *Staphylococcus aureus* Nor A efflux pump, Org. Biomol. Chem. 13 (2015) 5424–5431, http://dx.doi.org/10.1039/C5OB00246J.
[19] K.E. Whalen, K.L. Poulson-Ellestad, R.W. Deering, D.C. Rowley, T.J. Mincer, Enhancement of antibiotic activity against multidrug-resistant bacteria by the efflux pump inhibitor 3,4-Dibromopyrrole-2,5-dione isolated from a *Pseudoalteromonas* sp, J. Nat. Prod. 78 (2015) 402–412, http://dx.doi.org/10.1021/np500775e.
[20] R. Chovanová, J. Mezovská, Š. Vaverková, M. Mikulášová, The inhibition the Tet(K) efflux pump of tetracycline resistant, Lett. Appl. Microbiol. 61 (2015) 58–62, http://dx.doi.org/10.1111/lam.12424.
[21] A.E. González-Ramírez, M.E. González-Trujanob, S.A. Orozco-Suárezc, N. Alvarado-Vásquezd, F.J. López-Muñoza, Nerol alleviates pathologic markers in the oxazolone-induced colitis model, Eur. J. Pharm. 776 (2016) 81–89, http://dx.doi.org/10.1016/j.ejphar.2016.02.036.
[22] L. De Fazio, et al., Dietary geraniol by oral or enema administration strongly reduces dysbiosis and systemic inflammation in dextran sulfate sodium-treated mice, Front. Pharm. 7 (2016) 38, http://dx.doi.org/10.3389/fphar.2016.00038.
[23] H.M. Barreto, et al., Effect of *Lippia origanoides* H.B.K. essential oil in the

resistance to aminoglycosides in methicillin resistant *Staphylococcus*, Eur. J. Integr. Med. 6 (2014a) 560–564, http://dx.doi.org/10.1016/j.eujim.2014.03.011. Available from:.

[24] M.G. Peixoto, et al., Toxicity and repellency of essential oils of *Lippia alba* chemotypes and their major monoterpenes against stored grain insects, Ind. Crops Prod. 71 (2015) 31–36, http://dx.doi.org/10.1016/j.indcrop.2015.03.084.

[25] A. Kedia, B. Prakash, P.K. Mishra, N.K. Dubey, Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities, Int. J. Food Microbiol. 1 (7) (2014) 168–169, http://dx.doi.org/10.1016/j.ijfoodmicro.2013.10.008.

[26] F.B. Holetz, et al., Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases, Mem. Inst. Oswaldo Cruz (2002) 1027–1031, http://dx.doi.org/10.1590/S0074-02762002000700017.

[27] H.M. Barreto, E.C. Silva Filho, E.O. Lima, H.D.M. Coutinho, M.F.B. Morais-Braga, C.C.A. Tavares, S.R. Tintino, J.V. Rego, A.P.L. Abreu, M.C.G. Lustosa, R.W.G. Oliveira, A.M.G.L. Citó, J.A.D. Lopes, Chemical composition and possible use as adjuvant of the antibiotic therapy of the essential oil of *Rosmarinus officinalis* L, Ind. Crops Prod. 59 (2014b) 290–294, http://dx.doi.org/10.1016/j.indcrop.2014.05.026.

[28] T.S. Almeida, J.T. Rocha, F.F.G. Rodrigues, A.R. Campos, J.G.M. Costa, Chemical composition, antibacterial and antibiotic modulatory effect of *Croton campestris* essential oils, Ind. Crops Prod. 44 (2013) 630–633, http://dx.doi.org/10.1016/j.indcrop.2012.09.010. Available from:.

[29] E.L. Souza, C.E.V. Oliveira, T.L.M. Stamford, M.L. Conceição, N.J. Gomes Neto, Influence of carvacrol and thymol on the physiological attributes, enterotoxin production and surface characteristics of *Staphylococcus aureus* strains isolated from foods, Braz. J. Microbiol. 44 (2013) 29–35, http://dx.doi.org/10.1590/S1517-83822013005000001.

[30] A.L. Mihai, M.E. Popa, *In vitro* activity of natural antimicrobial compounds against *Aspergillus* strains, Agric. Agric. Sci. Procedia 6 (2015) 585–592, http://dx.doi.org/10.1016/j.aaspro.2015.08.092.

[31] A. Ahmad, A. Viljoen, The in vitro antimicrobial activity of *Cymbopogon essential oil* (lemon grass) and its interaction with silver ions, Phytomedicine 22 (2015) 657–665, http://dx.doi.org/10.1016/j.phymed.2015.04.002.

[32] D.W. Al Abbasy, N. Pathare, J.N. Al-Sabahi, S.A. Khan, Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.), Asian. pac. J. Trop. Dis. 5 (2015) 645–649, http://dx.doi.org/10.1016/S2222-1808(15)60905-7.

[33] I.B.E.H. Ali, M. Chaouachi, R. Bahri, I. Chaieb, M. Boussaïd, F. Harzallah-Skhiri, Chemical composition and antioxidant, antibacterial, allelopathic and insecticidal activities of essential oil of *Thymus algeriensis* Boiss. et Reut, Ind. Crops. Prod. 77 (2015) 631–639 doi: 2015.09.0460926-6690.

[34] A.H. Ammar, J. Bouajila, A. Lebrihi, F. Mathieu, M. Romdhane, F. Zagrouba, Chemical composition and in vitro antimicrobial and antioxidant activities of *Citrus aurantium* L. flowers essential oil (Neroli Oil), Pak. J. Biol. Sci. 15 (2012) 1034–1040, http://dx.doi.org/10.3923/pjbs.2012.1034.1040.

[35] H.J.D. Dorman, S.G. Deans, Antimicrobial agents from plants: antibacterial activity of plant volatile oils, J. Appl. Microb. 88 (2000) 308–316, http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x.

[36] R.L. Van Zyl, S.T. Seatlholo, S.F. Van Vuuren, A.M. Viljoen, The biological activities of 20 nature identical essential oil constituents, J. Essent. Oil Res. 18 (2006) 129–133 (accessed 13.04.16), http://cat.inist.fr/?aModele=afficheN&cpsidt=18019096.

[37] M.E.I. Badawy, E.I. Rabea, N.E.M. Taktak, M.A.M. El-Nouby, The antibacterial activity of chitosan products blended with monoterpenes and their biofilms against plant pathogenic bacteria, Scientifica (2016) 1–10, http://dx.doi.org/10.1155/2016/1796256.

[38] M. Friedman, P.R. Henika, R.E. Mandrell, Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica*, J. Food Prot. 10 (2002) 1545–1560 doi: 1111/1750-3841.12021.

[39] K. Knobloch, A. Pauli, B. Iberl, H. Weigand, N. Weis, Antibacterial and antifungal properties of essential oil components, J. Essent. Oil Res. 1 (1989) 118–119, http://dx.doi.org/10.2174/0929867033457719.

[40] P. Suppakul, J. Miltz, K. Sonneveld, S.W. Bigger, Antimicrobial properties of basil and its possible application in food packaging, J. Agric. Food Chem. 51 (2003) 3197, http://dx.doi.org/10.1021/jf021038t.

[41] N.A. Kasim, et al., Molecular properties of WHO essential drugs and provisional biopharmaceutical classification, Mol. Pharm. 1 (2004) 85–96, http://dx.doi.org/10.1021/mp034006h.

[42] A. Ahmad, A. Khan, N. Manzoor, Reversal of efflux mediated antifungal resistance underlies synergistic activity of two monoterpenes with fluconazole, Eur. J. Pharm. Sci. 48 (2013) 80–86, http://dx.doi.org/10.1016/j.ejps.2012.09.016.

[43] F. Guilhelmelli, N. Vilela, P. Albuquerque, L.S. Derengowski, I. Silva-Pereira, C.M. Kyaw, Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance, Front. Microbiol. 4 (2013) 353, http://dx.doi.org/10.3389/fmicb.2013.00353.

[44] B.D. Schindler, P. Jacinto, G.W. Kaatz, Inhibition of drug efflux pumps in *Staphylococcus aureus*: current status of potentiating existing antibiotics, Future Microbiol. 8 (2013) 491–507, http://dx.doi.org/10.2217/fmb.13.16.