Annals of West University of Timişoara, ser. Biology, 2017, vol. 20 (2), pp.201-210

THE ANTIBACTERIAL EFFECT OF *MELALEUCA ALTERNIFOLIA* (TEA TREE) EXTRACTS

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Received 24 October 2017; accepted 18 December 2017

ABSTRACT
Tea tree oil is the essential product optained from Melaleuca alternifolia, with antiviral, antibacterial, antifungal and anti-inflammatory properties. The antimicrobial effect of the Melaleuca alternifolia extract was tested using standardized bacterial strains: Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes and Enterococcus faecalis) and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Legionella pneumophila). The working methods were: the diffusion method (Kirby Bauer) which allows the determination of the minimum inhibitory concentration (CMI) and the spectrophotometric method to determine the minimum bactericidal concentration (CMB). Based on the inhibition rates, the antimicrobial effect of the Tea Tree Oil (TTO) extract decreases as regards the strains used, as follows: Salmonella enteritidis > Streptococcus pyogenes > Escherichia coli > Legionella pneumophila > Enterococcus faecalis > Pseudomonas aeruginosa > Staphylococcus aureus. The inhibition rate values determined upon application of the TTO extract confirm the results obtained for the inhibition rays. The obtained results strongly demonstrate the antibacterial properties of the TTO extract and highlight its use for other strains of pathogenic bacteria
KEY WORDS: *biological active compound, extract, bacteria, Melaleuca alternifolia*

INTRODUCTION

The Tea Tree Oil (TTO) is an essential oil, extracted from the leaves of *Melaleuca alternifolia,* a species native to Australia. The TTO has antiseptic properties and is used as a topic treatment in folk medicine for healing wounds and to prevent local infections. *M. alteniflora* belongs to Family Myrtacee, a tall shrub, with leaves linear in shape and that contains secretory glands used to extract this etheric oil; the flowers occur in white masses and the fruits are woody in structure (Cheel, 1924).

The research focused on the therapeutic properties of essential oils dates back in history and in time became increasingly popular because many synthetic based medications have nowadays a wide array of undesired side effects, such as toxicity to leaver and kidneys. Finding alternative medication became a serious business during the last decades. The essential

oils, which represent by-products of plants were investigated for their bioactive properties and potential therapeutic use, such as anti-inflammatory, anticancer and septic effects (Astani *et al,* 2010; D'Arrigo *et al,* 2010; Greay *et al,* 2010).

The oil extracted from *M. alteniflora* is made from terpene hydrocarbons, mostly monoterpenes but equally aromatic compounds with antibiotic, fungicide, antiviral and anti-inflammatory properties. Moreover, the indigenous aborigines from Australia used this compound in their traditional medicine to treat cough, various forms of cold, sower throat and skin rushes (Carson *et al,* 2006). The TTO is considered a sort of universal remedy against acnes, skin rushes, herpes, burns, bug bites and mycoses (Hammer *et al,* 2006).

The chemical composition of TTO was intensively studied by Carson & Riley (1995), who showed that the compound extracted from the leaves and branches of *M. alternifolia* with antibiotic properties is made mostly of terpinen-4-ol. Carson *et al.* (2006) reported the complex structure of TTO, comprising mostly monoterpenes, sesquiterpenes and other related compounds.

The mechanisms behind the antibiotic properties of TTO was tested on *E. coli* and it was proved that this compound acts mainly as an inhibitor of cell respiration and by decreasing the cell membrane permeability (Cox *et al*, 1998). De Prijck *et al.* (2008) pointed out the destruction of various bacterial strains, such as *E. coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* following exposure to a mixture of TTO and jojoboa oil. Moreover, the TTO was studied by employing transcription profiles for a better understanding of the antibiotic mechanisms (Lachenmeier, 2008). This research showed that the antibiotic action is in fact manifold, by targeting various components of the wall cell. This result was in line with other bodies of evidence of other researchers, who described the antibiotic properties of TTO that denatures the proteins and modifies the structure and functionality of cell membranes.

Therefore, from all plants considered nowadays as having antibiotic properties, the tea tree stands out, mostly because of the oil extracted from its leaves. Studies underlining its antibiotic effects were carried out on the genera *Staphylococcus sp.* and *Streptococcus sp.* (Carson & Riley, 1994). Moreover, the antibiotic efficiency of TTO for acne was equally investigated. As far as the antibiotic efficiency is concerned, the TTO-L diminished significantly the incidence of acne, of *Propionibacterium acnes*, *S. aureus* and by reducing the swells. The TTO acted as very efficient antibiotics but as far as the safety of this treatment is concerned, a percentage less than 5 % is considered more adequate and safe for its treatment (Lee *et al,* 2013).

Another study followed the effects of TTO *in vitro* against several bacterial strains cultivated from the wounds situated on patient legs, but equally against standard strains of *S. aureus* (ATCC 25923). The conclusion of this study was that the TTO has antibiotic properties *in vitro* against bacterial strains isolated from these wounds that proved to be resistant to classic antibiotic exposure (Portilho Falci *et al,* 2015).

The aim of this experiment was to assess if the TTO is efficient in diminishing the activity of several standard bacterial strains (some of them never tested before from this perspective) and if the inhibitory activity takes place on at least some of the tested bacteria. If the experiment would indicate the antibiotic effect of TTO, this could have far-reaching

consequences on industrial scale, for biologic and cleaning products to give just two examples, because would not manifest negative effects on human health or on the environment.

MATERIALS AND METHODS

The tested biologic material

The TTO (commercial form) was analysed by GC-MS and the active component was identified: α-Terpinen-4-ol. The TTO comprised terpinen-4-ol in four different concentrations: C1=4%, C2=8%, C3=16% and C4=32%.

Tested microorganisms

The efficiency of TTO in various concentrations was tested on seven standardised bacterial strains, as follows: *Staphylococcus aureus - ATCC 25923*, *Salmonella enteritidis - ATCC 13124*, *Streptococcus pyogenes - ATCC 196415*, *Escherichia coli - ATCC 8739*, *Enterococcus faecalis - ATCC 29212*, *Pseudomonas aeruginosa - ATCC 10145*, *Legionella pneumophila - ATCC 33152.*

a. **Assessments of minimal inhibitory concentration (MIC).** Assessment of antibiotic activity of the TTO was undertaken by using the Kirby-Bauer diffusion method that allows the estimation of the minimal inhibitory concentration (MIC) based on the inhibition distances (mm) occurring in the agar media. The growing media used in this experiment was Plate-Count-Agar (Sigma-Aldrich). The media and the wafers were autoclaved (T=121° C, 2.1 atmospheres) before the experiment. From the bacterial culture aliquots of 50µL were applied on Petri dishes. On each growing media10 µl of TTO were applied and afterwards the bacterial plates were incubated at 37 ºC for 24 h - 48 h. The test was carried out in triplicates. The zone of inhibition was then measured using a scale, and the antimicrobial activity was calculated (Vander & Vlietnck, 1991).

b. **The cell viability assays with TTC (2,3,5-trifeniltetrasol chloride).** Following the presence of bacteria, the TTC is reduced to formazan dye, of red colour, that indicates the activity and viability of cells (Eloff, 1998; Ianovici *et al,* 2008; Ianovici *et al,* 2010). This method is based on the capacity of surviving bacteria following the exposure to various concentrations of TTO to absorb the TTC and generate a specific colour.

The microbial cells from the liquid growing media had a concentration of 10^7 UFC/ml. On each replicate, an aliquot of 100 µl was applied, along with 50 µl of TTO. The ELISA plates were incubated for six hours at 37°C and 200 rpm speed to facilitate the development of bacterial colonies. Following the incubation period, an aliquot of 10 µl of TTC solution (0,5% concentration) was applied in each of the 96 wells. The plates were again incubated for two hours at 37°C to allow the interaction of bacterial colonies with the TTC. A microspectrophotometer (Tecam Sunrise) was used to read the concentrations at 460 nm wave length. The assessment of inhibition rate to TTO exposure was based on the following formula:

Inhibition rate (%) = [(Absorbance control- Absorbance treatment)/Absorbance treatment] x 100

RESULTS AND DISCUSSIONS

Following incubation, the MIC was calculated for each bacterial strain by considering the inhibition distance (mm) and the concentration of the TTO. The average and standard deviation for each treatment were calculated afterwards. The graphs present the mean inhibition distances and their ± standard deviation.

The strain of *Staphylococcus aureus* presents an increased sensibility following TTO exposure, mainly for the first three concentrations, with average inhibition distances of 7.75±0.077 mm (c1), 6.50 mm (c2) and 5.25±0.052 mm (c3), respectively. For concentration c4 the average inhibition distance was 3.00±0.010 mm, although it can be concluded that as far as this concentration is concerned, the sensitivity to this compound is somehow moderate (Fig. 1).

The TTO proved to be a very efficient antibiotic on *S. aureus* meticilino-resistant strains (MSSA), along with other isolated strains of staphylococci (i.e. coagulase-negative staphylococci (CoNS) from biofilms developed around wounds, Carson and Riley, 1995). Previous studies revealed that terpinen-4-ol is the main active compound against bacteria. Our results are therefore in line with those of Cox et al. (2000) on the antibiotic effect of TTO over a standardised strain of *S. aureus* NCTC 8325.

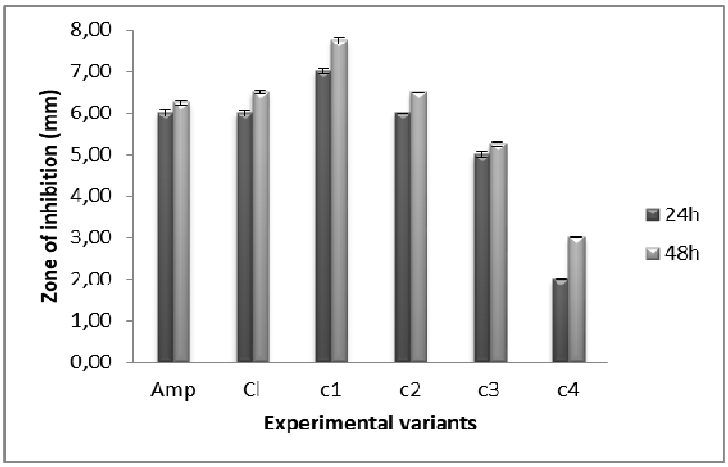

FIG. 1. The antibiotic effects of TTO on the strain *Staphylococcus aureus*

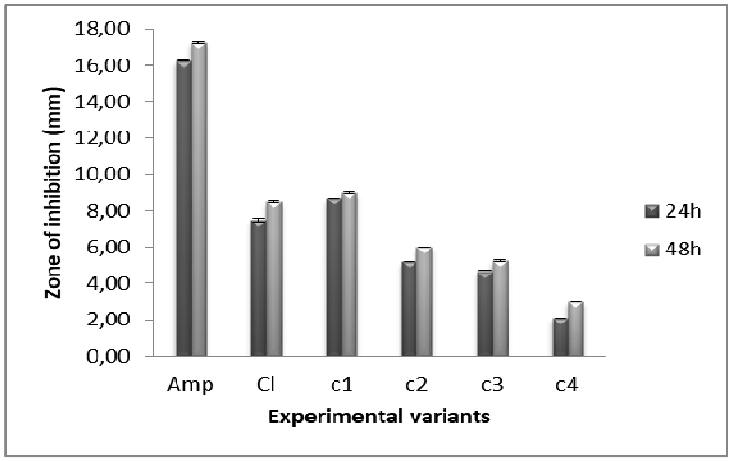

FIG. 2. The antibiotic effect of TTO on *Salmonella enteridis* strain

The TTO proved to be less efficient compared to control antibiotics, but we can conclude that the strain of *Salmonella enteridis* is sensitive to certain concentrations of TTO, mainly c1 and c2, where the average inhibition distances were 9 and 6.00 mm, respectively. According to these results, it can be concluded that *Salmonella enteridis* is sensitive to the former two concentrations and intermediary sensitive for the latter two concentrations of TTO (Fig. 2).

Streptococcus pyogenes is a strain with an intermediary resistance for the former two concentrations, by employing mean inhibitory distances of 5±0.073 mm and 4 mm, respectively, but resistant to the latter two concentrations of TTO (Fig. 3). Other previous studies confirmed the antibiotic effects of TTO on various bacterial strains of the genus Streptococcus *sp*. These effects employed the exposure of these oils to other bacterial strains, such as: *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Streptococcus mutans* and *Streptococcus sobrinus*. Along with the TTO, other essential oils were employed in previous studies: the manuka, eucalyptus, lavender and romarinus oils, for each one of them the MIC being assessed. The essential oils generally inhibited the growth of tested bacteria. The MIC's showed that mostly the lavender oils act as bacteriostatic agents, whilst the rest as typical antibiotics. The TTO and manuka oil were found to be great inhibitors for *P. gingivalis* growth. All these tested oils inhibited *S. mutans*. This study showed that, from all essential tested oils, the Manuka oil, but mostly the TTO stands out as great antibiotic potential products (Takarada *et al*, 2003).

E. coli showed sensitivity towards TTO exposure for the former two concentrations, with mean inhibition distances of 6±0.055 mm and 5 mm, respectively. The sensitivity of *E. coli* decreases with the concentrations of TTO (c3 and c4), showing a moderate antibiotic response with mean inhibitory distances of 2.75±0.058 mm and 2.5±0.021 mm, respectively (Fig. 4). Several previous studies tested the growth of *E. coli* to TTO, revealing the underpinning mechanisms by which this compound acts against bacterial strains. The results from the current study therefore are in accordance to those obtained by another group of scientists, which confirmed the antibiotic effect of TTO on standard strains of *E. coli* AG100 by decreasing the permeability of bacterial wall cells (Cox *et al*, 2000).

As far as the *Enterococcus faecalis* response to TTO is concerned, one can notice an intermediary sensitivity only to the first concentration (c1), with a mean inhibition rate of 3±0.007 mm. To other employed concentrations of TTO, however, this species proved to be extremely resistant (Fig. 5). *Pseudomonas aeruginosa* represents a bacterial strain with increased resistance to all employed concentrations of TTO in this experiment. The values of CMI strongly support this statement (Fig. 6). Other previous studies found that this compound, however, has some antibiotic effects, either alone, either in combination with other essential oils on standard strains of *Pseudomonas aeruginosa* ATCC 9027 (Mayaud *et al*, 2008).

Instead, *Legionella pneumophila* showed a certain sensitivity to the former two employed concentrations of TTO, with a mean inhibition rate of 6.5±0.058 mm and 6±0.022 mm, respectively. The moderate sensitivity was noticed to exposure to c3 and c4 concentrations, by employing inhibition rates of 3.75 mm and 3 mm, respectively (Fig. 7).

The inhibition rate is assessed by assay tests, that allow the identification of alive microorganisms and by employing the calculation formula to estimate the mortality rate.

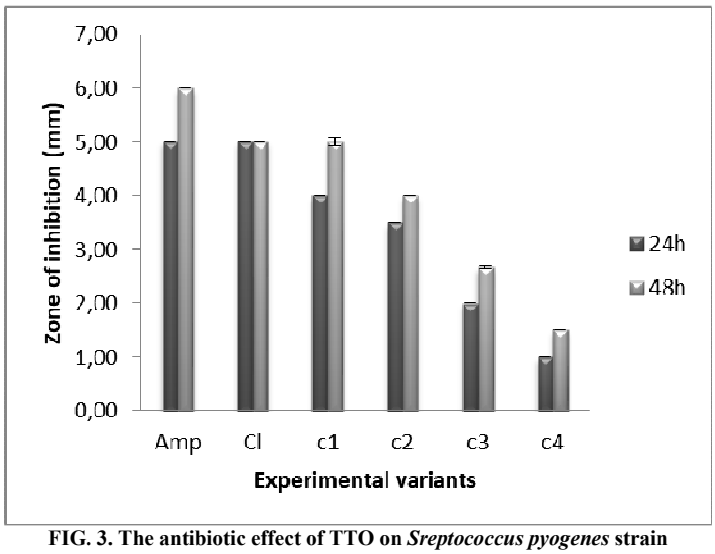

FIG. 3. The antibiotic effect of TTO on *Sreptococcus pyogenes* strain

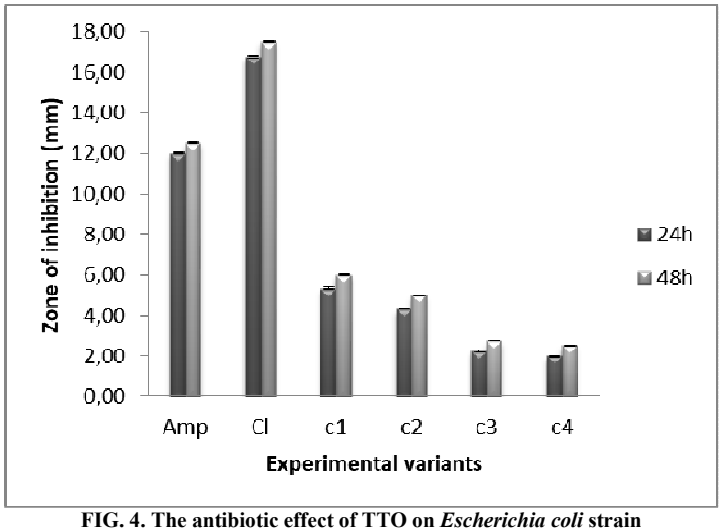

FIG. 4. The antibiotic effect of TTO on *Escherichia coli* strain

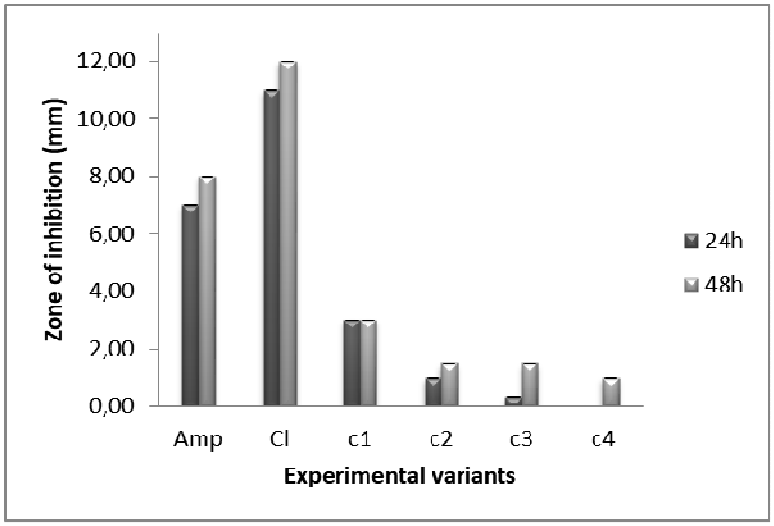

FIG. 5. The antibiotic effect of TTO on *Enterococcus faecalis* strain

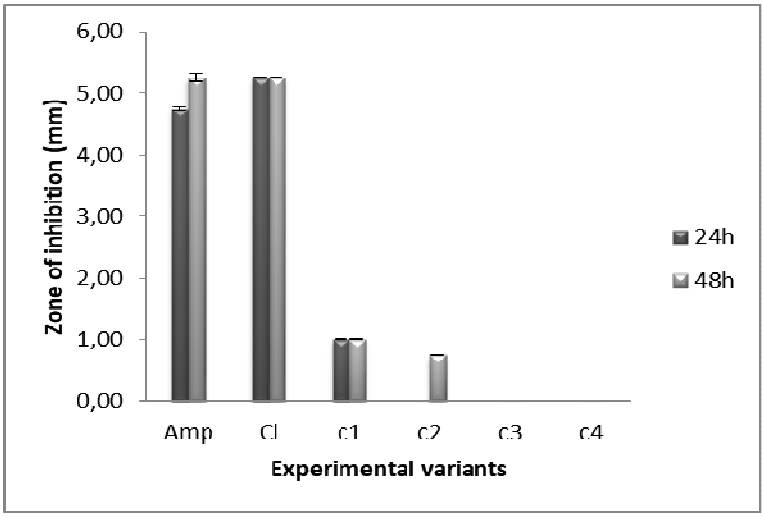

FIG. 6. The antibiotic effect of TTO on *Pseudomonas aeruginosa*

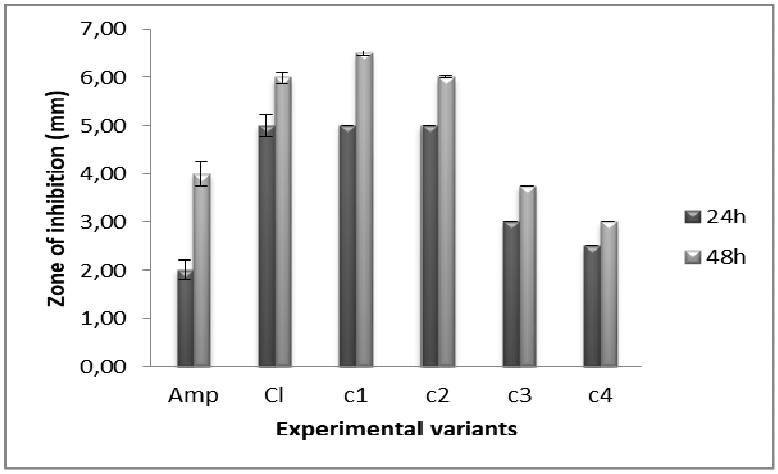

FIG. 7. The antibiotic effect of TTO on *Legionella pneumophila*

For *Staphylococcus aureus,* the inhibition rate to the first three concentrations of TTO were way above the threshold of 50%: 84.84% (c1), 77. 27% (c2) and 62.93% (c3), respectively. At c4 the inhibition rate was only 23.20% (Fig. 8). Therefore, it can be concluded that the TTO is efficient against *Staphylococcus aureus*, fact proved as well by the values of inhibition distances. The results of a previous study confirmed that the TTO is very efficient against staphylococci, even at low dosages. However, the repeated exposure of TTO at sub-lethal concentrations decreases the efficiency of this product. Therefore, the therapies employing TTO's against stafiloccoci infections can have a low efficiency (McMahon et al, 2007; McMahon et al, 2008).

As far as the strains of *Salmonella enteridis* towards TTO are concerned, one can notice that the sensitivity is noticeable only for the first two concentrations, with inhibition rates of 74.94% (c1) and 68.83% (c2), respectively. Following exposure to c3 and c4, the inhibition rates were way below the threshold of 50 %, fact that lead us to conclude that the efficiency of this product against this bacterium is reduced (Fig. 8).

The inhibition rate towards TTO for the strain of *Streptococcus pyogenes* decreased with the TTO concentration, from a maximum value of 92.48% down to 20.20%. In the same line with the previous examples, it can be noticed that the efficiency of TTO is increasing directly with its concentration. For the standardised strains of *Escherichia coli*, the inhibition rate to TTO varied between 59.90% and 25.20%. At values only slightly above the threshold of 50 % it can be concluded that this strain presents an intermediary sensitivity (Fig. 8).

Enterococcus fecalis is very resistant to TTO. This fact was noticed by analysing the inhibition rate, which at a rate significantly below 50 % indicates a low efficiency following exposure to this product. Similar with the previous example, the strain of *Pseudomonas aeruginosa* was again very resistant following exposure to TTO. The inhibition rates were again significantly below 50 %, indicating a similar low efficiency of this product (Fig. 8).

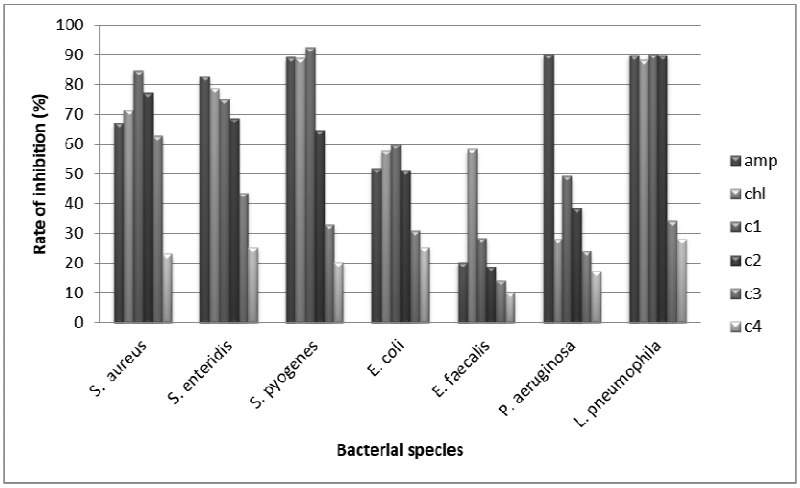

FIG. 8. The antibiotic effect of TTO on bacterial strains

The strain of *Legionella pneumophila* instead proved to very sensitive to TTO, but only for the former two concentrations, by employing inhibition rate of 90.10% and 89.64%, respectively, values which are comparable to those obtained following antibiotic exposure (Fig. 8). The latter two concentrations of TTO, however, proved to be less efficient in stopping the growth of this bacterial strain.

The potential of TTO to inhibit the cell respiration and to increase the microbial wall permeability suggest that its lethal effects are above all the result of metabolic processes inhibation with a localisation in the cell's membrane and by disrupting the chemosynthesis. The reaction of the strains of *E. coli*, *S. aureus* and up to a point for *C. albicans* to TTO can be explained by the amplitude of the effects at cell level as induced by this monoterpene (terpinol 4-ol). However, as *C. albicans* is concerned, the absence of TTO stimulates the efflux of K^+, hence a different mechanism at membrane level as compared to other bacteria (Cox *et al*, 2001).

CONCLUSIONS

Based on the inhibition distances, the antibiotic effect of TTO decreased for the used bacterial strains as follows: *Salmonella enteritidis* > *Staphylococcus aureus* > *Legionella pneumophila* > *Streptococcus pyogenes* > *Escherichia coli* > *Enterococcus faecalis* > *Pseudomonas aeruginosa*. The values of their inhibition rates following exposure to TTO confirms the results obtained from inhibition distances. These results proved the antibiotic properties of TTO and underlined its applicability for other pathogens.

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