Journal of
Complementary Medicine & Alternative Healthcare

Juniper Publishers
key to the Researchers

Short Communication
Volume 2 Issue 1 -March 2017
DOI: 10.19080/JCMAH.2017.02.555577

J Complement Med Alt Healthcare

Essential Oils in the Environmental Bacteria Control: The *Legionella Pneumophilacase*

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Submission: February 03, 2017; **Published:** March 31, 2017

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Abstract

Antimicrobial resistance is nowadays one of the major problems afflicting public health. In this context, essential oils (EO) have been widely investigated for their antibacterial activity, and due to their high volatility they may represent a valuable support in countering the environmental spread of pathogens microorganisms. Among the most worrying widespread-environment bacteria, *Legionella pneumophila* represent the causing agent of a serious and sometimes lethal type of pneumonia, and of the Pontiac Fever, an influenza-like illness. In spite of this, until now the anti-legionella activity of EO has been relatively poorly investigated. The purpose of this work was to characterize by GC-MS and to test by the disk-diffusion method the anti-*Legionella p.* activities of n = 48 EO. The obtained results show that a good number of EO (n = 6) provided inhibition-zones superior than 17.6 mm, while for n=13 it was higher than 22.6 mm. Although it was difficult to find a direct correlation among the constituents and the anti-Legionella activity of the tested EO, it was possible underlying that all the EO bearing phenolics or aromatic compounds shown the maximal anti-microbial activity, as well as the EO containing α-, β-pinene and D-limonene. Although the synergistic or agonistic contribution of the less abundant components to the predominant compounds should be further investigated, this study provides a clear evidence that the aero-dispersed essential oils may represent an additional level of health surveillance to counteract legionnaires' disease.

Keywords: Legionellosis, Essential oil, Antimicrobial activity, Disk-diffusion

Introduction

It is well known that antimicrobial resistance represents today a major public health problem, and aside the minimization of the unnecessary prescribing or over prescribing of antibiotics, the investigation of complementary and appropriate infection control procedures represents an interesting therapeutic opportunity. Among the alternative strategies to those involving conventional sanitizing agents, the use of essential oils (EO) represents an experimental frontier in terms of safety, efficacy and patient's compliance to antimicrobial treatments. The EO antimicrobial capacity have been extensively evaluated by several *in vitro* studies, which established their inhibitory activity against pathogenic bacteria, fungi, molds and yeasts [1].

Among the most concerning environmental pathogenic bacteria, *Legionella pneumophila* (Aerobic, Gram-) is the causative agent of important clinical syndromes in human beings: a pneumonia called *Legionnaires*' disease and a self-limited flu-like illness named Pontiac fever. Legionella is ubiquitous in freshwater environments where it survives as intracellular parasite of free-living protozoa and it can be associated with biofilms [2]. In Europe, Legionella species are one of the most frequent causative agent of pneumonia cases occurring in community, health care institutions, also requiring admission to intensive care units [3]. Outbreaks of legionellosis have been mostly associated to artificial contaminated environments such as building water distribution systems, cooling towers, spas, and similar. Water distribution systems and aerosol-producing medical devices are the main sources of health care-associated legionellosis.

With increasing awareness of legionellosis associated with spas and bathtubs, their regular clean-up and disinfection become essential.

Although a variety of treatments with natural compounds was evaluated for their antibacterial properties against *L. pneumophila* in addition to superheating and chlorine disinfections, few authors focalized their attention to the antimicrobial efficacy of EO: Chang et al. [4] tested the anti-Legionella activity of *Cryptomeria japonica* and *Cinnamomum osmophloeum* EO, stating a strong antimicrobial activity of Cinnamomum and its main compound, Cinnamaldehyde, while Mondello et al. [5] evaluated the susceptibility of *L. pneumophilia*

to *Melaleuca alternifolia* EO. Chaftar and colleagues investigated the activity of 6 Tunisian plant EO by the microdilution assays, founding a strong antimicrobial activity for *Juniperus phoenicea* and *Thymus vulgaris* EO, while, Dunkic et al. [6] evaluated the sensitivity of *L. pneumophila* to *Satureja cuneifolia* EO.

As a part of our phytochemical investigation, we previously reported the effectiveness of an essential oils blend aero-dispersion in reducing the environmental microbial contamination in a nosocomial health-care house [7].

With this communication we report the evaluataion of the *in vitro* antimicrobial activity of a large panel of GC-MS characterized commercial EO (Esperis S.p.A., Milan) against *L. pneumophila* (ATCC33152), considering that up to now, a so wide-ranging evaluation has not yet been reported in literature.

Conclusion

Table 1: Essential Oil activity against *L. pneumophila* in the disc-diffusion tests.

Essential Oil	Activity*
Abies alba	res
Angelica archangelica	res
Anthemisnobilis L.	res
Artemisia vulgaris	res
Betula alba	res
Citrus bergamia	res
Laurusnobilis	res
Zingiber officinalis	res
Zingiber officinalis	res
Chamomilla matricaria recutita L.	1
Helichrysum italicum	1
Pinus sylvestris	1
Pinus sylvestris	1
Pistacia lentiscus	1
Rosmarinus officinalis	1
Salvia officinalis L.	1
Salvia sclarea	1
Citrus paradisii seeds	1
Foeniculum vulgaris	1
Illicium verum	1
Myrtus communis	1
Ocimum basilicum	1
Origanummajorana	1
Pinus pumilio	1
Piper nigrum	1
Melaleuca viridiflora	1
Melaleuca cajeputii	1
Eucalyptus globulus	1
Carum carvi L.	2
Coriandrum sativum	2
Eugenia caryophillus C.	2
Lavandula angustifolia	2
Lavandula latifolia	2
Melissa officinalis	2
Abies siberica	3
Cinnamomum zelanycum Blume	3
Citrus aurantium bitter, peel oil	3
Citrus aurantium bitter, flos oil	3
Cupressus sempervirens	3
Juniperus communis	3
Lippia citriodora	3
Melaleuca alternifolia	3
Mentha piperita	3
Myristica fragrans	3
Origanum vulgare	3
Satureja hortensis	3
Thymus serpillum	3
Thymus vulgaris	3

*res: resistant;1: 14mm<zone inhibition<17,5; 2: 17,6 mm< zone inhibition<22,5 mm; 3: zone inhibition> 22,6 mm.

As reported in Table 1, a large number of the tested EO were effective in counteracting *L. pneumophila*. Some of these OE displayed predominant constituents, but it was difficult to find a direct correlation between the chemical composition of the GC-MS characterized EO and their anti *L. pneumophila* activities. Nevertheless, taking into account their characterized chemical components, it was possible to make some relevant remarks: according to the results of Chang et al. [4] the most active EO (zone inhibition > 22,6 mm) were those richest in

(i) Phenolic or aromatic compounds (carvacrol, thymol, myristicine, cinnamaldehyde in *Thymus spp., Origanum vulgare, Myristica fragrans, Satureja hortensis* and *Cynnamomun zelanicum* EO); in

(ii) D-limonene (*Citrus aurantium* bitter peel and petitgrain oil, *Melissa officinalis* and *Lippiacitriodora* OE);

(iii) inα- and β-pinene, as demonstrated by the results of Cupressus sempervirens, Juneperus communis and Abies siberica OE. β-linalool, the characteristic constituent of Coriandrum sativum and the *Lavandula spp*. OE, seems to

How to cite this article: Celia H, Andrew F L. Why Cam Should Pay Heed to Potential Transference and Counter-Transference. J Complement Med Alt Healthcare. 2017; 2(1): 555577. DOI: 10.19080/JCMAH.2017.02.555577.

bear a mild antimicrobial activity. A strong activity vs *L. pneumophila* was shown also by the *Melaleuca alternifolia* OE, as shown by Mondello et al. [5] whereas *M. viridiflora* and *M. cajeputii* shown a very small inhibition zone (< 17,5 mm). Otherwise, it seems that the antimicrobial activity of some of the mentioned major compounds could benegatively modulated from the contribution of minor compounds: this is the case of *Citrus bergamia* and *Citrus paradisii* EO (characterized by D-limonene) or of *Abies alba,* Pinus pumilio and Pinus sylvestris EO (characterized by α- and β-pinene), which have shown little or no effect.

As often happens, these interactions between the OE components and their contribution to the EO biological activities, should be further studied, but the presented results clearly indicate for the first time that a large number of OE may represent a useful tool as a contrast agent for the control of legionella.

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DOI: 10.19080/JCMAH.2017.02.555577

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