“Antibacterial Effects of Oregano Essential Oil and its potential Applications”.

Abstract.

Essential oils are commonly used in food industry, due that they possess antioxidative and antimicrobial properties. They are few essential oils that have been used in medicine, due to its potent antibacterial activity against intrahospital pathogens. Oregano essential oil (OEO) have experimentally shown potent antibacterial effect on nosocomial Gram-positive bacteria, therefore it can be very useful in hospital environments, where they are many bacterial pathogens, which are the etiological agents of nosocomial infections and most of them are resistant to several antibiotics. **Objective:** the aim of this study was to determine antimicrobial effect of OEO on most frequent bacterial intrahospital pathogens: MRSA, MRSE comparatively to selected ATCC bacterial reference strains. **Methods.** This experimental study investigates the antibacterial action of oregano (*Origanum vulgare*) essential oil (OEO) on two human pathogens: *Staphylococcus aureus* (SA) and *Staphylococcus epidermidis* (SE). Here, we used OEO against one of the most prominent antibiotic-resistant bacterial strains: methicillin-resistant SA (MRSA *mecA*+ and *mecA*-), methicillin-resistant SE (MRSE *mecA*+) and reference strains: *S. aureus* ATCC 700699, *S. epidermidis* ATCC 359845 and *E. coli* ATCC 25922. Bactericidal effects of the OEO on these bacteria were mainly evaluated using undiluted and four serial dilutions in coconut oil: 1:10, 1:100, 1:200, 1:400. **Results.** OEO, undiluted and 4 serial dilutions showed potent antibacterial activity against all strains tested. In conclusion, this OEO could be used as an alternative in medicine. The ability of OEO to inhibit and kill clinical Multi-Drug-Resistant (MDR): MRSA and MRSE strains, highlights it’s potential for use in the management of drug-resistant MDR infections in hospitals wards.

Introduction.

Essential oils in addition to having aromatic properties they also have outstanding therapeutic properties; therefore, they are widely used in the pharmaceutical, food, perfume industries and more recently in medicine. Oregano EO have been described to have several benefits for human health, it may support gastrointestinal, respiratory, and skin health. These properties were once recognized in ancient Greece where they were often used for treating bacterial infections on the skin or in wounds, and it was also employed to protect food from bacteria. There have been recognized several disinfectant and antimicrobial properties of OEO: antioxidant, antiviral, antibacterial, antifungal, antiparasitic, anti-inflammatory, digestive, emmenagogue and anti-allergenic substance [1,2,3,4]. The oregano EO consisted mainly of oxygenated monoterpenes (20%); corresponded to the phenol compounds: carvacrol and thymol (80%) as the main components. Due to its high concentration of phenols compounds, that OEO possesses disinfectant properties [1,5,6,7]

Antimicrobial resistance is a major health concern worldwide. A narrowing of the antibiotic development pipeline and resurgence in public opinion towards 'natural' therapies have renewed the interest in using essential oils as antimicrobial agents [8,9,10].

There have been several reports of antimicrobial effects of OEO on several human and animal pathogens, mainly Gram-positive and Gram-negative bacteria, such as: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus cereus, Clostridium perfringes, Salmonella typhimurium*, *Klebsiella pneumoniae*, *Shigella sonnei. S. flexneri, Yersinia enterocolitica, Enterobacter cloacae*; *Vibrio sp., Listeria monocytogenes* y *Bacillus subtilis.,* [1,3,5,6]. There are scarce reports on the mechanisms of action of OEO, most of the active antimicrobial compounds have been identified as: thymol and carvacrol components. It seems that the main initial carvacrol action is on the integrity of both layers, cytoplasmic membrane and cell wall, therefore altering cell

permeability, the thymol mode of action is as growth inhibitor [3,5,6] Antimicrobial activity of OEO have also been documented against other microorganisms: virus, molds and parasites [1,3].

Objective: the aim of this study was to determine *in vitro* the potential antimicrobial effect of commercial *Origanum vulgare* EO on most frequent bacterial intrahospital pathogens: MRSA, MRSE comparatively to selected ATCC bacterial reference strains.

Methods.

To perform this study, three clinical staphylococci strains were isolated from hospitalized patients with Nosocomial Infection (NI) and three reference strains were chosen from the American Type Culture Collection "ATCC" (*S. aureus* ATCC 700699, *S. epidermidis* ATCC 359845 and *E. coli* ATCC 25922). All three clinical staphylococci strains (MRSA*mecA+*, MRSA*mecA- and* MRSE*mecA+*) and all bacterial reference strains were characterized in the laboratory by conventional (phenotypic analyses) and by molecular methods (genotypic analyses) by PCR detection of *coa* and *mecA* genes. These clinical strains were isolated in a period since 2010 to 2013 from 3 newborns, with nosocomial infection at the National Institute of Perinatology "INPer" (Instituto Nacional de Perinatología "Dr. Isidro Espinosa de los Reyes", SSA). The isolates came from two different infection sites: blood (2 strains) and membrane conjunctive (1 strain) (Table 1).

Antimicrobial activity of OEO was tested against three clinical staphylococci strains (MRSA*mecA+*, MRSA*mecA- and* MRSE*mecA+*) comparatively to three reference selected strains from the American Type Culture Collection "ATCC" (*S. aureus* ATCC 700699, *S. epidermidis* ATCC 359845 and *E. coli* ATCC 25922). Effects of the OEO on bacteria were

mainly evaluated using undiluted and 4 serial dilutions of the oregano oil in coconut oil diluent (1:10, 1:100, 1:200 and 1:400). The assay was performed with bacterial suspension of each strain adjusted to a 0.5 McFarland Standard (~$1.5x10^8$ Colony Forming Units, "CFU"). This standardized bacterial suspension of each strain tested, clinical and reference, was inoculated in a tube containing undiluted and serial 4 serial dilutions of OEO. The mixture was homogenized for 30-60 seconds on tube Vortex device (Stuart Scientific, CO., LTD., UK.) and the OEO was allowed to act for 15 minutes. Immediately after, an aliquot of each tube mixture was inoculated on a Petri dish and a melted BHI culture media maintaining at 50-55 °C in a water bath was poured on a duplicate dish. These mixtures with melted culture media were homogenized by gentle circle movements to the right and to the left. Petri dishes with solidified media were incubated at 36 $\pm$ 1 °C for 24 hours. CFU were estimated using a Colony Counter (Stuart Scientific, CO., LTD., UK.)

Results.

Phenotypic and genotypic laboratory results of all three clinical staphylococci strains (MRSA*mecA+*, MRSA*mecA- and* MRSE*mecA+*) and all three reference strains (*S. aureus* ATCC 700699, *S. epidermidis* ATCC 359845 and *E. coli* ATCC 25922) are shown in Table 1.

TABLE 1.- PHENOTYPIC AND GENOTYPIC ANALYSIS OF ALL CLINICAL STAPHYLOCOCCI STRAINS AND ALL ATCC REFERENCE STRAINS USED.

HOSPITAL*	ID[1]-CODE	INFECTION SITE	HOSPITAL WARD	Staphylococci Strains	Genotype [3]*coa* [4]*mecA*	Phenotype[5] COT MT Cfx
INPer 550	FEH-RN**	Conjunctive membrane	ICU[2]	Staph. aureus	+ -	+ + -
INper 722	ANA-RN	Blood	ICU	Staph. epidermidis	- +	- - +
INPer 883	URM-RN	Blood	ICU	Staph. aureus	+ +	+ + +
ATCC[6]	-700699	----	----	Staph. aureus	+ +	+ + +
ATCC	-359845	----	----	Staph. epidermidis	- -	- - -
ATCC	-25922	----	----	Escherhia coli	- -	- - -

*INPer= Instituto Nacional de Perinatología "Dr. Isidro Espinosa de los Reyes, SSA ". **RN= newborn. [1]ID= Identification. [2]ICU= Intensive Care Unit. Genotype: [3]*coa* = coagulase gene; [4]*mecA* = meticillin resistance gene. [6]ATCC= American Type Culture Collection. Biochemical Tests[5]: COT = coagulase; MT = mannitol fermentation; Cfx = cefoxitin resistance (30 µg- disk)

All positive control Petri dishes without OEO were shown countless CFUs (Table 2). Undiluted and serial dilutions (1:10, 1:100, 1:200 and 1:400) were shown no bacterial growth at all (Table 1). All bacterial strains, clinical and reference ATCC, were grown in the absence of OEO. All bacterial strains, clinical and reference ATCC, were inhibited in the presence of undiluted and 4 serial dilutions of OEO. The OEO showed an outstanding bactericidal effect (higher than a 1:400 dilution) against a wide spectrum of microorganisms, such as Gram-positive (*S.aureus, S. epidermidis,* MRSA and MRSE) and Gram-negative (E. coli) bacteria. All clinical bacterial strains (S. epidermidis *mecA*+, S, aureus *mecA*+ and *mecA*-) were completely inhibited by serial dilutions of OEO, since 1:10 until 1: 400**.** All ATCC reference bacterial strains (*S. aureus* # 700699, *S. epidermidis* # 359845 and *E. coli* # 25922) were completely inhibited by serial dilutions of OEO, since 1:10 until 1: 400**.** Further experiments are needed to precisely determine the ending point of bactericidal activity (>1:400 dilution).

TABLE 2.- ANTIMICROBIAL EFFECT OF OREGANO ESSENTIAL OIL ON CLINICAL AND REFERENCE BACTERIAL STRAINS.

BACTERIAL STRAIN	**SERIAL DILUTIONS**				
	UNDILUTED CONTROL +	**1:10**	**1:100**	**1:200**	**1:400**
S. aureus ATCC 700699	Countless UFC*	NO-growth	NO-growth	NO-growth	NO-growth
S. epidermidis ATCC 359845	Countless UFC*	NO-growth	NO-growth	NO-growth	NO-growth
E. coli ATCC 25922	Countless UFC*	NO-growth	NO-growth	NO-growth	NO-growth
INPer+ 550 *S. aureus mecA* (-)	Countless UFC*	NO-growth	NO-growth	NO-growth	NO-growth
INPer+722 *S.epidermidis mecA* (+)	Countless UFC*	NO-growth	NO-growth	NO-growth	NO-growth
INPer+ 883 *S. aureus mecA* (+)	Countless UFC*	NO-growth	NO-growth	NO-growth	NO-growth

UFC*= Colony Forming Units
BHI**= Brain Heart Infusion Media
INPer+ = Perinatology National Institute

Discussion.

There are several new compounds from natural products, such as OEO, to which antimicrobial activities so far have been detected [2]. There are many scientific reports on antimicrobial effects of essential oils (EOs) on diverse bacterial species, but scarce reports specifically on MRSA and MRSE strains [3,5,6,11]. *Staphylococcus aureus* and *S. epidermidis,* specifically MRSA and MRSE strains are the most frequently strains isolated from nosocomial infections worldwide [4,9]. Biofilm formation is one of the pathogenic mechanisms involved in medical device-related infections and is also responsible for antimicrobial resistance [6,8,12,13]. There are some scientific reports that show that clinical MRSE*mecA*+ and MRSA*mecA*+ strains carry a higher number of resistance determinants in

biofilm-producer strains [8,13,14]. Some EOs have shown antibacterial activity against gram-positive bacteria that cause community or nosocomial infection, and some of these EOs have been tested for skin antisepsis. Even more when an EO (Melaleuca alternifolia) is combined with an antimicrobial agent (tobramycin), there is a synergistic effect against multi-drug-resistant *S. aureus* [15]. Therefore, here we confirmed antibacterial activity of OEO against two specifically clinical staphylococci strains, having methicillin resistance genes (*S. epidermidis mecA*+, *S, aureus mecA*+ and *mecA*-), further more we comparatively demonstrated antibacterial effect of OEO to three ATTC reference bacterial strains (*S. aureus* # 700699, *S. epidermidis* # 359845 and *E. coli* # 25922). The antibacterial outstanding effect of OEO against these nosocomial bacterial pathogens seems very potent (>1:400). EOs in combinations can produce additive antimicrobial activity, and EOs in combination with other antimicrobials can improve antimicrobial effectiveness. Bacterial resistance to antiseptic solutions has increased globally. Recent studies have shown the activity of EOs as penetration enhancers for antiseptics and as restorers of antimicrobial activity against resistant species [2,16], The activity of EOs as penetration enhancers for antiseptics could be applied to prevent infections that are related to surgery and medical devices and to restore antimicrobial activity against resistant species [10,16,17]. EOs represent a source of natural antimicrobial substances and have the potential to be used in the hospital, in specific emergency wards such as intensive care units as a preservative to prevent bacterial growth [2,16,17].

EOs also possess bioactive properties with antibacterial activity that could be used directly in for cleaning purposes. Natural antimicrobials could be used alone or in combination with other preservation technologies [18]. Many EOs have the ability to reduce bacterial numbers, as the majority of EOs tested exhibit a considerable inhibitory capacity against pathogenic microorganisms [19].

Conclusions.

The analyzed OEO showed strong activity on the bacterial growth of all tested reference and clinical strains of Gram-positive *Staphylococcus* spp. and Gram-negative *Escherichia coli.*

There are several useful biological properties shown by EOs, since potent antimicrobial activities, synergistic, antiseptic and bioactive activities, that could be used alone or in combination with other antibiotics or chemical agents, to inhibit pathogenic microorganisms and several resistant species in several hospital wards.

EOs could also be evaluated in combination with disinfectants on contaminated surfaces.

It is likely that it will be more difficult for bacteria to develop resistance to the multi-component EOs than to common antibiotics that are often composed of only a single molecule.

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