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Comparative study on disinfection efficacy of *Thymus Vulgaris* and *Aloe Vera* extracts with commercial disinfectants, on bacteria isolated in nosocomial environment

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Abstract

Health concerns, environmental issues, resistance development of microbes and financial constraints drive hygienists to explore alternative disinfection methods to the commonly used, in order to address these issues. One possible solution may be the utilization towards that direction of materials used traditionally in food industry such as plants and herbs, directly consumed or used to flavour foods. *Thymus Vulgaris*, a plant with substantial antimicrobial activity, and *Aloe Vera*, a plant with great therapeutic capabilities, are examined in this study for their potential to be the main substance of new disinfection products, intended to be used in nosocomial environments. The extracts, obtained by hydrodistillation (thyme) and ethanol solution (Aloe), were evaluated through antimicrobial screening of their efficacy in comparison with commercial disinfectants, widely used in health care units. Their efficacy was tested against bacteria isolated from hospital environment, responsible for the half of nosocomial infections worldwide namely: *Methicillin Resistant Staphylococcus Aureus*, *Staphylococcus Aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*, diluted in deionized water and in reconstituted skim milk. The sensitivity evaluation was performed by broth dilution followed by viable count of the bacteria population after being subjected to different concentrations of the disinfectants with and without the presence of organic matter (skimmed milk). Bacteria were enumerated at time 0, 2, 5 and 10 minutes. Bacterial numbers were expressed as \log_{10} CFU ml⁻¹ and the log reduction was calculated. In the tested concentrations of the extracts promising results were obtained from the samples diluted in deionized water, especially from the *Thymus* extract. More than 2 log reduction was achieved by the thyme essential oil on four out of six tested bacteria populations from the 5.0 ml/L dilution. Similar but lower counts were obtained from the same dilution of ethanol extract of *Aloe Vera* ($1 < DR < 2$). However, in the presence of organic matter their antibacterial activity was greatly inhibited giving less than 1 log reduction for both extracts. Overall, comparing the tested commercial disinfectants with the natural extracts, the latter presented lower disinfection activity, which was expected taking into account the tested concentrations and their chemical complexity. These preliminary results showed that both extracts have the potential to be used as disinfectants and further studies should be conducted in higher concentrations in order to achieve 5 log reduction.

Keywords: Disinfection, agents, hospital, plant extract, Thymus Vulgaris, Aloe Vera, bacteria.

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1. Framework of analysis

1.1 Introduction

The past two decades numerous in vitro studies have been performed exploring the antimicrobial abilities of several medicinal plant extracts. Thyme's medicinal properties have attracted the scientific attention from the beginning, while *Aloe* plants have been found under the research scope only recently. The antimicrobial and disinfection capabilities has been documented in vitro for a large range of microorganisms, concerning thyme, and for a much smaller range, concerning *Aloe Vera*, providing with promising results in both cases. These results along with a variety of issues drive hygienists to explore the use of natural occurring disinfection agents in order to counter the drawbacks coming by the use of commercial and industrially synthesized disinfectants, widely used in health care units. Development of bacterial resistance to disinfection agents, environmental issues, health concerns for the users and the patients and the costly alternatives are the motivation factors towards the development of a range of disinfection products having as primary components plant extracts commonly used as food flavoring agents and in the food industry in general (Burt, 2004; Nychas, 1995; Abreu et al., 2013). Further the driving forces of innovation dictates to explore alternative materials to provide alternative solutions serving in a non-traditional way the growth of food-agricultural industry. Thus, the utilization of food materials to be used in alternative ways could serve that purpose, as the finding of new uses to old technologies take place for many years now into the car industry, for example (Trott, 2009).

Thymus V. antimicrobial abilities are mainly attributable to the presence of phenolic components in its essential oil (Burt, 2004). Thymol, carvacrol, linalool, p-cymene and γ -terpene are the main constituents of thyme's oil responsible for its disinfection potential through a variety of inhibition and killing mechanisms, which target on multiple sites the bacterial cell (Burt, 2004; Xu et al., 2008; Kačániová et al., 2012; Fong et al., 2011; Juven et al., 1994; Dorman and Deans, 2000; Alves-Silva et al., 2013; Lambert et al., 2001; Imelouane et al., 2009; Ballester-Costa et al., 2013). *Aloe Vera* antimicrobial effect is due to its main gel components which are anthraquinones, phenols and terpenoids, targeting mostly the bacterial cell wall and membrane (Pareek et al., 2013; Lawrence, Tripathi & Jeyakumar, 2009; Lu et al., 2011; Carol et al., 1996; Bhardwaj, Ballal & Velmurugan, 2012). However, their effectiveness is subjected to a number of factors such as temperature, pH and organic matter, with the later to be the most inhibitory factor for their disinfection potential (Juven et al., 1994; Burt, 2004).

Despite, though, the large amount of data gathered for each plant's antimicrobial potential, little is known about their efficacy on actual disinfection scenarios in nosocomial environments. This is due to the fact that the research around that matter was confined in in-vitro studies without simulating the variables of a hospital disinfection procedure. Based on the literature review conducted during this study, the plant extracts have not undergone evaluation of their disinfection potential in comparison with commercial products, especially when organic matter is present.

1.2 Aims and objectives

The purpose of this study is to evaluate the disinfection ability of two plant extracts, grown in Crete, from *Thymus Vulgaris* (essential oil) and *Aloe Vera* (ethanol extract) in comparison with commercial disinfectants, used in cleaning procedures in nosocomial units. The comparison is taking place in order to explore the potential of these extracts to constitute the primary components in future disinfectant products, intended to be used for the same purposes (non-critical surfaces in hospital environment). For that reason, the experimentation method is the same as the one used for the evaluation of industrially

synthesized disinfectants, while at the same time the materials try to simulate, as accurately as possible in a laboratory environment, the conditions occurring during disinfection of non-critical surfaces in hospitals.

1.3 Structure of the report

The literature review is structured according to a step-wise sequential explanatory mode starting with the general concept of surface disinfection by clarifying the need for it in health care units, food processing environments and expands on the potential beneficial effect on public health that disinfection of fresh foods surface may cause. Next, in an effort to create a frame wherein the different disinfection agents can be presented, the most important definitions that govern the disinfection field are quoted, followed by the major antimicrobial killing or inhibiting mechanisms. These mechanisms are observed during the application of chemical agents on microbes, which are explored and explained in a comprehensive manner. Then, the natural disinfection compounds/substances are analyzed based on literature review, correlating them with the aforementioned mechanisms. After these parts, where the reader gets to know the concept, the chemistry and the biological phenomena of disinfection, follows an argumentation based on scientific evidence found in the literature in order to support and explain the need for natural disinfectants. The literature review is then concluded by presenting the under evaluation plants, their characteristics and their chemical composition along with some data regarding their antimicrobial potential. Then, hopefully, the reader will be able to link together the disinfection principles, mechanisms and substances with the natural extracts of the under examination plants, acquiring all the needed theoretical background to proceed to the later parts of this study.

Concerning the experimentation laboratory work, the method followed was the broth dilution method with bacteria cell viable count, both in vitro and in presence of organic matter.

1.3.1 Limitations and Boundaries

This study will try to explore the disinfection capabilities of two natural extracts in comparison with industrial synthesized disinfectant agents in liquid form, with low selectivity, used only for inanimate/non-critical surfaces in nosocomial environments, upon bacteria species isolated from the same environment. Thus, a possible disinfectant product from these natural sources will be intended to be used for the same purpose. In addition to this potential use, other possible applications are mentioned, in order to argue, mostly, for the need of disinfection products from natural sources. These applications concern the decontamination of fresh or minimally processed foods' surfaces, intended to be consumed as they are harvested or cut, and the disinfection of food-contacting surfaces. However, because no food-borne bacteria will be used in the experimentation method, the correlation of natural extracts to the decontamination of food related surfaces will be based on literature findings and upon them the argumentation will be build.

Further, the experimentation methods include bacteria and no other kinds of microbes; hence the antimicrobial mechanisms of disinfection agents, mentioned in this study, will refer mostly to the effects upon bacteria cells. Concerning the conducted literature review of natural antimicrobial compounds, it is limited to the main antimicrobial agents found in nature and, at the same time, in the under study plants. The same way of thinking is applied on the literature review of the industrially synthesized agents, since the reviewed chemical substances are the main active components of the under evaluation commercial disinfectants. It is included, though, two more agents that are used extensively nowadays, which are the peroxide compounds and alcohols.

Concerning the experimentation method, it may not reflect in a high degree the complexity of a hospital scenario, since it was performed on a laboratory scale. However the

method evaluated the disinfectants on isolated bacteria found in nosocomial environment, both in vitro and in the presence of organic matter, in an effort to simulate that scenario as accurately as possible. Further, since the experimentation method was performed once, no statistical analysis of data took place. The obtained results from the plants' extracts were compared with the results of commercial disinfectants and the efficacy benchmark performance that EOF (the Greek drug authority) demand from a disinfectant in order to be approved. Based on that, the relative conclusions were extracted. Finally, the tested concentrations of natural extracts were limited to only 3 (1.0, 2.5, 5.0 ml/L) due to lack of financial resources.

1.3.2 Definitions & Terms

Disinfection - the process of killing pathogenic organisms or rendering them inert (Mosby's Medical Dictionary, 2013). More analytical, there are five elements in the definition of disinfection (1) removes infection, (2) kills, not just inhibits, microorganisms in the negative stage, (3) does not necessarily kills spores, (4) is ordinarily a chemical but it can be a physical agent and (5) is used only in inanimate objects, not on the human or animal body (Block, 2000).

Decontamination –the freeing of a person or object of some contaminating substance, e.g., war gas, radioactive material, chemicals, micro-organisms etc (Mosby's Medical Dictionary, 2013)

Disinfectant – A disinfectant is an agent that frees from infection usually a chemical agent but sometimes may be a physical one such as x rays or ultraviolet light that destroys disease-carrying or other harmful microorganisms but may not kill bacteria spores. It refers to substances applied to inanimate objects. (Block, 2000)

Commercial disinfectants – in this study are referred so the commonly used disinfection agents in nosocomial institutions.

Antimicrobial agents – are the physical (e.g. radiation) or chemical agents (both natural and industrial) that present a bacteriostatic or bactericidal activity.

Chemical agents – are regarded any industrial or natural agent that has antimicrobial activity

Natural disinfectants or natural disinfection agents – are the disinfection agents occurring from plants only, through any extraction process. This term is used where this distinction is necessary.

Industrially synthesized disinfection agents – are the chemical agents used for surface disinfection that are produced through chemicals reactions occurring in industrial environment. Examples are; Acids and esters, alcohols, aldehydes and aldehyde-releasing agents, halogens (including chlorine-releasing agents), metals, phenols and cresols, quaternary ammonium compounds and biguanides. Some of them, such as phenols, that occur, also, in nature and exist in natural disinfectants will be distinguished when any mention to them take place, by indicating that are industrially synthesized. When they are simply mentioned as “phenols” they should be comprehended by the reader as the natural occurring ones. This term is used where this distinction is necessary. (Russell, 2000)

Bacteriostatic – is an agent capable of inhibiting the growth or reproduction of bacteria or spores correspondingly (Segen's Medical Dictionary, 1992).

Bactericidal or sporicidal – in an agent that destroys bacteria or bacteria spores correspondingly (Segen's Medical Dictionary, 1992).

2. Theoretical Background

2.1 The benefits of surface disinfection

The effective use of disinfectants constitutes an important tool towards the prevention of hospital-associated infections (Rutala & Weber 2004). According to Breathnach (2005) patients may become infected with new organisms, usually from other patients, or more rarely from staff or the environment. Transient hand carriage by medical staff is thought to be the main route of spread (Breathnach, 2005). Spaulding (1968, cited in Rutala, 2004 p. 226) categorized the germicidal action aiming to prevent a risk of infection associated with the use of equipment or surfaces in hospitals into three categories: (1) noncritical, (2) semicritical, and (3) critical. The environmental nosocomial surfaces, which are the target of the disinfectants examined in this study, are considered noncritical items because they come in contact with intact skin (ibid, 1968). The other two categories refer to surfaces that potentially get in contact with open wounds (surgeries) or open wounds themselves, which need special treatment concerning their disinfection (ibid, 1968).

While noncritical surfaces have not been correlated directly in disease transmission and the discussion around that issue is controversial (Dettenkofera & Spencer; 2007, Hota, 2004), Rutala and Weber (2004) argues that these surfaces contribute to cross-transmission by allowing acquisition of transient hand carriage by medical personnel due to contact with an infected surface or by patient contact with the same surfaces. Thus, these contaminated surfaces may contribute to transmission of epidemiologically important microbes such as *Staphylococcus aureus* and *Acinetobacter* (Hota, 2004). According to Donskey (2013) many researchers have concluded that inanimate surfaces near infected patients usually get contaminated with the aforementioned bacteria and this contamination can lurk for hours up to weeks, depending on the condition of the surface, serving as a reservoir or source of pathogenic microbes in hospitals, but the precise role of the environment in the transmission of diseases has not been fully delineated (Rutala & Weber 2004). Despite that, the Centers for Disease Control and Prevention (CDC) in the United States recommends in their Isolation Guidelines that all non-critical surfaces, which include the bedside equipment and environmental surfaces (e.g., bed rails, bedside tables, carts, commodes, door-knobs, and faucet handles) should be disinfected since are indicated for certain pathogens (Rutala & Weber, 2004). Despite the lack of conclusive studies, CDC's recommendations are based on well-supported empirical data and facts (ibid, 2004). During the past decade, though, a growing number of evidence has accumulated suggesting that improvements in environmental disinfection may prevent transmission of pathogens and reduce health care-associated infections (Donskey, 2013). Albeit the quality of much of the evidence still remains suboptimal, a number of high-quality investigations now support environmental disinfection as a control strategy (Donskey, 2013).

Enumerating some of the microorganisms and the related dangers that are partially prevented/handled through disinfecting surfaces in hospitals, Hota (2004) cited; **(1)** viruses (influenza, parainfluenza, enteric viruses, hepatitis B virus, severe acute respiratory syndrome (SARS)-associated coronavirus), **(2)** fungi (*Candida albicans*, *Candida glabrata*, and *Candida parapsilosis*) and **(3)** bacteria (*Clostridium difficile*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *E. Coli*).

However, disinfection techniques aren't applied only to nosocomial environments in order to safeguard human health. Food industry dedicate a lot of recourses in order to protect consumers from food-borne illnesses, thus it can be easily conceptualized that decontamination of food contacting surfaces is a key step towards the production of safe foods. These food contacting surfaces may be food processing machinery and equipment, quality assurance laboratory environments, packages, etc (Burt, 2004; Valeriano et al., 2011). In food processing environments bacteria and vegetative microbes, pose a significant concern. Contaminated surfaces with spoilage and pathogenic bacteria, and cross-

contaminate food products cause reduced product shelf-life and diseases transmission (Valeriano et al., 2011). The World Health Organization in 2007 summarized some facts about food safety and foodborne illness. Trying to estimate the magnitude of the problem, they stated that despite the difficult to estimate the global incidence of foodborne diseases, it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases. A great proportion of these cases can be attributed to contamination of food and drinking water (WHO, 2007). In addition, in industrialized countries, the percentage of the population suffering from foodborne diseases each year has been reported to be up to 30% (WHO, 2007). In the United States of America (USA), for example, around 76 million cases of foodborne diseases, resulting in 325,000 hospitalizations and 5,000 deaths, are estimated to occur each year (WHO, 2007). Of course the route of this problem is not only the poor or insufficient hygiene and disinfection procedures, but according to many researchers and WHO (2007), the cleaning and sanitizing of food processing environments can greatly contribute to the decrease of the previously mentioned statistical numbers (Burt, 2004; Negi, 2012; WHO, 2007).

In addition, recently scientists explore another possible application for surface disinfection techniques in the field of food sector. By applying disinfectants on the surface of fresh or minimally processed foods, such as vegetables, fruits and meat or fish cuts, achieve to inhibit microbial growth, reducing the microbial load or eliminate it completely. That way the cases of foodborne illnesses are reduced, increasing at the same time the preservation time (Burt, 2004; Negi, 2012).

2.1.1 Disinfection of surfaces

Disinfection of a surface occurs after the use of disinfectants which are chemical agents intended to be used on inanimate objects to inactivate/kill all pathogenic microorganisms (McDonnell & Russell, 1999, Block, 2000). Unlike antibiotics, which are chemotherapeutic drugs used, mostly, internally to control infections and which interact with specific structures or metabolic processes in microbial cells, disinfectants act non-specifically (low or non-selectively) against multiple targets (Bridier et al., 2014). The mode of action of disinfectants depends on the type of biocide employed, as has been extensively described in numerous reviews (McDonnell and Russell 1999; Bridier et al., 2014; Russell, 2000). Potential target sites in Gram-positive or Gram-negative bacteria are the cell wall or outer membrane, the cytoplasmic membrane, functional and structural proteins, DNA, RNA and other cell components (Bridier et al., 2014; Russell, 2000). Disinfection treatments are used in nosocomial, industrial, domestic or food processing environments to control the contamination of surfaces from microorganisms (Bridier et al., 2014; Tornuk et al., 2011; Papazoglou et al., 2012; Alsaimary & Mezaal, 2008; Nychas, 1995). Although these disinfection treatments kill most surface microorganisms, some may survive and cause substantial problems in terms of public health (Bridier et al., 2014).

2.2 Disinfection mechanisms

Antimicrobial agents may be of several different types either physical or chemical, as mentioned before, or, sometimes, a combination of the two. The response of microorganisms to adverse agents depends on the microorganism's type, the agent's nature and the intensity (e.g. concentration of a chemical, temperature of exposure) and duration of exposure of the cells (Russell, 2000). In this study only chemical agents and their disinfection mechanism are focused.

The different chemical agents achieve their lethal or inhibitory antimicrobial effect targeting different cellular parts of microorganisms and they can be grouped according to that precise targeting mechanism, as quoted in table 1.

Table 1: Cellular Targets of Antimicrobial Action (adopted by Russell, 2000)

Target	Agents	Effect
Cell wall	Enzymes, flavonoids*, tannins*	Inhibition of cell wall Attacks peptidoglycan
	Aldehydes	Interaction with –NH ₂ groups
	Anthraquinones*	Peptidase liberates N-terminal glycine and alanine
	Phenols **, Anionic surfactants, terpenoids*, PAA	High concentration: lysis
	Saccharides*	Osmotic effect leading to cell bursting
Outer membrane	EDTA,	Chelates cations, induces release of up to 50% of lipopolysacchride of outer membrane
	Polycations, e.g. polylysine, alkaloids* terpenoids*	Displace cations diffusion of nutrients through the cell membrane
Cytoplasmic membrane	Moist heat, phenols**, quaternary ammonium compounds, biguanides, parabens, hexachlorophene, terpenoids*	Leakage of low-molecular-weight material
Proteins	Quinones*, tannins*, alcohols**, iodophors	Affect protein synthesis in different ways Inactivation of proteins and loss of function
Nucleic acids	Dyes, alkylating agents, alkaloids*, iodophors, ionizing and ultraviolet radiations	Possible binding of agents to nucleic acids
Enzymes Or proteins	Simple phenols**, metal ions, chlorine	Reaction with sulfhydryl groups
	Alkylating agents, oxidizing agents, alkaloids*, Hydrogen Peroxide, PAA	May combine with or destroy DNA or RNA

(*) with asterisk are marked the agents found in plants' extracts

(**) with two asterisks are marked the agents found in plants' extract and are industrially synthesized.

2.2.1 Targeting bacteria cell wall synthesis

The bacterial cell wall synthesis is a complicate mechanism that is described thoroughly in Russell's chapter in Block's book in 2000. Here some very general elements of this mechanism are described in brief, in order to have a small insight of the antimicrobial activity of chemical agents upon bacteria cell wall.

The basic unit of the bacterial cell wall is composed of peptidoglycan, which confers mechanical rigidity on the cell and protects the delicate underline cytoplasmic membrane (Russell, 2000). In gram-positive bacteria, the wall consists of a thick layer of peptidoglycan interspersed with an acidic polymer, usually teichoic acid and in gram-negative bacteria that layer is much thinner (ibid, 2000).

Some of the antimicrobial agents that affect bacteria cell wall inhibit the synthesis of the aforementioned peptidoglycan or attack it damaging the mechanical structure of the wall. Other causing autolytic cell wall degradation after interacting with other components of the cell wall and finally, other agents induce lysis through osmotic phenomenon (Russell, 2000).

2.2.2 Membrane-active antimicrobial agents

A range of diverse chemical agents has been shown to attack the cytoplasmic membrane. Some of these affect both fungi and bacteria, whereas others have a selective action against yeasts and fungi (Russell, 2000). The main substances of the antimicrobial agents that disrupt the cytoplasmic membrane cause the leakage of intracellular materials, although other effects have also been reported. Phenols (both industrial and natural) and chlorhexidine, in low concentrations, have been found to cause cell lysis; polymixin promotes leakage of cytoplasmic proteins and other agents inhibit the synthesis of ATP or hydrolyzing it causing the structural damage of the membrane (Russell, 2000).

2.2.3 Inhibition of protein synthesis

Protein synthesis arises from polypeptide chains formed by amino acids linked together by peptide bonds. Synthesis occurs in the ribosomes (the protein “workshop” of the cell), made up of rRNA and protein. Each bacterial ribosome is assembled from two subunits: the smaller particle which contains RNA and 20 proteins, and the larger particle which contain RNA and approximately 34 proteins (Rapoport, 2007, Russell, 2000).

The antimicrobial agents targeting the protein synthesis activity of bacteria are divided into three groups according to disruption that cause to a specific point of the mentioned above synthesis procedure (Rapoport, 2007). Hence (and without further explanation of the mechanism, for more details see Block’s book “Disinfection, Sterilization and Preservation”), there are the agents that (1) inhibit the smaller’s ribosome’s subunit functions, (2) the agents that inhibit the larger ribosome’s subunit functions and (3) the agents that inhibit the translocation process that occurs during proteins elongation (Russell, 2000).

2.2.4 Agents acting on nucleic acids

Antimicrobial agents acting on nucleic acid (DNA and/or RNA) can do so by different mechanisms. These mechanisms include inhibition of the DNA synthesis, blocking of DNA transcription by RNA polymerase, inhibition of RNA synthesis, inhibition of DNA gyrase and, although of little clinical importance, alkylation, which is the biologic activity of alkylating agents reacting with nucleophilic groups (Russell, 2000).

2.3 Microbial resistance to chemical agents

In order to fully outline the complex mechanisms of antimicrobial activity of chemical agents, it is needed to talk about microbial resistance. Microorganisms are characterized as resistant when they are not killed by a disinfectant at a concentration used in practice, when they are not killed by a concentration of a chemical agent that kills the majority of cells in the culture, or when a strain is not killed by an agent that kills similar strains at a specific concentration (Russell, 2000). The main resistance mechanisms are discussed below.

2.3.1 Transferable resistance

Transferable resistance occurs when bacteria, which are exposed to agents, survive, creating mutations that confer drug/agent-resistant determinants into the bacteria DNA (Russell, 2000; Huang & Eells, 2011). This genetic material can be transferred from one bacteria to another in three ways: (1) by transduction, in which a transducing phage might “pick-up” a stretch of DNA containing a drug-resistant determinant and transfer this to another cell; (2) by transformation, in which DNA extracted from the cells of one strain may be absorbed by a second strain; or (3) by conjugation, in which cell-to-cell contact is necessary (Russell, 2000).

2.3.2 Inactivation of antimicrobial agents

Proteins, lipids, salts, pH, oxygen presence and temperature are factors which have the potential to affect the antimicrobial activity, both positively and negatively (Russell, 2000; Skandamis & Tassou, 2003; Gomez-Lopez, 2012). The inactivation mechanisms are more thoroughly investigated in paragraph 2.4.6

2.3.3 Permeability barriers

Permeability barriers concern the antimicrobial activity of antibiotics, but in the case of some gram-negative bacteria, could affect the efficiency of other chemical agent such as phenols and quaternary ammonium compound (Russell, 2000). This is due to the composition of the outer membrane of gram-negative bacteria, which is composed by lipopolysaccharide, proteins and lipid. It is believed that the presence of lipid in the cell is

related to the fact that gram-negative bacteria are more resistant than the gram-positive to antibacterial agents (Russell, 2000).

2.3.4 Bacterial biofilms

The process of bacteria adhesion is initiated by the binding of them with the surface by means of exopolysaccharide glycocalyx polymers. Then, the bacteria cells are divided within the glycocalyx matrix that bound them together (Russell, 2000). The development of adherent microcolonies leads eventually to the production of a continuous biofilm on the colonized surface. Bacteria that form these films are tent to be much more resistant to chemical antimicrobial agents than bacteria in batch-type cultures. There are two possible reasons for that: (1) physiologic changes in the cells and (2) penetration barriers presented by the formed matrix (Russell, 2000).

2.4 Natural antimicrobials from plants

Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness, founding literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* (Ciocan & Bara, 2007). The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s (Dorman & Deans, 1999). These antimicrobial properties of plants' chemical constituents have been assessed and reviewed extensively, concerning a wide variety of plants (Nychas, 1995; Ceylan & Fung, 2004; Ballester-Costa et al., 2013; Dorman & Deans, 1999). Some of the researchers refer to them in total, as volatile oils or more frequently, as secondary metabolites (Nychas, 1995; Dorman & Deans, 1999) or phytochemicals (Saviova, 2012).

Plant volatile oils are generally isolated from non-woody plant material by distillation methods and are variable mixtures of terpenoids and a variety of low molecular weight aliphatic hydrocarbons (such as natural phenols), acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally nitrogen- and sulphur-containing compounds, coumarins and homologues of phenylpropanoids (Dorman & Deans, 1999; Nychas 1995). These secondary metabolites just mentioned, have potential in medical procedures, applications in cosmetics, food preservation (both as product ingredient and as a food-surface disinfectant) and pharmaceutical industries (Tornuk et al., 2011; Papazoglou et al., 2012; Dorman & Deans, 1999; Nychas 1995). More specifically and related to this study, these plants' chemical constituents can potentially disinfect hard surfaces in nosocomial institutes, food industries and protect livestock and food from diseases, pests and spoilage (Abreu et al., 2013; Alsaimary & Mezaal, 2008; Dorman & Deans, 1999; Nychas, 1995; Brut, 2003). This can be conceptualized much easier when someone study their antimicrobial efficiency evaluation, which reveal the wide range of micro-organisms they act against, which will be analyzed more in depth in later parts of this study (Abreu et al., 2013; Dorman & Deans, 1999).

2.4.1 Phenolics and Polyphenols

Phenolic compounds are found widely in plants and in every plant part, where they have as primary goal to protect them from microbial infections (Savoia, 2012). They, also, have potential anti-oxidative properties along with anti-infective (Saleem et al., 2010). They are a large group of aromatic compounds, consisting of flavones, flavanoids and flavanols, quinones, tannins, polymeric phenolic substances and coumarins (Cowan, 1999; Negi, 2012).

2.4.1.1 Simple phenols and phenolic acids

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring (Cowan, 1999). Examples of that are the cinnamic and caffeic acids, common representatives of a wide group of phenylpropane-derived compounds found in plants (ibid, 1999). Common herbs, as it is thyme, contain caffeic acid, which inhibit the growth and

spread of viruses (Savioa, 2012), bacteria (Negi, 2012; Cowan, 1999; Papazoglou et al., 2012; Dorman & Deans, 1999; Nychas 1995), and fungi (Duke, 1985; Burt, 2004; Nychas, 1995)

From chemical point of view, it is thought that the phenols' relative toxicity to microorganisms is related to the site(s) and number of hydroxyl groups on the phenol group, since there are evidence showing that increased hydroxylation result in increased microbe-toxicity (Cowan, 1999; Nychas, 1995; Nychas, Skandamis & Tassou, 2003). The simple phenols called "catechol" and "pyrogallol", which both are hydroxylated phenols, are characteristic examples, which were observed to be toxic to microorganisms. Catechol has two -OH groups, and pyrogallol has three. In addition, other scientific evidence shows that more highly oxidized phenols are more inhibitory against microbes (Scalbert, 1991).

Considering the mechanisms responsible for phenolic toxicity to microorganisms, it is suspected that include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Cowan, 1999; Nychas, 1995 & 2003).

2.4.1.2 *Quinones.*

Quinones are aromatic rings with two ketone (organic compound with the structure $RC(=O)R'$) substitutions, found very commonly in nature (Cowan, 1999; Savioa, 2012). These compounds are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (De Ancos, 2006; Cowan, 1999)

Concerning the antimicrobial mechanism, quinones inactivate proteins leading to loss of their function (Cowan, 1999). More complex quinone derivatives are the anthraquinones, found in aloe plants, which have shown wide antimicrobial activity against both Gram-positive and Gram-negative bacteria (Hamman, 2008). Probable targets in the microbial cell are surface-exposed adhesins (biofilms), cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism (Cowan, 1999; Savioa, 2012).

2.4.1.3 *Flavones, flavonoids, and flavonols*

Flavones are phenolic structures containing one carbonyl group and the addition of a 3-hydroxyl group produce a flavonol (Cazarolli, 2008; Savioa, 2012). Flavonoids are also hydroxylated phenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring (Cazarolli et al., 2008). Since these phenolic substances are known to be synthesized by plants in order to encounter microbial infections (Kurek et al., 2011), it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a variety of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Cazarolli et al., 2008; Kurek et al., 2011; Nychas, 1995; Nychas, Skandamis & Tassou, 2003), without these antimicrobial mechanisms been conclusively proved (Cushnie & Lamb, 2011). More lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996; Cowan, 1999).

According to Cowan (1999), Savioa (2012) and Ciocan & Bara (2007) flavonoid compounds, worth mentioning, are catechins. These flavonoid compounds have been extensively researched due to their occurrence in oolong teas, presenting significant antimicrobial activity, placing them in the center of scientific research since 1980ies.

However, there are some conflicting findings as result of scientific research. Cushnie and Lamb (2011) in their article state that some scholars found that flavonoids lacking hydroxyl groups are more active against microorganisms than are those with the -OH groups. This result supports the notion that the target is the outer cellular membrane. However Sato et al. (1996) found the opposite effect. The more hydroxylation, the greater the antimicrobial activity. Thus, it is safe to say that it cannot be predicted the degree of hydroxylation in relation with the toxicity to microorganisms (Cowan, 1999; Cushnie & Lamb, 2011).

2.4.1.4 *Tannins*

Tannins are a group of polymeric phenolic substances found in almost every plant part characterized by antibacterial activity due to the inactivation of bacterial adhesins (biofilms), enzymes, cell wall and transport proteins (Savioa, 2012; Engels, Schieber & Gänzle, 2011). This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of ills (Cowan, 1999).

Concerning other antimicrobial mechanisms and more recent researches, Engels, Schieber & Gänzle in 2011 found that gallotannin-rich plant extracts presented antimicrobial activity against different bacteria, which can be attributed to their strong affinity for iron and to the inactivation of membrane-bound proteins.

2.4.2 **Terpenoids and Essential Oils**

The fragrance of plants is carried in the essential oil fraction, which is commonly referred to as a total essential oil (EO) (Nychas, 1995; Cowan, 1999). These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure (Cowan, 1999). They are called terpenes and their general chemical structure is $C_{10}H_{16}$. They can be found as component in plants' essential oils as monoterpenes, diterpenes, triterpenes, and tetraterpenes, as well as hemiterpenes and sesquiterpenes (Cowan, 1999; Kurek et al., 2011; Ciocan & Bara, 2007). Examples of common terpenoids are methanol, thymol, linalool and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids) (Cowan, 1999; Nychas 1995). Terpenes or terpenoids are effective against bacteria, fungi and viruses (Nychas, 1995; Nychas, Skandamis & Tassou, 2003; Cowan, 1999; Savioa, 2012; Ciocan & Bara, 2007; Kurek et al., 2011; Termentzi, Fokialakis & Skaltsounis, 2011; Negi, 2012; Dorman & Deans, 1999; Juven et al., 1993; Ceylan & Fung, 2004)

The mechanism of action of terpenes is not fully understood but is speculated to involve disruption of the cellular membrane, inhibition of ATPase activity, and release of intracellular ATP and other constituents of microorganisms (Termentzi, Fokialakis & Skaltsounis, 2011; Nychas, Skandamis & Tassou, 2003; Raybaudi-Massilia et al., 2009; Burt, 2004). Concerning more in general essential oils, it is believed that they may have, also, an antimicrobial effect by influencing the diffusion rate of nutrients through the cell membrane (Raybaudi-Massilia et al., 2009). One example of application of an essential oil as a disinfectant is the oil of basil, a commercially available herbal that was found to be as effective as 125 ppm chlorine in disinfecting lettuce leaves (Cowan, 1990). However, essential oils disinfection effectiveness was decreased when experiments were conducted in vivo and more specifically on meat or similar samples (Mondello et al., 2003; Dorman & Deans, 1999; Nychas, 1995).

But the essential oils (EOs) obtained from plant materials by distillation are not constituted only by terpenoids. EOs contains a mixture of compounds, which includes terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters, they are mainly used as food flavorings, functional components in pharmaceuticals or antimicrobial compounds and are classified as GRAS (generally recognized as safe) substances (Burt, 2004; Nychas et al., 2003; Lambert et al., 2001). Their complex chemical concentration leads to a range of antimicrobial mechanisms as shown in Figure 1.

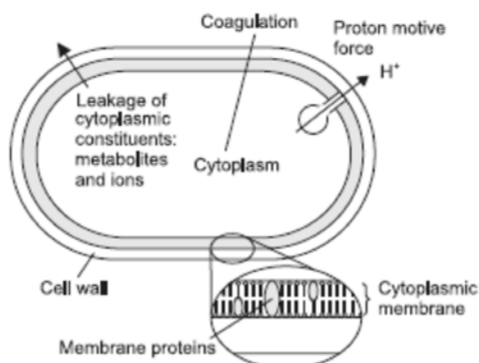


Fig. 1: Locations and mechanisms in the bacterial cell thought to be sites of action for EO components: degradation of the cell wall, damage to cytoplasmic membrane, damage to membrane proteins, leakage of cell contents, coagulation of cytoplasm and depletion of the proton motive force. (Adopted from Burt, 2004)

2.4.3 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. Several researchers have observed the antimicrobial activity of alkaloids found in plant or herb extracts (Savioa, 2012). Their activity is depended upon the chemical composition of the extracts and membrane permeability of the microbes (Medeiros et al., 2011). Diterpene alkaloids, commonly isolated from the plants of the Ranunculaceae group, have also antimicrobial properties (Rahman & Choudhary, 2011). For example, berberine, which is present in roots and stem-bark of *Berberis* species, is a hydrophobic cation widely used in traditional medicine due to its activity against bacteria, fungi, protozoa and viruses (Savioa, 2012). The antimicrobial mechanism of alkaloids can be summarized to their ability to accumulate in cells through the outer membrane and to the fact that they are excellent DNA intercalators because they target on RNA polymerase, gyrase and topoisomerase IV and on nucleic acid (Yi, Yu, Liang, Zeng, 2007; Iwasa et al., 2001).

2.4.4 Other Compounds

Many phytochemicals not mentioned above have been found to exert antimicrobial properties, but this study focus on reports of chemicals which are found in the essential oils or extracts of *Thymus Vulgaris* and *Aloe Vera* and are active in multiple instances against microbes and especially against bacteria. However, based on Cowan's (1999) review upon that matter It should be mentioned, that there are studies revealing the antimicrobial properties of other naturally occurring antimicrobial agents such as polyamines (in particular spermidine), isothiocyanates, thiosulfonates, saccharides and glucosides.

2.4.5 Factors affecting microbial action of natural antimicrobials

Proteins, lipids, salts, pH, oxygen presence and temperature are factors which have the potential to affect the antimicrobial activity of phenolics (Juven et al., 1993, Nychas, 1995; Nychas, Skandamis & Tassou, 2003). For example Juven et al. found that the total phenolic content in thyme essential oil had enhanced antimicrobial effect against *Staphylococcus aureus* and *Staphylococcus typhimuriurn* in anaerobic conditions and remained stable during long-term storage. According to the same researchers, the reason for the stronger antibacterial effect of thyme oil observed under anaerobic conditions might be related to the lower energy yields of bacterial metabolism under lower oxygen tensions and consequent increased sensitivity of the microorganisms to the oil toxicity.

Other researchers reported that the antimicrobial effects of BHA and TBHQ, which are phenolic compounds often added to foods to preserve fats, were influenced by the presence of different amounts of casein, which is a protein found naturally in milk (Nychas, Skandamis & Tassou, 2003; Board, 1995). An increase in protein content in the growth media influenced negatively the inhibitory effect of BHA and TBHQ against *St. aureus*,

Pseudomonas fluorescens and *Saccharomyces cerevisiae*. The antimicrobial effect of the phenols caused less than one log cycle decrease when the media had 3% protein content (Nychas, 1995). Explaining that inhibition Kostenbauder (2000) states that antimicrobial agents, in general, tend to bind with macromolecules like proteins, carbohydrates and nucleic acids decreasing the availability of the agent.

Concerning the effect of fats, Rico-Munoz and Davidson (1983, cited in Nychas, 1995 p. 76) observed that the effectiveness of the same phenols, as in the previous paragraph, was reduced by small amounts of corn oil in the growth media. Additionally, the effect of electrolytes, such as salt, to the inhibitory effectiveness of antimicrobial agents is important by effecting both microorganisms and agents (Kostenbauder, 2000). Salt affects the solubility of the aqueous solution of an antimicrobial agent, such as phenol, reducing the solvent's affinity. That increases the phenols' activity constituting them more efficient in a sodium chloride solution (Nychas, 1995; Kostenbauder, 2000).

pH is another factor affecting the microbial action of natural antimicrobials. Extremes of acidity or alkalinity can effectively limit the growth of microorganisms, pH 4.5 to 9 being a limiting range for many microorganisms (Kostenbauder, 2000). Moreover, the activity of antimicrobial agents that occur as different species within the pH range compatible with microbial growth may be profoundly influenced by relatively small changes in the pH of the medium (ibid, 2000). However, it is difficult to predict the antimicrobial activity when it comes to phenols. Nychas et al., (2003) reported different inhibition rates of BHA depending on the pH and the tested microorganism. Concerning thyme's oil antibacterial activity the optimum pH range is 5,5, because at low pH values, the thymol molecule is mostly undissociated, more hydrophobic, and may bind better hydrophobically with the membrane proteins and dissolve better in the lipid phase of the bacterial membrane (Juven et al., 1994)

Finally, temperature plays an important role in the activity of agents. The temperature dependency of the antimicrobial activity of an agent represents a complex situation. The observed effects of temperature change include terms for the temperature dependency of the microbe's growth rate and the temperature dependency of the microbe's thermal death rate, as well as the temperature dependency of the antimicrobial properties of the agent (Kostenbauder, 2000). From the above, it can be easily concluded that this factor is difficult to analyze and apply quantitatively, thus information regarding the activation energy that is required by an agent could be useful for practical applications (ibid, 2000). For example, if one wishes to use a disinfectant at a temperature considerably above room temperature, greater efficiency might be achieved by selecting an agent with large energy of activation (Kostenbauder, 2000). That way as the temperature rises, the agent's efficiency will increase (ibid, 2000).

2.5 Alternative disinfectants – preservatives

As "alternative", in terms of disinfectant and preservation methods, is defined as an unconventional or non-traditional disinfection or preservation procedure, technique or mean. Concerning the disinfection of nosocomial non-critical and semi-critical surfaces or equipment the traditionally or commonly used antimicrobial agents are chlorine and its derivatives compounds, iodophors, alcohols, nitrogen compounds such as formaldehyde compounds, peroxide compounds, industrially synthesized phenols, quaternary ammonium compounds, acid-anionic surfactant sanitizers and chlorhexidine (Abreu et al., 2013, Hota, 2004). The same can be applied to food-processing industries surfaces that contact food (Chorianopoulos et al., 2007). Regarding traditional preservation methods of foods, such as fresh vegetables and fruits, the most commonly used is washing with just water or chlorinated water, refrigeration, atmosphere modification (low temperature and humidity) and simple packaging or under vacuum (Gomez-Lopez, 2012). Meat and fish cuts, after their

processing (e.g simple cutting, salt or smoke addition) they are traditionally packaged in air-shield containers with inert atmosphere or under vacuum (Alcicek, 2011).

Nowadays, and for various reasons mentioned later, there is a shift towards the use of novel and innovative materials, techniques and procedures in order to disinfect nosocomial and food contacting surfaces or preserve food by eliminating surface contamination or microbial growth (Abreu et al., 2013; Gomez-Lopez, 2012). Among these innovations are disinfection techniques using steam vapors or hydrogen peroxide mist/vapors/plasma, UV light, thermal and non-thermal gas plasma, irradiation, ozone and nitrogen dioxide chambers (Abreu et al., 2013; Rutala & Weber, 2001; Schneider, 2013; Negi, 2012). Another innovative approach to the disinfection field is by developing and optimizing chemical antimicrobial agents. Examples are; oxidizing chemical formulations, sodium hypochlorite, polyhexamethylene-guanidine hydrochloride, which belongs to nitrogen compounds (guanidine family) reviewed previously, two-part disinfectants (silver and ethyl alcohol in the one part and hydrogen peroxide and peracetic acid in to the other, which is the activator of the disinfection solution that is mixed on site) and naturally occurring agents such as carvacrol, thymol, γ -Terpinene, p-Cymene, linalool and camphor, which can be used as hydrosols or in vapor and mist form (Abreu et al., 2013; Burt, 2004; Schneider, 2013; Amiri et al., 2013; Valeriano et al., 2012; Negi, 2012). These naturally occurring antimicrobials are found in plants' extracts that are under study by this paper and according to the literature have the potential to be used as disinfecting agents in nosocomial environments for non-critical/semi-critical surfaces or for food preservation and food-contacting surface sanitation purposes.

2.5.1 Why there is a need for them?

Contrasting disinfectants from plant sources and the traditional antimicrobial agents mentioned in the previous paragraph, several advantages and motivation factors can be identified in order to justify the pursuit of replacing the latter with the natural ones. In general, the growing negative consumer perception against synthetic compounds has led to the search for natural alternatives. In this context, essential oils (EOs) and plant extracts emerge as feasible alternative solution (Oliveira et al., 2010).

The most commonly mentioned motivator towards natural disinfectants across the literature is the negative environmental impact of the industrially produced agents (Dettenkofer & Spencer, 2007; Olmez and Kretzschmar, 2009; Tornuk et al., 2011). Western society appears to be experiencing a trend of "green" consumerism, desiring fewer synthetic products with a smaller impact on the environment (Smid and Gorris, 1999; Burt, 2004; Chorianopoulos et al., 2007). More specifically referring to nosocomial units, Daschner and Dettenkofer, back in 1997, had raised the alarm concerning the environmental impact of hospitals, which contribute significantly to environmental pollution and consumption of limited natural resources. The same researchers had calculated that in German hospitals the patients consume 450 liters of drinking water daily, most of which is used for cleaning and disinfecting processes including the water required for the production of disinfectants and detergents. The same concerns govern the disinfection procedures followed by the food industry regarding the equipment and machinery sanitation (Chorianopoulos et al., 2007).

An equally important motivator found in the literature concerns the health risks that occur during and after the use of an industrial synthesized agent. In general disinfectants may be hazardous to personnel and patients and require special safety precautions during handling and application (Dettenkofer & Spencer, 2007). Moreover, health risks concerning the formation of carcinogenic by-products are associated with disinfectant products containing chlorine (Gil et al., 2009). Other compounds, such as chloroxylenol, can cause contact allergies in some individuals and it is toxic if inhaled or ingested (Rhoades et al., 2013). Finally, some of them are caustic and can potentially cause skin, eye, mucous membranes or respiratory irritation, if not handled properly; one example is the peroxygen

compounds (Block, 2000; Abreu et al., 2013). Rubber gloves, masks, safety goggles, and protective clothing should be worn when handling concentrated HP, PAA, or any liquid peroxygen compound or solution (Block, 2000). Thus, Tornuk et al (2011) observed that in this context, many researchers investigated alternative sanitizing agents including essential oils or their components in order to replace the conventional ones and to compensate the drawbacks they have.

Additionally, the use of commercial disinfectants is impaired by the development of bacterial resistance, usually observed in nosocomial establishments (Amiri et al., 2013; Dettenkofer & Spencer, 2007). Conventional cleaning and disinfection regimes coupled with uncritical use of biocides, especially in low concentrations, may contribute to antimicrobial resistance dissemination, due to insufficient biofilm control or not complete eradication of microbial population (Valeriano et al., 2012; Dettenkofer & Spencer, 2007). One example could be the commonly used antimicrobial, triclosan, which is implicated in the development of bacteria's antibiotic resistance (Aiello et al. 2007). Resistance can be developed even against the most effective disinfectant. Several bacteria that produce certain metabolites, such as catalase, can be mutated, as they survive from exposure to H₂O₂, producing larger amounts of these metabolites, which inactivates the specific agent (Ukuku et al., 2012). This issue, also, interest the food industry (Chorianopoulos et al., 2007). Essential oils are capable of affecting biofilm formation due to their ability to decrease significantly bacterial adhesion and affect bacterial viability in biofilms (Filoche, Soma & Sissons, 2005). In addition, due to their multi-target antimicrobial mechanism, bacteria resistance can be counteracted, since bacteria tend to build up defenses against the most common to them threat (Burt, 2004; Barry-Ryan & Bourke, 2012)

Furthermore, there are secondary reasons for hospital epidemiologists and food technologists to lean towards natural antimicrobial versus the other alternatives to the traditional disinfection techniques (e.g. steam vapors, hydrogen peroxide mist/vapors/plasma, UV light, etc). Abreu et al (2013) and Rutala & Weber, 2001 mention as their disadvantage the high purchase and operational cost, the possible interaction with acids leading to corrosive phenomena, non-compatible surfaces or equipment and the ability of some to cause skin, eye and respiratory tract irritation.

More motivation factors towards the research and development of natural surface disinfectants can be found in the literature, if taken into account their possible application as foods' surface disinfectants aiming to food safety and the elongation of their shelf life, through the avoidance or delay of microbial growth. Firstly, consumers' demands are increasingly focusing on minimally processed food products, with less use of synthetic additives and at the same time without compromising food safety (Negi, 2012). There is therefore a need for new methods of making food safe which have a natural or "green" image (Burt, 2004). Secondly, the environmental and health risks of chlorine, used in vegetable and fruit washing, should be reduced (Tornuk et al., 2011). Also, the health risks, due to excessive salt consumption, can be avoided or reduced, if other additives replace the preservation effect that salt have on foods (Burt, 2004). Current novel preservation technologies of vegetables and meat/fish cuts, such as activated films, non-thermal treatments or irradiation may cause loss of organoleptic properties of foods and reduce consumer acceptability (Negi, 2012). Finally, It has been estimated that as many as 30% of people in industrialized countries suffer from a food borne disease each year, therefore, there is still a need for new methods of reducing or eliminating food borne pathogens respecting at the same time consumers' preferences (Burt, 2004).

Reflecting on all the above and after having established the antimicrobial capability of plant extracts and EOs, it can be easily assumed that the natural disinfection agents can potentially provide a solution to all the aforementioned issues related to disinfection of health care units and food preservation.

2.6 Plants' description

2.6.1 *Thymus Vulgaris*

2.6.1.1 *Botanological, morphological and general characteristics*

The common English word 'thyme' covers both the genus and the species most widely used, *Thymus vulgaris* L. (common thyme, garden thyme). From the aromatic and medicinal points of view, *T. vulgaris* is the most important species of *Thymus* and is widely used as a flavouring agent in foods, a culinary herb and as a herbal medicine (Stahl-Biskup, 2004).

Thymus vulgaris belongs to the *Labiata* family (*Lamiaceae*), subfamily *Nepetoideae*, tribe *Menthae*. The distribution of the genus can be described as Eurasian with the Mediterranean region, especially the Iberian Peninsula and northwest Africa, being the centre of the genus (Stahl-Biskup, 2004). It is a perennial subshrub, 10–30 cm in height with slender, wiry and spreading branches. The small leaves are evergreen, opposite, nearly sessile, oblong-lanceolate to linear, 5–10 mm long and 0.8–2.5 mm wide, grey-green, minutely downy and gland-dotted. The flowers are light-violet (figure 2), two-lipped, 5 mm long with a hairy glandular calyx (Stahl-Biskup, 2004).



Fig.2: *Thymus Vulgaris* flowers (hishtil.com/; loveofherbs.co.uk/)

The essential oil is responsible for the typical aroma of thyme. It is stored in glandular peltate trichomes situated on both sides of the leaves. They show a very typical anatomy with a gland head of 8–16 secretory cells sitting on one basal stalk cell. In the secretory cells the oil is produced and is secreted into the subcuticular space. If the cuticle is ruptured, e.g. by rubbing or grinding, the volatile oil spreads into the air. Dried plant material of thyme contains 1–2.5% of essential oil. (Stahl-Biskup, 2004)

Considering *Thyme's* production, some very general information are quoted here; Thyme is grown commercially in a number of countries for the production of essential oil, extracts, dried leaves and other applications. Thyme-producing countries are Spain, Portugal, France, Germany, Italy, the UK and other European countries, as well as North Africa, Canada and the USA (Prakash, 1990. cited in Stahl-Biskup, 2004 p. 303).

Successful growing of most thyme species is possible in any climate having a mean annual temperature from 7 to 20°C. It thrives in full sun, but also tolerates partial shade. The accumulation of essential oil depends on light. (Stahl-Biskup, 2004)

Drying is undoubtedly the most ancient and still the most widely used method of the fresh herb processing. In order to obtain stable products that will withstand long periods of storage without deterioration, the water content of thyme must be reduced to 8–10%. Drying is the most critical process because of the volatility and susceptibility to chemical changes of the contained volatile oil. (Stahl-Biskup, 2004)

Referring specifically to Thyme oil as a product or ingredient of other products, such as disinfectants, flavoring additives and preservatives, Fong et al., (2011) stated that it is natural, environmental friendly. Classified as a *Minimum Risk Pesticide*, it has low oral and dermal toxicity. Thyme oil as a food additive is Generally Recognized as Safe (GRAS) for

ingestion, in the United States. Moreover, a thymol-based disinfectant may not require a rinsing or wiping step for disinfecting surfaces, thus it can be safely used undiluted, minimizing water consumption. However, thymol is listed as a sensitizer and asthmagen by the Association of Occupational and Environmental Clinics (AOEC) (Fong et al., 2011).

2.6.1.2 *Thymus V. essential oil composition*

The total chemical composition of thyme is summarized by two main classes of secondary products, the volatile essential oil (Stahl-Biskup, 2002; Burt, 2004) and the non-volatile polyphenols (Vila, 2002 cited in Stahl-Biskup, 2004 p. 298). However, the proportions of constituents in the essential oils extracted from different plants may vary considerably (Ballester-Costa et al., 2013). This is caused, mainly, by intrinsic and extrinsic factors during cultivation and harvesting (Stahl-Biskup, 2004). These factors are local climate and environmental conditions (temperature, day length, sun, rain, etc.), season, geographical location, geological aspects and soil's nutrients (Viuda-Martos et al., 2008; Ballester-Costa et al., 2013). In addition, the parts of the plant and the method used to obtain the EO are of great importance considering the final chemical composition (Ballester-Costa et al., 2013).

Most of the volatiles present in thyme's essential oil belong to the monoterpene group with thymol as the main representative, which is responsible for the typical strong and spicy smell associated with thyme (Stahl-Biskup, 2004). Additional monoterpenes always accompany thymol, such as carvacrol, an isomerterpene phenol, as well as p-cymene and γ -terpinene (Stahl-Biskup, 2004; Hudaib et al., 2002). Moreover, the methyl ethers of thymol and carvacrol are often present (Stahl-Biskup, 2004). Further monoterpenes present in the thyme's EO are linalool, borneol, camphor, limonene, myrcene, β -pinene, trans-sabinene hydrate, α -terpineol and terpinen-4-ol. Sesquiterpenes are not very important in thyme oils, with the only worth mentioning to be β -caryophyllene (Stahl-Biskup, 2004; Hudaib et al., 2002).

In order to better illustrate the great variations in the chemical composition of thyme's essential oil from study to study, the findings of several similar papers are listed here. Ballester-Costa and her associates (2013) analyzed samples of *Thymus V.* essential oil indentifying 80 compounds, which represented 98.97% of the total EO's chemical composition. They found that the main component was linalool (44.00%) followed by terpineol-4 (11.84%), γ -terpinene (8.91%) and myrcene (6.89%). On the other hand, Imelouane et al. (2009), who studied the chemical composition of *T. vulgaris* EO from Morocco, obtained very different findings concerning the proportions of chemical constitutes. The main compounds identified were camphor (38.54%), camphene (17.19%) and α -pinene (9.35%). In addition, Viuda-Martos et al. (2010) analyzed *Thymus*'s EO from Egypt and resulted that its major constituents were thymol (32.23%), γ -terpinene (21.19%), and p-cymene (20.27%). The latter study is in agreement with the main body of literature publications, which states that the EO's chemical compositions is; thymol (30–55%), carvacrol (1–5%), which are phenols, p-cymene (15–20%), γ -terpinene (5–10%), which are hydrocarbons, linalool (1–5%), which is an alcohol, and, in smaller percentages (0.5–1.5%), borneol, α -terpineol, terpinen-4-ol, trans-sabinene hydrate, which are alcohols, camphor, which belongs to ketones and limonene, myrcene, a-pinene and b-caryophyllene (1–3%), which belongs to the hydrocarbons group (Stahl-Biskup, 2004; Daferera et al., 2000). Figure 3 shows the chemical structure of the commonly identified constitutes of EO's of *Thymus V.*

Greek *Thymus Vulgaris* chemical composition is similar to the compositions reported in to the main body of scientific publications. Two studies verify that and their finding are presented in table 2

Table 2: The amount of the main components present in the samples of *Thymus vulgaris* essential oil (expressed as % of the total composition)

Components	Manou et al., 1998		Daferera et al., 2000
	Sample no. 1 %	Sample no. 2 %	
Thymol	38,60	42,98	63,6
p-Cymene	28,70	23,09	23,5
γ-Terpinene	5,86	9,14	4,3
Carvacrol	9,83	2,23	2,2
Linalool	5,08	5,76	-
α-Pinene	2,44	1,95	1,0
Myrcene	1,23	1,53	-
β-Caryophyllene	1,55	1,53	1,3

Except, though, the aforementioned factors explaining the variations in the EO's chemical composition, De Lisi et al. (2011) adds one more. The variations in *T. vulgaris* EOs chemical composition could be attributed to the different intra-specific chemotypes of this species. Thus, the significant differences in the above research results could be attributed there, since the materials and methods used were similar. For example in the study of Ballester-Costa et al. (2013), the researchers analyzed a type of *Thymus V.* that had a chemotype characterized by a high amount of linalool.

2.6.1.3 *Thyme's essential oil antibacterial activity-mechanism*

In order to highlight the importance of antimicrobial activity of thyme, Ceylan and Fung (2004) state in their paper that the oil with the widest spectrum of antibacterial activity was found to be from thyme, followed by oils from oregano, clove, nutmeg, black pepper and geranium. Further, from the mentioned in the previous part, it can be observed that the *Thymus V.* EO contain a large number of different groups of chemical compounds, thus it is most likely that their antibacterial activity is not attributable to only one antimicrobial mechanism but that there are several targets on the cell, as it can be seen in Figure 2, thus a short elaboration upon the major components and their antimicrobial capability (Table 3) and mechanisms will follow (Burt, 2004; Fong et al., 2011; Kačániová et al., 2012). Concerning the factors affecting thyme's oil antimicrobial activity see paragraph 2.3.5.

Thymol & Carvacrol

Thymol and carvacrol are the chemicals in the essential oil that are believed to present the most antimicrobial activity (Burt, 2004). They have been demonstrated to cause an increase in permeability of the cytoplasmic membrane to ATP of gram-negative bacteria outer membrane and releasing lipopolysaccharides (Burt, 2004; Xu et al., 2008; Kačániová et al., 2012). In addition, carvacrol and thymol can cause distortion of the cell's membrane physical structure, leading to expansion, destabilization and increase of membrane fluidity, which increase passive permeability (Burt, 2004). This takes place because the two phenols can interact with the cell membrane by dissolving into the phospholipid bilayer (ibid, 2004). Moreover, they can cause reduction in the proton motive force, and decrease in intracellular levels of adenosine triphosphate (ATP) (Xu et al., 2008; Fong et al., 2011). More specifically considering thymol, it has been hypothesized that it forms hydrophobic bonds with the membrane proteins of *Staph. Aureus*, changing that way the membrane's permeability characteristics (Burt, 2004).

p-cymene

P-cymene is hydrophobic and causes swelling of the cytoplasmic membrane to a greater extent than does carvacrol (Burt, 2004). By itself is not an effective antibacterial (Juven et al., 1994; Dorman and Deans, 2000; Burt, 2004), but when combined with

carvacrol functions synergistic (Ultee et al., 2000b, cited in Burt, 2004 p. 241). Also, p-cymene owns its efficiency due to the fact that facilitates transport of carvacrol across the cytoplasmic membrane since it dissolves in the lipid bilayer of bacteria outer membrane (Ultee et al., 2000b, cited in Burt, 2004 p. 241)

Table 3: Antibacterial spectrum of Thyme against bacterial strains. Adopted from Ceylan & Fung, 2004 and updated with recent findings or older, which were missed from the authors of the original table.

Spice	Bacterial Species Inhibited	Bacterial Species Not Inhibited	References
Thyme	<i>Acinetobacter calcoaceticus</i>	<i>Listeria innocua</i>	Aktug and Karapinar 1986 *
	<i>Actinobacillus</i>	<i>L. monocytogenes</i>	Arras and Grella 1992*
	<i>Aerobacter aerogenes</i>	<i>Moraxella</i> sp.	Azzouz and Bullerman 1982*
	<i>Aeromonas hydrophila</i>	<i>Micrococcus</i> (Sarcina)	Beuchat 1994*
	<i>Achromobacter denitrificans</i>	<i>Micrococcus luteus</i>	Deans and Ritchie 1987*
	<i>Alcaligenes faecalis</i>	<i>Micrococcus flavus</i>	El-Kady <i>et al.</i> 1993 *
	<i>Bacillus anthracis</i>	<i>Mycobacterium phlei</i>	Farag <i>et al.</i> 1989b *
	<i>Bacillus cereus</i>	<i>Pantoea</i> sp	Huhtanen 1980 *
	<i>Bacillus subtilis</i>	<i>Proteus vulgaris</i>	Kivanc and Akgul 1986*
	<i>Beneckea natriegens</i>	<i>Pseudomonas aeruginosa</i>	Sheief 1984*
	<i>Brevibacterium linens</i>	<i>Pseudomonas fluorescens</i>	Bozin <i>et al.</i> , 2006
	<i>Brochothrix thermosphacta</i>	<i>Pseudomonas fragi</i>	Alves-Silva <i>et al.</i> , 2013
	<i>Campylobacter jejuni</i>	<i>Salmonella Paratyphi</i>	Cosentino <i>et al.</i> , 1999
	<i>Citrobacter freundii</i>	<i>Salmonella Pullorum</i>	Lambert <i>et al.</i> , 2001
	<i>Clostridium sporogenes</i>	<i>Salmonella Typhimurium</i>	Firouzi <i>et al.</i> , 1998
	<i>Enterococcus faecalis</i>	<i>Salmonella enteritidis</i>	Ballester-Costa <i>et al.</i> , 2013
	<i>Enterobacter aerogenes</i>	<i>Sarcina lutea</i>	Dorman and Deans 2000
	<i>Enterobacter amnigenus</i>	<i>Serratia marcescens</i>	Imelouane <i>et al.</i> , 2009
	<i>Enterobacter gergoviae</i>	<i>Staphylococcus albus</i>	Šipailieneė <i>et al.</i> , 2011
	<i>Erwinia carotovora</i>	<i>Staphylococcus aureus</i>	Stahl-Biskup, 2004
	<i>Escherichia coli</i>	<i>Staphylococcus faecalis</i>	
	<i>Flavobacterium suaveolens</i>	<i>Streptococcus faecalis</i>	
	<i>Fusobacterium</i>	<i>Streptococcus nasik</i>	
<i>Hafnia alvei</i>	<i>Shigella sonei</i>		
<i>Klebsiella pneumoniae</i>	<i>Shewanella putrefaciens</i>		
<i>Lactobacillus plantarum</i>	<i>Vibrio parahaemolyticus</i>		
	<i>Yersinia enterocolitica</i>		

With asterisk (*) are marked the references as are quoted in the original table from Ceylan's & Fung's (2004) work. The rest cited references add more bacterial species that the thyme's extracts present inhibitory or killing activity.

2.6.2 Aloe Vera

2.6.2.1 Botanical, morphological and general characteristics

The botanical genus of *Aloe* has been classified in the *Liliaceae* family, because it germinates from an original bulb in the same way as lilies. Other well known plants in this family are onions, garlic, and asparagus. (Bassetti & Sala, 2005)

The *Aloaceae* family contains approximately 350 varieties of the plant throughout the planet. The range spanned from the miniature type like *Aloe aristata*, larger-sized Aloes, and those having a cosmetic, curative value, such as *Aloe arborescens* Miller, *Aloe ferox*, *Aloe barbadensis* Miller *Vera*, *Aloe chinensis*, *Aloe saponaria*, and *Aloe succotrine* (Bassetti & Sala, 2005). *Vera* is a perennial that grows into the shape of a tuft, whose base is surrounded by a rosette of succulent and thorny-edged leaves with a spiral development (figure 3). (Bassetti & Sala, 2005)



Fig. 3: *Aloe Vera* plant fully grown (aloehellas.com/)



Fig. 4: *Aloe Vera*'s leaf incision revealing the inner gel (<http://www.aloehellas.com/>)

Aloe Vera has succulent, fleshy leaves of a mottled light green color and reaches maturity after four years, with average leaf length between 60 – 100 cm, base width from 7 to 13 cm and leaf weigh from one to two kilos. It produces an average of twelve to thirty leaves. Its leaves are rich in gel in comparison to the external cuticle or the skin encasing it (Figure 4). (Bassetti & Sala, 2005)

Several procedures may help preserve and stabilize the gel, but most of them either diminish or destroy the original characteristics and nutrients. However, few companies are able to manufacture a thick Aloe pulp, avoiding the use of any form of heat or enzyme treatment. Long and costly mechanical beating produces reasonable liquid fraction, which retains the important components of the fresh product. (Bassetti & Sala, 2005)

Aloe's gel or juice, are the main substance for many secondary products. Pure gel can be used internally, referring to direct consumption as a juice. Additionally, aloe's gel is used for external skin applications for various reasons, which include burning soothing, skin hydration etc. Further, cosmetics and cosmeceuticals use as base the aloe's gel in order to produce, face-creams, repair creams, shampoos, bathfoams and in order to provide UV protection attributes to other cosmetics. (Bassetti & Sala, 2005)

Although, across the literature the aloe's antibacterial properties have been studied in some extent, the potential of a surface disinfection product from it, have not attracted much attention.

2.6.2.2 *Aloe Vera*'s extract composition

Large fluctuations are observed in *Aloe Vera* gel's composition found in the literature, but they have been explained by the fact that the gel's composition is significantly influenced by factors like aloe type, season, soil and cultivation routines. (Bassetti & Sala, 2005; Hamman, 2008)

Aloe is made up of a vast range of compounds which can be divided into three large groups, but the main ingredient is water (98,5%-99,5%). The first group, complex sugars (with acemannan as primary polysaccharide), are constituents of the leaf's gel. Next are the anthraquinones, contained in the outermost part of the skin and last are the several chemical substances mentioned in table 4. (Bassetti & Sala, 2005)

Among major constituents of *Aloe Vera*, besides water, is anthraquinone glycoside called aloin or aloe emodin, along with barbaloin, C-glucoside, aloesin, aloesone and emodian (Hamman, 2008; Deshpande, 2010; Moghaddasi, 2010). Anthraquinones are phenolic compounds, quinone derivatives (Rodríguez, Martín & Romero, 2013). The percentage of these varies from 4.5 to 30% depending on aforementioned factors (Deshpande, 2010; Bassetti & Sala, 2005; Hamman, 2008). Regarding polysaccharides, glucomannan, mannose, galactose, xylose and arabinose are the most commonly found in aloe vera gel or juice. In addition aloetic acid, emodin crygeminic acid, crysophenic acid, galactronic acid, amylase, along with some other steroids, organic acids, enzymes, aminoacids, saponins and minerals like calcium (4.7%),

sodium (1.43%), potassium (6.6%), chloride (12.2%) and manganese (0.01%) are present (Deshpande, 2010; Bassetti & Sala, 2005).

Table 4. Summary of the chemical composition of *A. vera* leaf pulp and exudates. Adopted by Hamman (2008)

Class	Compounds
Anthraquinones/anthrones	Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose
Chromones	8-C-glucosyl-(2'-O-cinnamoyl)-7-O-methylaloediol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol, 8-C-glucosyl-7-O methylaloediol, 8-C-glucosyl-noreugenin, isoaloesresin D, isorabaichromone, neoaloesin A
Enzymes	Alkaline phosphatase, amylase, carboxypeptidase, catalase, cyclooxygenase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, superoxide dismutase
Inorganic compounds	Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium, zinc
Phenols and Miscellaneous including organic compounds and lipids	Pyrocatechol, p-Coumaric, 1,8-cineole Arachidonic acid, γ -linolenic acid, steroids (campesterol, cholesterol, β -sitosterol), triglycerides, triterpenoid, gibberillin, lignins, potassium sorbate, salicylic acid, uric acid
Non-essential and essential amino acids	Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, lysine, methionine, proline, tyrosine, valine
Proteins	Lectins, lectin-like substance
Saccharides	Mannose, glucose, L-rhamnose, aldopentose
Vitamins	B1, B2, B6, C, β -carotene, choline, folic acid, α -tocopherol

2.6.2.3 *Aloe Vera's* antibacterial activity-mechanism

As an antibacterial agent, Aloe Vera liquid have shown to have a wide range of effectiveness against Gram positive and Gram negative bacteria (Pareek et al., 2013; Lawrence, Tripathi & Jeyakumar, 2009). The chemical constituents that are proposed by the literature to exhibit the antibacterial activity attributed in Aloe Vera gel are anthraquinones, saponins, phenols, terpenoids and enzymes (Pareek et al., 2013; Lawrence, Tripathi & Jeyakumar, 2009; Carol et al., 1996). More in specific, 1,8-cineole (monoterpenoid), pyrocatechol (phenol), aloin (anthraquinone) and superoxide dismutase (enzyme) are responsible for a range of antibacterial mechanisms (Carol et al., 1996; Bhardwaj, Ballal & Velmurugan, 2012; Lawrence, Tripathi & Jeyakumar, 2009). The mechanisms attributed to terpenoids and phenols are well covered in previous sections of this study. According to Lu et al., (2011) anthraquinones' antibacterial mechanism is attributed to a similar mechanism as described for phenolic compounds; e.g. increased membrane permeabilization, loss of structural integrity of cell wall and cytoplasmic membrane, and leakage of intracellular contents. Other researchers however, have described a different mechanism. Comini et al. (2011) and Montoya et al. (2011) stated that anthraquinones produce bacterial photoinactivation through a mechanism that allows their intercalation between the nucleic acid bases. Another proposed mechanism has the same final output as the one before, but this is reached through a photodynamic photosensitization, acting mainly through the generation of reactive oxygen species (ROS). Finally, superoxide dismutase enzyme transforms O_2 into hydrogen peroxide (H_2O_2), known for its antibacterial capability as it was discussed previously (Carol et al., 1996; Bhardwaj, Ballal & Velmurugan, 2012).

Further, during the antimicrobial activity determination of aloe extracts, the results depend greatly on the solvent that it is used to dissolve in the aloe's gel. Ethanol, acetone and water are commonly used in the research projects found in literature. However the results are contradictory. Barandozi (2013) reported that the maximum antibacterial activity was observed in acetone extract followed by ethanol extract, with last the aqueous. On the other hand, Bawankar et al., 2013 reported that the maximum antimicrobial activity was

observed in ethanol extract followed by the aqueous and at last with little or no activity was the acetone extract. This could be attributed to the largely different chemical constituents that each aloe gel bears and are extracted by the different solvents (Bawankar et al., 2013).

Regarding Aloe's Vera antibacterial activity, only few studies have been performed. The plant's antimicrobial agents were reported to effectively inhibit the growth, greatly reduce or kill several bacteria as shown in the following table.

Table 5: Inhibited Bacterial Species by ethanol extract of Aloe Vera gel.

Plant	Inhibited Bacterial Species	Not-Inhibited Species	References
Aloe Vera	Aggregatibacter	Proteus vulgaris	Pareek et al., 2013
	actinomycetemcomitans	Pseudomonas aeruginosa	Barandozi, 2013
	Bacillus cereus	Salmonella typhosa	Lawrence, Tripathi & Jeyakumar, 2009
	Bacillus subtilis	Serratia marcescens	Mariappan & Shanthi, 2012
	E. coli	Sigella sonie	Gontijo et al., 2012
	Enterobacter aerogenes	Staphylococcus epidermidis	Irshad, Butt & Younus, 2011
	Enterococcus faecalis	Streptococcus Aureus	Cock, 2008
	Helicobacter pylori	Streptococcus mutans	
	Klebsiella pneumonia	Streptococcus pyogenes	
	Mycobacterium tuber.		

3. Materials and Methods

3.1 Plant extracts

3.1.1 *Thymus Vulgaris* essential oil

Thymus Vulgaris essential oil was purchased from Cretan Herbal Chem S.A., a local producer providing with essential oils and other extracts. *Thymus Vulgaris* leaves, stem and flowers were harvested at the end of March from Kasteli area, Iraklion, Crete, Greece (altitude 355m). The oil was extracted by hydro-distillation using a Clevenger-type apparatus during 30 min. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate. The origin and the purity of oil were confirmed by the quality certificates supplied by the company and the samples were treated under the mildest conditions to ensure the stability of the extract composition. According to the manufacturer the oil consisted approximately (major components): Thymol 35%; p-cymene: 20-25%; γ -terpinene: 8-10%; carvacrol: 5-8%.

Suspensions of the above mentioned extract was prepared in concentrations of 1.0, 2.5, 5.0 ml/L, by dispensing appropriate amounts in 1L sterile deionized water. Then the suspension was thoroughly mixed by shaking vigorously for 5 min at room temperature before use.

3.1.2 *Aloe Vera* extract

Aloe Vera ethanol dried extract was purchased by the same company. The Aloe leaves were harvested in April in the same region as previously in thyme. Then the inner leaf gel was removed, minced and homogenized using a blender. For the preparation of ethanol extract, the leaf gel was dried in the oven at 80 °C for 48 h and then powdered. Twenty grams of this powder was soaked in 200ml of ethanol for 24 h. The content was then filtered through Whattmann filter paper no. 1 and the filtrate was evaporated to dryness. The suspensions were prepared as previously described for thyme by adding 1.0, 2.5 and 5.0 g/L.

3.2 Test Bacteria

The bacteria species included in this study were isolated strains of *methicillin resistant Staphylococcus aureus (MRSA)*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. These strains were isolated from the University Hospital of Heraklion clinical environment, by the microbiological laboratory of University Hospital of Heraklion. The specific species were

selected, firstly, based to their epidemiological characteristics. They are predominant nosocomial pathogens, causing nosocomial infections responsible for the half of the infections in intensive care units (Huang & Eells, 2011). These along with *Enterococcus faecium* are often referred to as E.S.K.A.P.E. highlighting their importance for the nosocomial hygienists. (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *enterobacter* species) (Huang & Eells, 2011). An additional reason for their inclusion to this study is that they are able to develop resistance to commonly used disinfectants (Valeriano et al., 2012).

More specifically, *S. aureus* is the leading cause of hospital-acquired infections as identified by the National Healthcare Safety Network (NHSN) in USA, with the prevalence of *S. aureus* infections continuing to increase worldwide (Huang & Eells, 2011). Moreover its significant antimicrobial resistance results in frequent disinfection and treatment failures with severe outcomes as well as dramatic increases in total healthcare costs (ibid, 2011). Concerning the selection of *Enterobacteriaceae* family species, represented here by *E. Coli* and *Klebsiella pneumoniae*, is explained by the fact they are the second most common cause of nosocomial-acquired infections, with main mechanism of transmission the contact with contaminated surfaces (Pitout, 2011, Abreu et al., 2013). Further, many studies have documented the contamination of sinks and sink drains by *P. aeruginosa* in nosocomial environments, contributing to the spread of this bacteria (Hota et al., 2009). Finally, *Acinetobacter baumannii* is recognized to be among the most difficult antimicrobial-resistant gram-negative bacilli to control and treat with prolonged periods of survival under a wide range of environmental conditions in health care units (Maragakis & Perl, 2008). In the following table (6) a summarization of the clinical relevant nosocomial pathogens are presented with some characteristics highlighting their importance as environmental sources of hospital-acquired infections, regarding the mode of transmission, the time length that they can survive being a potential source of contamination and the disease/symptoms they cause in a hospital environment.

Secondly, the specific bacteria strains were included in this study because they were isolated from a nosocomial environment and did not come from a culture collection. The reason for that was to replicate more accurately the reality of nosocomial disinfection scenarios, because the culture collection strains respond differently to the presence of disinfectants in comparison with clinical isolated strains (Abreu et al., 2013). Furthermore, the evaluation of disinfecting agents was performed using both Gram (+) and Gram (-) bacteria of multiple species in order for the experimentation to be representative of what occurs in clinical practice.

Table 6: Characteristics of the selected bacteria species (adopted by Abreu et al., 2013)

Bacteria	Mode of transmission	Length of survival	Disease/symptoms
<i>Acinetobacter baumannii</i> (Gram -)	extensive environmental contamination	33 days on plastic laminate surface; 3 days to 5 months on dry inanimate surfaces	pneumonia and bloodstream infection
<i>Escherichia coli</i> (Gram -)	ingestion of contaminated food, water or milk; person-to-person transmission	1.5 h to 16 months on dry inanimate surfaces	blood and urinary tract infection
<i>Klebsiella pneumoniae</i> (Gram -)	contact with contaminated surfaces and objects, medical equipment and blood products	2 h to >30 months on dry inanimate surfaces	urinary tract infections, pneumonia, septicemias and soft tissue infections
<i>Pseudomonas aeruginosa</i> (Gram -)	contamination from tap water and different medical devices	6 h to 16 months on dry inanimate surface; 5 weeks on dry floor; 7 h on glass slides	lung and urinary tract infection
<i>Staphylococcus aureus</i> , including MRSA	contact with the organism in a purulent lesion or on the hands;	<i>S. aureus</i> can remain virulent for 10 days on dry surfaces;	blood, skin and respiratory tract

(Gram +)	burn units extensively contaminated	MRSA can survive for 7 days to 9 weeks on dry inanimate surfaces and 2 days on plastic laminate surfaces	infection, septicaemia and death
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3.3 Preparation of the inoculums

The lyophilized clinical bacterial strains in beads, that were obtained from the microbiological laboratory of University Hospital of Heraklion, were subcultured three consecutive times for 18 h in 35.5 °C in Brain Heart Infusion (BHI) broth (Oxoid; Cheshire, England). The last subculture was centrifuged and the bacterial cells were re-suspended in saline to the original number of cells (10^6 - 10^7 cfu/ml). The cells were counted in duplicate BHI agar plates after vortexing for 2 minutes in neutralizing broth and plating.

3.4 Commercial Disinfectants

Disinfectants of three different producers were provided by the University Hospital of Heraklion categorized, below, based on their active ingredient (next are quoted their abbreviations used in results):

1. Iodophors (Company A, B, C) - same
2. Acid sanitizers (Company A, B) - Acid
3. Hypochlorite sanitizer (Company A, B) - Hypochlorite
4. Quaternary Ammonium sanitizers (Company A, B, C) - QAC
5. Quaternary Ammonium sanitizer and acid (Company A) - QAA
6. Phenolic sanitizer (Company A) - Phenol

3.5 Antimicrobial screening

The method followed for the antimicrobial evaluation of the tested disinfection compounds is the broth dilution method with bacteria cell viable count, both in vitro and in presence of organic matter.

The first step of the antimicrobial screening of plant extracts and commercial disinfectants is the preparation of neutralizing broth, which include the dissolve of 2g lecithin, 10ml Tween 80, 9.5g sodium chloride, 1g sodium thiosulfate, and 37 g BHI broth powder (OXOID) in 1000 ml distilled water. After dissolving the ingredients by mild heating, the preparation was sterilized for 15 min at 121° C and aseptically dispensed in sterile test tubes in appropriate amounts. Neutralizing broth is used to terminate the bactericidal effect of disinfectants on the bacteria population (Sutton, 2010). After the application of a disinfectant in a bacterial culture for a specific amount of time; the remaining cells must be recovered and counted. However, residual disinfectant in the recovery agar could artificially depress the recovery of viable cells, and so it is important to neutralize this residual activity to get accurate counts of survivors (Sutton, 2010). Thus the dispensation of the solution containing the bacterial population with the disinfection substance is done at the chosen time on a neutralizing broth. At the same time the neutralizing broth must not have an effect on the bacteria population.

Then, in order to examine the effect of neutralizing broth on bacterial survival, amounts of 9.9 ml of neutralizing broth was mixed with 0.1 ml of bacterial cell suspension in saline and ten-fold serial dilutions in the same broth were immediately prepared and plated on BHI agar. The plating was repeated after 30 min of contact time of the bacteria with the neutralizing broth.

The ability of the neutralizing broth to inactivate each disinfectant was evaluated by mixing 8.9 ml of neutralizing broth and 0.1 ml of bacterial cell suspense for 1 min and then adding 1ml of 400 ppm disinfectant in distilled water, mixing for another 15 min and then plating on BHI agar to estimate the number of bacterial survivors. Plating was repeated after 10 min.

In addition, the bactericidal effect of deionized water was evaluated on the selected strains by dispensing 10 ml on pre-counted bacteria populations, applied for several contact times (10, 20, 30, 40 min). The specific evaluation was performed during a previous study that took place in the same laboratory by other researchers as part of the research project that this study is part of (Panoulis, 2014).

In order to test the compounds' bactericidal activity, first 1 ml of either deionized water or reconstituted skim milk (OXOID) was added to 9 ml of test compound solution in deionized water (prepared as was described in 3.1 paragraph). After vortexing, 1 ml of the bacterial preparation was added and the suspension was vortexed again. After 2, 5 and 10 min of exposure, a series of ten-fold dilutions were prepared in neutralizing broth and plated on BHI agar. The plates were incubated for 48 h at 37° C and the decimal reduction (DR) was defined as \log_{10} counts of the initial inoculums minus the \log_{10} counts of the surviving cells.

Finally, the hypochlorite disinfectants were both tested for amount of available chlorine (ppm) by the chlorine drop count test kit (HACH; Colorado, USA) according to the manufacturer's instruction in order to verify the available chlorine content and ensure the disinfectant ability of the compounds

4. Results

4.1 Material validation

Regarding the validation of the materials used in the experimentation procedure all results were as expected. The effect of neutralizing broth on bacterial survival was tested by exposing the cells' population to the broth at levels of 10^6 - 10^7 . The initial counts and the counts after 30 min of exposure to the broth did not differ, indicating that the broth was sterile and didn't have any bactericidal activity. Further, the effect of neutralizing broth on the disinfectants was tested by suspending a cell population in the broth, while 400 ppm of each disinfectant was added into the broth. After 15 min of exposure the number of viable cells of the test organisms did not change. Therefore, the neutralizing broth was assumed to be effective in neutralizing the bactericidal activity of disinfectants. Moreover, concerning the validation of chlorine compounds and their disinfection activity, the results from the HACH test kit (Colorado, USA) regarding the available chlorine, were the same as the manufacturers claimed. Thus, these disinfection compounds didn't have reduced bactericidal capability, resulting from low levels of available chlorine. Finally, deionized water has been found not able to reduce the counts of populations in contact times under 20 minutes.

4.2 Antimicrobial screening of commercial disinfectants

Through the method described in the corresponding paragraph, the effectiveness of the selected disinfectants was tested at the manufacturers recommended concentrations and application times, in the absence or presence of organic matter.

Table 7 shows the DR of the tested organisms at the recommended concentrations and times of exposure of the disinfectants in deionized water. It was evident that all disinfectants were effective (> 5 DR) at the recommended dilutions and exposure times.

Table 7: DR of the tested organisms at the recommended concentrations and times of exposure to the disinfectants in deionized water or the effective concentration at certain times of exposure

Disinfectant		Recommended		Tested		MRSA	St. A.	Acin. B.	E. Coli	Ps. A	Kleb. p.
		PPM	MIN	PPM	MIN						
Iodophor	A	12.5	2	12.5	2	>6.16	>7.41	>7.18	>7.50	5.16	>7.66
	B	100	2	100	2	5.72	>7.52	5.55	6.61	>7.30	>7.4
	C	50	>5	50	7	5.66	7.64	>6.85	6.24	6.0	5.27
Acid	A	100	2	100	2	5.79	6.59	5.02	4.97	6.32	5.97
	B	200	2	200	2	>6.01	6.63	5.63	5.55	7.97	6.80
Hypochlorite	A	100	2	100	2	>6.16	6.76	5.27	>7.90	5.53	5.70
	B	200	>2	200	3	>7.44	7.08	7.24	>6.18	>7.38	6.76
QAC	A	100	2	100	2	6.44	6.76	6.26	6.38	5.23	5.26
	B	150	2	150	2	5.32	6.91	6.36	5.10	>7.36	6.08
	C	200	2	200	2	5.38	7.03	>6.85	6.63	6.92	6.61
QAA		200	2	200	2	5.28	>7.55	5.60	5.91	>7.38	5.92
Phenolic		100	2	100	2	5.6	5.26	6.24	7.35	>7.38	>7.25

The antimicrobial screening results of commercial disinfectants on the tested organisms at the recommended concentrations and times of exposure in 0.91% skim milk are expressed in DR in table 8. Specifically, in presence of organic matter only Iodophor B and the phenolic compound were effective against all bacterial species at the recommended concentration and exposure times. QAC A was effective against 3 out of 6 species while acid B and the combined QAC and the acid disinfectant were effective against 2 out of 6 organisms. Iodophor A, acid A, both hypochlorite preparations, and QAC b and C were not effective at the recommended concentrations.

Table 8: DR of the tested organisms at the recommended concentrations and times of exposure to the disinfectants on 0.91% skim milk or the selected concentration at certain times of exposure

Disinfectant		Recommended		Tested		MRSA	St. A.	Acin. B.	E. Coli	Ps. A	Kleb. P.
		PPM	MIN	PPM	MIN						
Iodophor	A	12.5	2	12.5	2	>7.51	3.83	3.06	>4.91	>4.05	>4.41
	B	100	2	100	2	>6.29	>7.44	>7.34	>7.73	7.56	>7.41
	C	50	>5	50	7	<1.00	<1.00	1.97	<1.00	<1.35	1.69
Acid	A	100	2	100	2	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
	B	200	2	200	2	4.91	6.14	4.64	6.48	4.84	5.97
Hypochlorite	A	100	2	100	2	2.91	0.46	0.87	0.73	0.36	0.47
	B	200	>2	200	3	3.77	1.01	1.62	<1.00	0.71	0.54
QAC	A	100	2	100	2	>6.7	>6.75	>6.58	3.74	3.71	2.19
	B	150	2	150	2	2.83	2.50	3.41	<1.00	2.52	<1.00
	C	200	2	200	2	1.78	3.63	>6.85	>6.74	1.08	1.68
QAA		200	2	200	2	<1.00	<1.00	>7.12	<1.00	<1.00	>7.34
Phenolic		100	2	100	2	>7.06	>7.42	>7.12	>7.79	>7.20	>7.45

4.3 Antimicrobial screening of Thyme oil and Aloe Vera preparations

Thyme essential oil was prepared in three different concentrations and was applied for three different contact times (2, 5, 10 min) on the populations of the six bacterial strains tested (10^6 - 10^7 cfu/ml) in sterile deionized water. Table 9 summarize these findings expressed in DR, which are calculated through the subtraction of the viable cell count, after the oil application, from the bacterial cell counts obtained when no oil was present. The higher bactericidal activity was achieved with the higher oil concentration in the higher

application time. Specifically, in 4 out of 6 bacterial strains the DR achieved was more than 2 and in the rest was close.

Table 9: Decimal reductions of the tested bacteria after treatment with thyme oil

Treatment	Concentration (ml/L)	Contact time (min)	Initial viable counts					
			MRSA	St. A.	Acin. B.	E. Coli	Ps. A	Kleb. p.
Thyme essential oil	0	2	6.48	6.51	6.46	6.54	6.40	6.36
		5	6.46	6.51	6.43	6.50	6.38	6.41
		10	6.41	6.52	6.50	6.57	6.51	6.43
			Population reduction log ₁₀ cfu after contact time					
	1.0	2	0.11	0.10	0.06	0.26	0.10	0.14
		5	0.09	0.15	0.16	0.31	0.21	0.24
		10	0.13	0.22	0.37	0.56	0.49	0.43
	2.5	2	0.30	0.40	0.38	0.41	0.33	0.31
		5	0.37	0.46	0.34	0.55	0.27	0.4
		10	0.51	0.75	0.68	0.77	0.80	0.62
	5.0	2	1.30	1.45	1.34	1.35	1.18	1.25
		5	1.61	1.84	1.71	1.90	1.46	1.57
		10	2.16	2.11	2.02	2.13	1.83	1.94

The log₁₀ reductions for the plant extract treatment were calculated as follows:
(Initial count – viable count = population reduction)

Table 10 summarize the findings on the effect of three different concentrations of Aloe Vera powdered methanol extract (1.0, 2.5, 5.0 ml/L) after three different contact times (2, 5, 10, min) with the population of the six tested bacterial strains. Similar results were obtained as before in thyme oil, however the decimal reductions were lower.

Table 10: Decimal reductions of tested bacteria after treatment with Aloe extract

Treatment	Concentration (g/L)	Contact time (min)	Initial viable counts					
			MRSA	St. A.	Acin. B.	E. Coli	Ps. A	Kleb. p.
Aloe Vera dried ethanol extract in distilled water	0	2	6.36	6.41	6.39	6.50	6.42	6.33
		5	6.40	6.44	6.49	6.55	6.39	6.40
		10	6.56	6.54	6.52	6.64	6.49	6.48
			Population reduction log ₁₀ cfu after contact time					
	1.0	2	0.22	0.19	0.10	0.16	0.20	0.14
		5	0.36	0.42	0.38	0.41	0.32	0.29
		10	0.55	0.64	0.50	0.60	0.49	0.46
	2.5	2	0.48	0.67	0.41	0.39	0.41	0.26
		5	0.70	0.8	0.69	0.51	0.48	0.45
		10	1.04	1.18	0.81	0.80	0.74	0.70
	5.0	2	0.65	1.39	0.57	0.89	0.54	0.53
		5	1.14	1.06	0.89	1.06	0.67	0.78
		10	1.87	1.90	1.34	1.42	1.09	0.97

The log₁₀ reductions for the plant extract treatment were calculated as follows:
(Initial count – viable count = population reduction)

When the extracts were dispensed to the bacterial cultures in the presence of skim milk, the results showed that the thyme's oil bactericidal effect suffered great reduction. The higher concentration in the maximum application time that before had >2 DR, in the presence of organic matter is reduced almost by one DR, almost in half.

Table 11: Decimal reductions of the tested bacteria after treatment with thyme oil in the presence of organic matter

Treatment	Concentration (ml/L)	Contact time (min)	Population reduction log ₁₀ cfu after contact time					
			MRSA	St. A.	Acin. B.	E. Coli	Ps. A	Kleb. p.
Thyme essential oil	0	2	6.40	6.36	6.48	6.31	6.28	6.30
		5	6.44	6.28	6.54	6.38	6.40	6.44
		10	6.51	6.54	6.60	6.55	6.60	6.58
			Population reduction log ₁₀ cfu after contact time					
	1.0	2	0.24	0.14	0.16	0.10	0.00	0.10
		5	0.44	0.18	0.42	0.24	0.22	0.34
		10	0.51	0.59	0.58	0.51	0.49	0.58
	2.5	2	0.37	0.24	0.38	0.20	0.08	0.2
		5	0.61	0.28	0.49	0.33	0.40	0.39
		10	0.83	0.69	0.70	0.75	0.70	0.83
	5.0	2	0.60	0.41	0.48	0.27	0.18	0.25
		5	0.80	0.48	0.69	0.54	0.48	0.64
		10	1.23	1.26	1.21	1.03	0.95	1.08

The log₁₀ reductions for the plant extract treatment were calculated as follows:
(Initial count – viable count = population reduction)

The bactericidal effect of Aloe Vera extract was also reduced, but the results showed that the reduction was smaller than the one presented by thyme oil, if we take into account the fact that initially Aloe extract had smaller antimicrobial effect and resulted with almost the same. In the case of *E. Coli* the Aloe extract was more bactericidal than Thyme oil in the presence of organic matter.

Table 12: Decimal reductions of tested bacteria after treatment with Aloe Vera extract in the presence of organic matter

Treatment	Concentration (g/L)	Contact time (min)	Population reduction log ₁₀ cfu after contact time					
			St.A. (a)	St. A. (b)	Acin. B.	E. Coli	Ps. A	Kleb. p.
Aloe Vera dried ethanol extract in distilled water	0	2	6.36	6.40	6.44	6.28	6.48	6.39
		5	6.39	6.41	6.48	6.32	6.48	6.51
		10	6.50	6.55	6.51	6.57	6.54	6.60
			Population reduction log ₁₀ cfu after contact time					
	1.0	2	0.14	0.24	0.33	0.10	0.30	0.15
		5	0.19	0.35	0.47	0.21	0.28	0.37
		10	0.32	0.43	0.40	0.49	0.43	0.56
	2.5	2	0.48	0.48	0.44	0.23	0.36	0.35
		5	0.58	0.60	0.67	0.32	0.38	0.57
10		0.79	0.91	0.81	0.67	0.51	0.78	
5.0	2	0.56	0.53	0.63	0.26	0.46	0.39	
	5	0.68	0.66	0.86	0.57	0.48	0.71	
	10	1.12	1.03	1.07	1.15	0.66	0.80	

The log₁₀ reductions for the plant extract treatment were calculated as follows:
(Initial count – viable count = population reduction)

Generally, the results showed that *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were more resistant to the bactericidal effect of the two extracts, presenting the lowest decimal reduction to their populations. These results were repeated both in the presence and absence of organic matter.

5. Discussion

Summarizing the literature review findings around plants' extracts much information were gathered explaining their antimicrobial performance during the tests. First, their

chemical composition greatly influence their antibacterial capabilities and their chemical composition is greatly influenced by several factors, such as agricultural practices, climate, soil and chemo-type, partially explaining the differences observed among the extracts studied from different researchers regarding their antimicrobial potential (Nychas, 1995; Burt, 2004; Ballester-Costa et al., 2013; Stahl-Biskup, 2004). Secondly, the wide range of microbial-cell target sites that these extracts aim for is believed to be responsible for the inhibition of bacterial resistance development, along with the wide range of bacteria they are capable of killing (Burt, 2004; Nychas, 1995). However, in any case of bactericidal mechanism attributed to natural extracts, the killing substances always interact with the bacterial cell wall and membrane constituting them more effective against Gram + strains than Gram - (Nychas, 1995). In addition, plant extract disinfection potential is greatly reduced by the presence of protein or lipid loads (Mondello et al., 2003; Dorman & Deans, 1999; Nychas, 1995). Finally, the natural extracts are more effective against food borne pathogens and the isolated microbes from clinical practices are not behaving the same as the microbe coming from culture collections against disinfection agents (Burt, 2004; Fong et al., 2011; Abreu et al., 2013).

Proceeding to the discussion of the results obtained from this study, the test bacteria strain populations used here (isolated in the nosocomial environment) were substantially reduced from both *Thymus Vulgaris* oil and *Aloe Vera* extract. The decimal reductions achieved by the different concentrations and application times showed that the extracts have the potential to achieve ≥ 5 DR, similar to the commercial disinfectants, when higher concentrations will be used. As shown by the work of Fong et al., 2011, thyme oil achieved 5 DR against *E. coli* in a concentration similar to the one tested here (0,5% resulted in 2 DR), although the *E.coli* strains were isolated from human faeces and food sources and not from hospital environments. Additionally the same decimal reduction was achieved against *Staph. Aureus* but in a much higher concentration (2,5%), thus a comparison is not possible since the tested oil concentration against *St. Aureus* performed here was 5 times less. The differences between their results and the ones obtained here can be attributed mainly to the differently chemical compositions of thyme oil and the different sources of bacteria strains. However, based on unpublished results (Panoulis, 2014) obtained from research projects of the Laboratory of Food, Water and Environmental Microbiology in UoC, the maximum bacterial load that a contaminated surface in hospital environment normally bears is $10^2 - 10^3$. This finding along with the results obtained from this study indicate that the thyme essential oil can adequately reduce the bacterial load to levels of just few thousands cells.

The 5 DR goal can be more easily achieved, possibly, against the MRSA, *Staph. aureus*, *Acinetobacter baumannii* and *Escherichia coli*. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were more resistant to the extracts' disinfection abilities due to the fact that they are Gram negative bacteria, which in general are more resistant to disinfectants, form biofilms, which set penetration barriers (Russell, 2000), and more specifically they are more resistant in contrast to the other Gram (-) bacteria tested here, due to their cell wall lipid structure/composition (Nychas, 1995). However, this resistance was not an issue for the industrially synthesized disinfectants, indicating that these natural antimicrobial agents are not as effective against gram negative, biofilm forming bacteria with increased resistance.

Besides, a possible disinfectant produced from the tested plant extracts will not be intended to be used in surfaces that bear contaminants other than microbe load. This proposal is supported by the results of the antimicrobial screening in the presence of organic matter. Since the antimicrobial efficacy was hampered by the skim milk dilution, as proposed by the literature (Mondello et al., 2003; Dorman & Deans, 1999; Nychas, 1995), it is suggested that a surface with organic load (e.g. blood, urine, lipids etc) must be pre-treated with a cleaning procedure before disinfection with products from natural sources. Yet, the commercial disinfectants presented similar results in the presence of skimmed milk.

Only iodophors and phenolic compounds were effective against all bacterial strains under these conditions. From that it can be suggested that the potential products from natural materials can be used on inanimate surfaces with the same way as the rest disinfectants that failed to achieve 5 DR, yet are extensively used in nosocomial institutions.

Comparing the industrially synthesized disinfectants with the plants' extract it can be easily observed that the natural ones were less effective although the overall concentration of the tested solutions were higher (12.5 – 200 ppm against 5ml/L=5000ppm). However, the concentrations of active components in the plant extracts were much lower than the aforementioned dilution, explaining partially that difference (Burt, 2004; Ballester-Costa et al., 2013; Ceylan & Fung, 2004). Additionally, antagonistic effects may occur among the chemical substances contained in the extracts that potentially lower their disinfection ability (Ceylan & Fung, 2004; Burt, 2004).

Furthermore as it was expected, based on the extracts' chemical composition and the antimicrobial activity, Thyme was more effective than *Aloe*. This could be attributed on the main constituents of thyme (thymol, carvacrol, linalool) against the main constituents of *Aloe* gel (anthraquinones), suggesting that the first mentioned phenolics are more antimicrobial active, nonetheless no solid explanation can be given since the antimicrobial mechanisms of anthraquinones are not yet fully understood (Carol et al., 1996; Lawrence, Tripathi & Jeyakumar, 2009). Moreover, the smaller reduction on the antimicrobial efficacy that Aloe extract suffered when organic matter was present in comparison with the Thyme oil, indicate that Aloe may be provide a disinfection solution against biofilm forming bacteria.

Generally the extracts presented greater antibacterial activity against Gram positive bacteria as compared to Gram negative as suggested by the literature review. The phenolic contents in Aloe extract (anthraquinones and simple phenols) and in thyme oil (terpenoids) although they present antimicrobial activity in both Gram (+) and (-) they seem to be more effective against Gram positive (Hamman, 2008; Nychas, 1995). Additionally, concerning the ethanol extract used in this study and the controversial data found in the literature regarding the Aloe gel antimicrobial activity when extracted with ethanol, the findings here are in line with the findings of Bawankar et al., 2013 that report substantial antimicrobial activity of Aloe ethanol extracts.

Further, the lack of similar studies that used the same screening method, nosocomial isolated bacterial strains and tested the antimicrobial efficacy in the presence of organic matter, isn't allowing to proceed further the discussion by comparing their findings with the findings here. Although, a lot of researches have been performed upon the bactericidal and bacteriostatic activity of *Thymus Vulgaris* essential oil few of them used the broth dilution method with bacteria cell viable count and none tested the oil in the presence on organic matter, as resulted from the literature review performed here. In addition, in the mass majority of papers the tested bacteria came from a culture collection, thus even if the methods and materials are the same as it this research the results can be comparable, because the culture collection strains respond differently to the presence of disinfectants in comparison with clinical isolated strains (Abreu et al., 2013). Regarding the antimicrobial activity of *Aloe Vera* extracts the available literature are much less, since the specific plant attracted the scientific attention only recently with much effort to be dedicated to its medicinal properties and not so much to its antimicrobial effects.

6. Conclusions

Although the results from the performed antimicrobial screening can be characterized as initial concerning the potential of these extracts to be used for actual disinfection purposes in nosocomial environments, several conclusions can be drawn with relative safety. Firstly, the tests showed clearly the potential they have as possible

disinfectants. The low concentrations they were tested along with the relative high decimal reductions they achieved to the bacteria populations in the deionized water dilutions showed that at least constitute a potential component of a future natural disinfection product. Of course extensive further research must be performed in order to scientifically prove that potential, but these results encourage in any case future research attempts and coupled with the need for innovations into the food/agricultural field, their study can be characterized as necessary, since they can potentially promote its interests.

Secondly, in comparison with the commercial disinfectants the results concluded that they were much for efficient than the natural extracts in the tested concentrations. However, the potential benefits from them along with the drawbacks, that each commercial disinfectant possess, and the general concern for environmental protection, could be important motivators towards the pursuit of developing such products, which could, eventually, safeguard and promote the public health.

7. Proposals for future research

Regarding the continuation of the present research, the same plant extracts should be screened antimicrobial with other methods, estimating the minimum inhibitory and bactericidal concentration, along with the concentration that present the maximum antimicrobial effect against the specific bacterial strains. Additionally, more bacterial strains and other microorganism should be included in future researches against more plant extracts with promising antimicrobial attributes.

A very interesting thing to explore would be the antimicrobial efficacy and the possible synergistic effects between these two plant extracts. The high water content of Aloe gel could be the water based solution that the thyme's essential oil could be diluted in, resulting, possibly, to a disinfection solution with enhanced antimicrobial activity, both in presence and absence of organic matter.

Another field of study that these extracts deserve to be examined is the potential preservation effect they offer, when used as decontaminant of fresh or minimally processed food surfaces. Initially, the research should be conducted in-vitro and include food borne pathogens and spoilage microorganisms in food matrices. In a next level the tests should be performed in-vivo upon actual food products and storage conditions.

Finally, the disinfection activity of these extracts should be examined in food production environments aiming to disinfect elements of the environments itself, following similar experimentation methods.

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10. Popular scientific summary

New disinfectants for hospitals from Thyme and Aloe Vera; Can they be as good as the chemical ones?

Although effective, the disinfectants used in hospitals nowadays are coming with a number of drawbacks. Their negative environmental impact, the health hazards they bear for patients and hospital personnel along with other issues such as the development of bacterial resistance and budget restraints; push hygienists to find new, alternative ways to disinfect hospital environments. One possible way may be the use of thyme essential oil and Aloe Vera gel extract. Here the two extracts were tested against bacteria commonly found in hospital environments, which are responsible for over the half of the infections acquired by patients. The results showed that they have the potential to disinfect hospital surfaces, but in high concentrations and not in the presence of organic materials, such as proteins and fats. Although, the specific plant extracts performed lower than the chemical disinfectants, the low tested concentrations and their chemical composition, which can vary greatly, can explain that differences. Specifically when the chemical disinfectants managed to reduce in average the bacteria population by $>5 \log_{10}$ at their recommended concentrations, the thyme oil achieved a reduction of $2.1 \log_{10}$ in a solution of only 0.5% oil concentration. Aloe Vera, on the other hand, achieved lower reductions ($1,8 \log_{10}$), but it was remarkably active against specific bacteria strains.

These findings could initiate the development of disinfection products that are based completely or partially on natural materials, which are mostly used as food or food flavourings, introducing new innovative ways of their use. This could mean the opening of new market segments to the agricultural and food industry. Natural disinfectants may serve as an additional tool to protect public health, contributing to the reduction of hospital infections. These infections lead to increased rates of deaths among patients, especially in ICUs, prolongation of treatment time and as a consequence the health care costs are greatly increased. Also, they provide an alternative to the traditional chemical disinfectants that it is not as expensive or toxic as other alternative disinfection methods, such as ozone and steam. Additionally, natural disinfectants may serve public health from another post. They can disinfect environments and equipments used for food manufacturing and handling, resulting to the minimization of food borne illnesses. Finally, their application may be expanded to the disinfection of foods' surfaces leading to prolonged preservation times and to all the associated benefits.

The first step to evaluate the disinfection potential of Thyme and Aloe Vera was to select the microorganisms that they were going to be tested against and the chemical disinfectants to compare with. Six bacteria were isolated from an actual hospital environment in order to better imitate the conditions the disinfectants face in real disinfection scenarios. These bacteria were, then, cultivated and when they reached the wanted population, they were dispensed in small testing tubes along with de-ionized water or skimmed milk and an amount of the natural extracts. After certain times the bacteria populations were counted on plates in order to see how many were killed by the extracts. The addition of skimmed milk was done in order to replicate the scenario, where the disinfectants are applied on hospital surfaces where blood or other organic tissues are present and see how they will perform. This whole experiment was repeated for the chemical disinfectants and the obtained results were compared with the results from the plant extracts.

Of course much more are need to be done in order to come up with an effective disinfectant from natural sources. First, higher concentrations must be tested against a wider range of microorganisms. And secondly, different extracts of the same type of plants, but from different geographical regions, agricultural practices and climates, need to be evaluated, because their chemical composition may vary considerably.

