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„Biological Properties of Terpinen-4-ol and α-Terpineol“

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# Abstract

Terpineols are naturally occurring unsaturated monocyclic monoterpenoid tertiary alcohols. There are five common isomers of terpineols, namely alpha-, beta-, gamma-, delta- and terpinen-4-ol, of which terpinen-4-ol and α-terpineol are the most common terpineols found in nature. Terpinen-4-ol is considered as the major active component (30 % to 40 %) of *Melaleuca alternifolia* (tea tree oil), whereas α-terpineol can be isolated from various essential oils, such as pine oil. Both, terpinen-4-ol and α-terpineol are believed to be important commercial products and play an important role in the industrial field. Moreover, it is well known that α-terpineol has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics and flavors.

In addition, in the last few years terpinen-4-ol and its isomer α-terpineol attract a great interest of more and more scientific studies, as they exert different and a wide range of biological properties on human and animals. The focus of this review, which comprises the scientific studies mainly from 2000 up to 2014, is directed to the biological effects as the antimicrobial, anti-inflammatory, antioxidative, anticancer, anticonvulsant, antiulcer, antihypertensive, skin penetration enhancing properties of both terpinen-4-ol and α-terpineol and their use in the medicinal field.

Collectively, the medicinal application and the use of terpinen-4-ol and α-terpineol in the pharmaceutical industry has become very popular and in consequence they are gaining more and more importance in the future in this field.

# Zussamenfassung

Terpineole sind natürlich vorkommende, ungesättigte monocyclische monoterpenoide, teritäre Alkohole. Es gibt fünf strukturisomere Terpineole: alpha-, beta-, gamma-, delta- und Terpinen-4-ol, von denen Terpinen-4-ol und α-Terpineol die häufigsten vorkommenden Terpineole in der Natur sind. Terpinen-4-ol ist der Hauptbestandteil (30% bis 45%) des Teebaumöls (*M. Alternifolia*), α-Terpineol kann aus verschiedenen ätherischen Ölen isoliert werden, z.B. Terpentinöl. Terpinen-4-ol and α-Terpineol sind eigentlich wichtige Handelsprodukte, die eine große Rolle im Bereich der Industrie spielen. Zusätzlich ist es schon lange bekannt, dass α-Terpineol einen äußert intensiven Geruch nach Flieder aufweist, infolgendessen wird α-Terpineol in Kosmetischen Produkten, Seifen und in Parfums verwendet.

Terpinen-4-ol und dessen Isomer (α-Terpineol) gewinnen in den letzten Jahren ein großes Interesse von immer mehr wissenschaftlichen Studien, da sie verschiedene biologische Eigenschaften und Wirkungen auf Mensch und Tier ausüben. Der Schwerpunkt dieser Arbeit, in der die wissenschaftlichen Berichte von 2000 bis 2014 zusammengefasst worden sind, liegt vor allem auf den biologischen Wirkungen wie antimikrobielle, entzündungshemmende, antioxidative, anti-tumor, antikonvulsive, anti-ulcus, antihypertensive, ebenso auf der Eigenschaften von Terpinen-4-ol bzw. α-Terpineol und deren künftige Verwendung im medizinischen Bereich, wie Z.B. Hautpenetrationsförderung und als Insektenschutzmittel.

Zusammenfassend sind die medizinischen Anwendungen und Auswirkungen von Terpinen-4-ol und α-Terpineol in der pharmazeutischen Industrie sehr populär geworden, daher werden sie in der Zukunft in diesem Bereich mehr und mehr an Bedeutung gewinnen.

# Abbreviations

**BHA** Butylated hydroxanisole

**CCCP** Carbonyl cyanide m-chlorophenylhydrazone

**CVD** Cardiovascular disease

**DA** Dopamine

**DMSO** Dimethyl sulfoxide

**DOCA** Deoxycorticosterone-acetate

**DPPH** 2,2-Diphenyl-1-picryl-hydrazyl

**DSC** Differential scanning colorimetry

**EDTA** Ethylenediaminetetraacetic acid

**EDC** (1-Ethyl-3-(3-dimethylamino) propyl) carbodiimide

**EEG** Electroencephalographic

**ELISA** Enzyme-linked immunosorbent assay

**eNOS** Endothelial nitric oxide synthase

**EOAZ** Essential oil of *Alpinia zerumbet*

**EOs** Essential oils

**ERK** Extracellular signal-regulated kinase

**FMLP** N-formyl-methionyl-leucyl-phenylalanine

**FT-IR** Fourier transform infrared spectroscopy

**FLU** Flumazenil

**GABA** Gamma-aminobutyric acid

**HSV** Herpes simplex virus

**IL-1** Interleukin-1

**IL-2** Interleukin-2

**IL-4** Interleukin-4

**IL-6** Interleukin-6

**IL-8** Interleukin-8

**IL-10** Interleukin-10

**iNOS** Induciable nitric oxide synthase

**L-NAME** L-nitro arginine methyl ester

**LPS** Lipopolysaccharide

**MAP** Mean aortic pressure

**MAPK** Mitogen-activated protein kinase

**MBC** Minimum bacterial concentration

**MDCK** Madin-Darby canine kidney

**MES** Maximal electroshock seizures

**MFC** Minimum fungicidal concentration

**MIC** Minimum inhibitory concentration

**MRSA** Methicillian-resistant *Staphylococcus aureus*

**NF-κB** Nuclear factor kappa β

**NOS** Nitric oxide synthase

**NSCLC** Nonsmall cell lung carcinoma

**ORAC** Oxygen Radical Absorbance Capacity

**PBS** Phosphate-buffered solution

**PBMCs** Peripheral blood mononuclear cells

**$PGE_2$** Prostaglandin $E_2$

| | |
|---|---|
| **PI** | Propidium iodide |
| **PMA** | Phorbol 12-myristate 13-acetate |
| **PMBN** | Polymyxin B nonapeptide |
| **SC** | Stratum corneum |
| **α- T** | Alpha terpineol |
| **T-4-ol** | Terpinen-4-ol |
| **TNF-α** | Tumor necrosis factor-alpha |
| **TRPV1** | Transient receptor potential vanilloid 1 |
| **TTO** | Tea tree oil |
| **VFA** | *In vitro* fermentation assay |

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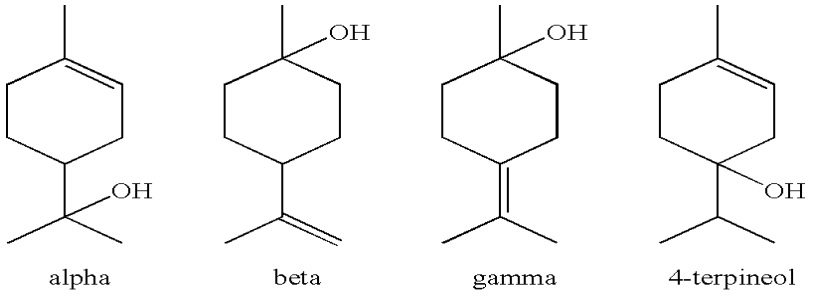
# 1. Introduction

## 1.1. Terpineols:

Terpineols are naturally occurring unsaturated monocyclic mono-terpenoid alcohols [1], and can be found in flowers such as narcissus and freesia, in herbs, such as marjoram, oregano, rosemary and in the oil expressed from the peels of lemons. Reports on the level of terpenoids in oils occasionally vary considerably and one wonders how much this is due to variation in the plants and to variations in the isolation process since terpineols could also be an artifact [2]. Terpineols have a pleasant odor similar to lilac and are common ingredients in perfumes, cosmetics and flavors. In addition, terpineols are an interesting group of terpenic alcohols on account of their wide range of biological properties [3].

There are five common isomers of terpineols; alpha-, beta-, gamma-, delta- and terpinen-4-ol (T-4-ol).




4 Terpineol isomers

α- and β- Terpineol occur in optically active forms and as a racemate. Both, α-terpineol and T-4-ol are the most important commercial products and they occur in a large number of essential oils. On the other hand, β-, γ- and δ- terpineols do not occur widely in nature [1].

## 1.2. Terpinen-4-ol (T-4-ol)

Terpinen-4-ol occurs as (+)-, (-)- and racemic terpinene-4-ol in many essential oils, such as *Melaleuca alternifolia* Cheel. (Myrtaceae) (tea tree oil). T-4-ol possesses a variety of medicinal properties such as antiviral, antibacterial, antifungal effects as well as antioxidant and anti-inflammatory activities. Terpinen-4-ol is found also in lavender oil and its racemic form can be isolated from pinus and eucalyptus species [1].

Terpinen-4-ol is a colorless liquid with a spicy, nutmeg like, woody-earthy odor and also with a pleasant note similar to lilac [1]. The nutmeg like odor is due to the presence of terpinen-4-ol in a high concentration in the essential oil of the evergreen tree *Myristica fragrans,* L.(Myristicacea*)* which is the main source of spices as nutmeg and mace [1, 4]. Additionally, T-4-ol is considered as a fragrance chemical possessing a pleasing earthy-green note with a slightly peppery-woody undernote. Therefore, it is believed that terpinen-4-ol possesses a desirable effect in all fragrance compositions to enhance naturalness and diffusiveness [5].

## 1.3. α-Terpineol (α-T)

α-Terpineol is also a monocyclic monoterpene tertiary alcohol which can be isolated from a variety of sources such as cajeput oil, pine oil and petitgrain oil [3]. α-T is a colorless, crystalline solid, smelling of lilac [1], and is an optically active monoterpenoid that occurs naturally in the (+)-, (-)- and (±) forms. The presence of natural racemic mixtures of α-T were discovered in geranium oils and in Morio-Muscat-wine aroma. α-T enantiomers which are found in the *Myrtaceae* family, in citrus and lavender oil, were separated by the means of a two-columned coupled system and a mixture of two chiral phases, respectively [6].

Because of its pleasant odor similar to lilac, α-T is widely used in the manufacture of cosmetics, soaps, perfumes, antiseptic agents and is

considered one of the most frequently used fragrance compounds as well as its acetate and other simple esters of α-T are also used in perfumery and flavourings. Therefore, the most important reaction for the fragrance industry is its esterification particularly the acetylation to terpinyl acetate [1, 2, 7]. In addition, α-T possesses a wide range of biological activities which attract a great interest in the medicinal field [7].

Terpineols, especially the most commonly used compounds as T-4-ol and α-T, exert different and a wide range of biological actions on humans, animals, and also plants. They are not only popular fragrance ingredients used in perfumes, cosmetics and household cleaning products as well as being used for flavoring food and drink, but also they possess various important biological and medicinal properties [8, 9]. These medicinal properties are being practiced upon since ancient times in human history, due to their presence in different types of essential oils, which were traditionally used. Scientists from all over the world are trying to characterize the range of these biological activities of terpineols which include antimicrobial, antiviral, anticancer, antioxidant, anti-inflammatory, antiprotozoal and antimutagenic ones [10].

## 2. Important biological properties of terpinen-4-ol (T-4-ol):

Tea tree oil (TTO), or melaleuca oil, is a volatile essential oil derived mainly from *Melaleuca alternifolia* Cheel. (Myrtaceae), which is native to southeast Queensland and the northeast coast of New South Wales, Australia. TTO is incorporated as an active component in many topical formulations used in treatment of cutaneous infections. It is also widely available over the counter (OTC) as a remedy for various ailments [8]. The essential oil is steam distilled from the Australian native plant *M. alternifolia* and it is used usually topically due to its great antimicrobial and anti-inflammatory effects [11].

TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their related alcohols, and its composition is regulated by the international standard ISO 4730:2004. TTO has a relative density of 0.885 to 0.906. It is only sparingly soluble in water and can be miscible with nonpolar solvents. The MICs of tea tree oil are typically between 0.125 and 2 % (vol/vol), and bactericidal activity is largely attributable to nonspecific membrane effects. The composition of TTO, which is commercially sold, is regulated by an international standard for "Oil of Melaleuca–terpinen-4-ol type". It sets maxima and/or minima for 14 components of the oil (Table 2.1.), in which T-4-ol is considered as its major active component (30% to 45%). Six varieties, or chemotypes of *M. alternifolia* have been known, each producing an oil with a distinct chemical composition. They are classified into a T-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes. The T-4-ol chemotype is the one used in commercial TTO production [8, 11].

**Table 2.1** "Composition of *M. alternifolia* (tea tree) oil" (adapted and newly drawn from Carson *et al*. 2006 [8]).

| ***Component*** | ***Typical Composition*** | ***Composition ISO 4730 range %*** |
|---|---|---|
| Terpinen-4-ol | 40.1 | ≥ 30 |
| γ-Terpinene | 23.0 | 10-28 |
| α-Terpinene | 10.4 | 5-13 |
| 1,8-Cineole | 5.1 | ≤15 |
| Terpinolene | 3.1 | 1.5-5 |

| | | |
|---|---|---|
| ρ-Cymene | 2.9 | 0.5-12 |
| α-Pinene | 2.6 | 1-6 |
| α-Terpineol | 2.4 | 1.5-8 |
| Aromadendrene | 1.5 | Trace-7 |
| δ-Cadinene | 1.3 | Trace-8 |
| Limonene | 1.0 | 0.5-4 |
| Sabinene | 0.2 | Trace-3.5 |
| Globulol | 0.2 | Trace-3 |
| Viridiflorol | 0.1 | Trace-1.5 |

TTO and T-4-ol have been shown to possess many biological activities [4]. T-4-ol as the most abundant component of TTO is likely a mediator of the *in vitro* and *in vivo* efficacy of TTO [12]. Although the biological properties of TTO are increasingly well characterized, limited data are still available on the toxicity and safety of this oil. Anecdotal evidence from almost 80 years of use suggests that topically used TTO is relatively safe, and that adverse effects are considered to be very low, occasional and self-limiting. Published data indicate that this oil may be toxic if ingested in higher doses and at high concentrations can also cause skin irritation. Moreover, allergic reactions to this oil occur mainly in predisposed individuals and may be due to the different oxidation products that are formed by exposure of TTO to light and/or air. Adverse reactions may be reduced by avoiding ingestion, applying only the diluted form of TTO topically and using an oil which has been stored correctly. However, TTO and its constituents are not genotoxic, it was indicated

according to the available limited ecotoxicity data that the oil is toxic to some insect species but further studies are needed [13].

## 2.1. Antimicrobial activity

Of all of the properties claimed for TTO, its antimicrobial activity has received the most attention. The antimicrobial activity of TTO is attributed mainly to T-4-ol and α-T which possesses a lower effect than T-4-ol. Consequently, the antimicrobial activity of TTO can be optimized, a lower limit of 30 % and no upper limit were set for the content of T-4-ol. Crushed leaves of "tea tree" were used to be inhaled to treat colds and coughs. They were also sprinkled on wounds, after applying a poultice. In addition, tea tree leaves could be soaked to make an infusion which can be used in treatment of skin ailments or sore throat. The first reports of its microbial activity were published in the 1920s and 1930s by Penfold [8].

### 2.1.1. Antibacterial activity.

TTO shows a broad spectrum of inhibitory activity against various Gram-positive and Gram-negative bacterial pathogens as well as yeast [10, 14]. It was also proven, that T-4-ol displays a greater efficacy than TTO. T-4-ol is also effective against drug sensitive as well as drug resistant strains [10]. Many findings indicate that TTO can be less active *in vitro* than T-4-ol alone and also suggest that the presence of a non-aqueous phase in TTO formulations may limit the microbial availability of its active component [15].

A broad range of bacteria have shown susceptibility to TTO, these are summarized in Table 2.2. Most bacteria are susceptible to the antibacterial effect of TTO at concentrations of 1-0 % or less, however, MICs higher than 2 % have been reported for organisms such as commensal skin staphylococci and micro-cocci, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, and drug resistant *P. aeruginosa*.

TTO and its major component T-4-ol are bactericidal in nature, although they may be only bacteriostatic at lower concentrations [8].

**Table 2.2.** **"**Susceptibilty data for bacteria tested against *M. alternifolia* Oil**" (**adapted and newly drawn from Carson *et al*. 2006 [8]).

| ***Bacterial species*** | ***% (vol/vol)*** ***MBC*** | ***% (vol/vol)*** ***MIC*** |
|---|---|---|
| *Acinetobacter baumannii* | 1 | 1 |
| *Actinomyces viscosus* | > 0.6 | 0.6 |
| *Actinomyces* spp. | 1 | 1 |
| *Bacillus cereus* | | 0.3 |
| *Bacteroides* spp. | 0.06-0.12 | 0.06-0.5 |
| *Corynebacterium* sp. | 2 | 0.2-2 |
| *Enterococcus faecalis* | >8 | 0.5->8 |
| *Enterococcus faecium* | 0.5-1 | 0.5-1 |
| *Escherichia coli* | 0.25-4 | 0.08-2 |
| *Fusobacterium nucleatum* | 0.25 | 0.6->0.6 |
| *Klebsiella pneumoniae* | 0.25 | 0.25-0.3 |
| *Lactobacillus* spp. | 2 | 1-2 |

| | | |
|---|---|---|
| *Micrococcus luteus* | 0.25-6 | 0.06-0.5 |
| *Peptostreptococcus anaerobius* | 0.03->0.6 | 0.2-0.25 |
| *Porphyromonas endodentalis* | 0.025-0.1 | 0.025-0.1 |
| *Porphyromonas gingivalis* | 0.13->0.6 | 0.11-0.25 |
| *Prevotella* spp. | 0.03 | 0.03-0.25 |
| *Prevotella intermedia* | 0.003-0.1 | 0.003-0.1 |
| *Propionibacterium acnes* | 0.5 | 0.05-0.63 |
| *Proteus vulgaris* | 4 | 0.08-2 |
| *Pseudomonas aeruginosa* | 2->8 | 1-8 |
| *Staphylococcus aureus* | 1-2 | 0.5-1.25 |
| *S. aureus* (methicillin resistant) | 0.5 | 0.04-0.35 |
| *Staphylococcus epidermidis* | 4 | 0.45-1.25 |
| *Staphylococcus hominis* | 4 | 0.5 |
| *Streptococcus pyogenes* | 0.25-4 | 0.12-2 |
| *Veillonella* spp. | 0.03-1 | 0.016-1 |

The activity of T-4-ol against antibiotic resistant bacteria has attracted considerable interest, with methicillin-resistant *S. aureus* (MRSA) receiving the most attention thus for. In addition, the vaporized TTO can also inhibit bacteria such as *Mycobacterium avium* ATCC 4676, *Haemophilus influenza, E. coli, S. pneumonia and S. pyogenes. Moreover,* several anecdotal reports suggest that the aerosolized TTO may help in reducing hospital-acquired infections but no specific data [8, 10].

**Mechanism of antibacterial action:**

The mechanism of action of TTO against bacteria has been partly elucidated. Assumptions about its mechanism of action were made on the basis of its hydrocarbon structure and attendant lipophilicity and hence its easily permeability through the cell wall and cell membrane [8, 10]. Since hydrocarbons partition itself preferentially into biological membranes and disrupt their vital functions, T-4-ol was also presumed to behave in this manner. This premise is further supported by data showing that TTO permeates into the modal liposomal system [8]. It is stated by several scientists that T-4-ol increases cytoplasmic membrane fluidity and permeability, disturbs the order of membrane embedded proteins, inhibits cell respiration, and alters the ion transport process [16]. Interactions of TTO components with polysaccharides, fatty acids, and phospholipids render the bacterial membranes more permeable, so that loss of ions and cellular contents lead to cell death. Similarly, interference in proton pump activity, loss of membrane integrity, leakage of the cellular contents can result in loss of viability. Other important mechanisms of action include denaturation of cytoplasmic proteins and inactivation of cellular enzymes leading to bacterial cell death [10, 17].

**a) Treatment of *S. aureus* with TTO and its major component terpinen-4-ol.**

*S. aureus* is one of the most important Gram-positive bacteria in humans, causing localized or generalized septic infections [16]. *„ Infections*

*caused by the bacterial pathogen S. aureus are attributed to a majority rate comparable to the deaths caused by HIV/AIDS, tuberculosis and viral hepatitis combined in the United States. The relatively high incidence of infections caused by multiple antibiotic-resistant methicillin-resistant S. aureus (MRSA) strains remains a major concern within the medical community"*[18]. MRSA strains usually colonize the anterior noses of hospital patients and healthy individuals and cause epidemics in hospital [16]. For instance, in 2005, it was estimated that about 14 million healthcare visits for suspected skin and soft tissues infections were only caused by *S. aureus* in which MRSA could cause a high percentage of these infections. *S. aureus* and MRSA are susceptible to TTO and T-4-ol and has been known that TTO is also efficacious in the decolonization of MRSA carriers [18, 19]. Loughlin *et al.* studied the antimicrobial activity of T-4-ol and TTO against clinical skin isolates of MRSA and coagulase-negative Staphylococci (CoNS) and their toxicity against human fibroblast cells were examined by using broth microdilution and quantitative *in vitro* time-kill test methods. As a result, T-4-ol exhibited significantly a greater bacteriostatic and bactericidal activity than TTO against both CoNS isolates and MRSA. T-4-ol has been shown to be non-toxic against human fibroblasts over the 24h test period [20].

In conclusion, these results showed that T-4-ol is more potent than its crude oil against MRSA and CoNS isolates with neither agents exhibits toxicity to fibroblast cells. Therefore, T-4-ol can be used as a single agent in several products formulated for topical treatment of MRSA infections [20].

TTO is successfully used worldwide in nursing and to treat certain local bacterial infections, because it reveals a high antibacterial activity against *S. aureus in vitro* and *in vivo* [16]. The assumptions regarding the mechanism of action of TTO when treated with *S. aureus* have been based on the nature of its major component T-4-ol. In a study, *S. aureus* cells were killed by TTO and its components in the stationary phase of

growth. Generally, organisms in this growth phase are less sensitive to be injured than those in the exponential one. Since antimicrobial agents which affect cellular synthetic processes often have little effects on organisms in the stationary phase of growth it is revealed by these results that the principal target of TTO is not a macromolecular synthetic process. The failure of TTO or T-4-ol and α-T by measurement of the optical density at 620 nm to lyse *S. aureus* cells indicates that their primary mechanism of action is not due to a gross cell wall damage. Furthermore, treatment–induced release of membrane-bound cell wall will induce lysis by autolytic enzymes eventually. Although the activation of autolytic enzymes is believed to be responsible for this effect, cell wall weakness and subsequent rupture of the cytoplasmic membrane due to the osmotic pressure may also affect lysis [18, 19]. *„ S. aureus suspensions when treated with TTO or particularly with T-4-ol lost significant 260-nm-absorbing material, suggesting that nucleic acids were lost by means of a damaged cytoplasmic membrane. Sublethal injury of microbial cell membranes may alter their permeability and affect the membrane's ability to osmoregulate the cell adequately or to exclude toxic materials. Consequently, the loss of tolerance to salts or other potentially toxic compounds may be exploited to reveal membrane damage in sublethally injured bacteria. Treatment of S. aureus with TTO or T-4-ol significantly reduced the ability of the survivors to form colonies on media containing NaCl. Additionally, T-4-ol was found to be unable to lyse S. aureus and the suggested membrane damage occured by the appearance of mesosomes and a loss of cytoplasmic material. These lesions have been reported previously after treatment with antimicrobial agents such as vancomycin"*[19].

The original premise was that T-4-ol acts on the microbial membranes, leading to the loss of 260-nm-absorbing material, the formulation of mesosomes, increased susceptibility to NaCl and the loss of cytoplasmic material when treated with TTO and T-4-ol. Additionally, another study supports this idea showing that treatment of *S. aureus* with

TTO induces the loss of potassium ions, promotes the uptake of propidium iodide and inhibits respiration. [8, 19].

**b) Treatment of *E. coli* with TTO and T-4-ol.**

*„E. coli is a Gram-negative, rod-shaped bacterium which is commonly found in the lower intestine of warm-blooded organisms (endotherms). E. coli and other facultative anaerobes constitute about 0.1 % of gut flora, and the fecal-oral transmission is the major route by which pathogenic strains of the bacteria cause disease"*[21].

In *E. coli*, when it is treated with TTO or T-4-ol, detrimental effects on potassium homeostasis (stimulated the leakage of intracellular $K^+$ ions), respiration, morphology, and ability to exclude propidium iodide have been observed [8]. In an electron microscopic study, *E. coli* cells cultivated in the presence of TTO or T-4-ol (MIC: 0.25%) showed cytoplasmic losses similar to *S. aureus*, as well as the formation of extracellular blebs [16]. Moreover, a modest loss of 280-nm-light absorbing material has been reported. Lysis also occurs in *E. coli* treated with TTO and its major component T-4-ol, in contrast to the absence of whole-cell-lysis found in *S. aureus* treated with TTO. On this basis, TTO compromises the functional and structural integrity of bacterial membranes [8, 19].

**c) Treatment of *Pseudomonas aeruginosa* with TTO and T-4-ol.**

*Pseudomonas aeruginosa*, a Gram-negative bacterium, is an opportunistic pathogen notable for its high level of resistance to antimicrobial agents and it remains a major causative agent of nosocomial infections [22, 23]. *P. aeruginosa* is less susceptible than most bacterial species to TTO and T-4-ol, with MICs ranging from 1 to 8%, compared to a range from 0.06 to 0.5 % (v/v) for *E. coli*, *S. aureus* and *Streptococcus spp*. [22, 24]. Several mechanisms may faciliate this reduced susceptibility, including reduced outer membrane permeability and active efflux systems [24].

The activity of TTO and T-4-ol against *P.aeruginosa* may be enhanced by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) which depolarizes the cytoplasmic membrane [22]. The pretreated *P. aeruginosa* cells with outer membrane permeabilizers such as EDTA or polymixin B non-apeptide (PMBN) are supposed to be more susceptibile to the bactericidal effects of TTO and T-4-ol [8]. However, it is thought that the exact mechanism of action of PMBN is by binding to lipopolysaccharide (LPS) without causing its release. In contrary, EDTA is able by means of chelating divalent actions to disrupt their integrity of the outer membrane and releasing LPS. As a result, the pre-treatment with PMBN or EDTA and their role in enhancement of the bactericidal activity of TTO and/or T-4-ol indicate that at least one or more target sites for this oil lie within the cell [24].

*„ In general, P. aeruginosa and Pseudomonas spp. are known for their involvement in nosocomial infections and their incidence of resistance to antibiotics. Complementary or alternative treatments for Pseudomonas skin and wound infections that fall outside the realm of conventional antibiotics are needed. TTO is emerging as an alternative antimicrobial agent that is safe for topical applications. A large number of products containing TTO or T-4-ol as active antimicrobial agent are available for wound management or hand-washing containing 5-10% (w/v) TTO. Given that the $MBC_{90}$ of TTO for P.aeruginosa was approximately 4 %, it is possible that the use of such topical agents in both the treatment of wounds and other skin washing situations may be beneficial in preventing and reducing infection and transmission"*[23].

**d) Antimicrobial activity of TTO and T-4-ol against *Campylobacter jejuni*.**

*Campylobacter jejuni* is a Gram-negative bacterium considered to be a common cause of human gastrointestinal illness, and a precursor of gastric ulcer resulting in bloody diarrhea, abdominal cramps, fever and vomiting [25]. Although in most subjects, the mortality rate is low and the

illness simply can be treated with antibiotics, an infection in the elderly and in young children may be severe. Moreover, Guillain-Barré syndrome and reactive arthritis are rare post-infection complications of this illness [26]. Chickens are healthy carriers of *C. jejuni* and have been reported to be the main vehicle of human infection based on epidemiological evidence [27]. Once a chicken is infected in a commercial poultry flock, *C. jejuni* spreads rapidly to all birds in the flock. Prevalence of *C. jejuni* in the chicken carcass can be high due to contamination from intestinal contents during slaughter [28]. Several strategies have been employed to control or reduce the spread of *Campylobacter* spp. in poultry including biosecurity measures, competitive explosion in the gut, vaccination and bacteriophage therapy, but none of these attempts proved to be effective in commercial flocks. Furthermore, there is a rising public concern regarding the use of antibiotics as a feed additive in animal production systems. In response to such concerns, the European Union banned the use of antibiotics as a growth promoter in animal nutrition as a part of their regulation (EC) 1831/2003 in 2006 [29]. However, it has been reported that the withdrawal of antibiotics from animal foodstuffs can lead to reduction of animal productivity and an increase in the incidence of some bacterial diseases. In this regard, replacement compounds for antibiotics are urgently needed and the use of plant extracts in foodstuffs could be used as a strategy to reduce food-borne pathogens including *C. jejuni* [30].

TTO and T-4-ol show an antimicrobial activity against *C. jejuni*, *campylobacter coli*. According to Kurekci *et al*., using broth microdilution and disc diffusion assay, it was reported that, among the single compounds, T-4-ol showed the highest antimicrobial activity towards *Campylobacter* spp. Based on the antimicrobial activity of T-4-ol and α-tops (α-terpineol + cineole + terpinen-4-ol) which was tested using an *in vitro* fermentation assay that was developed to provide a suitable and controlled environment for the growth of chicken caeca microbiota including *Campylobacter* spp., it was proven that at a concentration of

0.05 % of all of these compounds demonstrated antimicrobial activity against *C. jejuni*. T-4-ol and α- tops had also no effect on gas production or VFA (*in vitro* fermentation assay) at the highest concentration tested (0.05 %). However, a higher dose level revealed that T-4-ol at the concentration of 0.1% reduced the total anaerobic bacteria in an *in vitro* fermentation system using pig jejuna flora [31].

In summary, TTO and T-4-ol possess strong anti-campylobacter activity without adversely affecting the fermentation potential of the chicken-caeca microbiota. It was found that T-4-ol has the potential to control *C. jejuni* colonization and abundance in poultry [31].

**e) Antibacterial activity of TTO and T-4-ol against bacteria from the respiratory tract.**

TTO and T-4-ol exert an antibacterial activity against many bacterial strains. TTO is one of the essential oils which can be used for the treatment of bacterial respiratory tract infections due to its secretolytic and secretomotoric properties. In many cases, several viral infections such as common colds and include sinusitis, tonsillitis, pneumonia and bronchitis may also develop into bacterial respiratory tract infections [16]. TTO and T-4-ol show a considerable antimicrobial activity against bacteria such as *S. pneumonia, S. pyogens,* and *Haemophilus influenza*, which are the most frequently isolated bacteria from the respiratory tract [10, 16].

**f) Antibacterial activity of TTO and T-4-ol against *Mycoplasma pneumonia*.**

*Mycoplasmas* are known as bacteria without a rigid cell wall. *M. pneumoniae* is spread all over the world causing frequent atypical courses of pneumonia, especially in adults between 30 and 35 years and children between 5 and 15 years. Moreover, various diseases as lung inflammations, arthritis, myocarditis, polyneuritis and other chronic

diseases may also result in causing pneumonia. The typical "pear shape" is the most common morphological shape of *M. pneumoniae* with the presence of a tip structure at only one end of the cell. It was found that when *M. pneumonia* was treated with TTO or T-4-ol, it loses their typical "pear-shaped" appearance and becomes rounded. On the other hand, the integrity of the cell membrane was not impaired by TTO. As a result, it was proven that all *Mycoplasma* species show high susceptibility against TTO and T-4-ol [16].

**Table 2.3.** "Susceptibility of different *Mycoplasma* species against TTO" (adapted and newly drawn from J. Reichling *et al.,* 2009 [16]).

| ***Bacteria*** | ***MIC, 10 %*** |
|---|---|
| *Mycoplasma pneumoniae* (2 isolates) | 0.01 |
| *Mycoplasma hominis* (26 isolates) | 0.06-0.12 |
| *Mycoplasma fermentans (6 isolates)* | 0.01-0.06 |

## 2.1.2 Antifungal activity

Comprehensive investigations of the susceptibility of fungi to TTO and terpinen-4-ol have only recently been completed [8]. It was found that TTO and T-4-ol exhibit strong antimicrobial properties against fungal biofilms [32]. Early data were largely limited to the susceptibility of *Candida albicans*, which was a commonly chosen model test organism, to TTO. But recent data show that a range of yeasts, dermatophytes, and other filamentous fungi are susceptible to TTO and T-4-ol, too.

**Table 2.4** "Susceptibility of different fungal species against TTO and T-4-ol  (adapted and newly drawn from C. Carson *et al.,* 2006 [8]).

| **Fungal species** | **% (vol/vol)**<br><br>**MFC** | **% (vol/vol)**<br><br>**MIC** |
|---|---|---|
| *Altenaria* spp. | 0.06-2 | 0.016-0.12 |
| *Aspergillus flavus* | 2-4 | 0.31-0.7 |
| *A. fumigatus* | 1-2 | 0.06->2 |
| *A. niger* | 2-8 | 0.016-0.4 |
| *Blastoschizomyces capitatus* | | 0.25 |
| *Candida albicans* | 0.12-1 | 0.06-8 |
| *C. glabrata* | 0.12-0.5 | 0.03-8 |
| *C. parapsilosis* | 0.12-0.5 | 0.03-0.5 |
| *C.tropicalis* | 0.25-0.5 | 0.12-2 |
| *Cladosporium* spp. | 0.12-4 | 0.008-0.12 |
| *Crytococcus neoformans* | | 0.0015-0.06 |

| | | |
|---|---|---|
| *Epidermophyton flocossum* | 0.12-0.25 | 0.008-0.7 |
| *Fusarium* spp. | 0.25-2 | 0.008-0.25 |
| *Malassezia furfur* | 0.5-1.0 | 0.03-0.12 |
| *Microsporum canis* | 0.25-0.5 | 0.03-0.5 |
| *M. gypseum* | 0.25-0.5 | 0.016-0.25 |
| *Penicillium* spp. | 0.5-2 | 0.03-0.06 |
| *M. sympodialis* | 0.06-0.12 | 0.016-0.12 |
| *Rhodotorula rubra* | 0.5 | 0.06 |
| *Saccharomyces cerevisiae* | 0.5 | 0.25 |
| *Trichophyton mentagrophytes* | 0.25-0.5 | 0.11-0.44 |
| *T. rubrum* | 0.25-1 | 0.03-0.6 |
| *T. tonsurans* | 0.12-0.5 | 0.004-0.016 |

| | | |
|---|---|---|
| *Trichosporon* spp. | 0.12 | 0.12-0.22 |

Generally by using different test methods, it was investigated that the fungicidal concentrations approximately range from 0.12 to 2% and MICs range between 0.03 and 0.05%. Notably, the minimal fungicidal concentrations (MFCs) of only *Aspergillus niger* was reported to be as high as 8%. According to subsequent assays, it was proven that germinated *Candida* is considerably more susceptible to TTO and T-4-ol than nongerminated *Candida*, proposing that the intact *Candida* wall confers a significant protection. In addition, it has also been demonstrated that the vapors of TTO is able to affect sporulation and inhibit fungal growth [8].

Studies investigating the mechanism of antifungal action of T-4-ol have focused almost exclusively on *C. albicans* [8]. In general, *Candida* species are currently the most common cause of fungal infections worldwide and the first Polish multicentre Candidaemia study revealed that the most frequent fungal pathogens is *C. albicans* [33, 34]. *„Many fungal species are harmless commensals or endosymbionts of hosts including humans. However, when mucosal barriers are disrupted or the immune system is comprised they can invade and cause disease. In the last 30 years there has been a significant increase in the incidence of fungal infections in humans"*[35]. A number of factors has been involved in this increased occurrence of fungal disease. Specific conditions of the organism and in particular during predisposing situations, such as diabetes, pregnancy, genetic factors and the increased and widespread use of certain medical practices, namely immunosuppressive therapies, invasive surgical procedures and the use of cortisones, contraceptives, estrogen and in particular broad-spectrum antibiotics are significant [33].

Members of *Candida* species cause significant problems in medicine and in many industrial branches also. In order to prevent

*Candida* spp. development, TTO and other essential oils can be frequently used as natural, non-toxic, non-pollutive and biodegradable agents with a broad spectrum of antimicrobial activity. TTO and T-4-ol can cause changes in cell and colony morphology, as well as in the metabolism of *C. albicans* [33].

**Oral candidiasis**

*C. albicans* is known as an opportunistic infectious microorganism, forms oral biofilms, which causes diseases such as oral candidiasis, which are difficult to treat with conventional antifungal agents [32, 36]. Because of the strong antimicrobial properties of T-4-ol against fungal biofilms, it has safety advantages over the complete essential TTO and may be suitable for prophylaxis and treatment of established *oropharyngeal candidosis*. *C. albicans* is susceptible to TTO, α-T and T-4-ol with an $MIC_{50}$ of 0.5, 0.25 and 0.25 % respectively. These three compounds also showed potent activity against the 69 biofilm-foaming strains, of which both T-4-ol and α-T demonstrated rapid kill kinetics [32].

*„C. albicans, especially Candida cells with hyphal growth, cause the oral candidiasis by invading oral mucosal tissues; the Candida-infected lesions are often accompanied by pain in the tongue, redness and swelling which greatly lower the quality of life of the patient"*[36]. Oral treatment with TTO and T-4-ol decreased the number of *Candida* cells found in the oral cavity and also improved *Candida* infected lesions on the tongue's surface, as well as it is suggested that the oral treatment with T-4-ol only inhibits the accumulation of inflammatory cells at the local sites in *Candida* infected tongue mucosa [37]. Thus, TTO and T-4-ol suppress hyphal growth of *C. albicans* at low concentrations, serve in oral candidiasis therapy, as well as possess anti-inflammatory activity [36, 38].

**Vaginal candidiasis**

The antifungal properties of TTO have been used with reference to the treatment of vaginal candidiasis. *C. albicans* is a prime agent of acute and recurrent forms of vulvovaginitis. Since T-4-ol has a significant curative effect on vaginal candidiasis *in vitro*, Mondello *et al.* demonstrated that *in vivo* T-4-ol could control *C. albicans* vaginal infections. It was found that T-4-ol is the most likely mediator of TTO *in vitro* and *in vivo*. $MIC_{50}$ and $MFC_{90}$ for T-4-ol were found to be lower than of those of TTO. Important to note that the $MICs_{90}$ were always lower even for resistant *C. albicans* strains. As a result, T-4-ol shows a rapid cytocidal acticity and it holds promise for the treatment of vaginal candidiasis, and particularly the azole-resistant forms [12].

**Mechanism of antifungal activity**.

Similar to the results found for bacteria, when treated with TTO and/or T-4-ol. They also alter the permeability of *C. albicans* cells [8]. The treatment of *C. albicans* with 0.25 % TTO caused after 30 min a reasonable uptake of propidium iodide [39], and after six hours a loss of 260-nm-light-absorbing materials and a significant staining with methylene blue has occurred. TTO and T-4-ol also alter the permeability of *C. glabrata* [40].

TTO also inhibits respiration in C. *albicans* in a dose-dependent manner [41], namely after treatment with 0.1% TTO by approximately 95% and after treatment with 0.25 % TTO by about 40%. Furthermore, at a concentration of 0.023 % TTO, the respiration rate of *Fusarium solani* is inhibited by 50% [8]. TTO can also inhibit glucose-induced medium acidification by *C. glabrata, C. albicans,* and *Saccharomyces cerevisiae* [40]. This medium acidification takes place by the expulsion of protons by the plasma membrane ATPase, which is filled by ATP acquired from the mitochondria. This inhibition indicates that the plasma and/or mitochondrial membranes are affected adversely. These results are in correspondence with a suggested mechanism of antifungal action, by which TTO causes damage or changes to the functioning of fungal

membranes [8]. Further studies have revealed that the treatment of *C. albicans cells* with diethyl stilbestrol inhibit the plasma membrane ATPase and as a result, they have a higher susceptibility to TTO than to control cells [40]. Probably the plasma membrane ATPase plays a role in protecting cells against the destabilizing or lethal effects of TTO [8].

According to the results of two different reports, it was shown that the formation of germ tube in *C. albicans* was completely inhibited in the presence of TTO at concentrations 0.25 and 0.125%. Furthermore, it was noticed that treatment with 0.125% TTO caused a trend of blastospores accompanied by a significant alteration in their morphologies from singly budding to multiply budding ones over the 4-h test period [42, 43]. Interestingly, it was also suggested that the inhibition of germ tube formation is reversible, due to the ability of cells to form germ tube after TTO being removed [43]. However, there was a noticeable delay in germ tube formation proposing that TTO exerts a post anti-fungal effect [8].

T-4-ol is reported to block *C. albicans* in S-phase of cell cycle due to its cell cycle inhibitory activities against *C. albicans* [10] on account the volatility and the hydrophobic properties of this alcohol. One solution was found to the problem to immobilize T-4-ol in a pharmaceutical carrier which plays an essential role for its stability and antibiofilm activity [44]. Lipid nanoparticles are alternative drug carriers and they have more advantages for drug loading in comparison to other particular carriers, such as the biodegradability and good tolerability. T-4-ol was immobilized in lipid nanoparticles by film sonication technology. The mechanism to improve the targeting ability to *C. albicans*, is by using the ability of the negative charges which are found on the cell surface of *C. albicans* to attract the positive charged nanoparticles after their modification with glycine. In consequence, the bactericidal activity of T-4-ol may increase against the biofilm. Meanwhile, the binding of these drug-related nanoparticles to the biofilm surface is by means of EDC (1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide) as a coupling reagent.

T-4-ol can be released gradually into the interior of cells by diffusion. Moreover, the antibiofilm activity of T-4-ol loaded lipid nanoparticles was verified to occur because of its capability to disrupt the structure of the cell membrane and blocking the respiration chain by means of succinate dehydrogenase inhibition which is bound to the inner cell mitochondrial membrane [44].

**Tinea pedis**

Since there has been an increasing interest in the use of natural therapies, TTO and T-4-ol attract a great interest and are already widely available in Australia for treatment of superficial infections such as tinea pedis. *„Tinea pedis is a dermatophyte infection of the feet or toes affecting 10 % of the population at any given time. It is most commonly caused by Trichophyton rubrum, T. mentagraphytes and Epidermophyton floccosum, and appears to be related to occlusive footwear. Tinea pedis occurs as one of four clinical cariants: intertriginous, populosquamous, vesicular and acute ulcerative. Chronic, intertriginous tinea pedis is characterized by a scaling and fissuring of the lateral toe webs caused by dermatophyte invasion of the stratum corneum; macerated, erosive infections may follow as a result of secondary overgrowth of commensal bacteria, including micrococcaceae (usually staphylococci), aerobic coryneforms and Gram-negative organisms"*[45]. The minimum inhibitory concentration of TTO for *T. rubrum* is 1.0% (v/v) and *T. mentagrophytes* 0.3-0.4% (v/v) [45, 46].

Satchell *et al*. demonstrated that 10% TTO cream including T-4-ol was clinically effective in improving the tinea. On the other hand, the mycological cure rate was not found to be much better than placebo. Therefore, the concentrations of TTO have been increased to 25% and 50% of TTO prepared in solution instead of a cream due to the immiscibility of TTO in aqueous media, in order to be more able to improve the mycological cure rate. This solution was applied twice daily to affected areas for 4 weeks and, as a result, a clinical response was

marked in 68% of the 50% TTO group and 72% of the 25% TTO, compared to 39% in the placebo group. Therefore, 25% TTO is recommended for the treatment of patients with tinea pedis due to the antifungal activity of T-4-ol [45].

**Onychomycosis**

Flores *et al.* suggested that TTO and T-4-ol are effective in treating onychomycosis. The antifungal efficacy of nano-capsules and nano-emulsions containing TTO was evaluated against *T. rubrum* in two different *in vitro* models of dermatophyte nail infection. As a result fungal free areas were obtained 2.88 ± 2.08 $mm^2$, 14.59 ± 2.01 $mm^2$, 40.98 ± 2.76 $mm^2$, and 38.72 ± 1.22 $mm^2$ for the nano-capsules containing TTO, nano-emulsions containing TTO, emulsion and untreated nail, respectively. The ability of the formulations to reduce *T. rubrum* growth was demonstrated by using nail infection models. As a result, the inclusion of oil in nano-capsules was found to be the most efficient way [46].

**Dandruff**

Dandruff appears to be related to the yeast *Pityrosporum ovale*. Since the antifungal properties of T-4-ol and TTO are well known, it was suggested to use this antifungal activity against *P. ovale* and in the treatment of dandruff. 5% TTO shampoo was used daily for 4 weeks in a comparison to a placebo group, and showed 41% improvement compared to 11% in the group of placebo. Reasonable improvements were also statistically noticed in the total severity score, the total area of involvement score, as well as the itchiness and greasiness compounds of the patients. As a conclusion, 5% TTO appears to be effective and well tolerated in the treatment of dandruff [47].

### 2.1.3 Antiviral activity

*„A virus is a small infectious particle (20-300 nm) that is able to infect cells of another living organism, in which it can be replicate itself. Viruses cannot reproduce on their own: a virus is composed of genes and a protein coat and some have an envelope of fat that surrounds them. Viruses can lead to infections, which provoke an immune response that usually eliminate the infecting virus"*[9]. Several viruses resist therapy or prophylaxis rather than other microorganisms, therefore, the infectious viral diseases remain till now an important worldwide problem. Nowadays, there are only very few effective antiviral drugs available for the treatment of viral diseases. Consequently, the need to find new compounds with not only intracellular but also extracellular antiviral activities is increasing more and more. The evaluation of *in vitro* antiviral activities of natural and synthetic compounds are identified by using several methods. These methods depend mainly on the inhibition or reduction of plaque formation, inhibition of cytopathic effects, and the reduction in the yield of virus, as well as other viral functions as selected host cell culture [16].

**a) Herpes simplex:**

*Herpes simplex* virus type 1 (HSV-1) and type 2 (HSV-2) [DNA viruses] are two members of the herpesvirus family, Herpesviridae, causing many common viral infections among humans. For instance, herpetic keratitis, mucocutaneous herpes infections, neonatal herpes and herpetic encephalitis. The particles of HSV-1 or HSV-2, after a primary infection, are carried via sensory nerve endings by retrograde transport to the ganglia, where the virions stayed at a latent range till the development of reactivation occur by different stimuli [16]. The activity of TTO and its main component T-4-ol was examined against *Herpes simplex* virus (HSV) [48]. It was found that T-4-ol exhibits a significant efficacy in the

treatment of recurrent HSV infections, by inhibition of the entry of influenza virus into host cells [10, 49].

T-4-ol exhibits a significant efficacy and shows an antiviral activity against HSV-1 and HSV-2. It was investigated that T-4-ol may inhibit 50 % of plaque formation with a TTO concentration 0.0009 % for HSV type 1 (HSV-1) and 0.0008 % for (HSV-2), relative to controls which are not treated with TTO. This study also shows that the HSV-1 titers is lowered by 98.2% and also HSV-2 titers by 93.0% at a higher TTO concentration of 0.003% [8]. In addition, T-4-ol is a useful antiviral agent which acts on specific steps of viral biosynthesis. It inhibits specific process in viral replication cycle so that little or no viral progeny is produced [16]. Therefore, the application of TTO or T-4-ol at different stages in the virus replicative cycle which is characterized by a complex sequence of different steps which offers opportunities to these antiviral agents to intervene, may have a great influence on free virus. Moreover, a slight reduction in the formation of plaque was observed, after using these agents during the adsorption period [48]. Minami *et al.* evaluated the activity of TTO and/or T-4-ol against HSV-1 in Vero cells and it was found again, that TTO and/or T-4-ol exert most of their viral activities on free virus, where a complete inhibition of plaque formation occur by using 1% TTO and reduction of plaque formation by approximately 10% by using 0.1% TTO. On the other hand, the plaque formation was not considerably affected when the Vero cells prior to virus addition are pretreated or posttreated with 0.1% TTO after viral infection absorption [50].

These studies revealed that the human pathogen HSV is susceptibile to the inhibitory action of TTO and T-4-ol [16] and indicate their ability to interfere with viral envelope structures, so that the entry of virus into the host cells is prevented [10]. In general, it is indicated that TTO and T-4-ol may act against both enveloped and nonenveloped

viruses, although the range of the nonenveloped viruses tested so far is very limited [8].

TTO shows also efficacy (6% TTO gel) in the treatment of recurrent *Herpes labialis*. In addition, systemic therapy with antivirals such as, Famiciclovir, Aciclovir, or Valaciclovir can inhibit or at least shorten the duration of attacks, but the availability of these agents are limited due to their costs. The antiviral activity of TTO and T-4-ol against HSV, the aetiological agent of RHL, was examined to prove that after treatment with TTO the median time to re-epithelization was found to be 9 days compared to 12.5 days with placebo, showing some advantages to TTO [51]. TTO which possesses little threat of inducing resistance to systemic antiviral agents might be a potentially useful cheaper alternative, acceptable to patients [16].

**b) Influenza virus**

*„Influenza is an infectious disease caused by the influenza virus which is a RNA virus of the family Orthomyxoviridae. Influenza spread around the world in seasonal epidemics with estimated three to five million cases of severe illness and 250,000 to 500,000 deaths per annum"*[52]. In the 20$^{th}$ century, four influenza pandemics resulted in more than 20-50 million deaths. Therefore, infections caused by influenza virus remain one of the most common causes of mortality worldwide. 2009 H1N1 virus has caused over 17,000 reported deaths. In consequence, drugs and vaccines against a H1N1 virus infection are urgently needed [53]. This virus has developed a resistance to commercially available anti-influenza drugs, e.g. it is resistant to the neuraminidase (NA) inhibitor Oseltamivir which can interfere with the enzymatic activity of the NA of the influenza virus [54]. Recently, it has been reported that TTO and T-4-ol exert an antiviral activity on influenza virus which has been principally attributed to this alcohol [55].

Garozzo *et al.* indicated in two different studies that T-4-ol, mainly rather than α-T, exhibited an inhibitory effect on influenza A/PR8 subtype H1N1 virus replication at doses below the cytotoxic dose [55, 56]. The anti-influenza virus assay was based on the inhibition of the virus-induced cytopathogenicity [9]. The results of these studies demonstrated an interference in an early step of the viral replication: thus, the viral replication was significantly inhibited if TTO was added within 2h of infection. The infective center assay was the method used in order to study the influence of TTO and T-4-ol on the virus adsorption step, indicating that TTO did not interfere with the virus cellular attachment. It was also found that TTO did not inhibit the influenza virus neuraminidase activity. The effect of TTO and T-4-ol on acidification of cellular lysomes by vital staining with acridine orange indicates that TTO inhibits the influenza virus growth in MDCK cells by acidification of the intralysomal compartment that could inhibit viral uncoating [56].

In another study, the antiviral activity of TTO concentrate was examined by its inhibition of cytopathic effects. T-4-ol could combine with the membrane fusion site of haemagglutinin. *„The presence of haemagglutinin (HA) on the surface of influenza virus particles is a major viral membrane glycoprotein molecule, which is synthesized in the infected cell as a single polypeptide chain precursor with a length of approximately 560 amino acid residues and subsequently cleaved by an endoprotease into two subunits called HA1 and HA2 and then covalently attached by the disulfide bond. Thus, T-4-ol could prevent influenza virus from entering the host cells by disturbing the normal viral membrane fusion procedure"*[49].

## 2.2 Antiprotozoal activity

Various protozoal diseases such as chagas disease, amoebiasis, leishmaniasis, giardiasis, trichomoniasis and malaria, caused by

*Trypansoma cruzi, Entamoeba histolytica, Leishmania sp., Giardia lambila, Trichomonas vaginalis* and *Plasmodium sp*., respectively, are important public health problems. Use of available antiprotozoal drugs is limited due to drawbacks such as side effects, emergence of drug resistance and requirement of prolonged use [10]. New options for treatment of protozoal diseases are being looked for, due to the various side effects of antiprotozaol drugs, TTO is one of these options used in treatment of protozoal diseases, because of its antiprotozoal activity [8, 10]. A publication of Mikus *et al.* showed that TTO causes about 50% reduction in growth of the protozoa *Tryptanosoma brucei* and *Leishmania major* at concentrations of 0.5 mg/ml and 403 mg/ml, respectively. Furthermore, this activity is significantly attributed to T-4-ol which is responsible for the *in vitro* death of *T. brucei* trypomanstigotes [57]. Another study indicated that TTO at concentration 300 mg/ml was able to kill all *T. vaginalis cells*. Based on this result, TTO and T-4-ol may be also effective in treating *T. vaginalis* infections [58].

## 2.3 Antiectoparasitic (acaricicdal) activity

T-4-ol exerts not only antibacterial and antifungal activities, but also it has been attributed to possess a miticidal activity [59]. Such a wide range of antimicrobial effects of this alcohol has been shown against several microbes responsible for hospital-acquired infections and ocular surface infections including MRSA, Methicillin-sensitive *S. aureus* (MSSA), coagulase-negative *Staphylococci* (CoNS), and *P. aeruginosa* [11, 60, 61]. As well as its acaricidal activity against ectoparasits such as its role in exerting *Demodex* mite-killing effects [59], its activity against *Sarcoptes scabiei var hominis* [62] and also against *Pediculucs capitis* (head lice) [63].

**a) Demodex mites**

*Demodex* mites (*Demodex folliculorum* and *Demodex brevis*) are the most common ectoparasites infesting the pilosebaceous unit of the skin. Unknown to many, *Demodex* prevalence increases with age and is observed in 84% of the population at age 60 and 100% of the population over the age 70 [59]. Uncontrolled skin *Demodex* infestation (demodicosis) has been implicated in several diseases in the skin, including rosacea, with papulopustular skin lesions and perifollicular inflammatory infiltrate [64, 65]. The eye is not easily approachable to daily hygiene like the rest parts of the body because it is surrounded with several protruding body parts such as the nose, the brow, and the cheek. Therefore, once demodicosis occurs in the face, it will spread and flourish in the eyelids. It has been estimated that demodicosis is the most common, but often overlooked, cause of 29% to 74% of eyes with chronic blepharitis [66], which constitutes 37% and 47% of the patients seen in clinical practices of ophthalmologists and has a high incidence in elderly population [59].

TTO is effective in reducing *D. mite* counts and ocular surface inflammation associated with blepharitis, conjunctivitis, and keratitis [67]. According to the previous study, Tighe *et al.* identified that T-4-ol as the most potent ingredient of TTO based on the dose-dependent survival time measured by an *in vitro* killing assay. T-4-ol is primarily responsible for TTO's Demodex killing effect because of its relative abundance and relative potency. Not surprisingly, T-4-ol was the only ingredient that exhibits a killing effect at a concentration as low as 1 % in mineral oil. Furthermore, the potency of this alcohol at a given concentration was greater than TTO at an equivalent concentration. For example, 5 % T-4-ol exerted an average survival time of 32.1 ± 6.6 minutes similar to 34.7 ± 4.3 minutes of 25 % TTO. Thus, it is highly plausible to promote the potency of killing *Demodex* mites by using T-4-ol alone [59].

Additionally, T-4-ol also possesses anti-inflammatory properties by suppressing superoxide production and pro-inflammatory cytokines [68], evidenced by a notable resolution of ocular irritation and inflammatory signs and vision improvement in some patients presumably due to reduction of corneal inflammation and neovascularization [67]. Collectively, these data support the inclusion of T-4-ol as the most active pharmaceutical ingredient in future formulations to treat various cutaneous and ocular diseases resulted from demodicosis which may be accompanied or not by concomitant fungal or bacterial infections.

**b) *Sarcoptes scabiei var hominis***

*„Scabies is a worldwide ectoparasitic disease of skin caused by the itch mite Sarcoptes scabiei. It is a major problem in many developing countries, related primarily to poverty and overcrowding. Despite the availability of topical acaricides, individuals often transmit the disease to others before receiving therapy. Preventing scabies has become a priority in many of these communities, as the intensely itching lesions engender significant morbidity, often becoming secondarily infected with group A Streptococcus. Prevention of the spread of scabies in these at-risk populations is based on mass community treatment"*[62]. Mass treatment of people in these endemic communities by using acaricides caused an environment for developing drug resistance or tolerance. The drug resistance is very difficult to manage, as many documents show that Crotamiton®, Lindane®, and benzyl benzoate fail in treatment, as well as 5% Permethrin® develops likely a resistance [69]. Moreover, other serious infestation may also rarely appear, known as crusted scabies. This type of scabies can be identified by the presence of huge number of mites beneath scabs and also the appearance of exfoliative crusts on different parts of the body. Crusted scabies is significantly more contagious than ordinary scabies, due to the presence of huge burden of mites [62].

Although TTO and T-4-ol are well known for their various traditional medicine uses for bruises, insect bites, and skin infections,

there is little information on their antiectoparasitic activity. One of the antiectoparasitic activities of T-4-ol is the acaricidal activity against *Sarcoptes scabiei var hominis* [62]. According to the results of this study, 5% TTO and its active component T-4-ol were highly effective in reducing mite survival times. Interestingly, 5% TTO showed the best effect against all scabies mites which were dead within only 3 hours. However, T-4-ol alone took 11.5 hours for 100% mortality. This alcohol alone exerted a reasonable effect on the viability of the scabies mites, which appears similar to that when combined together with 1,8-cineole and α-T. This indicates that both α-T and 1,8-cineole were not considered to be effective against the scabies mite [62].

Since ectoparasitic infections are primarily controlled by topical drugs, TTO and T-4-ol could be effective novel agents for the treatment of scabies, as demonstrated by the fast *in vitro* killing time [62], and their effectiveness *in vivo* when combined with benzyl benzoate [69].

**c) Pediculus capitis**

*„Head lice infestation is caused by Pediculus humanus capitis De Geer, belonging to the family Pediculocidea. The head louse is a hematophagous animal that survives by sucking the blood, several times a day (every 2-3 h). The female P. humans capitis (3-4 mm) is the most important vector of infection, since it lives 30 days after fertilization and lays 8-10 eggs per day for a total of 50-300 eggs during its lifetime"* [70, 71]. The transmission may be direct, from one head to another when they are very close, or indirect through clothing [72]. The infestation caused by *P. capitis* is either symptomatic or asymptomatic. In symptomatic cases, itching occurs at variable rates in high percentage of patients [73]. In developed and undeveloped countries is the infestation of head lice considered to be one of the most common emerging social problems. Using various alternatives, such as EOs, have been nowadays suggested

in order to treat and prevent this parasitic infestation, due to a notable loss of many long-used insecticidal compounds to their efficacy in treatment of this infestation, which resulted from increasing of louse resistance [74]. TTO and T-4-ol show a great efficacy against lice and its eggs. TTO can be used alone and in combination with nerolidol (ratio 1:1 and 1:2) [63]. Campli *et al.* monitored the ovicidal activity by microscopic inspections for 15 days. It has been shown that TTO was more effective than nerolidol against head lice with 100 % mortality at 30 min. and 1 % concentration. TTO can exert its action in a mechanical way. The active component of TTO, namely T-4-ol, can cause death of head lice by its passing through the cuticle of louse then up to the trachea resulting in suffocation of it. The results of this study, indicates a promising scenario for using either TTO alone due to its pediculocidal effect or in combination with nerolidol as effective alternative for treating pediculosis [63].

## 2.4 Anti-inflammatory activity

*„Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. It can be classified as either acute or chronic and involves a cascade of biochemical events comprising the local vascular system, the immune system, and different cell types found in the injured tissue. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. Chronic inflammation concerns a progressive change in the types of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissues"*[75].

The evidence of the anti-inflammatory effect of TTO and T-4-ol was supported by several recent reports. In the last decade, numerous *in*

*vitro* studies have indicated that this alcohol can influence a range of immune responses, both *in vivo* and *in vitro* [8]. Then modest water-soluble components of TTO [76] can suppress the pro-inflammatory mediator production via inhibition of the lipopolysaccharide-induced production of the inflammatory mediators, namely interleukin-1β (IL-1β), IL-10 and tumor necrosis factor alpha (TNF-α) at approximately 50% by human peripheral blood monocytes and at about 30% that of prostagalndin $E_2$ after 40 hours [68]. By lymphocytes and monocytes interferes TTO with the cytokine secretion and also increases IL-4 and IL-10 expression and decreases the production of IL-2. TTO inhibited peripheral blood mononuclear cells (PBMC) proliferation, as revealed by reduction in IL-2 secretion and at a concentration of 0.1% directly increased the secretion of the anti-inflammatory cytokine IL-4 and increased IL-10 secretion at 0.01%. This finding offers a novel perspective for the therapeutic use of TTO and T-4-ol in inflammatory diseases [77].

As reported, the model used for tissue macrophages was the human peripheral blood monocytes [68]. Several mediators are produced by these cells including the central mediators of inflammation, IL-1β and TNF-α upon activation with molecules as LPS. In addition, IL-8, IL-10, and $PGE_2$ are also considered to be other important monocyte/macrophage-derived mediators of inflammation. These molecules can damage tissue together with other products of activated macrophages or activate other cells in order to produce pro-inflammatory mediators. And after further examination of the water-soluble fraction of TTO, (T-4-ol, α-T, 1,8-cineole as the main components) [76], it was found that only T-4-ol was able to suppress to 40-50% after 40 hours the production of cytokines such as TNF-α, IL-1β, IL-8, IL-10, and prostaglandin $E_2$ by lipopolysaccharide-activated human monocytes [68, 78]. Nogueira *et al.* also emphasized the ability of T-4-ol to suppress the production of inflammatory mediators (IL-1ß, IL-6 and IL-10) in LPS-stimulated human macrophages. On the other hand, it was shown that T-

4-ol has no effect on the production of TNF-α [79]. This result contradicts to the published report of Hart *et al.* [68]. Additionally, T-4-ol also suppressed the superoxide production [76]. However, the mechanism of action of α-T does not interfere with the cytokine production, but rather suppresses only the production of superoxide [80]. Furthermore, it was shown that T-4-ol significantly suppressed FMLP- and LPS- but not the PMA-stimulated superoxide production whereas α-T was active in this manner also by stimulating the PMA superoxide production. 1,8-Cineole exerted no effect, thus suggesting the potential for selective regulation of cells types by these components during the inflammation [76]. In contrast, another study found that TTO and T-4-ol may help by both stimulated neutrophils and monocytes in reducing the production of reactive oxygen species (ROS) and they also stimulate by means of nonprimed neutrophils and monocytes the production of ROS [81].

Nogueira *et al*. also investigated via an *in vitro* system the immunomodulatory properties of T-4-ol and α-T with lipopolysaccharide (LPS) from several bacterial species, acting as activators of TLR4 or TLR2/4 in human macrophages [79]. A couple of trials to eliminate the pathogen starts upon activation of Toll-like receptor (TLR), by their interacting ligands, by means of releasing cytokines, chemokines and antimicrobial peptides [82]. Moreover, NF-κB signalling is particularly responsible upon TLR activation for the expression of inflammatory cytokines [83]. Therefore, the inhibition of NF-κB activation using several strategies have attracted a great interest, because they aim by interfering with the cytokine network at modulating the development of inflammatory disease [82, 83]. Furthermore, T-4-ol is a potent modulator of pro-inflammatory cytokine production by macrophages, mediated by interfering with NF-κB, upon activation of TLR4 and TLR2/4 [79].

Many studies identify specific mechanisms by which TTO acts *in vivo* to diminish the normal inflammatory response. Brand et al. postulated that *in vivo* topically applied TTO has been shown to modulate

the edema and to reduce it associated with the efferent phase of a contact hypersensitivity response in mice. This activity was attributed primarily to T-4-ol and α-T [84]. Another report by these authors also indicated the ability of T-4-ol to reduce immediate type of hypersensitivity in mouse after histamine injection. As a result, the topical application of T-4-ol significantly suppresses histamine-induced ear swelling in murine ears [78].

According to another study, the inhibitory activities on the inflammatory response exerted by T-4-ol occur due to a significant reduction in vasodilation and plasma extravasation associated with histamine-induced inflammation in humans, where α-T showed anesthetic properties [85]. Histamine receptors have been identified on both endothelial cells and sensory nerves and it has been documented that histamine causes vasodilation and an increase in microvascular permeability (wheal and flare responses) by two different effects, either indirect effects on the sensory nerves activity or direct effects on the former cells [85, 86]. In this later performed study [85], the components of TTO that modulate histamine-induced wheal and flare in human skin and their mechanism of action were examined. As a result, it was found that T-4-ol, but not 1,8-cineole or α-T, is responsible for the immunoregulatory effects of TTO on human skin, because only T-4-ol significantly reduced both the wheal and flare following histamine injection.

In addition, T-4-ol, was without effect on sensory nerves but modulated vasodilation and plasma extravasation [85], and this alcohol induced vascular smooth muscle relaxation in the deoxycorticosterone-acetate-salt hypertensive rat [87]. In contrast, the inhibitory effects of T-4-ol in skin models of inflammation in rat and human were attributed to the modulation of a post-terminal endothelium-mediated vasodilation. On the other hand, anesthetic and anti-edema properties are contributed to α-

T. In addition, a direct and indirect effect are associated with these properties on the microvascular responses of the blister base [85].

*„In summary, this monoterpene alcohol reduced substance P-induced microvascular changes and protein extravasation by a direct nitric oxide-mediated effect on the microvasculature, without sensory nerve involvement, whereas α-T regulated both pre- and post-sensory nerve terminals. In human skin, T-4-ol applied 10 minutes after histamine injection, but not α-T or 1,8-cineole, regulated the developing wheal and flare suggesting that the histamine-induced responses in human (at the dose 50 µl of 330 µM histamine) are in large part determined by histamine directly affecting the vasculature via post-terminal-mediated events"* [85].

TTO is now used around the world in many cosmetics, medicinal and dental products. The anti-inflammatory properties of TTO and especially T-4-ol, show also a great effect on dental plaque and chronic gingivitis. A TTO containing gel used twice daily on existing gingival inflammation has a significant reduction in PBA and GI scores, however, TTO did not reduce plaque scores. In other words, TTO/ T-4-ol caused a significant decrease in the gingival inflammation without a concomitant reduction in plaque scores. This indicates that anti-inflammatory is the suggested mechanism of action rather than antibacterial. This monoterpene alcohol which is known to have lipophilic properties, facilitates its diffusion through epithelium and is able to be readily absorbed after topical application showing anti-inflammatory properties to inflamed gingival tissues. This may prove to be a useful non-toxic adjunct to chemotherapeutic periodontal therapy [88].

## 2.5 Antioxidant activity

The antioxidant effect of T-4-ol remains unclear till now. Previous studies in which the role of this alcohol as anti-inflammatory agent indicated that it can suppress the production of inflammatory mediators [68], helps to decrease the production of reactive oxygen species (ROS) by both stimulated neutrophils and monocytes [81], and is also able to suppress the superoxide production by agonist-stimulated monocytes [76]. Based on these results, it is suggested that T-4-ol possesses a potential antioxidant activity, as well as a free radical scavenging activity [10].

During normal cellular activities, reactive oxygen species (ROS) are produced by several processes which occur inside of cells. Hydrogen peroxide ($H_2O_2$), hydroxide radical ($OH^-$), and superoxide ion ($O_2^-$) are the most common ROS. These previous compounds can damage lipids and cellular proteins or form DNA adducts which may be able to develop the carcinogenic activity. This process only occurs when these compounds are found in a high enough concentration. The purpose of an antioxidant is trying to prevent ROS concentrations within a cell from reaching a high- level which may promote the carcinogenic activity and cause a damage [89].

T-4-ol and TTO may not act only as an anti-inflammatory mediator by means of its antioxidant activity by reducing ROS production, but also may protect the organism efficiently by minimizing the occurrence of cell proliferation. In addition, they do not affect their capacity to secrete anti-inflammatory cytokines [77].

*„ ROS are a potential double-edged sword in disease prevention and promotion. Whereas generation of ROS once was viewed as detrimental to the overall health of the organism, advances in research have shown that ROS play a crucial role in normal physiological processes including response to growth factors, the immune response, and the apoptotic elimination of damaged cells. Notwithstanding these beneficial functions, aberrant production or regulation of ROS activity has been demonstrated to contribute to the development of some*

*prevalent diseases and conditions, including cancer and cardiovascular disease CVD"*[89].

TTO and T-4-ol are suggested to be regarded as potential antioxidant agents due to the suppression of production of ROS and their free radical scavenging activity. Therefore, they can be considered as a promising therapy in the future for prevention and treatment of several diseases. However, further studies are needed, particularly *in vivo,* in order to obtain a better understanding of their protective effects against oxidative stress [77].

## 2.6 Anticancer (antitumor) activity

One of the most difficult challenges in chemotherapy is treatment of malignant cell growth leading to cancer. *„Cancer, also known as a malignant tumor or malignant neoplasm, is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. There are over 100 different known cancers that affect humans. The most common types of cancer in males are lung cancer, prostate cancer, colorectal cancer and stomach cancer, and in females, the most common types are breast cancer, cervical cancer. Skin cancer other than melanoma would account for at least 40 % of cases for both men and women"*[90]. Many studies highlight the potential anticancer activity of TTO and T-4-ol [91, 92, 93]. T-4-ol shows an antitumor activity against human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamicin-resistant cells [91], as well as against aggressive murine tumor cell lines, AE17 mesothelioma and B16 melanoma, and fibroblasts cells L929 [92, 93]. In another Australian study [94], the toxicity of TTO and T-4-ol was evaluated on five human cell lines after an estimating period of 4 h and 24 h incubation. Susceptibility of the cell lines differed significantly to the toxicity of TTO and its main component showing 50% killing at a concentration of 0.28%

after 4h for HeLa (epithelioid), Hep G2 (hepatocellular carcinoma) cells and K562 (chronic myelogenous leukaemia). In addition, 50% killing of Molt-4 (lymphoblastic leukaemia) and CTVR-1 (B cell-derived from bone marrow of a patient with acute myeloid leukaemia) cell lines occurred at 0.06% TTO over 4 h. The $IC_{50}$ was observed after 24 h to be 0.27% for Hela cells, 0.03% for K562 cells, Molt-4 cells and CTVR-1 cells, and 0.002% for Hep G2 cells [94].

**a) Human Melanoma M14 Wt cells**

Melanoma is a type of skin cancer which forms from melanocytes (pigments containing cells in the skin). In women, the most common sites are the legs, and melanomas in men are most common on the back [95]. *„Cutaneous melanoma is a highly invasive and metastatic tumor, highly refractory to chemotherapy. Melanoma cells are known to exhibit both in vitro and in vivo a high level of intrinsic resistance to various cytostatic agents"*[91]. Moreover, classical multidrug resistance (MDR) phenotype is acquired by the melanoma cells after following a drug treatment. This MDR phenotype is characterized by a reduction in accumulation of the intracellular drug and an increase in the resistance index. Several reports propose the great role of MDR-related protein (MRP1) and specific drug-transporter proteins, including P-glycoprotein (P-gp) in the demonstration of the MDR phenotype in melanoma cells [96]. Various trials were performed by many *in vitro* and *in vivo* studies in order to reverse the drug resistance phenotype and to improve the chemotherapeutic strategies to be more creative and effective against MDR tumors. An inhibition of the P-gp molecule activity is induced competitively by means of MDR modulators, such as calmodulin inhibitors, calcium channel blockers (e.g. verapamil), and immunosuppressive agents. Based on several factors, it was unfortunately observed that using of these compounds *in vivo* expresses a lot of obstacles. Among these factors: (1) the decrease of the bioavailability, due to binding of these modulating substances to certain macromolecules (e.g. serum proteins), (2) toxicity of

the inhibitory concentration of modulators to patients suffering from heart block or hypotension etc., (3) the sensitivity of hematologic malignancies to MDR modulators more than soild tumors. Therefore, the search for innovative therapeutic approaches based on the use of new substances is gaining more interest in clinical oncology [91].

Calcabrini *et al.* examined the potential anti-tumoral activity of TTO and T-4-ol against human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamicin-resistant cells (M14 ADR) [86]. In this paper the human melanoma M14 WT and M14 ADR cells were shown to be able to grow at the concentrations ranging between 0.005 and 0.03% TTO. Moreover, the two higher concentrations 0.02% and 0.03% TTO and 0.01% T-4-ol appeared to be strongly inhibitory for the growth of both parental M14 WT and resistant M14 ADR cells. Both TTO and T-4-ol alone were able to induce caspase-dependent apoptosis of melanoma cells and the result confirmed that this effect was more evident in the resistant variant cell population, where the results of T-4ol experiments indicated that these resistant cells, in comparison to sensitive ones, are more susceptible to this alcohol [91].

In addition, freeze-fracturing and scanning electron microscopy analysis suggested that the effect of this monoterpene alcohol and TTO was mediated by their interaction with plasma membrane and subsequent reorganization of membrane lipids. Moreover, P-gp was found to be unable to protect against TTO-or T-4-ol- stimulated apoptosis. The higher susceptibility to T-4-ol action was demonstrated by dead P-gp-positive (M14 ADR) cells. That means that T-4-ol and TTO are apparently able to overcome P-gp mediated resistance to the caspase-dependent form of apoptosis. In conclusion, the growth of M14 melanoma cells is suggested to be impaired by T-4-ol and TTO. Furthermore, both of them may also exert greater effect on their resistant variants and help to overcome this resistance to caspase-dependent apoptosis presented by P-glycoprotein-positive tumor cells [91].

**b) AE17 mesothelioma, B16 melanoma, and fibroblasts cells**

Greay *et al*. also investigated the *in vitro* activity of TTO and T-4-ol against two aggressive murine tumor cell lines, AE17 mesothelioma, and B16 melanoma and fibroblasts cells by evaluating antiproliferative efficacy in a dose- and time-dependent manner. The results indicated that AE17 cells were more susceptible to TTO and T-4-ol than B16 cells. Interestingly, cytotoxic doses of T-4-ol are significantly less efficacious against non-tumor fibroblast cells [92].

As a result, it was found that T-4-ol and TTO induced necrotic cell death coupled with low level apoptotic cell death in both tumor cell lines, by means of changes in AE17 cells including plasma membrane, disruption of mitochondrial crista, nuclear envelope disturbance, and loss of electron density within the cytoplasm, nucleus. On the other hand, by using MTT assay it was investigated that B16 cells show a higher resistance to T-4-ol than AE17. The lower levels of cell death by apoptosis and necrosis proposed the involvement of other potential mechanisms in the *in vitro* anticancer activity. In addition, both TTO and T-4-ol induced an inhibitory effect by eliciting G1 cell cycle arrest. Specifically, significantly greater G1 cell cycle arrest was observed in B16 cells when compared with AE17 cells. According to the results of this study, TTO and T-4-ol have a significant anti-proliferative activity against AE17 mesothelioma and B16 melanoma at doses non-cytotoxic in non-tumor fibroblast cells. Both TTO and T-4-ol induce AE17 and B16 cells death by primary necrosis, low level apoptosis, as well as the ability to induce cell cycle arrest. The potential anticancer activity of T-4-ol is highlighted by many observations that revealed that both TTO and this monoterpene alcohol are able to induce cell death as well as inhibiting aggressive tumor cells growth. Therefore, it may be a feature of a promising anticancer and clinical chemotherapeutic agent [92].

Further investigations demonstrated a topical antitumor efficacy of TTO and T-4-ol formulations in immunocomponent tumor bearing

mice. Moreover, Greay *et al*. had recently revealed for the first time that TTO and T-4-ol possess *in vivo* antitumor activity and a potential use as topical antitumor agents which can be used in skin cancer treatment [93].

Using of topical chemotherapy as a single therapy or in a combination with surgical excision of skin cancers expresses a safe and an alternative anticancer treatment. Many topical treatments such as the thymidylate synthase inhibitor 5–Flurouracil (5-FU) and the immune modifier Imiquimod have already shown successful and effective results in treating actinic keratosis and superficial basal cell carcinoma (sBCC). But on the other hand, long treatment (more than 6 weeks) is restricted because it may be accompanied by systemic toxicity. Further, Greay *et al.* also examined a TTO-combination with an effective carrier DMSO once daily for 4 daily topical treatments [93]. DMSO is widely used as an efficient penetration enhancer and may act by lowering skin resistance or increasing drug partitioning [97]. These treatments of subcutaneously implanted AE17 mesotheliomas were sufficient to induce a period of significant regression and growth inhibition of these aggressive, chemo-resistant tumors. DMSO was required for TTO-induced tumor eradication, although it has alone an antitumor effect [93]. According to several reports, the penetration effect of TTO through human epidermis was found to be within the range of 1.1-4% TTO. After 4 h of TTO application, about 98% of the oil is evaporated and only T-4-ol is the component which can penetrate the skin [98].

Additionally, the differential effect between the two tumor cell types appears to be similar, as topical TTO regressed AE17 tumors and retarded the growth of subcutaneously implanted B16-F10 cells during the treatment period. In conclusion, TTO and T-4-ol are responsible for the antitumor efficacy, because they are able to penetrate the skin. Moreover, various factors render TTO and T-4-ol to be ideal candidates for treatment of skin cancer, such as the lack of systemic activity associated with normal body weight within the treatment period, normal

liver histology and no difference in serum alkaline phosphatase or aspartate aminotransferase. On this basis, TTO and this monoterpene alcohol have the potential for a cost effective, self-administrated skin cancer treatment [93].

**c) Human non-small cell lung cancer**

*„Lung cancer is the leading cause of cancer-related deaths worldwide. Among lung cancers, nonsmall cell lung carcinomas (NSCLC) account for approximately 80% of lung cancer cases"*[99]. Although the early detection of the disease and its treatment increase the survival percentage, rapid recurrence and progression of the lung cancer are considered to be the obstacle on the way of some patients to reach a complete healing. In consequence, new therapeutic methods are urgently required in order to help in the treatment of this disease [100]. Based on previous reports, it was suggested that the antitumor activity of T-4-ol is caused by means of triggering caspase-dependent apoptosis in the cells of human melanoma or necrotic cell death induction and cell-cycle arrest on mouse mesothelioma and melanoma cell lines without affecting normal cells [91, 92]. The results of a recent study, which investigated the anticancer effects of this monoterpene alcohol on A549 and CL1-0 human lung adenocarcinoma cells, demonstrate for the first time that T-4-ol induces apoptosis also in human lung cancer cells *in vitro* and *in vivo*. A decrease in IAP family proteins XIAP and hence a survival were observed following T-4-ol treatment, notably, it was found that this alcohol induced dependently apoptosis in p53 [100].

*„Apoptosis and necrosis are two typical types of cell death. Apoptosis is characterized by several biochemical criteria such as changes in mitochondrial membrane permeability, caspase signalling activation, internucleosomal DNA cleavage, and the release of intermembrane mitochondrial proteins. In contrast, necrosis is characterized mostly in negative terms by the absence of apoptosis parameters, such as caspase activation. Recently, accumulative evidence*

*has indicated that the disregulation of apoptosis contributes to carcinogenesis"*[101]. In consequence, the ability of tumor cells to respond to apoptosis is the main factor which can influence their susceptibility to chemotherapeutic compounds. It was also suggested that apoptosis was involved in T-4-ol rather than necrosis inducing A549 and CL1-0 cell death, due to the presence of annexin V+/PI- activated forms of caspase-9 and casepase-3 and PARP cleavage. Furthermore, the mitochondrial pathway of apoptosis is believed to be involved upon the treatment with this alcohol, because of increasing in cytochrome-c release into the cytosol and decreasing in mitochondrial membrane potential [100].

Further investigations indicated an increase in the protein expression of apoptotic Bax and a significant reduction in that of anti-apoptotic Bcl-2, XIAP, and survivin within 24 h after treatment with T-4-ol. These results indicated the occurrence of T-4-ol induced mitochondrial mediated apoptosis. Another finding suggests that p53 is a tumor-suppressor protein, protects against cancer by regulating the 02cellular response to DNA damage, apoptosis, and oncogene activation. The presence of fractional p53 acts as the key factor in switching necrosis to apoptosis in human NSCLC. As a result, T-4-ol is most effective against cancer with fractional p53 [100]. Cell-cycle arrest is a common cause of cell growth inhibition [102]. Further results indicated that the exposure of A549 and CL1-0 cells to T-4-ol caused $G_2$/M phase arrest. The difference in type of cell-cycle arrest elicited by this alcohol might be due to species or to cell-type differences [100].

In conclusion, the results revealed that the mechanism of T-4-ol induced apoptosis in NSCLC is caspase-dependent mitochondrial dysfunction. It was also suggested that the ability of T-4-ol to increase the susceptibility of NSCLC cells to apoptosis induction is depending on the occurrence of down regulation in the protein expression of Bcl-2, XIAP and survivin after treatment with this alcohol. Interestingly, T-4-ol

induced apoptosis in NSCLC is p53–dependent. Moreover, intra-tumoral injection of this alcohol, as emphasized by TUNNEL assay, induced apoptosis causing a significant inhibition in the growth of S.C. A549 xenografts. Collectively, these data highlight the molecular mechanisms occurring during T-4-ol-induced apoptosis on NSCLC, suggesting that this monoterpene alcohol is a promising anticancer drug for NSCLC [100].

## 2.7 Anticonvulsant activity

T-4-ol can be obtained not only from TTO but also from several plants such as *Alpinia zerumbet* [103], *Tanacetum cademum* [104], and other several aromatic plants species [105]. This alcohol exerts a broad range of pharmacological actions, such as antiulcer, antihypertensive, and antioxidant [103, 106, 107], in addition to its antimicrobial activities which were previously explained (section 2.1). T-4-ol shows also an anticonvulsant activity according to the study of De Sousa *et al.* which evaluated the profile of this alcohol in the CNS and its possible anticonvulsant activity [108].

Epilepsy is a chronic neurological disease characterized by spontaneous recurrent seizures that affects approximately 1 % of the world population. Nearly 30 % of patients with epilepsy do not respond to any form of pharmacological treatment [109]. For these patients, currently available antiepileptic drugs are ineffective and have significant undesirable effects. Therefore, numerous studies have been directed at the discovery of new pharmacological strategies using novel compounds to treat epilepsy. In the above mentioned study [108], the anticonvulsant activity of T-4-ol (200mg/kg) was investigated in convulsion animal models (mice) and a significant decrease in the spontaneous motor activity at 30, 60 and 120 min after administration was observed. A significant dose-dependent increase, by using this monoterpene alcohol,

occurred in the duration of sleep in mice at doses of 100 and 200 mg/kg. In addition, a pretreatment of mice with T-4-ol causes a significant elevation in the latency of pentylenetetrazole (PTZ)-induced convulsions at concentrations 100, 200 and 300 mg/kg. The induced seizures of picrotoxin (PIC) are also inhibited by using T-4-ol at doses of 200 and 300 mg/kg and in another model (maximal electroshock seizures [MES]), it decreased the tonic hind convulsions percentage at the dose of 300 mg/kg. This alcohol may also be effective in blocking generalized tonic clonic partial and generalized clonic seizures [108]. These data are in agreement with the results obtained in mice administrated with α-T which is also effective in PTZ and MES models [110]. From these results, it was concluded that the depressant effect on the CNS and significant anticonvulsant activity probably is due to interaction with GABA receptors [108].

Further investigations examined the effects of T-4-ol on convulsions induced by mercaptopropinoic acid (3-MP) and seizures induced by pentylenetetrazole (PTZ) via behavioral and electroencephalographic methods. The molecular mechanisms involved in these effects were investigated too, especially by electroencephalographic (EEG) recordings. T-4-ol is a potent anticonvulsant that inhibits chloride ion channels activated by γ-amino-butyric acid $GABA_A$ receptors. Because of the importance of $GABA_A$ receptor as a target for many anticonvulsant drugs, the participation of the benzodiazepine site of this receptor in the anticonvulsant effect of the T-4-ol was tested in the presence of Flumazenil (FLU), a selective antagonist for this benzodiazepine site of the $GABA_A$ receptor. Furthermore, T-4-ol decreased the sodium current through voltage dependent sodium channels and thus its anticonvulsant effect can be related to changes in neuronal excitability because of modulation of these channels [111].

## 2.8 Antihypertensive activity

T-4-ol is also the major component of the species *A. zerumbet* or *A. speciosa* (Blume) D. Dietr., (Zingiberaceae) EO. Using this medicinal plant as a tea is traditionally spread in treatment of arterial hypertension [112]. *„ Hypertension is classified as either primary (essential) hypertension; about 90-95 % of cases are categorized as "primary hypertension" which means high blood pressure with no obvious underlying abnormal causes. The remaining 5-10 % of cases categorized as secondary hypertension which are attributed to other conditions that affect the kidneys, arteries, heart or endocrine system"*[113]. Lahlou *et al.* indicated that intravenous administration of T-4-ol caused immediate blood pressure reduction in a dose-dependent manner in normotensive rats. In this study, the cardiovascular effects of i.v. treatment with the essential oil of *A. zerumbet* (EOAZ) and T-4-ol were investigated. Also the ANS involvement in the improvement of EOAZ-and T-4-ol induced changes in mean aortic pressure (MAP) and heart rate (HR) was assessed. Bolus injections (i.v.) of EOAZ (1 to 20 mg/kg) elicited an immediate and dose-dependent decrease in MAP, as well as T-4-ol at a dose (1 to 10 mg/kg). These results indicate that the hypotensive effects of this alcohol were significantly greater than those evoked by the same dose of EOAZ. These results have shown that i.v. treatment with EOAZ in either anaesthetized or conscious rats induced an immediate and significant hypotension, an effect which is highly attributed to the actions of T-4-ol. The occurrence of this hypotension effect is suggested to be independent on the presence of an operational sympathetic nervous system. Therefore, it is proposed that the EOAZ and this monoterpene alcohol may be direct vasorelaxant agents [103].

Lahlou *et al.* also investigated in another study that the antihypertensive effects of T-4-ol in an experimental model of by deoxycorticosterone-acetate (DOCA)-salt made hypertensive rats and also here the MAP was decreased in a dose-related manner. Notably,

hypotensive responses to both T-4-ol and EOAZ at the same doses (1-10 mg/kg) were compared together giving a significantly greater hypotensive effect to T-4-ol than EOAZ. The maximal percentage decreases in MAP is considerably enhanced by treatment with DOCA-salt. T-4-ol was able to induce a concentration-dependent vasorelaxation. Based on these results, a significant reduction in the blood pressure occurred in conscious, DOCA-salt made hypertensive rats by means of i.v. treatment with this monoterpene alcohol in a dose-dependent manner. In comparison to un-nephroectomized controls, this action was observed to be enhanced. A noticeable increase by means of induced vascular smooth muscle relaxation is suggested to be more related to this enhancement than to an enhanced sympathetic nervous system activity [87].

## 2.9 Antiulcer activity

Another important activity of T-4-ol is its antiulcer effect. Matsunaga *et al.* showed that the essential oil from the leaves Tateyamasugi *Cryptomeria japonica* (Cupressaceae) which contains T-4-ol as an active component exhibits strong inhibitory activity on ulcerations induced by several mediators, including HCl/ethanol, HCl/aspirin, water immersion stress and pylorus ligation [106]. *„A peptic ulcer is a distinct breach in the mucosal lining of the stomach (gastric ulcer) or the first part of the small intestine (duodenal ulcer), as a result of caustic effects of acid and pepsin in the lumen. The most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. A gastric ulcer would evoke epigastric pain during the meal, as gastric acid production is increased as food enters the stomach"*[114]. In the first above mentioned study, the antiulcer constituents of the essential oil of *C. Japonica* were separated by means of distillation and chromatography and it was found that T-4-ol is the most potent compound against ulcers

[106], due to its potency in reducing the secretion of gastric juice and output of acid, and also by lowering the pepsin activity [115].

## 3. Conclusion

T-4-ol, a naturally occurring monocyclic monoterpenoid alcohol, is found in different essential oils obtained from several aromatic plants, T-4-ol is considered as the active component (30%-45%) of *Melaleuca alternifolia*

(TTO). It was also proven, that this alcohol of *M. alternifolia* oil particularly displays a greater efficacy than its oil [4, 8].

T-4-ol exerts different and a wide range of biological properties on human and animals [8]. It plays an important role against many bacteria such as *E. coli. P. aeruginosa, C. jejuni, S. aureus* [19, 23, 31], as well as against the methicillin-resistant *S. aureus* (MRSA) which attracts considerable interest nowadays [11]. Several studies reported on the antifungal activity of this alcohol against various yeasts, dermatophytes and other filamentous fungi as *C. albicans* [32, 36]. However, 5 % TTO and T-4-ol may be useful in topical applications such as in the treatment of dandruff [47]. Moreover, the antiviral activity of this monoterpene alcohol shows a great efficacy against mainly (HSV-1) and (HSV-2) [48], as well as influenza virus [49].

One of the most important biological activities of T-4-ol is its anti-inflammatory activity, as it was found that it is able to supress the production of cytokines such as TNF-α, IL-1β, IL-8, IL-10 and PGE2 [68, 79]. In addition, the lipohilic properties of T-4-ol facilitates its diffusion through epithelium and it is able to be absorbed after topical application, as well as it proves to be a useful non-toxic adjunct to chemotherapeutic periodontal therapy [88]. This alcohol shows also a potential antioxidant activity, as well as a free radical scavenging activity [10], because it helps to decrease the production of reactive oxygen species (ROS) by both stimulated neutrophils and monocytes [81], and it is also able to suppress the superoxide production by agoinst-stimulated monocytes [76]. Based on the previous results, it was shown that T-4-ol can be a potential anticancer drug, as it is able to impair the growth of M14 melanoma cells [91], to induce apoptosis in NSCLC in p53-dependent manner, as well as to inhibit the growth of two aggressive murine tumor cell lines (AE17 mesothelima and B16 melanoma) in a dose-and time dependent manner [92, 100]. T-4-ol also exerts a significant anticonvulsant activity due to an

interaction with GABA receptors and a depressent effect on the CNS, as well as a significant protection against induced seizures [108, 111].

T-4-ol is not only the major constituent of TTO, but also of many other essential oils such as the essential oil of the species *A. zerumbet*, which is well known in the treatment of arterial hypertension due to its antihypertensive effect [112]. Also it was revealed that i.v. treatment with this alcohol decreased dose-dependently the blood pressure in conscious by DOCA-salt made hypertensive rats [103]. Another biological activity which belongs to T-4-ol is the antiulcer activity, as it inhibits strongly the ulceration induced by several mediators, including HCl/ethanol, HCl/aspirin, water immersion stress and pylorus ligation [106, 115]. Therefore, T-4-ol can be considered to be a promising agent in the future in the treatment of many diseases, due to its various biological activities which attracted the interest of many studies.

## 4. Various biological activities of α-terpineol (α-T):

α-T, a volatile monoterpenoid alcohol, is the major component of essential oils of several species of aromatic plants (e.g. *Origanium*

*vulgare L.* and *Ocimum canum Sims*.) which are widely used for medicinal purposes [116]. α-T is relatively cheap and an abundant aroma compound; therefore it is widely used in food, cosmetics and household products and it possesses a wide range of biological properties too. Various reports showed that this alcohol exhibits an antiproliferative effect on human erythroleukaemic cells [117], as well as anti-inflammatory properties [118], as it was found to be a potent inhibitor of superoxide production [76]. And many studies have been reported that α-T can enhance the permeability of skin to lipid soluble compounds [119].

## 4.1 Cardiovascular and antihypertensive effects

Systemic arterial hypertension and cardiovascular diseases increase the risk of mortality and morbidity worldwide [120, 121]. Arterial hypertension is considered to be the major risk factor for both heart attack and generally stroke [122]. *„In fact, it has been shown that blood pressure levels are strongly and directly related to the relative risks of stroke and heart disease. Endothelial dysfunction in hypertension triggers an imbalance between the production and release of these factors, increasing the generation of reactive oxygen species and diminishing NO synthesis and bioavailability. L-arginine is the precursor of NO synthesis by NO synthase (NOS), an enzyme that exists in three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (eNOS)"*[116]. Furthermore, inhibition of NOS activity and then NO biosynthesis by means of L-arginine analogues administration such as L-nitro arginine methyl ester (L-NAME) leads to hypertension [123, 124]. Accordingly, many reports were designed to investigate the cardiovascular and antihypertensive effects i032tnduced by α-T in rats with hypertension induced by L-NAME [116, 125]. The NOS inhibitor L-NAME has been used extensively as a mean of inducing hypertension in animal models [116].

Sabino *et al.* examined the effect of α-T on haemodynamic parameters which was evaluated by the treatment of non-anaesthetized rats once a day with different doses of α-T (25, 50 or 100 mg/kg/day) for one week. The results have indicated that the induction of a marked hypotensive effect in rats occurred by oral administration of α-T. Hypotension may be exerted due to a decrease in peripheral vascular resistance. The beneficial effects of α-T on isolated rat's mesenteric from L-NAME–induced hypertensive rats were demonstrated, and as a result, α-T in a concentration–dependent manner, relaxed the endothelium-intact mesenteric rings precontracted with phenylephrine and depolarization with KCl. Furthermore, α-T-induced relaxation was not considerably reduced by the mechanical removal of the endothelium in phenylepherine precontracted mesenteric rings. According to these results, it was proposed that the vasorelaxant activity of α-T is endothelium-dependent, and that this alcohol blocks $Ca^{+2}$ entry through voltage-dependent $Ca^{+2}$channels, which is involved in the mechanism by which relaxation can be produced. Further results indicated that α-T was able to inhibit contractions induced by the cumulative addition of phenylephrine without endothelium preparations suggesting that this alcohol could exert its activity on vascular smooth muscle contractile machinery [116].

Several mechanisms for an endothelium-independent vasodilation are being involved in its relaxant activities of vascular smooth muscles. Among these mechanisms: (a) inhibition of agonist-mediated release of $Ca^{+2}$ from intracellular stores, (b) blockage of extracellular $Ca^{+2}$influx by transmembrane $Ca^{+2}$ channels, (c) inhibition of the contractile apparatus and (d) opening of $K^{+}$ channels. By means of two kinds of transmembrane $Ca^{+2}$channels: receptor-operated $Ca^{+2}$channels (ROCC) and voltage-operated $Ca^{+2}$channels (VOCC) occur the influx of extracellular $Ca^{+2}$ [126]. These results indicated that α-T significantly attenuated the concentration induced by $CaCl_2$, indicating that this alcohol can inhibit vasoconstriction induced by extracellular $Ca^{+2}$ influx through VOCC [116]. It is also known that the $Ca_{v\ 1.2}$, which is considered as a $Ca_v$L

subtype present in various smooth muscle cells (VSMCs), is the main voltage–operated calcium channel found in VSMCs. The $Ca_{v\ 1.2}$ (voltage-gated calcium channel $\alpha_1$ subunit) is a subtype of L-type calcium channel ($Ca_vL$), which is found in different cell types such as myocytes, smooth muscle myocytes and they are responsible for the excitation-contraction coupling, hormone release, and regulation of transcription as well as synaptic integration [127]. In summary, the reduction of calcium influx occurred through the voltage-sensitive $Ca_vL$ channels may result in a decrease in vascular resistance which is attributed to α-T leading to hypotension induction. [116].

As conclusion, α-T-induced hypertension and vasorelaxation are mainly mediated by releasing NO and activating the NO-cGMP pathway. In addition, oral administration of α-T was able to reduce mean arterial pressure and in mesenteric artery rings it induced a vascular endothelium independent vasodilatation, showing alternations in biochemical parameters which indicate an antioxidant effect as well. These data indicate that the ability of this alcohol to decrease the arterial pressure is mainly depending on restoring the enzymatic antioxidants in L-NAME-induced hypertensive rats and reducing the vascular resistance [116, 125].

## 4.2 Antioxidant activity

*„Antioxidants, such as vitamins, enzymes or $Fe^{+2}$, etc. are able to neutralize free radicals. They exert a health-enhancing effect on the human organism because they protect cells from oxidative damage"*[9]. Oxidative stress has an important influence on the development and progression of many diseases, such as cardiovascular diseases, inflammation, neurodegenerative diseases and aging processes. In addition, oxidative stress is mainly characterized by the presence of high bioavailability of reactive oxygen species (ROS) [128]. α-T shows an antioxidant activity, as it was previously mentioned that it is able to

suppress the superoxide production by agonist-stimulated monocytes but not neutrophils [76]. *„The antioxidant action of α-T reflects its capacity to act as a preservative in food, cosmetics and pharmaceutical products, preventing oxidative degeneration of their components"*[129].

Arterial hypertension can be developed from oxidative stress and is believed to result from systemic damage in different target tissues by oxygen free radicals. Non-enzymatic antioxidants (e.g. reduced glutathione) and antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase) are the factors which are used to help the performance of intracellular defense against active oxygen species [130]. Reduction of Catalase and glutathione peroxidase in L-NAME-treated rats were observed when compared with L-NAME control groups. However, this observed decrease in antioxidant enzymes activities returned again after the administration of α-T to be much nearer to those in normal control rats. Based on these data, α-T proved to possess a potential antioxidant activity against free radicals causing injury [116].

α-T exerts an antiproliferative effect, therefore, it can be used in the prevention or even treatment of cancer. The antiproliferative capacity of α-T can be measured using two methods: 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH), which is a simple and accurate indirect method determining scavenging potential of free radical, and the second method is Oxygen Radical Absorbance Capacity (ORAC) which is used as a direct method in order to determine the ability of lipophilic and hydrophilic substances, via hydrogen atoms transfer, to resist the oxidation reactions with peroxyl radicals. Results revealed that α-T showed very low antioxidant activity in DPPH assay, but it could be compared to commercial antioxidants in the ORAC asssay. As it was shown that this alcohol demonstrated a potential antioxidant capacity against peroxyl radicals. Moreover, α-T also exerted cytostatic activities which were found to be very effective against six human cancerous cell lines, such as prostate, breast, lung, leukemia and ovarian, especially

against breast adenocarcinoma (MCF-7) and chronic myeloid leukemia (K-562). In a range of 181-588 μM the impressing results also revealed that α-T with an antioxidant potential similar to BHA, which is considered to have a potential protective activity in foodstuffs, acts as a natural preservative [129]. Thus, this alcohol attracts the interest for further research than can culminate in its use as a functional additive, as well as in its role in cancer-prevention *in vivo*. Hereafter, *in vivo* assays must be performed to confirm the antioxidant potential of α-T.

## 4.3 Anticancer activity

*„Cancer is characterized by uncontrolled growth of cells disregarding the normal limits, by invasion and, in the worst case, by metastasis, the expansion of the disease to another non-nearby organ by lymph or blood"*[9]. α-T is a bioactive component of *Salvia libanotica* essential oil extract and has shown antitumor activity [119]. *S. libanotica* (sage) is a species endemic to the Eastern Mediterranean. It has been shown that both induction of cell cycle arrest and apoptosis in human colorectal cancer cells occurred depending on the synergistic action of its three bioactive components: α-T, camphor and linalyl acetate, via caspase activation, mitochondrial damage (cytochrome C release), and PARP cleavage [131].

The link between development of cancers and chronic inflammation is found to be related with the activation of the transcription factor NF-κB. Since several types of human tumors express mainly NF-κB, blocking this factor was proposed to increase its sensitivity to the action of anti-tumor agents or stopping the proliferation caused by tumor cells [132]. Hassan *et al*. proved that α-T acts as a potential anticancer agent by suppressing NF-κB signalling. The cytotoxicity of α-T towards 14 different human tumor cell lines representing different haematological and non-haematological malignancies was evaluated *in vitro*, where α-T

exerted a considerable cytotoxic effect on the cell line of the small cell lung carcinoma, representing a tumor-specific activity. Interestingly, the effective cytotoxic activity of α-T renders this alcohol a promising effect for treatment of patients with drug-resistant tumors, due to the limited effects of resistance represented by α-T [119]. The risk of toxicity against normal lymphocytes is reduced, due to the suggested slight tumor selectivity of this alcohol, helping as an important feature in many of the cytotoxic drugs which are clinically used [133]. *„Treatment with α-T induces cell cycle arrest and apoptosis in the cell line tested in a dose- and time-dependent manner. The results suggest that cell cycle phase arrest by α-T may depend on drug concentration at the shorter exposure time. This finding is consistent with α-T which showed that it is active in including cell cycle changes if combined with linalyl acetate rather than if used alone in colorectal tumor cells"* [119].

Hassan et al. also demonstrated that the inhibition of the NF-κB translocation and activity in tumor cells was exerted by the anticancer activity of α-T in a dose dependent manner, as indicated by means of the two NF-κB assays. Moreover, the response of NF-κB expression to α-T treatment and other related genes as IL-1R1, IL-1β, ITK, AKT1S1, EGFR, IFNG, BAG1 and TNIK was indicated via microassay analysis showing significant down regulation. Furthermore, the probable influence of α-T on kinases was examined by using the cell-free assay representing a modest inhibitory effect on AKT, JNK1, JNK2 and IKK beta kinases. The supposed correlation of α-T with AKT kinase and NF-κB inhibitors is attributed to this moderate inhibition of AKT and IKK beta kinases. In addition, the release of cytochrome C due to the disruption of the mitochondrial membrane potential cannot be ignored as an extra cytotoxic mechanism for α-T and may be also the reason which helps in induction of apoptosis in colon cancer cell lines, when linalyl acetate and camphor combined together with this alcohol [119,131]. On the other hand, the antifungal activity exerted by α-T is also represented by the

uncommon structure of mitochondria of the fungi and its cell membrane disruption [134].

Based on the results of many experiments, α-T appears to inhibit the growth and induces cell death in tumor cells by a mechanism that involves inhibition of NF-κB activity and translocation in a dose dependent manner by means of two NF-κB assays, and also is able to down regulate many NF-κB related genes expression such as IL-1β and IL1R1. [119]. It was also indicated that linalyl acetate and α-T exhibit synergistic anti-proliferative effects. Treatment using this potential combination showed significant suppression of basal and TNF-alpha-induced NF-κB activation using DNA binding assays. Moreover, IκB-α degradation and inhibition of p65 nuclear translocation are found to be in correspondence with this suppression. As a result, it is indicated the anticancer activity of α-T is partly mediated by the suppression of NF-κB activation, suggesting its use in a combination with linalyl acetate with chemotherapeutic agents to induce apoptosis [135].

## 4.4 Antinociceptive activity

*„Another important activity which is correlated to α-T is the antinociceptive activity. A nociceptor is a sensory receptor that responds to potentially damaging stimuli by sending nerve signals to the spinal cord and brain. The anti-nociceptive effect is a reduction in pain sensitivity made within neurons when endorphin or a similar opium-containing substance combines with a receptor"*[9]. One of the most important symptoms of inflammatory disease is pain. Sanitation of primary afferent nociceptors can result in allodynia and/or hyperalgesia, known as hypernociception in animal models [136]. The main function of pain is avoiding the damage of tissue stimuli via activating the spinal reflex withdrawal mechanisms. Thus, it helps in protecting the tissues of the organism from damaging. In acute pain conditions, pain exists for a

while even after healing the injury. On the other hand, chronic pain conditions can be explained by the presence of typical inflammation and neuropathy [137]. Moreover, available antinociceptive drugs show low efficacy to relieve painful conditions in patients and possess numerous side effects [138]. Therefore, natural products showing fewer side effects exert promising therapeutic activities in developing of new drugs which can be able to manage certain chronic pain conditions [139].

Golshani *et al*. reported that the essential oil of *Dracocephalum Kotschyi Boiss* (Labiatae), containing α-T as an active component, possesses antinociceptive properties [140]. Therefore, many experiments based on these results took place to investigate the antinociceptive effect of this alcohol. The results of another study revealed that α-T possesses both peripheral and central analgesic properties. α-T produced significant ($p<0.01$ or $p<0.001$) analgesic effects by reduction at the early and late phases of paw licking and reduced the acetic acid-induced writhings reflex in mice. Those effects are probably in relationship to the inhibition in the peritoneal fluid levels of $PGE_2$ and $PGF_{2\alpha}$ with the release inhibition of substance P and other inflammatory molecules, such as serotonin, histamine, bradykinin and prostaglandins [141].

It has been investigated that glutamate plays an important role in transmitting the nociceptive signals from the peripheral nervous system to the spinal cord, mainly the dorsal horn. Moreover, glutamate injection provoked nociceptive responses, which are mediated by neuropeptides (Substance P) releasing from C fibers and due to activation of glutamate receptors [i.e., N-methyl-D-aspartate acid (NMDA)] that can stimulate the production of a variety of intracellular second messengers. These are NO, then pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-1β, which act synergistically in the excitation of the neurons [142]. Trink *et al*. indicated that the intravaginal treatment with α-T, one of the main components of *Artemisia princeps* Pamp (Asteraceae) essential oil (APEO), significantly decreased viable *G.*

*vaginalis* and *C. albicans* germs in the vaginal cavity by inhibition of the expression of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), COX-2, iNOS. Based on these results, α-T most potently inhibited the expression of proinflammatory cytokines and NF-κB activation [143]. Additionally it was found that spinal, supraspinal, peripheral sites of action are involved in the induced nociceptive response by glutamate which is mainly mediated by both non-NMDA and NMDA receptors [144]. Thus α-T produces an inhibition of the nociception induced by glutamate [141]. The anti-inflammatory activity of α-T was assessed in another study, where α-T showed inhibition of bovine cyclooxygenase-1 and 2 (COX-1 and COX-2). α-T exerted selective COX-2 inhibition, where its $IC_{50}$ values against COX-1 and COX-2 were 5.14 mM and 0.69 mM, respectively. This indicated that this alcohol showed higher COX-2 activity inhibition than Aspirin®, which is the most popular NSAID [145].

Sakurada *et al*. suggested that the capsaicin-induced pain model examines substances which act on pain of neurogenic origin. Furthermore, capsaicin can be defined as an extracted neurotoxic substance from red pepper, resulting in irritation of the skin when applied or injected into animals causing a painful sensation and subsequent desensitization to chemically induced pain. Many reports have revealed that capsaicin provokes the release of neuropeptides, Nitric oxide (NO), excitatory amino acids (glutamate and aspartate), and proinflammatory mediators and also helps in transmission of nociceptive information to the spinal cord [146]. The analgesic action of α-T was presented by Le Bars *et al*. involving the supraspinal as well as the spinal components by the utilization of the hot plate test [147]. The results suggested that α-T (only at a higher dose) has a central analgesic effect, due to the occurrence of time response delay during a hot plate test, when mice were exposed to a nociceptive stimulus [141].

According to Poole *et al*., releasing primary hypernociceptive mediators are believed to be stimulated by a cascade of cytokines and not directly by means of inflammatory stimuli [148]. Mechanical hypernociception is induced by carrageenan (CG) using this cascade of cytokines. TNF-α is the first cytokine to be set free and subsequently triggers the release of other cytokines such as IL-1β [149]. This can lead to a neurogenic inflammation which contributes to the inflammatory process resulting in central and peripheral hyperalgesia. Moreover, the α-T's antinociceptive activity indicated that the development of this mechanical hypernociception is inhibited by pre-systemic treatment with this alcohol at doses (25, 50 or 100 mg/kg. i.p.). A similar action was also noticed upon prostaglandin $E_2$ ($PGE_2$) and dopamine (DA) administration, where it was observed that α-T was able to maintain the baseline nociceptive threshold and significantly inhibited the neutrophil-influx in the pleurisy model [137]. These results may conclude that the synthesis of compounds, such as eicosanoids which is correlated with the inflammatory process, is inhibited by α-T possibly by means of suppressing NF-κB signalling [119]. α-T (1, 10 and 100 μg/ml) also significantly reduced ($p<0.01$) nitric oxide (NO) production in macrophages stimulated by lipopolysaccharides (LPS) *in vitro* [137].

In summary, the data collected so far provide information about the antinociceptive and anti-inflammatory properties of α-T which attract great pharmaceutical interest in developing new clinically drugs which can be useful in managing and controlling painful and/or inflammatory disease [137, 141].

## 4.5 Antiulcer activity

*„Peptic ulcer is one of the most common gastrointestinal diseases. Gastric ulcers are generally caused by a disruption in the balance between aggressive factors (pepsin and hydrochloric acid) and mucosal*

*defensive factors, such as blood flow, mucus and biocarbonate secretion. In recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources"*[150].

As α-T is an isomer of the monoterpene alcohol T-4-ol which possesses anti-ulcer activity [106], it was also of interest to evaluate and present the anti-ulcer activity of α-T. Years ago, the gastroprotective activity of α-T was determined in the two ethanol-and indomethacin-induced ulcer models in rats. In the ethanol-induced ulcer model, the oral administration of α-T furnished gastroprotective activity, by reduction of the gastric lesions. Stimulation of defense mechanisms (cytoprotective effect) is the suggested mechanism of action of drugs showing gastroprotective activity against ethanol-induced gastric lesions, rather than inhibition of aggressive ones (antisecretory effect). The indomethacin-induced gastric lesions are also decreased by means of an oral treatment with α-T, but a considerable inhibition ($p<0.01$) was noticed only at concentrations 30 mg and 50 mg/kg. This result shows that this alcohol exerts its gastroprotective action in a dose-dependent manner [150]. Moreover, there is a relationship between gastric acid and the gastric lesion formation which is induced by indomethacin. Gerkens *et al.* proposed that the indomethacin-induced lesion formation is attributed to the decrease of gastric mucosal blood flow [151].

Pretreatment with indomethacin (10 mg/kg) did not inhibit the gastroprotective action of α-T on ethanol-induced ulcers. Based on this result, an increase in prostaglandin synthesis is not believed to be involved in the gastroprotective action of α-T at a concentration 50 mg/kg. On the other hand, the secretion of gastric acid can be inhibited by either proton pump inhibitors and/or histamine $H_2$ receptor antagonists, which represent the currently used drugs in order to treat ulcers. However, α-T has not changed proton concentration values, pH and the gastric volume after pylorus ligation, indicating that its gastroprotective action is not suggested to be due to gastric secretion inhibition. On this

basis, this alcohol exerts its gastroprotective effect probably by means of cytoprotective mechanisms which need further investigations to be more explained [150].

## 4.6 Anticonvulsant and sedative activity

About 450 million people, as reported by the WHO, suffer from many problems during their lives, such as neurological, mental or behavioural disturbance [152]. Epilepsy can be defined as a plenty of disorders accompanied by recurrent spontaneous seizures, caused by several complex mechanisms including different neurotransmitter systems as GABA and cholinergic system. Despite of using more efficient and modern anticonvulsant drugs to treat epilepsy patients worldwide, seizures still considered to be unmanageable in more than 20% of the cases. Furthermore, most of the currently used antiepileptic drugs are obtained by means of chemical synthesis, such as benzodiazepines and succinimides. Therefore, recent studies on monoterpene compounds such as α-T have been required to examine their pharmacological aspects in order to develop new anticonvulsant drugs with lower side effects and more advantages than that of the currently used compounds [153].

De Sousa *et al.* investigated the anticonvulsant activity of α-T. The results of this study indicated that the latency to pentylenetetrazole-induced convulsions is increased by treatment with α-T at concentrations 100 and 200 mg/kg and the incidence of hind-limb extension produced by MES is reduced at concentrations 200 and 400 mg/kg in a dose dependant manner [110]. Another study analyzed the therapeutic effect of α-T as a relaxing drug and tranquilizer. The data showed that this alcohol increased the sleep time of the mice indicating a sedative property, due to the suggested action on central mechanisms affecting the inhibition of the metabolism of pentobarbital or the regulation of sleep in mice. In other

words, α-T exhibited a depressant effect on the pentobarbital-induced sleep test, indicating a sedative property [154].

## 4.7 Anti-bronchitis activity

*„Chronic obstructive pulmonary disease (COPD) is a chronic obstructive lung disease and is frequently found in well-developed countries due to the issue of aging population. COPD can lead to the restriction of lung function"*[155, 156]. The current treatment options for COPD are very limited and their side effects of treatment frequently noted is Cushing Syndrome caused by long term steroid use [157]. Many COPD patients finally need lung transplants and the survival outcome is still poor even when patients undergo lung transplantations [158]. Despite improvement with regard to pharmacy and drug invention the occurrence of COPD and mortality related to COPD continues to rise [159]. Clearly, efforts to stop smoking to reduce air pollution and to control pneumonia could be the appropriate prevention methods to limit deterioration in cases of COPD. However, there are no other useful ways to attempt to cure the COPD; thus it remains the leading cause of death throughout the world [160]. Therefore, prevention of the occurrence of COPD is the important issue to address, by not only the above mentioned methods but also by the inhibition of IκB-kinase beta (IKK2) which is linked to COPD occurrence [161, 162].

Tsou *et al*. investigated the effect of α-T against COPD. The top 3 traditional Chinese medicine (TCM) compounds were found to be sinapic acid-4-O-sulphate, kaempferol and α-T belonging to the TCM herbs *Magnolia officinalis*, *Bupleurum Chinese* and *Bursaphelenchus xylophilius,* respectively [163]. α-T and its herb exert an antimicrobial effect and in particular prevent infections that originate from periodontopathic and carcinogenic bacteria [164]. As a result, it was indicated that the above mentioned top 3 TCM compounds can have an

effect on IKK2 inhibition and prevent exacerbation and disease progression with regards to COPD [163].

## 4.8 Skin penetration enhancing activity

Over the last 2-3 decades, the skin has become an important route for the administration of drugs for topical, regional or systemic action. The skin however has evolved as a physical and biochemical protective barrier which prevents the loss of water from the body, and guards against entry into the body of external toxic chemicals and infections agents, thereby maintaining homeostasis. The role of the skin as a barrier to the external environment renders the absorption and transdermal delivery of most drugs problematic. The stratum corneum (SC) which is the outermost layer of the skin and comprised of keratin-rich cells embedded in multiple lipid bilayers has been considered the rate-determining structure governing precutaneous absorption of permeants. Therefore, most of drugs are not able to penetrate the SC or to be delivered through it [165]. *„Many strategies have been employed to enhance dermal and transdermal delivery. These include the use of chemical penetration enhancers, preparation of supersaturated drug delivery systems, electrically driving molecules through the tissues by iontophoresis, and physically disrupting the skin structure by electroporation or sonophoresis "*[166].

Delivery of drugs via the skin has numerous advantages, like non-invasiveness, potential for continuous or controlled delivery, and potential for delivery of certain classes of drugs that are not amenable for the administration via other routes of the drug delivery. Penetration enhancers have therefore frequently been used in the field of transdermal drug delivery research and various types of penetration enhancers with different modes of action [165]. Transdermal delivery of drugs promises many advantages over oral or intravenous administration such as

decreasing the side effects, improving patients compliance, first-pass effect elimination, sustained drug delivery and interruption of the drug treatment if required [167], though human skin provides an effective barrier to the permeation of most drugs in the form of SC [168, 169]. Many factors have a great influence on the dermal absorption such as skin type, the origin (human, animal), environmental factors, as well as the physicochemical activities of the tested substance and delivery systems [170]. Transdermal therapeutic systems offer a more reliable mean of administering drug through the skin by various physical, chemical, biochemical, supersaturation and bioconvertable prodrug enhancement strategies [171].

Out of these strategies, a popular technique is the use of chemical permeation enhancers, which alters reversibly the permeability barrier of the SC. α-T is considered one of the chemical enhancers, which is currently believed to improve solubility within the SC or increase lipid fluidity of the intracellular bilayers [165, 171]. Many studies have been reported that α-T appears to be acceptable as a promising skin penetration enhancer as indicated by following advantages [170]:

1. High percutaneous enhancement ability,

2. less toxic with low irritancy potential,

3. reversible effect on the lipids of SC, and

4. the absence of toxicity.

Several studies suggest that the activity of α-T as an enhancer is a result of disrupting the intracellular lipid bilayers. Evidence from skin electrical conductivity measurements suggests that this alcohol may create polar pathways across the SC for ions and polar drug penetration. In addition, results from electron paramagnetic resonance have demonstrated that α-T can fluidize the SC lipids and weaken the hydrogen-bonded network of

the polar interface of the SC [167, 172, 173]. The mechanism of action of this alcohol appears to be difficult, depending on the nature of permeants (e.g. hydrophilic or lipophilic). Furthermore, α-T is an alcoholic monoterpene with a high degree of unsaturation and appears to be a better candidate for enhancing the permeation of hydrophilic drugs such as, e.g. 5-flurouracil by increasing the diffusion of the drug in the SC [165, 171]. *„The interaction of α-T with SC lipids and keratin can be elucidated with instrumental methods such as Fourier transform infrared spectroscopy (FT-IR) and differential scanning colorimetry (DSC). The FT-IR provides the information about the molecular and conformational changes of lipids and proteins, whereas the DSC provides information about their thermotropic behaviour"*[167].

As skin penetration enhancer, α-T has been employed directly or in combination with co-solvents such as propylene glycol or ethanol. Synergistic activity has been reported between α-T and propylene glycol as well as between α-T and ethanol [167, 172]. It was reported that the *in vitro* permeation of haloperidol (HP), an antipsychotic drug, is increased through human skin by using α-T at concentration 5% w/v in 100% propylene glycol (PG). Haloperidol is a lipophilic drug and may play an important role in developing the transdermal dosage form. Since HP is clinically needed to be found in a long-acting formulation in order to avoid psychosis relapse, it was required to use as skin penetration enhancer α-T and as co-solvent PG to increase the permeation of HD [167].

Narishetty *et al*. investigated the effect of this monoterpene alcohol and other various oxygen-containing monoterpenes, such as 1,8-cineole, menthol, methone, pulegone and carvone for the *ex vivo* permeation of zidovudine (AZT), the first approved and wide clinically used anti-HIV substance, in vehicle (66.6 % ethanol in water) across rat skin. Based on the result of this study, it was indicated that a hydrogen bonding interaction is formed by α-T with the ceramide head group of SC

lipids and a subsequent reduction in the skin barrier property has occurred [172].

According to many skin penetration studies using the skin of hairless mice and excised animal skin, it was found that α-T was effective in enhancing the skin penetration of model permeants, such as caffeine [174] and 5-Flurouracil [175] respectively. α-T exerted an effective penetration enhancing activity for hydrocortisone percutaneously and also increased the permeation between 3.9-fold and 5-fold, and this alcohol was the most active compound among several other compounds to increase the delivery of triamcinolone acetonide [170, 174].

The use of local anesthetics in combination with penetration enhancers could overcome the barrier properties of the skin to epicutaneous penetration of local-anesthetic drugs. Lidocaine is a topical anesthetic agent with low skin permeability which cannot penetrate adequately to the intact skin. On the other hand, the ideal topical anesthetic agent is one that provide 100 % anesthesia in a short period of time, is effective on the intact skin without systemic side effects, and invokes neither pain nor discomfort [176]. The authors of this study investigated the effects of some permeability enhancers such as polysorbate 80, polysorbate 20, dimethylsulfoxide (DMSO), tert-butyl cyclohexanol (TBCH), and α-T in different concentrations on the percutaneous permeation of lidocaine. According to the results of this study, α-T showed the best permeability enhancing effects on the lidocaine penetration through the skin. Since this alcohol is a relatively safe compound, its incorporation in low concentration into local anaesthetic cream formulations can be recommended. α-T exerts the best effect at a concentration of 2.5%, as it is believed that it is able to produce eutectic mixtures with lidocaine and then could increase the thermodynamic activity of lidocaine in the relevant formulation [176].

Interestingly, Fang *et al.* found that the best method used to enhance the curcumin permeation is pretreatment of rat skin with 5% α-T

in an ethanolic solution for 1 h [177]. Curcumin exhibits various biological properties such as antioxidant, anticancer, anti-inflammatory and wound healing [178]. Therefore, several disorders can be treated using curcumin, such as tumors and proinflammatory chronic diseases [179, 180]. Because of the insufficient aqueous solubility and bioavailability of curcumin, it is not widely used in the clinical field for treatment of cancer and other diseases. The low bioavailability of curcumin is attributed to several factors including rapid metabolism, the poor absorption and rapid systemic elimination [181]. In another study, three terpenes, namely α-T, 1,8-cineole and limonene, were used to compose an oil phase of the microemulsions which provide another promising alternative for the dermal and transdermal delivery of both hydrophilic and lipophilic drugs. Their effects on curcumin skin delivery were evaluated using neonate pig skin mounted on a Franz diffusion cell. The results indicated that the curcumin retained in the skin increased in the order limonene > α-T > 1,8-cineole [166]. Additionally, it was reported that α-T was used as a transdermal enhancer for buspirone hydrochloride, an anxiolytic, in hairless mouse skin [182]. Moreover, Jain *et al.* showed that the effect of this alcohol on imipramine hydrochloride (IMH) permeation in the ethanol (EtOH): W (2:1) system. By means of unjacketed Franz diffusion cells, permeation studies of IMH were performed through rat skin. Based on the results of this study, it was found that α-T is an effective permeation enhancer for IMH [183].

## 4.9 Insect repellent activity

*„Some facts indicate that the use of synthetic chemicals to control insects and arthropods raises several concerns as to the environment and human health. So, there is a growing demand for alternative repellents or natural products. These products possess good efficacy and are environmentally friendly. Essential oils from plants belonging to several*

*species have been extensively tested to assess their repellent and even insecticidal properties as valuable natural resources"*[9]. In a study [184], the chemical composition and insecticidal and repellent activity of the essential oil of the aerial parts of *Artemisia rupestris L*. against the booklice *Liposcelis bostrychophila* Badonnel was determined. The principal compounds in the essential oils were α-terpinyl acetate (37.2 %), α-T (10.1 %), linalool (7.6 %), followed by T-4-ol (3.9 %) [184].

Booklice (*L. bostrychophila* Badonnel) have a widespread distribution infesting domestic premises, manufacturing factories, raw material stores and historical documents in museums [185]. Booklice are believed to be nuisance pests but they do not bite and cannot transmit diseases. Due to the presence of more damaging post-harvest primary pests and the small size of booklice, they are often disregarded and are generally considered as secondary pests. The results of the previous study, indicated that α-T exhibited a strong contact toxicity (LD50 = 140.30 μg/cm$^2$) against booklice. It was found that α-T showed strong repellency also against *L. bostrychophila*, while T-4-ol exhibited only weak repellency. It was concluded that this essential oil and its most active compounds α-T and α-terpinyl acetate have great potential for development into natural insecticides or fumigants as well as repellents for control of insects in stored grains [184].

# 5. Conclusion

α-T is a monocyclic monoterpene tertiary alcohol with a pleasant odor similar to lilac. Therefore, it is widely used in the manufacture of perfumes, cosmetics, soaps, antiseptic agents used is considered one of the most frequently used fragrant compounds [1]. In addition, α-T possesses a wide range of biological activities which attract a great interest in the medicinal field [7].

The cardiovascular and the antihypertensive effects of α-terpineol were investigated in several studies. Based on these results, it was indicated that oral administration of α-T was able to reduce the mean arterial pressure and endothelium independent vasodilatation. Moreover, α-T is able to restore enyzmatic antioxidant in L-NAME-induced hypertensive [116, 125].

Additionally, α-T shows an antiproliferative (antioxidant) activity, which can be used in the prevention or even treatment of cancer, as it was found that this alcohol demonstrated a potential antioxidant capacity effect against different human cancerous cell lines (breast, lung, prostate, ovarian and leukemia). α-T inhibits the growth and induction of cell death in tumor cells by means of an inhibition of NF-κB activity [119, 129].

The antinociceptive activity is one of the most important biological activities which is correlated to α-T. It was indicated that this alcohol produced significant analgesic effects by reduction at the early and late phases of paw licking and reduced the acetic acid-induced writhings reflex in mice (formalin and writhing tests, respectively). Those

effects are probably in relationship to the inhibition in the peritoneal fluid levels of $PGE_2$ and $PGF_{2\alpha}$ and to the release inhibition of substance P and other inflammatory molecules [141]. However, α-T exerted also a selective COX-2 inhibition (0.69mM), therefore, it is believed that this alcohol showed higher COX-2 activity inhibition than Aspirin® [145]. α-T might be potentially interesting in the development of new drugs for the management of painful and/or inflammatory diseases, as well in the development of novel therapies for COPD [163].

Several studies have reported that this alcohol also possesses antiulcer activity. The results suggested that it presented a gastroprotective activity by reduction the gastric lesions at the doses 10, 30 and 50 mg/kg without the involvement of gastric acid secretion inhibition or increase in prostaglandin synthesis [106,150]. Furthermore, α-T shows anticonvulsant and sedative activities via a depressant effect on the pentobarbital-induced sleep test [154]. In addition, it increased the latency to convulsions induced by pentylenetetrazole and decreased the incidence of hind limb extension produced by MES in a dose related manner [110].

Another important biological activity which belongs to α-T is its promising effect as a chemical skin penetration enhancer, which is currently believed to improve the solubility within the stratum corneum or to increase the lipid fluidity of the intracellular bilayers [165, 171]. In addition, the insect repellent activity of this alcohol attracts the interest of many scientists. It was reported that α-T found in the essential oil of *A. rupestris* exhibited a strong contact toxicity against booklice. Therefore, it is suggested that α-T is a potential agent for development into natural insecticides or fumigants, as well as repellents for control of insects in stored grains [184].

# 6. References

[1] Bauer K, Garbe D, Surburg H. **(2001)** *Common Fragrance and Flavor materials*. Wiley, New York, 1-269.

[2] Sell C. **(2003)** *A Fragrant Introduction to terpenoid chemistry.* The Royal Society of Chemistry, Cambridge, UK, 1-307.

[3] Başer KHC, Buchbauer G. **(2010)** *Handbook of essential oils.* Taylor and Francis group, New York, US, 1-949.

[4] Chooluck K, Singh RP, Sathirakul K, Derendorf H. **(2013)** Plasama and dermal pharmacokinetics of terpinen-4-ol in rats following intravenous administration. *Pharmazie*, 68, 135-140.

[5] Sell C. **(2006)** *The Chemistry of fragrances.* The Royal Society of Chemistry, Cambridge, UK, 1-321.

[6] Singh G. **(2007)** *Chemistry of Terpenoids and Carotenoids.* Discovery Publishing House, New Delhi, India, 1-157.

[7] Bhatia SP, Letizia CS, Api AM, **(2008)** Fragrance material review on alpha-terpineol. *Food and Chemistry Toxicology, 46(11),* 280-285.

[8] Carson CF, Hammer KA, Riley TV **(2006)** *Melaleuca alternifolia* (Tea tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiology Reviews, 19(1),* 50-62.

[9] Adorjan B, Buchbauer G. **(2010)** Biological properties of essential oils: an update review. *Flavour and Fragrance Journal, 25,* 407-426.

[10] Raut JS, Karuppayil SM. **(2014)** A status review in the medicinal properties of essential oils. *Industrial Crops and Products, 62,* 250-264.

[11] Carson CF, Hammer KA, Riley TV. (**2012)** Effect of *Melaleuca alternifolia* (tea tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility. *Antimicrobial Agents and Chemotherapy, 56,* 909-915.

[12] Mondello F, De Bernardis F, Girolamo A, Cassone A, Salvatore G. **(2006)** *In vivo* activity of terpinen-4-ol, the main bioactive component of *Melaleuca alternifolia* cheel (tea tree) oil against azole susceptible and resistant human pathogenic Candida species. *BMC Infectious Diseases, 6,* 158.

[13] Hammer KA, Carson CF, Riley TV, Nielsen JB. **(2006)** A review of *Melaleuca alternifolia* (tea tree) oil. *Food and Chemistry Toxicity, 44,* 616-625.

[14] Harrewijin P, Van Oosten AM, Piron PG. (**2001**) *Natural terpenes as messengers*. Kluwer Academic Publishers, Dordrecht, The Netherlands, 1-425.

[15] Cox SD, Mann CM, Markham JL. **(2001)** Interaction between components of the essential oil of *Melaleuca alternifolia*. *Journal of Applied Microbiology, 91(3)*, 492-497.

[16] Reichling J, Schnitzler P, Suschke R, Saller U. **(2009)** Essential oils of Aromatic plants with antibacterial, antifungal and cytotoxic properties -an overview. *Forschende Komplementärmedizin*, *16(2),* 79-90.

[17] Bakkali F, Averbeck S, Averbeck D, Idaomar M. **(2008)** Biological effects of essential oils a review. *Food and Chemical Toxicology, 46*, 446-475.

[18] Cuaron JA, Dulal S, Song Y. **(2013)** Tea tree oil-induced transcriptional alternations in *Staphylococcus aureus*. *Phytotherapy Research, 27,* 390-396.

[19] Carson CF, Mee BJ, Riley TV. **(2002)** Mechanism of action of *Melaleuca alternifolia* determined by time-kill lysis, leakage and salt tolerance assay and electron microscopy. *Antimicrobial Agents and Chemotherapy, 46(*1), 1914-1920.

[20] Loughlin R, Gilmore BF, McCarron PA, Tunney MM. **(2008)** Comparison of the cidal activity of tea tree oil and terpinen-4-ol against clinical bacterial skin isolates and human fibroblast cells. *Applied Microbiology*, *46(4),* 428-433.

[21] Wikipedia, the free encyclopaedia. Available at: http// de.m.wikipedia.org/wiki/Escherichia coli/ [4 June 2015].

[22] Papadopoulos CJ, Carson CF, Chang BJ, Riley TV**. (2008)** Role of the MexAB-OprM efflux pump of Pseudomonas aeruginosa in tolerance to tea tree oil and its monoterpene components terpinen-4-ol, 1,8-cineole and α-terpineol. *Applied and Environmental Microbiology, 74(6),* 1932-1935.

[23] Papadopoulos CJ, Carson CF, Hammer KA, Riley TV. **(2006)** Susceptibility of Pseudomonas to *Melaleuca alternifolia* (Tea tree) oil and components. *Journal of Antimicrobial Chemotherapy, 58(2):* 449-451.

[24] Longbahom CJ, Carson CF, Hammer KA, Mee BJ, Riley TV. **(2004)** Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree)

oil is associated with the outer membrane and energy dependent cellular process. *Journal of Antimicrobial Chemotherapy, 54,* 386-392.

[25] Zilbauer M., Dorrell N, Wren BW, Bajaj-Elliot M. **(2008)** Campylobacter jejuni-mediated diseases pathogenisis. *Transactions of the Royal Society of Tropical Medicine Hygiene. 102(2),* 123-129.

[26] Nachamkin I, Allos BM, Ho T. **(1988).** Campylobacter species and Guillian-Barre syndrome. *Clinical Microbiology Reviews, 11(3)*, 555-567.

[27] Müllner P, Collins-Emerson JM, Midwinter AC. **(2010)** Molecular epidemiology of Campylobacter jejuni in a geographically isolated country with a uniquely structured poultry industry. *Application Environmental Microbiology, 76(7)*, 2145-2154.

[28] Pointon A, Sexton M, Dowsett P, Saputra T. **(2008)** A baseline survey of the microbiological quality of chicken portions and carcasses at retail in two Australian states (2005 to 2006). *Journal of food protection, 71*(6), 1123-1134.

[29] Castanon JIR, **(2007)** History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science, 86*, 2466-2471.

[30] Andreasen CB, Spickler AR, Jones BE. **(2005)** Swedish antimicrobial regulations and their impact on food animal production. *Journal of American Veterinary Medical Association, 227(1),* 41-45.

[31] Kurekci C, Padmanabha J, Bishop-Hurley SL. **(2013)** Antimicrobial activity of essential oils and five terpenoid compounds against *Campylobacter jejuni* in pure and mixed culture experiments. *International Journal of Food Microbiology, 166,* 450- 457.

[32] Ramage G, Milligan S, Lappin DF, Sherry L, Sweeney P, Williams C, Bagg J, Culshaw S. **(2012)** Antifungal, Cytotoxic and Immunomodulatory properties of tea tree oil and its derivative

components: Potential role in management of oral Candidiasis in cancer patient. *Frontiers in Microbiology, 3,* 220.

[33] Rajkowska K, Kunicka-Styczyńska A, Maroszyńska M, Dąbrowska M. **(2014)** The effect of thyme and tea tree oil on morphology and metabolism of *Candida albicans*. *Acta Biochimica Polonica, 61(2),* 305-310.

[34] Nawrot U, Pajączkowska M, Fleischer M, Przondo-Mordarska H. **(2013)** Candidemia in polish hospitals-1 multicentre survey. *Mycoses, 56(5)*, 576-581.

[35] Lass-Flörl C. **(2009)** The changing face of epidemiology of invasive fungal disease in Europe, *Mycoses, 52*, 197-205.

[36] Ninomiya K, Hayama K, Ishijima SA. **(2013)** Suppression of inflammatory reactions by terpinen-4-ol, a main constituent of tea tree oil, in a Murine Model of oral Candidiasis and its suppressive activity to cytokine production of macrophages *in vitro*. *Biological and Pharmaceutical Bulletin, 36(5),* 838-844.

[37] Ninomiya K, Maruyama N, Inoue S. **(2012)** The essential oil of *Melaleuca alternifolia* and its main component, terpinen-4-ol protect mice from experimental oral candidiasis. *Biological and Pharmaceutical Bulletin, 35(6)*, 861-865.

[38] Ninomiya K, Hayama K, Ishijima SA. **(2013)** Effects of inhibitory activity of mycelial growth of Candida albicans and therapy for murine oral candidiasis by the combined use of terpinen-4-ol and middle-chain fatty acid. *Yakugaku Zasshi, 133(1),* 133-140.

[39] Cox SD, Mann CM, Markham JM. **(2001)** Determining the antimicrobial actions of tea tree oil. *Molecules, 6*, 87-91.

[40] Hammer KA, Carson CF, Riley TV. **(2004)** Antifungal effects of *Melaleuca alternifolia* oil and its components on Candida albicans,

Candida glabrata and Saccharomyces cerevisiae. *Journal of Antimicrobial Chemotherapy, 53(6)*, 1081-1085.

[41] Cox SD, Mann CM, Markham JL, Bell HC. **(2000)** The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of Applied Microbiology, 88(1)*, 170-175.

[42] D´auria FD, Laino L, Strippoli V, Tecca M, Salvatore G., Battinelli L, Mazzanti G. **(2001)** *In vitro* activity of tea tree oil against *Candida albicans* mycelial conversion and other pathogenic fungi. *Journal of Chemotherapy, 13(4),* 377-383.

[43] Hammer KA, Carson CF, Riley TV. **(2000)** *Melaleuca alternifolia* (tea tree) oil inhibits germ tube formulation by *Candida albicans. Medical Mycology, 38(5),* 355-362.

[44] Sun L, Zhang C, Li P. **(2012)** Characterization, Antibiofilm, and Mechanism of Action of Novel PEG-Stabilized Lipid Nanoparticles Loaded with Terpinen-4-ol**.** *Journal of Agriculture and Food Chemistry, 60(24),* 6150-6156.

[45] Satchell A, Saurajen A, Bell C, Barnetson R. **(2002)** Treatment of interdigital tinea pedis with 25% and 50% tea tree oil solution: A randomized, placebo-controlled, blinded study. *Australian Journal of Dermatology, 45(3),* 175-178.

[46] Flores FC, De Lima JA, Riberio RF, Alves SH, Rolim CM, Beck RC, De Silva CB. **(2013)** Antifungal activity of nanocapsule suspensions containing tea tree oil on the growth of *Trichophyton rubrum***.** *Mycopathologia, 175,* 281-286.

[47] Satchell A, Saurajen A, Bell C, Barnetson R. **(2002)** Treatment of dandruff with 5% tea tree oil shampoo. *Journal of the American Academy of Dermatology, 47(6),* 852-855.

[48] Schnitzler P, Schön K, Rcichling J. **(2004)** Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. *Pharmazie, 56*, 343-347.

[49] Li X, Duan S, Chu C, Xu J. **(2013)** *Melaleuca alternifolia* concentrate inhibits *in vitro* entry of influenza virus into host cells. *Molecules, 18*, 9550-9566.

[50] Minami M, Kita M, Nakaya T. **(2003)** The inhibitory effect of essential oils on herpes simplex virus type-1 replication *in vitro***.** *Microbiology and Immunology, 47(9)*, 681-684.

[51] Carson C, Ashton L, Dry L, Smith D, Riley T. **(2001)** *Melaleuca alternifolia* (tea tree) oil gel (6%) for the treatment of recurrent herps labialis. *Journal of Antimicrobial Chemotherapy, 48(3),* 450-451.

[52] World Health Organization Influenza (Seasonal) Available online:[23August2012].
http://www.who.int/mediccentre/factsheets/fs211/en/index.

[53] Girard MP, Tam JS, Assossou OM, Kieny MP. **(2010)** The 2009 a (H1N1) influenza virus pandemic: A review. *Vaccine, 28(31)*, 4895-4902.

[54] Ujike M, Shimabukuro K., Mochizuki K., Obuchi M. **(2010)** Oseltamivir-resistant influenza viruses A (H1N1) during 2007–2009 influenza seasons, Japan**.** *Emerging Infectious Diseases***,** *16*(6), 926-935.

[55] Garozzo A, Timpanaro R, Bisignano B, Furneri PM, Bisvignano G, Castro A. **(2009)** *In vitro* antiviral activity of *Melaleuca alternifolia* essential oil. *Applied Microbiology, 49(6*), 806-808.

[56] Garozzo A, Timpanaro R, Stivala A, Bisigmno G, Castro A. **(2010)** Activity of *Melaleuca alternifolia* (tea tree) oil on influenza virus A/PR/8: study on the mechanism of action. *Antiviral Research, 89(1),* 83-88.

[57] Mikus J, Harkenthal M, Steverding D, Reichling J. **(2000)** *In vitro* effect of essential oils and isolated mono- and sesquiterpenes on Leishmania major and Trypanosoma brucei. *Planta Medica, 66(4)*, 366-368.

[58] Viollon C, Mandin D, Chaumont JP. (**1996**) Activities antagonists, in vitro, de quelques huiles essentielles et de composes naturels volatils vis á vis de la croissance de Trichomonas vaginalis. *Fitoterapia, 67*, 279-281.

[59] Tighe S, Gao YY, Tseng SCG. **(2013)** Terpinen-4-ol is the most active ingredient of tea tree oil to kill *Demodex* mites. *Translational Vision Science and Technology, 2(7),* 2.

[60] Thomasen NA, Hammer KA, Riley TV, Van Belkum A, Carson CF. **(2013)** Effect of habituation to tea tree (*Melaleuca alternifolia*) oil on the subsequent susceptibility of *Staphylococcus* spp. to antimicrobials, triclosan, tea tree oil, terpinen-4-ol and carvacrol. *International Journal of Antimicrobial Agents, 41(4)*, 343-351.

[61] Hidron AL, Edwards JR, Patel J. **(2008)** NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the national healthcare safety network at the centres for disease control and prevention, 2006-2007. *Infection Control and Hospital Epidemiology, 29(11),* 996-1011.

[62] Walton SF, Mckinnon M, Pizzulto S, Dougall A, Williams E, Currie BJ. **(2004)** Acaricidal activity of Melaleuca alternifolia (tea tree) oil: in vitro sensitivity of sarcoptes scabiei var hominis to terpinen-4-ol. *Archives of Dermatology, 140(5),* 563-566.

[63] Campli DE, Bartolomeo DS, Pizzi DP. **(2012)** Activity of tea tree oil and nerolidol alone or in combination against Pediculus capitis (head lice) and its eggs**.** *Parasitology Research, 111(5),* 1985-1992.

[64] Basta-Juzbasic A, Subic JS, Ljubojevic S**. (2002)** Demodex folliculorum in development of dermatitis rosaceiformis steroidica and rosacea-related diseases. *Clinics in Dermatology, 20(2)*, 135-140.

[65] Baima B, Sticherling M. **(2002)** Demodicidosis revisited. *Acta Derm Venerol, 82*, 3-6.

[66] Garbacewicz A, Udziela M, Grytner-Ziecina B. **(2010)** Demodex infections in general Polish population, in patients suffering from

blepharitis, and among people who work with microscopes. *Klinika Oczna, 112*, 307-310.

[67] Gao YY, Pascuale MA, Elizondo A, Tseng SC. **(2007)** Clinical treatment of ocular demodecosis by lid scrub with tea tree oil. *Cornea, 26(2),* 136-143.

[68] Hart PH, Brand C, Carson CF. **(2000)** Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. *Inflammation Research, 49(11),* 619-626.

[69] Walton SF, Myerscough MR, Currie BJ. **(2000)** Studies *in vitro* on the relative efficacy of current acaricides for *Sarcoptes scabiei* var. *hominis*. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 94(1),* 92-96.

[70] Burgess IF. (**2004**) Human lice and their control. *Annual Reviews of Entomology, 49*, 457-481.

[71] DPDx - Laboratory Identification of Parasitic Diseases of Public Health Concern, http://www.cdc.gov/dpdx/pediculosis/index.html [29 November 2013].

[72] Lenung AK, Fong JH, Pinto-Rojas A. **(2005)** Pediculus capitis. *Journal of Pediatric Health Care, 19(4),* 369-373.

[73] Chosidow O. **(2000)** Scabies and Pediculosis. *The Lancet, 355(9206),* 819-826.

[74] Hunter JA, Barker SC. (**2003**) Susceptibility of head lice *(Pediculus humanus capitis)* to pediculicides in Australia. *Parasitology Research, 90(6),* 476-478.

[75] De Silveria R, Andrade L, De Sousa D. **(2013)** A review on anti-inflammatory activity of monoterpenes. *Molecules, 18,* 1227-1254.

[76] Brand C, Ferrante A, Prager RH, Riley TV, Carson CF, Finaly-Jones JJ, Hart PH. **(2001)** The water-soluble components of the essential oil of

*Melaleuca alternifolia* (tea tree oil) suppress the production of superoxide by human monocytes, but not neutrophils, activated in vitro. *Inflammation Research, 50(4),* 213-219.

[77] Caldefie-Chezet F, Fusillier C, Jarde T, Laroye H. **(2006)** Potential anti-inflammatory effects of *Melaleuca alternifolia* essential oil on human peripheral blood leukocytes**.** *Phytotherapy Research, 20*, 364-370.

[78] Brand C, Townley SL, Finlay-Jones JJ, Hart PN. **(2002)** Tea tree oil reduces histamine-induced oedema in murine ears. *Inflammation Research, 51*, 283-289.

[79] Nogueria MN, Aquino SG, Rossa JS, Spolidorio DM. **(2014)** Terpinen-4-ol and alpha-terpineol (tea tree oil components) inhibit the production of IL-1β, IL-6 and IL-10 on human macrophages. *Inflammation Research, 63*, 769-788.

[80] Abe S, Maruyama N, Hayama K, Ishibashi H. **(2003)** Suppression of tumor necrosis factor-alpha-induced neutrophil adherence responses by essential oils. *Mediators of Inflammation, 12(6),* 323-328.

[81] Caldefie-Chezet F, Guerry M, Chalchat JC. **(2004)** Anti-inflammatory effects of Melaleuca alternifolia essential oil on human polymorphonuclear neutrophils and monocytes. *Free Radical Research, 38(8)*, 805-811.

[82] Eskan MA. **(2008)** TLR4 and S1P receptors cooperate to enhance inflammatory cytokine production in human gingival epithelial cells. *European Journal of Immunology, 38*(4), 1138-1147.

[83] Souza JAC, Junior CR, Garlet GP, Nogueira AV, Cirelli JA. **(2012)** Modulation of host cell signalling pathways as a therapeutic approach in periodontal disease. *Journal of Applied Oral Science, 20*(2), 128-138.

[84] Brand C, Grimbaldeston MA, Gamble JR. **(2002)** Tea tree oil reduces the swelling associated with the efferent phase of a contact hypersensitivity response. *Inflammation Research, 51*(5), 236-244.

[85] Khalil Z, Pearce AL, Satkunanathan N. **(2004)** Regulation of wheal and flare by tea tree oil: complementary human and rodent studies. *Journal of Investigative Dermatology, 123*, 683-690.

[86] Clough G. **(1999)** Experimental models of skin inflammation. *Clinical and Experimental Allergy, 29(3)*, 105-108.

[87] Lahlou S, Interaminense LF, Leal-Cardoso J, Durate GP. **(2003)** Antihypertensive effects of the essential oil of *Alpinia zerumbet* and its main constituent, terpinen-4-ol, in DOCA-salt hypertensive conscious rats. *Fundamental and Clinical Pharmacology, 17(3),* 323-330.

[88] Soukoulis S, Hirsch R. **(2004)** The effects of a tea tree oil-containing gel on plaque and chronic gingivitis**.** *Australian Dental Journal, 49(2),* 78-83.

[89] Seifried HE, Anderson DE, Milner JA, Fisher EI. **(2007)** A review of the interaction among dietary antioxidant and reactive oxygen species. *The Journal of Nutritional Biochemistry, 18(9),* 567-579.

[90] Wikipedia, the free encyclopaedia. Available at: http://en.wikipedia.org/wiki/Cancer [26 May 2015].

[91] Calcabrini A, Stringaro A, Toccacieli L, Meschini S, Marra M, Colon M, Salvatore G. **(2004)** Terpinen-4-ol, the main component of *Melaleuca alternifolia* (tea tree) oil inhibits the *in vitro* growth of human melanoma cells**.** *Journal of Investigative Dermatology, 122*, 349-360.

[92] Greay SJ, Ireland DJ, Kissick HT, Levy A, Beilharz MW, Riley TV, Carson CF. **(2009)** Induction of necrosis and cell cycle arrest in murine cancer cell lines by *Melaleuca alternifolia* (tea tree) oil and terpinen-4-ol. *Cancer Chemotherapy and Pharmacology, 65(5),* 877-888.

[93] Greay SJ, Ireland DJ, Kissick HT, Heenan PJ, Carson CF, Beilharz MW, Riley TV. **(2010)** Inhibition of established subcutaneous murine tumour growth with topical *Melaleuca alternifolia* (tea tree) oil. *Cancer Chemotherapy and Pharmacology, 66(6),* 1095-1102

[94] Hayes AJ, Leach DN, Markham JL. **(1997)** *In vitro* cytotoxicity of Australian tea tree oil using human cell lines. *Journal of Essential Oil Research, 9(5),* 575-582.

[95] Wikipedia, the free encyclopaedia. Available at: http:// en.m.wikipedia.org/wiki/Melanoma [3 June 2015].

[96] Molinari A, Calcabrini A, Meschini S, Stringaro A. **(2000)** Induction of P-glycoprotein expression on the plasma membrane of human melanoma cells. *Anticancer Research, 20(4),* 2691-2696.

[97] Williams AC, Barry BW. **(2012)** Penetration enhancers**.** *Advanced Drug Delivery Reviews, 64,* 128-137.

[98] Cross SE, Russell M, Southwell I, Roberts MS. **(2008)** Human skin penetration of the major components of Australian tea tree oil applied in its pure form and as a 20% solution *in vitro*. *European Journal of Pharmaceutics and Biopharmaceutics, 69(1),* 214-222.

[99] Parkin DM, Bray F, Ferlay J, Pisani P. **(2001)** Estimating the world cancer burden: Globocan 2000. *International Journal of Cancer, 94(2),* 153-156.

[100] Wu CS, Ch YJ, Chen JW, Shieh JJ, Huang CH. (**2012**) Terpinen-4-ol induces apoptosis in human nonsmall cell lung cancer *in vitro* and *in vivo*. *Evidence-Based Complementary and Alternative Medicine, 2012,* 1-13.

[101] Kaufmann SH, Gores GJ. **(2000)** Apoptosis in cancer: cause and cure**.** *BioEssays, 22(11),* 1007-1017.

[102] Leake R. **(1996)** The cell cycle and regulation of cancer cell growth. *Annals of the New York Academy of Sciences, 784*, 252-262.

[103] Lahlou S, Galindo CA, Leal-Cardoso JH, Fonteles MC, Duarte GP. (**2002**) Cardiovascular effect of the essential oil of *Alpinia Zerumbet* leaves and its main constituent, terpinen-4-ol in rats: role of the autonomic nervous system. *Planta Medica, 68(12),* 1097-1102.

[104] Özek G, Özek T, Işcan G, Başer KHC, Hamzaoglu E, Duran A. (**2007**) Composition and antimicrobial activity of the essential oil of *Tanacetum cadmeum* (Boiss.) heywood subsp. *orientale* grierson**.** *Journal of Essential Oil Research, 19(4),* 392-395.

[105] Pascual ME, Slowing K, Carretro E, Sánchez MD, Villar A. **(2001)** Lippa: traditional uses, chemistry and pharmacology: a review. *Journal of Ethnopharmacology. 76(3),* 201-214.

[106] Matsunaga T, Hasegawa C, Kawasuji T, Suzuki H, Saito H. **(2000)** Isolation of the antiulcer compound in essential oil from the leaves of Cryptomeria japonica. *Biological and Pharmaceutical Bulletin, 23(5),* 595-598.

[107] Gourine N, Yousifi M, Bombarda I, Nadjemi B, Stocker P, Gaydou EM. **(2010)** Antioxidant activities and chemical composition of essential oil of *Pistacia atlantica* from Algeria. *Industrial Crops and Products, 32(2),* 203-208.

[108] De Sousa DP, Nobrega FF, De Morais LC, De Almeida RN. **(2009)** Evaluation of the anticonvulsant activity of terpinen-4-ol. *Zeitschrift für Naturforschung, 64*, 1-5.

[109] Rodenburg R, Wagner JL, Austin JK, Kerr M, Dunn DW. **(2011)** Psychosocial issues for children with epilepsy**.** *Epilepsy and Behaviour, 22(1),* 47-54.

[110] De Sousa DP, Quintans-Júnior L, De Almeida RN. **(2007)** Evolution of the anticonvulsant activity of α-terpineol. *Pharmaceutical Biology*, *45(1),* 69-70.

[111] Nóbrega FF, Salvadori MG, Masson CJ. **(2014)** Monoterpenoid terpinen-4-ol exhibits anticonvulsant activity in behavioural and electrophysiological studies. *Oxidative Medicine and Cellular Longevity, 2014,* 1-9.

[112] Santos MR, Moreira FV, Fraga BP, De Sousa DP, Bonjardim LR, Quintans-Júnior L. **(2011)** Cardiovascular effects of monoterpenes: a review. *Brazilian Journal of Pharmacognosy, 21(4),* 764-771.

[113] Wikipedia, the free encyclopaedia. Available at: http://en.m.wikipedia.org/wiki/hypertension. [2 June 2015].

[114] Wikipedia, the free encyclopaedia. Available at: http://en.m.wikipedia.org/wiki/peptic ulcer. [3 June 2015].

[115] Oliveria FD, Andrade LN, De Sousa EB, De Sousa DP. (**2014**) Anti-ulcer activity of essential oil constituents. *Molecules, 19(5)*, 5717-5747.

[116] Sabino CK, Ferreria-Filho ES, Mendes MB, Da Silva-Filho JC. **(2013)** cardiovascular effects induced by α-terpineol in hypertensive rats. *Flavour and Fragrance Journal, 28(5),* 333-339.

[117] Lampronti I, Saab AM, Gambari A. **(2006)** Antiproliferative activity of essential oils derived from plants belonging to the Magnoliophyta division. *International Journal of Oncology, 29(4),* 989-995.

[118] Held S, Schieberle P, Somoza V. **(2007)** Characterization of alpha-terpineol as an anti-inflammatory component of orange juice by in vitro studies using oral buccal cells. *Journal of Agricultural and Food Chemistry, 55(20),* 8040-8046.

[119] Hassan SB, Muhtasib HG, Goeransson H, Larsson R. **(2010)** Alpha Terpineol: a potential anticancer agent which acts through suppressing NF-κB signalling. *Anticancer Research, 30(6),* 1911-1920.

[120] Mayet J, Hughes A. **(2003**) Cardiac and vascular pathophysiology in hypertension. *Heart, 89*, 1104-1109.

[121] Wang G, Zhang Z, Ayala C. **(2010)** Hospitalization costs associated with hypertension as a secondary diagnosis among insured

patients aged 18–64 Years. *American Journal of Hypertension, 23(3),* 275-281.

[122] Nguelefack TB, Mekhfi H, Dongmo AB, Dimo T, Watcho P. (**2009**) Hypertensive effects of oral administration of the aqueous extract of Solanum torvum fruits in L-NAME treated rats: Evidence from in vivo and in vitro studies**.** *Journal of Ethnopharmacology, 124(3),* 592-599.

[123] Moncada S, Higgs EA. **(2006)** The discovery of nitric oxide and its role in vascular biology. *British Journal of Pharmacology, 147,* 193-201.

[124] Fernandes-Santos C, Mendonca LD, Mandarim-de-Lacerda CA. **(2006)** Beneficial effects of angiotensin II AT1 blocker on cardiovascular adverse remodeling due to nitric oxide synthesis blockade. *International Journal of Morphology, 24(3),* 309-318.

[125] Ribeiro TP, Porto DL, Menezes CP, Antunes AA. **(2010)** Unravelling the cardiovascular effects induced by α-terpineol: A role for the nitric oxide–cGMP pathway. *Clinical and Experimental Pharmacology and Physiology, 37(8),* 811-816.

[126] Jones RD, Pugh PJ, Jones TH, Channer KS. **(2003)** The vasodilatory action of testosterone: a potassium-channel opening or a calcium antagonistic action. *British Journal of Pharmacology, 138(5),* 733-744.

[127] Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. **(2005)** International union of pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. *Pharmacology Reviews, 57(4),* 411-425.

[128] Touyz RM. **(2000)** Oxidative stress and vascular damage in hypertension. *Current Hypertension Reports, 2(1)*, 98-105.

[129] Bicas JL, Neri-Numa IA, Ruiz AL, De Carvalho JE, Pastore GM. **(2011)** Evaluation of the antioxidant and antiproliferative potential of bioflavors. *Food and Chemical Toxicology, 49(7),* 1610-1615.

[130] Saravankumar M, Raja B. **(2011)** Veratric acid, a phenolic acid attenuates blood pressure and oxidative stress in L-NAME induced hypertensive rats. *European Journal of Pharmacology, 671(1-3),* 87-94.

[131] Itani WS, El-Banna SH, Hassan SB, Larsson RL, Bazarbachi A, Gali-Muhtasib HU. **(2008)** Anti colon cancer components from Lebanese sage (Salvia libanotica) essential oil: Mechanistic basis. *Cancer Biology and Therapy, 7 (11),* 1765-1773.

[132] Garg A, Aggarwal BB. **(2002)** Nuclear transcription factor-kappaB as a target for cancer drug development. *Leukemia, 16(6),* 1053-1068.

[133] Lindhagen E, Rickardson L, Elliott G, Leoni L, Nygren P, Larsson R, Aleskog A. **(2007)** Pharmacological profiling of novel non-COX-inhibiting indole-pyran analogues of etodolac reveals high solid tumour activity of SDX-308 in vitro. *Investigational New Drugs, 25(4),* 297-303.

[134] Park MJ, Gwak KS, Yang I, Kim KW, Jeung EB. **(2009)** Effect of citral, eugenol, nerolidol and alpha-terpineol on the ultrastructural changes of Trichophyton mentagrophytes. *Fitoterapia, 80(5),* 290-296.

[135] Deeb SJ, El-Baba CO, Hassan SB, Larsson RL, Gali-Muhtasib HU. **(2011)** Sage components enhance cell death through nuclear factor kappa-B signalling. *Frontiers in Bioscience, 3,* 410-420.

[136] Parada CA, Vivancos GG, Tambeli CH, De Queiróz Cunha F, Ferreira SH. **(2003)** Activation of presynaptic NMDA receptors coupled to NaV1.8-resistant sodium channel C-fibers causes retrograde mechanical nociceptor sensitization. *Proceeding of the National Academy of Sciences of the United States of America, 100(5),* 2923-2928.

[137] De Oliveira M, Marques R, De Santana M, Santos A. **(2012)** α-Terpineol reduces mechanical hypernociception and inflammatory response. *Basic and Clinical Pharmacology and Toxicology, 111*, 120-125.

[138] Mendell JR, Sahenk Z. **(2003)** Painful sensory neuropathy. *The New England Journal of Medicine, 348,* 1243-1255.

[139] De Sousa DP. **(2011)** Analgesic-like activity of essential oils constituents. *Molecules, 16(3),* 2233-2252.

[140] Golshani S, Karamkhani F, Monsef-Esfehani HR, Andollahi M. **(2004)** Antinociceptive effects of the essential oil of *Dracocephalum kotschyi* in the mouse writhing test. *Journal of Pharmacy and Pharmaceutical Sciences, 7(1),* 76-79.

[141] Quintans-Júnior LJ, Oliveria M, Santana MF, Santana MT, Guimaraes A, Siqueria J, De Sousa D, Almeida R. **(2011)** α-Terpineol reduces nociceptive behavior in mice**.** *Pharmaceutical Biology, 49(6),* 583-586.

[142] Ribas CM, Meotti FC, Nascimento FP, Jacques AV, Dafre AL. **(2008)** Antinociceptive effect of the *Polygala sabulosa* hydroalcoholic extract in mice: Evidence for the involvement of glutamatergic receptors and cytokine pathways. *Basic and Clinical Pharmacology and Toxicology, 103(1),* 43-47.

[143] Trink HT, Lee IA, Hyun YJ, Kim DH. **(2011)** Artemisia princeps Pamp. Essential oil and its constituents eucalyptol and α-terpineol ameliorate bacterial vaginosis and vulvovaginal candidiasis in mice by inhibiting bacterial growth and NF-κB activation. *Planta Medica, 77(18),* 1996-2002.

[144] Beirith A, Santos AR, Colixto JB. **(2002)** Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. *Brain Research, 924(2),* 219-228.

[145] Kawata J, Kameda M, Miyazawa M. **(2008)** Cyclooxygenase-2 inhibitory effects of monoterpenoids with a p-methane skeleton. *International Journal of Essential oil Therapeutics, 2(4),* 145-148.

[146] Sakurada T, Matsumura T, Moriyama T, Sakurada C, Ueno S, Sakurada S. **(2003)** Differential effects of intraplantar capsazepine and ruthenium red on capsaicin-induced desensitization in mice. *Pharmacology Biochemistry and Behavior, 75(1),* 115-121.

[147] Le Bars D, Gozariu M, Cadden SW. **(2001)** Animal Models of Nociception. *Pharmacological Reviews, 53(4),* 597-652.

[148] Poole S, De Queiróz Cunha F, Ferreira SH. **(1999)** Hyperalgesia from subcutaneous cytokines. *Progress in Inflammation Research,* 59-87.

[149] Lorenzetti BB, Veiga FH, Canetti CA, Poole S. **(2002)** Cytokine-induced neutrophil chemoattractant 1 (CINC-1) mediates the sympathetic component of inflammatory mechanical hypersensitivity in rats. *European Cytokine Network, 13(4)*, 456-461.

[150] Souza RH, Cardoso MS, Menezes CT, Silva JP, De Sousa DP, Batista JS. **(2011)** Gastroprotective activity of α-terpineol in two experimental models of gastric ulcer in rats. *DARU Journal of Pharmaceutical Sciences, 19(4),* 277-281.

[151] Gerkens JF, Shand DG, Flexner C, Nies AS, Oates JA, Data JL. **(1977)** Effect of indomethacin and aspirin on gastric blood flow and acid secretion. *The Journal of Pharmacology and Experimental Therapeutics, 203*, 646-652.

[152] WHO. The World Health Report. Mental Health: New Understanding New Hope; WHO: Geneva, Switzerland, **2001**.

[153] De Almeida RN, Agra MD, Maior FN, De Sousa DP. **(2011)** Essential oils and their constituents: Anticonvulsant activity. *Molecules, 16(3),* 2726-2742.

[154] De Sousa D, Raphael E, Brocksom U, Brocksom T. **(2007)** Sedative effect of monoterpene alcohols in mice: a preliminary screening. *Zeitschrift für Naturforschung, 62(7-8),* 563-566.

[155] Taffet GE, Donohue JF, Altman PR. **(2014)** Considerations for managing chronic obstructive pulmonary disease in the elderly. *Clinical Interventions in Aging, 9,* 23-30.

[156] Vestbo J, Hurd SS, Agusti AG. **(2013)** Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease**.** *The American Journal of Respiratory and Critical Care Medicine, 187(4),* 347-365.

[157] Martinez FJ, Danohue JF, Rennard SI. **(2011)** The future of chronic obstructive pulmonary disease treatment—difficulties of and barriers to drug development. *The Lancet, 378(9795),* 1027-1037.

[158] Bustacchini S, Chiatti C, Furneri G, Lattanzio F, Mantovani IG. **(2011)** The economic burden of chronic obstructive pulmonary disease in the elderly: results from a systematic review of the literature. *Current Opinion in Pulmonary Medicine, 17(1),* 35-41.

[159] Najafzadeh M, Marra CA, Lynd LD, Sadatsafavi M, FitzGerald JM. **(2012)** Future impact of various interventions on the burden of COPD in Canada: A dynamic population model. *PloS ONE 7(10).*

[160] Ozyilmaz E, Kokturk N, Teksut G, Tatlicioglu T. **(2013)** Unsuspected risk factors of frequent exacerbations requiring hospital admission in chronic obstructive pulmonary disease. *International Journal of Clinical Practice, 67(7),* 691-697.

[161] Banerjee A, Kozial-white C, Panettieri Jr. R. **(2012)** p38 MAPK inhibitors, IKK2 inhibitors, and TNFα inhibitors in COPD. *Current Opinion in Pharmacology, 12(3),* 287-292.

[162] Adcock IM, Chung KF, Caramori G, Ito K. **(2006)** Kinase inhibitors and airway inflammation. *European Journal of Pharmacology, 533(1-3),* 118-132.

[163] Tsou YA, Huang HJ, Lin WW, Chen CY. **(2014)** Lead screening for chronic obstructive pulmonary disease of IKK2 inhibited by traditional Chinese medicine. *Evidence-Based Complementary and Alternative Medicine, 2014,* 1-16.

[164] Park SN, Lim YK, Freire MO**. (2012)** Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. *Anaerobe, 18(3),* 369-372.

[165] Songkro S. **(2009)** An overview of skin penetration enhancers: penetration enhancing activity, skin irritation potential and mechanism of action. *Songklanakarim Journal of Science and Technology, 31(3),* 299-321.

[166] Liu CH, Chang FY, Hung DK. **(2011)** Terpene microemulsions for transdermal curcumin delivery: Effects of terpenes and cosurfactants. *Colloids and Surfaces B: Biointerfaces, 82(1),* 63-70.

[167] Vaddi HK, Ho PC, Chan SY. **(2002)** Terpenes in propylene glycol as skin-penetration enhancers: Permeation and partition of haloperidol, fourier transform infrared spectroscopy, and differential scanning calorimetry. *Journal of Pharmaceutical Sciences, 91(7),* 1639-1651.

[168] Ahad A, Aqil M, Kohli K. **(2011)** Role of novel terpenes in transcutaneous permeation of valsartan: effectiveness and mechanism of action. *Drug Development and Industrial Pharmacy, 37(5),* 583-596.

[169] Ahad A, Aqil M, Kohli K. **(2011)** Interactions between novel terpenes and main components of rat and human skin: Mechanistic view for transdermal delivery of propanol hydrochloride. *Current Drug Delivery, 8(2),* 213-224.

[170] Herman A, Herman AP. **(2014)** Essential oils and their constituents as skin penetration enhancer for transdermal drug delivery: A review. *Journal of Pharmacy and Pharmacology, 67(4),* 473-485.

[171] Patil UK, Saraogi R. **(2014)** Natural products as potential drug permeation enhancer in transdermal drug delivery system. *Archives of Dermatological Research, 306(5),* 419-426.

[172] Narishetty S, Panchagnula R. **(2003)** Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action. *Journal of Controlled Release, 95(3),* 367-379.

[173] Dos Anjos JL, Alonso A. **(2008)** Terpenes increase the partitioning and molecular dynamics of an amphipathic spin label in stratum corneum membranes. *International Journal of Pharmaceutics, 350(1-2),* 103-112.

[174] Godwin DA, Michniak BB. **(1999)** Influence of drug lipophilicity on terpenes as transdermal penetration enhancers. *Drug Development and Industrial Pharmacy, 25(8),* 905-915.

[175] Williams AC, Barry BW. **(1991)** Terpenes and the Lipid–Protein–Partitioning Theory of Skin Penetration Enhancement. *Pharmaceutical Research, 8(1),* 17-24.

[176] Mohammadi-Samani S, Jamshidzadeh A, Montaseri H, Rangbar-Zahedani M, Kianrad R. **(2010)** The effects of some permeability enhancers on the percutaneous absorption of lidocaine. *Pakistan Journal of Pharmaceutical Sciences, 23(1),* 83-88.

[177] Fang JY, Hung CF, Chiu HC, Wang JJ, Chan TF. **(2003)** Efficacy and irritancy of enhancers on the in-vitro and in-vivo percutaneous absorption of curcumin. *Journal of Pharmacy and Pharmacology, 55(5),* 593-601.

[178] Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. **(2006)** Multiple biological activities of curcumin: A short review. *Life Sciences, 78(18),* 2081-2087.

[179] Kuttan G, Kumar KB, Guruvayoorappon C, Kuttar R. **(2007)** Antitumor, anti-invasion, and antimetastatic effects of curcumin**.** *Advances in Experimental Medicine and Biology, 595*, 173-184.

[180] Menon VP, Sudheer AR. **(2007)** Antioxidant and anti-inflammatory properties of curcumin. *Advances in Experimental Medicine and Biology*, *595,* 105-125.

[181] Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. **(2007)** Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics, 4(6),* 807-818.

[182] Al-Khalili M, Meidan V, Michniak B. **(2003)** Iontophoretic transdermal delivery of buspirone hydrochloride in hairless mouse skin. *The American Association of Pharmaceutical Scientists, 5(2),* 61-71.

[183] Jain AK, Thomas NS, Panchagnula R. **(2002)** Transdermal drug delivery of imipramine hydrochloride: I. Effect of terpenes. *Journal of Controlled Release, 79(1-3),* 93-101.

[184] Liu XC, Li YP, Li HQ, Deng ZW. **(2013)** Identification of repellent and insecticidal constituents of the essential oil of *Artemisia rupestris* L. aerial parts against *Liposcelis bostrychophila* Badonnel. *Molecules, 18,* 10733-10746.

[185] Liu ZL, Zhao NN, Liu CM, Zhou L. **(2012)** Identification of insecticidal constituents of the essential oil of *Curcuma wenyujin* rhizomes active against *Liposcelis bostrychophila* Badonnel. *Molecules, 17(10),* 12049-12060.

# 7. CURICULUM VITAE

## CHRISTINA KHALEEL

### PERSONAL INFORMATION

- **Date of Birth** 30.11.1987
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### EDUCATION

- **Sep. 1993 - Jun. 1998** Saint Joseph Language Elementary school – Egypt.
- **Sep. 1998 - Jun. 2004** Saint Joseph Language High school – Egypt.
- **Sep. 2004 - Jun. 2009** Bachelor degree in Pharmaceutical sciences, Faculty of pharmacy – Cairo Uni.
- **Since 2013** Notification of Pharmacy degree - Vienna Uniersity – Austria.

### EXPERIENCE

- **Jul. 2008** Practical training at PFIZER – Egypt.
- **Sep. 2008 – Nov. 2008** Marketing and sales skills at American University- Egypt.
- **Jul. 2009 – Sep. 2009** General training at Astra Zeneca – Egypt.

- **Nov. 2009 – Nov. 2010** Pharmacist at Mina Habib´s Pharmacy- Egypt.
- **Dec. 2010 – Aug. 2012** Owner and manager of Mina Habib´s Pharmacy – Egypt.
- **Apr. 2014 – Jun. 2014** Cell culture laboratory training on Osteoblasts and osteoclasts, Vienna University – Austria.

## LANGUAGES

- **Arabic** Mother tongue
- **English** Excellent ( IELTS British council )
- **German** Excellent
- **French** Good

## COMPUTER SKILLS

- **Microsoft Office** Very good (ICDL License - German University in Cairo – Egypt.