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Abstract

Oxidative stress contributes to the development of different kind of disorders in human body such as diabetes, cardiovascular diseases, gastrointestinal disorders, carcinogenesis and many others. Speaking of oxidative stress it is important to notice that it is caused by high concentration of free radicals, which are highly reactive species. Once present, free radicals react with biomolecules such as proteins, lipids and nucleic acids, affect their function, change their structure or inactivate them. Following the investigations of antioxidative characteristics of essential oils and their single compounds, published in many scientific articles worldwide, this work presents an overview of current findings. Different research methods have shown that essential oils are highly active and able to scavenge free radicals. Also, many scientific articles emphasize their role as natural antioxidants.

Abstract

Oxidativer Stress trägt zur Entwicklung von verschiedenen Arten von Erkrankungen im menschlichen Körper wie Diabetes, Herz-Kreislauf-Erkrankungen, Magen-Darm-Erkrankungen, der Karzinogenese/Krebsentstehung, usw. bei. Dabei ist wichtig zu betonen, dass oxidativer Stress durch eine hohe Konzentration an freien Radikalen (die hoch reaktiv sind) verursacht wird. Einmal vorhanden, reagieren freie Radikale mit Biomolekülen wie Proteinen, Lipiden und Nukleinsäuren, beeinflussen ihre Funktion, verändern ihre Struktur oder inaktivieren diese. Die ätherischen Öle können die Konzentrationen der freien Radikale verringern und als Antioxidantien wirken. Mit Bezug auf die in vielen wissenschaftlichen Artikeln weltweit veröffentlichten Untersuchungen der antioxidativen Eigenschaften der ätherischen Öle und ihrer Einzelverbindungen wird in dieser Arbeit ein Überblick über die aktuellen Ergebnisse präsentiert. Die verschiedenen Forschungsmethoden haben gezeigt, dass ätherische Öle hochaktiv und in der Lage sind, freie Radikale abzufangen. Viele wissenschaftliche Arbeiten heben ihre Funktion als natürliches Antioxidans hervor.

1 Introduction 5
1.1 Free radicals 5
1.1.1 Reactive oxygen species (ROS) 6
1.1.2 Reactive nitrogen species (RNS) 7
1.2 Synthesis of ROS 8
1.3 Positive and side effects of free radicals 9
1.4 Oxidative stress 10
1.5 Antioxidants 11
1.6 Antioxidant activity 11
2 New test methods 12
2.1 FRAP 12
2.2 Ferric ion reducing antioxidant power assay 13
2.3 CUPRAC assay 13
2.4 DMPD assay 14
3 Antioxidant properties of essential oils 14
4 Essential oil effect on different enzyme function and free radicals formation 30
5 Conclusion 35
6 Reference 37
7 Curriculum vitae 49

1 Introduction

Essential oils have found wide spread use in various domains of everyday human life (Bassolé et al., 2012; Mimica-Dukić et al., 2010). They are applied as a flavoring agent (Prakash et al., 2013) in medicinal and industrial purposes, and their usage is still increasing because of their safe status, great consumer compliance and multifunctional administration. Most of them are famous for their antimicrobial, carminative, spasmolytic, antiviral, and many others properties. Essential oils show radical scavenging activity, what qualifies them as natural antioxidants. When compared with synthetic ones, essential oils show less toxic side effects, what makes them as an important substitution in food preservation (Bassolé et al., 2012; Miguel, 2010; Mimica-Dukić et al., 2010; Aidi Wannes et al., 2010).

Essential oils are a heterogeneous group of organic substances produced by plants. They have low molecular weight, oily consistency and can be liquid or solid at room temperature, and can be obtained by steam distillation or squeezing out the peels of citrus fruits. They are synthesized as secondary metabolites by all plant organs: flowers, stems, seeds, leaves, fruits, roots, etc. and can possess different colors like yellow, blue, brownish red or green. Their chemical composition and quality vary from different environmental conditions (Bassolé et al., 2012; Karakaya et al., 2011; Miguel, 2010; Aidi Wannes et al., 2010).

1.1 Free radicals

Free radicals are a highly unstable species with a single electron in their atomic electron shell. Because of their attempt to establish the balance, they bind electrons from other atoms or molecules, whereby they trigger biochemical instability what mostly ends up with successive chain reaction (Ríos-Arrabal et al., 2013) and oxidation of lots of biomolecules such as proteins, lipids and DNA. Under physiological condition, free radicals are formed during ATP production in mitochondria or as metabolic products in

liver cells. They are also formed in muscle cells during exercise or in phagocytes during immune reaction. Physiologically they show an essential function in protecting organism from infection or in keeping the regular metabolism of cells, however their occurrence in high concentrations represent a significant risk to human health (Juranek et al., 2013; Ríos-Arrabal et al., 2013).

1.1.1 Reactive oxygen species (ROS)

A **superoxide** free radical ($O2^{-\bullet}$) is produced from an enzyme called NADPH oxidase mostly in mitochondrion (Ríos-Arrabal et al., 2013). It is less reactive than a hydroxyl radical ($^{\bullet}OH$), but much more selective. Its lifetime is not longer than few seconds in biological systems, and it reacts with another superoxide molecule (self-dismutation reaction) to form a hydrogen peroxide. Superoxide also reacts with a **nitric oxide** to form a **peroxynitrite**, a very potent oxidant that belongs to **reactive nitrogen species** (Basak et al., 2013; Juranek et al., 2013; Kalyanaraman, 2013; Miguel, 2010).

A radical-radical reaction between two superoxide radicals leads to formation of oxygen and **hydrogen peroxide** by superoxide dismutase (SOD). Hydrogen peroxide is rapidly destroyed in presence of UV radiation or ferric or cupric ions resulting in formation of **hydroxyl radical** ($^{\bullet}OH$). On the other hand, antioxidant enzymes such as catalase and glutathione peroxidase degrade hydrogen peroxide (Kalyanaraman, 2013; Miguel, 2010; Narotzki et al., 2013). Hydroxyl radical is highly reactive with life time of about 10^{-9} s and there is no selectivity for this radical which reacts with the most nearest cell compounds (Kalyanaraman, 2013). Although not being a free radical, **singlet oxygen** belongs to reactive oxygen species, because it rapidly reacts with cell proteins, nucleic acids and unsaturated lipids causing oxidative damage. One of the most frequent side effects is increased photosensitivity in the present of light. Singlet oxygen has a half-life of only 10 μs and it has no unpaired electron in comparison to oxygen that we breathe, leaving an empty orbital to be occupied by electrons of other molecules (Kalyanaraman, 2013). In addition, binding of a proton to an oxygen molecule forms a **hydroperoxide**

radical ($HO_2^{-\bullet}$) (Ríos-Arrabal et al., 2013). Eventually, myeloperoxidase/H_2O_2 dependent oxidation of chloride anion forms **hypochlorous acid** (HOCl, or bleach), which is also a reactive oxygen species but less active than hydroxyl radicals (Kalyanaraman, 2013; Miguel, 2010).

Superoxide anion	$O_2 \xrightarrow[Oxidaze]{NADPH} O_2^{-\cdot}$
Hydrogen peroxide	$O_2^{-\cdot} \xrightarrow[SOD]{O_2^{\cdot}} H_2O_2$
Hydroxyl radical	$H_2O_2 \xrightarrow{Fenton\ Reaction} HO^{\cdot}$
Hydroperoxide radical	$O_2 \xrightarrow{H^+} HO_2^{-\cdot}$

Table 1. Reactive oxygen species (Ríos-Arrabal et al., 2013)

1.1.2 Reactive nitrogen species (RNS)

Free radicals such as **nitric oxide** ($NO^{\bullet}$), **peroxynitrite** ($ONOO^-$), the radical **nitrogen dioxide** ($NO_2^{\bullet}$) and **nitrite** (NO_2^-) belong to the **reactive nitrogen species** (RNS) group. Nitric oxide synthetase (NOS) enzyme produces nitric oxide from L-arginine. Peroxynitrite is formed by the reaction of nitric oxide with a molecule of superoxide. An intermediator in this reaction is nitrogen dioxide ($NO_2^{\bullet}$), which reacts with nitric oxide forming the **anhydride nitrous** acid (N_2O_3) (Miguel, 2010; Ríos-Arrabal et al., 2013).

To the NOS family belong endothelial (eNOS), neuronal (nNOS), mitochondrial (mtNOS) and inducible (iNOS) forms, whose activities are based on NADPH and

calmodulin (Ríos-Arrabal et al., 2013). Endothelial NOS modulates cancer related processes, such as cell death, angiogenesis, invasion etc., so it possesses significant importance in tumor development. Cyclic guanylate monosphosphate (cGMP) and guanylyl cyclase (sGC) mediates many biochemical reaction of nitric oxide (Miguel, 2010; Ríos-Arrabal et al., 2013).

Nitric oxide	L-Arginine $\xrightarrow[NOS]{}$ $NO^{\cdot}$
Peroxynitrite	$NO^{\cdot}$ $\xrightarrow{O_2^{-\cdot}}$ $ONOO^-$
Anhydride nitrous	$NO^{\cdot}$ $\longrightarrow$ N_2O_3
Nitrogen dioxide	$NO_2^{\cdot}$

Table 2. Reactive nitrogen species (Ríos-Arrabal et al., 2013)

1.2 Synthesis of ROS

Physiologically, free radicals are produced during a lot of metabolic reactions in human body, where 80% of oxygen that we breathe are consumed by mitochondria, while only 5% of oxygen are converted into hydroxyl radicals and superoxide. In contrast to this, lack of oxygen inhibits mitochondrial respiration and ATP production, resulting in increased free radicals synthesis. Reactive oxygen species are also formed by the cytochrome P-450 system and oxidative enzymes such as NAD(P)H oxidases, endothelial xanthine oxidase, myeloperoxidases and arachidonate oxygenases (Juranek, et al., 2013; Serviddio et al., 2013). In hypoxia stages and cytosolic calcium overload,

xanthine oxidase can be activated resulting in elevated ROS synthesis. Consequence of this is mostly an irreversible tissue damage. On the other hand elevated ROS synthesis activates neutrophils which increase further ROS formation. Leucocytes and macrophages also produce reactive oxygen species, protecting the body against virus and bacteria. Hydroxyl radicals are the most synthesized radicals during metabolism of prostaglandins and lipids. Metabolism of drugs and flavoring agents also increase ROS synthesis (Juranek, et al., 2013).

1.3 Positive and side effects of free radicals

Under physiological conditions, many of biochemical reactions are forced by free radicals. Endogenous substances such as cholesterol, steroidal hormones and prostaglandins are mediated by free radicals. Hydroxyl radicals, for example, are involved in hydroxylation of amino acids needed for collagen biosynthesis. ROS regulate gene transcription, maintain cell homeostasis, activate apoptosis and protect against cancer. They also increase skeletal muscle endurance after physical exercise. When speaking of immune system, one of the most important functions is protecting the body from diverse infections and tissue insults. Moderate ROS concentrations guarantee maintenance of human health (Juranek, et al., 2013). However, sustained exposure to free radicals can trigger various tissue injuries, where the most frequent targets are proteins, DNA and lipids (Juranek, et al., 2013; Kannan et al., 2013; Ríos-Arrabal et al., 2013). They also generate tumor development and contribute to cancer metastasis (Ríos-Arrabal et al., 2013).

ROS/RNS role in the carcinogenesis		
· DNA damage · Genetic instability · Cell cycle/ Repair/ cell death	· Inflammation · Cellular transformation · Proliferation · Apoptosis	· Tumor promoting · Angiogenesis · Immune response · Metastasis

Table 3. Role of free radicals in the tumor development (Ríos-Arrabal et al., 2013)

1.4 Oxidative stress

Conditions of increased free radicals formation and their decreased degradation are defined as oxidative stress. These kind of cytosolic conditions contribute to great disorders and damages in human organism resulting in degradation of basic biomolecules such as lipids, nucleic acids and proteins, what can end up with genotoxic effects (Narotzki et al., 2013; Mimica-Dukić et al., 2010; Kalyanaraman, 2013; Ríos-Arrabal et al., 2013; Saleh et al., 2010).

The imbalance in free radical concentration mostly comes in stages of hypoxia events. On the other side, ROS formation elevates with age. And finally, defects in expressing antioxidant genes, reduced activity of antioxidant enzymes or lack of dietary antioxidants increase free radical levels leading to oxidative stress (Juranek, et al., 2013; Kalyanaraman, 2013; Narotzki et al., 2013). Prolonged exposure to oxidative stress has

the consequence in formation of many diseases such as diabetes, heart and liver diseases, arthritis, Alzheimer's disease, and many others (Bhalla et al., 2013; Li Fu et al., 2010; Narotzki et al., 2013)

1.5 Antioxidants

Antioxidants degrade free radicals and protect the body from oxidation. There are three different types of defense mechanism, to which belong antioxidative enzymes, low molecular non-enzymatic antioxidants and repair mechanisms. Catalase, superoxide dismutase, glutathione peroxidase and peroxidase belong to antioxidative enzyme, while non-enzymatic antioxidants include ascorbic acid, tocopherols, glutathione, ubiquinone, beta-carotene, etc. Repair enzymes degrade impaired biomolecules and produce new ones. To this group belong enzymes such as proteases, ligases, polymerases and phospholipases (Juranek, et al., Kannan et al., 2013; Kalyanaraman, 2013; 2013; Ríos-Arrabal et al., 2013). Glutathione maintains redox balance in hypoxia conditions or in case of increased ROS and NO• formation during tumor invasion. Its reduced state, GSH, catches ROS, which is lately recycled by the glutathione-reductase enzyme (GRd) (Ríos-Arrabal et al., 2013). Lipid peroxyl radicals are formed in lipid peroxidation processes and a lipophilic Vitamin E easily scavenges these radicals terminating the chain reaction. On the other hand, vitamin C is water-soluble and scavenges superoxide and hydroxyl radicals. This cytosolic antioxidant recycles membrane bounded vitamin E, increasing its total antiradical activity (Kalyanaraman, 2013; Lebold and Traber, 2013).

1.6 Antioxidant activity

Antioxidant activity is defined as a property of antioxidants to neutralize any free radicals. Generally speaking of antioxidants, their activity is required to act as a

hydrogen donor. On the other hand, the aromatic ring plays a significant role and especially phenolic hydroxyl groups enhance the inhibition of oxidation (Gülcin, 2011). Antioxidant activity is also a measure for substance ability to prevent a free radical concentration increment, oxidative stress and risk for development related diseases. And for purpose of measuring antioxidant activity, many assays are applied such as DPPH assay, ABTS assay, β-carotene bleaching test, ferric and cupric reducing power, linoleic acid assay, etc. (Akrout et al., 2011; Chabir et al., 2011; Jia et al., 2010; Serrano et al., 2010).

Many studies assess the antioxidant activity of the essential oil to be used as a natural source of antioxidants. Generally, plant species are rich in phenolic compounds, flavonoids, tannins, lignans, etc., all able to protect human health. Single compounds of essential oils donate protons to highly reactive radicals, inactivate it and prevent possible damage. Various studies propagate essential oils as a great source of antioxidants and an ideal substitution of synthetic ones, such as butylated hydroxytoluene and butylated hydroxyanisole, which are defined as highly potent antioxidants but also with high carcinogenicity and other toxic properties as side effects (Bhalla et al., 2013; Li Fu et al., 2010; Mimica-Dukić et al., 2010; Iqbal et al., 2013; Messaoud and Boussaid, 2011; Aidi Wannes et al., 2010).

2 New test methods

(Addition to those already described by Buchbauer (2010) in the Handbook of Essential oils-Science, Technology and Applications, Taylor & Francis).

2.1 FRAP

Ferric ion reducing antioxidant power (FRAP) assay represents reducing antioxidant activity of substances, which reduce colorless oxidized Fe^{3+} to a blue-colored Fe^{2+} ion. FRAP reagent is made from tripyridyltriazine (TPTZ) and ferric chloride and sodium acetate (pH 3.6) and is used as a solvent in this test. To assess the reducing power of essential oils, FRAP reagent is mixed with essential oil diluted in ethanol, and after reaction at room temperature during four minutes, its absorbance is measured at 593 nm with UV–visible spectrophotometer. 50% Ethanol is used as a negative control. The reducing capacity of the essential oil is compared with a standard solution, which is prepared from ferrous sulfate and TPTZ. The ferric reducing power is presented as μmol Fe^{2+}/g of sample (Serrano et al., 2010; Teixeira et al., 2013).

2.2 Ferric ion reducing antioxidant power assay

This assay measures the electron transfer reaction where the ferric salt represents the oxidant, while the reducing power is investigated with a mixture of the diluted essential oil, potassium hexacyanoferrate (III) (2.5 ml, 10 mg/ml) and phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6). Then, trichloroacetic acid (2.5 ml, 0.1 gm/l) should be added at room temperature after 30 minutes of incubation at 50°C. 2.5 ml of this solution needs to be added to a mixture of 0.5ml of ferric chloride and 2.5ml of water, finally to be measured at 700 nm in a spectrophotometer (Gülcin, 2011; Mossa and Nawwar, 2011; Teixeira et al., 2013). The positive control is ascorbic acid, and a negative is ethanol (Teixeira et al., 2013). Trolox can also be used as a positive control (Mossa and Nawwar, 2011). The ferric reducing power is expressed in μmol ascorbic acid g^{-1} of the sample (Teixeira et al., 2013). Elevated absorbance represents higher reducing power of antioxidants (Gülcin, 2011).

2.3 CUPRAC assay

Reduction of Cu^{2+} to Cu^{+} is used in this assay to investigate the reducing power of an essential oil. For this reason, the essential oil sample is mixed with a mixture made from 0.25ml ethanolic neocuproine solution (7.5 · 10-3 M), 0.25ml $CuCl_2$ solution (0.01M) and 0.25 ml CH_3COONH_4 buffer solution (1M). This mixture is filled up to 2ml with distilled water and measured against blank at 450 nm. Results were compared with those of positive controls, BHA and α-tocopherol. As in the FRAP assay, the increase of absorbance indicates a high reduction ability (Gülcin, 2011; Tel et al., 2010).

2.4 DMPD assay

Also the DMPD (dimethylphenylendiamin) assay is used for measurement of radical scavenging activity of essential oils, because DMPD radicals can be neutralized by taking protons from single compounds of the applied essential oil. DMPD reagent contains dissolved DMPD in water and acetate buffer (pH, 5.3). After adding of 0.05M ferric chloride ($FeCl_3$) and essential oil sample to DMPD reagent, the absorbance was measured at 505 nm. Acetate buffer is to be used as a blank solution (Gülcin, 2011).

3 Antioxidant properties of essential oils

Thyme species (Lamiaceae) are well known aromatic herbs used not only as spice, but for treatment of different diseases too (Jamalia et al., 2012). *Thymus vulgaris* L. has been applied for different indication in human life such as dry cough, bronchitis, and digestive problems (Grosso et al., 2010; Tsai et al., 2011). Thyme essential oil contains thymol, carvacrol, p-cymene, γ-terpinene and linalool, where the thymol and carvacrol are present in relatively high percentage, up to 41.6% and 7.9%, respectively (Aazza et al., 2011; Asbaghian et al., 2011; Grosso et al., 2010). Thymol and carvacrol are

phenolic compounds with estimated antioxidant activity (Aazza et al., 2011). They are considered to be responsible not only for antioxidant features of thyme oil, but for antimicrobial and anesthetic as well (Jamalia et al., 2012). By DPPH measurement thymol showed an IC_{50} of 0.051 mg/ml, and by TBARS an IC_{50} of 0.172 mg/ml, whereas the corresponding IC_{50} values for Carvacrol were IC_{50} of 0.052 mg/ml and 0.276, respectively (Aazza et al., 2011). *T. vulgaris* essential oil exhibited the IC_{50} value for scavenging the DPPH radical of 0.1 μg/ml (Tsai et al., 2011), and the antioxidant activity for preventing lipid oxidation in TBARS assay was 0.116 mg/ml (Aazza et al., 2011). The ß-carotene/linoleic acid assay showed an IC_{50} value of 0.14μg/ml and revealed an even better activity than the synthetic antioxidant BHT (Tsai et al., 2011; Aazza et al., 2011; Wei and Shibamoto, 2010; Viuda-Martos et al., 2010).

The essential oils of *T. serpyllum* L., *T. maroccanus* Ball., *T.pallidus* Batt., *Thymus broussonetii* Boiss., *T. ciliatus* Desf., *T. satureioides* Coss. , *T. leptobotrys* Murb. contain monoterpenes, and thymol and carvacrol are present with highest percentage. These compounds are followed by p-cymene, γ-terpinene, linalool and borneol. Samples were collected in southern and southwestern Morocco and were used for isolation of essential oil by hydrodistillation. While the oil of *T. serpyllum* had no activity in the DPPH assay, the highest radical scavenging activity showed *T. leptobotrys* and *T. maroccanus* oils. In comparison to this, hydroxytoluene (BHT) and quercetin showed higher radical scavenging. Keeping step with this, *T. leptobotrys* oil obtained the highest ferric reducing capacity with a half maximal effective concentration (EC_{50}) of 19.24 mg/ml. The second potent oil was *T. maroccanus* with EC_{50} of 139.5 mg/ml. Taking the oil composition into account, a strong linear correlation between the antioxidant activity and the phenolic ($r=0.980$, $p=0.001$) or carvacrol content was observed ($r=0.911$, $p=0.011$). For *T. capitatus* L. oil is reported to be rich with carvacrol (62 –83%) and it was estimated to have a ferric reducing capacity of EC50 312.5 mg/ml (Jamalia et al., 2012).

Thymol, p-cymene, and gamma-terpinene were major components of *T. proximus* Serg. and *T. marschallianus* Will. essential oil, growing wild in Xinjiang. In dose depended manner, both essential oils showed results between 12000 and 20000 μg/ml in reducing power assays, while the results of positive control BHT and thymol were better than

from oil. Results in DPPH assay were also concentration depended. *T. proximus* essential oil exhibited IC_{50} value of 3326 μg/ml, while the *T. marschallianus* essential oil had a slightly lower IC_{50}. Thymol showed the best DPPH radical scavenging acitivty better than BHT (Jia et al., 2010). Ichrak et al. (2011) investigated antioxidant and antibacterial activity of *T. pallidus* Coss. ex Batt. and *T. satureioides* Coss. essential oils obtained by steam hydrodistillation. *T. pallidus* essential oil revealed to have camphor (29.8%) as a major compound, followed with dihydrocarvone, borneol and camphene 17.6%, 7.6% and 7.5% respectively. *T. satureioides* possessed borneol, carvacrol and β-caryophyllene as most content (29.5%, 9.1% and 8.2% respectively). *T. pallidus* and *T. satureioides* essential oils exhibited strong antioxidant activity (Ichrak et al., 2011).

Artemisia arborescens L. (Asteraceae) is a Mediterranean plant used in a traditional medicine especially in skin diseases. Areal parts collected in February were used for hydrodistillation to obtain an essential oil which was consisted mostly of chamazulene (52%). ABTS and DPPH assay were applied, where the ABTS assay showed significantly better results than DPPH test. IC_{50} value for *A. arborescens* essential oil was 21.9 μg/ml, while for Trolox 15.1 μg/ml. DPPH assay showed the IC_{50} for *A. arborescens* essential oil to be higher than 200.0 μg/ml, while it was for Trolox only 4.36 μg/ml. FRAP assay was also applied and showed for the essential oil to possess 147.7 mmol TE/g of essential oil (Ornano et al. 2013). *A. campestris* L. possesses antiseptic and antioxidant activity. β-pinene and limonene were the major constituents analyzed by GC-MS. Three tests were applied for investigating antioxidant activity of essential oil obtained from areal parts of this plant: ABTS, DPPH and β-carotene bleaching method. All tests showed moderate antioxidant activity (Akrout et al., 2011).

Melaleuca (Myrtaceae) is a native genus of Australia and has found spread use in medicinal and cosmetic purposes. Essential oils extracted by steam distillation from Melaleuca species are mostly composed of 1,8- cineole, α-pinene, β-pinene, terpinen-4-ol, and they possess antimicrobial and antioxidant properties. Melaleuca oil is a commercial name for oil extracted from leaves of *M. armillaris* Sm. with 1,8-cineole (85.8%) as main compound, followed by camphene, and α-pinene as constituents also in major concentration, but to lower extent. This oil showed a better effect on radical

scavenging in the ABTS than in DPPH assay. Vitamin C was a reference with a higher antioxidant activity for ABTS and DPPH assay (Chabir et al., 2011).

Pino et al. (2010) investigated the chemical composition and antioxidant features of leaf and fruit essential oil of *M. lucandendra* L. GC-MS (gas chromatography/ mass spectrometry) revealed for the leaf oil forty-one volatile compounds where the main one were 1,8-cineole (43.0%), α-pinene (5.3%), α-terpineol (7.0%), limonene (4.8%), and viridiflorol (24.2%), while for fruit oil sixty-four compounds were identified with viridiflorol (47.6%) as the most present compound, followed with globulol (5.8%), guaiol (5.3%), and α-pinene (4.5%) also as major ones.

The DPPH radical scavenging capacity of leaf essential oils solutions with concentrations of 0.3 mg/ml was between 9.9 and 76.6 % with an EC_{50} value of 2.4 mg/ml, while 0.2 mg/ml fruit essential oil had a scavenging capacity in between 6.1 and 78.8 % and an EC_{50} value of 2.3 mg/ml. Ascorbic acid was used as a positive control. The second method was the TBARS assay which showed the extent of lipid degradation inhibition in dose dependent manner. BHT was used as a positive control. Concentrations of the volatile leaf oils from 20 to 250 mg/ml lead to 7.54 to 60.66% inhibition of lipid peroxidation. Fruit volatile oil showed 9.46 to 61.89 % inhibition of lipid peroxidation for the same concentrations as for the leaf oil. The third test was the ABTS assay with Trolox as a reference. The results were 448 and 565 mM for the leaf and fruit volatile oils, respectively. In comparison to this, volatile oils of *Origanum vulgare* L. and *Ocimum basilicum* L. had lower antioxidant power with 1105 mM and 997 mM, respectively. In all three tests, both *M. leucadendra* essential oils (obtained from leaves and fruits) showed significant antioxidant capacity, while the oil obtained from fruits had the higher one. Although major compounds such as 1,8-cineole, α-terpineol, α-pinene, limonene, globulol, and guaiol may contribute to the wanted activities, it is hard to tell whether only those among the whole mixture are responsible for the results. It is possible that the trace constituents also contribute to the antioxidant activity (Pino et al., 2010).

Eucalyptus camaldulensis Dehnh. belongs to a eucalyptus species mostly common in Sardinia, Italy. Its aerial parts are used for isolation of essential oil by steam distillation,

whose chemical composition was investigated with help of GC-ITMS (ion trap mass spectrometry) and GC-FID (flame ionization detection). Thirty-seven compounds were found to be present in the essential oil, where the major one were p-cymene, 1,8-cineole, spathulenol, β-phellandrene and cryptone, whose chemical presence varied during the vegetative stage of single samples. DPPH radical scavenging assay was carried out, showing the values in between 0.5 and 5.8 mmol/l (Barra et al., 2010). Herzi et al. (2013) found in this oil 1,8-cineole (45.7%) and p-cymene (17,1%) as a major components. He also proved by ABTS assay an antioxidant activitiy of 183 mg/l as IC_{50} value, whereas in the DPPH assay revealed an IC_{50} of 1146 mg/l. GC-MS and GC-FID analysis showed that the main compounds of *E. cinerea* F. Muell. essential oil were 1,8-cineole and p-menth-1-en-8-ol, 64.9% and 88.2% respectively. DPPH and ABTS assays were also applied (Herzi et al., 2013). The antioxidant capacity and chemical content of the essential oils obtained from *E. cinerea* and *E. camaldulensis* were influenced by the extraction technique, whereas the supercritical carbon dioxide extract (SCE) furnished better results than the essential oil obtained by hydrodistillation (HD). In *E. gracilis* F. Muell. oil 26 compounds were found with the major components 1,8-cineole with 77.9%, and then cis-sabinol, p-cymene and *α*-pinene, 4.2%, 4.5% and 2.3% respectively. This essential oil showed a low DDPH activity and the ABTS assay furnished better results (Yvon et al., 2012).

DPPH and ABTS tests were carried out for the essential oil of *E. oleosa* F. Muell. too. Using steam distillation, essential oils were obtained from different aerial parts: stems, leaves, flowers and fruits, and main compounds were 1,8-cineole, spathulenol and γ-eudesmol. DPPH showed a moderate to low antioxidant capacity, where the fruit essential oil had highest activity. Second active oil was the one obtained from flowers. The ABTS assay showed the best activity for the leaves essential oil, followed by the stem essential oil. Fruits and flowers also showed only moderate ABTS scavenging activity (Marzoug et al., 2011). Hassine et al. (2012) showed for the first time the antioxidant activity of *E. gilli* Maid. essential oil. For this reason the IC_{50} was demonstrated to be 163.5 mg/l and 94.7 mg/l in DPPH and ABTS measurement, respectively. This activity may be due to the presence of oxygenated monoterpenes hydrocarbons and monoterpenes, which possess moderate activity compared to phenols

and ascorbic acid. In comparison to *E. gilli* which had good results, *E. radiata* Benth. possessed only low antioxidative property in the ABTS assay (Hassine et al., 2012).

Azza et al. (2011) tested the antioxidative activity of some commercial essential oils. Various *in vitro* tests were applied for investigating essential oils of *Thymus vulgaris*, *Eucalyptus globules* Labill., *Foeniculum vulgare* Mill., *Cupressus sempervirens* L. and *Citrus aurantium* L (Aazza et al., 2011). *T. vulgaris* showed the best lipid peroxidation inhibition among other oils having IC_{50} value of 0.116 mg/ml in TBARS method, whereas *C. limon* oil had the lowest one. The activity of the *T. vulgaris* oil is due to its phenolic compounds thymol and carvacrol, which showed the greatest antioxidant activity among all other essential oil compounds used in test as standards. In contrast to that, limonene possessed a low activity per se, IC_{50} 3.346 mg/ml, explaining the low results of *C. limon* essential oil which is rich in limonene. *E. globulus* essential oil showed that its activity increased with the increment concentration. It also showed not higher IC_{50} values than other essential oils, with the best IC_{50} result of 1.109 mg/ml. *E. globulus* oil contained 1,8-cineole and limonene, 38% and 55% respectively, and both of these compounds showed lower activity in comparison to *E. globulus* oil. However, 1,8-cineole yielded an IC_{50} value of 9.360 mg/ml explaining that a synergistic effect is responsible for the lower the IC_{50} value of this essential oil. *F. vulgare* essential oil was better than *E. globulus* essential oil. On the other hand, *C. aurantium oil* showed no difference in lipid peroxidation inhibition than *F. vulgare* essential oil. Trans-anethol, an essential oil single compound characterized by high antioxidant activity was present up to 70% in *F. vulgare* oil, what explains its inhibitory activity, compared with *C. aurantium* mostly containing linalool. Keeping in step with thymol, carvacrol and trans-anethol, a compound with also good activity is δ-3-carene. δ-3-carene is highly present in *C.sempervirens*, explaining its inhibitory activity of 0.766 mg/ml. (Aazza et al., 2011). *T. vulgaris* also showed the best results in DPPH scavenging assay, whereas the essential oils of *E. globulus* and *F. vulgare* showed the weakest ones, not even able to reach the IC_{50} value (Aazza et al., 2011; Miguel et al., 2010; Senatore et al., 2013). Ferric reducing features of the oils *T. vulgaris* were also the best among other essential oils, in contrast to *F. vulgare* with the weakest one. Following the *T. vulgaris* oil, *C. limon* oil also showed great reductive performance of Fe (III) ion, in comparison to

other assays where it furnished mostly the weakest one (Aazza et al. 2011; Senatore et al., 2013). The distillation time influences the essential oil quality and the antioxidant capacity of *F. vulgare* (Zhelkazkov et al., 2013).

Citrus genus is one of the largest subunit of Rutaceae family with wide spread use, mostly applied for its antibacterial, antifungal and antioxidant effects (Campelo et al., 2011). *Citrus maxima* Merr. and *Citrus sinensis* L. essential oils are used because of their antifungal and anti-aflatoxigenic properties, having moderate antioxidant activity in DPPH assay (Singh et al., 2010). *Citrus aurantium* L. (bitter orange) belongs to the *Citrus species* commonly used as an aromatic plant and its essential oil is mostly popular because of its antimicrobial and antioxidant features. Fresh flowers of this species collected from North East of Tunisia were analyzed for its chemical composition and antioxidant acitivty. Limonene was most present up to 27%. While (*E*)-nerolidol α-terpineol, α-terpinyl acetate and (*E*)-farnesol were also present, but in lower concentrations (Ammar et al., 2012; Hsouna et al., 2013). Ability to scavenge DPPH radicals was highly present (IC_{50} of 1.8μg/ml), whereas β-carotene bleaching inhibition showed an IC_{50} value of 15.3μg/ml. These results approved its wide usage, proposing it to be used in food preservation and pharmaceutical purposes as well (Hsouna et al., 2013). In ABTS assay the IC_{50} value was determined to be 672 mg/l (Ammar et al., 2012). Sarrou et al. (2013) investigated the antioxidant activity of *C. aurantium* too, using peel, flower and leaves of plants growing in Greece. Upon 31 different constituents, most common were limonene, linalool, and α-terpineol, whereas *trans*-β-ocimene, and β-pinene were also present, but in lower concentrations. The highest antiradical activity in DPPH assay had the essential oil of old leaves (94.36%). Flower oil had double lower value with 53.98%, while young leaves and peel oil had similar results, 22.79% and 19.29%, respectively (Sarrou et al., 2013). The antioxidant activity of *C. aurantium* leaves oil was also measured according the season´s variations of its content on essential oil single compounds. Fresh leaves were collected in Nabel, Tunisia during January, April, July, September and November between 8 and 10 a.m. Among 46 compounds of the essential oil obtained by hydrodistillation, mostly present were linalool, linalyl acetate and *α*-terpineol. The tests showed that the best activities in

DPPH and ABTS assays were registered for the essential oil obtained in July (Ellouze et al., 2012). *C. limon* L. leaves oil showed significant scavenging effects in TBARS assay, representing itself as a potent inhibitor of lipid oxidation against NO and hydroxyl radicals. Trolox was used as a reference. Although concentrations increment exhibited increase of *C. limon* essential oil activity, low concentrations inhibited NO scavenging effect. In comparison to NO radicals, the essential oil yields a strong antiradical activity of hydroxyl radicals, what is probably due to high concentrations of limonene (52.8%). Geranyl acetate and trans-limonene-oxide were also present in the oil and contributed to its activity (Campelo et al., 2011).

Cuminum cyminum L. (Apiaceae) is a common used spice with wide spread distribution in Asia and Mediterranean countries (Hajlaoui et al., 2010). Tunisian *C. cyminum* had been hydrodistillated and its essential oil was analyzed by means of GC and GC–MS. Four tests were applied for investigating of antiradical features of this essential oil. The first one was DPPH assay with an antioxidative value of IC_{50} 31 mg/ml. BHT was used as a reference with a lower IC_{50} of 11.5 mg/ml. The second applied system used to assess the superoxide scavenging activity was PMS-NADH-NBT. The coupling reaction of PMS (phenazine methosulphate) and NAD (nicotinamide adenine dinucleotide) produces a superoxide anion, which is able to reduce NBT (nitroblue tetrazolium). An absorbance decrease suggests consumption of superoxide anion. The third method was used for measuring reducing power. The essential oil showed a better activity in comparison to the BHT. The fourth test applied was the β-carotene bleaching test and here *C. cyminum* essential oil showed a better inhibition than the control (20 and 75μg/ml respectively). All four tests confirm the capacity of *C. cyminum* oil, rich with cuminaldehyde and γ-terpinene, to be able to neutralize the reactive species and to prevent lipid oxidation (Hajlaoui et al., 2010).

Origanum vulgare L. (Oregano, Lamiaceae), is an aromatic herb traditionally used as spice in Mediterranean countries. It is also applied to treat gastrointestinal and respiratory disorder. Because its main essential oil compounds such as thymol, carvacrol, γ-terpinene and β-fenchyl, oregano possess antioxidative and antibacterial

properties. Its antioxidant capacity was measured with FRAP and DPPH assay. *O. vulgare* essential oil exhibited moderate ferric reducing power with 38.5μmol/g in FRAP assay, while it revealed only a poor antioxidant activity in DPPH assay. Even ethanolic, hot water and cold water extracts showed better results in comparison to the essential oil in the DPPH assay, where the hot water extract had the best one. In contrast to this, the essential oil furnished the best results in the FRAP test. Oregano essential oil showed 16.3 mg/g of total phenolic content (Teixeira et al., 2013). The DPPH test of *O. vulgare* L. spp. *vulgare* essential oil dissolved in extra virgin oil showed the antioxidative capacity of 65.93%, mostly due to its antioxidative essential oil constituents thymol and carvacrol (Asensio et al., 2011). *O. majorana* L. essential oil mostly composed from 4-terpineol, γ-terpinene, α-terpinene and trans-sabinene showed radical scavenging activity in concentration dependent manner (10-160 mg/ml). The DPPH assay of it had the IC_{50} value of 58.67 mg/ml, in comparison to the reference Trolox with a double lower value of 23.95 mg/ml. The Fenton-reaction was used for measuring the hydroxyl radical scavenging effect of this essential oil in comparison to Trolox as a reference, where the 160 mg/ml of the *O. majorana* essential oil showed an IC_{50} value of 67.11mg/ml. The reference was still better in inhibiting activity. At the same concentration of 160 mg/ml, the essential oil was able to scavenge H_2O_2 radicals (IC_{50} value of 91.25 mg/ml), while Trolox had 51.30 mg/ ml. Reducing power of Fe^{3+} was measured too and showed for the concentration of 160 mg/ml from essential oil and reference values of 78.67 and 42.22 mg/ml, respectively. Lipid peroxidation was induced with H_2O_2, and various essential oil concentrations were also used in this test for measurement of malondialdehyde inhibition, 10 to 160 mg/ml. Higher concentrations showed better activity, with IC_{50} of 68.75 mg/ml and 52.72 mg/ml for the essential oil and Trolox, respectively, but in all tests the essential oil possessed lower radical scavenging activity than its reference (Mossa and Nawwar, 2011). *O. vulgare* L. ssp. *hirtum* rich with thymol, carvacrol and p-cymene possesses significant antioxidative properties. The total antioxidant activity in ABTS assay of the essential oil, obtained by conventional hydrodistillation, was 97.45%, and it yielded higher values than Trolox. Its antioxidant capacity was also measured by the DPPH assay where it showed a 87.09%-activity, lower than the oil obtained by solvent-free microwave extraction (SFME) with

a great activity of 91.97%. Keeping in step with this, linoleic peroxidation inhibition was also high with 90.43% (Karakaya et al., 2011). The essential oil of *O. vulgare* L. subsp. *glandulosum*, collected from three different localities of Tunisia (Krib, Bargou and Nefza), was consisted mostly of p-cymene, thymol, γ-terpinene and carvacrol. Antioxidative property for oils from all three localities ranged from 59 to 80 mg/L in DPPH assay (Mechergui et al., 2010). The essential oil of *O. acutidens* L. consisted of mostly carvacrol, p-cymene, γ-terpinene and β-caryophyllene. It showed strong antioxidant activity in the DPPH test and 65% of inhibition in the β-carotene linoleic acid assay. BHT was the positive reference with 125 mg/ml and 100% respectively (Goze et al., 2010). Oregano leaves oil of *O. compactum* L. contained carvacrol, thymol and p-cymene as major components among its 46 identified constituents, with 36.4%, 29.7% and 24.3% respectively. In the ABTS test oregano essential oils revealed an IC_{50} value of 2.0 mg/L, showing an antioxidant activity as strong as vitamin C. On the other hand, in DPPH assay oregano oil revealed an IC_{50} value of 60.1 mg/L, while carvacrol and thymol yielded 0.4 and 0.7 mg/L, respectively (Babili et al., 2010). *O. syriacum* L. tested within TBARS assay showed a significant percentage of inhibition with 85.79% (Vuida-Martos et al., 2010).

Fresh and dry rhizomes of *Curcuma longa* L. (turmeric, Zingiberaceae) possess potent anti-arthritic effects (Funk et al., 2010), and were used for isolation of the essential oil by hydrodistillation followed by testing its antioxidant activity. GS-MS revealed a slight difference in the composition between fresh and dry rhizome essential oil. Fresh rhizome essential oil was mostly composed from β-turmerone, aromatic-turmerone and α-turmerone, while the dry rhizome oil contained aromatic-curcumene, α-santalene and aromatic-turmerone (Singh et al., 2010). DPPH, metal chelating and lipid peroxidation assays were used to assess the antiradical capacity of turmeric oil. BHT and BHA were used as positive controls. Turmeric essential oil showed in all tests better results than references, and fresh rhizome oil showed higher antioxidant activity than dry rhizome oil. Presence of curcumin, ar-turmerone and α-turmerone explains a good radical scavenging activity of these essential oils. α- and β-turmerone are particularly present in fresh rhizome oil, explaining its higher antioxidative capacity. This explains that drying of the rhizomes leads to a loss of its active compounds. Therefore is advisable that fresh

rhizomes should be applied in pharmaceuticals and other purposes (Singh et al., 2010). Liju et al. (2011) showed mild DPPH scavenging activity of turmeric essential oil (IC_{50} 1 000 mg/ml). On the other side, lipid peroxidation inhibition was better with an IC_{50} value of 400 mg/ml. Capacities to scavenge superoxide and hydroxyl radicals were 135 mg/ml and 200 mg/ml, while the ferric reducing one was 5mM for 50mg of turmeric oil (Liju et al., 2011). Essential oils of *C. wenyujin* Y.H.Chen et C.Ling and *C. longa* L. were tested for their activities too, and in DPPH assay, it was found for them to possess strong activities, showing *C. wenyujin* oil with a higher potential than *C. longa* oil (Zhao et al., 2010). *C. aromatica* Salisb. essential oil was able to scavenge DPPH radicals with an IC_{50} value of 14.45μg/ml (Al-Reza et al., 2010).

Satureja khuzestanica Jamzad (Lamiaceae) essential oil (SKE) possesses many beneficial properties for human health against diabetes or cardiovascular diseases. Essential oil of this endemic plant of Iran showed strong antioxidant activity (Bagheri et al., 2013; Hashemi et al., 2012), with good results in the DPPH assay possessing IC_{50} of 5.30 ng/ml. BHT as a positive control had an IC_{50} value of 3.89 ng/ml. The total antiradical capacity of SKE essential oil was 3.20 nmol of ascorbic acid equivalents/g SKE by means of the phosphomolybdenum method (Bagheri et al., 2013). LDL oxidation may lead to arterial cell damage, platelet aggretation and even to diabetes mellitus deterioration. SKE oil is supposed to decrease LDL oxidation, so it was tested trough continuous monitoring of formation of conjugated dienes (CD) in LDL. $CuSO_4$ was used for induction of LDL oxidation and it was estimated that it induces formation of conjugated dienes for about six-fold in comparison to the control LDL. Vitamin E is used as a positive control for inhibition of the oxidation. For SKE it is found to have a dose dependent activity. A concentration of 5μg/ml increased the lag time for about 33.33%, while for concentrations of 100μg/ml and 200μg/ml the lag time increment was 67% and 100% respectively, which points out the strong peroxidation inhibition capacity of SKE. For the positive control the lag time increase was found to be 111% for concentration of 100 μmol/L (Bagheri et al., 2013). Lipid peroxidation inhibition was tested with help of TBARS assay. Secondary metabolite concentrations of the lipid oxidation were measured by malondialdehyde (MDA) formation analysis. Tests showed

that MDA production was significantly inhibited by SKE and vitamin E, where the SKE showed dose depended activity in the dosage of 50, 100 and 200μg/ml (Bagheri et al., 2013). *S. khuzestanica* essential oil inhibited oxidation of sunflower oil stored ad 60°C, mostly due to its main compound carvacrol (Hadian et al., 2011). All concentrations of SKE (0.02%, 0.04%, 0.06% and 0.08%) showed antioxidant activity on peroxide value, while the DPPH assay revealed IC_{50} of 31.5μg/ml (Hashemi et al., 2012). *S. thymbra* L. essential oil exhibited high antioxidative capacity, having ability to scavenge DPPH radicals by IC_{50} 0.0967 mg/ml. Essential oil composition of this plant, growing wild in Libya, mostly consisted of γ-terpinene, p-cymene, carvacrol and thymol. *S. thymbra* essential oil also showed better results than thymol and γ-terpinene. On the other side its results were more or less equal to butylated hydroxyanisole (BHA). *O. vulgare* essential oil, also contains much γ-terpinene and thymol, had shown better results than the positive control BHA (Giweli et al., 2012). Mediterranean *S. montana* L. is famous because of its fungicidal, antiviral and antimicrobial properties. This annual plant yields an essential oil rich of carvacrol, thymol and carvacrol methyl ether, all famous for their antioxidant activity. In comparison to ethanol and water extract, the essential oil of *S. montana* showed the lowest antioxidant activity in DPPH assay. In the FRAP assay this essential oil showed better results (Serrano et al., 2010).

Kavoosi et al. (2013) tested two essential oils of different plants for their antioxidant activity, *Carum copticum* L. (Ajowain, Apiaceae) and *Ferula assafoetida* L. (Apiaceae). Both of them are used in traditional medicine for their sedative, analgesic, antitussive, antihypertensive, digestive and other properties. Both are potent antioxidants in concentration dependent manner (Kavoosi et al., 2013). Scavenging activities for reactive oxygen species (ROS) and reactive nitrogen species (RNS) demonstrated by IC_{50} values of 40 and 45μg/ml for *C. copticum* essential oil, and IC_{50} 130 and 150μg/ml for *F. assafoetida* essential oil, respectively. IC_{50} values for hydrogen peroxide radicals were 60μg/ml for *C. copticum* EO and 160 μg/ml for *F. assafoetida* EO. TBARS assay was also applied with results of IC_{50} values of 50μg/ml and 155 μg/ml for *C. copticum* and *F. assafoetida* essential oil, respectively. Ascorbic acid was used as a positive control (Kavoosi et al., 2013). Carum oil as major components contains thymol, p-

cymene and γ-terpinene, whereas the ferula oil possessed β-pinene, 1,2-dithiolane and α-pinene as major constituents. These differences may explain the different antioxidant behavior of these two essential oils, particularly showing carum more effective than ferula essential oil (Kavoosi et al., 2013). Inhibitory concentrations (IC_{50}) for total radical scavenging capacity were between 40 and 60 μg/ml of carum oil and 130 and 160 μg/ml of ferula oil. Carum and ferula essential oils displayed a potent and concentration dependent ROS, RNS, H_2O_2, and TBARS scavenging activities (Kavoosi et al., 2013). Another paper reported on the DPPH assay of *C. carvi* L. and *Coriandrum sativum* L. essential oils to have good antioxidant property (Samojlik et al., 2010).

Zataria multiflora Boiss. (Lamiaceae) has been used in traditional medicine for many years and is endemic in Iran (Sharififar et al., 2012). Five ecotypes of *Z. multiflora* were used for testing the antioxidant activity of their essential oils. According to the different phytogeographic occurrence in Iran, names given to these ecotypes were names of towns where they were collected, including Hajiabad, Farashband, Yazd, Najafabad and Poldokhtar. With help of GS/MS it was found that these essential oils have small differences in the concentration of essential oil constituents. Among all, thymol was the most abundant compound in all essential oils ranging from 27.1% to 64.8%, followed by carvacrol, γ-terpinene, thymol methyl ether and carvacrol methyl ether (Karimian et al., 2012; Saei-Dehkordi et al., 2010). The best activity in DPPH assay was assessed with the Najafabad sample (IC_{50} 19.7 μg/ml). The Yazd sample was the second one, then Farashband and Hajiabad samples followed, while the Poldokhtar sample showed the lowest activity on account of the fact that thymol and carvacrol were less present. Ascorbic acid and BHT were used as references, showing that Najafabad sample possessed similar activity as BHT (Saei-Dehkordi et al., 2010).

Pakistan *Rosmarinus officinalis* L. essential oil (Lamiaceae) contains 1,8-cineole (38.5%) as a main compound. This essential oil, famous for its antioxidant and antibacterial (Beretta et al., 2011; Sirocchi et al. 2013) properties, was able to reduce DPPH radical and to inhibit lipid peroxidation in linoleic acid system. On the other hand 1,8-cineole showed poor activity, suggesting that the effects of the essential oil was due to synergistic effect of other compounds contented in the oil, such as camphor,

limonene, camphene and linalool (Hussain et al., 2010). Zaouali et al. (2010), on the other hand, tested antioxidant activity of two *R. officinalis* varieties: *typicus* Batt. *and troglodytorum* Maire., endemic to Tunisia. Essential oils of both varieties yielded positive results in DPHH assay, ferric reducing (FRAP) assay and β-carotene bleaching test. *R. officinalis* var. *troglodytorum* showed higher activity in DPPH assay compared with the var. *typicus*. The IC_{50} values in β-carotene bleaching test were 1.1 μl/ml for var. *troglodytorum* and 3.2 μl/ml for var. *typicus* essential oils, showing that these essential oils possess a significant lipid peroxidation inhibition capacity. Although the presence of α-thujene, camphor and linalyl acetate as essential oil constituents may contribute to the obtained results, it is believed that the synergistic effect of all compound is responsible for the antioxidative results (Zaouali et al. 2010).

D-Borneol is a major compound of *Cinnamomum burmannii* Nees leaves oil (Lauraceae), being present up to 78.6%. Other constituents are bornyl acetate, (-)-spathulenol and eucalyptol. The oil was investigated for its antiradical properties in DPPH and ABTS assays and showed moderate scavenging activity, however weaker than the positive control BHT. Although concentration dependent, maximal scavenging rates were 58.89% for ABTS radicals and 21.71% for DPPH radicals (Al-Dhubiab, 2012). *C. glaucescens* Hand.-Mazz. is used in antifungal, insecticidal and antiaflatoxin purposes. DPPH test was applied to assess the antioxidant activity of *C. glaucescens* essential oil and BHT was used as a positive control (Prakash et al., 2013).

Clove oil form *Eugenia caryophyllata* Thunb. or *Syzygium aromaticum* L. (Myrtaceae) consist mostly eugenol (Arenas et at., 2011), which is used for food flavoring and posses antiseptic, antibacterial, sedative and antifungal properties. The total antioxidant activity of 15μg/ml eugenol was 96.7%, whereas among the positive controls the highest activity was BHT with 99.7%, while the other controls Trolox, α-tocopherol, BHA were less effective. Eugenol showed high reducing capacity among the ferric and cupric ions. In both tests eugenol furnished better results than controls, however all results were dose-dependent. Eugenol also showed significant scavenging activity for DPPH stabile radicals with an IC_{50} value of 16.06 μg/ml, representing itself as the best one among

positive controls compared with the IC_{50} values for BHA, BHT, α-tocopherol, and Trolox. Keeping in step with the DPPH assay, eugenol also showed the best results in comparison to controls among DMPD radicals (N,N-dimethyl-p-phenylenediamine) (Gülcin, 2011). GS-MS analysis showed for the *Syzygium cumini* L. (Myrtaceae) leaves essential oil to contain α-pinene, β-pinene and trans-caryophyllene as major compounds. The total phenolic content of essential oil in the DPPH assay showed 55.87% scavenging activity at the concentration of 50μg/ml. On the other hand, ferric reducing power in FRAP method was 0.47 μg/100 mg of essential oil (Amal et al., 2013).

For investigation of the antioxidant activity also was applied *Leucas virgata* Balf.f. (Lamiaceae) essential oil, collected from Island Soqotra (Yemen). By GC-MS this oil was analyzed and 43 constituents, mostly oxygenated monoterpenes (champhor and fenchon, followed by borneol), were detected. Among oxygenated sesquiterpenes, β-eudesmol and caryophyllene oxide were found. *L. virgata* essential oil showed 31% of scavenging activity in DPPH assay for 1 mg/ml of essential oil (Mothana et al. 2013).

Laurel essential oil was distilled from *Laurus nobilis* L. (Lamiaceae) leaves, which is mostly used for flavoring and preservative purposes, and it was identified to have 29 different compounds, where the major were 1,8-cineole, 1-(*S*)-α-pinene, and *R*-(+)-limonene (Basak et al., 2013; Ramos et al. 2012). Laurel essential oil have low antioxidant activity (Basak et al., 2013; Ramos et al. 2012). Basak et al. (2013) investigated the potential of laurel essential oil to inhibit hydrogen peroxide, superoxide, and hydroxyl radicals. The DPPH scavenging activity and lipid peroxidation inhibition also were measured. *In-vitro* Fe^{3+}-ascorbate-EDTA-H_2O_2 system was applied for determination of hydroxyl radicals, which ore one of the most reactive oxygen species. Tests showed for the oil to possess low IC_{50} values, representing it as a good scavenger for hydroxyl radicals. Curcumin and BHT were positive controls 1,8-cineole, *R*-(+)-limonene and 1-(*S*)-*α*-pinene also proved to be hydroxyl radical scavengers, among which 1,8-cineole had the major activity. The superoxide radical inhibiting test presented laurel essential oil as the strongest superoxide scavenger among the controls curcumin, ascorbic acid and BHT. Upon testing some single compounds of this essential oil, 1,8-cineole showed the highest scavenging activity. Laurel oil was also the most

active antioxidant against hydrogen peroxide. Positive controls were curcumin, BHT and ascorbic acid in the hydrogen peroxide scavenging test. In lipid peroxidation assay and in DPPH test, it was assessed for laurel oil to be better than the controls (Basak et al., 2013).

Corsican *Limbarda crithmoides* L. ssp. *longifolia* (Asteraceae) essential oil was investigated and it was found to contain 65 constituents, among which the major were p-cymene, α-pinene, thymol-methyl-ether, 2,5-dimethoxy-p-cymenene, 3-methoxy-p-cymenene, and α-phellandrene. In DPPH and ABTS assay this essential oil showed positive results. A moderate reducing power of ferric ion was measured with FRAP assay and was attributed to the essential oil (Andreani et al., 2013).

Mentha longifolia L. (Lamiaceae), also called wild mint, has been used against different infections and gastrointestinal disorders for many years. Using GC-MS, 19 compounds were identified, with piperitenone oxide, borneol, germacrene D, and β-caryophyllene as major constituents. Different tests such as linoleic acid assay, β-carotene bleaching test and DPPH assay were applied in order to investigate the antioxidant property of mint, growing wild in dry region of Pakistan. In DPPH assay *M. longifolia* essential oil showed a scavenging activity (IC_{50} 21,8μg/ml) while the linoleic-acid-peroxidation-inhibition test had revealed antioxidant activity of 37.3%. On the other hand, *M. longifolia* essential oil showed poor activity the in linoleic acid assay (Iqbal et al., 2013).

Essential oils obtained from needles of *Pinus densiflora* Siebold et Zucc. needles and *Pinus thunbergii* Parl. (Pinaceae) possess antiinflammatory, anticarcinogenic and antioxidant properties. *P. densiflora* essential oil is rich in camphene, α-limonene and α-pinene. The *P. thunbergii* essential oil consists of δ-3-carene, α-terpinolene and β-phellandrene. For investigation of the antioxidant activity the DPPH assay and nitrite-scavenging activity assays were proposed. The DPPH assay of *P. densiflora* and *P. thunbergii* essential oils exerted antioxidant activity of 120 and 30 μg/ml (IC_{50}) respectively. On the other hand *P. densiflora* and *P. thunbergii* EO revealed a nitrite-scavenging activity higher than fifty percent (Park and Lee, 2011).

Piper officinarum C. DC. (Piperaceae) essential oils were obtained from leaves and stems by steam distillation. Leaf oil mostly consisted of β-caryophyllene, α-pinene, limonene, β-selinene and sabinene. The stem oil was mostly composed of β-caryophyllene, limonene and α-pinene and in comparison to the leaf oil it possessed higher concentrations of α-phellandrene and linalool. In the DPPH assay, the essential oils showed low antioxidant activity with an IC_{50} value of 777.4μg/ml. The β-carotene linoleic acid test yielded better results for the essential oil with peroxidation inhibition of 88.9%. In comparison to this essential oil, BHT revealed 95.5% peroxidation inhibition (Salleh et al., 2012).

4 Essential oil effect on different enzyme function and free radicals formation

Many studies have shown that essential oils and their single compounds exert an effect on various enzymes, mostly linked to free radical production. Infections, different kind of inflammations, tumor cells, and macrophages are the greatest cause of reactive species production and oxidative stress. They all exhibit their effect through activation of different enzymes and cytokines such as cytochrome P-450 system and oxidative enzymes such as NAD(P)H oxidases, endothelial xanthine oxidase, myeloperoxidases and arachidonate oxygenases (Juranek, et al., 2013; Serviddio,G; et al., 2013) or iNOS, COX-2, TNF-α, IL-6 etc (Chou et al, 2013; Valente et al., 2013). On the other hand, antioxidative enzyme expression will be induced during oxidative stress too. To this group of enzymes belong catalase, superoxide dismutase, glutathione peroxidase and peroxidase (Juranek, et al., Kannan et al., 2013; Kalyanaraman, 2013; 2013; Ríos-Arrabal et al., 2013). It is estimated that many essential oils can exhibit their efficacy not only by antioxidative properties, but also via possibility to inhibit oxidative enzymes and cytokines or to enhance antioxidative enzyme reactions. For example, an essential

oil compound (-)-α-bisabolol, obtained by direct distillation of the essential oil from *Vanillosmopsis erythropappa* Sch. Bip. (Asteraceae), is able to increase the SOD activity, which is responsible for forming hydrogen peroxide (Rocha et al., 2011). *Achillea millefolium* L. (Asteraceae) is a popular traditional plant in many countries. Also called yarrow, this plant is applied for ages in folk medicine to treat different kinds of inflammations, skin diseases and gastrointestinal disorders. *A. millefolium* essential oil (AM-EO) is rich in artemisia ketone, linalyl acetate, camphor and 1,8-cineole, all famous for their antioxidative, antimicrobial and anticancer activities. Various infections, tissue damages or other health disorders stimulate an inflammatory response, followed by free radical formation. Macrophages are mostly responsible for immune response and thought to be one of the main sources of free radical production and inflammation. For this reason, RAW 264.7 macrophages were applied for investigation of *A. millefolium* essential oil (AM-EO) activity and its capacity to inhibit production of free radicals and inflammation, when pretreated with lipopolysaccharide, a bacterial toxic molecule responsible for activation of macrophages and inflammation processes. Firstly, it was assessed that essential oils have no effect on RAW 264.7 macrophages viability. When pretreated with 80 μg/ml of AM-EP, NO production decreased to 35%. Lipopolysaccharides (LPS) are significant agents of oxidative stress. They not only activate NO production, but activate formation of superoxide anion and malondialdehyde (MDA) as well. They influence GSH concentration and induce DNA damage. AM-EO decreased superoxide anion formation at any concentration added. Generally, superoxide promotes oxidative stress and cell death of healthy cells during inflammation too. 20μg/ml are able to inhibit 58%, while 40 μg/ml and 80 μg/ml concentrations were able to decrease superoxide formation in that manner, similar to untreated macrophages with LPS. AM-EO was able to inhibit lipid peroxidation too, being able to decrease formations of the MDA and GS. Also the DNA damage was less present in AM-EO treated RAW 264.7 macrophages. The effect of this essential oil on antioxidant enzymes was also measured, which are activated from cells in inflammations conditions. SOD, CAT and GPx activities were reduced at all concentration of AM-EO added, proving that AM-EOs activity is obtained from its antioxidative property, and not via activating antioxidative enzymes. INOS, COX-2,

TNF-α, IL-6 also influence an increase of free radicals and inflammation. iNOS activates NO synthesis and COX-2 convert arachidonic acid into prostaglandins, while pro-inflammatory cytokines IL-6 and TNF-α are expressed during inflammation. Expression of these inflammatory agents is highly decreased after pretreatment of macrophages with AM-EO, indicating that this essential oil has a significant protective properties during oxidative stress. On the other hand HO-1 expression levels, which suppress ROS formation and protect the body from inflammation by inhibiting NO function, are low after cell pretreatment with this essential oil, what is due to the fact that AM-EO suppresses oxidation via its own antioxidant property (Chou et al, 2013).

The aerial parts of *Oenanthe crocata* L. (Apiaceae) were used for hydrodistillation to obtain an essential oil, which mostly contained sabinene, trans-β-ocimene, and cis-β-ocimene. Although the root of *O. crocata* is highly toxic, its leaves and flowers are used in traditional medicine for dermatological and brain related diseases. Nitric oxide scavenging activity was measured and the essential oil presented itself as a potent NO scavenger and it also inhibited the expression of nitric oxide synthase (NOS). Already 0.04 μl/ml of essential oil are able to decrease nitrite oxidation by 30%. NO scavenging activity of sabinene was also measured and it was less potent than that of the essential oil. High levels of NO during some inflammation are mostly due to iNOS. After pretreatment of macrophages with this essential oil, nitrite production was reduced from 211.1% to 103%, indicating the high potency of *O. crocata* essential oil to inhibit the iNOS (Valente et al., 2013).

Macrophages were used for generation of superoxide radicals by PMA (phorbol-12-myristate-13-acetate) and for measurement of turmeric oil scavenging activity. It was found for turmeric oil to inhibit an increase of superoxide radical concentration. Turmeric oil effect also was tested on antioxidant enzymes in blood and serum of mice. After administration of turmeric oil it was found for enzymes such as catalase, superoxide dismutase, glutathione and glutathione reductase, to be significantly increased, particularly by a concentration of 500 mg/kg after treatment over 30 days. Mice liver tissues were also used for estimation of turmeric oil activity on antioxidant enzyme induction. Increased enzyme levels were found to be for glutathione peroxidase,

superoxide dismutase. Even glutathione and GST (glutathione-S-transferase) levels were elevated. Catalase and glutathione reductase remained unaltered (Liju et al., 2011).

Zataria multiflora Boiss. (Lamiaceae) is traditional plant used in Middle East for carminative, analgesic and antimicrobial purposes. Only growing in warm parts of Afghanistan, Pakistan and Iran, *Z. multiflora* essential oil has a high presence of thymol and carvacrol, explaining its strong antioxidant property. It is also proven for this essential oil to be able to reduce hydrogen peroxide (H_2O_2) and nitric oxide (NO) production and it is believed to react as radical scavenger and inhibitor of related oxidative enzymes. It is also believed that carvacrol and thymol alone inhibit NO synthase (NOS) and NADH oxidase (NOX). H_2O_2 and NO are formed in human monocytes via glucose degradation, especially when high glucose concentrations are present. Major compounds of *Z. multiflora* essential oil determined by GS-MS were carvacrol, thymol, and p-cymene with percentage. Linalool and γ-terpinene were also present but to lower extent of 9.6% and 8% respectively. The ABTS assay was used to identify reactive oxygen inhibitory capacity of this essential oil, showing an IC_{50} value of 5.7μg/ml. Reactive nitrogen scavenging activity was also measured in the NO scavenging assay. Thymol and carvacrol showed a better activity, while linalool, γ-terpinene and p-cymene were inactive. Human monocytes were used for testing NO and H_2O_2 production. When incubated with glucose, the significant increase was found not only for these reactive species but also for NOS and NOX. After incubation of cells with 20 mM of glucose, the NO production was 263 nM and after pretreatment with 1, 10 and 100 ng/ml of *Z. multiflora* essential oil the NO production significantly decreased to 100 ng/ml. Using the same concentrations by carvacrol and thymol, pretreatment of monocytes showed reduction of NO in similar manner. Thymol was slightly better in comparison to carvacrol with 152 nM. Linalool, γ-terpinene and p-cymene revealed no difference to NO concentration after pretreatment of monocytes before incubation. The same affects were measured on NOS activity changes after pretreatment with *Z. multiflora* essential oil and its single constituents. Linalool, γ-terpinene and p-cymene caused no change in NOS activity. *Z. multiflora* essential oil also showed significant reduction of H_2O_2 production in glucose-stimulated monocytes. At a concentration of 100 ng/ml thymol extended similar result with 63nM, while carvacrol showed itself as

the better one with 59nM at the same concentration. Linalool, γ-terpinene and p-cymene had no effect on H_2O_2 production. Effects on NOS production in monocytes were also present after pretreatment with *Z. multiflora* essential oil, thymol and carvacrol. Like in all tests, p-cymene, linalool and γ-terpinene did not alter NOS activity (Kavoosi and Teixeira da Silva, 2012a). This author group investigated *Z. multiflora* essential oil activity on nitric oxide and hydrogen peroxide production in macrophages, stimulated with lipopolysaccharide (LPS). *Z. multiflora* essential oil reduces NO concentration in macrophages. After pretreatment, it showed a clear reduction of different concentrations of this essential oil. Pretreatment of macrophages with 1, 10 and 100 ng/ml essential oil lead also to a significant reduction of H_2O_2 radicals. Reduction of reactive nitrogen species and NADH oxidase were also present. 1, 10 and 100 ng/ml of this essential oil inactivated reactive nitrogen species (NOS) and furnished the reduction of NADH oxidase for the same concentrations as for other radicals. Thymol and carvacrol were positive controls and showed in all test significant reduction too. p-Cymene on the other hand had no effects on radicals and enzyme concentrations change after its pretreatment of macrophages (Kavoosi et al., 2012b).

Eugenol and methyl eugenol are the main compounds of the essential oil of *Ocimum sanctum* L., which is able to decrease serum lipids. *O. sanctum* leaves oil is used for measurement the antioxidative and antihyperlipidamic properties in rats, which were on cholesterol diet. Lipid peroxide concentration was assessed in TABRS assay and it was found for this essential oil to decrease thiobarbituric acid levels in heart and liver. In heart tissue, it is also shown for the oil to exert a decreasing effect on superoxide dismutase (SOD) and glutathione peroxidase (GPx) without effecting catalase (CAT). On the other hand GPx, SOD and CAT were not impacted in liver tissue (Suanarunsawat et al., 2010)

5 Conclusion

Speaking of an antioxidant generally, its activity is required to act as a hydrogen donor and neutralize free radicals. On the other hand, aromatic rings play a significant role and phenolic hydroxyl groups enhance the inhibition of oxidation (Gülcin, 2011).

Essential oils comprise many different single constituents in different concentrations. An antioxidant effect is mostly attributed to compounds with highest presence. However investigations have shown that minor constituents also contribute to the essential oil activity, what concludes that single compounds are on one hand highly active substances and show already in small concentration great activity and on the other hand they contribute by their synergistic effect to the property of the essential oil (Victoria et al., 2012). For example, essential oils obtained from *M. leucadendra* leaves and fruits showed significant antioxidant capacity in DPPH, TBARS and ABTS assay. However, it is hard to tell whether the antioxidant activity results from presence of 1,8-cineole, α-terpineol, α-pinene, limonene, globulol, and guaiol as major constituents, or from a synergistic effect. On the other hand, trace constituents could also contribute to the activity (Pino et al., 2010).

Different kind of methods, such as DPPH assay, ABTS assay or β-carotene bleaching test or many others, already mentioned in previous text, are used to assess the antioxidative properties of essential oils and their single compounds. Being one of the main topics in many studies for many yaers, essential oils have shown themselves as very potent substances in decreasing high free radical concentrations and in decreasing their formations. Many scientific articles have shown how antioxidant activity is of special importance for protecting cells against DNA damage and mutagenesis, cancer invasion and inhibition of bacterial growth and infections (Gülcin, 2011). Following this, they are good in preventing different human diseases and food spoilage. Some of them have pleasant aroma and are used as a flavor or as fragrant agents. Especially because of their low or even non side effects properties their popularity elevate and they show themselves as a potent replacement for synthetic antioxidants having a bright

application spectrum in pharmacy and food industry (Bassolé et al., 2012; Mimica-Dukić et al., 2010; Prakash et al., 2013; Aidi Wannes et al., 2010).

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