

DIPLOMARBEIT

Titel der Diplomarbeit

„Biological Properties of Some Volatile Phenylpropanoids“

verfasst von

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angestrebter akademischer Grad

Magistra der Pharmazie (Mag.pharm.)

Wien, März 2015

Studienkennzahl lt. Studienblatt:

A449

Studienrichtung lt. Studienblatt:

Diplomstudium Pharmazie

Betreut von:

Univ. Prof., Dr. Phil., Mag. Pharm.
Gerhard Buchbauer

Acknowledgment

First of all, I would like to express my sincere gratitude to Mr. Univ. Prof. Dr. Phil. Mag. Pharm. Gerhard Buchbauer, for his full support and expert guidance. I would like to show appreciation for giving me the opportunity to do and finish my thesis. It was a great honor to work with you.

I would like to thank all my friends and colleagues, for all of the unforgettable moments and for making the studying much easier, more interesting, as well as challenging.

Finally, many thanks to my parents, my sister and my boyfriend for their encouragement, understanding and patience. Thank you for always being there and for pushing me further than I thought I could go.

Thank you all for letting me fulfill my dream!

Abstract

Plants and their extracts are the new field of interest for many scientists and also of some pharmaceutical industries. In order to provide more information for their usage in the prevention and treatment of diseases many clinical trials and researches are being carried out. In this review the biological activities and the mechanism of action of volatile phenylpropanoids found in essential oils are presented. The aim of this overview is to show that volatile PPs, found in EOs, can exert many of those biological activities which are generally attributed to EOs. Almost all of the PPs possess antimicrobial, anti-inflammatory and anticancer activities. These are related to the different substitution of the phenylpropane molecule. For each isolated group not only one, but more pharmacologically activities can be credited.

Abstract

Pflanzen und ihre Extrakte stellen für viele Forscher, wie auch für einen Teil der Pharma-Industrie, ein neues Interessen-Gebiet dar. Um mehr Informationen über ihre Anwendung in der Prävention und Heilung von Krankheiten zu erlangen, werden viele Studien und Versuchsreihen veröffentlicht. In diesem Review werden die biologischen Aktivitäten und Mechanismen der Aktionen der flüchtigen PPs, gefunden in EOs, präsentiert. Das Ziel dieses Überblicks ist es aufzuzeigen, dass flüchtige PPs, gefunden in EOs, viele jener biologischen Aktivitäten aufweisen, die generell EOs zugeordnet werden. Fast alle PPs verfügen über antimikrobielle, antiinflammatorische und antikanzerogene Aktivitäten. Dies ist den verschiedenen Substituenten des Phenylpropan-Moleküls zuzuschreiben. Jeder isolierten chemischen Gruppe ist nicht nur eine sondern sind mehrere pharmakologische Aktivitäten zuzuordnen.

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Introduction

There are more than half a million plants all around the world, but only about 15% of them are known for their pharmaceutical activity and have been phytochemically examined and only 6% have already a scientifically proven biological activity. This makes the nature an important source of novel drugs as natural alternatives.

EOs are derived from plants and possess a wide range of biological activities. EOs are concentrated liquids consisting of volatile low molecular compounds such as alcohols, aldehydes, acyclic esters and lactones, derivatives of mono- and sesquiterpenes, phenylpropane and also simple alkanes and alkenes. Parts of plants, such as leaves, roots, peels, fruits, barks can be used as extraction source. Among various extraction methods only the steam distillation is allowed to be used [1-3]. Plants are able to produce the primary metabolites (amino acids and carbohydrates) essential for survival and by means of glycosylation, methylation and hydroxylation to turn them into the secondary ones, used as defense from different pathogens, predators and abiotic stimuli [4].

The plant's secondary metabolites are the volatile organic compounds (VOCs) that are found in the EOs. Phenylpropanoids (PPs), terpenes (isoprenoids, terpenoids) and nitrogen-containing compounds like alkaloids and heterocyclic aromatics are the three major classes of secondary metabolites. Among them PPs are the largest ones [4]. They are named after their chemical structure, which consists of a six carbon aromatic phenol group and a three carbon propene tail, present since the very beginning of the PPs

synthesis [2]. These organic molecules are part of the plant defense system against wounding, herbivores, UV and infections. They are synthesized in the shikimic pathway from the amino acid phenylalanine as primary metabolite that under the influence of PAL can be converted into cinnamic acid that is the basis structure for the further development of other volatile and non-volatile PPs. Besides their presence in food, perfumery and cosmetic industry they were used since ancient time in traditional medicine. Especially their antimicrobial, anti-inflammatory, anti-cancer and antioxidant activities are in the limelight. Nowadays scientists try to discover the mechanism of action of the active compounds and this led to increased interest of the pharmaceutical industries in PPs as the potent natural alternatives for the treatment of many diseases [2,4,5].

Some volatile phenylpropanoids identified in essential oils

1'-Acetoxychavicol acetate

1'-Acetoxychavicol acetate has been originally isolated from the rhizomes and seeds of *Languas garanga* L.Willd. or also known as *Alipinia garanga* L. Willd., belonging to the Zingiberaceae family. Many reports and reviews revealed that this PP possesses significant anti-tumor-promoting activity. Furthermore, 1'-acetoxychavicol acetate can exert anti-inflammatory, antioxidant and antiviral activity [6].

Anticancer activity

It is capable to suppress the NF- κ B, a protein responsible for cytokine production and cell survival, and to inhibit *in vivo* and *in vitro* the cellular

growth of cancer cells. It has chemo preventive effects and can be used against rat oral carcinomas. Therefore, 1'-acetoxychavicol acetate could be a novel strategy for the treatment of multiple myeloma patients [7,8].

Anethole

Anethole (1-methoxy-4-benzene-(1-propenyl)) is a monoterpene isomer mainly found in the EO of anise (*Pimpinella anisum* L., Apiaceae), star anise (*Illicium verum* Hook.F., Illiciaceae), and sweet anise (*Foeniculum vulgare* Mill., Apiaceae) and is with more than 90% the major constituent in the EO of these plants. It was also identified in very low concentrations in the EO of basil (*Ocimum basilicum* L., Lamiaceae), cilantro (*Coriandrum sativum* L., Apiaceae) and lemon balm (*Melissa officinalis* L., Lamiaceae) [9]. The smell of anethole is the cause of the classic anise flavor. Because of the double bond present in anethole, there are two isomers: cis and trans-anethole. Both isomers possess different physical properties [10].

Trans-anethole is the isomer responsible for most of the properties given to the EO of star anise, such as: anti-carcinogenic, anti-oxidative, anti-nociceptive, anti-inflammatory, antifungal, antimicrobial and antiviral [11]. Furthermore, Tognolini et al. [12] have shown that anethole can also exert an impact on the blood coagulation. Antiplatelet and clot destabilizing activity were observed [12].

Anticancer activity

The international Agency for Research on Cancer has declared that there were over 14 million new cancer cases and 8.2 million cancer deaths in 2012. By 2030 is expected that the number will reach 26 million new cancer cases and 17 million deaths per year. The fast progression and the increasing number of deaths made cancer the most aggressive killer worldwide. During

the last 10 years, many synthetic chemotherapeutic agents have been used in order to fight cancer. The expectation was not completely fulfilled despite the costs for their development. Therefore, many scientists constantly are trying to develop new, more effective, and not so expensive anticancer drugs [13].

Since the beginning of human history plants have been used for the treatment of human diseases and they were the basis for the derivation of chemical compounds. In the last few decades natural products have begun to receive more attention in the cancer treatment as a novel cancer preventive. It has been proven that they can inhibit different stages of tumorigenesis and in the same time to suppress inflammatory processes. Nowadays 60% of the drugs used for cancer treatment are derived from natural compounds. The plant kingdom is currently the most significant source of common alternatives for cancer treatment [13].

Apoptosis is a type of programmed cell death and is responsible for the elimination of unwanted cells. It is mediated via *extrinsic* (initiated by stimulation of death receptors) and *intrinsic* mitochondrial pathways. The activated death receptors in the extrinsic pathway can induce the production of the DISC (death-inducing signal complex) and activate caspase-8 (initiator enzyme involved in programmed cell death), which induces the caspase-cascade and cell death. The intrinsic pathway is initiated by DNA-damage and causes disruption of mitochondrial membrane and pro-apoptotic proteins are set free in the cytosol. Then a complex is being formed that can further activate caspase-9 (initiator enzyme involved in apoptosis). Deregulation of the apoptotic program is linked to the pathogenesis of many diseases including cancer [14].

Anethole has a cytotoxic effect on fibrosarcoma tumor, breast cancer, cervical carcinoma hepatocytes and Ehrlich ascites tumor. The antiproliferative effect of anethole is determined by its ability to inhibit the induction of NF- κ B, activator protein 1 (AP1), c-jun N-terminal kinase (JNK) and mitogen activated

protein kinase. Additionally, it also inhibits the activity of metalloproteinases (MMP-3 and MMP-9) and increases the activity of MMP inhibitor TIMP-1 [7].

Anti-inflammatory and analgesic activity

Inflammation is a stereotypical physiological response to noxious stimuli that can cause tissue and cell damage. It is the protective mechanism of organism against harmful stimuli. The role of inflammation is to initiate pathogen killing as well as tissue repair processes and to help restoring the homeostasis at infected or damaged sites. There are two types of inflammation: acute and chronic. The acute inflammation is a consequence of leukocytes infiltration to the site of the injury or the infection. At the begin of the inflammation, inflammatory mediators are being released from the macrophages and the mast cells in order to restore homeostasis. The infiltration of inflammatory cells into compartments where they are not normally found in such a high concentration leads to the amplification of the inflammatory response. When the inflammation is acute it is not so dangerous, because the negative feedback mechanism is already involved and the cause will be rapidly removed. The inflammation is chronic when the body cannot find the appropriate response toward the noxious stimuli and the body mechanism has failed to regulate itself. Chronic inflammations are an indication for the progression of the disease. Many diseases, such as asthma, atopic dermatitis, cancer, peridontitis and rheumatism are linked with this type of inflammation. The goal of the inflammation therapy is to decrease the number of inflammatory mediators. Lately, science became more interested in the anti-inflammatory potential of the EOs. Various phenolic structures, such as the PPs have been identified to modify and suppress the body inflammatory response. The data obtained from the different studies suggest that the volatile PPs in EOs, on account of their wide variety of mechanisms of action, possesses a great potential as novel drugs for the inflammation treatment [5,15].

It is believed that anethole modifies the voltage gated L-type Ca^{2+} channels and on this way inhibits the LPS-induced (lipopolysaccharid found in the outer membrane of Gram-negative bacteria) inflammatory mediators production such as $\text{TNF-}\alpha$ (tumor necrose factor) and $\text{IL-1}\beta$ (cytokine). At both concentrations 10 or 50 mg/kg this important volatile PP has significantly suppressed the cytokine production. The effects were almost similar as seen with ketoprofen. Therefore, it's usage could be the novel strategy for treatment of inflammatory diseases caused by Gram-negative bacteria [11]. Furthermore, Da Silviera e Sa et al., [5] reported that anethole in mice can suppress the inflammatory response not only in acute but also in persistent inflammatory models and can decrease the level of migrated leukocytes, NO and PGE_2 in the inflammatory exsudates. Moreover, anethole also has the capacity to inhibit $\text{I}\kappa\text{B-}\alpha$ degradation and to block the $\text{NF-}\kappa\text{B}$ activation. These findings are suggesting anethole's possible therapeutical involvement in treatment of inflammation in humans [5].

Its peripheral antinociceptive effect is also linked with the decreased release of prostaglandin E_2 , nitric oxide and other inflammatory mediators like cytokines. Anethole can additionally decrease the secretion of bradykinin, histamine and serotonin, peptides that elevate the pain sensitivity, and in this manner to amplify the analgesic effect. It's anti-nociceptive activity is especially important for the medication of peridontitis. Major concern in the pain treatment is when the analgesic effect causes other undesired effects like sedation, because it can interfere with the results in the test of nociception and lead to false statements. Treatment with different doses of anethole did not alter motor activity and no sedative effect was shown. For all these properties Anethole may represent an interesting natural alternative in inflammatory and painful diseases [9,11].

Antioxidant activity

During normal physiological function free radicals are continuously produced as a result of normal metabolic processes. In stress conditions increases the amount of these reactive oxygen species. Free radicals possess an unpaired electron in an oxygen atom, for example: superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), perhydroxy radicals ($HO_2^{\bullet}$). Besides the radicals, there are also non-radical species like singlet oxygen (O_2), ozone (O_3) and peroxide (H_2O_2) [16,17]. These highly reactive molecules have been modulated by enzymatic and non-enzymatic antioxidants. Antioxidants are an important part of the oxidative stress treatment. They can modulate ROS concentrations through reacting with free radicals, chelating free catalytic metals and scavenging oxygen. Alterations in their activities in organism lead to an imbalance between radical-generating and radical-scavenging effect and oxidative stress development. The consequences are damages on cellular and extracellular constituents, as carbohydrates, lipids, proteins and nucleic acids. This correlates to the development and progression of different human diseases including cancer, arteriosclerosis, neurodegenerative disorders as Alzheimer and aging processes [16,18,19].

EOs, especially the ones with phenolic volatile compounds, because of their high reactivity with peroxy radicals, have emphasized antioxidant properties. They can be absorbed through skin and are topically applicable. Volatile PPs demonstrate its activity directly by scavenging of ROS or by breaking the chain of peroxy radicals [19].

Trans-anethole is a small molecule that can be easily absorbed through skin, because of its lipophilic character. By means of its non-toxic, non-sensitizing and non-genotoxic properties it is often used as an antioxidant. Its activity depends on the conjugate double bonds. Lately, a correlation between anethole's antioxidant activity and anti-ageing was discovered. On molecular level oxidative stress activates NF- κ B, an oxidant sensitive transcriptional factor, responsible for MMP-2 expression, enzyme that degrades collagen in

skin fibroblasts and leads to skin aging. Anethole is able to suppress directly the lipid-peroxidation and to chelate the Zn-ion from matrix metalloproteinases (MMPs). At a concentration of 1 mM can completely block NF- κ B activation [19].

Antiplatelet activity

Blood is a bodily fluid in animals that is circulated in the body through blood vessels by the pumping action of the heart. It delivers necessary substances as oxygen and nutrients to every cell of the body and transports waste products away from the same cells. Among red and white blood cells, platelets are important parts of the blood. They are able to collate and to prevent the blood loss from blood vessel injuries. The formed clots are only preferred within injured blood vessels. The clotting process is specified as dangerous when it happens in the health vessels and is not reversible. It can lead to constipation and block the bloodstream within the vessels. That means that oxygen will not be transferred to all of the body cells. If it is not treated in time it can lead to heart attack, stroke and pulmonary embolism. Nowadays, many efficient synthetic blood thinners, such Aspirin[®] or heparin, are licensed with this indication. Beside their efficiency, they also have many side effects that can cause greater risk than effect. Many researches throughout the years have shown that plants can be the source for the development of such anticoagulants. The advantage of natural alternatives against synthetic drugs is that they cost less, they are mostly safe and can be as effective as the synthetic ones [20].

Tognolini et al. [12] have explored the anti-aggregator activity of EOs containing PPs and phenols on platelets. The EOs were extracted by steam distillation and analyzed with GC/FID and GC/MS. The plasma from guinea pigs was centrifugated in order to obtain platelet rich plasma (PRP). The potency of the EOs was estimated in the platelet aggregation assay and the clot retraction assay and presented in form of IC₅₀ [12].

In the platelet aggregation assay the coagulation agonists ADP, arachidonic acid and U46619 (thromboxan A2 agonist) were added. The tests were performed within 3 hours, in order to avoid the platelet inactivation. The results are presented in Table 2. Anethole from the EO of *Foeniculum vulgare* (L.) Mill, (Apiaceae) possesses the highest activity against arachidonic acid, the pre-step for the further production of thromboxan A2, an important coagulation factor in the intrinsic coagulation. Its activity is comparable to the one of acetylsalicylic acid. On the other hand, it can also suppress the blood coagulation induced by ADP, (adenosin 5-diphosphate) pathway that is responsible for the formation of thrombus by recruiting platelets and leukocytes to the primary layer of collagen adhering platelets. Moreover, anethole has also influence on the coagulation caused by U46619, the thromboxan A2 agonist and on the clot retraction ($IC_{50}=180\mu g/ml$) [12,21]. Clot retraction is the "shrinking" of a blood clot after a number of days, the edges of the blood vessel wall at the point of injury are slowly brought together again to repair the damage [22].

Asarone

Asarone (2, 4, 5-trimethoxy-1-propenylbenzene) is an ether that can be found as α -(*trans*) and β -(*cis*) isomer types in certain plants such as *Acorus calamus* L. (Acoraceae), *A. gramineus* Solander (Acoraceae) and some other [23]. α -Asarone is mainly found in the EO of *A. gramineus* and exerts anticoagulant, anti-inflammatory, antioxidant, anticonvulsant, antitumor and antiviral activity. Recently it was also found that α -asarone protects neurons from amyloid-beta induced neurotoxicity and exhibits an impact on the processes in CNS and immune system. Because of its wide spectrum of effects there are many products, such as capsules, injections and tablets available on the market [24]. In one comparative study the cytotoxicity and genotoxicity of both of the isomers were examined. The observed results have shown that α -

asarone, isolated from plants of the genus *Asarum*, because of its stronger metabolism possesses higher cytotoxicity. The advantage compared to β -asarone is the absence of genotoxicity [25]. β -Asarone (cis-1-propenyl-2,4,5-trimethoxy-benzene) can mainly be found in the EO of *A. tatarinowii* Schott (Acoraceae) and has been traditionally used in China for the treatment of depressions. This indication is based on its capacity to pass easily the blood brain barrier and to induce neurogenesis [26]. Besides its neuroprotective function it has also cytotoxic activity, but lower compared to α -asarone. Unger et al. [25] have found that β -asarone on account of the additional metabolism and the epoxide formation possesses side effects like genotoxicity. This makes them less appealing for further investigations than α -asarone [25].

Anticancer activity

Both pharmacologically active isomers, α -asarone and β -asarone, isolated from the rhizomes of *A. gramineus* had showed minimal cytotoxicity ($IC_{50} < 30 \mu M$) in 4 human tumor cell lines: non-small cell lung adenocarcinoma (A549), ovarian cancer cell (SK-OV-3), skin melanoma (SK-MEL-2) and colon cancer cell (HCT-15) [7].

Anti-inflammatory activity

Asarone is another example of a volatile PP in an EO that is known to possess anti-inflammatory properties. It's mechanism is based on the inhibition of certain enzymes in the arachidonic acid pathway. The extract from *Daucus carota* L. (Apiaceae) seed, that mainly contains asarone, has shown an inhibitory effect on the prostaglandin H endoperoxide synthase-I (46.2%) and prostaglandin H endoperoxide synthase-II (64.4%) at 100 $\mu M/mL$, both enzymes in the arachidonic acid pathway [5].

Anticonvulsant activity

Epilepsy is a chronic neurologic disorder linked with spontaneous occurring seizures that result from changes in the neurotransmitter systems as the glutamatergic, cholinergic and GABA-ergic system. Although many effective drugs are available for its treatment and epilepsy is mostly held under control, seizures remain refractory in 20% of the cases. Herbs have been used since ancient times in traditional medicine to treat convulsions and epilepsy. Recently some aromatic plants, especially their volatile oils have attracted the science's attention and encouraged investigate further their pharmacological activity. Epilepsy attack develops because of the imbalance of glutamate and GABA level [27].

Scientists found the aromatic plant *A. gramineus* especially effective in the treatment of epilepsy. The EO extracted from the rhizomes of this plant, mainly containing α -asarone can block the NMDA receptors on cultured cortical neurons which results with decreased glutamate level. Furthermore, the inhaled EO before seizures has an influence on GABAergic system and can delay the appearance of convulsion. This is because of the α -asarone capacity to inhibit the activity of GABA (gamma aminobutyric acid) transaminase and to increase the level of GABA-neurotransmitter. The EO of *A. tatarinowii* possesses also an anticonvulsive activity. Although it failed to prevent the occurrence of seizures, later it was found that it can delay and prevent the convulsion-related damage of the GABAergic neurons [27].

Asaraldehyde

Asaraldehyde (2, 4, 5-trimethoxybenzaldehyde) is a PP and an active constituent of *A. gramineus* rhizome, often used in Asian countries to improve memory and learning and to induce sedation and analgesia. The most important property of asaraldehyde is the high antifungal activity, especially against the resistant fungi as *Candida* sp. Moreover, this PP can also inhibit COX- II

enzyme and suppress inflammation. It was also reported that asaraldehyde possesses low cytotoxic and antiepileptic activity [7,28]

Cinnamyl alcohol

Cinnamyl alcohol (3-phenylprop-2-en-1-ol) is a volatile PP derived by reduction of CIN. It is identified in storax, Peru balsam and in the leaves of cinnamon and is mainly used as perfume in the cosmetic industry [29].

Cinnamaldehyde

Cinnamaldehyde (3-phenyl-2-propenal) is an aldehyde possessing an α -, β - unsaturated olefinic substituent [30]. The natural product is trans-CIN. It was isolated from cinnamon EO in 1834 by Dumas and Péligot and synthesized in the laboratory by Chiozza in 1854. CIN is an active constituent naturally found in the bark and leaves of cinnamon trees, such as *Cinnamomum cassia* Nees (Lauraceae) and *C. zeylanicum* J.Presl (Lauraceae). The commercially available Cinnamon is a brown powder derived from the inner side of the bark of *C. cassia*. The concentration of CIN in *Cinnamomum* oils differs depending on the plant part from where it is isolated. Anyway, with more than 80% it is the major component of cinnamon bark EO and gives to it the typical flavor and odor. Since ancient times in China, India, Bangladesh, Sri Lanka and Vietnam it has been used to treat cold, influenza, fever and other inflammatory diseases [31,32].

This aldehyde has been investigated for a long time for its biological and pharmacological properties. It has been identified to have antioxidant, antibacterial, anti-inflammatory, hypoglycemic, immune-modulatory, anti-mutagenic and anti-tumorigenic activities [30]. Furthermore, over the years a lot of reports were published describing the main volatile compound of the

cinnamon EO as the one responsible for the anti-diabetic and anti-obesity activity of cinnamon [33]. Due to the rapid absorption, metabolism and excretion, the effect of CIN, its acid and alcohol are species-, gender- and dose-independent [30].

Antibacterial activity

Nowadays the incidence of infectious diseases, especially in the economy undeveloped countries, is much higher and slowly it becomes a prominent global problem. Although the production of anti-bacterial and antifungal drugs, or commonly known as antimicrobials, dates back to 1940 the number of infection diseases constantly increases [34]. The reason is the higher number of antibiotic resistant pathogenic microorganisms. The current higher-levels of antibiotic-resistant bacteria are attributed to the widespread use and misuse of antibiotics [35]. EOs possess promising activities and since decades they are known for their antibacterial properties. This led to increased interest for their usage as natural alternatives and the EOs containing PPs have gained importance [2].

Volatile PPs, so to speak phenolic structures, found in the EOs, exert significant antimicrobial activities against bacteria. Their activity depends on free hydroxylic groups and the substitutions on the aromatic ring. Generally, Gram-negative bacteria can develop resistance to EOs easier than the Gram-positive. The reason is the additional outer membrane over the peptidoglycan layer in the Gram-negative bacteria that render them permeable only for hydrophilic solutes. Gram-positive bacteria consist only of peptidoglycan layer and therefore the penetration of lipophilic molecules is easier. That means that the phenolic compounds of the EOs can easily penetrate and exert an effect on both the cell wall and the cytoplasm [2].

CIN possesses significant antibacterial properties. The mechanisms of action are depending on the used concentration. At very low concentrations it inhibits the enzymes that are involved in the cytokines interaction. At higher

concentrations it inhibits the ATPase and lethal doses of CIN finally can perturb the bacteria-membrane. As eugenol, also this volatile aldehyde can modify the lipid profile of the membrane [2]. Further detailed molecular studies for the development of ligands with a similar structure to the cinnamic-skeleton are of great significance for the solution of the problem of drug-resistant microbial pathogens [34].

Volatile PPs have also shown activity against *Mycoplasma hominis*, a human mycoplasma species responsible for bacterial vaginosis, pelvic inflammatory disease and pyelonephritis. Because of its increased resistance against antibiotics that are directed to inhibit the cell wall synthesis, to find an alternative became a challenge for scientists. The highest effect against *M.hominis* was observed with cinnamon bark oil ($MIC_{90} = MBC_{90} = 500 \mu L/mL$), containing the main compound *trans*-CIN. The antimicrobial mechanism is based on the capacity to bind on proteins and to inhibit amino acid decarboxylases. Furthermore, it was discovered that *C. zeylanicum* extract can exert its antibacterial strong effect also on other pathogenic microorganisms such as *Haemophilus influenza*, *Streptococcus pyogenes*, *S. pneumonia*, *S. aureus* and *E. coli* [35].

Anticancer activity

There are many reports that have shown that CIN can exert antiproliferative effects on various types of cancer cells, especially in human hepatoma cells, the sixth most common neoplasm. CIN is capable of engaging both intrinsic (mitochondria-mediated) and extrinsic (death receptor-mediated) apoptosis. In the apoptotic effect of CIN are involved the mitochondria and the pro-apoptotic molecules of the Bcl-2 family like Bax, Bak, Bid and Bad. CIN induces the accumulation of hepatoma cells in the S-Phase which is associated with increased expression of the pro-apoptotic protein Bax and ROS formation. The cleavage of targets such as Bid and PARP (poly-(ADP-ribose) polymerase) furnishes an increased cytochrome c leakage which leads to

activation of caspase-3 and caspase-8, both initiator-apoptose enzymes, and induces cell death. It is also important to mention that simultaneously treatment with antioxidants, such as vitamin E, can suppress the release of the apoptotic factors and decrease the cytotoxic activity. CIN has also effect on the mitogen-activated protein kinases (MAPKs), which plays an important role in inflammation, cell proliferation, cell differentiation and cell death. The c-Jun N-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs) and p38 belong to this group. The treatment with CIN activates JNK, p38 and ERK kinases and amplifies the cell death. Additionally, CIN mechanism, involved in the apoptose especially of liver cancer cells, is the up-regulation of CD95 protein, a type 1 transmembrane receptor expressed on tumor cells. The death pathway is initiated by linking of CD95 ligand on the receptor and is followed by the formation of death-inducing signaling complex that triggers the activity of different caspases and the production of death substrates. The multi-specific anti-hepatoma effect of CIN should be further explored, because due to the currently findings, it can be a novel strategy for the liver-cancer treatment [36].

Anti-inflammatory activity

The EO of the leaves of *C. cassia* and CIN act in a few ways against inflammatory mechanism and show a significant anti-inflammatory effect as well *in vivo* as *in vitro*. They both inhibit TNF- α , interleukin IL-1 β , IL-6 and ROS in LPS-stimulated cells and monocytes. They also decrease the expression of inflammation enzymes as inducible nitric oxide synthase (responsible for a large amount of NO production), cyclooxygenase-2 and microsomal prostaglandin-E synthase. However, they do not only decrease the amount of inflammatory mediators, but also increase the mRNA expression and the production of anti-inflammatory mediators like IL-10 and transforming growth factor- β . The effect of the EO of *C. cassia* was slightly higher than the one of CIN. If one considers the fact that elevation of NO and iNOS are linked with higher inflammation than he can understand why it is superior compared

to CIN. The potent inhibition is the result of the presence of eugenol and its additional inhibitory effect on NO and iNOS [32].

Anti-obesity and anti-hyperglycemic activity

Nowadays, especially in developed countries, the number of people affected by obesity has reached an alarming rate. Excess weight has a negative impact on the health and can induce many cardiovascular diseases, diabetes and cancer. CIN can exert anti-obesity and anti-hyperglycemic effect and is a significant natural alternative in glycemia and weight management. This volatile phenylpropanoid is an agonist on the TRPA1 (transient receptor potential-ankyrin receptor) that is expressed not only in sensory neurons, but also in the gastrointestinal tract. Its activation can cause modifications in gut motility, gastric emptying, cholecystokinin secretion in small intestine and serotonin release from intestinal enterochromaffin cells. Furthermore, TRPA1 also affects the metabolism as an enhancer of energy expenditure by means of thermoregulation. After administration of CIN, TRPA1 expression will be up-regulated and the anti-obesity effect will be amplified. Last, but not least CIN can decrease the production of ghrelin, an orexigenic hormone that is a potent stimulator of feeding [33]. Camacho et al. [33] have reported that ghrelin and TRPA1 co-localize in the same enterochromaffin cells in duodenum and that CIN via TRPA1 activation can partially decrease the ghrelin secretion. This “hungry” hormone affects not only the food intake, but also the glucose homeostasis process. It can increase the glycogenesis and glucogenolysis and down-regulate the glucose-induced insulin production, which results with higher insulin sensitivity and increased number of insulin receptors. This effect is especially desirable in the treatment of Diabetes Mellitus Typ II. Noteworthy is also the presence of TRPA1 in pancreatic β -cells. *In vitro* was observed basal insulin release by Ca^{2+} influx. *In vivo* this statement was not confirmed [33].

Antiplatelet activity

Tognolini et al. [12] have shown that the EO extracted from *Ocotea quixos* Lam.Kosterm. (Lauraceae), that contains both of the CIN isomers, cis and trans, has an influence on the blood coagulation process, in suppressing the coagulation induced by AA, ADP and U46619. Especially noteworthy is the low inhibitory concentration ($IC_{50}=19\mu\text{g/ml}$) and the high capacity to destabilize clot retraction [12].

Cinnamic acid

Cinnamic acid (3-phenylprop-2-enoic acid) is an aromatic, carboxylic acid (C6-C3) that can be mainly found in green plants covalently bound to the cell walls and in the reproductive organs of flowering plants. Cinnamic acid is formed via shikimate pathway, through L-phenylalanin deamination by phenylalanine ammonia lyase. By the influence of other enzymes different secondary metabolites, such as coumarins, flavonoids, isoflavonoids, PPs are produced from them.

The term “cinnamic” is linked with the different cinnamon species. Besides in *C. zeylanicum*, *C. burmannii* NEES and other *Cinnamomum* species this phenylpropanoid can also be found in coffee beans, tea, cocoa, mate, spinach, potato and tomato. The increased interest of science about this acid led to the development of many medicaments e.g. Ozagrel[®], imidazol para-substituted cinnamic acid, that it is used for the treatment of ischemic acute stroke. Other therapeutically important cinnamic acid-containing molecules are Cinromide[®] and Piplartine[®]. In the different clinical trials and reviews also anti-cancer, antioxidant, anti-inflammatory, antimicrobial, anti-atherogenic and anti-tubercular activities of this volatile phenylpropanoid, have been observed. Despite it's pharmacological activity, cinnamic acid is often used in pharmacophore models in order to modify the solubility, permeability and other parameters for the observed drug [34].

Antibacterial, antitubercular and antifungal activity

The cinnamic skeleton is an important moiety displaying fungal and bacterial growth inhibition. Honey and propolis, both bee products, are being used since ancient times because of their antimicrobial properties. The analysis of their content has shown the presence of cinnamic compounds like cinnamic acid and their esters. The antibacterial activity of cinnamic acid on Gram-positive and Gram-negative bacteria is very weak, and the required MIC values are even higher than 5.0 mM. The effect on the fish pathogens like *Aeromonas hydrophila*, *A. salmonicida* and *Edwardsiella tarda*, with MIC higher than 5.6 mM is comparable to the one on the Gram-positive and Gram-negative bacteria [34].

In 19th century during the tuberculosis epidemic, cinnamon and storax balsam extracted from *Liquidambar orientalis* Mill, (Hamamelidaceae) have been used for its treatment. Later on it was discovered that both of this plants contain cinnamic acid. Further investigations have shown that the presence of free carboxylic acid and the α,β -unsaturation in its chemical structure are the prerequisite for the activity against the tuberculosis-causing bacteria, *Mycobacterium tuberculosis*. Guzman [34] has reported that a MIC from 270 to 675 μM is needed to inhibit the growth of *M. tuberculosis* H₃₇Rv, in accordance to the results of Rastogi et al. [37]. Another important fact is the difference in the activity of both isomers. Comparing the minimum inhibitory concentrations *trans*-cinnamic acid with MIC=2 mM, is 120 times less active than the *cis*-isomer with MIC=16.9 μM [34].

Cinnamic acid possesses besides the antibacterial and antitubercular also antifungal activities. With a MIC value of 405 μM against *C. albicans* a similar effect comparable to the one against *M. tuberculosis* can be obtained. Only low antifungal activities were observed against *A. flavus* and *A. terreus* but a significantly higher effect (MIC values of 844 μM) on *A. Niger*. Recently it was reported that the inhibition of the enzyme responsible for the aromatic

detoxification, namely benzoate-4 hydroxylase, is the one responsible for the antimicrobial activity, however this thesis was declined later, because this enzyme can only be found in the fungi and not in bacteria, and cinnamic acid clearly has proven anti-bacterial effects [34].

Anticancer activity

Cinnamic acid has the capacity to inhibit the proliferation of utero-cervical carcinoma, leukemia, colon adenocarcinoma, glioblastoma, melanoma, prostate, lung carcinoma, osteogenic sarcoma cells, May Coy cells, Hep G2 cells and kidney epithelial (VERO) cells. Cinnamic acid has an influence on the cell cycle, it causes shortened G₂-M period, lengthened cell cycle and inhibits the cell proliferation in utero-cervical carcinoma (U14). In the human colon cells (Caco2) this acid modulates the cell phenotype by stimulating sucrose and aminopeptidase N activity, while inhibiting alkaline phosphatase activity. Cinnamic acid can also reduce the invading capacity of melanoma cells and modulate the expression of genes included in the tumor metastasis, such as collagenase typ IV and tissue inhibitor metalloproteinase 2. It results with cell differentiation followed by morphological changes and increased melanin production [7].

Anti-inflammatory activity

Another cinnamic molecule with a significant anti-inflammatory activity is cinnamic acid. This volatile phenylpropanoid can be found in the EO from the rhizome of *Panax Ginseng* C.A. Meyer (Apiaceae), a very popular plant in Asian countries. Recent studies have shown that cinnamic acid is responsible for the anti-inflammatory and neuroprotective activity of its EO. Cinnamic acid exerts its activity by inhibiting the O₂ generating response, caused by noxious stimuli. There is an assumption that this effect is based on its ability to reduce the calcium mobilization, a consequence of the activation

of the immune-responsive cells. This causes further activation of the kinase cascade that lead to cell proliferation [5].

Coumarin

Its chemical structure (2H-1-benzopyran-2-one) (less commonly known as inner ester of o-hydroxy cinnamic acid) possesses a system of a fused benzene with a α -pyrone ring. Coumarins are a large class of phenolic substances, actually more than 1300 that can be found in plants, bacteria and fungi. Originally they were found in the tonka bean (*Dipteryx odorata* Wild, Fabaceae). Today coumarins are identified in about 150 different species distributed over nearly 30 different families. The most common ones are Apiaceae, Clusiaceae, Caprifoliaceae, Guttiferae, Nyctaginaceae, Oleaceae, Rutaceae and Umbelliferae. Coumarins can be found in all parts of the plants, but in fruits at the highest level. Bael fruits (*Aegle marmelos* L. Corrêa, Rutaceae), *Tetrapleura tetraptera* Schum. & Thonn.Taub., (Fabaceae) than in tonka beans *D. odorata* and *C. inophyllum* L., (Clusiaceae) followed by the roots (*Ferulago campestris*, Apiaceae), leaves *Murraya paniculata* (L.)Jack, (Rutaceae), *Phellodendron amurense* Rupr., (Rutaceae) and latex of the tropical rainforest tree *C. teysmannii* var. *inophylloide* L., (Calophyllaceae), green tea and other foods such as chicory. Interestingly, coumarins are also parts of cassia oil, cinnamon bark oil and lavender oil. Their amount can vary and it depends on the environmental conditions and seasonal changes. Nowadays large number of coumarins has been identified. This led to their classification in six types, due to the different chemical structures: Simple coumarins, furano coumarins, dihydro furano coumarins, phenyl coumarins and bi-coumarins. Each group shows biological properties, depending on the substitution pattern. That explains the antiplatelet activity of bi-coumarins and the antibacterial and antitubercular activity of dihydro furano coumarins. The wide pharmacological spectrum of coumarins has an essential impact on

human pathophysiological processes. Their most common pharmacological activities are anti-inflammatory, anticoagulant, anticancer, anti-hypertensive, anti-hyperglycemic and antioxidant effects. Other important biological properties of coumarins are antibacterial, antifungal, antiviral, anti-tubercular and anti-convulsant activities. Methoxalen from the seeds of *Ammi majus* L. (Umbelliferaceae) has an inhibiting effect on Cytochrom P450, which is responsible for the metabolism of drugs. Even single doses can inhibit CYP 2A6 activity [38].

Antibacterial activity

Coumarins show low antibacterial activities. But the one with a long chain hydrocarbon substitution, such as ammosesinol, shows a good effect on Gram-positive bacteria, such as *Bacillus megaterium*, *Micrococcus luteus*, *M. lysodeikticus* and *S. aureus* [38].

Anticancer activity

Grandvittin, agasyllin, aegelinol benzoate and osthol from *Ferulago campestris* L. (Apiaceae) can exert a cytotoxic effect against the A549 lung cancer cell line. Osthol is an effective coumarin for the cancer treatment, because of its capacity to inhibit matrix metalloproteinase-s promoter and enzyme activity, thus suppresses the migration and invasion of breast cancer cells. Esculetin despite its antioxidant activity can also exert a very low and insignificant cytotoxic effect. Chartreusin can suppress the proliferation of B16 melanoma, P388 leukemia and murine L1210 cell lines. Interestingly, also the coumarins extracted from cassia leaf oil have shown certain anticancer activity [38].

Anticonvulsant activity

Esculetin (6,7-dihydroxy-coumarin) because of the influence on the GABAergic neurons decreases the seizure response. It can increase the GABA-

level and exert an anticonvulsant effect, a sedation and myorelaxation. Imperatorin (ED_{50} =167-290 mg/kg) and osthole (ED_{50} =253-639 mg/kg) are another coumarins with anticonvulsant activity, and they reveal effect comparable to the one of valproate (a synthetic drug used for epilepsy treatment). These two coumarins are promising agents against seizures. A comparable study has revealed that actually the coumarins with C-8 substituted psoralene ring (imperatorin) exert a stronger effect than the ones that have a substituted chain on C-5 position as bergapten [38,39].

Anti-hyperglycemic activity

Diabetes mellitus is a chronic disease related with high level of blood glucose and can potentially cause cardiovascular morbidity and nerve damage. Thus, the maintaining of blood glucose level is of great significance for the treatment. Coumarin derivatives containing pyrazoline have been found to exhibit antidiabetic activities. 3-(4-((Z)-(5,6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl)-1-p-tolyl-1H-pyrazol-3-yl)-2H-chromen-2-one and 3-(4-((Z)-(5,6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl)-1-(4-methoxy phenyl)-1H-pyrazol-3-yl)-2H-chromen-2-one have shown the greatest activity. Furthermore, fraxidin by inhibiting the formation of inducible nitric oxide synthase can down-regulate the blood glucose level [38,40]

Anti-inflammatory activity

Coumarin's anti-inflammatory properties are mainly used for the treatment of edema. They stimulate proteolysis, enzyme production and phagocytosis and on this way they remove protein and edema fluid from injured tissues. Imperatorin and esculetin are examples of coumarins used with this indication. Imperatorin has an impact on the arachidonic acid pathway and inhibits the cyclooxygenase-2 and inducible nitric oxide synthase (iNOS), which are responsible for the production of prostaglandins and NO. Esculetin compared to imperatorin blocks not only COX, but also LOX enzymes [38].

Antioxidant activity

The intracellular production of radicals is associated with activation of the enzyme xanthine oxidase (XO), able to convert the molecular oxygen in superoxide. Esculetin and its derivate 4-methylesculetin can inhibit the XO and suppress the free radical production. The hydroxyl group in esculetin binds to XO enzymes and inhibits the undesirable conversion. Furthermore, fraxin extracted from *Weigela florida* BUNGE A.DC. (Caprifoliaceae), can protect the cells against the H₂O₂ induced oxidative stress and exert a radical scavenging effect at high concentration (0.5 mM). Grandvittin, agasyllin, aegelinol benzoate and osthol are other examples for coumarins with antioxidant properties. 3, 7-dihydroxy coumarin derivatives have shown antioxidant activity, comparable to the one of Vitamine C [38,41].

Elemicin

Elemicin (5-allyl-1,2,3-trimethoxybenzene) is another volatile phenylpropanoid that can be found together with myristicin in the EO of nutmeg, *Myristica fragrans* Houtt (Myristicaceae). It is believed that actually myristicin is the compound responsible for the hallucinogenic effect of the nutmeg. It was also identified in the methanolic extract of the roots of *Asiasarum sieboldii* L. (Aristolochiaceae) [5].

Anti-inflammatory activity

Elemicin has the capacity to inhibit the lipoxygenase-5 induced fatty acid conversion in leukotrienes and thus to suppress the inflammation response. This inflammatory cytokine is also responsible for the pathological symptoms in asthma. Elemicin's capacity to down-regulate the leukotrien level makes it a promising natural alternative for the treatment of this respiratory disease [5].

Estragole

Estragole (1-allyl-4-methoxybenzene) is an isomer derived from anethole and belongs to the volatile PPs too. It is a chemical constituent of the EOs of many aromatic plants as *Artemisia dracunculus* L. (Asteraceae), *Leonotis ocymifolia* (PERS.) R.Br. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), *Croton zehntneri* Pax & K.Hoffm. (Euphorbiaceae), *Pimpinella anisum* L. (Apiaceae), *Illicium anisatum* Hook F. (Schisandraceae) and *Foeniculum vulgare* Mill. (Apiaceae). *In vivo* and *in vitro* experimental assays have shown that estragol affects CNS and through reducing the neuronal excitability exerts sedative and anticonvulsant activities. As already mentioned estragol is derived from anethole, for which important similarities in their activities has been described. They both are involved in inflammation and immunological processes, including the inhibition of all effects of TNF α . Furthermore, an anesthetic, antiplatelet, antioxidant and antimicrobial activity has been observed. Because of its intensive scent it can be used in perfumes, alcoholic beverages and tea preparations [7,42-44]

Antiplatelet activity

Estragol, with 70.1%, the main compound of *A. dracunculus*, is mainly responsible for the antiplatelet activity of its EO. Estragol, because of the phenyl core linked to a propenyl chain, shows structural similarity and correlation to eugenol, myristicin and elemicin which also possess an antiplatelet activity. These results are suggesting that the common activity is ascribed to the presence of the phenylpropan moiety. Estragol compared to the above mentioned volatile PPs, has significantly higher activity against the AA, ADP and U46610 induced coagulation. On the other hand, estragol can suppress the blood coagulation by inhibiting the clot retraction. In the clot retraction assay of the EO extracted from *A. dracunculus* L., was observed a significant effect (IC₅₀=126 μ g/mL), anyway lower than the one with CIN [12].

Eugenol

Eugenol (4-allyl-2-methoxyphenol) is a volatile phenylpropanoid mainly found in the EOs from *Pimenta racemosa* Mill. J.W.Moore (Myrtaceae), *Cinnamomum Verum* J.Pressel (Lauraceae) and *Syzygium aromaticum* L.Merr. & L.M.Perry (Myrtaceae) [45]. Cloves are the flower buds of *S. aromaticum* L.Merr. & L.M.Perry, also known as *Eugenia caryophyllata* L. and *E. aromatica* L. They are collected in the months of February and October, then dried carefully and after separation from their peduncles are ready to use. Cloves are not only used in perfumery, cosmetics and as food flavors in cooking, but also in traditional medicine for the treatment of asthma, various allergic disorders and toothache. The wide usage of cloves since ancient times in Australia and Asian countries made it a big target in the pharmaceutical industries. Pharmacologically important is the light yellow or colorless fluid extract from the buds. The different biological activities are attributed to eugenol, the major compound of the clove EO (>70%). It was reported that eugenol shows antimycotic, antibacterial, antifungal and insecticidal effects. At low concentrations also remarkable anti-inflammatory and antioxidant activities were observed, whereas in higher concentrations it can induce apoptosis of human cancer cells. Furthermore, the eugenol extracted from *S. aromaticum* can also induce the S-transferase enzyme that is responsible for the detoxification in intestines and liver. Recently it was put emphasis on its antiviral properties against *Herpes Simplex* and *Hepatitis C Virus* and the anticonvulsive effect in tonic seizures [46]. Interestingly, Mishra and Singh [47] have shown that different doses of eugenol have diverse effects on the testosterone level. While higher doses (30 and 60mg/kg) led to reduction the testosterone production, lower doses (15mg/kg) increased the testosterone level [47]. Singh et al. [46] also reported that there are few side effects that should not be neglected during the treatment with clove oil. If ingested or injected in large amounts it can cause Acute Respiratory Distress Syndrome, Fulminant Hepatic Failure and CNS-disorder. The lethal oral dose of clove has

been reported to be 3.752 g/Kg body weight [46]. Nowadays, the antimicrobial, anti-inflammatory, analgesic and antifungal activity of eugenol made clove oil a promising natural alternative for the treatment of oral cavity in the dental medicine [48].

Antibacterial, larvicidal and antifungal activity

Eugenol is another volatile phenylpropanoid with a distinct antimicrobial activity. Its mechanism is based on the ability to modify the fatty acid profile of different bacteria, to alter the membrane and to influence the transport of ions and ATP [2]. Rodriguez et al. [48] have observed the effect of eugenol-consisting EO on *S. mutans*, a bacterium which is responsible for the dental caries. The EO was obtained by hydrodistillation from the flower buds of *S. aromaticum* and analyzed by thin layer chromatography. Eugenol was determined as the active and major compound (67.5%) of the clove oil by mean of identification via Wiley 7N.1. database. In order to estimate the clove oil efficiency the MIC (minimal inhibitory concentration) and MBC (minimum bactericidal concentrations) were determined. MBC is defined as the lowest concentration of the oil necessary to kill 99% of the initial inoculums after 24 hours of incubation. The MIC and MBC were 125 and 250 $\mu\text{L/mL}$, respectively [48]. Moreover, Rodriguez et al. [48] also reported an effect of eugenol against *C. albicans*, *S. aureus* and *Actinomyces viscosus* [48].

Medeiros et al. [49] have reported about the larvicidal activity of eugenol against *Aedes aegypti*, the cause for dengue fever, an infectious disease that causes very high fever and can even have an impact on the CNS. It is especially present in the Amazon region and can be transmitted by mosquitoes of the *Aedes* genus. The results have shown that eugenol, compared to the aqueous and methanolic extracts from clove, *Eugenia caryophyllata* Thunberg, attained with $\text{LC}_{50}=3.6$ mg/ml the highest larvicidal effect on *A. aegypti* larvae at 48h [49].

Over the last two decades not only bacteria, but also fungi have been classified as major cause of human infections. Because of the enormous impact on mortality and morbidity the interest for the antifungal properties of volatile PPs increased [45]. Not only eugenol, but also its derivatives have shown high antifungal activity against *C. albicans*, non-*albicans* spp., *Cryptococcus neoformans* and dermatophytes. The eugenol derivative 4-allyl-2-methoxy-5-nitrophenol was the one with the highest activity against these strains. By means of the Ergosterol Assay, a test that detects the binding of derivatives to ergosterol of the fungal membrane, it was discovered that this compound is not effective in this manner. No changes on cell were identified in the presence of different concentrations (50 μ L/mL-250 μ L/mL). On the other hand, with the Cellular Leakage Assay it has been proven that this eugenol derivative produces fungal membrane damage and causes a release of intracellular components. The amount of these compounds is estimated by measuring the absorption at 260 nm. The higher absorption indicates that the amount of released intracellular compounds has increased. The results showed 22% fungal membrane damage at 1xMIC concentrations and 71% at 4xMIC. As a conclusion Carrasco et al. [45] reported that the antifungal effect has been attained by disruption of the fungal membrane and not by binding on membrane's ergosterol [45].

Anticancer activity

Eugenol has a promising antitumor effect. It can induce cell deaths in many tumor and cell types including mast cells, breast adenocarcinoma, melanoma cells, leukemia, colon carcinoma, cervical carcinoma, prostate cancer, submandibular gland adenoma carcinoma, human dental pulp cells, human gingival fibroblasts, and epidermoid carcinoma cells derived from human submandibular glands. Eugenol decreases the expression of anti-apoptotic genes for Bcl-2, COX-2, IL-1b and reduces the secretion of pro-inflammatory cytokines. It can also up-regulate the expression of pro-apoptotic

genes like Bax, p53 and active caspase-3 in the cell lines and induce tumor growth delay. Moreover, eugenol's capacity to hold the cells in the replication phase (S-phase) and to inhibit the repair of DNA damage or to activate apoptosis in cases of massive DNA damage is mainly responsible for its cytotoxic effect. It is important to mention that its antioxidant effect amplifies the antiproliferative activity, but the dose plays an important role. It is interesting to know the fact that eugenol in lower concentrations (5-10 μ M) induces the ROS production. On the other hand, in higher concentrations (500 μ M) it inhibits the ROS production [7].

Anti-inflammatory activity

A lot of investigations have been performed in order to determine the anti-inflammatory mechanism of eugenol. Previous studies have revealed that eugenol exerts an inhibitory effect on thromboxan A₂, cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), all parts of the arachidonic acid pathway. As a consequence the production of the inflammatory mediators leukotriene and prostaglandin will decrease. Furthermore, it was discovered that PAF, the phospholipid factor responsible for the platelet aggregation, is not only a thrombosis but also an inflammatory mediator. It can induce additionally arachidonic acid liberation that is further metabolized by COX and LOX. This leads to an increased level of thromboxane A₂ and prostaglandins. *In vitro* studies have shown that eugenol in dose-dependent manner inhibits the metabolism of arachidonic acid by COX and LOX pathway and can also suppress the PAF and AA-platelet aggregation. The high anti-inflammatory effect of eugenol was also reported in a *in vivo* study. The pre-treatment with 25, 50 and 100mg/kg of this compound has significantly reduced the carrageenan induced paw edema dose-dependently. The results were compared to Aspirin[®]. Interestingly eugenol has shown even higher effect than this synthetic drug. Moreover, eugenol has also the ability to decrease the production of pro-inflammatory cytokines such as TNF-alpha and IL-6 that are

being produced in non-parenchyma cells. This effect can be used for the treatment of different liver injuries. Last but not least, the decreasing effect of eugenol on the leukotriene level can be used for the treatment of other important and common inflammatory diseases as asthma, allergic rhinitis and rheumatoid arthritis [5].

Antiplatelet activity

The EO of *O. basilicum* can inhibit the blood coagulation. Its activity is based on the presence of eugenol and its capacity to decrease the liberation of COX-enzyme in the arachidonic acid pathway. Consequently, thromboxan-A2 level, mediator responsible for the platelet aggregation, will decrease. Moreover, eugenol influences the PAF activity that beside inflammation and proliferation can also stimulate the blood coagulation by inducing the AA liberation. In the platelet aggregation assay, the pharmacological activity of eugenol was confirmed. The aggregation induced by AA and the thromboxan A2 agonist was inhibited. As expected no effect was observed on the ADP-induced platelet aggregation and in the clot retraction assay [5,12].

Hydroxychavicol

Hydroxychavicol (4-prop-2enylbenzene-1,2-diol) is found in high concentrations in *Piper betle* L. (Piperaceae), which leaves are used for the production of betel quid. Antimicrobial, antioxidant, anti-inflammatory, cytotoxic and anti-platelet activities are attributed to hydroxychavicol [50,51].

Antibacterial activity

Hydroxychavicol (HC) that is isolated from the chloroform extraction of aqueous extract of *P. betle* leaves has shown significant antimicrobial activity against the oral cavity pathogens. The exerted activity is equally

effective on Gram-negative anaerobic periodontal pathogens, Gram-positive carcinogenic bacteria and noncarcinogenic early-colonizer bacteria [50]. Sharma et al. [50] have reported that HC can exert bactericidal activity against *Streptococcus mutans*, *Enterococcus faecium*, *E. faecalis*, *S. sanguis*, *Actinomyces viscosus*, *Haemophilus. actinomycetemcomitans*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. The antimicrobial effect of HC is presented in form of MIC and MBC. The MIC range is between 62.5 and 500 µg/mL and MBC was found to be twofold greater than the inhibitory concentration. Another important finding in this study is the HC effect on bacteria protected with biofilm, as *S. mutans* ATCC 25175 and *A. viscosus* ATCC 15987. Despite the inhibition of the biofilm formation, HC can also reduce the pre-formed biofilm. It is assumed that this phenylpropanoid probably works through the disruption of the permeability barrier of microbial membrane structures [50].

Anticancer activity

Hydroxychavicol has an impact on the mitochondria and induces its cytotoxic effect by means of changes in the membrane potential at an early stage and lipid peroxidation at a later stage. The onset of cytotoxicity depends on the initial and residual concentrations of HC [7].

2’Hydroxy-cinnamaldehyde

2’Hydroxy-cinnamaldehyde [3-(2-hydroxyphenyl)-2-propenal] is derived from CIN. An *in vivo* immune-modulatory activity is being observed [7].

Isoeugenol

Isoeugenol (4-propenyl-2-methoxyphenol) is a volatile phenylpropanoid derived by isomerisation from eugenol. It occurs in two isomers, namely trans-isoeugenol and cis-isoeugenol. Because of the different structure, they also differ in the aggregate condition, the trans-isomer is crystalline and the cis-isoeugenol is liquid. Mainly this phenylpropanoid can be found in Ylang-Ylang (*Cananga odorata* Lam. Hook.f. & Thomson (Annonaceae)) and in lower amounts in clove, nutmeg and parsley [52]. It can be used as a flavoring agent in baked food, chewing gums and non-alcoholic beverages. However, it should be used carefully, because a certain carcinogenic activity was observed, especially in the male rats. Clearly it was shown that isoeugenol causes rarely occurring mammary and thymoma gland carcinoma. Furthermore, the increased risk for hepatic adenoma and carcinoma is also attributed to isoeugenol. On the other hand, its carcinogenic activity in the female rats is less aggressive. Its usage has only led to increased risk of histiocytic sarcoma [7,52].

Methyleugenol

Methyleugenol (4-allyl-1,2-dimethoxybenzene) is a volatile phenylpropanoid in the EOs from flowers, stems and roots in over 450 plant species. In the EOs of several species, such as *C. cordatum* Kosterm. (Lauraceae) and *Croton malambo* H.Karst. (Euphorbiaceae) can be found more than 90% methyleugenol. However, the content can vary depending on the plant tissue and the harvest time. Methyleugenol is derived from eugenol. It is the precursor for the synthesis of elemicin and myristicin. Methyleugenol has also influence on human health especially in the treatment of cancer, inflammation and cerebral ischemic injury. On the other hand, because of its mutagenic and carcinogenic properties it can have diverse side effects. Tan et al. [53] have shown that orally administrated high doses of methyleugenol can cause hepatic neoplasm [53].

Antibacterial and antifungal activity

The effect of various concentrations of the EO from the leaves of *Pelargonium odoratissimum* L. (Geraniaceae) has been examined. Methyleugenol is the main compound in the EO. It makes about 96.8% of the volatile fraction. The targets were *Aspergillus Flavus* CML 1816, *A. carbonarius* 1815 and *A. parasiticus* CMLA 817 fungi and *Escherichia coli* ATCC 25 992 and *Staphylococcus aureus* ATCC 25923 bacteria. The EO was isolated by steam distillation and analyzed by means of GC/MS and GC/FID (flame ionization detector). This volatile phenylpropanoid is the one responsible for the antifungal and antimicrobial activity of the EO. The results from the study of Andrade et al. [54] are listed in Table 1. In a dose-dependent manner a significant inhibition of the mycelial growth was observed for the three toxigenic fungi *A. Flavus*, *A. parasiticus* and *A. carbonarius*. On the 7th day of the treatment, 100% inhibition with the 0.5 µl/ml concentration was observed, only *A.flavus* showed a small growth. Thus the EO showed significant antifungal properties. On the other hand, methyleugenol has shown very low antibacterial activity against the bacteria *E. coli* and *S. aureus*. The EO from the leaves of *P. odoratissimum* acted inhibitory on the growth of *E. coli* at a concentration of 100 µL/mL. And the greatest effect was noticed at 300 µL/mL. This EO exerts even a lower effect on *S. aureus*, certain inhibition was observed on concentrations > 200 µL/mL and at a concentration of 500 µL/mL the greatest inhibition occurred. To sum up, methyleugenol possesses significant antifungal properties on the fungi of the *Aspergillus* species and low antibacterial activity against the bacteria *E. coli* and *S. aureus* [54].

Mycelial growth (cm)				Inhibition halo (cm)	
Concentration of the EO (µL/mL)	<i>A.flavus</i>	<i>A.carbonarius</i>	<i>A.parasiticus</i>	<i>S.aureus</i>	<i>E.coli</i>
0.10	3.90	1.80	2.50	-	-
0.25	2.15	1.10	1.65	-	-
0.50	0.30	0.00	0.00	-	-
1.00	0.00	0.00	0.00	-	-
2.00	0.00	0.00	0.00	-	-
50	-	-	-	0.00	0.00
100	-	-	-	0.00	0.65
200	-	-	-	0.50	0.75
300	-	-	-	0.50	0.85
400	-	-	-	0.60	0.90
500	-	-	-	0.80	0.95

Tabelle 1. Mycelial growth of *A. flavus*, *A. carbonarius*, *A. parasiticus* fungi and *S. aureus* and *E. coli* bacteria in the presence of different concentrations of the *P. odoratissimum* EO (adapted from Andrade et al. [54] and newly drawn).

Anticancer activity

The structural similarity of this substance to eugenol has been used as an advantage in further pharmacological studies, in order to explore its therapeutic potential in cancer treatment. It was found that methyleugenol produces cytotoxic effects in rat and mouse hepatocytes and leukemia [7].

Anti-inflammatory activity

The eugenol-similar structure, methyleugenol, can also exert anti-inflammatory activity. Besides its antioxidant properties methyleugenol decreases the level of the pro-inflammatory cytokines (IL-1beta, IL-6 TNF-

alpha) and inhibits the iNOS-enzyme. Additionally, this phenylpropanoid has also an impact on the gene expression of anti-inflammatory cytokines IL-10 and TGF-beta. All these activities rendered methyleugenol to be a very important part of the cerebral Ischemia treatment [5].

Antiplatelet activity

The volatile methyleugenol present in the EO of *O. basilicum* can exert only a low and practically insignificant antiplatelet activity against the AA, ADP and U46619 induced platelet coagulation. Furthermore, it can decrease clot retraction by showing IC₅₀ of 220 µg/mL [12].

Methylisoeugenol

Methylisoeugenol (1,2-dimethoxy-4-propenylbenzene) is a natural occurring food flavor. It can be found as mixture of cis- and trans-isomers in the EOs of *Asarum arifolium* L. (Aristolochiaceae), *Cymbopogon javanensis* Spreng. (Poaceae) and others. It is the major compound in the EO (93.7%) from *Pimenta pseudocaryophyllus* Lindl. (Myrtaceae) leaf. Since ancient time these leaves have been used because of their calming properties. Nowadays the anxiolytic and antidepressant effects have been determined and are used for the treatment of mood disorders. Besides its effect on the CNS, methylisoeugenol even as food flavor can exert hypotensive and vasorelaxant activities and can economize the heart function [55-57].

Hypotensive and vasorelaxant activity

Methylisoeugenol can affect the cardiovascular system and induce hypotensive activity. Many heart parameters are influenced by the i.v. MIE administration. Significant dose-dependent (1.11, 2.25 or 4.50 mg/kg) decrease in the mean arterial pressure (-16.9, -19.0 or -27.2 mm Hg, respectively) was observed. Furthermore, the heart rate has also shown a dose-dependent increase

(17.4, 24.4 or 29.9 bmp, respectively). The oral administration of MIE (25 or 50 mg/kg) can reduce the systolic blood pressure (-13.6 or -16.6 mm Hg, respectively) without causing any alterations in the baroreflex sensitivity. The hypotensive and vasorelaxant activity of MIE are attributed to its influence on the calcium channels [55].

Anxiolytic and antidepressant activity

Mood disorders, a common psychiatric disease with life-time prevalence, are worldwide a great problem. The EO extracted from the leaves of *P. pseudocaryophyllus* Lindl. (Myrtaceae), consisting mainly of MIE (93.7%) has been reported to possess an anticonvulsant, anxiolytic and antidepressant activity. Behavioral alterations in the MIE pharmacological activity are dependent from the dose and the route of administration. There are no pharmacologically data regarding the anxiolytic and antidepressant activity of MIE, but the findings suggested the participation of serotonergic pathway [57].

Myristicin

Myristicin (1-allyl-3,4-methylenedioxy-5-methoxybenzene) is a volatile phenylpropanoid found in parsley, carrot, basil, cinnamon and nutmeg (*M. fragrans*). It has been widely used in the traditional medicine for the treatment of anxiety, diarrhea and stomachaches. Besides its carminative effect, an antibacterial, anti-inflammatory, anticancer and hepatoprotective activity of myristicin has also been observed [5].

Anticancer and hepatoprotective activity

Myristicin can cause cleavage of PARP, which is accompanied by accumulation of cytochrome c and activation of caspase-3. This apoptotic mechanism of myristicin can induce cytotoxicity in human neuroblastoma SK-

N-SH cells. Myristicin also increases the activity of glutathione S-transferase and NADPH quinone oxidoreductase, enzymes important for the detoxification in liver. Thus, myristicin was observed to show strong potential as an effective chemoprotective agent against cancer [7].

Anti-inflammatory activity

Myristicin possesses the capacity to decrease the intracellular calcium concentration. It is believed that this effect actually suppresses the liberation of the different inflammatory mediators, such as NO, interferon inducible protein-10, IL-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), MCP-3, macrophage inflammatory protein-1- α (MIP), MIP-1- β and leukemia inhibitory factor. This effect was observed in *in vitro* studies with three different myristicin concentrations (10, 25 and 50 μ M). The suppressive activity of myristicin on the production of inflammatory mediators like IL-6, LIF and IL-10 can have positive effects on the progression of the auto-immune diseases, such as encephalomyelitis, Crohn's disease and rheumatoid arthritis. The NO-decreasing effect of myristicin is important for the treatment of pathogenic-infections. These infections are associated with inflammations caused by an increased NO level [5].

Safrole

Safrole (4-allyl-1,2-dimethoxybenzene(methyleugenol)) is a volatile phenylpropanoid found in the EO of sassafras, basil, anise, nutmeg and pepper. It is a well known mutagenic and cancerogenic agent. It has attracted attention for further studies because it can induce cancerogenesis and on the other hand it shows also a certain anticancer activity. Furthermore, safrole isolated from *P. aduncum* L. (Piperaceae) possesses anti-inflammatory and analgesic effects [5,7].

Anticancer activity

In Taiwan, Sri Lanka, India, Indonesia and Vietnam a lot of betel quid is being consumed. Betel quid or Paan consists of leaves from pepper (*Piper betel*, Piperaceae) chewed together in a wrapped package along with the areca nut, which, by association, is often inaccurately called the "betel nut" and mineral slaked lime (calcium hydroxide). The increased prevalence of oral squamous cell carcinoma and oral submucous fibrosis in these Asian countries is assumed by dentists to be related to the consumption of high amounts of betel. A study carried out in Taiwan has been discovered that safrole in high concentration found in *P. betel* is the one responsible for the cancer development. A high concentration of near 0.5 mM safrole was found in the saliva of people consuming this stimulant on a daily basis [58]. On the other hand, safrole shows an antiproliferative effect on human tongue squamous carcinoma, primary human buccal mucosal fibroblasts, prostate cancer rat hepatocytes and leukemia. Its main effect is to increase the apoptotic factors Bid and Bax and to decrease the expression of Bcl-2 which leads to a higher ratio of Bax/Bcl-2, further to released cytochrome c, increased Apaf-1 levels and in the end activation of caspase-3 and caspase-9. Safrole shows a different mechanism in different cancer cells. The influence on the human tongue squamous SSC-4 cancer cells is mediated by a mitochondria- and caspase dependent signal pathway. In A549 lung cancer cells, safrole activates caspase-3, 8 and 9. Furthermore, in rat hepatocytes cell, the induced cell death is realised by the loss of mitochondrial membrane potential and the generation of oxygen radical species. Then the apoptotic mechanism in primary human buccal mucosal fibroblasts (BMFs) is the increased NF- κ B expression in fibroblasts, mediated by ERK activation and COX-2 signal transduction pathway. Furthermore, safrole causes increased release of Ca^{2+} from the endoplasmic reticulum which decreased cell viability. This is how it induces death in PC3 prostate cancer cells. SAFO (Safrole-2',3'-oxide) is derived from

safrole and is the most mutagenic metabolite. It can induce cytotoxicity and DNA strand breakage [7].

Antiplatelet activity

Tognolini et al. [12] have shown that safrole, present in the EO of *P. crasinervium* can affect the blood coagulation by inhibiting the activity of AA and ADP, biological active constituents that trigger the platelet aggregation [12].

Trans-Anethole oxide and Trans-Asarone oxide

trans-Anethole oxide (2-(4-methoxyphenyl)-3-methyl-oxirane) and trans-Asarone oxide (1-propenyl-2,4,5-(trimethoxybenzene)) are derived from trans-asarone and trans-anethole, respectively via dimethyldioxirane. These alkenyl benzene derivatives are used as natural fragrance and flavoring chemicals [59]. Kim et al. [59], have shown that mutagenic and carcinogenic activities are linked to this phenylpropanoid. Until now no significant pharmacologically effect is being reported [59].

Plant	Volatile Phenylpropanoid	%	Attack point			
			AA	ADP	U46619	Clot retraction
<i>Anthemis nobilis</i>	Methyleugenol	1.18	•	•	•	
<i>Artemisia dranunculus</i>	Estragol	70.12	•	•	•	•
<i>Foeniculum vulgare</i>	Anethole	75.83	•	•	•	•
<i>Ocimum basilicum</i>	Eugenol	12.32	•		•	•
<i>Ocotea quixos</i>	cis-CIN	0.39	•	•	•	•
	trans-CIN	27.81	•	•	•	•
<i>Piper crasinervium</i>	Safrole	2.59	•	•		

Table 2: Percent composition of PPs in the EO of *A. nobilis*, *A.dranunculus*, *F. vulgare*, *O.basilicum*, *O.quixos* and *P. crasinervium* and their activity in the Platelet Aggregation Assay (against AA, ADP and U46619-induced aggregation) and in the Clot Retraction Assay (adapted from Tognolini et al. [12] and newly drawn).

Conclusion

From ancient time plants have been exploited as resources in traditional societies, whereas they have been used as food and medicine. The potential health benefits have evoked scientist's interest for further investigations of their pharmacologically activities. In the last decades the number of natural products introduced into the market has reached more than 50%, which is a proof for their significant positive health effects.

PPs, a great group of plant secondary metabolites because of their wide presence in the traditional medicine, have fallen into the focus of nowadays researches. The aim of this work was to review the biological activities of a special group of PPs, namely the volatile ones, isolated from EOs. It is important to mention that the number of volatile PPs among all bioactive PPs is very large and there is no pharmacologically explanation for all of their activities. There are many PPs that show activities comparable to the ones of synthetic drugs. Trans-anethole, the main constituent in the EO of anise, star anise and sweet anise exerts an anti-inflammatory effect almost similar as seen with ketoprofen. Furthermore, this same PP has shown anti-platelet activity comparable to the one of acetylic salicylic acid. The activities of the EO and the separately isolated PPs have been tested. Compared to the variety of biological activities that are typical to the phenolic-containing-EOs, the single volatile PPs have shown prevailing antimicrobial, anti-inflammatory and cytotoxic activities. To most of the above listed PPs one or even more pharmacologically activities are attributed. E.g., cinnamic acid and cinnamaldehyde isolated from the plants of the *Cinammomum* species, such as *C.zeylanicum*, have shown similar activities, The cinnamic skeleton is an important moiety displaying anti-inflammatory, cytotoxic activity and fungal and bacterial growth inhibition. Because of their synergistic effects the EO extracted from *C. zeylanicum* can exert a greater effect, than the PPs

separately. Furthermore, for eugenol and its derivative methyleugenol, has been observed similar anti-inflammatory, cytotoxic, antimicrobial and antifungal activities. In addition, elemicin and myristicin, both products of methyleugenol have also shown the same pharmacological effects. It is important to mention that the anti-hyperglycemic activity of CIN and coumarins are of great significance as a novel therapy for the treatment of Diabetes Mellitus Typ II. Moreover, the PPs estragol, coumarins and methylisoeugenol possess neuroprotective properties and can also be of great significance for the treatment of epilepsy and mood disorders. Despite their influence on the CNS, they have also come up with a cardio protective function. Methylisoeugenol can dilate the blood vessels and exert vasorelaxant and hypotensive effects. Coumarins, by means of their antihyperglycemic and anti-hypertensive activities can also decelerate the progression of cardiovascular diseases. Anyway, not all of the isolated phenylpropanoids can exert pharmacological effects. Cinnamyl alcohol although derived from CIN, which is one of the most effective PPs, does not possess any considerable pharmacological activities.

Because of the wide presence of PPs in nature they play an important role and contribute to a variety of biological activities of these EOs.

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