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Abstract

Phase I metabolism mediated by cytochrome P450 (CYP) enzymes represents a major route of elimination of many drugs that can compete for the same CYP enzyme. The bioactivity of essential oils (EOs) and their flavor and fragrance constituents has been known since ancient times and like any other physiological process in the human body also the activity of CYP enzymes can be influenced (increased and/or decreased) by these natural compounds. This review discusses the effects of EO constituents on important drug metabolizing CYP enzymes. Exposure to EO constituents by commonly used products by cutaneous, oral, and inhalative routes, respectively, is outlined. Therefore, the impact of some important EO constituents on CYP enzymes is more described. Especially simultaneous application of EO constituents with drugs or excessive use of EOs and their constituents can lead to unexpected adverse effects.

Kurzfassung

Die Hauptroute für die Elimination von vielen Medikamenten stellt der von Cytochrom P450 (CYP) Enzymen durchgeführte Phase I Metabolismus dar. Da die Bioaktivität von ätherischen Ölen (ÄÖ) und deren Geruchs- und Geschmackskomponenten seit langem bekannt sind, und die Aktivität der CYP Enzyme wie jeder andere physiologische Prozess im menschlichen Körper durch zahlreiche Faktoren beeinflusst (erhöht und/oder erniedrigt) werden kann, behandelt dieser Review die Effekte von ÄÖ Inhaltsstoffen auf wichtige Medikamente metabolisierende CYP Enzyme. Es wird die kutane, orale und inhalative Aufnahmeroute von ÄÖ Inhaltsstoffen beschrieben. Besonders die gleichzeitige Anwendung von ÄÖ Inhaltsstoffen mit Medikamenten oder exzessive Anwendung von ÄÖ und deren Komponenten kann zu unerwarteten unerwünschten Auswirkungen führen.

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1. Introduction

The bioactivity of essential oils (EOs) and their flavor and fragrance constituents have been known since ancient times. Nowadays, EOs and their constituents are commercially important because of their properties, particularly for the pharmaceutical, food, household and cosmetic industries. EOs consist of a very complex mixture of bioactive compounds with low molecular weight, especially terpenes/terpenoids and aromatic and aliphatic compounds. Moreover, they often contain two or three major constituents in relatively high concentrations, whereas other components are only present in minor or in trace levels. The interaction of these chemical mixtures is very difficult to assess, but of biological importance, as increase of permeability means increase of activity. Studies on the biological activities of EO constituents have become increasingly important, as the demand for safe and natural alternative medicines has risen because nowadays consumers are concerned about the toxicity of synthetic chemicals.[1] In the course of these studies toxic properties of specific EOs, such as mutagenic, carcinogenic or central nervous system activity also became evident in some cases. Therefore, it cannot be assumed that natural products are generally safe,[2–4] but most EOs are known for their antimutagenic and anticarcinogenic effects. The main mechanisms involved in the biological activity of EOs and their components happen on cell level. Important damage to cellular membranes and mitochondrial dysfunction play an important role, as mitochondria are a major source of oxidation. Antioxidant and pro-oxidant, pro- and anticancer effects of EOs are concentration-dependent. If the antioxidant concentration is too weak to permeate through mitochondrial membranes, it cannot be converted into pro-oxidant, and therefore keep its activity and act as such.[5]

As already mentioned some lines above, EOs possess a lot of beneficial and therapeutically biological properties.[5–13] In these papers and reviews the mechanisms of the actions of EO-constituents on cells are discussed and their benefits for many medicinal targets emphasized. Naturally, among these manifold activities also the interaction of EO-constituents with CYP-enzymes became an interesting topic of studies recently, especially when EOs are concomitantly used with drug medication.[14] Here, Tisserand et al.[15] reported on EO-constituents that affect CYP 450 -enzymes either as inducers or inhibitors and discussed some important EO-drug interactions as well.

Tisserand's and Golos' paper,[14] finally, prompted us to perform this review on newly reported interactions of EO-constituents with CYP-enzymes.

In the course of the first steps of the interactions of a xenobiotic with the human organism – the pharmacokinetic phase – all xenobiotics, such as EO-constituents, comply in accordance with the following general pattern, namely **a**bsorption, **d**istribution, **m**etabolism and **e**limination (the so-called „ADME-principle"). The xenobiotics in the present case, mainly mono- and sesquiterpenoids, *„easily enter the human body by oral absorption, penetration through the skin, or inhalation, very often leading by distribution to measurable blood concentrations. A number of different enzymes, however, readily metabolize these compounds to more water-soluble molecules. Although nearly every tissue hast the ability to metabolize xenobiotics, the liver is the most important organ of drug biotransformation.[16] In general, metabolic biotransformation occurs at two major categories called Phase I and Phase II reactions.[17] Phase I concerns mostly cytochrome P450 (CYP)-mediated oxidation as well as reduction and hydrolysis. Phase II is a further step where a phase I-product is completely transformed to high water-solubility. This is done by attaching already highly water-soluble endogenous entities such as sugars (glucuronic acids) or salts (sulfates) to the phase I-intermediate and forming a phase II-product. It is not always necessary for a compound to undergo both phases I and II: indeed, for many terpenoids one or the other is enough to eliminate these volatile plant constituents"* (with special permission of the authors W. Jäger and M. Höferl).[18]

Positive or negative aspects of bioactive substances like EO constituents often depend on the metabolism in the human body. Already in 1962 this important liver enzyme, which shows a distinct absorption band at 450 nm, was detected by Omura and Sato.[16] These heme-containing CYP enzymes utilize two atoms of molecular oxygen and NADH/NADPH in order to form a water molecule together with the generation of a more polar metabolite of the xenobiotic which can be excreted more easily. These so-called phase I metabolism reactions include mainly hydroxylation reactions, besides de-alkylation and reduction reactions.[19,20]

Like any other physiological process in the human body the functioning of CYP enzymes can be influenced by numerous factors. On the one hand, induced CYP enzyme activity often follows long term application of bioactive substances like EO constituents and can

happen by increased expression, which is transcriptionally regulated by nuclear receptors that respond to xenobiotics. Commonly, activation of the pregnane X receptor(PXR, also known as NR1I2, SXR, or PAR) and constitutive androstane receptor (CAR; NR1I3) by xenobiotics like EO constituents can lead to an increased expression of CYP enzymes and can affect the metabolism of other xenobiotics.[21] Furthermore, the mechanism of up-regulation of CYP enzymes could be due to the presence of major constituents of EOs, which were found to induce δ-aminolaevulinic acid synthetase activity, the key enzyme involved in the synthesis of the heme moiety of CYP enzymes.[22] CYP inhibition can happen by different mechanisms. Reversible interactions can be a result of competition at the CYP active site and often affect only the first step of the CYP catalytic cycle. Irreversible or quasi-irreversible inhibitors act during and subsequent to the oxygen transfer step.[23] Of course, there can be made a gradation in potency of CYP inhibitors into potent or moderate inhibitors, which depends on the IC_{50} values of the tested CYP modulating EO constituents.[24]

Further influencing factors which affects functioning of CYP enzymes are for example sex related differences in the contents and catalytic properties of individual CYP enzymes.[25] Furthermore, there exists high inter-individual genetic variability of some hepatic CYP enzymes because of several well documented genetic polymorphisms, which can lead to either very high or low activity of CYP enzymes, and therefore to ultra-rapid, extensive, intermediate or poor metabolizers, respectively.[19] Lifestyle factors can also lead to alterations in CYP activity, for example smoking, consuming charbroiled food, or use of barbiturates effects induction of some CYP enzymes.[4,26] Stress or consumption of fruit juices like grapefruit, black mulberry or wild grape fruit juices appear to weaken the action of CYP enzymes.[27,28]

Numerous influencing factors of metabolizing CYP enzymes can result in far-reaching consequences. As some CYP isoforms (e.g. CYP2A, where 2 denotes the family, A the subfamily and an eventually further figure refers to the 1st, or 2nd or 3rd, … enzyme) can contribute to the activation of procarcinogens (e.g. aflatoxin B1, cyclophosphamide, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK = nicotine derived nitrosamine ketone)), induction of these CYP enzymes by EO constituents like (-)-linalool can increase the likelihood of cancer, whereas EO constituents, such as citral, geraniol or β-myrcene that inhibit these CYP isoforms may be useful as chemopreventive agents.[29–31]

Supporting this proposition, the results of an interesting study indicated that dietary *Zataria multiflora* essential oils show colon chemopreventive properties by modulation of CYP enzymes.[32] In reverse, that also means that EO constituents like the structurally related alkylbenzenes estragole, safrole or methyleugenole can be activated to their proximate carcinogens by CYP inducing factors.[4,26] Clear distinction should be made between CYP induced biotransformation of major EO components into bioactive metabolites like the alkylbenzenes estragole, safrole and methyleugenole mentioned above, and the interference of major EO components with CYP enzymatic activities (induction or inhibition of CYP enzymes by major EO components). The latter represent most of the EO constituents this review deals with.

CYP enzymes play an important role in the biotransformation of xenobiotic chemicals, and the metabolic clearance and toxic effects of different compounds might be modulated by a change in the activity of these CYP enzymes. Some examples are pointed out in the work of M. Golos et al.[14] Consulting the comprehensive review of S. Rendic[33] about drug metabolizing CYP enzymes it can be assessed in more detail, if degradation reactions of commonly prescribed drugs are possibly disturbed. This review discusses the effects of EO constituents on CYP enzymes and points out just some adverse health effects and possible interferences with the metabolic clearance of drugs that have been reported in scientific references.

2. Cutaneous Topical Products

EOs and their volatile components are worldwide used in cutaneous topical products such as solutions, ointments, emulsions, gels, cosmetic preparations and toiletries.[34]

Diluted EOs are used for ages to treat numerous diseases of the skin or the muscular skeletal system via massage. The term aromatherapy comes to mind because it is used to describe a wide range of practices involving odorous substances. Massaging with diluted EOs can be an important therapy for human wellbeing and health.[35]

Another therapeutic use of EO components are transdermal drug delivery systems. Nowadays one of the favored routes for therapeutically effective drugs is the transdermal route, due to several advantages like bypassing the first pass effect, better patient compliance and sustained release of drugs over a period of time.[36] There are many studies investigating the permeation enhancing effect of EO components, particularly of monoterpenes and phenylpropanoids on the permeation behavior of different skin delivered pharmaceuticals.[37] A great barrier for transdermal drug delivery is the stratum corneum, which is the outer most layer of the skin, therefore natural or synthetic penetration enhancers are commonly used in pharmaceutical formulations. It is well documented that natural terpenes possess higher enhancement activity on the permeation of drugs through the skin compared with synthetic penetration enhancers and that natural terpenes are a very safe and effective class of penetration enhancers.[38]

Especially extensive dermal application or additional utilization of EOs like having an aroma bath can lead to a relevant blood concentration of EO components.[37,39,40] Unfortunately, there are few experimental studies that deal with the human skin permeation behavior of EO components. We do not know much about the influence of cooperative interactions of individual EO components on the percutaneous EO absorption, which can lead to unexpected pharmacological effects.[37]

Due to the chemical structure and the lipophilic properties compounds of EOs are able to migrate from skin into blood circulation.[34,37,39,40] In addition the octanol-water-partition coefficient (log P) influences the skin permeation behavior just as well as molecular

features. Moderately polar components have a higher permeation rate than lipophilic compounds.[39] The EO components carveol, carvone, eugenol, fenchone, geraniol, limonene, linalool, methyleugenol, and verbenone are commonly used in massage oils, cutaneous topical products and permeation enhancers and have the potential to permeate the skin and have pharmacological effects.[36,37,39,40] These EO components and their influence on CYP enzymes are described in the following.

2.1. Carveol/Carvone

Humans are often exposed to monoterpene alcohols like carveol, as carveol is a product of carvone and limonene metabolism.[41] Unexpected interactions with drugs especially of narrow therapeutic range are of particular interest. In order to determine interactions of carveol and other monoterpene alcohols with anestethics, the effect of carveol on the metabolism of the short acting anesthetic propofol was examined. *In vivo* studies, which were made with male mice, showed a prolonged anesthesia time and increased blood propofol concentration when mice were pre-treated with carveol. These findings were determined in more detail with rat liver microsomes from adult male rats. Microsomes were incubated with propofol in the absence or presence of (-)-carveol and the results showed that carveol decreased the metabolism rate of propofol, which was mediated, among other mechanisms, by CYP2B and CYP2C. It can be expected that carveol leads to increased blood propofol concentration through competitive metabolism by CYP2B and CYP2C. Better understanding of these interactions requires additional research, preferably in human testing systems.[42]

Carvone, which can be metabolized via reduction by NADPH in human liver to carveol, also can be found in EOs of caraway, spearmint, and dill.[42] Interesting results had been found out in a study with recombinant human CYP-enzymes in *Trichoplusia ni* cells. Human CYP2C9 and CYP2C19 were determined as the principle enzymes in the metabolism of carvone to carveol, but it was not pointed out weather these CYP-enzymes were induced or inhibited. In the study occurred species related differences in the carvone metabolism, which underlines the fact that results obtained from animal studies cannot exactly be extrapolated to humans.[41]

2.2. Eugenol

The inhibition of CYPs by EO constituents can decrease the formation of toxic metabolites, as CYPs play an important role in procarcinogen activation.[22] Eugenol, which naturally occurs among others in clove EO, was reported to inhibit CYP1A1 and CYP1B1 activity. These CYP enzymes are key enzymes in the biotransformation of the carcinogen 7,12-dimethylbenz[α]anthracene (DMBA) to more carcinogenic metabolites. DMBA increases the expression of CYP1A1 and CYP1B1 by transcriptional activation. Human MCF-7 cells (a breast cancer cell line) co-treated with eugenol and DMBA showed significant dose-dependent suppression of the DMBA-induced CYP1A1 and CYP1B1 mRNA transcripts. Therefore, eugenol attenuates the formation of DNA adducts by DMBA by down-regulating the expression of CYP1A1 and CYP1B1. A hypothetic direct inhibition of CYP1A1 and CYP1B1 enzyme activity by eugenol was approved by testing microsomal fractions from livers of 3-methylcholanthrene (3-MC; CYP1 inducer)-treated rats.[43]

Furthermore, eugenol was found to decrease CYP2E1 activity, which was tested in adult male Wistar rats. Thioacetamide (TA) was administered to the rats, which induced liver injury. Among other markers of hepatic injuries the CYP2E1 activity was assessed and the rats pretreated with eugenol showed lower CYP2E1 activity levels and, interestingly, lower values of liver injury markers, as in comparison with rats exposed to TA alone.[44]

2.3. Fenchone

Fenchone is widely distributed in plants and is the major component of the EO of Bitter fennel with camphor like smell. (+)-Fenchone was reported to be metabolized by CYP2A6, which was determined in human liver microsomes and *salmonella typhimurium* cells expressing human CYP2A6.[45,46] Furthermore, CYP2B6 contributes to the (+)-fenchone metabolism, which was tested in human liver microsomes.[45] Further reports about the biotransformation of (-)-fenchone showed similar results. CYP2A6 and CYP2B6 are the principle enzymes in (-)-fenchone metabolism, which was also tested in human liver microsomes and recombinant enzymes.[47] Unfortunately, there was no information about inhibition or induction of these CYP-enzymes.

2.4. Geraniol

Geraniol is a major component of palmarosa oil (*Cymbopogon citratus*, Poaceae) and geranium oil (*Pelargonium x asperum*, Geraniaceae) and commonly used in everyday items as flavouring component. Tested in a human liver microsomal system, geraniol showed potent inhibition of CYP2B6. It is a fact that CYP2B6 mediates the metabolism of some important drugs such as bupropion, cyclophosphamide, efavirenz, sibutramine, and tamoxifen. The inhibitory effect of geraniol on CYP2B6-mediated bupropion hydroxylation was measured and as a result geraniol showed stronger inhibition as compared with the well-known CYP2B6 inhibitor thio-TEPA. Further results of the study suggest that geraniol is not a mechanism based but a competitive inhibitor. Given that CYP2B6 plays also a role in the activation of procarcinogens such as aflatoxin B1, cyclophoshamide, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone also known as NNK (nicotine-derived nitrosamine ketone), geraniol may be useful as a chemopreventive agent.[31]

In a further interesting study the CYP-mediated cutaneous metabolism of geraniol was described in order to investigate the allergenic activity of geraniol. As experimental setup geraniol was incubated with recombinantly expressed human CYP bactosomes (CYP1A1, 1B1, 2B6, 2E1, 3A5) in *E. coli,* Tebu-bio. These CYP enzymes represent a skin-like CYP cocktail, therefore, this system provides a convenient method for studies of cutaneous metabolism using the CYP isoforms occurring in the skin. Consistent with the findings mentioned above, CYP2B6 showed high activity in geraniol metabolism prior to CYP1A1 and CYP3A5. (CYP1B1 and CYP2E1 showed low activities.) Nevertheless, CYP1A1 and CYP3A5 were suggested to be responsible for the majority of cutaneous geraniol metabolism since they are constitutively expressed at significantly higher levels in human skin compared to CYP2B6. Summarizing, the allergenic activity of pure geraniol can be explained by the metabolic activation to sensitizing metabolites beside autoxidation.[48]

2.5. Limonene

Limonene is shown to be present in orange peel and other plants and is very commonly used in everyday items. Limonene is known to be nontoxic to the skin,[36] but has several

effects on the activity of CYP enzymes. Limonene was found to decrease CYP2B1 activity, which was tested in vitro in rat liver microsomes from female Wistar rats. The rats were pretreated with phenobarbital which induces CYP2B1 expression in the liver. The CYP2B1 overexpressing rat liver microsomes were incubated with pentoxyresorufin as substrate and limonene as inhibitor. As a result the CYP2B1-mediated pentoxyresorufin-O-dependentylase (PROD) activity was potently inhibited by limonene and the kinetic parameters suggested that inhibition was competitive and reversible.[29]

Furthermore, there has been reported a slight inhibition of CYP2B6 by limonene. Pooled human liver microsomes were incubated with bupropion in the presence or absence of limonene, as the bupropion hydroxylation is CYP2B6-dependent and a selective marker for CYP2B6 activity. The experiments in the presence of limonene showed between 70 % and 80 % of activity as in comparison with control activity, therefore, the inhibitory effect on CYP2B6 by limonene is not very strong.[31] Contrary to this result in another study with human liver microsomes it was found that CYP2B6 did not catalyze oxidation of limonene at significant rates.[25]

Research also showed controversial CYP3A4 modulation by limonene. As midazolam (MDZ) is a common probe to estimate CYP3A4 activity the effect of limonene on CYP3A4-dependant midazolam-1'-hydroxylation and 4-hydroxylation was determined in pooled human liver microsomes. Interestingly, limonene stimulated CYP3A4-dependant midazolam-1'-hydroxylation and inhibited CYP3A4-mediated formation of 4-OH-MDZ, but not as potent as the control stimulator α-naphthoflavone and the control inhibitor ketoconazole, respectively. The underlying mechanism for the differential effects of limonene is not yet known. As CYP3A4 is a complex heme-containing enzyme that shows non-Michaelis-Menten kinetics as well as opposed effects of several substrates one hypothesis suggests the existence of two or more possible midazolam-binding sites, with at least one favoring the formation of the 1-hydroxy metabolite and another favoring the 4-hydroxy metabolite.[49]

Another interesting study determined which P450s are the major enzymes in the oxidation of limonene. The study used specific P450 inhibitors and antibodies raised against purified human liver P450 enzymes, P450s isolated from *Escherichia coli* membranes to which human P450 isoform cDNAs have been introduced, human P450 enzymes

expressed in *Trichoplusia ni* cells, and human liver microsomes, respectively. The results showed that among eleven tested CYP isoforms CYP2C19 has the highest rates of catalyzing oxidation of limonene, followed by CYP2C9, 3A4, 2C8, and 2C18. The remaining forms of P450 including CYP1A1, 1A2, 1B1, 2A6, 2B6, and 2E1 were not active. Although CYP2C19 showed high metabolism rates it can be said that CYP2C9 is more important for catalyzing limonene oxidation, as the content of CYP2C9 in human livers is estimated to be 14-fold compared with CYP2C19. Unfortunately, although the study determined major enzymes in limonene metabolism it was not tested whether CYP isoforms were induced or inhibited.[25]

2.6. Linalool

With linalool, one has to distinguish enantiomers (3*S*)-(+)-linalool, also known as coriandrol, and (3*R*)-(-)-linalool, known as licareol, as they vary strongly in odor and biological activity. (+)-Linalool is the main component of the EO of *Coriandrum sativum* seeds and its smell is described as sweet, floral and herbaceous. (-)-Linalool is the main component of the EO of *Lavandula officinalis* flowers and has a woody, lavender-like aroma. Many biological activities of (-)-linalool were described, and the influence of (-)-linalool on the metabolic activity of CYP enzymes in rat liver microsomes was examined in order to predict possible herb-drug interactions. (-)-Linalool was subchronically administered to male Wistar rats and the metabolic activity of rat liver CYP2A, 2B, 2C6, 2C11 and 3A enzymes was assessed. Only the CYP2A-family which includes in rat CYP2A1, CYP2A2 occurring in hepatic tissue and CYP2A3 occurring in lungs, was significantly influenced by (-)-linalool. The CYP2A-dependant rate of 7α-hydroxylation of testosterone was significantly enhanced, which corresponds to increased metabolic activity of CYP2A. Few drugs are predominantly metabolized via CYP2A (coumarin, nicotine), whereas CYP2A has low importance for the biotransformation of e.g. halothane, methoxyflurane or valproic acid. As the rat CYP2A shows about 60 % homology in amino acid sequence to human CYP2A and the tested doses of (-)-linalool were very high, it was assumed that the possible influence of linalool on drug metabolizing enzymes is not clinically relevant. In order to confirm the results, further studies using other experimental systems like human liver microsomes are necessary. On the other hand it was found that CYP2A can metabolically activate a number of

carcinogens including nitrosamines and aflatoxins, and therefore, induction of CYP2A activity should be regarded with attention.[30]

Another study, screening for the inhibition of CYP2B6 showed a slight inhibition by linalool. Pooled human liver microsomes which were incubated with linalool showed only about 50 % activity of CYP2B6-catalyzed bupropion hydroxylation. Bupropion is often used as auxiliary substance in order to assess modulating effects on CYP2B6 activity. However, the results suggest that the inhibition of CYP2B6 by linalool is not clinically relevant.[31]

2.7. Methyleugenol

Methyleugenol is a natural ingredient of several herbs, including basil, nutmeg, tarragon, star anise, and fennel. Furthermore, methyleugenol is used as a flavoring substance in a wide variety of products of everyday use. Although methyleugenol formerly was classified as GRAS (Generally Recognized as Safe) newer research showed the carcinogenic potential of methyleugenol, especially at high average daily intake. Cytochromes P450 and other liver enzymes converse methyleugenol in several steps to a carbocation which can cause DNA adducts and may ultimately cause liver tumors. It was found that at low concentrations of methyleugenol CYP1A2 is the main enzyme involved in the bioactivation of methyleugenol to its carcinogenic metabolite 1'-hydroxymethyleugenol and that at high substrate concentrations CYP2C9 and 2C19 may also contribute to the bioactivation of methyleugenol. If these enzymes were either inhibited or induced by methyleugenol was not examined. These results were obtained from experiments using either recombinant CYP enzymes or human liver microsomes. The findings also showed 5-fold differences in 15 samples of human liver microsomes, which can be explained by genetic polymorphisms of CYP enzymes but also lifestyle factors that influence the activities of these enzymes. As genetic polymorphisms of CYP1A2, which accounts for 13 % of the total P450 content of the human liver, are very rare, it can be assumed that lifestyle factors that increase the activity of CYP1A2 are very important for the individual risks of the bioactivation of methyleugenol. For example, cigarette smoking, charbroiled food and cruciferous vegetables are known to induce CYP1A2, and barbiturates induce CYP2C enzymes. Therefore, these factors contribute to an increased risk of adverse effects of methyleugenol.[50]

2.8. Verbenone

The characteristic spicy and herbaceous smell of the EO of rosemary verbenone chemotype (*Rosmarinus officinalis var verbenone*aka *Rosmarinus officinalis ct. Verbenone)* comes from its main component verbenone.[51] The roles of CYP enzymes in the metabolism of verbenone were examined in a study of Miyazawa et al.[52] (-)-Verbenone was incubated with rat liver microsomes and with liver microsomes of different human liver samples. Recombinant rat and human CYP enzymes expressed in *Trichoplusia ni*cells were also used in order to test the catalytic activity in (-)-verbenone metabolism. CYP2A6 and 2B6 were shown to catalyze (-)-verbenone hydroxylation at high rates. Confirming these results, Anti-CYP2A6 IgG, (+)-menthofuran (CYP2A6-inhibitor) and triethylenethiophosphoramide (thioTEPA), an inhibitor of human CYP2B6, respectively, significantly reduced (-)-verbenone metabolism in human liver microsomes. CYP2A6 showed 4-fold higher catalytic activity than CYP2B6, considering that CYP2B6 is present at low levels in the human liver. Nonetheless, CYP-induction or inhibition was not determined in this study.[52]

3. Inhalative Products

It was proven that EO components can be well absorbed from the breathing air due to their volatility, and be detected in the blood in humans.[53] The use of EOs by inhalation tend to be daily for a period of days or weeks rather than in a single application, for example, either to induce psychological or physical effects,[27] or simply to enjoy the delicious odors of EOs by air fresheners. However, extensive use of inhalative EO components can possibly lead to unexpected pharmacological effects.

Several studies consistently showed that odors or fragrance compounds can produce specific effects on human neuropsychological and autonomic function.[54] For example α-pinene, an EO component, accumulated in the brain and liver of mice, when inhaled for 90 min/day for five days caused pharmacological effects. Indeed, anxiolytic activity could be observed, which can be attributed to EO components acting on olfactory receptors or brain receptors. Moreover, hepatic metabolism of the compound might be related to its therapeutic effects.[27]

Furthermore, there seems to be a difference if single or mixed EO components are inhaled. For example, the amount of α-pinene in brain and liver of mice was higher after mixed-component inhalation as compared with a single-component inhalation. This may be caused by the nasal mucus which can control the absorption of volatile compounds and metabolize them.[55]

There is a large number of EOs which show proven effects on the central nervous system or exert a positive effect on respiratory diseases because of antimicrobial activity. For example, cinnamon EO inhibited different types of pathogenic bacteria in the vapor phase, or inhalation of peppermint and rosemary EOs can significantly decrease stress related symptoms. Inhaled application of rose oil caused a 30 % decrease in adrenaline concentration and inhalation of chamomile EO reduced levels of adrenocorticotrophic hormone caused by stress in rat plasma.[35] Therefore, several main components of these volatile oils are closer described below.

3.1. Cedrol, β-cedrene, thujopsene

Cedrol, β-cedrene and thujopsene are the main components of cedar EO (from wooden parts of *Juniperus virginiana)* and occur ubiquitously in the wood of cedars and conifers. Cedar EO and its main components cedrol, β-cedrene and thujopsene show many various biological activities and due to the fresh, woody and balsamic aroma these compounds are used globally as traditional medicines, as well as in soaps, shampoos, fragrances, and cosmetics. Due to the prevalent use in our daily lives, the inhibitory effects of cedrol, β-cedrene or thujopsene on eight human CYP enzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4) were evaluated using pooled human liver microsomes.

Cedrol, β-cedrene, and thujopsene markedly inhibited CYP2B6-mediated bupropion hydroxylation, comparable with the selective CYP2B6 inhibitor, thioTEPA. These findings indicate that the co-application of cedrol, β-cedrene, and thujopsene with drugs metabolized by CYP2B6, such as antiretrovirals (efavirenz), anticancers (cyclophosphamide and ifosfamide), and antidepressants (bupropion) has to be carefully observed to avoid negative effects.

Cedrol and thujopsene significantly, and β-cedrene moderately blocked CYP3A-mediated midazolam 1-hydroxylation activity. Cedrol and thujopsene need to be used carefully in combination with drugs metabolized by CYP3A4 (e.g. amlodipine, atorvastatin, cyclosporine, clarithromycin, estradiol, felodipine, lovastatin, nifedipine, ritonavir, simvastatin, and tacrolimus) to avoid adverse interactions.

Concerning the remaining CYP-isoforms, cedrol moderately inhibited CYP2C8-, CYP2C9-, and CYP2C19-catalyzed activities, and thujopsene is assumed to be a mechanism-based inhibitor of CYP2C8-, CYP2C9-, and CYP2C19-mediated reactions.

It is important to note, however, that the inhibition of CYP2B6 and CYP3A4 activity *in vitro* does not necessarily has to be translated into drug-drug interactions in clinical situations. To predict a possible drug-drug interaction potential of cedrol, β-cedrene, and thujopsene, pharmacokinetic studies of these components and *in vivo* studies are required to determine the clinical relevance of CYP2B6 and CYP3A4 inhibition.[56]

3.2. Cinnamaldehyde

Cinnamaldehyde (trans-cinnamic aldehyde) is the main component of cinnamon or cassia EO (from the bark of *Cinnamomum Zeylanicum* or the branches with leaves from *Cinnamomum cassia)* and contributes to its flavor and aroma. Human exposure to cinnamaldehyde is common due to routine use of cinnamon or cinnamon EO. There is a growing use of cinnamon EO as a complementary treatment of diabetes mellitus to lower blood sugar or to benefit from its antimicrobial, anti-inflammatory, lipid-lowering, anticancer, and amyloid plaque-reducing effects. Therefore, research has been done in order to evaluate the effects of cinnamaldehyde on the important xenobiotic-metabolizing CYP-enyzmes CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4, using recombinant human enzymes and pooled human liver microsomes. Well-known substrates and positive control inhibitors were used for evaluating the effect of cinnamaldehyde on each iso-enzyme, and the results showed strong inhibition of CYP2A6, followed by a 10.5-fold higher IC_{50} value for CYP2E1. IC_{50} values for all other CYPs were at least 15.8-fold higher than the IC_{50} value for CYP2A6, and substantial inhibition of CYP2D6 was not observed. Furthermore, the type of inhibition has been investigated, as mechanism based inhibition possesses great importance on clinically relevant herb-drug interaction because of long lasting effects, compared with competitive/reversible inhibition. The findings suggested a mechanism based inhibition, as well as a competitive component. Furthermore, cinnamaldehye could be bioactivated by CYP2A6 to a reactive metabolite that covalently binds to the enzyme. The effects on CYP2A6 are important to know, as this enzyme is a major clearance route for nicotine and letrozole, which is an aromatase inhibitor used to treat breast cancer. Moreover, the extensive genetic diversity of CYP2A6 can contribute to differences in smoking behavior, or lead to great differences in steady-state plasma concentration of letrozole.[57]

3.3. Camphor

Camphor which is one of the major constituents of the EOs from the wood of *Cinnamomum camphora* or from the leaves of *Rosmarinus officinalis* is often used to treat coughs and colds in children and adolescents. Nevertheless, camphor is a potentially toxic compound and there is a risk inducing neurological disorders, especially convulsions, in infants and small children after ingestion of camphor containing products.

The CHMP (EMA Committee for Medicinal Products for Human) contraindicated the use of suppositories containing camphor in children under 30 months old.[2] CYP enzymes have been shown to change compounds to more polar and sometimes more reactive or toxic metabolites. Thus, it was examined how (-)-camphor is metabolized by CYP enzymes in human liver microsomes. Eleven recombinant human P450 enzymes expressed in baculovirus-infected insect cells were tested for their catalytic activities to catalyze (-)-camphor oxidation. CYP2A6 was found to hydroxylate (-)-camphor, whereas CYP1A1, 1A2, 1B1, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 did not show catalytic activity in the metabolism of (-)-camphor. Experiments with human liver microsomes, anti-CYP2A6 antibodies and (*R*)-(+)-menthofuran, an inhibitor of CYP2A6, confirmed these findings. Compared with former studies made with rats, rabbits and dogs the current results suggest that there are species related differences in (-)-camphor metabolism. There was no information about induction or inhibition of CYP2A6.[58]

3.4. Safrole

Safrole and other alkylbenzenes are important constituents of herbs such as cinnamon, nutmeg, anise star, tarragon, sweet basil, sweet fennel, or anise vert. As safrole is genotoxic for humans, it was already banned as an additive in food by the U.S. Food and Drug Administration and by the Council of the European Communities. Safrole can be converted to its proximate carcinogen by 1`hydroxylation by CYP enzymes, followed by the conversion to 1′-sulfooxysafrole by sulfotransferase enzymes. Cleavage of the sulfate moiety leads to an electrophilic carbocation, which can covalently bind to macromolecules and cause DNA damage. Due to the main role of CYP enzymes in the first step of the bioactivation route of safrole, the activity of CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4, which are important isoenzymes present in the human liver and involved in drug and xenobiotics metabolism, was studied. Safrole was incubated with supersomes, expressing these enzymes at a high level, genetest microsomes, and with microsomes obtained from 15 individual human livers. As a result, among the tested CYP-enzymes, only CYP2B6, 2C8, and 3A4 showed almost no 1`hydroxylation activity, whereas CYP2C9 showed high activity, followed by CYP2D6 and 2E1. The CYP2C subfamily accounts for 20 % of the total CYP content in the human liver, whereas CYP2D6 accounts for 1.5 % and CYP2E1 for 7 % of the total CYP content, which underlines the great importance of CYP2C9 in safrole metabolism.

CYP2A6 also plays an important role in the bioactivation of safrole, although in the human liver it is a minor enzyme and only accounts for approximately 4 % of the total CYP content, but it is known to be involved in the bioactivation of various precarcinogens, like aflatoxin B1 and N-nitrosamines. Genetic human polymorphisms, which are known for CYP2C9, 2A6 and 2D6 and can lead to poor or ultrarapid metabolizers, play a great role in the risk assessment of safrole. Besides that, lifestyle factors might also influence the susceptibility for safrole genotoxicity, more precisely, ethanol induces CYP2E1 in both rats and humans and barbiturates induce, among others, CYP2C enzymes. Summarizing, CYP2C9, 2A6, 2D6, and 2E1 are the most important isoenzymes in the bioactivation of safrole, but for a risk assessment of the carcinogenic effects of safrole special attention has to be paid to high risk patients due to the excessive use of alcohol or barbiturates or polymorphisms leading to ultrarapid metabolizer phenotypes.[4]

3.5. Cadinene

Cadinene is a collective name for a number of isomeric hydrocarbons and is a major part in the wood EOs of juniper and other woods, and to a small extent in cassia cinnamon (chinese cinnamon). Due to the use of cassia cinnamon in herbal medicines such as cinnamon capsules for type II diabetes mellitus patients or the intensive use of cassia cinnamon EO or other essential wood oils can lead to an over-exposure to cadinene. This should be regarded with attention, as in a study with hepatic microsomes of rat it was found out by SDS-PAGE and immunoblotting that cadinene induces rat CYP2B1 and 3A2, however, to a smaller extent than the well known inducer phenobarbital. CYP1A1, 2A2, 2C11, 2E1, and 4A2, which were also tested, showed no induction. CYP2B metabolizes many xenobiotics such as benzphetamine, 7-ethoxycoumarin, and aminopyrine. CYP2B and 3A hydroxylate testosterone and other steroids, which play many roles in maintaining homeostasis. Therefore, it was postulated in the study that the inhalation of cadinene affects the hepatic metabolism of endogenous and exogenous compounds.[59]

3.6. α-Pinene

α-Pinene is a widely encountered monoterpene in nature. As the name implies, α-pinene is a main constituent in the EO of pine wood and many other conifers, as well as in the EO of rosemary or sage. Furthermore, α-pinene is one of the primary constituents of turpentine, which is commonly used as a solvent. As mentioned in the beginning of this chapter, α-pinene can be enriched in human brain and liver tissues after inhalation, so the assessment of the effect of α-pinene on metabolizing CYP-enzymes is of high interest. α-Pinene was examined concerning both inhibition or induction of CYP2B1. The induction studies were made with rat liver microsomes from male Sprague-Dawley rats, which were injected intraperitoneally with α-pinene, phenobarbital (inducer of CYP2B1), or only corn oil once a day for five days. The results showed that α-pinene leads to significantly increased levels of CYP2B1, which was tested by SDS-PAGE and immunoblotting and measured photometrically.[59] Interestingly, the inhibition study showed significant inhibition of CYP2B1 activity, which seems to be a converse outcome, but enzyme induction happens on gene level and leads to higher amounts of the enzyme, which does not exclude the fact that enzyme activity could also be inhibited by the same substance. Moreover, the inhibition study used a different experimental setup and detecting methods. The effect of α-pinene on the activity of pentoxyresorufin-O-dependentylase (PROD), a selective marker for CYP2B1, was determined in a pool of liver microsomes prepared from female Wistar rats. Pentoxyresorufin was used as a model substrate for CYP2B1-dependent enzymes, and the results showed that (-)- and (+)-α-pinene were potent *in vitro*- and possibly also *in vivo*-inhibitors of PROD activity.[29]

3.7. Terpineol

Terpineol naturally occurs among others in rosemary oil, cajuput oil, or pine oil, and is usually a mixture of the four isomers α-, β-, γ-terpineol and terpinen-4-ol, with alpha-terpineol as the major constituent. Terpineol has a pleasant odor similar to lilac and has been employed in the manufacture of perfumes, cosmetics, soaps and antiseptic agents. Due to the common use of terpineol studies were undertaken in which the inhibitory effects of terpineol on the activities of ethoxyresorufin-O-deethylase (EROD), a marker for CYP1A1, and methoxyresorufin-O-demethylase (MROD), a marker for CYP1A2, and pentoxyresorufin-O-dependentylase (PROD), a marker for CYP2B1, were determined in a

pool of rat liver microsomes. The rat had been treated with β-naphthoflavone and phenobarbital, respectively, as β-naphthoflavone induces CYP1A-subfamily and phenobarbital induces CYP2B1 in rat liver microsomes. Due to the overexpressed enzymes it is easier to obtain significant results concerning inhibition. The results from this study indicated that terpineol is a rather selective inhibitor of CYP2B1, but does not cause any decrease of EROD (CYP1A1) and MROD (CYP1A2) activity. These findings suggest that terpineol may alter the biotransformation of drugs and toxicants (cyclophosphamide, barbiturates, DDT, bromobenzene and others) which are substrates for CYP2B1 monooxygenases.[60]

3.8. Terpinen-4-ol

Terpinen-4-ol is widely distributed in plants and can be found in the EOs of cinnamon, marjoram or tea tree oil. Studies using recombinant human CYP enzymes and liver microsomes of human samples were made to obtain more information about the absorption or excretion of (-)- and (+)-terpinen-4-ol. For (-)-terpinen-4-ol, among eleven tested CYP-enzymes CYP2A6 was the major enzyme in the oxidation process. Moreover, menthofuran, an inhibitor of CYP2A6, inhibited oxidation of (-)-terpinen-4-ol.[61] To investigate the metabolism of (+)-terpinen-4-ol, studies were undertaken using recombinant human CYP-enzymes and human liver microsomes. Deviating from its enantiomer, among eleven tested CYP-enzymes CYP1A2, 2A6 and 3A4 showed catalytic activity in the oxidation process of (+)-terpinen-4-ol. Interestingly, in the inhibition experiments only (+)-menthofuran and ketoconazole, inhibitors known to be specific for CYP2A6 and 3A4, respectively, showed inhibition of (+)-terpinen-4-ol-metabolism, whereas α-naphthoflavone, a selective CYP1A2-inhibitor, did not exhibit considerable inhibitory effects. Due to these findings CYP2A6 and 3A4 can be regarded as major enzymes in (+)-terpinen-4-ol-metabolism.[62]

3.9. Borneol

Borneol is often the main constituent of the EOs of medicinal herbs such as rosemary or plants of the genus *Micromeria* and *Pinus*. Studies were carried out to determine whether dietary monoterpene alcohols like borneol interact with the commonly used short acting intravenous anesthetic propofol and could be used to prolong the anesthetic effect by

inhibiting propofol metabolism in animals. As propofol undergoes rapid biotransformation to various metabolites in human liver catalyzed by the CYP enzymes CYP2B and 2C, the findings were also used to assess the effect of borneol on the activity of the mentioned CYP enzymes. The effects of pre-treatment with borneol on the pharmacokinetics of propofol were studied. The *in vivo* studies showed that the anesthetic effect of propofol and propofol blood concentration was increased when mice were pre-treated with borneol. However, it had been found out in experiments with rat hepatocytes that borneol is a poor substrate or inhibitor of the mentioned CYP enzymes CYP2B and 2C. In fact, the anesthetic prolonging effect of borneol is primarily due to the inhibition of propofol glucuronidation.[42] Another study confirmed these findings when human liver microsomes were incubated with CYP2B6-dependently metabolized bupropion in the presence or the absence of borneol. The presence of borneol showed an about 60 % activity of bupropion hydroxylation as compared with control activity. This suggests that borneol is only a weak inhibitor of human CYP2B6.[31]

3.10. Rose oxide

The two isomers cis- and trans-rose oxide can be isolated from rose oil and are widely used as food flavor, in perfumes and cosmetics, because they have the characteristic odor properties for rosy notes. As there had been little information about the absorption and excretion of cis- and trans-rose oxide in the human body, the metabolism by CYP enzymes in human liver microsomes and recombinant human CYP enzymes was examined. The activities of cis- or trans-rose oxide metabolite formation were compared with the activities of known CYP-catalyzed marker reactions. The results showed that among eleven forms of human recombinant CYP enzymes expressed in insect cells CYP2B6 had the highest catalytic activity for forming the metabolites of cis- and trans-rose oxide, followed by CYP1A2 and 2C19. CYP enzymes including CYP1A1, 1B1, 2A6, 2C8, 2C9, 2D6, 2E1 and 3A4 had very low activities or activities below the limit of detection. The study did not include information about inhibition or induction of the CYP enzymes that are involved in the metabolism of cis- and trans-rose oxide.[63]

3.11. α-Bisabolol, Chamazulene, cis-, trans-Spiroether

α-Bisabolol is among bisabololoxides A and B, farnesene, chamazulene, and cis- and trans-spiroether one of the main constituents of chamomile EO from *Matricaria recutita* flower heads. *M. recutita* is beneficial as a sedative, spasmolytic and anti-inflammatory agent and is also very commonly used to relieve skin disorders such as psoriasis, eczema and acne, and to alleviate fever, bronchitis, cough and the common cold. However, adverse effects or drug interactions are reported for simultaneous and excessive use of chamomile products. The reasons for these interactions are mainly unknown.[64,65] Studies with human recombinant CYP-enzymes investigated the inhibitory properties of *M. recutita* volatile oil and its major bioactive constituents on clinically relevant CYP-enzymes CYP1A2, 2C9, 2D6, and 3A4. The effects were measured fluorometrically. Testing of the crude EO should indicate whether the overall activity was explainable by the tested compounds or caused by other constituents. Bisabololoxides A and B and farnesene did not show any considerable inhibitory potential against the mentioned CYP enzymes. Thus, the remaining main constituents α-bisabolol, chamazulene, cis-, and trans-spiroether were also closely looked at in the following. α-Bisabolol was found to be a potent inhibitor of CYP2D6, and a weak inhibitor of CYP2C9. Chamazulene showed potent inhibitory potential against CYP1A2, 2D6, and 3A4. Spiroethers were the most active individual compounds against CYP1A2 and 3A4 among the tested substances. As several chemotypes of *M. recutita* are known, differing significantly in their EO composition, certain metabolic factors (e.g. unspecific interactions with proteins, competition between co-administered drugs, and enzyme induction due to chronic intake) cannot be covered in *in vitro*-tests with recombinant CYP-enzymes, and clinically significant pharmacokinetic interaction cannot be totally confirmed. However, the results of the mentioned study clearly suggest that herbal products containing chamomile may have the potential to inhibit the metabolism of certain co-administered drugs like warfarin, morphine, or cyclosporine.[24,64,65]

4. Tobacco Products

Cigarettes, E-Cigarettes and Smoke Less Tobacco (SLT) which includes chewing tobaccos, snuff tobaccos and dissolvable tobaccos are widely used because of the addiction to nicotine.[66] However, it has to be considered that tobacco products also consist of many additives like flavorings and enhancers (e.g., cocoa, licorice, menthol, fruit extracts), humectants (e.g., propylene glycol, glycerol, sorbitol), various sugars and ammonium compounds which can have unexpected pharmacological effects.[67] Following the topic of this review, we also focus here on the EO compound menthol.

4.1. Menthol ((1R,3R,4S)-(-)-Menthol)

(-)-Menthol is a main constituent of various mint oils and is widely used as fragrance ingredient in food (candies, chewing gum), but also in cosmetics, shampoos, soaps, household cleaners, and especially as cigarette flavoring ingredient.[2]

It has been shown that smokers who use mentholated cigarettes report smoking 35 % fewer cigarettes per day than smokers of not mentholated cigarettes. Paradoxically, the former had significantly higher rates of smoking-related illnesses and lung cancer due to higher urine levels of the tobacco specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK = nicotine derived nitrosamine ketone) as compared with smokers of non-menthol cigarettes. The molecular mechanism behind was not clear, a possible reason whereby (-)-menthol could increase the health risk could involve the cooling effect of (-)-menthol and increased duration or deepness of inhalation due to (-)-menthol. Another important mechanism could include the inhibition of nicotine metabolism by (-)-menthol thereby enhancing the toxicity of mentholated cigarettes.[68]

The primary addictive agent nicotine is efficiently oxidized to cotinine by CYP2A6 which is primarily a hepatic enzyme and by CYP2A13 which is expressed in the respiratory tract.[66,68] CYP2A6 and CYP2A13 also catalyze the activation of the Nicotine-derived nitrosamine ketone (NNK) also known as 4--1--1-butanone, one of the key tobacco-specific nitrosamines with carcinogenic potential. CYP2A13 is a 200-fold more efficient

catalyst than CYP2A6, and CYP2A13 plays a very significant role in the induction of lung cancer in smokers.[66]

It has been also reported that (+)-menthol inhibits CYP2A6 and CYP 2A13 activity in human liver microsomes. Coumarin is a well-known substrate for probing CYP2A activity. The 7-hydroxylation of coumarin is mediated both by CYP2A6 and CYP2A13 and was significantly inhibited by (+)-menthol in human liver microsomes.[66,68] Using (+)-menthofuran and anti-CYP2A6-antibody, typical inactivators of CYP2A6-dependent catalytic activity in human liver microsomes, a significant inhibition of the oxidation of (+)- and (-)-menthol in human liver microsomes was observed. This is a further proof of CYP2A6-dependent metabolism of (+) and (-)-menthol.[69]

Blocking nicotine metabolism can reduce the extent of smoking, and similarly, the inhibition of NNK metabolism could mitigate exposure to activated NNK. Therefore, all modifications of CYP2A6 and CYP2A13 activities seem to be critical to understand the influence of these enzymes on tobacco consumption and carcinogenesis. The results mentioned above (inhibition of the CYP enzymes CYP2A6 and CYP2A13) appear to suggest that menthol cigarettes may be potentially less harmful than non-menthol cigarettes, which is in contrast to the assumed enhanced toxicity of mentholated cigarettes mentioned above.[66]

Also, a slight inhibition of CYP2B6-catalyzed oxidations by (-)-menthol has been reported. A study in a human liver microsomal system used bupropion as testing substance, as it is known that the bupropion hydroxylation is CYP2B6-dependent and a selective marker for CYP2B6 activity. To be exact, the CYP2B6-catalyzed bupropion hydroxylation in presence of (-)-menthol showed only about 30 % of control activity. But the assessment of the clinical relevance of CYP2B6 inhibition by (-)-menthol requires *in vivo* studies. Adverse effects may occur in patients who take drugs such as bupropion, cyclophosphamide, efavirenz, sibutramine, or tamoxifen, as CYP2B6 is important in the metabolism of these substances.[31]

5. Food and Dietary Supplement Products

There is a considerable exposure of humans to orally consumed EO components. On the one hand, this is due to their natural occurrence in a huge variety of plants and products made from them, for example spices or herbal supplements,[21,49] and on the other hand, it is the consequence of the very common use of EO constituents as flavoring food additives or natural food preservatives.[70]

A variety of spices is widely consumed on a daily basis by many people, for example by the Asian population, yet little is known about the influence of spices on CYP-mediated drug bioavailability. Given the wide substrate specificities of the different CYP-enzymes, undesirable therapeutic outcomes can result from co-administration of potent drugs with EO components that are substrates, inhibitors, or inducers of the proteins. In order to gain more knowledge about interactions between spices and CYP-enzymes, studies with commonly consumed spices were undertaken.[49] For example, *Ocimum basilicum* EO showed antifibrogenic potential in rats with carbon tetrachloride (CCl_4) induced liver fibrosis. In the study it is postulated that one of the underlying mechanisms is the total blockade of CCl_4 induced down regulation of CYP2E1, the CYP isoform responsible for detoxification of CCl_4.[71] As the compositions of whole spices could be highly variable, being influenced by plant species, as well as the conditions of growth, postharvest processing, and storage, pure components were of interest.[49]

Of further concern is the increasing use of complementary and alternative medicines particularly herbal medicines, where EOs and their components are often used in a crude or concentrated form. For instance, in soft capsules like Tavipec®, GeloMyrol®, or Lasea® crude EOs are the active ingredients.

Although natural products are difficult to patent, a new patent was filed which uses peppermint EO as part of the active principle. The invention concerns an optimized cannabis-based aphrodisiac and mood enhancer that contains peppermint oil in the range of 0.05 % to 3.0 % of a single dose. The CYP inhibitory effect of peppermint oil is used to prolong the time of bio-activity of tetrahydrocannabinol (THC) *in vivo*.[72]

Unfortunately, many users do not report herbal supplement use to their physicians, and regulations for herbal supplements in the United States do not require surveillance or the reporting of adverse events by the manufacturer to the FDA. The data concerning adverse interactions of herbal supplements are derived from case reports and series, retrospective databases, and progressive registries. In the United States, contrary to pharmaceuticals, herbal supplements are not required to be evaluated for the effect on CYP-enzymes before reaching the market. Concurrent use of pharmaceuticals with herbal supplements, as well as the use of multiple herbal supplements, can lead to drug-drug interactions, herb-drug interactions, and hepatotoxicity.[21] There has already been published a comprehensive review, where the most commonly used herbal supplements in the United States are discussed, that inhibit CYP-enzymes, or induce CYP-expression, and those whose toxicity is mediated by the CYP-system.[21]

In the following, we discuss some constituents of important spices like pepper, curry leaf, estragon, fennel, thyme, coriander, and lemon grass and their ability to modulate the activities of drug-metabolizing CYP-enzymes.

5.1. α-Humulene

The best-known source of α-humulene is hop, but this EO component is widespread in nature, for example in pepper. It has various biological activities which could be beneficial for human health, so α-humulene has a certain potential to be used in therapy of various diseases in the future. Because of the common use of α-humulene on the one hand and only limited previous findings about its ability to modulate the activities of CYP-enzymes on the other hand, inhibition studies with α-humulene were carried out. Enzyme activities of CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4 were assayed in human and rat liver microsomes, which were incubated with specific substrates in the presence or absence of α-humulene. From all the enzymes tested, only CYP1A2 and 3A and partly 2B, both human and rat microsomal fractions were inhibited by α-humulene. In the experiments with human microsomes, the specific CYP1A2 inhibitor α-naphthoflavone and CYP3A4 inhibitor ketoconazole were used for comparisons of the inhibitory effect. The results showed that α-humulene acted as weak inhibitor toward CYP1A2 activity, and inhibited CYP3A/2B very strongly. In humans, α-humulene can only be considered as CYP3A inhibitor, where the inhibitory effect was comparable to

that of the specific inhibitor ketoconazole. As CYP3A metabolizes about 50 % of all drugs, its inhibition by α-humulene might well result in adverse interactions.[73]

5.2. β-Caryophyllene, β-Caryophyllene oxide

β-Caryophyllene and β-caryophyllene oxide are structurally related with α-humulene and often occur together in the same plants. β-Caryophyllene is one of the ubiquitous EO components in well-known aromatic plants such as curry leaf, cloves, basil, oregano, sage, peppermint, ginger, cannabis, pine trees, etc. The β-caryophyllene derivative β-caryophyllene oxide is present in curry leaf, lemon balm, caraway, cloves, hop, basil, oregano, lavender, rosemary, cinnamon, etc. In spite of the widespread occurrence of both compounds, as well as the common use and great importance, information about their inhibitory effect on CYP enzymes remains limited. As β-caryophyllene and β-caryophyllene oxide have promising biological activities and a certain potential to be used in therapy of various diseases in the future, the inhibitory effect on CYP enzymes was studied in rat and human *in vitro*. Enzyme activities of CYP2A6, 2B6, 2C9, 2C19, 2D6, and 2E1 were not significantly affected. The two CYP-mediated activities of Ethoxyresorufin-O-deethylase (EROD) (which is ascribed mainly to CYP1A2) and Benzyloxyresorufin-O-debenzylase (BROD) (which is ascribed mainly to CYP3A4 and only partly to CYP2B1), were significantly inhibited in rat and human liver microsomes. β-Caryophyllene and β-caryophyllene oxide showed only weak inhibition of CYP1A2 activity. The specific CYP1A2 inhibitor α-naphthoflavone inhibited EROD activity by 92 %, but the same amount of β-caryophyllene and β-caryophyllene oxide by only 10 % and 18 %, respectively. On the other hand, strong inhibition of CYP3A4 activity was observed. Compared with the well-known CYP3A4 inhibitor ketoconazole, which inhibited BROD activity by 66 %, the inhibitory effect of the two compounds was similar: β-caryophyllene inhibited BROD activity by 57 % and β-caryophyllene oxide by 65 %. The type of inhibition by β-caryophyllene oxide (as the selected representative with the strongest effect) was competitive in human but non-competitive in rat liver microsomes. This inter-species difference demonstrates a certain limitation regarding drug interaction studies in experimental animals.[73]

5.3. Estragole

Estragole naturally occurs in herbs such as tarragon, basil, fennel, and anise and is also often used as flavoring substance. Excessive consumption of estragole should be avoided, regarding that the Scientific Committee on Food (SCF) of the European Union concluded that estragole is genotoxic and carcinogenic, due to its bioactivation to a carbocation that can form DNA-adducts and liver tumors. On the other hand, there is the opinion of the Expert Panel of the Flavor and Extract Manufacturers' Association (FEMA) of the United States, which concluded that the present exposure to estragole from food (mainly spices and added as such) does not pose a significant cancer risk. In order to better estimate the risks associated with the consumption of estragole, enzymes that catalyze the different biotransformation steps were investigated. Estragole was incubated with recombinant or pooled human liver microsomes. Specific CYP enzyme inhibitors or their respective antibodies for CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4 were used. As a result, especially CYP1A2 and 2A6 appeared to be active in estragole hydroxylation, and also CYP2C19, 2D6, and 2E1 showed a moderate activity. However, it was concluded that CYP2B6, 2C9, and 3A4 does not contribute to estragole hydroxylation in the human liver. Because CYP1A2 is an important enzyme in hydroxylation of estragole and the related alkylbenzene methyleugenol, and CYP2A6 is an important enzyme in hydroxylation of estragole and safrole, competitive effects can be expected. As these alkylbenzenes have carcinogenic potential, herb-based exposure to them should be regarded with attention, since in herbs such as anise, basil, and nutmeg, all three alkenylbenzenes are present. Furthermore, there are known polymorphisms for CYP1A2 and 2A6, which leads to decreased/deleted or induced activity of these enzymes, and therefore, to decreased or increased bioactivation of estragole. Lifestyle factors like cigarette smoking and the consumption of charbroiled food and cruciferous vegetables increase the activity of CYP1A2, and therefore, can increase bioactivation and thus the genotoxicity of estragole.[26]

5.4. α-Terpinene

The terpines, which includes α-terpinene, β-terpinene, γ-terpinene and terpinolene, are a group of isomeric monoterpenes with the same carbon framework, but they differ in the position of carbon-carbon double bonds. α-Terpinene is the main important isomer and

naturally occurs in fennel, cardamom, marjoram and tea tree EOs, and other natural sources. It can be industrially produced, and due to its pleasant lime like odor, it is mainly used to improve industrial products. Taking into consideration that α-terpinene can modulate the metabolic clearance and toxic effects of different compounds by inducing a change in the activity of CYP enzymes, the inhibitory effect of α-terpinene on Pentoxyresorufin-O-dealkylase (PROD) activity (which is ascribed mainly to CYP2B1) was studied in rat liver microsomes from female Wistar rats. The results indicated that O-dealkylation of PROD by rat microsomes was significantly inhibited by α-terpinene, so it can be considered as an *in vitro*– and possibly, also an *in vivo*–inhibitor of CYP2B1. Moreover, α-terpinene may interfere with the biotransformation of drugs toxicants – such as cyclophosphamide, barbiturates, bromobenzene and others – which are substrates for CYP2B1 monooxygenase.[29]

5.5. Carvacrol

Carvacrol, a phenolic compound of the EOs of thyme, oregano, winter or summer savory, was demonstrated to exert multiple pharmacological effects. For example, it has promising anti-inflammatory properties due to its suppression of COX-2 expression and therefore can decrease lifestyle related diseases.[74] In order to better evaluate co-administration of carvacrol and other xenobiotics that mainly undergo CYP-mediated metabolism, a combination of chemical inhibition studies in human liver microsomes and assays with recombinant CYP isoforms were made. Nine recombinant CYP isoforms (1A2, 2A6, 2B6, 2C8, 2C9, CYP2C19, 2D6, 2E1, and 3A4) were employed to identify the CYP isoforms involved in the metabolism of carvacrol. It was found that two metabolites were formed by CYP enzymes, and the results showed that CYP1A2, 2A6 and 2B6 could catalyze the formation of the metabolites. The involvement of other CYP isoforms in the metabolism of carvacrol was negligible.[19] Given the fact that CYP2A6 is an important drug-metabolizing enzyme, attention should be paid to potential interactions. Moreover, due to inter-individual variability in hepatic CYP2A6 expression (30-fold), and many known CYP2A6 polymorphisms that significantly influence the function of this CYP isoform, different pharmacokinetic and clinical outcomes within people after administration of carvacrol can be predicted.[19,75]

5.6. Thymol

Thymol is a phenolic compound with a similar structure to carvacrol, and can be found in numerous aromatic plants, especially in thyme, and is responsible for the strong flavor of this herb. Due to its strong antiseptic properties it is widely used in medicine and therefore, the metabolism of thymol was investigated in pooled human liver microsomes. As thymol has been demonstrated to undergo phase I metabolism, the aim of the study was to investigate the role of various CYP isoforms (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) in the phase I metabolism of thymol. Results from experiments with these recombinant human CYP enzymes suggested that CYP1A2, 2A6 and 2B6 could catalyze the metabolism of thymol, similar to its isomer carvacrol.[19] As around 3 % of the drugs metabolized by CYP enzymes involve CYP2A6, and environmental factors, such as compounds found in food, cigarettes, or hormones, can affect CYP2A6 expression, especially the metabolism of thymol by CYP2A6 is of importance.[75]

5.7. β-Myrcene

β-Myrcene commonly occurs in a large variety of useful plants, for example, as a minor component of the EOs of coriander, lemongrass, verbena and others. But it is commonly used for production of flavorings and fragrances. β-Myrcene was recently in the focus of some studies concerning carcinogenicity, based on its high production volume and high level of human exposure, with the result that there was clear evidence of carcinogenic activity of β-myrcene in male rats and mice.[76] On the other hand, β-myrcene did not show evidence of mutagenic activity, moreover, it has been reported that β-myrcene showed antimutagenic properties in mammalian cells due to inhibiting certain forms of CYP enzymes required for activation of premutagens and precarcinogenes.[77] In order to evaluate the inhibitory effects of β-myrcene on CYP2B1, which is involved in the activation of some genotoxic substances, *in vitro* inhibition studies with liver microsomes prepared from female Wistar rats were performed. Pentoxyresorufin was used as a model substrate for CYP2B1-dependent enzymes, as pentoxyresorufin-O-dealkylase activity (PROD) is mainly ascribed to CYP2B1. As a result, β-myrcene caused a strong concentration-related decrease in microsomal PROD activity, the type of inhibition seemed to be competitive. Furthermore, only a slight inhibitory effect has been observed on EROD activity, which is a CYP1A1-dependent reaction.[29] The results are supported by

another study, where it was shown that a single oral dose of β-myrcene prolonged pentobarbital sleeping time when given to rats one hour prior to the barbiturate, which can be lead back to CYP2B1 inhibition. In contrast, the repeated administration of β-myrcene reduced the pentobarbital sleeping time, suggesting that microsomal enzyme induction occurred.[78] In order to evaluate this hypothesis, the induction of CYP2B1 and 1A1 was studied with rat liver microsomes from female Wistar rats. Activitiy of PROD (CYP2B1 dependent) was strongly increased, whereas EROD activity (CYP1A1-dependent) was only slightly changed, which suggest that β-myrcene induces CYP2B1 but not CYP1A1, when administered repeatedly. Further, densitometric quantitation confirmed that CYP2B1/B2 apoprotein levels in liver microsomes from β-myrcene treated rats were higher than in microsomes from control rats. It seems that β-myrcene is a natural substrate for CYP2B1, and therefore, depending on frequency and duration of administration, it is a competitive inhibitor and inducer of this isoenzyme.[79] Furthermore, investigations were undertaken to evaluate the effect of β-myrcene on CYP3A4 activity *in vitro* in human liver microsomes, as CYP3A is a major CYP enzyme responsible for metabolizing more than 50 % of clinical drugs in the human liver. In more detail, the CYP3A4-mediated metabolism of midazolam was evaluated. There are two metabolic pathways – the 1`-hydroxylation and the 4-hydroxylation, respectively – in midazolam metabolism by CYP3A4. The results showed that β-myrcene enhanced both pathways, but not as potent as the control stimulators α-naphthoflavone and testosterone, respectively, so it was postulated that β-myrcene is a moderate stimulator of CYP3A4 activity in human liver microsomes.[49]

5.8. Citral

Citral, a natural combination of two isomeric aldehydes namely, the isomers geranial (α-citral) and neral (β-citral) is mainly contained in lemongrass EO (from *Litsea citrata*).[80] As it has been shown that citral has various biological activities, the effect of citral on drug-metabolizing CYP enzymes was in the focus of some interesting studies.[29,31,49,80] Concerning modulation of CYP3A4 activity by citral, opposing results were found. A study with rat liver microsomes from male Sprague-Dawley rats, which were fed a pelleted diet and administered citral by gavage for two weeks, revealed that the testosterone 6β-hydroxylation activity (which was ascribed mainly to CYP3A4) was significantly reduced.[80] A study in a pooled human liver microsomal system used

midazolam as a common probe to estimate CYP3A4 activity showed that the CYP3A4-mediated 1'-hydroxylation and 4-hydroxylation of midazolam were significantly enhanced and inhibited, respectively. The opposing effects on these two metabolic pathways or the differential outcomes of the two mentioned studies[49,80] cannot be easily explained, as CYP3A4 is a very complex enzyme, and there is to date no firm consensus on the mechanism for the differential effects of CYP3A4 modulators.[49] Furthermore, several studies concerning modulation of CYP2B1 activity were undertaken. A study with rat liver microsomes from male Sprague-Dawley rats, which were fed a pelleted diet and administered citral by gavage for two weeks, came to the result that CYP2B1 activity (indicated by PROD) was not significantly affected by citral.[80] On the other hand, in a study with rat liver microsomes from female Wistar rats, that also used PROD as selective marker reaction for CYP2B1 activity, moderate inhibition of CYP2B1 could be observed.[29] A possible reason for the differential outcomes could be that the female Wistar rats were treated with phenobarbital to induce CYP genes. No significant difference was found in CYP2C, 1A2, 2D, and 2E1 activities.[80] Another study with the focus on inhibition of CYP2B6 activity used the probe drug bupropion in a human liver microsomal system, as bupropion hydroxylation is mainly catalyzed by CYP2B6. The results showed that citral is a potent inhibitor of CYP2B6. Given the fact that CYP2B6 can also contribute to activation of procarcinogenes like aflatoxin B1, cyclophosphamide, or NNK, citral may be used as chemopreventive agent.[31]

5.9. 1,8-Cineole

1,8-Cineole, a monoterpene cyclic ether also known as eucalyptol, is widely distributed in plants and a major component in eucalyptus oil from *Eucalyptus globulus,* tea tree oil, or EOs from peppermint, rosemary, or sage. It is extensively used as flavoring and fragrance substance due to its characteristic fresh and camphor-like odor, in pharmaceutical preparations, e.g. in soft capsules like GeloMyrol® as cough suppressant, and many other applications. It should be regarded with attention that 1,8-cineole is reported to induce neurological disorders, especially convulsions, in infants and small children.[2] Research was done concerning the metabolism of 1,8-cineole by CYP enzymes, which included induction, inhibition and metabolism experiments, respectively.[49,59,81,82]

The induction study made with hepatic microsomes from male Sprague-Dawley rats used testosterone 2β-, 6β-, 16α- and 16β-hydroxylation activities as marker reactions for CYP2B1 and 3A2 activities, which were found to be significantly increased. The levels of these isoenzymes were measured by immunoblotting and were found to be considerably higher, which confirmed the results that 1,8-cineole induces CYP2B1 and 3A2 activity in rat liver microsomes.[59] These data were confirmed by Debersac et al.[83] The group found out that the EO of *Rosmarinus officinalis* leaves, rich in 1,8-cineole, strongly induced CYP2B1 and 2B2 in male SPF Wistar rats.[83]

The metabolism studies focused on conversion of 1,8-cineole by liver microsomes of rats and humans and by recombinant CYP enzymes in insect cells in which human CYP cDNAs had been introduced. Ten forms of human CYP enzymes expressed in insect cells were used to determine which isoforms the major catalysts for the hydroxylation of 1,8-cineole in humans were. CYP3A4 had the highest activity, followed by CYP3A5, 2B6, and 2A6. Other CYP enzymes including CYP1A1, 1A2, 2C9, 2C19, 2D6, and 2E1 had very low activities or activities below the limit of detection. In more detail, CYP3A4 exhibited the highest activity for 1,8-cineole hydroxylation, the rate catalyzed by CYP3A5 was about one-fourth of that catalyzed by CYP3A4. The rates observed using human liver microsomes and recombinant CYP3A4 were very high among other CYP3A4 substrates reported so far.[81] Based on these results further research concerning 1,8-cineole hydroxylation by CYP3A4 and 3A5 was performed yielding concordant outcomes. Moreover, this study succeeded in proving metabolites of 1,8-cineole converted by CYP3A4 and 3A5 in human urine.[82] As the metabolism studies are lacking information on whether CYP3A4/5 enzymes are induced or inhibited, respectively, another study that used a pooled human liver microsomal system can be invoked. Midazolam 1-hydroxylation and 4-hydroxylation, which are dependent on CYP3A4, were used as marker reactions. Both were moderately inhibited by 1,8-cineole.[49] All these results lead to the conclusion that 1,8-cineole is a very effective substrate for CYP3A enzymes in rat and human liver.

5.10. α-Thujone

Thujone occurs in nature as a variable mixture of α-thujone and β-thujone in a number of plants, among others in the EO of spices like some junipers or sage. The addition of pure

thujone to foods is not permitted in the United States, whereas the content of thujone is restricted in the European Union, because of the toxicity and potential epileptogenicity of thujone in infants and small children at high doses.[2] On the other hand, the content of thujone in sage and sage oil (which can be up to 50 % thujone) is not restricted, as they can be found on the Food and Drug Administration's list of generally recognized as safe (GRAS) substances. The effect on CYP enzymes that catalyze the metabolism of α-thujone is of interest and a study was undertaken to characterize the metabolism of α-thujone in human liver preparations *in vitro*. The metabolism was studied including ten CYP enzymes (CYP1A1/2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, 3A4, 2C19) by different marker reactions, and enzyme activities were measured in the presence or absence of α-thujone. All studied CYPs displayed some, but very low, activity. CYP2A6 followed by CYP3A4 were the key enzymes responsible for α-thujone hydroxylation, and to a lesser extent CYP2B6 and 2D6. Interestingly, α-thujone only inhibited CYP2A6- and 2B6-associated activities. CYP2A6 exhibits a rather wide interindividual variability, some general polymorphisms, and inducibility by common inducers. It would be interesting to find out whether individuals with CYP2A6 deletions are more susceptible to the CNS effects of thujone, and whether CYP3A4 and other enzymes are able to compensate for the lack of the CYP2A6 enzyme.[84]

5.11. Myristicin

Myristicin can be found in the EO of nutmeg, parsley, or black pepper, and is mainly responsible for the psychoactive and toxic properties of nutmeg when used at doses much higher than usual in cooking. It should be regarded with attention that myristicin is used as flavoring agent for example in nonalcoholic beverages. Due to the interesting pharmacological profile of myristicin a study aimed to examine the effect of myristicin on the expression of CYP enzymes in rat liver microsomes. Male Sprague-Dawley rats were administered myristicin before preparing the hepatic microsomes. The myristicin treatment significantly increased EROD, BROD, and to a small extent aniline-4-hydroxylation activities, which are marker reactions for CYP1A1/2, CYP2B1/2 and CYP2E1, respectively. Further experiments suggest that myristicin is an inducer of CYP1A1/2 and CYP2B1/2 via increasing mRNA levels, except the mRNA level of CYP2E1 was not increased and appeared to proceed through posttranscriptional regulation.[3] Further studies were made to investigate the metabolism of myristicin by

CYP enzymes. Human liver microsomes and the techniques of correlation with marker activities, selective chemical inhibition, immunoinhibition, and enzyme reconstitution were used. The results collectively suggested that CYP3A4 plays a major role in the oxidation of myristicin. CYP1A2 also contributes to the metabolism, albeit to a low extent. CYP1A2 can play an important role in human liver microsomes with high CYP1A2 and low CYP3A4 activities, as expression of CYPs 3A4 and 1A2 have been reported to be highly variable among human liver samples. Moreover, both CYP3A4 and 1A2 are known to contribute to the metabolism of a wide variety of chemicals, including drugs and carcinogens. Adverse effects when given in association with other xenobiotics can be expected, for instance, the accumulation of myristicin due to decreased oxidation may enhance psychopharmacological effects.[85]

6. Overview of the Results

EO component	Effect on CYP450 enzymes	Experimental setup
1,8-Cineole	Induction of 2B1 and 3A2[59]	RLM (from male Sprague-Dawley rats)
	Metabolism by 3A4, followed by 3A5[81]	Human recombinant CYP enzymes expressed in insect cells
	Moderate inhibition of 3A4[49]	HLM
Borneol	Inhibition of 2B and 2C[42]	RLM
	Weak Inhibition of 2B6[31]	HLM
Cadinene	Induction of 2B1 and 3A2[59]	RLM
Camphor	Metabolism by 2A6[58]	HLM, human recombinant CYP enzymes
Carvacrol	Metabolism by 1A2, 2A6 and 2B6[19]	HLM, human recombinant CYP enzymes
Carveol	Metabolism by 2B and 2C[42]	RLM (from adult male rats)
Carvone	Metabolism by 2C9 and 2C19[41]	Human recombinant CYP enzymes expressed in *trichoplusia ni* cells
Cedrol	Strong inhibition of 2B6 and 3A4[56]	HLM
Chamazulene	Inhibition of 1A2, 2D6 and 3A4[24]	Human recombinant CYP enzymes
Cinnamaldehyde	Strong inhibition of 2A6, weak inhibition of 2E1[57]	HLM, human recombinant CYP enzymes
Cis-, Trans-Rose oxide	Metabolism by 2B6, followed by 1A2 and 2C19[63]	Human recombinant CYP enzymes expressed in insect cells
Cis-, Trans-Spiroether	Inhibition of 1A2 and 3A4[24]	Human recombinant CYP enzymes

Citral	Inhibition of 3A[80]	RLM (from male Sprague-Dawley rats)
	Inhibition/Induction of 3A4[49]	HLM
	Moderate inhibition of 2B1[29]	RLM (from female Wistar rats, PB-treated)
	Strong inhibition of 2B6[31]	HLM
Estragole	Metabolism by 1A2 and 2A6[26]	HLM
Eugenol	Inhibition of 1A1 and 1B1[43]	Human MCF-7 cells; RLM (3-MC treated)
	Inhibition of 2E1[44]	RLM (from male Wistar rats)
Fenchone	Metabolism by 2A6 and 2B6[45–47]	HLM; human recombinant CYP enzymes expressed in *salmonella typhimurium* cells
Geraniol	Inhibition of 2B6[31]	HLM
	Metabolism by 2B6, followed by 1A1 and 3A5[48]	Human recombinant CYP bactosomes expressed in *E. coli*
(+)-Limonene	Inhibition of 2B1[29]	RLM (from female Wistar rats, PB-treated)
	Inhibition of 2B6[31]	HLM
	Inhibition/Induction of 3A4[49]	HLM
	Metabolism by 2C19, followed by 2C9[25]	HLM; human recombinant CYP enzymes expressed in *trichoplusia ni* cells and *E. coli*, respectively
Linalool	Slight inhibition of CYP2B6[31]	HLM
(-)-Linalool	Induction of 2A[30]	RLM (from male Wistar rats)
Menthol	Inhibition of 2A6 and 2A13[68,69]	HLM
Methyleugenol	Metabolism by 1A2, followed by 2C9 and 2C19[50]	HLM; human recombinant CYP enzymes

Myristicin	Induction of 1A1/2, 2B1/2, 2E1[3]	RLM (from male Sprague-Dawley rats)
	Metabolism by 3A4, followed by 1A2[85]	HLM
Safrole	Metabolism by 2C9, followed by 2D6, 2E1, and 2A6[4]	HLM, human recombinant CYP enzymes
(-)-Terpinen-4-ol	Metabolism by 2A6[61]	HLM, human recombinant CYP enzymes
(+)-Terpinen-4-ol	Metabolism by 2A6 and 3A4[62]	HLM, human recombinant CYP enzymes
Terpineol	Inhibition of 2B1[60]	RLM (β-naphthoflavone and PB-treated)
Thujopsene	Strong inhibition of 2B6 and 3A4[56]	HLM
Thymol	Metabolism by 1A2, 2A6, and 2B6[19]	HLM, human recombinant CYP enzymes
Verbenone	Metabolism by 2A6, followed by 2B6[52]	RLM; HLM; rat and human recombinant CYP enzymes expressed in *Trichoplusia ni* cells
α-Bisabolol	Strong inhibition of 2D6, weak inhibition of 2C9[24]	human recombinant CYP enzymes
α-Humulene	Strong inhibition of 3A/2B, weak inhibition of 1A2[73]	RLM; HLM
α-Pinene	Induction of 2B1[59]	RLM (from male Sprague-Dawley rats, PB-induced)
	Inhibition of 2B1[29]	RLM (from female Wistar rats)
α-Terpinene	Inhibition of 2B1[29]	RLM (from female Wistar rats)
α-Thujone	Metabolism by 2A6, followed by 3A4; Inhibition of 2A6 and 2B6[84]	HLM
β-Caryophyllene	Strong inhibition of 3A/2B, weak inhibition of 1A2[73]	RLM; HLM
β-Caryophyllene oxide	Strong inhibition of 3A/2B, weak inhibition of 1A2[73]	RLM, HLM

β-Cedrene	Strong inhibition of 2B6, moderate inhibition of 3A4[56]	HLM
β-Myrcene	Strong inhibition and induction of 2B1, weak inhibition and induction of 1A1[29,79]	RLM (from female Wistar rats)
	Moderate induction of 3A4[49]	HLM

7. Discussion

As presented before, many commonly used EO components were tested to have an inducing and/or inhibiting effect on the activity of metabolizing CYP enzymes. The large variety of assays used to detect inhibiting or inducing properties of EO components makes it difficult to assess the clinical relevance of the different studies. To illustrate this point, the diversity of the studies complicating comparability is discussed here.

First of all, the majority of the studies are *in vitro* studies, but *in vitro* inhibition/induction of CYP enzymes does not necessarily need to be translated into *in vivo* drug interactions. Therefore, further *in vivo* studies are needed.

A better approach to pharmacological impacts of EO components in humans deliver experimental setups consists of using human liver microsomal systems and human recombinant CYP enzymes instead of the commonly used rat liver microsomes. Although often similar results are obtained with liver cells of both species, there exist inter-species differences regarding the action of potential inhibitors toward individual enzymes or low homology in amino acid sequence of CYP enzymes.[30] This demonstrates a certain limitation concerning drug interaction studies in experimental animals.[41,73] Furthermore, rats were often treated with CYP gene inducers like β-naphthoflavone (inducer of the CYP1A subfamily), phenobarbital (inducer of CYP2B1), or 3-methylcholanthrene (3-MC) (inducer of CYP1) to raise the amount of CYP isoenzymes in order to get significant results more easily, which may influence the outcomes.

Even though inducing CYP subfamilies or testing separated selected CYP enzymes makes it easier to detect inhibiting/inducing properties of EO components, these investigations do not indicate the physiological amount of the different CYP subfamilies in the human liver which reflect admittedly high interindividual differences. Only few studies worked with physiological CYP cocktails as a model system of human metabolism.[48]

Furthermore, it should be mentioned that in most cases only selected CYP enzymes were tested which play important roles in metabolism of commonly prescribed drugs. This

does not exclude that other CYP enzymes may be affected, too. Moreover, most studies are about inhibition of CYP activity by EO components and their metabolism, but it has to be considered that substrates of CYP enzymes can act both as inducer and inhibitor, like e.g. β-myrcene.[79] Referring to this, there is further need of studies concerning the induction of CYP activity by EO components.

Finally, we want to point out that all afore discussed interactions of EO-constituents with CYP-enzymes do not consider that an EO consists of more than two or three main compounds. Using an EO for such an experiment the entirety of all molecules (in small as well as in trace amounts) is involved in this CYP-interaction and yet we do not know which share each constituent contributes to the final outcome. Such studies would be worth to be performed.

8. Conclusion

CYP-mediated metabolism represents a major route of elimination of many drugs, which can compete for the same CYP enzyme. Therefore, drug interaction by mutual inhibition between drugs and EO components which affects CYP activity is almost inevitable, although likelihood of interaction rises when EO components are administered simultaneously over a long period, or EOs and their components are used extensively. As metabolic clearance and toxic effects of different compounds might be modulated (increased or decreased) by an EO component-induced change in the activity of CYP enzymes, drug interaction with serious clinical consequences will arise the smaller the difference is between toxic and effective concentration of a drug.

9. Abbreviations

3-MC	3-Methylcholanthrene
BROD	Benzyloxyresorufin-O-dealkylation
EO(s)	Essential Oil(s)
EROD	Ethoxyresorufin-O-deethylation
HLM	Human liver microsomes
MROD	Methoxyresorufin-O-demethylase
NNK	Nicotine derived nitrosamine ketone (= 4-(methylnitrosamino)-1-(3-pyridyl)-1-buanone
PB	Phenobarbital
PROD	Pentoxyresorufin-O-dealkylation
RLM	Rat liver microsomes

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