Thujone, a widely debated volatile compound: What do we know about it?

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Abstract Thujone is a volatile monoterpene ketone of plant origin which is produced by several plants that are frequently used for flavoring foods and beverages. The use of thujone and thujone-containing plant parts for human consumption is currently regulated by the European Parliament and Council and the European Medicines Agency. The best known neurotoxic effects are connected to the GABA-gated chloride channel, where α -thujone is a modulator roughly two to three times as potent as the β isomer. Based primarily on in vitro experiments, genotoxicity and carcinogenic properties of thujones have also been detected in parallel with antimutagenic and immune-modulatory effects. Some of the controversial effects seem to be strongly dose-dependent. Data on antidiabetic and antimicrobial activities of thujones may show new ways to use them. This review also describes the main steps of the biosynthetic route of thujones and their occurrence in the plant kingdom. The accumulation of these compounds seems to be more abundant in some plant families (e.g. Asteraceae or Cupressaceae) than in others. Four species (*Artemisia absinthium*, *Salvia*

officinalis, *Tanacetum vulgare*, *Thuja occidentalis*), characterised by a large intraspecific chemical variability, have been evaluated in detail from chemotaxonomical aspects. Experimental results show that the phenotypic manifestation and quantity of thujones in the essential oils depend on the plant organ and its developmental phase. Besides, weather conditions and growth habitat might also influence the ratios, as well as the possibly unique responses of the individual species. Unfortunately, comparison and an exact evaluation of the references is hampered by very diverse methods of treatment and analysis; sometimes reliability itself seems questionable. In order to optimise the safe use of thujone-containing preparations, it would be necessary to do further systematic studies from the plant biological, toxicological and pharmaco-kinetic points of view.

Keywords *Artemisia* · Chemotype · Essential oil · Food safety · *Salvia* · *Tanacetum* · Toxicity · *Thuja*

Abbreviations

EMA	European Medicines Agency
EO	Essential oil
GABA	Gamma-aminobutyric acid
GC	Gas chromatography
GPP	Geranyl diphosphate
HMPC	Committee on Herbal Medicinal Products
IUPAC	International Union of Pure Applied Chemistry

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NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
SCF	Scientific Committee on Food
SS	Sabinene-synthase

Materials and search strategy

This review is focused on the most characteristic facts and important up-to-date scientific information of the much debated toxic volatile monoterpene compound thujone, which might even gain pharmacologic significance in the future. Beside the human aspects, the article presents the biosynthetic route and occurrence of thujone in the plant kingdom, as well as the influencing factors of its variability. 172 published references have been cited in this review. First of all, we evaluated studies obtained from electronic databases according to a search strategy designed to retrieve abundant and new data. The review included databases such as Google Scholar, PubMed, Science Direct, SciFinder, Web of Science, etc. Additionally, other online sources (Research Gate, National Center for Biotechnology Information (NCBI), Springer Nature Open Access, Wiley Online Library, etc.) were used. In these cases no date restrictions were fixed. Besides, the bibliographies of the available publications were checked for additional references like reports, dissertations, etc. We also carried out a manual search in the Abstract books of International Symposium on Essential Oils (ISEO), International Society for Horticultural Science (ISHS) conferences, Breeding Research on Medicinal and Aromatic Plants (BREEDMAP) and Gesellschaft für Arzneipflanzenforschung (GA) in the last 10 years. Additionally, a physical search was carried out using all volumes of Journal of Essential Oil Research (JEOR) and available monographs at the library of Szent István University. The search was focused on English literature references and in some cases on German ones. The information gained from different sources was compared and evaluated to determine which facts and results have been confirmed, put into the practice, improved, extended, or even corrected.

In the present paper—if not explained otherwise—the thujone percentages mentioned refer to the ratio of

this compound in the essential oil as GC area percentage.

Chemical characteristics of thujone and its biosynthesis

The monoterpene ketones, α -thujone (3-thujone) and β -thujone (3-isothujone) (Fig. 1) are natural substances found in plants, commonly used for flavoring of foods and beverages (Lachenmeier and Uebelacker 2010). They are constituents of essential oils (EO). Their IUPAC name: (1S, 4R, 5R)-4-methyl-1-(propane-2-yl) bicyclo [3.1.0] hexan-3-one. In addition to the naturally occurring (–)- α -thujone and (+)- β -thujones, two other enantiomeric forms are known: (+)- α -thujone and (–)- β -thujone.

Till now, the biosynthesis and genetic regulation of the thujone pathway has been studied in detail basically only in two species: *Salvia officinalis* (European Parliament and Council 2008) and *Thuja plicata* (Foster et al. 2013) both of them accumulating mainly α -thujone. Additionally, there are some older data indicating thujone biosynthesis in tansy (*Tanacetum vulgare*).

The synthesis of thujone type compounds (thujane skeleton) (Fig. 2) starts from the general precursors geranyldiphosphate (GPP) (Lichtenthaler 1999) and neryl-diphosphate through a four-step biosynthetic pathway (Schilmiller et al. 2009).

The first monoterpene in this route is sabinene, the formation of which is catalyzed by the enzyme sabinene-synthase (SS). Sabinene has been demonstrated to be the precursor of thujone in *A. absinthium*, *T. vulgare* and *S. officinalis* (Karp and Croteau 1982).

Fig. 1 Thujones

Fig. 2 Proposed biosynthesis of α - and β -thujones in *Thuja plicata*

This enzyme is considered as a ‘hotspot’ of chemotype evolution in sage (Grausgruber-Gröger et al. 2012). SS in *Thuja plicata* is supposed to be regulated by a single genomic locus (Foster et al. 2013). The latter authors proposed it as a candidate for marker-assisted selection in breeding because of its basic significance as a precursor of thujones. While the biosynthesis of other monoterpenes from the GPP precursor is frequently under transcriptional control (Schmiderer et al. 2010; Xie et al. 2008), in sage (*S. officinalis*) no direct correlation could be found between mRNA level and that of the sabinene end product, which reflects a more complex genetic/metabolomic regulation (Grausgruber-Gröger et al. 2012).

From sabinene, thujone is formed in three consecutive steps (Fig. 2). The next biosynthetic step leads from sabinene to either (+)-*trans*-sabinol in the case of gymnosperm *T. plicata* (Gesell et al. 2015) or (–)-*cis*-sabinol in the angiosperm *S. officinalis* (Karp and Croteau 1982). Gesell et al. (2015) identified CYP750B1 and CYP76AA25 as the enzymes catalyzing the hydroxylation of (+)-sabinene to *trans*-sabin-3-ol. The role of the first enzyme is essential and it seems to be strongly substrate specific, while the CYP76AA25 has a broader substrate spectrum and its transcript profiles were not well correlated with thujone accumulation (Gesell et al. 2015). Earlier, Karp and Croteau (1982) suggested (–)-*cis*-sabinol to be the intermediary of the same pathway in sage; however, it is not known if the synthesis in *Artemisia* and other species is going on through *trans*- or *cis*-sabinol. In some manuscripts the presence of *trans*-sabinol has been demonstrated in the EO of *A. absinthium* (Blagojević et al. 2006; Judzentiene and

Budienne 2010); therefore it might be the candidate as an intermediary here.

After sabinol, only the (+) isomer of sabinone is mentioned as the next intermediary in thujones’ biosynthesis. Its formation is an NADPH and oxygene dependent hydroxylation. Based on investigations of sage and tansy, Dehal and Croteau (1987) suggested that dehydroxigenases exhibit a significant degree of specificity for “substrate-groups”.

From sabinone, the formation of the thujones is an NADPH dependent stereoselective reduction. The corresponding enzymes of this transformation and the formation of the different isomers of thujone (α - or β -thujones), has not yet been cleared up either. Based on the references, some species accumulate only one of them, while in other plants both isomers may be present (see below, “Occurrence of thujone in aromatic plants, chemotaxonomic aspects” and “Intraspecific variability of thujone content in selected aromatic plants” sections).

Regulatory aspects of thujone containing products

There is an open debate on the effect of monoterpene thujone on human health. As for the potential benefits and/or harms of thujone, we should mention the special history of wormwood, which is connected to *absinth*, a popular strong spirit aromatised by *Artemisia absinthium*. At the beginning of the twentieth century, the production of *absinth* was prohibited in several countries as its consumption was associated with a range of severe adverse symptoms called *absinthism*, including convulsions, blindness,

Table 1 Estimation of the thujone exposure via flavoring in food from Amberg-Müller (2007)

Foods/beverages	Proposed limit (mg/kg)	Intake (µg/person/day)		Intake (µg/kg bw/day)	
		Mean	97.5th percentile	Mean	97.5th percentile
Sage-flavoured sausages	10	175.3	536.6	2.5	7.7
Stuffing	10	74.0	204.3	1.1	2.9
Herb vinegar	5	12.6	86.9	0.2	1.1
Other meat dishes	10	389.3	1148	5.7	15.3
“Sage Derby” cheese ^a	10	128.6	128.6	1.4	1.4
Salad dressings	10	55	152	0.9	2.5
Vermouth	2	44.2	177.9	0.7	2.3
Liqueurs and bitters	10	99.7	474	1.6	5.9
Total (foods and beverages) (excl. sweets) ^b		244.8	911.4	3.6	13.5
Sweets ^b	50	195.8	900.2	3.0	13.4
Total (foods and beverages) (incl. sweets) ^b		263.5	984.7	3.9	14.2

^aOnly 1 consumer^bTwo intake estimates were made, including and excluding the contribution from sweets

hallucinations and mental deterioration (Lachenmeier et al. 2006a; Padosch et al. 2006). There are even some detailed cases reported in the literature from the very early times until recently. It was supposed that thujone was to blame for those cases (Weisbord et al. 1997). The study of Dettling et al. (2004) on 25 healthy subjects who consumed thujone-containing alcoholic beverages showed that the simultaneous administration of alcohol and high concentrations of thujone (100 mg/l) may severely affect attention performance.

Later, numerous investigations proved that thujone plays a minor or non-existent role in the clinical picture of *absinthism* (Lachenmeier et al. 2006b). According to these authors, thujone should not be the main reason for the symptoms, but adverse effects are predominantly caused by high alcohol concentration (> 50 vol%) that may lead to—and might have led to—major health and social problems, both today and in the eighteenth century. Strang et al. (1999) also suggested that acute alcohol intoxication was the main reason for the syndrome of *absinthism*. It could be supported in a pilot study of Kroner et al. (2005), who examined blood concentrations of both thujone and ethanol after the consumption of *absinthe*. They detected high blood alcohol concentrations (> 1 g/l) but—as expected—no thujone in the blood samples.

Recently, the species of *Artemisia absinthium*, *Salvia officinalis* and other thujone-containing flavoring plants are permitted in foods, because of the

removal of the general limit regulation. However, according to the regulation of the European Parliament and Council (2008), thujone in chemically pure form is not allowed to be added to foods, while it may be indirectly introduced into foods by using plants containing thujone. In addition, the European Medicines Agency (EMA) also proposed a daily maximum intake of thujone in *Absinthii herba*, which was set at 3.0 mg thujone/day/person as acceptable for a maximum duration of 2 weeks in the wormwood (*A. absinthium*) monograph (EMA/HMPC 2008a). During assessments of sage (*S. officinalis*), a low level of thujone in sage leaf preparations is preferable, and a daily intake of 5.0 mg thujone/day/person is acceptable for a maximum duration of 2 weeks (EMA/HMPC 2008b). Nevertheless, some other food products (Table 1) such as sweets, salads dressings, or alcoholic drinks may contain thujone in considerable amounts (Amberg-Müller 2007).

Biological activities and new ways for beneficial utilization of thujone

Thujone-containing species are frequently used as natural remedies in ethnobotanical applications. Wormwood, as a well-known species containing thujone, has been used in folk remedies in Europe as a gynecological agent for abortion and to induce

menstruation. Even now it is still used in Yemen to alleviate the pains associated with parturition (Rätsch 2005). According to Tan et al. (1998), wormwood has been used as traditional medicine in Asia due to its antimalarial, antiviral, antitumor, spasmolytic and other effects. Essential oil of *T. occidentalis* containing thujone as a major compound, has been widely used in folk medicine for the treatment of hepatoprotection, bronchial catarrh, rheumatism, psoriasis, and uterine carcinomas (Küpeli Akkol et al. 2015; Dhiman et al. 2012). In homeopathy, the extract of *T. occidentalis* containing about 65% α -thujone, is used as mother tincture in preparations against a number of diseases (Torres et al. 2016). Thuja preparations with thujone were regularly used by the American Indian traditional healers. The soup and the tea prepared from the inner bark of the soft twigs could relieve constipation and headache (Ellingwood 1919). It has been also used for the treatment of polyps, birthmarks, wounds, and as a painkilling and anthelmintic remedy (Pudelek et al. 2019).

Biological activities of thujone have now been justified by modern instruments, pharmacological and clinical investigations. The mechanism of neurotoxicity of thujones has been widely investigated in animals, cultured neuronal cells and expressed receptors. Thujones are modulators of the GABA-gated chloride channel where α -thujone is about twofold to threefold more potent than β -thujone. The neuronal effect was declared to be completely reversible (Pelkonen et al. 2013). Czyżewska and Mozrzyms (2013) proved that the effect of (α -thujone may depend on the subtype of GABA_A receptors because of their varying sensitivity. Besides, cannabinoid CB1 and serotonin 5-HT₃ receptors were also suggested as potential targets of thujone. Thujones are rapidly metabolized. According to in vitro pharmacokinetic animal studies, 7-hydroxy-(α -thujone is the main metabolite of (α -thujone, and 4-hydroxy- β -thujone is that of the other isomer. In human tissues CYP2A6 seems to be the principal metabolic enzyme of thujones (Pelkonen et al. 2013). As hydroxy-thujones were detected in larger concentrations in the brain, their potential importance in the neurotoxicity has been suggested (Höld et al. 2000).

Risk assessment and setting of limit values are difficult as data originates from studies under widely varying conditions. Thujone at 25 mg/kg showed behavior changes, mortality, weight loss, decrease in

the organ weight and alterations in both hepatic and renal functions of 6–8 week-old mice (Siveen and Kuttan 2011a). In single dose toxicity tests 45 mg/kg body weight was determined as the i.p. LD₅₀ (Höld et al. 2000) and 230 mg/kg b.w. as oral LD₅₀ value of thujones in mice (Pelkonen et al. 2013). Long term administration of sub-convulsive doses may lead to chronic toxicity. According to the latter authors, the best estimate for maximum daily intake by humans in food or herbal products is around 3–7 mg/day.

Beside neurotoxicity, thujones may show genotoxic and carcinogenic properties; however, antimutagenic and anticarcinogenic effects can appear, too (Nikolić et al. 2015). Most likely, these effects depend on the investigated cell type, genetic background, experimental setup and concentrations applied. A 20-h treatment of monkey Vero cells with 1–4 mM of thujone indicated antigenotoxic effects in lower dosages, but severe genotoxicity in the higher concentrations (Nikolić et al. 2011).

In coincidence with the widespread ethnobotanical application of thujone-containing drugs, in the recent period several positive physiological effects of thujone have been demonstrated. There is some data on the possible sophisticated utilizations as well. Especially interesting are the potential immuno-modulatory and antitumor activities. Recently, Küpeli Akkol et al. (2015) revealed the anti-carcinogenic potential of α -thujone. This compound inhibited the proliferation of glioblastoma cells and angiogenesis, and in a rat model it stimulated an anticancer immune response (Torres et al. 2016). Immuno-modulatory properties of thujone have been determined by Siveen and Kuttan (2011a) on both humoral and cell mediated immune systems as well as on solid tumor development. The same authors demonstrated inhibition of melanoma metastasis by thujone in a mouse model where pulmonary metastasis was induced by B16F-10 melanoma cells in mice (20–25 g, 6- to 8-week-old males), with special emphasis on the mechanism of action (Siveen and Kuttan 2011b). An in vitro study by Biswas et al. (2011) showed the pro-apoptotic and cytotoxic effect of both the crude ethanolic extract of *Thuja occidentalis* and the thujone-rich fraction separated from it, on the A375 cell line. Küpeli Akkol et al. (2015) proved that the *T. occidentalis* essential oil and its active component, α -thujone demonstrate a beneficial effect in the treatment of polycystic ovary syndrome (PCOS) in a rat model. Later, Zhou et al.

(2016) found that α -thujone stimulates anticancer immune response. This compound enhances the proliferation of CD3AK (anti-CD3 antibody induced activated killer) cells and cytotoxicity to colon cancer cell line, especially at a dose of 0.15 $\mu\text{mol/l}$. A recent in vitro study demonstrated promising effects of α -thujone against glioblastoma cells whose viability and invasive potential were decreasing. In a dose-dependent manner, α -thujone inhibited carcinoma cell proliferation and acted as a pro-apoptotic, presumably through the induction of oxidative stress in GBM cells (Pudełek et al. 2019).

Cytotoxicity (IC_{50} : 1.0–2.8 mM) and inhibition of cell proliferation after thujone administration was also detected in other human cell lines by Nikolić et al. (2015). At the same time, low doses of thujone may activate the DNA repair mechanism and act as antigenotoxic agents.

Alkhateeb and Bonen (2010) identified the antidiabetic properties of thujone (0.01 mg/ml) in an in vitro study, showing that the compound improved insulin sensitivity in skeletal muscle. Thujone in a dosage of 5 mg kg^{-1} administered orally for 28 consecutive days was found to correct the lipid profile (cholesterol and triglycerides) in diabetic rats (Baddar et al. 2011). Sivropoulou et al. (1997) indicated that the two thujone isomers showed variable degrees of antimicrobial activity against the bacteria tested. In addition, thujones exhibited significant antiviral activity against *Herpes simplex* virus in vitro.

The strong antimicrobial effect of thujone compounds and thujone-containing EOs may be promising in further pharmaceutical applications (Tsiri et al. 2009). The same effect may be used for the antimicrobial decontamination of buildings (Hudson et al. 2011).

Occurrence of thujone in aromatic plants, chemotaxonomic aspects

Based on references of the last 5 decades, it appears that the most frequently mentioned species concerning the thujone content of their EOs are common sage (*Salvia officinalis* L.), tansy (*Tanacetum vulgare* L.), white cedar (*Thuja occidentalis* L.) and wormwood (*Artemisia absinthium* L.). These plants belong to three different families: Lamiaceae, Asteraceae and Cupressaceae. Thus, interestingly, they do not seem to

be in any special taxonomic relationship with each other. Therefore, the question arises whether the formation of thujone(s) may be considered as a chemotaxonomic marker.

The Lamiaceae family contains a lot of very popular and economically important EO producing plants, like lavender, mint, thyme, lemon balm, rosemary, oregano, basil, etc. In these genera, thujone is rarely mentioned—if at all—as an EO constituent. As for lavandin and lavender oils, thujones are basically not known and have never been reported as components above 1% (Angioni et al. 2006; González-Rivera et al. 2016; Hassiotis et al. 2014; Lis Balchin 2002 etc.). In mint (*Mentha*) species, traces of thujone have been mentioned for several species like *M. aquatica*, *M. arvensis*, *M. rotundifolia* (Azimova and Glushenkova 2012a, b, c), *M. piperita* (Taherpour et al. 2017). Nevertheless, thujones are very rare in mints (Lawrence 2007; Salehi et al. 2018) and have been detected among the EO constituents in considerable concentrations only in a few exceptions, like in samples of *M. longifolia* from the Tajikistan area: 0.3–3.2% of both (α and β thujones (Sharopov et al. 2012). *Ocimum* species are usually free of thujone (Carović-Stanko et al. 2010; Grayer et al. 1996; Hiltunen and Holm 2006; Nurzyńska-Wierdak 2012 etc.) although in rare cases it was detected in some samples, e.g. traces (0.36%) in an ornamental variety (Carovic-Stanko et al. 2010) or in Albanian varieties up to 6.6% (Cheliku et al. 2015). As further examples, there are no references to the presence of thujones in the EO of *Melissa officinalis* (e.g. Seidler-Łożykowska et al. 2017; Németh-Zámboriné et al. 2019); or *Perilla frutescens* (e.g. Chauhan et al. 2013; Ghimire et al. 2017; Yu et al. 1997).

On the other side, in the genus *Salvia* several species are characterised by thujone accumulation. It is found not only in the widely known *S. officinalis* (e.g. Craft et al. 2017; Grausgruber-Gröger et al. 2012), which is discussed here in detail later on, but in many others like *S. fruticosa* (Karousou et al. 1998), *S. triloba* (Longaray Delamare et al. 2007), *S. pilifera* (Kelen and Tepe 2008), etc. However, no detectable amounts or only traces of thujones were found in other species, like *S. sclareoides* (Sepahvand et al. 2014), *S. aramiensis* (Kelen and Tepe 2008), so it can not be established that thujones would be universal compounds of the genus *Salvia*.

It should be mentioned that thujone may accumulate in some less frequently used and investigated species of the Lamiaceae family, too. For example, higher amounts were detected in *Plectranthus* species, where α -thujone accumulated up to 28% of the EO (Mota et al. 2014) and in *Phlomis ferruginea* in 5.5% (Formisano et al. 2006). Similarly, α -thujone was detected by Kaurinovic et al. (2010) in 2.1–3.3% in samples of *Marrubium peregrinum*, nevertheless even the accumulation rate of total volatiles was very low (0.09–0.14% DW.).

In the Asteraceae family, thujone is a frequent component of yarrow (*Achillea*) species. From 35 species evaluated in summary reports, 12 contained either (α - or β -thujone or both in the EO, and in five species also as a major compound (Németh 2005; Kindlovits and Németh 2012; Turkmenoglu et al. 2015). At the same time, it should be mentioned that the number of studies on the individual species is varying and in several cases both thujone-containing and thujone free samples of the same species have been reported. Thus, in spite of the large number of investigations on yarrow EOs, until now the presence of thujone as a universally occurring component in any of the species can not be declared.

Similarly, in the genus *Artemisia*, numerous species are able to synthesize thujones. Considerable (above 20% of the EO) proportions of thujone were described in *A. arborescens* (Militello et al. 2012), *A. herba-alba* (Sbayou et al. 2014), *A. pontica* (Talzhhanov et al. 2005), *A. judaica* (Saad and Abdelgaleil 2014), *A. porrecta* (Tétényi 1970), *A. santolinifolia* (Khalilov et al. 2001), *A. tridentata* (Tétényi 1970), and *A. vulgaris* (Misra and Singh 1986; Blagojević, et al. 2006). Lower concentrations were mentioned in *A. anomala* from China (Zhao et al. 2013). The distribution and ratio of the two isomers varies on a large scale and unfortunately, some older sources do not mention the isomeric form at all. The review of Abad et al. (2012) lists furthermore *A. arborescens*, *A. distans*, *A. frigida*, *A. fukudo*, *A. kulbadica*, *A. lavandulaefolia*, *A. pontica*, *A. scoparia*, *A. sieberi* and *A. spicigera* as species containing either (α - or β -thujone as a major component in the EO. At the same time they found that some accessions of the same species may accumulate other major compounds and only traces of thujones. Other references ascertain the large intraspecific variability of wormwood species concerning the presence of thujones among their volatiles. Both thujone-

containing and thujone-free samples were detected in *A. campestris* from Lithuania (Judzentiene and Budiene 2014), *A. molinieri* from France (Masotti et al. 2003) and *A. vulgaris* from Lithuania (Judžentien and Buzelytė 2006). The best known species, *A. absinthium* shows an extremely high variability, too (Nguyen et al. 2018c). Thus, similar to yarrow species, the overall presence or absence of thujones in any of the wormwood species can not be established. At the same time, several representatives of the large *Artemisia* genus have not been investigated so far.

Thujones also appear frequently in the EO accumulating species of the genus *Tanacetum*, either as a major component like β -thujone (up to 82%) in *T. vulgare* (Keskitalo et al. 2001) α -thujone up to 70% in *T. argyrophyllum* (Akpulat et al. 2005) or as minor ones like β -thujone in 3.6% in *Tanacetum aucheranum* and (α -thujone in 1.1% in *T. chiliophyllum* (Salamci et al. 2007). In parallel, thujone-free species are also known in large number, among others *T. annuum* (Greche et al. 2000) and the pharmaceutically most important species *T. parthenium* (Akpulat et al. 2005). Data on the intraspecific variability of these plants concerning EO and thujones are scarce except *T. vulgare* (see “[Intraspecific variability of thujone content in selected aromatic plants](#)” section).

Beside the most frequently mentioned genera and species of the Asteraceae family, thujone may appear in other taxa, too. Recent references mention e.g. 32% α -thujone in *Cotula cinerea* (Ghouti et al. 2018), 51.8% α -thujone and 14.6% β -thujone in *Anthemis mixta* (Tadrent et al. 2016), 84% β -thujone in *Senecio chrysanthemoides* (Mengi et al. 1995) or 5.7% α -thujone may appear in some accessions of *Waldheimia glabra* (De et al. 2017). Characteristically, these thujone-accumulating species of the family are not closely related to each other, either botanically or geographically.

Among gymnosperms, the Cupressaceae family consists of several species accumulating volatiles especially in genera *Cupressus*, *Juniperus*, and *Thuja*. However, concerning their thujone-producing potential, these taxa are rather different. In the genus *Cupressus*, thujone has not been identified in any of the following species which were subjects of phytochemical investigations: *C. arizonica* (Ali et al. 2013), *C. atlantica*, *C. chengiana*, *C. doclouxiana*, *C. guadalupensis*, *C. macnabiana*, *C. sachmeriana* (Pierre-Leandri et al. 2003), *C. tonkinensis* (Thai

et al. 2013), *C. torulosa* (Padalia et al. 2013), *C. funebris* (Carroll et al. 2011; Pierre-Leandri et al. 2003), and *C. sempervirens* (Mazari et al. 2010; Ulukanli et al. 2014).

In terms of our study, the genus *Juniperus* presents a much larger intrageneric and intraspecific variability. In numerous species, like *J. chinensis* (Carroll et al. 2011), *J. communis* (Carroll et al. 2011; Lohani et al. 2010), *J. excelsa* (Unlu et al. 2008), *J. phoenicea* L. (Mazari et al. 2010) or *J. saltuaria* (Wedge et al. 2009), *J. virginiana* (Chelsey et al. 2015), and *J. scopulorum* (Zheljazzkov et al. 2013) thujones either appear at most in traces, or not at all. Based on the reports, *J. foetidissima* shows a large intraspecific variability as both α - or β -thujone may be present as main compounds, but depending on the origin there are also accessions free of thujones (Tunalier et al. 2002) or accumulating only traces of them (Asili et al. 2010). Similarly, samples of the Chinese *J. squamata* var. *fargesii* may contain β -thujone in a high proportion (Adams et al. 1996) or being free of it entirely (Wedge et al. 2009). Only 1.1% thujone was detected in *J. indica* by Lohani et al. (2010), while 25% β -thujone was the main compound of the same species in the analysis of Adams and Chaudhary (1996). As comparison studies show, there does not seem to be a clear connection between the EO composition and the origin of the samples: *J. cedrus* from the Canary Islands, *J. brevifolia* from the Azores, *J. communis* from Eastern Europe, *J. conferta* from Japan, *J. formosana* and *J. navicularis* from Portugal, *J. oblonga* (from Albania, Turkey and Iran), and *J. oxycedrus* from Asia were all free of thujones (Adams 1998). Interestingly, the biosynthetic precursor of the thujones, sabinene, appears frequently as a main constituent in *Cupressus* and *Juniperus* EOs (Padalia et al. 2013; Pierre-Leandri et al. 2003; Zheljazzkov et al. 2013).

Similarly, the genus *Thuja* seems to exhibit a wide diversity in its EO composition. According to Tsiri et al. (2009) the major component of both *T. occidentalis* and *T. plicata* was α -thujone (30–77%), with considerable concentrations of β -thujone (2–9%); although characteristic differences were registered among cultivars of both species. On the other hand, leaves of *T. orientalis* L. (syn. *Biota orientalis* (L.) Endl. or *Platycladus orientalis* (L.) Franco) either from the Himalaya (Guleria et al. 2008) or from Syria (Khubeiz et al. 2016) as well as cones of the same

species from Nigeria (Ololade et al. 2014) each provided a thujone-free oil, although in a sample from London thujones were described as major components (Banthorpe et al. 1973). Data on the volatiles of the other *Thuja* species are spotty. The Japanese *Thuja standishii* may contain a small amount (1–2%) of thujone (Banthorpe et al. 1973) while the endangered Chinese *Thuja sutchuenensis* produces thujone-free oil (Lei et al. 2010).

In Cupressaceae, thujone is not exclusively synthesized in the above mentioned plant families, but appears in other taxa, too. In addition to this family, Gildemeister and Hoffmann (1963) listed others, such as Pinaceae (genus: *Tsuga*), Rutaceae (genus: *Boronia*), and Verbenaceae (genera: *Aloysia*, *Acantholipia*) as accumulating thujones in the EOs.

Intraspecific variability of thujone content in selected aromatic plants

According to the available references, the content of thujone compounds in EOs displays a significant intraspecific variation even in the most well known thujone accumulating species like garden sage, white cedar, tansy and wormwood. This variation might be due to several factors such as genetic potential, ontogenetic factors, plant organs or environmental effects—as demonstrated in the next section. Table 2 summarizes the information of the thujone isomers regarding their presence and concentration in EOs of these most common thujone containing species. It should be mentioned that the sampling, the analytical methods and quantification are different in many cases, therefore most of the data basically represent ages and intervals in comparison.

It can be established that one of the isoforms usually dominates, and its presence seems to be a characteristic of the species. While sage and white cedar generally contain more α -thujone, tansy and wormwood usually contain more β -thujone, although contradictory examples can also be found. The occurrence of either of the isomers without detection of at least traces of the other one has been very rarely published in these species (e.g. wormwood: Judzietiene and Budiene 2010 or tansy: Goudarzi et al. 2015).

A review of Nguyen and Németh (2016) has summarized the concentrations of thujones in *A. absinthium* oils, which ranged between 36.8 and

Table 2 Intraspecific variability of thujone content in four characteristic thujone containing species

Species	Plant part	α -Thujone (per cent of GC area)	β -Thujone (per cent of GC area)	Origin of sample	Reference
<i>Artemisia absinthium</i>	L	23.0–25.4	49.5–66.8	Belgium	Nguyen et al. (2019)
	F	15.9–24.5	51.5–60.9	Belgium	Nguyen et al. (2019)
	AP	0.5	10.1	Canada	Lopes-Lutz et al. (2008)
	L	1.1–2.5	20.9–48.6	Croatia	Juteau et al. (2003)
	L&F	0.2–1.8	14.0–43.2	Croatia	Juteau et al. (2003)
	AP	2.4	64.6	Estonia	Orav et al. (2006)
	AP	1.0–1.8	2.1–85.2	Germany	Nguyen et al. (2018c)
	AP	4.5	38.7	Greece	Orav et al. (2006)
	AP	2.2	40.6	Italy	Orav et al. (2006)
	AP	0.9	18.6	Iran	Rezaeinodehi and Khangholi (2008)
	AP	3.2–23.5	tr.–30.7	Lithuania	Deiml et al. (2004)
	AP	tr.–36.8	tr.–48.9	Lithuania	Judzentiene and Budiene (2010)
	L	39.7	7.3	Morocco	Derwich et al. (2009)
	AP	20.8	13.7	Russia	Khalilov et al. (2001)
	AP	12.7	7.6	Siberia	Orav et al. (2006)
	AP	3.4	33.1	USA	Tucker et al. (1993)
	L&F	0.9	69.9	USA	Nin et al. (1995)
<i>Salvia officinalis</i>	L	40.9	5.6	Brazil	Porte et al. (2013)
	F	39.0	4.0	Canada	Perry et al. (1999)
	F	37.0	3.0	Denmark	Perry et al. (1999)
	AP	22.8	9.9	Egypt	Ahl et al. (2015)
	F	32.0	8.0	Germany	Perry et al. (1999)
	F	11.0	11.0	Hungary	Perry et al. (1999)
	L	16.7–24.7	4.4–8.6	Hungary	Nguyen et al. (2019)
	F	9.8–14.2	2.9–3.4	Hungary	Nguyen et al. (2019)
	F	39.0	4.0	Italy	Perry et al. (1999)
	AP	9.1–25.1	1.1–5.0	Iran	Mirjalili et al. (2006)
	AP	1.2–3.7	0.1–9.9	Jordan	Abu-Darwish et al. (2013)
	L	41.0	3.0	New Zealand	Perry et al. (1999)
	AP	10.3–49.7	1.5–44.9	Serbia	Jug-Dujaković et al. (2012)
	AP	28.2	5.1	Serbia	Radulović et al. (2017)
	AP	22.8–41.7	6.1–15.6	Spain	Cutillas et al. (2017)
	F	7.0	17.0	Switzerland	Perry et al. (1999)
	AP	15.7–25.2	5.3–7.1	Tunisia	Ben Farhat et al. (2016)
	L	21.4	3.9	Tunisia	El Euch et al. (2019)
<i>Thuja occidentalis</i>	L	38.4–64.4	2.9–10.7	Poland	Lis et al. (2016)
	L	30.4–40.5	6.5–9.0	Slovakia	Svajdlenka et al. (1999)
	L	49.75	3.14	Turkey	Küpeli Akkol et al. (2015)
	L	39.0–56.0	7.2–9.0	USA	Kamdern et al. (1993)

Table 2 continued

Species	Plant part	α -Thujone (per cent of GC area)	β -Thujone (per cent of GC area)	Origin of sample	Reference
<i>Tanacetum vulgare</i>	AP	0.76	91.7	Argentina	Gallino (1988)
	F	0–9.3	0–81.9	Finland	Keskitalo et al. (2001)
	AP	0.06–0.5	9.9–44.0	Iran	Goudarzi et al. (2015)
	F	49.1	78.4	Lithuania	Judzentiene and Mockute (2005)
	L	34.1	75.4	Lithuania	Judzentiene and Mockute (2005)
	F	0.3–0.7	55.3–76.0	Netherlands	Hendriks et al. (1990)
	L	0.5–0.9	47.4–67.9	Netherlands	Hendriks et al. (1990)
	AP	0–73.5	0–97.7	Norway	Rohloff et al. (2004)
	L	9.5–26.1	2.5–16.6	Romania	Mureşan (2016)
	AP	0.9	66.6	Serbia	Radulović et al. (2017)
	AP	0.9	1.0–9.2	Serbia	Stevović et al. (2011)

AP, aerial parts (not closely defined); F, flower; L, leaf

39.6% (α -thujone) and from 10.1 to 69.9% (β -thujone). Numerous descriptions and monographs regard thujone as the most characteristic constituent of wormwood EO (Foster et al. 2013, Meschler and Howlett 1999). However, wormwood is a chemically very diverse species concerning EO composition (Nguyen and Németh 2016). Nguyen et al. (2018c) evaluated 120 individuals of 12 accessions of wormwood of different origin and detected “pure” thujone chemotypes only in four accessions, while 72.5% of the individuals were described as free of thujones. It was established that thujone accumulation is not a universal characteristic of this species and in some populations even individual variability may exist.

Similarly, the major components of EO of *S. officinalis* are α -thujone (varying from 1.2 to 45.8% of the oil) and β -thujone (accumulating between 1.0 and 40.1% of the oil) as summarized by Kintzios (2003). At the same time, in some samples other mono- or sesquiterpene compounds may dominate. Based on these, five major chemotypes were defined by Craft et al. (2017), including α -thujone/camphor chemotype; α -humulene/ α -thujone chemotype; β -thujone/ α -thujone/camphor chemotype; 1,8-cineole/camphor chemotype; sclareol/ α -thujone. Interestingly, however, there is no reference, where thujones were not present at least in lower concentrations. This is definitely different from the polychemism of wormwood.

In *T. vulgare*, the thujone chemotype seems to be widespread and has been reported from different regions (Gallino 1988; Németh et al. 1994; Rohloff et al. 2004). A review by Lawrence (2000) indicated that the commercial essential oils of tansy are mostly of the thujone type. Rohloff et al. (2004) demonstrated the intra-population variability where the -thujone chemotype dominated (22 out of 40 plant individuals) and two individuals rich in α -thujone were also detected. Sorsa et al. (1968) described a south-to-north decline in the frequency of the thujone chemotype in wild populations in Europe. Thujone was also the predominant component in the essential oil of *Tanacetum vulgare* with the proportions of α -thujone (19%) and β -thujone (58%) (SCF 2002). However, in tansy, more than 40 chemotypes have been reported, among them many without thujones in the EO (Németh et al. 1994; Németh-Zámbori 2015).

In *Thuja occidentalis*, thujone is characteristically the dominant compound of the EO and even the name of the genus may be the source of the name of this compound. Although the α isomer is always represented in higher amounts, the β -thujone is also present (Table 2). The intraspecific chemical variability seems to be relatively low and basically quantitative, as there is no reference to any white cedar EO free of any thujones. In this respect this species is similar to sage, but different from tansy and wormwood.

The effect of biotic and environmental factors on the manifestation of chemism

The stage of development can be a determinant for the accumulation of secondary compounds during plant life (Németh 2005). Concomitantly, the composition of the essential oil including thujone may undergo major changes. During the plant development of wormwood (*A. absinthium*), the ratio of both main compounds (α -thujone and β -thujone) reached its highest value (66.8% and 25.4%, respectively) at floral budding and decreased after the flowering stage (Nguyen et al. 2018a). The results of Nguyen et al. (2018a) are in agreement with the findings of Carnat et al. (1992), who have reported that the content of thujone decreased during the ontogenetic phases. Perry et al. (1999) have proved that the total concentration of thujones (mainly *cis*-thujone) in *S. officinalis* oils differed during the vegetative period: the thujone content of the EO decreased sharply and significantly from over 40% to 25% after the flowering stage. From Iranian sage studied by Mirjalili et al. (2006), α -thujone (25.1%) was a major component of ripened fruit oil collected in the last phenological stage; however, in earlier developmental phases this component was found in only 9.1–13.2%. Ben Farhat et al. (2016) also reported that the percentages of thujone varied depending on the phenological period and reached the highest level at the fruiting stage. Nguyen et al. (2019) registered the highest level of α -thujone accumulation in sage leaf oils at the vegetative stage and budding floral stage (23.2–24.7%) and decreased significantly during the flowering period while in flower oils, the peak of this component was detected at the floral budding stage (14.2%) with no significant changes after that. It seems to be a general phenomenon that the accumulation of volatile compounds reaches a peak at the earlier developmental phases, usually at the beginning of flowering, and decreases sharply after flowering and during seed ripening (Mohammadi et al. 2015; Németh et al. 2007). Decreasing ratios of α -thujone during development in the EO of *S. officinalis* L. were also reported by Santos-Gomes and Fernandes-Ferreira (2001) and Shadi and Saharkhiz (2016). Similarly, in the case of tansy, Wolf et al. (2012) have reported that β -thujone (55–79%) was the main compound found in both introduced or native tansy in North America. The highest amount of α -thujone (44%) was detected in

Iranian tansy in two separate stages: in the leaf rosette stage and the beginning of fruit set (Goudarzi et al. 2015). Németh et al. (1994) described a slight increase of thujone content in the shoots of tansy from 42% after sprouting out to 62% during seed ripening phase. The dynamics of volatile content in the case of thujone accumulation may also be in connection with the development of oil glands and an intensive biosynthesis of volatile molecules due to their specific roles in ecological adaptation and other physiological mechanisms of the plants (Guitton et al. 2010; Németh et al. 2001).

Based on these data, ontogenesis is an important factor concerning thujone ratio in the EO. Most frequently, the level of thujone components is highest in the first period of the shoot formation; however, the degree of change depends strongly on the species. Therefore, the timing of harvest seems to be crucial to obtaining raw material with either high or low levels of thujones.

Just as chemosyndromes of the plants may vary not only according to ontogenetic phases but also by plant parts (Németh-Zámboi 2015), the thujone content varies on a large scale, too. In wormwood, Judzentiene and Budiene (2010) determined higher ratios of thujone in flowers (5.3–10.4%) than in leaves (0.0–8.9%). Findings by Nguyen et al. (2019) indicated that the ratio of both thujone isomers show higher amount in leaves than in flowers in both wormwood (*A. absinthium*) and sage (*S. officinalis*). Furthermore, a study in Portugal by Santos-Gomes and Fernandes-Ferreira (2001) indicated that α -thujone content in the oil of stems, leaves and flowers of *S. officinalis* represented about 55, 30, and 18% of the total oil, respectively. Similarly, in *S. officinalis* samples collected from wild regions in China, the percentage of β -thujone in leaves (14.9%) was higher than in flower oils with 6%. Nevertheless, the ratio of α -thujone in flowers was 3 times higher than that in the leaves (Li et al. 2015). In the case of *Tanacetum vulgare* most data refer to a higher thujone ratio in the flowers than in the leaves. Hendriks et al. (1990) found similar thujone concentrations both from tansy leaves (47.4–67.9%) and flowers (55.3–76.0%). In the investigations of Judzentiene and Mockute (2005) β -thujone ratios in flower and leaf oils of tansy were found to be from 24.3 to 11.1%, respectively. Similarly, earlier reports described that β -thujone in

flowers was higher compared with leaf oil in a Dutch accession (Hendriks et al. 1990).

It can be established that the results of most studies on wormwood and sage show a higher ratio of thujone in leaves than in flowers, although contradictory findings have also been published. In the case of tansy, thujone usually was measured in higher ratios in flowers than in leaves. The differences might represent the different ecological roles of these monoterpenes for different species.

According to the references, thujone content and the actual proportion of its two isomers may vary on a large scale also depending on the region or habitat the sample originates from. α - and β -thujones were detected in wormwood oil in 18.6% and 23.8%, respectively, in a study on collected wild Iranian plants (Rezaeinodehi and Khangholi 2008). In Serbian natural populations of the same species, β -thujone was the absolute major component, representing up to 63.4% of the total oil isolated from the aerial parts, while α -thujone occupied only 0.4% (Blagojević et al. 2006). In sage, Perry et al. (1999) classified 3 groups of different accessions from New Zealand as having high (39–44%), medium (22–28%) and low (9%) total thujone content as well as three chemotypes with different proportions of α - and β -thujones (α/β 10:1, 1.5:1, and 1:10). Jug-Dujakovic et al. (2012) have examined the EO composition of 25 populations of Dalmatian sage growing in Croatia and detected three quantitative chemotypes with major compounds of α -thujone (15.2–33.9%) and β -thujone (3.9–37.8%). A ratio of thujone in sage oils originating from different regions (Albania, Mexico and California) varied from 17.2 to 27.4% (α -thujone) and from 3.8 to 6.0% in the case of β -thujone (Craft et al. 2017). *T. vulgare* samples from Argentina mainly contained β -thujone (91.6%) (Gallino 1988) but Wolf et al. (2012) also reported very high levels (55–79%) from wild tansy plants collected in the United States. Khalilov et al. (2001) have indicated that α -thujone (30.4–40.5%) and β -thujone (6.5–9.0%) were main compounds in each EO sample of *Thuja occidentalis* originating from four regions in Slovakia. In case of this species, from several other regions the chemical profile with the dominant α -thujone compound was found to be similar: e.g. samples from the United States (Kamdern et al. 1993), Poland (Lis et al. 2016) and Turkey (Küpeli Akkol et al. 2015).

Unfortunately, the collection site and the origin of the sample may represent both genetic variability and inherited differences together with the eventual effects of the environment and changing phenotype due to external circumstances. Genetic and environmental effects can not be separated in these cases; therefore the real background of the above mentioned information and other findings must remain undetected.

Studies in controlled environmental conditions can reveal the primary factor influencing the content and composition of the EOs (Németh-Zámbori 2015), however these experiments are scarce. Gholami et al. (2005) reported that there were significant differences in the compositions of EOs obtained from plants grown under different conditions. Thujone in field grown wormwood oil reached its highest amount (60% and 5.5% of α -thujone and β -thujone respectively), followed by wormwood grown in a greenhouse with 41% of α -thujone and 3.0% of β -thujone, respectively. Interestingly, no thujone content could be detected in the case of wormwood grown in vitro (Gholami et al. 2005). A study of wormwood by Nguyen et al. (2018b) has indicated that the different temperature and light conditions created in two climatic chambers influenced the EO composition of wormwood. β -thujone was 3.3% in the oils at lower temperature with less light intensity, while this component was absent in wormwood grown under elevated temperature and high light conditions (Nguyen et al. 2018b). Under different growth conditions, considerable quantitative changes occur in the EO composition, which may influence the quality of the drug production.

Conclusion

It can be established that the production of thujone isomers is relatively widespread among volatile accumulating species. There are some plant families (e.g. Asteraceae or Cupressaceae), and genera within them (e.g. *Artemisia*, *Achillea*, *Thuja*, *Salvia*, etc.) where the presence of thujones in several species is frequent. Nevertheless, even in many of these species, the formation of thujone is not a universal phenomenon, but a considerable chemical variability has been demonstrated with thujone-containing and thujone-free intraspecific accessions or individuals. Based on the available information, the thujone-containing taxa or intraspecific accessions do not seem to have closer

taxonomic or geographical relationships with each other. Among the best known thujone-containing species, *A. absinthium* (wormwood) and *T. vulgare* (tansy) especially show huge intraspecific chemical diversity while *S. officinalis* (garden sage) and *T. officinalis* (white cedar) are more uniform and—according to the available references—thujone-free samples have not been described as yet.

Therefore, the formation of thujones seems to be the result of a special biosynthetic route, a route which is active in different plant species not necessarily related to each other. Furthermore, it is interesting to observe that the indirect precursor of thujone, the compound sabinene, is one of the most widespread monoterpene compounds in the EOs of a huge number of different, nonrelated plants. The presence of thujone, therefore, might be the result of an independent co-evolution of genes acting in pathways after formation of sabinene only in a restricted number of even non-related species. As another alternative, the thujone pathway may exist in many species containing sabinene, but the expression of the corresponding genes is repressed due to different metabolomic interactions leading to the lack of thujones. The latter assumption has been concluded for the accumulation of volatile terpenoids of the Lamiaceae family and might also be a first step explanation for the intraspecific variability experienced in several species. The question for thujones is still open due to the lack of appropriate and sufficient information.

In thujone producing genotypes, the accumulation of thujone is influenced by different external and internal factors. It can be established that changes have been detected during ontogenesis, as well as differences in the thujone level between plant organs harvested at the same time. Besides, environmental conditions (temperature, light) seem to be influencing factors, too. Nevertheless, systematic studies under controlled climatic conditions are still lacking for most of the relevant species.

We should emphasize that an exact evaluation of the references is hampered by the fact that methods of sampling, EO production, and analysis are very diverse and unfortunately sometimes reliability seems to be questionable. Older references frequently did not distinguish between the two thujone isomers, either.

Further research is suggested to clarify the thujone pathway concerning genetic regulation and its metabolomic connections, including isomerisation of the

thujones. This should be supplemented by studies on the presence of intermediates like sabinol, sabinone and related compounds in EOs; and additional investigations on the intraspecific variability of the thujone accumulating species. In order to regulate the content of thujone in the plant material, we need more data on the physiological role or other roles of thujone in the plants, too. Likewise, discovering and justifying further areas of human application for both thujones—and their safety considerations—are serious questions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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