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Review article

Sources of variability of wormwood (*Artemisia absinthium* L.) essential oil

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ABSTRACT

Artemisia absinthium L. is a medicinal and aromatic bitter herb frequently used in traditional medicine as antimicrobial agent since ancient times. The important active constituents, essential oil and bitter substances have attracted the interest of several researchers and producers throughout the world. The use of this herb as a source of natural products and the alcoholic beverage absinthe has recently experienced a revival after a period of prohibition. The composition of the essential oil exhibits a large intraspecific variability. Besides the most well known β-thujone, at least 17 other major compounds were described in the oil, among others myrcene, sabinene, sabinyl acetate, epoxyocimene, chrysanthenol, chrysanthenyl acetate, etc. Until now, both "pure" chemotypes and "mixed" chemotypes have been defined. Drugs originating from different regions often show great variability in quality. Nevertheless, most references do not characterize correctly the source of the plant material, therefore it is difficult to divide the roles of the genotype and other influencing factors.

The essential oil composition might change also during the ontogenesis, nevertheless there are only a few samples investigated in different chemotypes. The thujones seem to be varying during the vegetation period, as well. Although the organic differences have only scarcely been investigated, it seems that monoterpenes predominate in aerial parts, while the essential oil of the roots shows characteristically high ratios of monoterpenic and aliphatic esters. The role of environmental effects on the composition of wormwood oil needs further confirming data. Compared to hydrodistillation, other extraction methods resulted in significantly different compositions. According to some references, even the presence of thujones could be influenced by the extraction method.

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1. Materials and search strategy

This review is focused on the most important facts and scientific information on *Artemisia absinthium* concerning primarilyirst of all its chemical composition with special respect on the essential oil.

71 published references were cited in this review, mainly during the 25 past years. Two independent reviewers screened the retrieved records for potentially relevant articles and extracted data. The review was continuously updated in response to recommendations from reviewers, then was resolved discrepancies and reached a consensus on the final results by these reviewers.

We evaluated first of all studies obtained from electronic databases according to a search strategy designed to retrieve abundant and new data for our review. The review included databases such as: PubMed, Web of Science, Research gate, Google Scholar, Sci-hub etc. In this case no date restrictions were fixed. These databases were carefully selected to allow the identification of reports, dissertations, and gray literature in addition to journal articles. Besides, the bibliographies of the available publications were checked for additional references. We carried out also a hand searching in the Abstract books of ISEO (International Symposium on Essential Oils), ISHS conferences, BREEDMAP (Breeding Research on Medicinal and Aromatic Plants) and GA (Geseallschaft für Arzneipflanzenforschung) in the last 12 years. Additionally, a hand search was carried out using all of the volumes of JEOR (Journal of Essential Oil Research) 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., Unpublished material was selected by scans of evidence-based websites or by discussion with experts involved in international research on MAPs. The information gained from different sources was compared and evaluated which facts and results have been confirmed, put into the practice improved, extended, or even corrected.

2. Characteristics and significance of *Artemisia absinthium* L.

The genus Artemisia is member of the Compositae (Asteraceae) family and it is a large, diverse genus distributed in the temperate and cold regions of Eurasia and North America (Wang, 2004). *Artemisia absinthium* (Wormwood) is native to Europe and can be found over temperate Asia northwards to Lapland, Karelia and Southern Siberia, but it has become naturalized in North and South America and New Zealand, as well (Maw et al., 1985). The plant has been known by various names as wormwood/bitter wormwood (in English), grande absinthe (in French) or Wermut (in German) (Wright, 2003). It grows from 40 to 150 cm in height (Maw et al., 1985) with a woody, hardy rosette and high, branching stem covered by white hairs, and the leaf twigs are silvery hoary on both surfaces. Flowers of yellow colour are produced from July to October, are small and globular (Goud and Swamy, 2015). The use of this herb as a source of natural products has attracted the interest of many researchers and producers. The special utilization of wormwood in the spirit absinthe has gained the highest reputation, but it plays an important role as flavouring agent of some other alcoholic beverages, as well. *Absinthe* was produced in French-speaking Switzerland in the late 18th century and in the late 19th century. Absinthe became the most popular spirit drink in Europe called "green fairy" (Lachenmeier et al., 2006a,b). Vogt (1981) described that the hour of absinthe was between five and six o'clock when Parisians gathered to sit outside of the cafés and drink their customary glasses of this green, anise-flavored spirit. Nearly all of the 33.000 bar and cafés were filled with patrons sipping absinthe in that time and it was noted that "the sickly odor of absinthe lies heavy on the air" in the old village of Montmartre (France) (Holstege et al., 2002). By the rising interest in anise-based spirits as well as increased promotion and advertising, the production of Pernod's absinthe climbed up to 125.000 l in 1896. The annual per capita French consumption of absinthe grew dramatically in the period from 1875 to 1913 and the production reached 239.492 hectoliters in 1913, representing 60 l per inhabitant in France (Padosch et al., 2006). That is why the 1890s was called the *Absinthe decade* (Baker, 2001). However, at the beginning of the 20th century, the production of this bitter spirit was prohibited in several countries as it was blamed for a range of severe symptoms, called absinthism. After many toxicological studies in the following decade, nevertheless, the adverse effect of wormwood could not be justified undoubtedly. Therefore, the regulation today is a limitation of thujone content (35 mg/l) in alcoholic beverages, among others in absinthe (EU Council Directive, 1988). Because of this change in food safety policy, the popularity of absinthe has risen again. In 2003, 89 types of absinthe brands were distributed in Germany through the internet, the majority of them having been produced in the Czech Republic, Spain, France and Germany (Lachenmeier et al., 2006a,b).

Besides its significance in flavouring, wormwood has a long history of therapeutic use both in folk medicine and in modern pharmacology. The essential oil of this plant has been used in anthelmintic, anti cold, anti-inflammatory and antimicrobial preparations and for its antiseptic, antidepressant, digestive, carminative, stimulant, choleretic and tonic effects (Goud and Swamy, 2015; Watson and Preedy, 2008)

3. Main active ingredients of wormwood

Various secondary metabolites and other products have been isolated from *Artemisia absinthium*, the most important being the essential oil obtained from glands on the aerial parts. Because of high concentrations of volatile terpenes, especially in leaves and flowers, the essential oil of this species has strong aromatic smell.

References on the content of essential oil indicate different levels depending on the origin of the sample. Orav et al. (2006) obtained 0.1–1.1% essential oil from plant material coming from different European regions. Relatively high concentrations (1.10%-1.46%) were determined from wormwood collected in Tunisia (Msaada et al., 2015). Plant material from Cuba contained 1.25% essential oil (Pino et al., 1997), while the one from Greece gave a yield of 0.31% (Basta et al., 2007).

The colour of the oils has been also reported from different literatures. Hydrodistillation of dried wormwood drug resulted in a dark blue essential oil (Msaada et al., 2015). However, according to Pino et al. (1997), the oil extracted from the dried leaves and flowering tops of *A. absinthium* varied from dark green to brown or dark brownish green. Obviously, the colour of the oil is in connection with its composition.

The essential oil of *A. absinthium* is usually known and reported to be rich in bicyclic monoterpene thujone which, therefore may be considered as the most characteristic constituent of wormwood oil (Juteau et al., 2003), (Meschler and Howlett, 1999). Both isomeric forms, α- and β-thujones, were described in wormwood oil, but the concentration of β-thujone is usually higher than that of α-thujone. However, the actual proportion may change on a large scale. α- and β-thujones were detected in 18.6% and 23.8%, respectively in a study on wild collected Iranian plants (Rezaeinodehi and Khangholi, 2008). In Serbian natural populations, β-thujone was the absolute major component representing up to 63.4% of the total oil isolated from the aerial parts of wormwood, while α-thujone occupied only 0.4% (Blagojević et al., 2006). Lachenmeier and Nathan-Maister (2007) reviewed 24 references regarding to the wide variations of thujone content in wormwood oil. Based on these, they reported that mean proportions of α- and β-thujones in the essential oil of *A. absinthium* were 5.8% and 12.5%, respectively.

Other major compounds of *A. absinthium* volatile oil observed in several studies are myrcene, sabinene, linalool, *cis*-epoxyocimene, chrysanthenyl acetate and *trans*-sabinyl acetate. A study by Ariño et al. (1999c) indicated that the most abundant composition in all analyzed samples from Spain was *cis*-epoxyocimene. Llorens-Molina and Vacas (2015) found also *cis*-epoxyocimene (49.3–71.5%), *cis*-chrysanthenyl acetate (7.6–18%) and linalool (0.7–10.4%) as the main compounds in samples collected in Teruel, Spain. *cis*-epoxyocimene (24.6%) was also detected in an Italian material by Mucciarelli et al. (1995). However, according to Judzentiene et al. (2009), *trans*-sabinyl acetate (8.8-55.2%) as dominant compound was found in 15 investigated oil samples of Wormwood from wild growing sites in Vilnius city (Lithuania). Besides thujones, β-pinene was the second most important constituent of Iranian wormwood essential oil (Rezaeinodehi and Khangholi, 2008). In addition, Martin observed *cis*-epoxyocimene, chrysanthenol, and chrysanthenyl acetate as major compounds (Martín et al., 2011). Table 1 provides an outlook on the most frequently detected major compounds of the essential oil of wormwood.

The bitter taste and the activity of the herb are ascribed to several sesquiterpene lactones. Wright (2003) has reported that the stimulant property of *A. absinthium* is caused by the bitter substances anabsinthin (sesquiterpene lactone) and absinthin (dimer of sesquiterpene lactone) present in plant extracts.

The phytochemical study of Ashok and Upadhyaya (2013) on hexane and alcoholic extracts of wild Indian wormwood showed the presence of various chemical constituents, i.e. carbohydrate, glycoside, oils and fats, saponins, phytosterols, proteins and amino acids, tannins, phenolic compounds and flavonoids. In detail, the ratio of starch, sugar, tannin and total phenolic contents of the leaves were 11.66%, 6.38%, 0.20% and 2.78%, respectively.

Wormwood contains also fatty acids. Palmitic (33.39%), arachidic (26.2%), linoleic (27.5%), lauric, myristic, steric and oleic acids which have been detected in the lipid fraction of volatile oils of leaf (0.22%), flower (0.35%), and herb (0.3%) by TLC (Wasim Ahmad, 2010). Nikhat et al. (2013) reported that the seeds contain a mixture of 9-hydroxy-trans, trans, 10, 12-octadecadienoic acid and 13-hydroxy-trans, trans, 9, 11-octadecadienoic acid in the ratio of 2:1.

4. Physiological and therapeutic effects

In traditional medicine, *Artemisia absinthium* is believed to treat mental exhaustion and nervous depression, otalgia, chronic fever, anaemia, amenorrhoea, etc. (Wasim Ahmad, 2010). According to Guarrera (2005) and Wake et al. (2000), wormwood has been used in folk medicine as an antispasmodic, febrifuge, stomachic, cardiac stimulant, anthelmintic agent and for the restoration of declining mental function and inflammations of the liver.

It is a folk remedy in Asia, as well and a long list of medicinal uses have been attributed to wormwood, including antimalarial, antiviral, antitumor, spasmolytic and others (Tan et al., 1998). In China, *A. absinthium* has commonly been used as an aromatic substance and additive to rice wine (cf. Sake) and to grape wine, while it was an additive to beer in ancient Egypt.

It is still being used in Yemen to alleviate the pains associated with parturition (Rätsch, 2005). According to the same author, wormwood is one of the most important gynecological agents for abortion and to induce menstruation or delivery in European folk medicine. In tea form, it is consumed primarily for stomach pains, against lack of appetite, feelings of fullness, gallbladder problems, vomiting, and diarrhea. Moreover, in homeopathy, wormwood is used in accordance with medical descriptions to treat epilepsy and nervous or hysterical spasms (Rätsch, 2005).

Although obviously not all of the traditional uses could be justified by modern tools, pharmacological and clinical investigations, there are a lot of data on the possible sophisticated utilizations. In a study at Yale University, patients from five locations in Germany with Crohn's disease were administered an herbal blend containing wormwood herb (3 × 500 mg/day), or placebo, for a ten week period. It was observed that patients consuming the herb blend were able to get free of steroids – the conventional treatment of this disease – and the treatment improved their mood and quality of life (Omer et al., 2007). Similar findings were published by Krebs et al. (2010) administering 3 × 750 mg dried powdered wormwood for 6 weeks in addition to their basic Crohn's disease therapy. In animal experiments, Bora and Sharma (2010) detected neuroprotective effects of wormwood on focal ischemia and reperfusion-induced cerebral injury and this finding was ascertained by Lachenmeier (2010), too.

As many other plants, wormwood represents a rich source of antioxidants which may support healing of skin wounds (Bora and Sharma, 2011; Craciunescu et al., 2012). A study on oxidative stress conducted by Kharoubi et al. (2008) has indicated that oral administration of wormwood extract (200 mgkg^{-1} body weight) for 11 weeks to rats stimulated the activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase. The results of this study also suggested that *Artemisia absinthium* extract had a protective role against lipid peroxidation. Similarly, Altunkaya et al. (2014) reported that the essential oil of *A. absinthium* showed significant antimicrobial activity against 6 bacteria and 2 yeasts in disk diffusion trial.

The benefits of this plant are not limited to human health; its efficacy is also known in veterinary therapies. As a result of a study by Tariq et al. (2009), wormwood extract may be a natural alternative to commercial drugs for addressing intestinal invaders in sheep. Wormwood's essential oil has been shown to have insecticidal activity (Kordali et al., 2005) and repellent effects against fleas, flies and mosquitoes (Erichsen-Brown, 1979). It is suggested that the essential oil of this plant might play an important role in the development of natural or biological insecticides, thus, contributing to the reduction of environmental pollution by chemicals.

5. Adverse effects and legislation

There is relatively few knowledge on agro-technical aspects, while primarily processing and its influence on drug quality has been the target of more studies. To the contrary of accumulated scientific results, there is still a frequent uncertainty or even negative feeling in the world in connection to wormwood and its application. The effect of monoterpene thujone on human health is controversially discussed. The syndrome called absinthism originates from the name of the plant. Symptoms of so-called absinthism included convulsions, blindness, hallucinations and mental deterioration (Lachenmeier et al., 2006a,b). As a consequence of its supposed negative effects on human health, absinthe, the bitter spirit containing wormwood, was banned for a period at the beginning of the 20th century (Padosch et al., 2006).

There are some exact cases reported in literature from the very early times till recent periods. Smith (1863) described a case of a salesman who was insensible, convulsed, foaming at the mouth and showed a tendency to vomit due to using wormwood oil. An other recent case reported by Weisbord et al. (1997) from the U.S. was a 31-year-old man who had purchased from a website and consumed 10 ml of wormwood essential oil used in aromatherapy, what he assumed was the spirit absinthe. A few hours after ingesting this oil, the patient became listless, suffered seizures and finally devel-

Table 1
Major essential oil (EO) components (> 10% of EO) in *Artemisia absinthium* reported in different references.

Compound	Plant part	Origin	Ratio (% E.O.)	References
Bornyl acetate	R	Spain (W)	21.1	Llorens-Molina and Vacas (2015)
	AP	Cuba (W)	23.0	Pino et al. (1997)
Caryophyllene-oxide	AP	Greece (W)	25.5	Basta et al. (2007)
Camphor	AP	Italy (W)	17.1	Mucciarelli et al. (1995)
	AP	Iran (W)	14.8	Nezhadali and Parsa (2010)
Chamazulene	AP	Turkey (W)	17.8	Kordali et al. (2005)
	AP	Tunisia (W)	39.9	Msaada et al. (2015)
cis-Chrysanthenol	FT	France (W)	69.01	Carnat et al. (1992)
	AP	Spain (C)	15.7	Martín et al. (2011)
cis-Chrysanthenyl acetate	L	Spain (W)	32.6	Ariño et al. (1999a)
	FT	Spain (W)	43.0	
	AP	France (W)	33.6	Juteau et al. (2003)
	AP	Tajikistan (W)	17.9	Sharopov et al. (2012)
trans-Chrysanthenyl acetate	AP	Italy (W)	21.6	Mucciarelli et al. (1995)
p-Cymene	AP	Iran (W)	10.3	Nezhadali and Parsa (2010)
	AP	Greece (W)	16.8	Basta et al. (2007)
cis-Epoxyocimene	L	Spain (W)	44.7	Ariño et al. (1999a),
	FT	Spain (W)	37.3	
	AP	Italy (W)	24.6	Mucciarelli et al. (1995)
	AP	Croatia (W)	30.8	Juteau et al. (2003)
	L	France (W)	49.7	
	AP	Russia (P)	21.1	Orav et al. (2006)
	L&F	France (W)	54.4	Chialva et al. (1983)
	AP	Tajikistan (W)	17.9	Sharopov et al. (2012)
α-Fenchene	R	Spain (W)	23.7	Llorens-Molina and Vacas (2015)
Myrcene	AP	Tajikistan (W)	22.7	Sharopov et al. (2012)
	FT	France (W)	10.4	Carnat et al. (1992)
	AP	Canada (W)	10.8	Lopes-Lutz et al. (2008)
	AP	Estonia (P)	29.9	Orav et al. (2006)
	AP	Moldova (P)	38.9	
	AP	Scotland (P)	18.0	
	R	Spain (W)	29.2	Llorens-Molina and Vacas (2015)
	R&AP	Turkey (W)	44.3	Altunkaya et al. (2014)
Neryl butanoate	AP	France (P)	13.9	Orav et al. (2006)
Sabinyl acetate	AP	Croatia (W)	13.5	Juteau et al. (2003)
	AP	Canada (W)	26.4	Lopes-Lutz et al. (2008)
	AP	Lithuania (W)	51.3	Judzentiene and Budiene (2010)
	AP	Siberia (P)	31.5	Orav et al. (2006)
	AP	France (P)	84.5	
	AP	Belgium (P)	18.6	
	AP	Estonia (P)	70.5	
	AP	Armenia (P)	34.2	
Sabinene	AP	Hungary (P)	18.1	Orav et al. (2006)
	AP	Estonia (P)	25.3	
Terpinen-4-ol	L&F	United State (C)	11.6	Nin et al. (1995)
	AP	Italy (C)	28.8	
α-Thujone	AP	Lithuania (W)	36.8	Judzentiene and Budiene (2010)
	L	Morocco (W)	39.6	Derwich et al. (2009)
β-Thujone	AP	Greece (P)	38.7	Orav et al. (2006)
	AP	Estonia (P)	64.6	
	AP	Iran (W)	18.6	Rezaeinodehi and Khangholi (2008)
	L	Croatia (W)	48.6	Juteau et al. (2003)
	AP	Canada (W)	10.1	Lopes-Lutz et al. (2008)
	AP	Lithuania (W)	48.9	Judzentiene and Budiene (2010)
	L&F	United State (C)	69.9	Nin et al. (1995)
	AP	United State (P)	33.1	Tucker et al. (1993)
	AP	Iran (W)	35.1	Morteza-Semnani and Akbarzadeh (2005)
β-Pinene	AP	Iran (W)	23.8	Rezaeinodehi and Khangholi (2008)

- AP: aerial parts; F: flowers; FT: flowering tops; L: leaves; R: roots.
- W: wild collected material; C: cultivated variety; P: purchased on the retail market.

oped rhabdomyolysis and the acute renal failure. It was supposed that thujone was to blame for the symptoms (Weisbord et al., 1997). The study of Dettling et al. (2004) on 25 healthy subjects who consumed alcoholic beverages of different amounts of thujone content shown that the simultaneous administration of alcohol and high concentrations of thujone (100 mg/l) may severely affect attention performance.

On the contrary, numerous investigations proved that thujone plays none or only a minor role in the clinical picture of absinthism (Lachenmeier et al., 2006a,b). According to these authors, thujone should not be the main reason of the symptoms, but absinthism is predominantly caused by high alcoholic concentration (>50 vol%) that may lead and might have led both today and in the 18th century to major health and social problems. Strang et al. (1999) also suggested that acute alcohol intoxication was the main reason leading to the syndrome of absinthism. Similarly, in a pilot study of Kroner et al. (2005) blood concentrations of both thujone and ethanol were examined after the consumption of absinthe. They detected high blood alcohol concentrations (>1 g/l) but – as expected – no thujone in the blood samples.

For bitter spirit drinks, such as absinthe, a maximum limit of 35 mg/kg thujone was introduced in the EU Council Directive

88/388/EC. According to Max (1990), in a typical recipe for absinthe, 2.5 kg of wormwood were used in preparing 100 l of absinthe. This is equivalent to 4.4 mg wormwood oil or 2–4 mg thujone per drink, which would be far below the level at which acute toxicity effects were observed. Even chronic administration of 10 mg kg^{-1} thujone to rats does not alter spontaneous activity or conditioned behaviour. Pharmaco-dynamical and toxicological studies revealed that even by consuming a relatively high amount of absinthe and reaching a blood alcohol concentration of 2.5 g/l it might mean only a thujone level of approximately 3.5 mg (0.005 mg/kg body weight). In a recent study, Lachenmeier and Uebelacker, 2010 provided evidence that the current EU limits assure a sufficient protection for consumers. Based on the last mentioned studies, it seems to be acceptable that thujone content in the spirit absinthe is hardly able to cause the characteristic syndromes of absinthism and these symptoms may easily be confused with those of chronic alcoholism.

Therefore, after a nearly century-long prohibition, absinthe has seen resurgence after recent de-regulation in many European countries. However, it should be added, that the huge majority of the mentioned studies have focused especially on thujone as the potentially toxic agent of the spirit. In addition, for the time being, no studies on the differences between the effects of α- and β-thujones with regard to the symptoms called absinthism are available. Besides, other compounds have seldom been investigated, thus the question on the source of potential health problems does not seem to be fully closed even today.

6. Variability sources of wormwood essential oil

Studies have shown that *A. absinthium* displays a significant intraspecific variation in the terpene constituents of the essential oil. Besides the genetic potential and inherited properties of the accession, many other factors, such as growing conditions, stage of development, organic differentiation and harvesting time may affect the constituents of the essential oil to a great extent (Müller-Riebau et al., 1997). Our review presents and evaluates the background and influencing factors of its variability in order to recognise and control themfor a well-established utilization of the species.

6.1. Origin of raw materials

The chemical constituents of essential oils from wormwood have been intensively studied in different countries around the world and different chemotypes have been reported in literature (Table 1).

Frequently, "pure" chemotypes and "mixed" chemotypes (when the plants contain two or more components in higher proportions) have been defined. As an example, Chialva et al. (1983) determined "pure" chemotypes including *cis*-epoxyocimene, sabinyl acetate and β-thujone types and "mixed" ones such as β-thujone + *cis*-epoxyocimene, β-thujone + sabinyl acetate, *cis*-epoxyocimene + chrysanthenyl acetate + sabinyl acetate ones, etc.

Nin et al. (1995) defined the samples from the United States (69.7%), from Germany (49.8%) and from Italy (49.2%) as characteristic β-thujone chemotypes. According to Juteau et al. (2003) pure thujone chemotype plants (48.6% oil) were found in Croatia, as well.

Chemotypes with the presence of thujone together with other major compounds were observed in several studies. Besides the above mentioned accessions in the study of Chialva et al. (1983), Rezaeinodehi and Khangholi (2008) reported β-thujone + β-pinene chemotypes in Iran, Tucker et al. (1993) determined β-thujone (33.11%) + α-sabinyl acetate (32.75%) types in the United States. In Lithuania, "mixed" thujone chemotypes were indicated as the oil contained predominantly 18.0–71.7% thujones (both α- and β-ones) together with β-sabinyl acetate (in 5.6-23%) (Judzentiene and Budiene, 2010).

Surprisingly, in several other cases thujone could not be demonstrated among the main components of the oil or was totally missing in the samples. Orav et al. (2006) described sabinene + myrcene type samples from items purchased at retail pharmacies in Estonia, while Carnat et al. (1992) reported a *cis*-chrysanthenol type population from Auvergne (France). In Cuba, bornyl acetate type plants could be identified with 23% bornyl acetate and only 0.29% thujone (Pino et al., 1997). Mixed types with the main components of caryophyllene oxide + p-cymene + 1,8-cineole were described by Basta et al. (2007) from wild populations in Greece, while *cis*-epoxyocimene and *cis*-epoxyocimene + chrysanthenyl acetate chemotypes were determined by Ariño et al. (1999c) in Spain. These two latter ones were free of thujone. Similarly, wormwood from Tajikistan has been reported as a new chemotype with a myrcene and *cis*-chrysanthenyl acetate and very low concentration of thujone (0.4–7.3%) (Sharopov et al., 2012).

It can be established that the essential oil profile of wormwood is variable and thus raw material originating from different countries and different regions may show great differences in quality. Nevertheless, the majority of publications do not reveal the background of this variability and frequently not even the origin of the sample is correctly described. In this situation, it is almost impossible to determine the real significance of the genotype and discern it from any other biotic and abiotic factors.

Principally, thujone-free chemotypes offer an alternative for the production of food industrial items, beverages and spice products. However, there are no appropriate studies published on whether the use of these plant materials has any connection with flavour and aroma of the extract. The absence or the presence of even a minimal amount of thujones might have an important role in food processing sector in avoiding potential toxicity and keeping concentration limits.

6.2. Ontogenetic factors

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 may undergo major changes.

In a Lithuanian study the content of *trans*-sabinyl acetate dramatically increased from 0.8% at leaf stage to make up 52.6% of total oil during fruiting, while sabinene content dropped from 16.8% (first leaves) to 2.9% (fruiting stage) (Judzentiene and Budiene, 2010). Variation of essential oil composition of *Artemisia absintium* has been investigated in a Spanish native population, as well. According to the study of Llorens-Molina and Vacas (2015) bornyl acetate and neryl-isovalerate were detected in highest concentrations at the beginning of summer and showed a significant quantitative shift from this early vegetation period (June) till the winter shoots (January). In parallel, other compounds, such as camphor, borneol, *cis*-geraniol and geranyl acetate exhibited a significant increase from the vegetative phenological stage and reached the highest percentages at the period from August to October.

Fluctuations of the most important component, thujone, were investigated by Carnat et al. (1992) in several wormwood samples from wild growing site in Auvergne (France). The proportions of both α- and β-thujones and *cis*-chrysanthenol changed dramatically during the harvesting period from June to November. Data of Table 2 show that the content of thujones decreased while *cis*-chrysanthenol increased significantly in the mentioned period. Unfortunately, the July samples do not follow this general tendency, therefore it is questionable if any other factors like organic differ-

Table 2
Variations of thujone and *cis*-chrysanthenol proportions (% in EO) at the different harvesting times based on a 4 year trial (Carnat et al., 1992).

Constituents	Harvesting period					
	June	July	August	September	October	November
α-thujone	21.60	2.55	21.54	18.04	8.07	3.75
β-Thjone	25.90	3.75	28.33	22.70	10.80	4.22
Cis- chrysanthenol	15.70	54.90	19.67	24.46	51.03	69.01

entiation, presence of flowers or weather conditions could take a role in these changes.

Table 2 Variations of thujone and *cis*-chrysanthenol proportions (% in EO) at the different harvesting times based on a 4 year trial (Carnat et al., 1992)

6.3. Morphogenetic factors

The oil composition can be largely dependent on the plant part used either they are aerial parts (leaves, stems, flowers) or roots. As mentioned above, ontogenetic and morphogenetic factors are frequently very difficult to divide from each other as well as from outside influencing factors. To make firm conclusions, well designed experiments are necessary and these are practically missing in the case of wormwood till now. Comparison of underground and overground organs was carried out first by Blagojević et al. (2006) who indicated that the major constituents in the essential oil isolated from aerial parts of wormwood were β-thujone, *cis*-β-epoxyocimene, *trans*-sabinyl acetate, sabinene, and linalyl 3-methylbutanoate, while α-fenchene is the main component found in wormwood root oil. In the aerial parts the oil was dominated by monoterpenes which took 84,6% of the oil, while major compounds of the root oil were aliphatic esters in 64.5%. Interestingly, while the investigated genotype was obviously a thujone type with β-thujone contents up to 63.4%, this constituent could not be detected in root oil (Blagojević et al., 2006).

Similarly, a relatively high ratio of other compounds was detected in the roots of a wild growing population from Spain. According to research results of Llorens-Molina and Vacas (2015), a predominance of oxygenated monoterpenes (81.4-89.1%) was observed in aerial parts while the root essential oil showed high ratios of hydrocarbon monoterpenes (43.8–55.1%) and monoterpenic esters (36.6–41.5%). The major constituents found in aerial parts were *cis*-epoxyocimene (49.3–71.5%), (Z)-chrysanthenyl acetate (7.6–18%) and linalool (0.7–10.4%), while β-myrcene and α-fenchene were the main components of the root oils. Mostly quantitative, but significant changes were observed also inside the shoots, between leaves and flowering tips. The study of Riahi et al. (2013) indicated that two dominant compounds, such as chamazulene and β-thujone were found both in leaves and flowers. However, camphor was found only in flower oil in a proportion of 16.2% and it could not be detected in the leaves. To the contrary, leaf oil was characterized by bornan-2-one (17.33%), whereas flower essential oils were lacking of this compound.

6.4. Environmental factors

Environmental and weather conditions affect significantly the content and composition of the essential oils. In the case of wormwood, results of investigations carried out under controlled conditions are not available. Gholami et al. (2005) showed that there were significant differences among the compositions of oils obtained from *A. absinthium* grown *in vitro*, in greenhouse and under field conditions. While thujone was the predominant compound of the greenhouse and field grown plants (41% and 60%, respectively), this constituent was absent in the regenerated *in vitro* plantlets. Meanwhile, the regenerated plantlet oil was found to be rich in citronelyl-isovalerate (22%) and terpinyl-isobutyrate (11%), but these compounds were not present in the in vivo samples. Interestingly, α-copaene (in 27.5%) was detected only in the oil of greenhouse-grown plants.

To the first glimpse, the effect of habitat and environmental factors may be anticipated from the data of Orav et al. (2006), as well, who published the occurrence of several different chemotypes of wormwood. Large variability was observed among samples from Estonia, Scotland, Moldova, Hungary and other European countries. Unfortunately, based on the published data, the real effect of ecological factors cannot be concluded, because these research samples were collected from retail pharmacies. Thus, the origin of this difference might be any factor (original growing habitat, genotype, sample homogeneity, phenophase, plant organs, etc).

6.5. Extraction methods and storage conditions

It has been demonstrated in the case of several species that the essential oil content and composition may be largely influenced by the different methods used to extract and analyze the volatiles (Figueiredo et al., 2008). In order to get the most reliable picture on the composition of the intact plant, considerable efforts have been invested in research optimizing the extraction and analysis (Jean et al., 1992; Reineccius, 1993).

In order to determine the best extraction procedure for reaching optimal composition from *A. absinthium*, numerous extraction techniques have been investigated by different authors. According to a recent study, organic solvent extraction (OSE) provided the highest yield (23.81%) against supercritical fluid extraction (SFE) and hydrodistillation (HD) (Martín et al., 2011).

Different extraction methods may result not only in different yield but in changing composition of the extracts. Neither sabinene nor α-thujone were found in wormwood product extracted by direct steam distillation (DSD) method, while these compounds could be detected in extracts produced by microwave assisted process (MAP) and distillation in water (DW). Other compounds like a not identified sesquiterpene ($C_{15}H_{24}$) was present in DSD at 4.2%, however, it was absent in MAP and DW (Chiasson et al., 2001).

In another study, *cis*-epoxyocimene, chrysanthenol and chrysanthenyl acetate were the major constituents found both in hydrodistilled (HD) essential oils and supercritical fluid extracts (SFE). However, quantitatively, the extracts were different. *cis*-epoxyocimene was the major compound of HD oils and SFE extracts (22.2% and 39.5%, respectively), but it was a minor one in both OSE hexane oils and OSE ethanol oils (6% and 0.3%, respectively) (Martín et al., 2011).

According to the study of Arino et al. (1999b), the composition of wormwood volatile oil obtained by HSE (headspace extraction) showed an increase in the concentrations of most volatile compounds when compared to the SDE (simultaneous distillation-extraction) extract. Chamazulene – which is also a characteristic compound of only a few species, among others wormwood – only appeared in the oil produced by SDE method (0.18% oil in fresh sample). However, this constituent was absent in the remaining oils produced by HSE, MWE (microwave extraction) and USE (ultrasonic extraction) (Arino et al., 1999b).

It can be established that choosing the proper techniques for the isolation of wormwood oil must play an essential role in the appropriate utilization and obtaining the required quality.

About the effects of primary processing methods on wormwood samples, there are hardly any relevant data. Arino et al. (1999b) investigated the essential oil of samples produced by different drying methods. Treatments included fresh wormwood materials, freezing (fresh wormwood branches were stored for 4 weeks at −18 °C) and air-drying (fresh wormwood branches were allowed to dry at room temperature for about 1 week). The results indicated no significant differences in the composition of the oil extracted from fresh plants compared to plants being processed and between samples produced by two different processing methods.

Storage conditions, such as light, temperature and moisture status play an important role in maintaining the quality of essential oil containing drugs and of the oils themselves. Although there are some data on the effect of storage conditions on wormwood products, the available information is still scarce. According to a study by Blagojević et al. (2006), after one year of storage of the dried herb at ambient temperature ($25\,^{\circ}C \pm 2\,^{\circ}C$) in sealed container, not only the concentration of the oil decreased from 0.29 to 0.08% but the number of detected components dropped, as well. The ratios of some compounds, such as *cis*- and *trans*-linalool oxides, 1,8-cineole and neryl 2-methylpropanoate proved to be higher, while the percentages of sabinene and β-myrcene decreased after one year of storage.

Lachenmeier et al. (2006a,b) reported that the thermal exposure of absinthes at $50\,^{\circ}C$ from 5 to 25 h had no significant changes of the contents of either α- and β-thujone isomers could be detected. The results were the same both in the case of commercial absinthe and a self-produced "Swiss Absinthe of Pontarlier". In the same study, however, after using a treatment of ultraviolet (UV) light irradiation for 25 h, there was a decrease of β-thujone content in commercial absinthe from 9.7 mg/l to 1.8 mg/l, while the α-thujone content of both products and the β-thujone concentration of the self-produced absinthe did not change significantly. The authors have explained the difference by changing amounts of natural antioxidants extracted from the plant material.

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