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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 6 0l 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