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Review

The génépi *Artemisia* species. Ethnopharmacology, cultivation, phytochemistry, and bioactivity

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A B S T R A C T

Wormwoods (*Artemisia* species) from the génépi group are, along with Edelweiss, iconic plants of the Alpine region and true symbols of inaccessibility because of their rarity and their habitat, largely limited to moraines of glaciers and rock crevices. Infusions and liqueurs prepared from génépis have always enjoyed a panacea status in folk medicine, especially as thermogenic agents and remedies for fatigue, dyspepsia, and airway infections. In the wake of the successful cultivation of white génépi (*Artemisia umbelliformis* Lam.) and the expansion of its supply chain, modern studies have evidenced the occurrence of unique constituents, whose chemistry, biological profile, and sensory properties are reviewed along with the ethnopharmacology, botany, cultivation and conservation strategies of their plant sources.

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1. Introduction

Infusions of plants in alcohol are popular in both European and Asian countries, as exemplified by medicinal wines, mulled wines, and medicinal sakes like the Japanese Toso [1]. In this context, plants from the genus *Artemisia* have long been used to flavour alcoholic beverages, giving them a bitter taste and alleged tonic properties. Historically, the Roman *vinum absinthiatum* can be considered an early precursor of what is known today as vermouth [2], and the growing popularity of absinthe, now legal in Europe if complying with the regulatory limits for thujones in alcohol beverages (35 mg/L) [3], further testifies the interest of consumers for this type of beverages. The literature on plant-enriched alcoholic beverages is dominated by studies on absinthe wormwood (*A. absinthium* L.), and little systematic attention has so far been dedicated to the Alpine *Artemisia* species that are used for the production of the celebrated liqueur génépi [4]. One major reason is, undoubtedly, the very limited availability of these iconic plants of the Alpine landscape, since collection in the wild is, depending on the location, either forbidden or severely limited, and domestication has been achieved only recently. Cultivation has been at the basis of the recent growing popularity of génépi-based products (liqueurs, teas, syrups, candies, chocolates, cakes, jams, mustards), a trend that, in turn, has fostered studies aimed at the isolation of compounds useful as markers to fight adulteration. The literature on génépi is scattered and no review article has been published on this topic so far, providing a rationale for summarizing current knowledge on the botany, cultivation and phytochemistry of the Alpine wormwoods from the génépi group, as well as on the molecular- and sensory pharmacology of their constituents.

2. Ethnopharmacology

Limited systematic work has been done so far on the ethnopharmacology of the alpine region of Europe [5]. Local knowledge on medicinal and food plants has mainly been transmitted through oral tradition, and might therefore be in part lost due to the sharp decrease of population observed in the past century and the dramatic changes in lifestyle related to the demise of agriculture and other traditional activities of the alpine economy [5]. In this context, génépi is an exception, since its medicinal properties have been documented at least from the second half of the XVIII century. Thus, unlike absinthe, that was, and growingly is, essentially consumed as a recreational beverage, génépi and its source plants have always been associated with medicinal properties. This was vividly testified by the philosopher Jean Jacques Rousseau who, in a famous passage of his *Confessions*, described the death of the gardener Claude Anet, who had gone on an Alpine trip to collect génépi for a physician (Monsieur Grossi) [6]. Possibly because of the weather and the fatigue associated with reaching the habitat of the plant, the gardener, who should be credited for the life-long interest of Rousseau in plants and botany [7], came back with pleurisy. The condition could not be treated with génépi, considered at that time one of the remedies of choice for pleurisy, and the gardener eventually succumbed to the disease [*…ce pauvre garçon s'échauffa tellement, qu'il gagna une pleurésie, dont le Génipi ne put le sauver, quoiqu'il y soit, dit-on, spécifique* (a pleurisy that genipi could not relieve, though said to be specific in that disorder)] [8].

In folk medicine, génépis are used as thermogenic agents to fight cold, a use testified by the credence that when sheep eat these bitter plants, the night will be very cold [9]. The association of génépi with cold resistance might be related to the thick hairy layer that covers the plant, a protection against the low temperatures of its environment (as well as against the sun). Infusions of génépis are used to fight cold and fever and stimulate perspiration, while wines aromatized with these plants are believed to stimulate appetite, promote digestion, and fight mountain sickness [4]. Like other asteraceous plants, génépis are used topically as wound-healing agents and to resolve bruises, like in the “Vulnéraire Suisse” or “Falktranck” that was celebrated in the ancient European pharmacopoeias [4].

Bitter liqueurs became popular as tonic medicines in the XIX century, and remained so until the first half of the past century. During the prohibition time, Fernet Branca remained, in fact, the only spirit legally sold in US, to the point that an American distillery for its production was opened in New York City's Tribeca [10]. Because of the rarity of the plant and the difficulty of its collection, the large-scale industrial production of the liqueur génépi started relatively late compared to absinthe and other liqueurs, being first documented only in 1827–1840 at Maison Chavasse near Chambéry in Savoy, at that time part of the Kingdom of Piedmont [11]. During the second half of the XIX century, génépi enjoyed a stellar fame for its digestive properties, immortalized by the comment of De Amicis, the author of the children book *Heart* (*Cuore*), who enthusiastically defined it “un liquore di fiori di prato che farebbe digerire una bomba lessa” (a liqueur of field flowers that would make you digest a boiled bomb) [12]. The consumption of the liqueur declined with the ban of absinthe in the early XX century, but increased with the popularity of winter sports since the 1970s, with génépi becoming a popular après-ski drink. However, its consumption has so far been substantially limited to the Alpine environment, although it can be found in premium liqueur stores in all major European cities. Because of the thujone regulation, the commercialization of génépi in the USA is limited to products that are prepared from thujone-free chemotypes of the plants [3]. Over the past decade, the production of the liqueur génépi has started also in central Italy, since one of the alpine génépi species (*Artemisia eriantha* Ten.) also grows in the high regions of the Appenines, and cultivation experiments have been successfully carried out in the Abruzzo region of Central Italy [13]. Although there are many commercial producers of génépi, it is difficult to provide an estimation of the overall harvest of the plant, since home production is widespread in the Western Alpine regions. The traditional recipe follows the so called *rule-of-four*, meaning that each litre of 40% alcohol requires 40 g of sugar and 40 flower heads [4].

3. Botany, genetics and conservation

3.1. Botany

The etymology of the name génépi is controversial. It might derive from the Arpitan dialect *zhènépi* or *jnépi*, a name used for several *Artemisia* species in Savoy (France), but a derivation from the Latin *Dianae spicum*, meaning ‘Diana's ear’ in reference to the shape of the inflorescence, has also been proposed [14]. In the Alps, the name génépi is used to refer to five perennial and aromatic *Artemisia* species (Table 1), traditionally collected from wild populations to produce digestive liquors and herbal teas.

Artemisia umbelliformis Lam., also called Alpine wormwood or white génépi, is the most widespread and easy to cultivate. *A. genipi* Weber, the so called black génépi, is the favourite species of liquor producers. *A. eriantha* Ten. is not often used for génépi, growing mainly in the Mediterranean Alps and in the Apennines. *Artemisia glacialis* L. is the least aromatic species and is rarely used to produce génépi. Even more rarely

Table 1
The génépi *Artemisia* species and their current state of protection (allowed amounts of collection in parentheses).

Country	Region	*A. umbelliformis* Lam.	*A. eriantha* Ten.	*A. genipi* Weber	*A. glacialis* L.	*A. nivalis* Br.-Bl.	Sources
Italy		1 kg (fresh weight) per person	–	1 kg (fresh weight) per person	1 kg (fresh weight) per person	–	Royal Decree no. 772 issued in 1932
	Alto Adige	Total protection					[89]
Switzerland		Protected and classed as Least Concern (LC)	–	Protected and classed as Least Concern (LC)	Protected and classed as Least Concern (LC)		[90]
	Ticino			Near threatened (NT)			www.infoflora.ch
	Valais				Near threatened (NT)	Endangered (EN)	www.infoflora.ch
France		No protection at the national level, but only 100 flowering stems are allowed to be collected	No protection at the national level, but only 100 flowering stems are allowed to be collected	No protection at the national level, but only 100 flowering stems are allowed to be collected	No protection at the national level, but only 100 flowering stems are allowed to be collected	–	
	Alpes-Maritimes	Protected	Protected	Protected	Protected		Arrêté préfectoral du 18 juin 1996, Protection et réglementation de certaines espèces végétales/Article 2
	Isère	Protected	Strictly protected	Protected	Protected		Arrêté préfectoral n°93–295 du 21 janvier 1993: Protection des espèces végétales sauvages dans le département de l'Isère/Articles 2 et 3
	Alpes-de-Haute-de-Provence	Protected	Protected	Protected	Protected		Arrêté préfectoral n°95/1533 du 28 juillet 1995, [département des Alpes-de-Haute-Provence]: Réglementation de la cueillette de certaines espèces végétales sauvages/Article 4
	Hautes-Alpes	Limited to one handful. Destruction of underground parts, sale and purchase of material are forbidden	Limited to one handful. Destruction of underground parts, sale and purchase of material are forbidden	Limited to one handful. Destruction of underground parts, sale and purchase of material are forbidden	Limited to one handful. Destruction of underground parts, sale and purchase of material are forbidden		Arrêté Préfectoral modificatif N° 2008-185-7 du 03 juillet 2008. Réglementation de la cueillette de certaines espèces végétales protégées/Article 3 modifié
Germany		On the Red List	–	–	–	–	Bundesartenschutzverordnung vom 16. Februar 2005 (BGBl. I S. 258, 896), die zuletzt durch Artikel 10 des Gesetzes vom 21. Januar 2013 (BGBl. I S. 95) geändert worden ist
Austria		No protection	–	No protection	–	–	
Spain	Pyrenees, Cantabrian Mountains and Sierra Nevada	Extremely rare	–	–	–	–	
Slovenia		No information	–	No information	–	–	
Carpathians and Balkans		No information	No information	–	–	–	

used, the very aromatic and extremely rare *Artemisia nivalis* Br.-Bl. might simply be a glabrous mutant of *A. genipi* [15]. In addition, a recent ethnobotanical survey documented the inclusion of *Artemisia vallesiaca* All. and various species of *Achillea* (*Achillea nana* L., *Achillea erba-rotta* All. subsp. *moschata* Wulff., *A. atrata* L.) in the production of génépi in Valais (Switzerland) and in the Aosta Valley (Italy) [16].

All five génépi species are small caespitose, sericeous perennial plants (5–30 cm), with a woody rhizome and aromatic basal leaves arranged in rosette. The stem is usually unbranched with more or less pubescent leaves and bracts. Basal and stem leaves are petiolate or sessile, simple to 7-fid (1–8 mm long). The capitulae (3–7 mm across) are erect or slightly nodding with a glabrous or pubescent base, composed of 8–50 yellowish florets. The capitulum is heterogamous, with female outer flowers and hermaphroditic inner flowers, pollinated by insects. The fruits (achenes) are glabrous to hairy. These five species are typical of high mountain habitats (2000–3200 m) where they grow on moraines, screes, rocks and arêtes, with a partially overlapping distribution [17,18] (Table 1). The distinction between the various species of génépi is difficult: Table 2 provides a simplified dichotomous key to distinguish them morphologically, a task rendered more difficult by the existence of some rare interspecific hybrids with overlapping morphological features (*A. genipi* $\times$ *A. glacialis*; *A. genipi* $\times$ *A. umbelliformis*; *A. glacialis* $\times$ *A. umbelliformis*) that have been observed in Aosta Valley (Italy) and in Valais (Switzerland) [18]. A combination of genetic, genomic and phytochemical studies would certainly provide a better distinction tool.

3.2. Genetics

According to a caryological review, the basic chromosome number in the genus *Artemisia* is $x = 9$, with only two species presenting the less common $x = 8$, namely *A. glacialis* L. and *A. granatensis* Boiss [19]. The caryotypes of each species that are used to make génépi are given in Table 1. In *A. umbelliformis*, the majority of the populations show the unusual existence of a stable hypotetraploid cytotype with $2n = 34$, most likely due to a chromosomal fusion, whereas the eutetraploid level $2n = 36$ is limited to some French populations.

The taxonomy of *Artemisia* species has long been debated [20], and most sources have agreed with De Candolle's subdivision of the genus in two sections named *Abrotanum* Bess. and *Absinthium* DC. However, a molecular phylogeny study based on the internal transcribed spacers (ITS) of the ribosomal DNA gene of 25 *Artemisia* taxa supported the monophyly of the genus, and differentiated five main subgenus clades: *Absinthium*, *Artemisia*, *Seriphidium*, *Dracunculus* and *Tridentatae* [21]. Within the génépi species *A. umbelliformis* and *A. glacialis* were grouped in the section *Absinthium*, where also *A. genipi* was moved from the section *Artemisia*, where it had originally been placed on the basis of morphological studies. *A. eriantha* and *A. nivalis* were not included in this study, but it can be assumed that they would also belong to the *Absinthium* section.

3.3. Conservation

In the IUCN Red List of Threatened Species [22,23], *A. nivalis* is endangered, *A. genipi* and *A. eriantha* are in the Least Concern (LC) category, while *A. umbelliformis* and *A. glacialis* are not listed. Table 1 details the current state of protection of all five species in Europe, but there is a lack of information on the status, population and distribution in the Carpathian and Balkan mountains, and no information to confirm its presence in Slovakia or Poland. As with many plants on the Red List, domestication and cultivation are the best strategies to preserve natural populations.

4. Domestication, breeding and cultivation

4.1. Domestication

In Piedmont (Italy), Aosta Valley (Italy), Wallis (Switzerland) and Savoy (France), génépi has been traditionally collected to prepare herbal infusions, decoctions in milk, and, since a little more than a century, liqueur by maceration in alcohol [4]. The production of liqueurs has been steadily growing since the 1960s, and nowadays it is likely that several hundreds of kilogrammes of dried plants are processed every year into génépi [5]. However, the cultivation of génépi is currently unable to meet the high demand for these plants, and wild collecting is still a reality, although this is forbidden in Italy and in Switzerland and strictly regulated in France (Table 1). With the increase of the needs of liquor, perfume and cosmetic (hand creams, sunscreens or anti-ageing creams) industries, the cultivation of *A. umbelliformis* has become the only way to secure a renewable supply chain for this plant.

The first attempts to domesticate *A. genipi* and *A. umbelliformis* were carried out in the middle of the XX century in the Swiss Alps [24,25]. Later on, several attempts were also performed in Italy [26,27] and in France [28,29]. They were all eventually thwarted by a high mortality rate, probably due to the low altitude or to fungal diseases caused by *Puccinia*, *Pythium*, *Sclerotinia*, *Fusarium* or *Phomopsis* [30]. In 1989, a systematic project of domestication and selection from wild alpine populations of génépi was started by Agroscope in Switzerland, with the aim of obtaining robust cultivars with low thujone content that are adapted to organic production [31]. Out of the five génépi species, four were initially tested for cultivation with seeds collected from wild populations in the Swiss Alps (Wallis), where agronomic and phytochemical characteristics were evaluated for three years at altitudes between 1000 and 1600 m: *A. genipi*, *A. eriantha*, *A. glacialis* and *A. umbelliformis*. *A. genipi* and, to a lesser extent, *A. eriantha* did not resist to fungal diseases (mainly *Puccinia absinthii*) and produced low yields. *A. glacialis* showed both low yields and low aromatic properties, in accordance with its limited use in the preparation of liqueurs. Finally, *A. umbelliformis* turned out to be the only species that was suitable for domestication and breeding, showing genotypes with erect growth and a relatively high yield potential compared to the other four alpine génépi species [31] (Fig. 1). The essential oil of *A. umbelliformis* contains a high percentage (up to 70–80%) of thujone, just like some populations of *A. absinthium*, a compound whose toxicity has long been debated [32]. The concentration of thujone in alcoholic beverages is strictly regulated within the EU, with the current limitation being 35 mg/L in the final product. This limitation is controversial due to the uncertain toxicological status of thujone, but is still implemented. Therefore, the objective of the breeding programme was to obtain cultivars of *A. umbelliformis* with low contents of this compound.

Table 2
Simple dichotomous identification key of the génépi species (based on Illustrierte Flora von Mitteleuropa [91]; Flora Europaea [17] and Flora der Schweiz [18]).

Pubescent bottom of the flower head	Capitulae (3–10) at the top of the stem, each with 25–50 florets; glabrous corolla		*A. glacialis*
	Capitulae along the stem, often heaped at the top, each with 10–30 florets; corolla mostly hairy		*A. umbelliformis*
Glabrous bottom of the flower head	Capitulae 4–7 mm in diameter, nodding, leaning on one side, 20–50 florets; inner bracts with light edge; plant height 10–30 cm		*A. eriantha*
	Capitulae 2–4 mm in diameter, erect (but often with nodding ears), leaning in all directions, 8–20 florets; inner bracts with brown edge; plant height 5–15 cm	Plant felted grey and pubescent	*A. genipi*
		Plant glabrous	*A nivalis*

Fig. 1. Cultivation of *Artemisia umbelliformis* for génépi production goes through growing seedlings (a) and plantlets (b) before planting at a density of 10 plants/m^2 (c).

4.2. Breeding

Plants collected from four natural populations in Wallis, Switzerland (Mattmark, Simplon, Gornergrat and Valsorey) showed a significant phenotypical and phytochemical heterogeneity, which was consistent with previous observations in French populations [31,33] (Table 3) and which was very useful for successful breeding (Table 4). The most interesting plants came from Simplon and Mattmark regions connecting Wallis to Piedmont (Italy). Mattmark plants showed a low mortality rate, high yields and nearly no thujone, whereas the Simplon plants showed up to 70% thujone in their essential oils. Several elite clones with erect growth were selected in the Mattmark and Simplon populations and were propagated in vitro, as well as cultivated in separate fields for open pollination in order to create homogeneous cultivars. A very high degree of homogeneity was observed, also because a significant amount of self-pollination has been observed in *A. umbelliformis*, the capitulae being composed of hermaphroditic self-fertile central flowers and female-only peripheral flowers [31,32]. The cultivar obtained with the plants from Mattmark showing nearly no thujone was named 'RAC 12' (in reference to the old name of the Agroscope research centre, Recherche Agronomique Changins), while the one obtained with plants from Simplon showing a significant amount of thujone (60–70% of the essential oil) was named 'RAC 18', and later on 'RAC 10'. Recently, a PCR–RFLP method was applied to the thujone-free 'RAC 12' and the thujone-rich 'RAC 10' as well as to native plants from Piedmont using RsaI and TaqI restriction enzymes on the sequence of the 5S-rRNA-NTS gene spacer region, and it enabled to clearly distinguish the two chemotypes [34]. Thujone-rich native plants produced a single fragment of about 224 bp (NCBI GenBank accession no. EU816950), whereas thujone-free cultivated plants produced a single fragment of about 327 bp (NCBI GenBank accession no. EU816951). This 103 bp difference is not uncommon between chemotypes or cytotypes [35]. Another interesting ecotype, a so-called Occitan ecotype

Table 3
Mortality after 3 years of cultivation, yield of two harvest (one in the second and the other in the third year) and composition of the essential oil of four accessions of *Artemisia umbelliformis* Lam. in a field trial in Bruson (1100 m), Switzerland [after 31].

Analysed parameters	Accessions from four alpine sites in Switzerland			
	Mattmark	Simplon	Gornergrat	Valsorey
Mortality (%)	19	41	98	55
Yield (dw, g/m^2)	155	79	30	96
Essential oil composition (%)				
β-pinene	31	0	3	2
1,8-cineole	17	3	1	23
α-thujone	0	72	0	0
β-thujone	0	0	0	0
Borneol	5	3	0	20
Terpinen-4-ol	2	0	2	3
Trans-caryophyllene	2	0	1	2

Table 4
Yields, essential oil content and costunolide content in the floral stems of white génépi (*Artemisia umbelliformis* Lam. 'RAC12') depending on five harvest stages (in 2002 and 2003) [39,41].

Harvest stages[a]	Floral trusses yield		Essential oil content		Costunolide content	
	(g dw/m[b])		(mL/100 g dw)		(g/100 g dw)	
	2002	2003	2002	2003	2002	2003
Stage 1	48 ab[b]	43 b	1.31 ab	1.46 b	2.72 a	2.91 a
Stage 2	37 b	45 b	1.53 a	1.76 a	3.00 a	2.76 a
Stage 3	93 ab	64 b	1.08 b	0.71 c	2.78 a	0.93 b
Stage 4	87 ab	73 ab	0.61 c	0.41 c	1.26 b	0.75 b
Stage 5	102 a	94 a	0.43 c	0.41 c	1.16 b	0.56 b

[a] Harvest stages: Stage 1 = "just before flowering"; Stage 2 = "beginning of flowering"; Stage 3 = "full flowering"; Stage 4 = "end of flowering"; Stage 5 = "flowering over".
[b] Newman–Keuls test: different letters indicate statistically significant differences ($P < 0.05$) between harvest stages.

of *A. umbelliformis*, is also cultivated in Italy (Elva, Valle Gesso, Valle Stura, Val Chisone and Gran Paradiso).

4.3. Cultivation

A recent ethnobotanical study evidenced that liquors produced with the traditional home-made mix of *A. genipi* and *A. glacialis* were preferred by consumers compared to those prepared with a cultivated strain of *A. umbelliformis* [5], suggesting that breeding activities should continue and go beyond the issue of thujone contents by considering also the profile of bitter compounds. On the other hand, the income from wild gathering could reach a significant value [5], suggesting that the practise of cultivation needs capillary distribution in the Alpine region to replace collection.

Micropropagation of both *A. umbelliformis* and *A. genipi* by meristem [36] was first reported in France [33,36]. Field trials from in vitro plants have been carried out since 2006 by the Centre d'Expérimentation et de Recherche en Biotechnologies Végétales (CERBIOTECH, Gap, Hautes-Alpes) in order to test their adaptability to low altitude. In Switzerland, micropropagation of the mother plants of 'RAC 12', 'RAC10' and other selected clones of *A. umbelliformis* could be successfully carried out [32]. In vitro shoot proliferation was achieved using nodes segments cultured on Murashige and Skoog [36] medium supplemented with 0.88 μM benzyl adenine (BA) and 2.0 μM indole-3-acetic acid (IAA).

A. umbelliformis is the only Alpine génépi species that has been successfully cultivated to some extent in Alpine areas of Switzerland and of France and on a larger scale in the western Alps of Italy [37]. Cultivation of this species favours the protection of endangered habitats and the preservation of natural plant resources within the Alps. For a successful cultivation, specific conditions are required: the plant grows better at elevations above 1600 m, and requires drained soils and south facing exposition [38]. For field cultivation (Fig. 1), *A. umbelliformis* is commonly propagated by seeds, which are viable up to several years if stored in dry and cool conditions. The germination of fresh seeds is very fast and the rate is high: in an experiment carried out in Switzerland, about 90% of the seeds had germinated after 10 days [39]. This feature most likely helps alpine plants like *A. umbelliformis* to grow and bloom the same year during the short vegetation period at high altitude. Planting of seedlings gives better results than direct sowing. In a cultivation field, the optimal density of plantlets is around 10 plants/m^2 on three to four rows, in order to facilitate mechanical weed control and harvesting [40] (Fig. 1). Fields of *A. umbelliformis* are usually kept for three years, with floral stems being harvested on the second and third years only. Although weed removal costs can be reduced by using plastic covering, this is not recommended because there is a high risk of plants losses (Fig. 1). Indeed, plastic covering favours soil-borne diseases such as *Fusarium* sp., *Sclerotinia* sp. and *Rhizoctonia* sp. that cause serious damage to organically grown génépi, especially at low altitudes, in heavy and damp soils, as well as in fields that were previously planted of génépi, cereals or vegetables [41].

Fertilization recommendations have been established based on the exportation of N, P, K, and Mg for an estimated yield of 1500 kg dry weight per ha: 30 kg/ha N, 20 kg/ha P_2O_5, 40 kg/ha K_2O, 5 kg/ha Mg [42]. In addition, fertilization (especially with nitrogen) at the vegetation start in spring has proved to give better yields than fertilization after harvest, or than splitting the nitrogen amount between the two periods [39]. More recently, it has been reported that inoculation of native arbuscular mycorrhizal (AM) fungi from alpine grassland on *A. umbelliformis* and *A. glacialis* in greenhouse conditions significantly increased P concentration in shoots, and that the use of the highly mycorrhizal species *Trifolium pratense* as a companion plant impacted positively on mycorrhizal colonization of *A. umbelliformis*, which might help extending the lifespan of génépi cultivation fields [38]. However, this technique is not applied in field cultivation up to now, mainly because of difficulties in mechanical harvesting of the génépi.

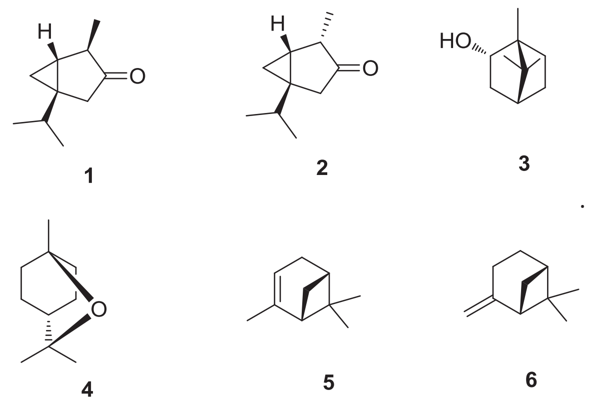
A. umbelliformis must be cultivated on well adapted sites in order to reach optimal yields. Soils with high proportion of sand have proven to favour plant development and yields [39] and to reduce the pressure of soil-borne diseases such as *Fusarium* sp., *Sclerotinia* sp. and *Rhizoctonia* sp. [37,41]. Therefore, it is recommended to cultivate white génépi in light soils with more than 60% of sand. The yields and the quality of the cultivar 'RAC 12' have shown to increase with altitude until 1550 m, probably thanks to better flower induction conditions as the altitude increases [39,41]. In terms of quality, the essential oil composition showed no clear correlation with altitude [41]. In contrast, the content of costunolide in the floral stems decreased as the altitude increased [39,41]. Costunolide is one of the compounds that are responsible for the bitter taste of génépi, potently interacting with the human bitter taste receptor hTAS2R46 [43]. Reduction of the costunolide content of the flower stems might be positive in order to reduce the intensity of the bitter taste of the liquors produced with the cultivar 'RAC 12'.

The optimal harvest stage is a critical issue in medicinal and aromatic plant cultivation, putatively affecting yield and phytochemical profile [39,44]. In génépi, a strong influence of the harvest stage was observed on the essential oil concentration in the floral stems (Table 3). At the beginning of flowering, the essential oil content of the thujone-free cultivar 'RAC 12' exceeded 5%. After 7–9 days, the essential oil content dropped by 30% in 2002 and by 60% in 2003. However, no significant variation in the chemical composition of the essential oil was observed in relation to harvesting stages. The kinetics of costunolide content was very similar to that of the essential oil, with a maximum at the beginning of flowering and a quick drop towards full flowering. At its maximal concentration, the costunolide content was very high, reaching about 3%. The harvest stage also had an important effect on floral stems yield that had doubled between the beginning and the end of the flowering period, reaching up to 100 g/m^2. Therefore, harvesting white génépi at the beginning of flowering is recommended to ensure product quality, even if yields are higher at the post-flowering stages. Therefore, prices for *A. umbelliformis* harvested at the beginning of flowering must be higher than at later stages in order to obtain a high quality product.

5. Phytochemistry

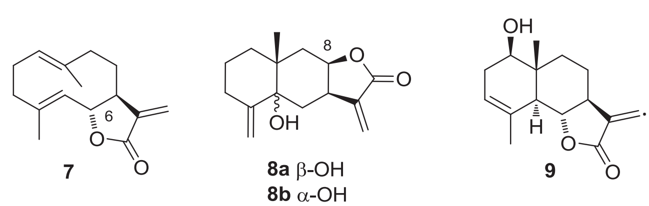
The essential oil (EO) of génépi, credited with spasmolytic, sedative and antiseptic properties, is mainly produced in leaves and flowers, and is characterized by very low isolation yields (0.1% on dry weight) and a significant percentage of thujone (45–76%) [45]. Over one hundred EO constituents have been characterized in *A. genipi* and *A. umbelliformis* [34,45,46], and the most abundant of them are used to distinguish chemotypes within a species or a group of species. Within the *Artemisia* species that are used to make génépi, four distinct chemotypes with distinct olfactory properties were identified as early as 1984 [47]:

a) Thujone (45–76%): This sabinane monoterpene exists in two diastereomeric forms, α-thujone or (+)-3-thujone (**1**), and β-thujone or (−)-3-thujone (**2**), with the β-form prevailing in génépis. The Agroscope cultivar 'RAC 10' is rich in thujone, with up to 60–70% [31]. Although absinth was banned in several countries because of the neurotoxicity of thujone, recent GC–MS studies have found a low content of this compound in absinth, concluding that it plays a negligible or only a minor role in the clinical picture of absinthism [48].
b) Borneol (**3**, up to 68%), with the woody camphoraceous note associated with this insect-repellent.
c) Cineol (**4**, ca. 25%)–borneol (**3**, ca. 21%), characterized by the spicy camphoraceous note of cineol (=eucalyptol)
δ) α-,β-Pinenes (**5**, **6**, respectively, 22–48%), with a pine resin note.



Intermediate chemotypes were also observed in GC profiles [46,47], suggesting a high degree of variability in the composition and in the organoleptic properties of the oils.

Artemisia species from the génépi group contain sesquiterpene lactones, responsible for the bitterness of the plant and possibly also involved in its bioactivity. Significant differences exist between the sesquiterpene lactones profile of various collections of plants classified as génépi, with *cis*-fused C-8 lactones and C-6 *trans*-fused lactones having an essentially vicariate distribution that apparently transcends the botanical classification [34]. Thus, the C-6 *trans*-lactone costunolide (**7**) is the major constituent of *A. genipi* [49] as well as the RAC 12 chemotype of *A. umbelliformis* [34], while telekin (**8a,b**)-type C-8-*cis* lactones are typical of *A. eriantha* and of the chemotypes of *A. umbelliformis* Lam. from the Southern Alps [50,51], with *A. glacialis* being totally devoid of compounds of this type [52]. A chemotype of *A. umbelliformis* containing very high concentrations of the C-6 *trans*-lactone santamarine (**9**) was found in the very first study on the non-volatile constituents of these plants [53], and this trait was also detected in a population of *A. genipi* from the Southern Alps [52]. In general, very high concentrations of lactones are present (up to 2% on dry plant material), and all compounds are of the exomethylene type, unlike lactones from wormwood, that are of the 11,13-dihydro type. Given the relatively small number of samples investigated, the variation in sesquiterpene lactones is really remarkable, and a systematic study of the distribution of sesquiterpene lactones in wild populations of the plants would probably identify additional chemotypes. Some sesquiterpene lactones are shared by different species, while others are unique to a population, like the sesquiterpene-monoterpene Diels–Alder adduct genepolide (see below), typical of the RAC 12 chemotype of *A. umbelliformis* [54].



From a structural standpoint, the presence of high amounts of the hydroperoxides **11a,b** in the Southern Alps chemotype of *A. umbelliformis* is interesting and might bear a relationship with the high intensity of solar exposure during the short period of vegetation. The plant contains a Δ^4-eudesmanolide (**10**) that could be converted by activated oxygen into a mixture of the two allylic hydroperoxides **11a,b** by an ene-type reaction, or, alternatively by [2π + 2π] cycloaddition into the dioxetane **12**, next fragmented to the seco-lactone umbellifolide (**13**) (Fig. 2).

These compounds have an interesting biological potential, being both electrophilic Michael acceptors and oxidants. Since Nrf2, the master switch of the intramolecular response to dangerous xenobiotics is sensitive to both electrophiles and oxidants [55], the hydroperoxides

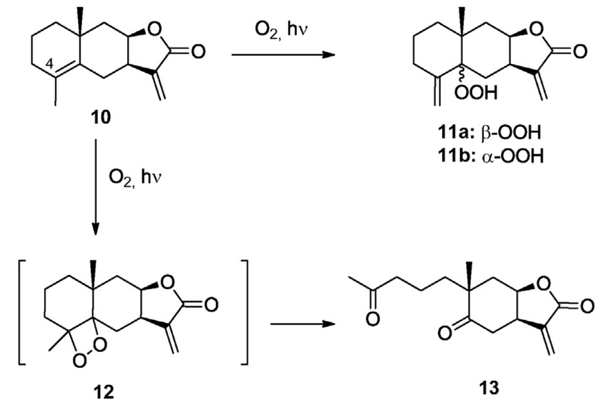
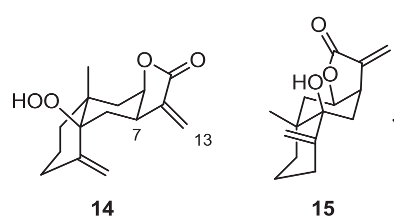


Fig. 2. Biogenetic relationships between the C-8 *cis*-fused sesquiterpene lactones of *A. umbelliformis.*

of exomethylen-sesquiterpene lactones are potentially capable of activating Nrf2 in a two-fold fashion, a molecular mechanism that might well fit the "tonic" reputation of génépi-based products.

A remarkable feature of the *cis*-decalin hydroperoxide **11a** is the conformation switch from a steroid-like to a non-steroid-like conformation upon reduction of the hydroperoxyl group [50]. This behaviour was first evidenced in solution, and is a remarkable example of the diagnostic power of the Samek rule on the relationship between the allylic coupling constant of the exomethylene protons and the conformation of the system [50]. Thus, the steroid-like conformation (**14**) associated with the *cis*-hydroperoxide is characterized by a small value (ca 1 Hz) of the allylic coupling $^3J_{7,13}$, while in the corresponding alcohol (epitelekin, **8a**) this coupling constant is higher (ca 3 Hz), as expected from a non-steroid like conformation (**15**). These differences are maintained also in the solid state, as evidenced by a crystallographic analysis of the hydroperoxide **11a** and its corresponding alcohol (**8a**, telekin) [56]. One possible explanation, backed up by ^{13}C NMR consideration, is related to an anomeric-type effect of the oxygen–oxygen bond on the π-olefin system, that favours a conformation with a *syn*-relationship between the exocyclic allylic double bond and the oxygen–oxygen bond [56].



The seco-eudesmanolide umbellifolide (**13**) is formally the product of dioxetane cleavage of the endocyclic olefin **10**, a minor constituent of the plant, and has been synthesized from artemisin [57]. The eudesmane olefin **10** is also the plausible precursor of the hydroperoxides **11a,b**, the major constituents of *A. umbelliformis* from the South-Western Alps and of *A. eriantha* (Fig. 3). Apart from the colour reaction with ferrous (II) thiocyanate related to the presence of the hydroperoxyl group, the hydroperoxyeudesmanolides **11a,b** also give a deep red colour with acids, while their corresponding alcohol **8a,b** do not give any colour under the same acidic conditions. This behaviour could be the result of a fragmentation of the eudesmane skeleton, with generation of a germacrane ketone related to tanacetols. This hypothesis was supported by the similarity of the visible spectrum of the red species generated from the hydroperoxides and tanacetol A (**16**) (Fig. 3) [58]. These colour reactions were used to localize histochemically the hydroperoxides in superficial glandular structures in various tissues of *A. umbelliformis* [58].

Considerable attention has been dedicated to hydroperoxides from *Artemisia* species, in the wake of the development of the seco-cadinane artemisinin as an anti-malarial drug. It has been suggested

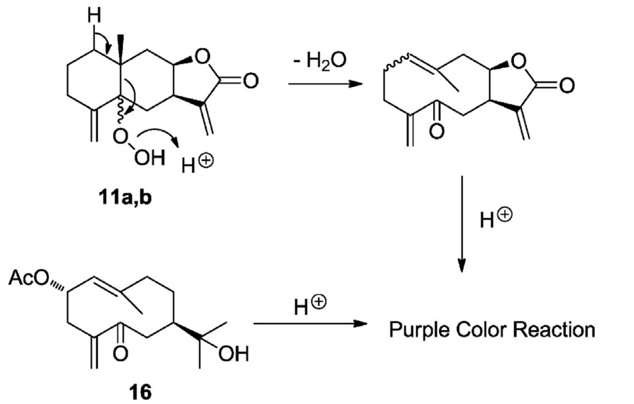
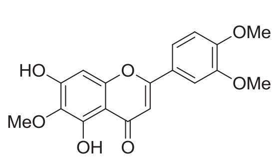


Fig. 3. Fragmentation of the hydroperoxides **11a,b** into germacrane ketones related to tanacetol A (**16**).

that the seco-cadinane artemisinin is an artefact of the post-harvesting oxidation of an unstable cadinane precursor [59]. Since the eudesmane olefin **10** is a stable compound, the accumulation of hydroperoxides by *A. umbelliformis* seems to be the result of a genuine enzymatic activity. During its brief vegetative period, the plant is indeed exposed to high solar irradiation, and the "organication" of reactive oxygen species (singlet oxygen) might represent a detoxification strategy.

Another interesting constituent of génépis is the sesterpene lactone genepolide (**17**), formally the Diels–Alder adduct between the sesquiterpene lactone costunolide and the monoterpene myrcene (**18**). Genepolide was isolated from the RAC 12 of white génépi (*A. umbelliformis*) [54], and is apparently the result of a genuine Diels–Alderase activity in the plant, since the parent precursors were virtually unreactive under a variety of conditions that promote cycloaddition reactions (heating, addition of Lewis acids) [54]. Myrcene is an unreactive diene, rather reacting as a dienophile in reverse electron-demand cycloadditions, as demonstrated in the biomimetic synthesis of some sesterpene Diels–Alder adducts by cycloaddition with an eudesmane dienone [60]. Conversely, the exomethylene lactone moiety of costunolide was shown to react with activated dienes of the artabsin-type to give in a regioselective way a dimeric lactone structurally related to arteminolides, a class of farnesyl-protein transferase inhibitors isolated from an Asian *Artemisia* species [54]. Interestingly, quaternarization at C-11 promoted the Cope-rearrangement of the germacradiene system, and moderate heating quantitatively transformed genepolide into its corresponding elemanolide **19**. This behaviour is typical of C-8 *cis*-lactonized germacranolides, that adopt a parallel conformation of the homoconjugated diene system that favours their interaction [61], but the configuration of the Cope adduct of genepolide indicates that the reaction proceeds via the crossed conformation of the cyclodecadiene system. The mechanistic rationale for this behaviour is therefore unclear.

All chemotypes of *A. umbelliformis*, *A. genipi* and *A. eriantha* investigated so far contain the lipophilic flavonoid eupatilin (**20**) [62]. Lipophilic flavonoids are typically accumulated in rutaceous plants, but can also be found in high concentrations (ca 0.1%) in some *Artemisia* species, like artemetin (**21**) from wormwood (*A. absinthium*) [62]. The concentration of eupatilin in génépis is much lower, in the range of 0.02% on dry weight, but the presence of a single compound is remarkable, since complex mixtures or methylated flavonoids are typically found in asteraceous plants [63].



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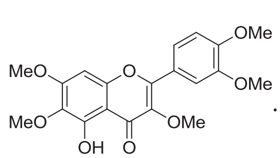

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6. Authentication

With the exception of *A. glacialis*, all the other *Artemisia* species from the génépi group show a distinct profile of essential oil and sesquiterpene lactones [34]. As already remarked, there is little overlapping between the isoprenoid profiles and the morphology-based taxonomy of these plants. *A. umbelliformis*, *A. genipi* and *A. eriantha* are closely related, and can hybridize, hampering a clear-cut distinction between them, as discussed in Section 3. On the other hand, based on the presence of thujone and a specific profile of sesquiterpene lactone, three basic chemical profiles seem to exist: thujone/C-6 *trans*-olides, eucalyptol/C6 *trans*-olide, and thujone/C-8 *cis*-olide. The overlapping between the chemotaxonomic and the phenotypic classification is poor, and genomic methods have been applied to address the complex classification of *Artemisia* species from the génépi group.

There is growing evidence that, unlike wines, where precipitation of hydrophobic proteins occurs easily with ageing, the higher alcohol contents and lower dielectric constant of aperitifs and liqueurs make them retain traces of proteins from the plants used in their preparation [64]. A proteomic analysis is therefore possible, and this approach was validated with the herbal liqueur Braulio [64]. It might similarly be possible to develop a proteomic profile of génépis to detect frauds, with proteotyping complementing the chemical analysis.

7. Bioactivity

7.1. Molecular targets

7.1.1. Bitter receptors

The bitter sesquiterpene lactones from génépi were instrumental to deorphanize the bitter receptor hTAS2R46, a previously orphan receptor without any known ligand, that was identified as their major sensory target [43]. Further investigations showed that this receptor is broadly tuned, recognizing not only bitter terpenoids like sesquiterpene lactones and clerodane diterpenoids, but also nitrogen compounds like strychnine and denatonium [65]. Structure–activity relationships emerged. Thus, reduction of the exomethylene lactone group is tolerated and is generally uninfluential for activity. On the other hand, the lactonization site is important, with C-6 *trans* lactones being generally more potent than C-8 *cis* lactones, an observation rationalizing the more bitter properties associated with costunolide-containing génépis compared to those containing telekin-type lactones, that were unable to interact with hTAS2R46 at a threshold of 10 000 nM. For costunolide (**7**), this threshold was 300 nM, similar to that of the diterpenoid marrubiin, while umbellifolide (**13**) was a weaker ligand, with an activation threshold of 10 000 nM. Molecular modelling studies suggested that the inactivity of telekin-type lactones (**11a,b**) might be due to the presence of the angular oxygen function at C-5 [43].

The activation of bitter receptors might, in principle, underlie the activity of génépi against airway infections, since hTAS2R46 is highly ectopically expressed in the nasal epithelium [66] and in the airways [67], where its activation stimulates, respectively, ciliar motion with secretion of the antibacterial gas NO (nasal epithelia), and relaxation (bronchial tissues), two activities useful to prevent infections and improve ventilation. It is not clear, however, how the traditional use of the plant in folk medicine, based essentially on herbal teas, could have delivered non-volatile compounds like sesquiterpene lactones to their potential sites of action in the airways. Bitter receptors are also expressed in the gastro-intestinal tract, where they mediate the liberation of intestinal hormones, and especially ghrelin, a messenger of hunger [68]. After an initial stimulation of hunger, a prolonged sensation of satiety follows, making it difficult to evaluate the overall impact of bitter compounds on weight management, although the short-term effect would be in line with the use of bitter aperitifs before meals to stimulate hunger.

7.1.2. Thiol trap-sensitive transcription factors

Exomethylene-γ-lactones are electrophilic compounds, and interact covalently with thiol-sensitive targets like the transcription factors NF-kB, STAT3, and Nrf2 [69]. In the case of NF-κB, the activity of the exomethylene-γ-lactones could be synergized by the flavonoid eupatilin (**20**), that also inhibits the activation of this transcription factor [70]. Methylated flavonoids have better absorption and metabolic stability compared to other classes of flavonoids [71]. Nevertheless, due to the low concentration of these compounds in génépi products, and to their overall limited intake, it is unlikely that the activity on these targets alone might be associated with any systemic activity. On the other hand, activation of NF-κB has been associated with gastric mucosal damage [72], and it does not seem unrealistic to associate the gastroprotective properties of génépi with a "local" intestinal inhibition of NF-κB activation. In a structure–activity study, the hydroperoxides of telekin (**11a,b**) and umbellifolide (**13**), all C8-*cis*-lactones, outperformed costunolide and other C-6 trans-lactonized compounds in terms of inhibition of TNF-α or PMA-induced activation of NF-κB [73]. As expected, genepolide (**17**) was inactive. Nevertheless, this compound outperformed exomethylene-γ-lactones in in vivo assays of anti-inflammatory activity [74], suggesting that a distinct set of yet-to-identify targets underlies its activity.

7.1.3. Thermo-TRPs

Interaction with TRPA1 (Transient Receptor Potential Ankyrin 1) has been demonstrated for the exomethylene-γ-lactone parthenolide [75], that behaves as a partial agonist and as a desensitizing agent, making TRPA1-expressing nerve terminals unresponsive to any stimulus and abrogating trigeminal nociceptive responses. Since parthenolide is structurally closely related to costunolide (**7**), the two compounds might share this activity. Interestingly, 1,8-cineol (**4**), a major constituents of the essential oil of both the thujone and the thujone-free chemotypes of *A. umbelliformis*, is a TRPM8 agonist [76], while (+)-borneol (**3**), a major volatile terpenoid of the thujone-free chemotype is a potent activator of another thermo-TRP, namely TRPV3 [77]. Taken together, these observations show that génépis contain both volatile and non-volatile modulators of thermo-TRPs, although it is unclear if this would, overall, result in a thermogenic activity, as claimed in the folk medicine.

7.2. Clinical targets

7.2.1. Gastric protection

In general, alcohol and alcoholic beverages have markedly different effects on gastric acid secretion, with non-alcoholic ingredients, and not alcohol, being actually responsible for the stimulatory gastric action of plant-based alcoholic beverages like beer, wine and liqueurs [78]. Remarkably, the increased secretion of gastric juices associated with these alcoholic beverages has been associated in folk medicine with gastroprotection [76], and this association seems especially well-founded for génépi. All wormwood from the génépi group that we have investigated (*A. genipi*, *A. umbelliformis*, *A. eriantha*) contain the lipophilic phenolic eupatilin (**20**) in concentrations ranging from 100 to 200 mg/kg [62]. Eupatilin is a potent anti-inflammatory agent, equipotent to indomethacine in the Croton-oil-induced dermatitis assay and with an overall in vivo topical anti-inflammatory activity qualitatively similar to that of hydrocortisone, and intermediate, in terms of potency, between those of steroid-and non-steroid drugs [79]. Eupatilin directly inhibits the production of leukotrienes by inhibiting the enzyme 5-lipo-oxygenase (5-LO), suppressing the genomic expression of leukotrienes and related pro-inflammatory enzymes by acting at the level of transcription factors like NF-kB and STAT3, with little direct activity on prostaglandin producing enzymes being observed [79]. Although no human data are available on the oral absorption of eupatilin, animal data evidenced a low absorption, with almost 70% of the dosage not absorbed by the gastro-intestinal tract [80]. It seems therefore unlikely that the low concentrations of eupatilin in génépis and their products can exert any systemic anti-inflammatory activity. On the other hand, topical activity at the intestinal level seems possible, especially in the light of the successful development of an eupatilin-containing herbal drug (Stillen®) as a gastro-protecting agent in South Korea. In the wake of the discovery that extracts from *Artemisia asiatica* Nakai exert potent cytoprotective and antiapoptotic effects in gastric and esophageal epithelial primary cells [81], a formulated ethanol extract containing 1 mg eupatilin/dosage as the active ingredient was developed as an anti-ulcer and gastroprotective agent [81]. The mucosal protective activity has been related to a combination of stimulation of mucus and bicarbonate secretion, local increase in prostaglandins and glutathione, and enhancement of mucosal blood flow [81]. Although the product is claimed to be validated by phase 3 and phase 4 investigations, no clinical study of Stillen® as a gastroprotectant is actually present in the indexed literature available from PubMed as of January 12, 2015. Assuming a concentration of eupatilin of 0.2 mg/g of génépi plant material and an excellent extraction with warm water, an intake of eupatilin in the range of 1 mg can be achieved in génépis herbal teas, but not in liqueurs, where other types of compounds might be responsible for this activity, in particular the exomethylene-γ-lactones that, just like eupatilin, inhibit pro-inflammatory transcription factors like NF-κB and that, because of their lipophilicity, might be better extracted in water-ethanol rather than in warm water.

7.2.2. Alcohol metabolism

Ethanol is metabolized into acetaldehyhde, a reactive compound that plays a major role in alcohol toxicity and that produces a strong and unpleasant aversive reaction, chronically damaging various organs, and eventually inducing cancer [82]. The plasma concentrations of acetaldehyde are regulated by the activity of two liver enzymes, with alcohol dehydrogenase (ADH) generating acetaldehyde from ethanol, and aldehyde dehydrogenase (ALDH) detoxifying it into acetic acid. ADH activity is also located in the upper digestive tract, locally controlling the amount of portal absorption of ethanol [82]. The activity of ADH and ALDH is under genetic control, and individuals with a mutant and less active ALDH are more susceptible to alcohol toxicity, as are those with elevated activity of ADH [83]. Various phenolics extracted from wood during the maturation of alcoholic beverages (vanillin, syringaldehyde, ellagic acid) inhibit ADH activity and depress alcohol metabolism, prolonging its half-life and resulting in a lower likelihood of overdrinking (but a prolonged drunkenness in case of overdrinking), a well know observation with alcoholic beverages [83]. On the other hand, some flavonoids are known to increase the activity of both ADH and ALDH, stimulating the detoxification of alcohol [83]. While these considerations are quite general for plant liqueurs, génépis contain non-phenolic compounds with a selective effect on alcohol absorption. Thus, a series of studies demonstrated that costunolide can dramatically affect the absorption of ethanol in rodents, being capable of strongly inhibiting its absorption at a 100:1 ethanol/costunolide weight ratio [84–86]. Assuming a 20% volume concentration of ethanol in génépi liqueurs, the use of ca 10 g of plant material, and the presence of up to 200 mg costunolide in this amount of plant material, ratios of this type are achievable in the stomach upon consumption of liqueurs. The mechanism of inhibition of alcohol absorption by costunolide has been extensively investigated, since the reduction of alcohol absorption is an important preventive strategy to prevent alcohol-related disorders. The activity has been related to the presence of an exomethylene-γ-lactone moiety, a common feature of all sesquiterpene lactones from the various génépis, and has been connected to a delay of gastric emptying [84]. Ethanol is absorbed slowly from the stomach, but rapidly from the small intestine, and has an intrinsic delaying activity of gastric emptying. Costunolide exacerbates it, and dilutes its concentration in the stomach by stimulating the production of gastric juices, overall avoiding the quick attainment of mind-altering blood concentrations of ethanol.

7.2.3. Diabetes

Eupatilin (**20**) was identified as the main active ingredient of *Artemisia princeps* (Japanese mugwort), a traditional treatment of diabetes in Japan and Korea [87]. In rodent experiments, extracts of this plant could enhance hepatic and plasma glucose metabolism, and a randomized, double-blind, placebo-controlled study confirmed the beneficial effects of the plant in the management of hyperglycemia, reducing the plasma levels of fasting blood glucose, HbA1c, and free-fatty acids. These effects were observed with dosages of extract corresponding to a daily intake of 15–30 mg eupatilin [87]. Interestingly, costunolide (**7**), a major constituent (>1%) of some génépis, showed potent anti-diabetic activity in the streptozotocin-induced diabetes model of the disease, with a dose-dependent effect on fasting glycemia, glycosidated haemoglobin (HbA1c) and plasma insulin concentrations, and with beneficial effects on serum total cholesterol, LDL cholesterol and triglycerides [88].

8. Conclusions

Génépi is currently a niche product in the market of alcoholic beverages, with a diffusion mostly limited to the Western Alpine region. However, given the growing interest in local food and the unique and pleasant sensory properties of this liqueur, the potential for a significant growth exists, including in the perfume and cosmetics industries. To implement this potential, cultivation of the source plants will become a necessity, as well as the development of analytical methods of authentication of both plant and biomass, and finished products. Many challenges still have to be addressed. Thus, the susceptibility to fungal diseases of the cultivated plants should be overcome by the selection of suitable cultivars and the optimization of the cultivation procedures, while the plague of adulteration requires the definition of mandatory rules for the qualification of plant biomass and finished products as génépi. Research carried out in the past decades highlights the concrete feasibility of overcoming the agronomical and analytical issues involved in the expansion of the currently very limited supply chain of the plant material, while the regulatory environment within the EU is definitely ripe for the definition of rigorous standards of authentication. Remarkably, several studies suggest that the bioactivity of various Alpine *Artemisia* species, and possibly of the liqueurs produced from them as well, is worth a serious pharmacological investigation, in accordance with the long tradition of medicinal use associated with these plants, while the peculiar sensory properties of génépi make it an attractive ingredient for the aromatization of a vast array of products, from chocolate to candies. Despite the medicinal potential of the génépi *Artemisia* species, only local intestinal effects are expected for génépi-based products like liqueurs, while systemic effects would requires more concentrated delivery forms, like teas or extracts. Taken together, these considerations qualify the Alpine *Artemisia* species from the génépi group not only as promising candidates for the valorisation of Alpine agriculture, but also as a source of interesting compounds for biomedical research.

Acknowledgements

This review is dedicated to the memory of don Giovanni Culasso (zio John, 1921-2004), who pioneered the cultivation of *A. umbelliformis* at Pietraporzio (CN, Italy) in the 1970s, paving the way to the first systematic phytochemical studies on this plant. We are also grateful to Dado Luciano for the picture of *A. umbelliformis* shown in the Graphical Abstract.

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