The *Achillea millefolium* group (Asteraceae) in Middle Europe and the Balkans: a diverse source for the crude drug Herba Millefolii

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Several species of the botanical genus *Achillea* (Asteraceae), which are commonly known as the *Achillea millefolium* group, comprise the crude drug Herba Millefolii. The essential oil-bearing plants are collected and used in Middle and Southeastern Europe, mainly for possessing anti-edematous, antiphlogistic and spasmylic activities. Medicinal indications of Herba Millefolii, which include digestive disorders and inflammations of skin and mucosa, are referred to essential oil, sesquiterpenes, alkaloids and flavonoids. The chemical composition varies strongly due to widespread geographical and ecological occurrence, different ploidy levels (di-, tetra-, hexa-, octoploid) and frequent hybridizations within the group but also with species from other *Achillea* sections. This paper reviews research on bioactive compounds of species from the *Achillea millefolium* group, including remarks on botany, ploidy and use in folk medicine.

**Key words**  *Achillea millefolium* group, Herba Millefolii, folk medicine, sesquiterpene lactones, polyploidy.

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**Introduction**

The word *<achillea>* refers to the famous Greek hero Achilles, who may have treated the wounded king Telephos with this herb.1) *<millefolium>* (transl.: thousand leaves) points at the characteristic multiple-pinnate leaves. According to the European Pharmacopoeia 2002,2) a minimum content of 0.02 % proazulenes (= a class of sesquiterpene lactones) in *Achillea millefolium* L. has to be proven for sufficient quality of Herba Millefolii, which is still mainly collected from wild resources. Due to antiphlogistic and spasmylic properties,3) the crude drug (= aerial parts) is used as tincture, infusion or decoct for digestive and intestinal disorders; externally as compress and bath for inflammations of skin, mucosa and as wound-healing remedy.4, 5) Tablets and juices of Herba Millefolii are also available as well as the extract is part of numerous combinatory preparations.6) Beside the medicinal applications, Herba Millefolii in Europe is used in bitters and cosmetic products. STAHL, an early researcher of essential oils from *Achillea*, mentioned already in 1952, that 90 % of Herba Millefolii samples from the market contain no proazulenes.6) Later it was revealed, that market samples frequently comprise several species of the genus *Achillea*, which differ clearly in morphology, ploidy and chemistry.7, 9) Most of these species have been summarized in the so-called *Achillea millefolium* group.10, 11) Unfortunately, still today *<Achillea millefolium* L.> is used synonymously for several species of the group. In recent years, pharmacognostical research was intensified in order to characterize single species and to elucidate bioactive components.

**Botany & Ploidy**

Plants of the *Achillea millefolium* group (Fig. 1) are herbaceous perennial, of variable length (up to 100 cm), with multiple-pinnate leaves; the small flower heads are arranged in corymbs (Fig. 2) with white, creamy-yellowish or pink rayflorists. The outline of leaf feathers and rayflorists was found to be of great systematic relevance.10, 11) Essential oil-bearing glands (Fig. 3) are found on stem, leaves and noticeable on ray- and discflorists within the aromatic-smelling flower heads. The *Achillea millefolium* group is distributed over the whole northern hemisphere, having its diversity center in Middle Europe and the Balkans.1) Generally, within the group 11 species are described in the floras and related publications for this area12-15) (see Tab. 1), which thrive from lowland (e.g. *A. setacea* up to alpine areas (e.g. *A. millefolium* subsp. *sudetica*). Furthermore, single species appear as different geographical and ecological types.8) Only few species belonging to the group are known for North America (*A. lanulosa* **Nut.**, *A. borealis* **Bong.**) and Central Asia [e.g. *A. asiatica* (L.) **Ser.**.]

The *Achillea millefolium* group is chemically and morphologically highly diverse, representing a polyploidy complex with di-, tetra-, hexa- and octoploid species.8, 9) Overevaluating the ploidy level results in heavily spreading features of morphology and chemistry, especially on the tetraploid level.16, 17) Ploidy has to be dealt as additional feature for systematic analysis.

For examining ploidy, usually fresh leaves are applied to flowcytometry by estimating the relative amount of DNA-content against a standard (usually diploid *A. cereta*).18, 19) Proazulene-containing species with antiphlogistic...
properties have been frequently found on the di- and
tetraploid level (see Tab. 1); findings of hexa- and octoploid
plants with proazulenes\(^{30}\) may be of hybridogenous origin.
Crossing experiments showed, that the ability of generating
antiphlogistic proazulenes is genetically inherited\(^{6,21}\) As a
complicating factor for taxonomic determination, hybridizations
within the polyploidy complex but also with other spec-
ies of the sectio Millefolium (\textit{A.crithmifolia}, \textit{A.nobilis},
\textit{A.clypeolata}, \textit{A.coaertata}) enable frequent gene flow, result-
ing in a broad spectrum of morphologically and chemically
heterogenous hybrids\(^{22-25}\).

As different species as well as proazulene-containing
and -non-containing plants can grow side by side in the
same habitat\(^{1}\) and considering their potential to hybridize,
the crude drug source for Herba Millefolii is in many as-
pects quite heterogenous. Nevertheless, the plant material is
still collected from wild resources on the Balkans mainly due to its very frequent natural occurrence in this area.

**Folk medicine**

The use of Herba Millefolii has a long tradition. The Greek DIOSKURIDES, who worked as military physician for the Romans in the 1st century A.D., described _stratiotes chiliphyllos_ (= _A. millefolium_ L.) as good for bleeds, old and fresh wounds and fistulae. In the 16th century, the plant was described in the famous herbal by _Boëck, Fuchs, Tabernaemontanus_ and later by their successors. In Middle European folk medicine, Herba Millefolii is said to be a haemostatic remedy (esp. for haemorrhoid and uterus bleedings) as well as it is used as _aromaticum amarum_ for digestive disorders. There is a vast number of other actions (e.g. carminative, choleric & cholagogue, tonifying, adstringent, antiseptic, antimicrobial, insect repellent, antirheumatic, emmenagogue, antisynergic, antiasthmatic in little children, antiperspirative) mentioned in modern herbals and phytotherapy manuals.

Recent ethnobotanical surveys in Europe confirmed the traditional use of Herba Millefolii in folk medicine. The crude drug is prepared and applied in various ways, as summarized in Tab. 2. In almost every survey, the botanical origin is described as _Achillea millefolium_ L. and therefore taxonomically inaccurate.

The use in Italian folk veterinary medicine as ointment for skin disorders and as wound healing herb was also revealed.

**Chemical compounds**

### A) Sesquiterpenes

The pharmaceutical interest of Herba Millefolii is directed towards the antiphlogistic proazulenes (see Fig. 4), a certain class of sesquiterpene lactones also present in the popular herbal drug Flos Chamomillae. They can be easily detected by simply heating some florets in a mixture of 60% chloroform and 85% phosphoric acid (2:1). During this process (and also by steam distillation), proazulenes are transformed to coloured chamazulenes. Proazulene-positive glands appear in blue, purple or black (see Fig. 3), whereas any other or missing colour indicates a different composition within the sesquiterpene lactones.

Proazulenes were generally found in 4 species of the _Achillea millefolium_ group (see Tab. 1). The authors of this paper found during field investigations of _Achillea_ on the Balkans mainly specimens of _Achillea collina_. Whereas Romanian populations were proazulene-positive like their Middle European counterparts, Bulgarian _A. collina_ showed different chemical patterns, often lacking proazulenes. Furthermore in a recent Bulgarian study, besides known sesquiterpene lactones new germacranolides and one new eudesmanolide are reported for _A. collina_. The latter compounds are unknown for Middle European _A. collina_.

Characteristic sesquiterpene patterns, which are neither affected by environmental and climatic conditions nor several micropropagation steps, were described for each species of the _A. millefolium_ group in Middle Europe by _Kubelka et al._ A large number of guaiololides was found especially in the proazulene-containing species of the _A._

<table>
<thead>
<tr>
<th>Botanical description</th>
<th>Local name</th>
<th>Locality</th>
<th>Part used</th>
<th>Formulation</th>
<th>Traditional use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>amelotu</td>
<td>Turkey</td>
<td>flowers</td>
<td>infusion</td>
<td>against diarrhea</td>
<td>Yesilada et al. 1993</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>bel ravnec</td>
<td>Bulgaria</td>
<td>aerial parts</td>
<td>infusion</td>
<td>adstringent, haemostatic</td>
<td>Leporatti &amp; Ivancheva 2003</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>odulina</td>
<td>Croatia</td>
<td>flowering tops</td>
<td>decoction</td>
<td>anti-obesity, digestive</td>
<td>Pieron et al. 2003</td>
</tr>
<tr>
<td><em>A. millefolium</em> L. s. str.</td>
<td>troneta, erba d’o marchese</td>
<td>Italy</td>
<td>flowering tops</td>
<td>tincture</td>
<td>digestive</td>
<td>De Feo &amp; Senateore 1993</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>millefoglio, erba livia</td>
<td>Italy</td>
<td>aerial parts</td>
<td>infusion</td>
<td>improve blood circulation, haemostatic, aid digestion, menstrual pain, bitter tonic, haemorrhoids</td>
<td>Leporatti &amp; Ivancheva 2003</td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td></td>
<td>France</td>
<td></td>
<td></td>
<td>stomachic, spasmylytic, diuretic, anti-inflammatory</td>
<td>Trouillas 2003</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td></td>
<td>Spain</td>
<td></td>
<td></td>
<td>hypercholesterolemia, immunostimulant</td>
<td>Agelet &amp; Valles 2003</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td></td>
<td>Sweden</td>
<td>herb</td>
<td></td>
<td>wounds, pain, inflammation, dysentery, headache, haemorrhoids</td>
<td>Tunon et al. 1995</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td>erba giegaia</td>
<td>Italy</td>
<td>leaf</td>
<td>ointment</td>
<td>wound healing in bovines</td>
<td>Uncini Manganelli et al. 2001</td>
</tr>
<tr>
<td><em>Achillea millefolium</em> L.</td>
<td></td>
<td>Italy</td>
<td></td>
<td></td>
<td>problems of skin and wounds in cattle</td>
<td>Viegi et al. 2003</td>
</tr>
</tbody>
</table>
millefolium group,\(^{47,48}\) whereas eudesmanolides, longipinene derivatives and germacranolides are typically detected in *A. pratensis*, *A. millefolium* subsp. *sudetica* and *A. pannonica*, respectively\(^ {49-51}\) (see Tab. 1).

To determine and quantify the main antiphlogistic sesquiterpenoids achillinic, 8α-tigloxy-artabas, 8α-angeloxy-artabasin, arglanin and santamarin in different Achillea species, GLASI et al.\(^ {52}\) developed an analysis system by means of HPLC in combination with TLC and MS.

**B) Essential oil.** Herba Millefolii contains 0.1 up to >1 % essential oil.\(^ {53}\) HOFMANN et al.\(^ {54}\) investigated the essential oils of three polyploids in the *Achillea millefolium* group by gas chromatography and found in total 149 components, of which 95 were identified. They recorded large differences in the proportions of mono- and sesquiterpenes, as well as other compounds in the essential oils of *A. collina* (4x), *A. millefolium* (6x) and *A. pannonica* (8x).

Because of the already mentioned overall high diversity within the *A. millefolium* group, phytochemical analysis of the essential oil is an important characterization tool. For distinguishing between species, it has been proven only to focus on selected mono- and sesquiterpenes.\(^ {55}\) Major terpenes of the proazulene-positive species (*A. asplenifolia*, *A. roseo-alba*, *A. ceratana, A. collina*) were shown to be sabine, β-pinene and β-caryophyllene, for the proazulene-free species especially 1,8-cineole and camphor, followed by α-pinene, camphene, p-cymene, γ-terpinene, α- and β-thujone, borneol, α-terpineol, bornyl acetate and elemol.\(^ {56,57}\)

In a further attempt to characterize single *Achillea* species and hybrids, the composition of the chiral monoterpenes α-pinene, β-pinene in the essential oil was assessed and found to be of different patterns, representing an additional marker for identification.\(^ {58,59}\) The enantiomeric distribution neither depends on method of extraction, nor on habitat or developmental stage.

**C) Flavonoids and others.** Major flavonoids are 7-O-glycosides and 7-O-malonylglycosides of apigenin and luteolin,\(^ {60}\) as well as rutin, saflachitoside and isoaflachitoside.\(^ {59,60}\) Flavonoids in species of the *Achillea millefolium* group were found to be of low chemotaxonomic relevance.\(^ {59}\) Capillary electrophoresis was applied for the rapid analysis of flavone-O- and -C-glycosides of *A. setacea*.\(^ {61}\)

As major nitrogen containing compounds, proline, stachydrine, the bitter-tasting betonicine, betaine and choline were isolated from aboveground parts of *A. collina*. TLC screening of the 11 species belonging to the *A. millefolium* group resulted in quantitative but no qualitative differences within these compounds.\(^ {62}\)

Tannins, coumarines, polycycltenes, amides and sterols have been also found in Herba Millefolii.\(^ {3,63-65}\)

**Bioactivity**

In 1969, GOEBL et al.\(^ {66}\) found for the aqueous extract from flower heads of *Achillea millefolium* an anti-inflammatory activity as measured by mouse paw edema. They presumed protein-carbohydrate complexes as active principles. Later, also the ethanolic extract of *Achillea millefolium* L. slightly reduced carrageenin foot edema in rats.\(^ {67}\) Anti-edematous activity of single sesquiterpene lactones in Herba Millefolii has been demonstrated by *Croton* oil ear test in mice.\(^ {68}\) Topical edema inhibition was shown for rupicoline B, 11,13-dehydro-deacetylmattacinin of *A. setacea*,\(^ {69,70}\) 8α-angeloyl-10-epi-artabas, 8-α-angeloyl-10-epi-3-oxa-artabasin of *A. asplenifolia*,\(^ {71,72}\) 8-α-tigloxy-10-epi-artabasin of *A. roseo-alba*,\(^ {73}\) achillinic of *A. collina*,\(^ {74}\) α-peroxyxichilofid, isopressin of *A. millefolium*,\(^ {75}\) 1,4-dihydroxy-germacra-5E-10(14)-diene of *A. pannonica*\(^ {76}\) and a lipophilic fraction from an aqueous extract of *A. pratensis*.\(^ {77}\)

Also for alkaloids, isolated from *Achillea millefolium* L., anti-inflammatory activity was recognized. Isobutylamine and piperideide derivatives appeared to be potent inhibitors of *in vitro* cyclooxygenase (COX) and 5-lipoxygenase, both key regulatory enzymes in inflammation processes.\(^ {78}\) Anti-inflammatory activity by inhibition of prostaglandin biosynthesis and PAF-induced exocytosis was found for water extracts.\(^ {35}\) Besides radical-scavenging and anti-inflammatory activity, the hydro-alcoholic extract of *Achillea millefolium* L. also showed cytotoxic effect on mouse B16 melanoma cells.\(^ {33}\)

Antioxidant and antimicrobial activities of the essential oil, ethanolic and methanolic extracts of single species of the *A. millefolium* group were recently confirmed.\(^ {74,76}\) The oil of a species described as *A. millefolium* subsp. *millefolium* showed activity against *Streptococcus pneumoniae, Clostridium perfringens, Candida albicans, Candida crusei, Mucobacterium smegmatis* and *Acinetobacter lwoffi*, while water-insoluble parts of methanolic extracts exhibited slight or no activity.\(^ {74}\) Also for essential oil from aerial parts of *Achillea setacea* inhibitory effects on growth of Clostridium perfringens, *Candida albicans* and *Acinetobacter lwoffi* were observed.\(^ {75}\) The ethanolic extract from aerial parts and the rhizome of *Achillea millefolium* L. was found to be active against *Bacillus cereus* and *Staphylococcus aureus* (only aerial parts).\(^ {78}\) On the other hand, aqueous extract of *A. millefolium* had no antibacterial activity on *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and *Escherichia coli*.\(^ {77}\)

The allergenic sesquiterpene lactone α-peroxyxichilofid had an inhibitory effect on *Plasmodium falciparum* in an *in vitro* model.\(^ {72}\)

Furthermore, three sesquiterpenoid esters of achimillic acid in the methanolic extract of *Achillea millefolium* L. (collected in Japan!) exhibited antitumor activity against mouse leukemia cells *in vivo*.\(^ {78}\)

Spasmolytic and choleric effect depending on several compounds (essential oil, flavonoids etc.) was also attributed to Herba Millefolii.\(^ {3,79}\)

Adverse effects: Herba Millefolii can cause contact dermatitis, for which allergenic α-methyleng-γ-lactone sesquiterpenoids [e.g. α-peroxyxichilofid in *A. millefolium* L. s. str. (see Fig. 4); rupicoline A and rupicoline B in *A. setacea*] are responsible.\(^ {72,80}\) Furthermore, photosensibilization after
oral intake was reported. For ethanolic extracts of flowers from *Achillea millefolium* L., an antispermatogenic effect on mice was observed.

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**References**


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**Fig. 4** Chemical structures of selected sesquiterpenes in *Herba Milletoflia*: proazulenes 1 - 8 (1: 8α-angeloyl-artabsin 2: 8α-tigloyl-artabsin 3: achillinc 4: 8-desacetyl-4-epi-matricin 5: 8-desacetyl-8-tigloyl-4-epi-matricin 6: 8-desacetyl-8-tigloyl-matricin 7: 2α,8α-dihydroxy-1α, 5α,6β,11β-guaiia-3,10(14)-dien-12,6-olide 8: 8α-acetoxy-2α-hydroxy-1α, 5α,6β,11β-guaiia-3,10(14)-dien-12,6-olide); chamaulene, which originates from proazulenes under separation of acetate, water and CO2 during heating processes (e.g. steam distillation); α-peroxyxachtifolid with allgenic α-methylene-γ-lactone ring.