Department of Pharmacognosy, University of Vienna, Vienna, Austria

Yarrow (*Achillea millefolium* L. s.l.): Pharmaceutical quality of commercial samples

B. Benedek, K. Rothwangl-Wiltschnigg, E. Rozema, N. Gjoncaj, G. Reznicek, J. Jurenitsch, B. Kopp, S. Glasl

Received May 31, 2007, accepted June 29, 2007

Ao. Univ.-Prof. Dr. Sabine Glasl, Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria
sabine.glasl@univie.ac.at

Pharmazie 63: 23–26 (2008) *doi: 10.1691/ph.2008.7646*

Yarrow (*Achillea millefolium* L. s.l.) is traditionally used against inflammatory and spasmodic gastro-intestinal complaints, hepato-biliary disorders, as an appetite enhancing drug, against skin inflammations and for wound healing due to its antiphlogistic, choleretic and spasmolytic properties. The main pharmacologically active principles were shown to be the essential oil (antimicrobial), proazulenes and other sesquiterpene lactones (antiphlogistic), dicaffeoylquinic acids (choleretic) and flavonoids (antispasmodic). In order to assess the pharmaceutical quality of the drug we evaluated the content of these bioactive compounds in 40 commercial drug samples. The essential oil and the proazulenes were analysed according to the European Pharmacopoeia, whereas the content of dicaffeoylquinic acids and flavonoids was determined by solid phase extraction (SPE)-HPLC. This comprehensive survey revealed that the quality of the drug material was very heterogenous, and only 50% of the samples met the standards of the European Pharmacopoeia. Moreover, this study gives information about the content of phenolic compounds in the drug and allowed to establish tentative reference values which may be used as additional parameters in the quality control of the drug.

1. Introduction

Aqueous and alcoholic extracts of yarrow (*Achillea millefolium* L. s.l.) are used in traditional European medicine in the treatment of inflammatory and spasmodic gastro-intestinal complaints, hepato-biliary disorders and as an appetite enhancing drug. Besides these internal applications the plant is also used externally against skin inflammations and for its wound healing effects (Willuhn 2002; Jurenitsch 1992). According to the "Volksmed-Datenbank", a database documenting the traditional use of medicinal plants in Austria, yarrow ranges among the eight most commonly used plants in Austria and is particularly applied against gynaecological and gastro-intestinal complaints (Gerlach et al. 2006). *Achillea millefolium* L. s.l. was positively reviewed by the German Commission E and the pharmacological actions of the plant were subsumed under "choleretic, antibacterial, adstringent and antispasmodic" (Blumenthal 1998). Moreover, several phytopharmaceuticals containing yarrow are on the market, particularly herbal tea mixtures. The indications of those products mainly comprise hepato-biliary and digestive complaints.

The pharmacological effects of *Achillea millefolium* L. s.l. are scientifically proven and could be attributed to different plant compounds: The essential oil mediates antimicrobial effects, the sesquiterpenes show antiphlogistic activity, the dicaffeoylquinic acids (DCQAs) exert choleretic effects and the flavonoids cause the spasmolytic properties of the plant (Simic et al. 2002; Candan et al. 2003; Karamanderes et al. 2002; Zitterl-Eglseer et al. 1991; Sosa et al. 2001; Kastner et al. 1993; Benedek et al. 2006; Lemmens-Gruber et al. 2006). Furthermore, the chemotaxonomic relevance of these plant constituents within the *Achillea millefolium* L. aggregate has been demonstrated (Kastner et al. 1992; Kubelka et al. 1999; Rauchensteiner et al. 2002; Benedek et al. 2007).

Yarrow is monographed in the European Pharmacopoeia where the drug (*Millefolii herba*) is defined as the "whole or cut, flowering tops of *Achillea millefolium* L." and should contain "not less than 2 ml/kg of essential oil and not less than 0.02 per cent of proazulenes, expressed as chamazulene, both calculated with reference to the dried drug" (Ph. Eur. 5th edition). In addition, we recently presented a SPE-HPLC/UV method that allows the quantification of the bioactive phenolic compounds (Benedek et al. 2007; Benedek 2007).

Hence, the aim of our present study was to assess the pharmaceutical quality of the drug by evaluating 40 commercial samples of *Achillea millefolium* L. s.l. regarding the content of all pharmacologically active compounds, namely essential oil, proazulenes, dicaffeoylquinic acids and flavonoids.

2. Investigations and results

The content of essential oil in the drug samples ranged from 0.50 to 5.88% with an average amount of 2.06% (for

Table: Specification of the commercial *Achillea* samples and content of essential oil, proazulenes, dicaffeoylquinic acids and flavonoids (in %)[a]

Sample no.	Origin	Herba/Flos	Essential oil	Proazulenes	DCQAs	Flavonoids
1	Poland (cultivation)	Herba	2.00	0.028	0.98	0.37
2	Poland	Flos	1.50	**0.017**	0.77	0.37
3	Poland	Herba	1.50	**0.016**	0.84	**0.28**
4	Bulgaria	Herba	2.75	0.034	1.15	0.61
5	Poland	Herba	2.00	**0.012**	0.71	0.67
6	Germany	Herba	1.75	0.033	0.83	0.39
7	Chile	Herba	0.50	0.027	0.67	0.68
8	Hungary	Herba	1.25	0.027	0.79	0.46
9	Hungary	Flos	3.80	0.088	0.75	0.58
10	Hungary	Flos	2.45	**0.001**	**0.53**	1.07
11	Chile	Flos	1.48	0.031	0.82	0.94
12	Germany	Herba	1.40	**0.000**	1.16	0.86
13	Germany	Herba	2.20	**0.000**	1.44	0.48
14	Poland	Herba	2.60	0.036	0.96	0.81
15	Germany	Herba	4.00	**0.018**	0.83	0.83
16	Poland	Flos	1.00	**0.007**	1.28	0.87
17	Hungary	Herba	1.25	0.022	1.15	0.45
18	Poland	Flos	1.75	0.037	1.60	1.11
19	Germany (cultivation)	Herba	5.65	0.173	1.33	0.42
20	Netherlands	Herba	0.55	**0.007**	0.94	**0.13**
21	Netherlands	Herba	1.65	**0.001**	**0.59**	**0.07**
22	Netherlands	Herba	1.15	**0.002**	0.94	0.31
23	Austria (cultivation)	Herba	5.88	0.113	0.76	0.35
24	Poland	Herba	1.75	**0.010**	**0.10**	**0.21**
25	Hungary	Herba	0.75	0.023	**0.51**	**0.28**
26	Hungary	Herba	2.25	**0.016**	0.62	0.74
27	Hungary	Herba	1.25	**0.018**	0.76	0.63
28	Macedonia	Flos	4.00	**0.001**	**0.55**	0.92
29	Albania	Flos	1.75	**0.002**	**0.56**	0.76
30	Bulgary	Flos	1.25	**0.003**	**0.55**	0.54
31	Italy (cultivation)	Herba	2.25	**0.010**	1.31	0.61
32	Slovakia	Herba	2.13	0.052	0.97	0.66
33	Austria (cultivation)	Herba	3.50	0.233	0.95	0.49
34	Bosnia-Herzegovina	Flos	2.13	0.036	0.78	0.73
35	Bosnia-Herzegovina	Flos	2.38	0.037	0.93	0.78
36	Bosnia-Herzegovina	Flos	2.50	0.040	0.98	0.77
37	Hungary	Herba	2.00	0.070	0.81	0.49
38	Czechia	Herba	0.50	**0.010**	**0.46**	0.81
39	Hungary	Herba	1.38	0.067	1.21	0.52
40	France	Herba	0.50	**0.000**	0.90	0.82

[a] Contents in bold do not correspond neither to the requirements of the Ph. Eur. (essential oil: 0.2%, proazulenes: 0.02%) nor to our tentative values for dicaffeoylquinic acids (0.60%) and flavonoids (0.30%)

all values see the Table). Considering the fact that the European Pharmacopoeia requires a minimum content of 0.2%, all samples were in accordance with this definition. Interestingly, the samples averagely contained far more essential oil (about tenfold) than required.

Regarding the proazulenes the concentration in the investigated drug material amounted up to 0.23% whereas three samples did not contain any proazulenes. The average content was 0.03%. Since the Ph. Eur. demands 0.02% proazulenes, exactly one half of the drug samples met this requirement.

The amount of DCQAs ranged from 0.10 to 1.60% (average: 0.87%), whereas the content of flavonoids amounted from 0.13 to 1.11% (average: 0.60%). Based on these data tentative reference values for the phenolic compounds were created: Given a minimum content of 0.60% DCQAs, 80% of the samples were meeting the standard. If a minimum of 0.30% flavonoids is defined, 35 out of 40 samples (88%) were within the limit.

Summing up, 50% of the commercial drug samples fulfilled the requirements of the Ph. Eur. regarding the content of essential oil and proazulenes. In addition, all these samples – except for one specimen (sample no. **25**) – also reached the tentative reference values of 0.60% DCQAs and 0.30% flavonoids.

3. Discussion

The European Pharmacopoeia defines four requirements for yarrow: plant origin (*A. millefolium* L.), appearance (whole or cut, flowering tops) and two limit values, one for the essential oil (0.2%) and the other for the proazulenes (0.02%). Regarding the proazulene content the investigated commercial samples showed striking variations and only 50% of the samples fulfilled the required criterion. On the one hand this phenomenon may be explained by the fact that yarrow is traded in different qualities, as "Herba" as well as "Flos", whereas the essential oil and proazulenes are concentrated in the glands of the ligules. The second and maybe even more evident reason is that the plant source is defined as "*A. millefolium* L.". According to new botanical definitions "*A. millefolium* L." comprises a group of several different species (Saukel and Länger 1992a, 1992b), and therefore it should be defined

more precisely as "*A. millefolium* L. sensu latiore (s.l.)". The respective species differ in their ploidy level and in their sesquiterpene lactone content. Diploid and some tetraploid species were shown to contain proazulenes which lack activated exocyclic methylene-groups. In contrast, other tetraploid, hexa- and octoploid species are characterised by sesquiterpene lactones with different skeletal structures (Rauchensteiner et al. 2002). They frequently exhibit exocyclic methylene-groups which may trigger type IV allergy after topical application (Merfort 2003). As the Ph. Eur. defines "*A. millefolium* L." as plant source on the one hand and requires a very low proazulene content of only 0.02% on the other hand, all these different species are traded. Consequently, big differences in the sesquiterpene pattern occur, which is reflected by the big variations of the proazulene content in the investigated samples.

The other evaluated classes of compounds, essential oil, DCQAs and flavonoids, showed much slighter variations than the proazulenes. However, a correlation between the contents of these four characters could not be observed in the investigated samples. Specimen **28** for example, which was declared by the provider as "cut yarrow flowers" and showed good optical appearance (high amount of flower heads, low amount of stalks), was one of the drugs with the highest contents of essential oil (4.00%) and flavonoids (0.92%), but showed very low values regarding the DCQAs (0.55%) and proazulenes (0.001%). Four samples (**9**, **19**, **23**, **33**), three of them originating from cultivation (**19**, **23**, and **33**), exhibited exceptionally high proazulene contents by more than 0.08% which is the fourfold of the required amount. This was also reflected by a high content of the essential oil, whereas none of the samples reached the average flavonoid content of 0.60%. However, all four samples were within the tentative flavonoid limit of 0.30%. Regarding the DCQAs, two of the four samples were above and two below the average value of 0.87%, but all of them reached the tentative DCQA limit of 0.60%.

Between the optical appearance of the drugs and the contents no correlation was detected. Some samples which complied with the requirement "whole or cut, flowering tops" exerted extremely low proazulene and DCQA contents (**28**, **29**, **30**). On the other hand, samples **7** and **8** reached more than 0.02% proazulenes, although their amount of stalks and leaves compared to the flower heads was far more than expected according to the definition of the Ph. Eur. The content of the phenolic compounds ranged far above the tentative limits, in sample **7** the flavonoid content was particularly high and amounted to 0.68%.

Our investigations showed that the suppliers may merchandise drugs with striking different quality, as for example samples **9** and **10** which were both provided by the same distributor. Sample **9** was characterised by remarkably high contents of essential oil and proazulenes, whereas in sample **10** particularly the amount of proazulenes was conspicuously low and likewise the DCQAs were below the tentative limit. Furthermore, we could show that if the samples do not reach the tentative limits regarding DCQAs (**10**, **21**, **24**, **25**, **28**, **29**, **30**, **38**) and flavonoids (**3**, **20**, **21**, **24**, **25**), they do not match – except for sample **25** – with the requirements of the Ph. Eur. either, because their proazulene content is too low.

To sum up, due to the present standards of the Ph. Eur. drug mixtures of species rich in proazulenes and proazulene-free species might be merchandised. This implies a very high variability of the traded plant material with a quality ranging between average to good, and all kind of sesquiterpene lactones may be expected in the drug "Millefolii herba", also those with allergic potential. Although sesquiterpenes with an exocyclic methylene group imply a risk for allergies after topical application, some of them were shown to exhibit also anti-edematous activity besides the undesirable allergenic effect (Kastner et al. 1993). Taking this into account and considering the fact that there is no statistical information about the incidence of contact allergy after the external use of plant remedies (Merfort 2003), the risk of adverse effects seems to be negligible. Nevertheless, if the quality of yarrow is desired to be homogenous, the drug is recommended to originate from cultivation. Respective samples exist and have been investigated (**19**, **23**, **33**) revealing remarkably high contents of proazulenes (0.173%, 0.113%, 0.233%) and essential oil (5.65%, 5.88%, 3.50%). These contents exceed the requirements of the Ph. Eur. by more than the five- and 15-fold, respectively. In addition, the values for the DCQAs and flavonoids were above the tentative limits in these samples. If botanically unequivocally defined azulenogenic species are used as drug a three- to 20-fold higher proazulene content (*A. asplenifolia* 0.07%, *A. ceretanica* 0.48%, *A. collina* 0.24%) and a 20- to 40-fold higher percentage of the essential oil (*A. asplenifolia* 4.25%, *A. ceretanica* 9.50%, *A. collina* 4.00%) may be expected (Rothwangl-Wiltschnigg 2004).

Based on these results we may state that the lower limits for proazulenes and essential oil in the Ph. Eur. are justified. However, higher levels would guarantee much bigger homogeneity among the traded products and would claim a drug that originates from cultivation. Moreover, a good quality of the drug implies the presence of DCQAs and flavonoids due to their choleretic and spasmolytic activity. Our tentative standard values of 0.60% (DCQAs) and 0.30% (flavonoids) might serve as additional parameters in the quality control of the drug.

4. Experimental

4.1. Plant material

40 commercial samples of yarrow were kindly provided by companies from Germany, Austria, France, Italy, the Netherlands, Great Britain and Bosnia-Herzegovina and are encoded with arabic numbers (**1**–**40**) in this paper. According to additional information from the providers, the major part of the samples represented wild-collected drug material supplied from Eastern European countries (Poland, Romania, Slovakia, Czechia, Hungary), whereas only few samples (**1**, **19**, **23**, **31**) were reported as cultivated plant material. In the Table the exact drug description (herba/flos) and the country of origin of all samples are indicated.

4.2. Quantification of essential oil and proazulenes

The analysis of essential oil and proazulenes was carried out according to the European Pharmacopoeia with slight modifications (Ph. Eur. 5th edition; Rothwangl-Wiltschnigg 2004). In brief, the content of essential oil was determined by steam distillation of 20.0 g cut drug with 500 mL water-ethylene glycol (1 + 9). After addition of 0.2 mL decalin instead of xylene in the graduated tube the distillation was carried out at a rate of 2 to 3 mL/min for 2 h. The content of proazulenes was determined after diluting the blue essential oil-decalin mixture to 50.0 mL by measuring the absorbance at 608 nm. Each drug sample was analysed in duplicate and mean values were determined.

4.3. Quantification of phenolic compounds

The content of dicaffeoylquinic acids (DCQAs) and flavonoids was analysed as previously described (Benedek et al. 2007; Benedek 2007). The validation of the method is documented in the PhD thesis of Benedek (Benedek 2007). Briefly, 1.00 g of powdered plant material was exhaustively extracted under reflux with 40% MeOH. The crude extract was fractionated by solid phase extraction on a C18-cartridge (Varian Mega BE, 5g, 20 mL). Elution with MeOH-water mixtures of decreasing polarity

yielded three fractions enriched in chlorogenic acid (eluted with water), DCQAs (eluted with 20% MeOH) and flavonoids (eluted with 80% MeOH), respectively. After HPLC analysis of the DCQA fraction using cynarin as internal standard the content of 1,3-, 3,4-, 3,5- and 4,5-DCQA was evaluated (Benedek 2007). HPLC analysis of the flavonoid fraction by internal standardisation with luteolin-3′,7-di-*O*-glucoside allowed the determination of the major flavonoids (rutin, luteolin-4′,7-di-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, schaftoside, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucuronide, luteolin-7-*O*-glucoside, luteolin-4′-*O*-glucoside and 6-OH-luteolin-7-*O*-glucoside and apigenin). In addition, minor flavonoids were summed up together with the nine major flavonoids to give the total flavonoid content (Benedek 2007). All samples were analysed twice and mean values were calculated.

Acknowledgement: We are thankful to Prof. Liselotte Krenn, Department of Pharmacognosy, University of Vienna, who helped us with the acquisition of the plant material.

References

Benedek B, Geisz N, Jäger W, Thalhammer T, Kopp B (2006) Choleretic effects of yarrow (*Achillea millefolium* s. l.) in the isolated perfused rat liver. Phytomedicine 13: 702–706.

Benedek B, Gjoncaj N, Saukel J, Kopp B (2007) Distribution of phenolic compounds in Middleeuropean taxa of the *Achillea millefolium* L. Aggregate. Chem Biodivers 4: 849–857.

Benedek B (2007) *Achillea millefolium* L. s. l. – Analysis of Phenolic Compounds and Biological Testing [dissertation]. Vienna: University of Vienna.

Blumenthal M (1998) The Complete German Commission E Monographs. Austin: American Botanical Council: 233–234.

Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HA (2003) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (*Asteraceae*). J Ethnopharmacol 87: 215–220.

European Pharmacopoeia, 5th edition. Strasbourg: EDQM; 2007: CD-ROM.

Gerlach S, Saukel J, Kubelka W (2006) Pflanzen in der österreichischen Volksmedizin – die „Volksmed-Datenbank". Sci Pharm 74: S. 36.

Jurenitsch J (1992) Achillea. In: Hänsel R, Keller K, Rimpler H, Schneider G (ed.) Hager's Handbuch der pharmazeutischen Praxis, Vol. 4, Drogen A-D, 5th ed., Berlin, pp. 45–54.

Karamenderes C, Karabay NÜ, Zeybek U (2002) Composition and antimicrobial activity of the essential oils of some *Achillea* L. species in Turkey. Acta Pharm Turc 44: 221–225.

Kastner U, Saukel J, Zitterl-Eglseer K, Länger R, Reznicek G, Jurenitsch J, Kubelka W (1992) Ätherisches Öl – ein zusätzliches Merkmal für die Charakterisierung der mitteleuropäischen Taxa der *Achillea millefolium*-Gruppe. Sci Pharm 60: 87–99.

Kastner U, Sosa S, Tubaro A, Breuer J, Rücker G, Della Loggia R, Jurenitsch J (1993) Anti-Edematous activity of sesquiterpene lactones from different taxa of the *Achillea millefolium* group. Planta Med 59: A 669.

Kubelka W, Kastner U, Glasl S, Saukel J, Jurenitsch J (1999) Chemotaxonomic relevance of sesquiterpenes within the *Achillea millefolium* group. Biochem Syst Ecol 27: 437–444.

Lemmens-Gruber R, Marchart E, Rawnduzi P, Engel N, Benedek B, Kopp B (2006) Investigation of the spasmolytic activity of the flavonoid fraction of *Achillea millefolium* s. l. on isolated guinea-pig ilea. Arzneimittel-Forsch 56: 582–588.

Merfort I (2003) Was man über Pflanzen und ihre kontaktallergene Wirkung wissen sollte. Z Phytother 24: 22–29.

Rauchensteiner F, Nejati S, Werner I, Glasl S, Saukel J, Jurenitsch J, Kubelka W (2002) Determination of taxa of the *Achillea millefolium* group and *Achillea crithmifolia* by morphological and phytochemical methods. I. Characterisation of Central European taxa. Sci Pharm 70: 199–230.

Rothwangl-Wiltschnigg K (2004) Analytik der Sesquiterpene zur Prüfung der pharmazeutischen Qualität von Schafgarbe [dissertation]. Vienna: University of Vienna.

Saukel J, Länger R (1992a) Die Achillea millefolium-Gruppe (Asteraceae) in Mitteleuropa, 1. Phyton 31: 185–207.

Saukel J, Länger R (1992b) Die Achillea millefolium-Gruppe (Asteraceae) in Mitteleuropa, 2. Phyton 32: 47–78.

Simic N, Palic R, Vajs V, Milosavljevic S, Djokovic D (2002) Composition and antibacterial activity of *Achillea asplenifolia* essential oil. J Essent Oil Res 14: 76–78.

Sosa S, Tubaro A, Kastner U, Glasl S, Jurenitsch J, Della Loggia R (2001) Topical anti-inflammatory activity of a new germacrane derivative from *Achillea pannonica*. Planta Med 67: 654–658.

Willuhn GT (2002) Millefolii herba. In: Wichtl M (ed.). Teedrogen und Phytopharmaka, 4th ed., Stuttgart, p. 399–403.

Zitterl-Eglseer K, Jurenitsch J, Korhammer S, Haslinger E, Sosa S, Della Loggia R (1991) Entzündungshemmende Sesquiterpenlactone von *Achillea setacea*. Planta Med 57: 444–446.