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Achillea millefolium L. s.l. – Is the anti-inflammatory activity mediated by protease inhibition?

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Abstract

Achillea millefolium L. s.l. is traditionally used not only in the treatment of gastro-intestinal and hepato-biliary disorders, but also as an antiphlogistic drug. As various proteases, for instance human neutrophil elastase (HNE) and matrix metalloproteinases (MMP-2 and -9), are associated with the inflammatory process, the aim of this study was to test a crude plant extract in *in vitro*-protease inhibition assays for understanding the mechanisms of anti-inflammatory action. Furthermore, two fractions enriched in flavonoids and dicaffeoylquinic acids (DCQAs), respectively, were also tested in order to evaluate their contribution to the antiphlogistic activity of the plant. The extract and the flavonoid fraction inhibited HNE showing IC_{50} values of approximately 20 μg/ml, whereas the DCQA fraction was less active (IC_{50} = 72 μg/ml). The inhibitory activity on MMP-2 and -9 was observed at IC_{50} values from 600 to 800 μg/ml, whereas the DCQA fraction showed stronger effects than the flavonoid fraction and the extract. In conclusion, the *in vitro*-antiphlogistic activity of *Achillea* is at least partly mediated by inhibition of HNE and MMP-2 and -9. After the recently described spasmolytic and choleretic effects the obtained results give further insights into the pharmacological activity of *Achillea* and confirm the traditional application as antiphlogistic drug.
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Keywords: *Achillea millefolium* L.; Anti-inflammatory activity; Human neutrophil elastase; Matrix metalloproteinases

1. Introduction

Aqueous and alcoholic extracts of yarrow (*Achillea millefolium* L.) are used in traditional European medicine internally in the treatment of gastro-intestinal and hepato-biliary disorders and externally in case of skin inflammations and for wound healing (Willuhn, 2002). Besides essential oil and sesquiterpenes phenolic compounds such as flavonoids and phenolcarbonic acids are a major group of plant constituents present in yarrow. Due to their high solubility in water and ethanol, those polar substances are completely extracted into teas and tinctures which are the traditional application forms of yarrow. Recently we demonstrated the spasmolytic activity of the flavonoids (Lemmens-Gruber et al., 2006) and the choleretic activity of the dicaffeoylquinic acids (DCQAs) from yarrow (Benedek et al., 2006). Moreover, the safety of the plant after chronic exposure was previously reported (Cavalcanti et al., 2006).

However, yarrow is not only applied due to its spasmolytic and choleretic effects, but also in the treatment of wounds and chronic gastro-intestinal and skin inflammations as an antiphlogistic drug similar to chamomile (Madaus, 1976; Fischer and Krug, 1984; Willuhn, 2002). While a topical anti-inflammatory activity of the sesquiterpenes was already shown being caused by inhibition of the arachidonic acid metabolism (Kastner et al., 1993), other mechanisms of action might also contribute to the antiphlogistic activity of the drug.

Various proteases, for instance human neutrophil elastase (HNE) and matrix metalloproteinases (MMPs), are associated with the inflammatory process.

Human neutrophil elastase is a serine protease primarily located in the azurophil granules of polymorphonuclear leukocytes and has a broad substrate specificity being capable not only of the enzymatic cleavage of elastin but also of various other extracellular matrix proteins, thus leading to degradation of the connective tissue. Under physiological conditions HNE activity is controlled by its endogenous inhibitor α_1-antitrypsin. The

Abbreviations: DCQA, dicaffeoylquinic acid; HNE, human neutrophil elastase; MMP, matrix metalloproteinase

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primary function of serine proteases released from neutrophils is the phagocytosis and eradication of microorganisms. However, neutrophils and their secreted proteases such as HNE are also present in inflammatory disorders without bacterial involvement. Recent investigations showed that HNE is not only an unspecific matrix-degrading enzyme and a microbial killer, but also plays an important role in cell signalling and contributes to the control of the inflammatory process (Bieth, 1998; Wiedow and Meyer-Hoffert, 2005).

Other proteases that are associated with the inflammation process are the matrix metalloproteinases MMP-2 (gelatinase A) and MMP-9 (gelatinase B). These zinc-dependent metalloendopeptidases are also involved in the degradation of extracellular matrix proteins such as collagens and gelatins whereas MMP-2 and -9 are the only metalloproteinases being capable of enzymatic cleavage of collagen type IV, the main component of the basal membrane. They are secreted primarily by fibroblasts and endothelial cells as inactive proenzymes into the extracellular space where they are proteolytically activated by other proteinases. Tissue inhibitors of metalloproteinases (TIMPs) regulate extracellular matrix turnover and tissue remodelling by controlling MMP activity (Murphy, 1998; Collier and Goldberg, 1998; Petrides, 1998). In the inflammatory process MMPs are secreted in response to both exogenous insults and inflammatory cytokines and facilitate leucocyte recruitment by extracellular matrix degradation and modulate cytokine and chemokine processing. As excess MMP activity following chronic inflammation or infection leads to persistence of inflammation, MMP inhibition is another possible mechanism for antiphlogistic activity (Elkington et al., 2005).

Besides the development of synthetic HNE inhibitors (Obayashi, 2005), different plant compounds were shown to possess inhibitory activity against HNE, namely phenolic compounds, sesquiterpene lactones, triterpenes, phytosterols and cinnamic acid derivatives (Löser et al., 2000; Melzig et al., 2001; Siedle et al., 2002; Mitaine-Offer et al., 2002). Moreover, some natural products were described as MMP inhibitors (Ende and Gebhardt, 2004; Jin et al., 2005; Seo et al., 2005).

Since the traditional application of yarrow comprises the treatment of skin and gastro-intestinal inflammations, we investigated the *in vitro*-inhibitory effect of a crude plant extract on the above-mentioned proteases. As prior studies indicated HNE and MMP-2 and -9 inhibition by phenolic compounds, two fractions enriched in dicaffeoylquinic acids and flavonoids, respectively, were additionally tested in order to assess their contribution to the protease inhibitory activity of the plant.

2. Materials and methods

2.1. Plant material

A commercial sample of the aerial parts of yarrow (*Achillea millefolium* L. s.l.) supplied by Aboca, Sansepolcro, Italy (sample 1D5787) was used for the preparation of the plant extract. A voucher specimen (Aboca 1) is deposited at the Department of Pharmacognosy, University of Vienna.

2.2. Extraction and fractionation

The powdered plant material (35 g) was extracted with 1500 ml of 20% (v/v) methanol p.a. under reflux for 30 min. After centrifugation, the residue was treated in the same way for another 20 min. Methanol was removed from the combined solutions by evaporation under reduced pressure at 40 °C and the remaining aqueous solution was lyophilisated yielding 12.7 g crude plant extract.

The crude extract (10 g) was fractionated by solid phase extraction on C18-cartridges (Varian, Mega BE-C18, 10 g, 60 ml) conditioned with 2 reservoir volumina (RV) methanol and 2 RV deionised water. After dissolving 10 g of crude extract in 300 ml of 20% (v/v) methanol p.a., 20 ml of the solution was applied on the cartridge. After purging with air for 10 min, the extract was eluted at a flow rate of 8 ml/min with 2 RV water, 2 RV 20% MeOH and 2 RV 80% MeOH. This procedure was repeated several times in order to fractionate the whole extract solution. Finally, the combined 20% MeOH fractions were dried under reduced pressure yielding a fraction enriched in dicaffeoylquinic acids (DCQAs; 725 mg), whereas the flavonoids were eluted with 80% MeOH yielding the flavonoid fraction (734 mg).

2.3. HPLC analysis

HPLC analyses were performed on a Perkin Elmer Series 200 instrument equipped with a column oven (25 °C) and an UV–vis detector set at a wavelength of 345 nm. As stationary phase a 5μ Hypersil BDS C18 (250 mm × 4 mm) column was used, the mobile phase consisted of water adjusted to pH 2.8 with acetic acid (A) and acetonitrile (HPLC-grade, Riedel-de Häen, purchased from Sigma–Aldrich, Germany) containing 0.8% acetic acid (B).

HPLC analysis of the dicaffeoylquinic acid (DCQA) fraction carried out by internal standardisation with cynarin revealed a total DCQA content of 48.8% as described before (Benedek et al., 2006).

The flavonoid fraction was analysed using luteolin-3′,7-di-*O*-glucoside (Extrasynthèse, Genay, France; purity 95.61%) as internal standard. After addition of 200 μl of internal standard solution (1 mg/ml) to 4 mg of the fraction and dilution to 1 ml with 80% methanol, 15 μl of the centrifuged solution was injected onto the HPLC column. Separation of the flavonoids was obtained by gradient elution (10–17% B in 30 min, 17–40% B in 10 min, 40–100% B in 1 min, 100% B in 5 min) at a flow rate of 1.2 ml/min. The total flavonoid content was estimated at 11.1% with apigenin-7-*O*-glucoside (3.1%), rutin (2.2%) and luteolin-7-*O*-glucoside (1.5%) as main flavonoids.

2.4. Test compounds

Besides the *Achillea* extract, the dicaffeoylquinic acid (DCQA) and the flavonoid fraction, pure compounds from *Achillea* sp., namely the flavonoids rutin (Merck, Germany), apigenin- and luteolin-7-*O*-glucoside (Extrasynthèse, France), quercetin (Sigma, Germany), luteolin (Extrasynthèse, France)

and the sesquiterpene lactone desacetylmatricarin, isolated from *Achillea* sp. and kindly provided by S. Glasl, Department of Pharmacognosy, University of Vienna (Glasl et al., 2002), were also tested in the elastase assay.

2.5. Elastase assay

Determination of elastase activity was performed with human neutrophil elastase according to Melzig et al. (2001). Briefly, 125 μl of substrate solution (1.4 mM *N*-methoxysuccinyl-ala-ala-pro-val-*p*-nitroanilide from Sigma, Germany, in Tris–HCl buffer (pH 7.5)), 60 μl of test solution (stock solutions of the test compounds were dissolved in 500 μl DMSO and diluted with Tris–HCl buffer (pH 7.5) to give the final sample concentrations) and 390 μl of Tris–HCl buffer (pH 7.5) were mixed and vortexed. After addition of 25 μl enzyme solution (approx. 0.18 U elastase from human neutrophils, EC 3.4.21.37, from Serva, Germany) the samples were incubated for 1 h at 37 °C. After the reaction was stopped by addition of 500 μl soybean trypsin inhibitor (Sigma, Germany; 0.2 mg/ml Tris–HCl buffer (pH 7.5)) absorbance was immediately measured at 405 nm.

As positive control we used GW311616A, purchased from Sigma, Germany.

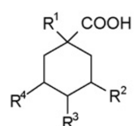
2.6. MMP assays

MMP assays were carried out using human MMP-2 and -9, respectively, purchased from Alexis Biochemicals, Switzerland, and universal protease substrate (resorufin-labeled casein) from Roche, Germany. When treated with proteases resorufin-labeled peptides are released from the substrate that cannot be precipitated by trichloroacetic acid. The concentration of resorufin-labeled peptides in the supernatant is equivalent to the proteolytic activity and can be measured fluorometrically (Schickaneder et al., 1988).

MMP-2 and -9 assays were carried out in the same way except for the enzyme concentrations: MMP-2 (approx. 1200 mU/mg) was activated by addition of 8 μl of 2.5 M aminophenylmercuric acid (APMA) to 2 μl of precursor enzyme solution and incubation at 37 °C for 90 min, whereas 1.5 μl of MMP-9 precursor solution (approx. 1875 mU/mg) was mixed with 6 μl of 2.5 M APMA and incubated in the same way.

After dilution with distilled water to 20 μl the activated enzyme solutions were mixed with increasing concentrations of the test solutions (10, 40, 60 and 80 μl of 2 mg/ml aqueous solutions of extract and fractions) and diluted with water to 100 μl. After addition of 50 μl of 0.4% substrate solution and 50 μl of incubation buffer (0.2 M Tris–HCl (pH 7.8), 0.02 M $CaCl_2$) the mixture was vortexed and incubated at 37 °C for 4 h. The reaction was stopped by addition of 480 μl of 5% trichloroacetic acid. After 10 min incubation at 37 °C and 5 min centrifugation, 600 μl of assay buffer (0.5 M Tris–HCl (pH 8.8)) was mixed with 400 μl of the supernatant. Fluorescence was read immediately at an excitation wavelength of 574 nm and an emission wavelength of 584 nm.

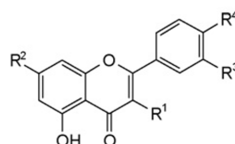
Na_2-EDTA·$2H_2O$ (10 mM) from Sigma, Germany, was used as positive control for both MMP assays.



3,4-DCQA R^1 = OH, R^2 = caffeoyl, R^3 = caffeoyl, R^4 = OH

3,5-DCQA R^1 = OH, R^2 = caffeoyl, R^3 = OH, R^4 = caffeoyl

4,5-DCQA R^1 = OH, R^2 = OH, R^3 = caffeoyl, R^4 = caffeoyl



rutin R^1 = O-rutinose, R^2 = OH, R^3 = OH, R^4 = OH

apigenin-7-O-glucoside R^1 = H, R^2 = O-glucose, R^3 = H, R^4 = OH

luteolin-7-O-glucoside R^1 = H, R^2 = O-glucose, R^3 = OH, R^4 = OH

Fig. 1. Chemical formulae of the dicaffeoylquinic acids (DCQAs) from the DCQA fraction and the main flavonoids from the flavonoid fraction.

2.7. Statistics

All assays were performed at least three times with duplicate samples. The influence of DMSO, which was used for the preparation of the test solutions for the elastase assay, on enzyme activity was considered in controls. Inhibition rates were calculated in percent to controls without inhibitors. IC_{50} values were determined from dose–effect curves by linear regression and are expressed as mean ± standard deviation (S.D.).

3. Results and discussion

The 20% methanolic extract from the commercial *Achillea* sample, the dicaffeoylquinic acid (DCQA) fraction, containing 48.8% DCQAs (for chemical structures, see Fig. 1), and the flavonoid fraction, containing 11.1% flavonoids with apigenin-7-*O*-glucoside, rutin and luteolin-7-*O*-glucoside as main compounds (see Fig. 1), all showed a dose-dependent inhibition of human neutrophil elastase (HNE) activity in the tested concentration range from 3 to 100 μg/ml (see Fig. 2). As shown in Table 1, the crude extract and the flavonoid fraction exhibited

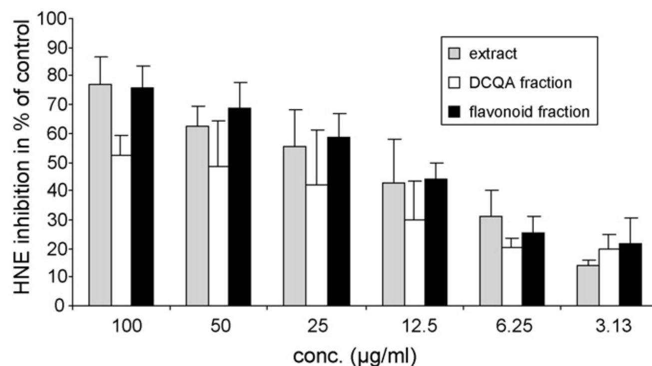


Fig. 2. Concentration-dependent inhibition of human neutrophil elastase by *Achillea* extract, DCQA and flavonoid fraction in % of control ($n = 3$).

Table 1
IC_{50} values (μg/ml) ± S.D. ($n = 3$) of *Achillea* extract, fractions and pure compounds in the elastase assay

Test compound	IC_{50} (μg/ml)
Achillea extract	23 ± 8.2
DCQA fraction	72 ± 16.7
Flavonoid fraction	20 ± 4.2
Luteolin	100 (=350 μM) ± 7.4
Quercetin	75 (=248 μM) ± 9.3
Rutin	>200[a]
Apigenin-7-*O*-glucoside	>200[a]
Luteolin-7-*O*-glucoside	>200[a]
Desacetylmatricarin	100 (=381 μM) ± 6.9
GW311616A (control)	0.04 (=100 nM) ± 4.1

[a] For rutin, apigenin- and luteolin-7-*O*-glucoside no activity higher than 20% was observed at the highest tested concentration (200 μg/ml).

equal inhibitory activities with IC_{50} values of 23 and 20 μg/ml, respectively, whereas the dicaffeoylquinic acid fraction was less potent (IC_{50} = 72 μg/ml).

In order to evaluate whether the flavonoids were responsible for the observed inhibitory effect, we screened different flavonoids from *Achillea* in the elastase assay. The three main flavonoids present in the crude extract and enriched in the flavonoid fraction, rutin, apigenin- and luteolin-7-*O*-glucoside, showed less than 20% inhibition at the highest tested concentration (200 μg/ml). For the flavonoid aglycons luteolin and quercetin we observed IC_{50} values of 100 μg/ml (350 μM) and 75 μg/ml (248 μM), respectively (see Table 1). For validation of our assay we used the orally bioavailable selective HNE inhibitor GW311616A (Macdonald et al., 2002) and measured an IC_{50} of 0.1 μM.

As the flavonoids seem to contribute only in a minor extent to the elastase inhibition of the extract and the flavonoid fraction, we tested a sesquiterpene lactone, desacetylmatricarin, which is also present in the extract and the fraction. Moreover, sesquiterpene lactones are known to possess elastase inhibitory properties (Siedle et al., 2002). Desacetylmatricarin inhibited HNE with an IC_{50} value of 100 μg/ml (=381 μM) which indicates a contribution to the elastase inhibiting activity, but still does not explain the potency of the extract and the flavonoid fraction.

These findings are, however, in contrast to our hypothesis that the phenolic compounds are the active principles of yarrow which was based on earlier studies from Melzig et al. (2001). In this paper both, flavonoids and dicaffeoylquinic acids, were shown to be potent HNE inhibitors.

Our current results indicate that a fractionation of the crude *Achillea* extract into fractions enriched in flavonoids and DCQAs, respectively, does not lead to an increase in the protease inhibitory effect as expected. Moreover, calculating the HNE inhibitory activity of the fractions at physiological concentrations shows that their effect only amounts to 58% of the crude extract's activity.

Hence, the activity of the extract might either be caused by additional, not further identified, plant compounds or might be mediated by the extract as a whole due to synergistic effects. This latter argument is consistent with the holistic approach of phytotherapy in that not single compounds but the extract as a multiple component mixture causes the desired effect.

Table 2
IC_{50} values (μg/ml) ± S.D. ($n = 3$) of *Achillea* extract and fractions in the MMP-2 and -9 assays

Test compound	MMP-2 inhibition (IC_{50} in μg/ml)	MMP-9 inhibition (IC_{50} in μg/ml)
Achillea extract	800 ± 8.3	800 ± 14.5
DCQA fraction	600 ± 5.1	700 ± 9.1
Flavonoid fraction	800 ± 9.2	900 ± 10.9

In the MMP-2 as well as in the MMP-9 assay the *Achillea* extract, the dicaffeoylquinic acid and the flavonoid fraction also exhibited a concentration-dependent protease inhibition, although at about 10-fold higher concentrations than in the elastase assay. While the extract inhibited both MMP-2 and -9 at IC_{50} values of 800 μg/ml, MMP-2 was stronger inhibited by the two fractions than MMP-9 (see Table 2). In both assays the DCQA fraction was more active than the crude extract and the flavonoid fraction showing IC_{50} values of 600 μg/ml in the MMP-2 and 700 μg/ml in the MMP-9 assay. These results suggest a contribution of the dicaffeoylquinic acids to the MMP inhibitory activity, as they are present in the DCQA fraction to almost 50%. For lack of isolated compounds we could not test the dicaffeoylquinic acids from *Achillea*, namely 3,4-, 3,5- and 4,5-DCQA, in the assays. Up to now no MMP inhibitory effects have been described for those compounds whereas for chlorogenic acid a MMP-9 inhibition was demonstrated recently (Jin et al., 2005). This compound is contained in *Achillea* as well, being present in the tested crude extract to 2.2%, but not in the DCQA fraction.

To sum up, the *Achillea* extract, the dicaffeoylquinic acid and the flavonoid fraction inhibited human neutrophil elastase as well as the matrix metalloproteinases MMP-2 and -9 *in vitro*. Those proteases are involved in chronic inflammatory skin conditions such as psoriasis and atopic dermatitis as well as in inflammatory bowel diseases like Crohn's disease and ulcerative colitis (Wiedow et al., 1992; Baugh et al., 1999; Gao et al., 2005). In addition, a recent study showed elevated levels of these proteases in chronic otitis externa that could be reduced by administration of protease inhibitors (Antonelli et al., 2005).

Inflammatory bowel diseases such as Crohn's disease and ulcerative colitis are characterised by acute and chronic inflammation and connective tissue defects. Initial inflammation leads to tissue damage and leucocyte infiltration characteristic of a wound-healing response, often with fibrotic hypertrophy, and typically following a recurrent disease cycle.

The role of up-regulated serine proteases and matrix metalloproteinases in colitis was clearly pointed out by Tarlton et al. (2000) who observed increased levels of trypsin and neutrophil elastase in a murine model of colitis. Moreover, a correlation between elevated serine protease levels and MMP-9 expression and MMP-2 and -9 activation was shown which suggests a relationship between serine protease penetration into gut tissue

and resident MMP activation. The resulting proteolysis leads to tissue damage and prevents regeneration and the resolution of inflammation. HNE and MMPs might thus be important targets for the treatment of inflammatory skin and bowel diseases.

Furthermore, Hirota et al. (2005) investigated the relationship between colitis and HNE and found significantly increased levels of HNE in ulcer areas. In addition, treatment with the orally active HNE inhibitor ONO-6818 reduced inflammation and progression of ulceration. Considering these facts, the HNE and MMP inhibitory activity of the *Achillea* extract can indeed be responsible for the antiphlogistic activity of the plant.

Taking into account that *Achillea* is traditionally used for skin and gastro-intestinal inflammations where resorption is not required for exertion of the effect, as the site of action is the colonic lumen and the skin surface, respectively, particularly the HNE inhibitory activity of the crude extract ($IC_{50} = 23\ \mu g/ml$) might be of therapeutical relevance. As the recommended daily intake of *Achillea* tea is three cups per day prepared with 1.5 g crude drug (Willuhn, 2002) one takes up 900 mg extract per day what might cause the antiphlogistic effect by inhibiting human neutrophil elastase and, to a minor extent, the metalloproteinase MMP-2 and -9.

Together with the spasmolytic effects caused by the flavonoid fraction (Lemmens-Gruber et al., 2006), the choleretic effects that are mediated by the DCQA fraction (Benedek et al., 2006) and the topical anti-inflammatory activity of the sesquiterpenes (Kastner et al., 1993), the protease-inhibitory activity adds new insights to the pharmacological effects of *Achillea millefolium* L. and confirms the traditional use of yarrow as antiphlogistic drug.

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References

Antonelli, P.J., Schultz, G.S., Cantwell, J.S., Sundin, D.J., Pemberton, P.A., Barr, P.J., 2005. Inflammatory proteases in chronic otitis externa. Laryngoscope 115, 651–654.

Baugh, M.D., Perry, M.J., Hollander, A.P., Davies, D.R., Cross, S.S., Lobo, A.J., Taylor, C.J., Evans, G.S., 1999. Matrix metalloproteinase levels are elevated in inflammatory bowel disease. Gastroenterology 117, 814–822.

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.

Bieth, J.G., 1998. Leucocyte elastase. In: Barrett, A.J., Rawlings, N.D., Woessner, J.F. (Eds.), Handbook of Proteolytic Enzymes. Academic Press, London, pp. 54–60.

Cavalcanti, A.M., Baggio, C.H., Freitas, C.S., Rieck, L., de Sousa, R.S., Da Silva-Santos, J.E., Mesia-Vela, S., Marques, M.C., 2006. Safety and antiulcer efficacy studies of *Achillea millefolium* L. after chronic treatment in Wistar rats. Journal of Ethnopharmacology 107, 277–284.

Collier, I.E., Goldberg, G.I., 1998. Gelatinase B. In: Barrett, A.J., Rawlings, N.D., Woessner, J.F. (Eds.), Handbook of Proteolytic Enzymes. Academic Press, London, pp. 1205–1210.

Elkington, P.T.G., O'Kane, C.M., Friedland, J.S., 2005. The paradox of matrix metalloproteinases in infectious disease. Clinical & Experimental Immunology 142, 12–20.

Ende, C., Gebhardt, R., 2004. Inhibition of matrix metalloproteinase-2 and -9 activities by selected flavonoids. Planta Medica 70, 1006–1008.

Fischer, G., Krug, E., 1984. Heilkräuter und Arzneipflanzen, seventh ed. Karl F. Haug Verlag, Heidelberg, pp. 220–221.

Gao, Q., Meijer, M.J., Kubben, F.J., Sier, C.F., Kruidenier, L., van Duijn, W., van den Berg, M., van Hogezand, R.A., Lamers, C.B., Verspaget, H.W., 2005. Expression of matrix metalloproteinases-2 and -9 in intestinal tissue of patients with inflammatory bowel diseases. Digestive and Liver Disease 37, 584–592.

Glasl, S., Mucaji, P., Werner, I., Presser, A., Jurenitsch, J., 2002. Sesquiterpenes and flavonoid aglycones from a Hungarian taxon of the *Achillea millefolium* group. Zeitschrift für Naturforschung 57c, 976–982.

Hirota, Y., Suzuki, M., Yamaguchi, K., Fujita, T., Katsube, N., 2005. Pathophysiological studies of trinitrobenzene sulfonic acid-induced colitis in Syrian hamsters (*Mesocricetus auratus*). Comparative Medicine 55, 45–54.

Jin, U.H., Lee, J.Y., Kang, S.K., Kim, J.K., Park, W.H., Kim, J.G., Moon, S.K., Kim, C.H., 2005. A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type of strong matrix metalloproteinase-9 inhibitor: isolation and identification from methanol extract of *Euonymus alatus*. Life Sciences 77, 2760–2769.

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 Medica 59, A669.

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. Arzneimittelforschung/Drug Research 56, 582–588.

Löser, B., Kruse, S.O., Melzig, M.F., Nahrstedt, A., 2000. Inhibition of neutrophil elastase activity by cinnamic acid derivatives from *Cimicifuga racemosa*. Planta Medica 66, 751–753.

Macdonald, S.J.F., Dowle, M.D., Harrison, L.A., Clarke, G.D.E., Inglis, G.G.A., Johnson, M.R., Shah, P., Smith, R.A., Amour, A., Fleetwood, G., Humphreys, D.C., Molloy, C.R., Dixon, M., Godward, R.E., Wonacott, A.J., Singh, O.M.P., Hodgson, S.T., Hardy, G.W., 2002. Discovery of further pyrrolidine trans-lactams as inhibitors of human neutrophil elastase (HNE) with potential as development candidates and the crystal structure of HNE complexed with an inhibitor (GW475151). Journal of Medicinal Chemistry 45, 3878–3890.

Madaus, G., 1976. Lehrbuch der biologischen Heilmittel, vol. 3. Georg Olms Verlag, Hildesheim, pp. 1911–1919.

Melzig, M.F., Löser, B., Ciesielski, S., 2001. Inhibition of neutrophil elastase activity by phenolic compounds from plants. Pharmazie 56, 967–970.

Mitaine-Offer, A.-C., Hornebeck, W., Sauvain, M., Zèches-Hanrot, M., 2002. Triterpenes and phytosterols as human leucocyte elastase inhibitors. Planta Medica 68, 930–932.

Murphy, G., 1998. Gelatinase A. In: Barrett, A.J., Rawlings, N.D., Woessner, J.F. (Eds.), Handbook of Proteolytic Enzymes. Academic Press, London, pp. 1199–1205.

Obayashi, H., 2005. Current synthetic inhibitors of human neutrophil elastase in 2005. Expert Opinion on Therapeutic Patents 15, 759–771.

Petrides, P.E., 1998. Binde- und Stützgewebe. In: Löffler, G., Petrides, P.E. (Eds.), Biochemie und Pathobiochemie, sixth ed. Springer, Berlin, pp. 734–748.

Schickaneder, E., Hösel, W., v.d. Eltz, H., Geuß, U., 1988. Casein-resorufon, a new substrate for a highly sensitive protease assay. Fresenius' Journal of Analytical Chemistry 330, 360.

Seo, U.K., Lee, Y.J., Kim, J.K., Cha, B.Y., Kim, D.W., Nam, K.S., Kim, C.H., 2005. Large-scale and effective screening of Korean medicinal plants for inhibitory activity on matrix metalloproteinase-9. Journal of Ethnopharmacology 97, 101–106.

Siedle, B., Ciesielski, S., Murillo, R., Löser, B., Castro, V., Klaas, C.A., Hucke, O., Labahn, A., Melzig, M.F., Merfort, I., 2002. Sesquiterpene lactones as inhibitors of human neutrophil elastase. Bioorganic & Medicinal Chemistry 10, 2855–2861.

Tarlton, J.F., Whiting, C.V., Tunmore, D., Bregenholt, S., Reimann, J., Claesson, M.H., Bland, P.W., 2000. The role of up-regulated serine proteases and matrix metalloproteinases in the pathogenesis of a murine model of colitis. The American Journal of Pathology 157, 1927–1935.

Wiedow, O., Meyer-Hoffert, U., 2005. Neutrophil serine proteases: potential key regulators of cell signalling during inflammation. Journal of Internal Medicine 257, 319–328.

Wiedow, O., Wiese, F., Streit, V., Kalm, C., Christophers, E., 1992. Lesional elastase activity in psoriasis, contact dermatitis and atopic dermatitis. The Journal of Investigative Dermatology 99, 306–309.

Willuhn, G., 2002. Millefolii herba. In: Wichtl, M. (Ed.), Teedrogen und Phytopharmaka, fourth ed. Wiss. Verl.-Ges, Stuttgart, pp. 399–403.