Mareike Maas, Summary

Eupatorium perfoliatum L.: Phytochemical characterization and functional *in vitro* investigations – Antiinflammatory, antiprotozoal and antiviral activities

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Boneset or thoroughwort (*Eupatorium perfoliatum* L.) is a medicinal herb from North America with a well documented traditional use for the treatment of fever, influenza, malaria, and rheumatoidal diseases. The current German Homeopathic Pharmacopoia (2010) contains a monograph about *Eupatorium perfoliatum* which is the basis for the application as homeopathic remedy.

In this work the phytochemical composition of different extracts of dried aerial parts of *E. perfoliatum* was characterized thoroughly. The cytotoxic, immunostimulating, antiinflammatory, anti-plasmodial and antiviral activities of different extracts as well as of isolated compounds were examined by *in vitro*-experiments.

The essential oil (1.8 mL/kg), obtained by steam distillation, contained *E*-Anethol und Carvon as main constituents.

From the 70 % methanolic extract eight caffeic acid derivatives (**KD1** to **KD8**) as well as five flavonoid glycosides (**FG1** to **FG5**) were identified (s. Table 1). The dicaffeoylglucaric acid derivatives **KD3**, **KD5**, and **KD6** represent natural products not described until now, belonging to an unusual class of compounds (depsides of hydroxy cinnamic acids with hexaric acids). These derivatives were shown to accumulate in the flowers of *E. perfoliatum*.

From the dichloromethane extract seven sesquiterpene lactones (**SL1** to **SL7**) were obtained including six newly described natural products. The free carboxylic acid in **SL1** and the unique mode of linkage in **SL3** represent novel structure elements of sesquiterpene lactones. Additionally, four flavonoid aglycons (**FA1** to **FA4**) were isolated from the dichloromethane extract. **FA2** was shown to be the major compound of the epicuticular fractions obtained from leave surface washes with chloroform. Nine triterpenes (**TT1** to **TT9**) together with three phytosterols (**St1** to **St3**) were identified in the dichloromethane extract by GC-MS (s. Table 1). Besides, several homologous fatty acids (C_{16} to C_{24}, odd and even numbered) and fatty alcohols (C_{18} to C_{26}, only even numbered) were identified.

A raw polysaccharide (RPS) was isolated by cold water extraction, ethanol precipitation and dialysation in yields of 1.1 % in relation to the dried plant material; besides, a content of 1.3 % low molecular fructans was identified. RPS was found to contain substantial endotoxin contamination, which was removed by polymyxin affinity chromatography prior to the use of the polysaccharide in functional tests.

The 70 % methanolic extract, the dichloromethane extract, the raw polysaccharide as well as the homeopathic mother tincture (ethanolic-aqueous extract according to the German Homeopathic Pharmacopoeia) were investigated for pharmacological activities in several *in vitro*-tests.

Concerning direct cytotoxicity against HaCaT keratinocytes only the dichloromethane extract exhibited cell activity reducing potential at higher concentrations (100 µg/mL). All other tested extracts did not influence cell vitality in the concentration range of 1 to 100 µg/mL.

Immunological activity of the test extracts (1 to 100 µg/mL) was not detected in different test systems (lymphocyte transformation test on PBMCs; tests on enhancement of phagocytic activity and of NO-production by murine RAW 264.7 macrophages).

The dichloromethane extract, the mother tincture, and the 70 % methanolic extract of *E. perfoliatum* (in order of decreasing activity) showed antiinflammatory activity against LPS-stimulated RAW 264.7 macrophages by inhibition of NO release (IC_{50} 19 µg/mL, 89 µg/mL, and > 100 µg/mL, resp.) due to inhibition of iNOS expression (Dot Blot) in non-toxic concentrations. Especially the dimeric guaianolide **SL3** and the flavonoid aglycon Eupafolin (**FA2**) exhibited prominent NO inhibiting activity (IC_{50} 16 µM and 6 µM, resp.).

The antiinflammatory activity of the dichloromethane extract was evaluated on genomic and protein level by microarray, real-time PCR and ELISA. Thereby the expression of several LPS-induced inflammatory genes was downregulated by the extract. Strongest downregulation (up to -44 % according to microarray results) was observed for the cytokines CSF-3, IL-1α, and IL-1β as well as for the chemokines CCL2, CCL22, and CXCL10. The cytokine TNF was downregulated moderately (-17 %). The pattern of downregulated genes suggests an involvement of NF-κB inactivation which remains still to be proven.

The dichloromethane extract also showed moderate antiprotozoal activity, with best efficacy against *Plasmodium falciparum* (IC_{50} 2.7 µg/mL, selectivity index (SI) 27.3). The dimeric guaianolide **SL3** exhibited highest activity against *P. falciparum* (IC_{50} of 2.0 µM, SI of 8.3) among the tested isolated compounds.

The ethanolic mother tincture showed promising antiviral activity against Influenza A-virus H1N1, compared to other plant extracts (IC_{50} 11.5 µg/mL, SI = 21.5).

Although the postulated immunostimulating properties of *E. perfoliatum* could not be confirmed, the detected activities can be seen as verification of the reported traditional uses of *Eupatorium perfoliatum* against inflammatory diseases, influenza, and malaria. The existing results suggest *E. perfoliatum* as an interesting topic for further *in vivo* and clinical studies.

Table 1: *Substances that have been identified in extracts of dried aerial parts of* E. perfoliatum*. For chemical structures see following chapters.* ★ *new natural product;* ◆ *reported as constituent of* E. perfoliatum *before.*

	identified substance
KD1	3-caffeoylquinic acid (neochlorogenic acid)
KD2	5-caffeoylquinic acid (chlorogenic acid)
KD3	2,4/3,5-dicaffeoylglucaric acid ★
KD4	caffeic acid
KD5	3,4-dicaffeoylglucaric acid ★
KD6	2,5-dicaffeoylglucaric acid ★
KD7	3,5-dicaffeoylquinic acid
KD8	4,5-dicaffeoylquinic acid
FG1	rutin ◆ (HABTEMARIAM, 2008; WAGNER *et al.*, 1972)
FG2	hyperoside ◆ (HABTEMARIAM, 2008; WAGNER *et al.*, 1972)
FG3	isoquercitrin
FG4	trifolin
FG5	astragalin ◆ (WAGNER *et al.*, 1972)
SL1	5*S*,6*R*,7*R*,8*R*,11*R*-2-oxo-8-tigloyloxyguaia-1(10),3-diene-6,12-olide-14-carboxylic acid ★
SL2	5*S*,6*R*,7*R*,8*R*,11*R*-2,14-dioxo-8-tigloyloxyguaia-1(10),3-diene-6,12-olide ★
SL3	5*S*,6*R*,7*R*,8*R*,11*R*,14*S*-14-hydroxy-2-oxo-14(4*S*,5*S*,6*R*,7*R*,8*R*,11*R*-8-tigloyloxyguaia-1(10),2-diene-6,12,2,14-diolid-4-yl)-8-tigloyloxyguaia-1(10),3-diene-6,12-olide ★
SL4	5*S*,6*R*,7*R*,8*R*,11*R*-14-hydroxy-2-oxo-8-tigloyloxyguaia-1(10),3-diene-6,12-olide ★
SL5	1*R*,5*R*,6*R*,7*R*,8*R*,10*S*,11*R*-2,14-dioxo-8-tigloyloxyguai-3-ene-6,12-olide ★
SL6	3α,14-dihydroxy-8β-tigloyloxy-6βH,7αH,11αH-germacra-1(10)*Z*,4*Z*-diene-6,12-olide ★
SL7	euperfolitin ◆ (HERZ *et al.*, 1977)
FA1	hispidulin
FA2	eupafolin
FA3	patuletin
FA4	kaempferol ◆ (WAGNER *et al.*, 1972)
TT1	β-amyron
TT2	lupenon
TT3	β-amyrin acetate
TT4	α-amyrin acetate
TT6	β-amyrin ◆ (HOOPER and CHANDLER, 1984)
TT7	α-amyrin ◆ (HOOPER and CHANDLER, 1984; DOMINGUEZ *et al.*, 1974)
TT8	lupeol ◆ (HOOPER and CHANDLER, 1984)
TT9	taraxasterol ◆ (HOOPER and CHANDLER, 1984)
St1	campesterol ◆ (HOOPER and CHANDLER, 1984)
St2	β-sitosterol ◆ (HOOPER and CHANDLER, 1984; DOMINGUEZ *et al.*, 1974)
St3	stigmasterol ◆ (DOMINGUEZ *et al.*, 1974)