Fukinolic acid and cimicifugic acids: a review

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Abstract During the last decades, the research on the biological activities of extracts from *Cimicifuga Actaea* species and *Petasites japonicus* as well as their active ingredients has been intensified. Besides terpenoids as dominant natural product group, hydroxycinnamic acid esters such as fukinolic acid and several cimicifugic acids have been isolated from *Actaea* and *Petasites* species and their chemical structures have been elucidated. Investigations on the biological properties of these hydroxycinnamic acid esters are currently undertaken and some compounds might be promising therapeutic tools. In this review, we have gathered information on the genera *Actaea* and *Petasites*, the occurrence of cimicifugic and fukinolic acids and some aspects of their biosynthesis. Furthermore, we have summarized the medicinal aspects of fukinolic acid and cimicifugic acids. In connection with the biological activities of these compounds, structural features of the hydroxycinnamic acid derivatives move into the focus. The position of the hydroxyl group at the aromatic rings and the introduction of an electron-donating moiety may be important for anti-inflammatory, antiviral, cytotoxic and vasoactive effects of these compounds.

Keywords *Actaea racemosa* (syn. *Cimicifuga racemosa* · Ranunculaceae) · Cimicifugic/fukinolic acids · Fukiic acid · Hydroxycinnamic acid esters · *Petasites japonicus* (Asteraceae) · Piscidic acid

Introduction

The use of herbs and herbal extracts for therapeutic aspects goes back a long time from a historical perspective. A multitude of plants have been used in traditional medicine, among them *Actaea* (syn. *Cimicifuga*) and *Petasites* species. Both species grow in the northern parts of our planet, namely Eastern Asia, North America and Europe. Different plant organs have been traditionally used for the treatment of common diseases such as gastroenteritis, bronchitis, postmenopausal symptoms, sore throat and headaches (Aydin et al. 2013; Chen et al. 2014; Henneicke-von-Zepelin 2017; Guo et al. 2017).

Various active compounds besides the hydroxycinnamic acid esters (fukinolic and cimicifugic acids) have been described from *Actaea* and *Petasites* species and their biological effects have been investigated. A major active group in *Petasites* are sesquiterpenes, especially petasin and its isomers that possess neuroprotective effects (Wang et al. 2013) as well as spasmolytic (Aydin et al. 2013) and anti-inflammatory activities (Thomet et al. 2001). Further

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metabolites are pyrrolizidine alkaloids (Kitajima et al. 2019 and literature cited therein), some of which have carcinogenic and hepatotoxic properties. Flavonoids (Kim et al. 2017), lignans (Min et al. 2005) and essential oil (Miyazawa et al. 2003) have been found in *Petasites japonicus* as well.

Actaea species are widely used in traditional Chinese medicine and have been traditionally used by Native Americans. This has been comprehensively reviewed by Guo et al. (2017). In the European Pharmacopoeia, an extract from rhizomes of *A. racemosa* is recommended as “Herbal medicinal product for the relief of menopausal complaints such as hot flushes and profuse sweating” (EMA/HMPC/48745/2017). The pharmacological properties of *Actaea racemosa* have very recently been compiled by Salari et al. (2021). The pharmaceutically most recognized compounds of *Actaea* species are triterpene glycosides with a cycloartane skeleton such as actein (acetyl-acteol-3-*O*- β -xyloside). Various structurally different triterpene aglyca have been identified: cimigenol, 25-dehydrocimigenol, acteol, 23-*epi*-acteol, 23-*O*-acetylshengmanol, hydroshengmanol, cimiramnoside, neocimigenol, isodahurinol, cimicidanol and cimicidol (Qiu et al. 2014). These compounds were reported to have cytotoxic, antiviral, anti-proliferative and anti-tumor activities (Watanabe et al. 2002; Sakurai et al. 2003, 2004). The triterpene glycosides are supposed to be the active compounds for the treatment of postmenopausal symptoms (Leach and Moore 2012). Some nitrogen-containing compounds have been identified as well, e.g. cimipronidine (a guanidine alkaloid), cyclocimipronidine or cimipronidine methyl ester, N_{ω} -methylserotonin, salsolinol or dopargine (Qiu et al. 2014). Nikolić et al. (2012) identified at least 73 nitrogen-containing primary and secondary compounds in *A. racemosa*, among them aporphine alkaloids, isoquinoline alkaloids, protoberberine alkaloids, protopine alkaloids, betains, guanidines, choline derivatives, β -carboline alkaloids, amides of hydroxycinnamic acids and pyridoxine derivatives. A comprehensive compilation of natural products from *Cimicifuga* species can be found in the review by Guo et al. (2017).

Apart from the already mentioned active specialized compounds of the genera *Actaea* and *Petasites*, hydroxycinnamic acid esters are becoming increasingly important. Since the first isolation of fukinolic acid from *P. japonicus* (Sakamura et al. 1969) its

medicinal properties have been investigated (Sakamura et al. 1973; Löser et al. 2000; Watanabe et al. 2007). Later, further hydroxycinnamic acid esters, congeners of fukinolic acid, have been isolated, mostly from *Actaea* species, and were named cimicifugic acids A–N in the order of their discovery (Gödecke et al. 2009).

According to current knowledge cimicifugic acids show a variety of pharmacological effects. The biological effects of cimicifugic acids and fukinolic acid comprise estrogenic, anti-tumor and collagenolytic activities (Kruse et al. 1999; Kusano et al. 2001; Jiang et al. 2008b), inhibition of hyaluronidase and cytochrome P450 enzymes (Iwanga et al. 2010a, b; Huang et al. 2010) and the influence on seed germination and seedling growth (Kusano et al. 1998). Furthermore, cimicifugic acids and fukinolic acid were found to possess anti-inflammatory (Löser et al. 2000; Wang et al. 2017b; Lee et al. 2019), antiviral (Powers and Setzer 2016; Ma et al. 2019), antioxidant properties (Burdette et al. 2002; Nuntanakorn et al. 2006, 2007; Watanabe et al. 2007) and additionally vasoactive (Noguchi et al. 1998) and miscellaneous other effects (Shimoda et al. 2006; Farnsworth et al. 2008). These will be further discussed in this review.

Although, a multitude of biological activities of fukinolic and cimicifugic acids have been published, there is currently no review that focusses on the importance of fukinolic acid and cimicifugic acids. The aim of this review is to shortly present the botanical aspects of plant species containing fukinolic acid and cimicifugic acids. Furthermore, the occurrence of hydroxycinnamic acid esters and their benzyltartaric acid moieties are summarized as well as the current knowledge of their biosynthesis. The main part will be information on the structural compositions of the acids that influence the pharmacological effects and the evaluation of the effectiveness of cimicifugic acids and fukinolic acid as potential drugs. To establish this review, we have searched the literature published in English or having at least an English abstract using SciFinder and Google Scholar and the following keywords: *Actaea*, *Cimicifuga*, *Petasites japonicus*, fukinolic acid, cimicifugic acid, fukiic acid, piscidic acid. To our knowledge, no relevant publication has been excluded.

Botanical aspects of *Actaea* species and *Petasites japonicus*

Petasites (Asteraceae) and *Actaea* (Ranunculaceae) species are distributed in the northern hemisphere, occurring in Eastern Asia, North America and Europe. Only those *Actaea* species that are officially of therapeutic use in China and Europe will be described here, even if there are further species containing cimicifugic acids and fukinolic acid. Furthermore, *Petasites japonicus* is presented here which has been introduced to Europe as an ornamental plant (Ożarowski et al. 2013) and, unlike other *Petasites* species (to our current knowledge), contains fukinolic acid.

Actaea species

Actaea species are native to subarctic, temperate and subtropical habitats of the northern hemisphere. The former genus *Cimicifuga* was included in the genus *Actaea* in 1998 due to molecular phylogenetic data showing a monophyletic clade of the former genera *Actaea*, *Cimicifuga* and *Souliea* (Compton et al. 1998). The genus *Actaea* now comprises about 30 species (World Flora Online <http://www.worldfloraonline.org>, accessed August 12, 2021). Different species of the genus *Actaea* (Ranunculaceae) are commonly used in the traditional Chinese herbal medicine, here still registered as the genus *Cimicifuga*. According to the Pharmacopoeia of China the official Cimicifugae rhizoma can include rhizomes of *Actaea foetida*, *A. heracleifolia* and *A. dahurica* (Guo et al. 2017). The quality of Cimicifugae rhizoma is controlled by the content of isoferulic acid (Shen et al. 2013). In Europe, only *A. racemosa* is of therapeutic relevance (European Union herbal monograph on *Cimicifuga racemosa* (L.) Nutt. rhizoma EMA/HMPC/48745/2017). *Cimicifuga* has got its name because of the unpleasant smell of the flowers that repels insects like leaf bugs: lat. *cimex* = bug and *fuga* = escape (*Cimicifuga* = bugweed) (Blaschek 2016). Most species are perennial and herbaceous plants that grow up to two meters and flower in late summer until early autumn.

The small, dense, white or creamy white flowers are arranged in a long, narrow, complex inflorescence. This inflorescence is characterized by numerous terminate clustered racemes that can vary from simple to spicate or branched. The cup consists of 4 or 5

petaloid white sepals that are orbovate-orbicular and caduceus. The flowers are dominated by the numerous stamens. *Actaea* species possess stems with various apical branches and carry large, long-stemmed pinnate compound leaves. The few yellow–brown seeds develop in autumn and are ellipsoid, narrow and flat. The dried rootstock of *Actaea* is used for extraction and pharmaceutical applications. The fibrous, cylindrical roots and creeping, crawling rhizomes are characterized by a dark brown color and robust consistence (Blaschek 2016; Guo et al. 2017).

Petasites japonicus

Petasites japonicus (Asteraceae) is native to Eastern Asia. The rhizomes, roots and leaves of *P. japonicus* are commonly used in the USA as dietary supplement. In Japan, the young petioles are used as legumes and in traditional medicine. The common name of the genus *Petasites* is butterbur, probably because the large leaves were used to wrap butter in them for storage (Lee et al. 2019). *P. japonicus* is a perennial dioecious herbaceous plant flowering during April and May. The plant is characterized by its basal relatively large rhubarb-like green leaves which develop after flowering. The leaves are smooth, the upper side of the leaf is glabrous, whereas the lower side is thickly covered by trichomes. The flowers are greenish-white and usually either male or female. The primary inflorescence is a flower head, while the secondary inflorescence is a raceme. The petioles are light grey, fuzzy and hollow and the plant possesses thick rhizomes (Sakai et al. 2008; Tys et al. 2015).

Biogenetic origin of hydroxycinnamic acid esters

The phenolic compounds considered in this review are specialized metabolites derived from the shikimate pathway, which gives rise to the aromatic amino acids L-phenylalanine and L-tyrosine (besides L-tryptophan). Both moieties of cimicifugic and fukinolic acids are derived from these two amino acids (probably with exception of cimicifugic acid H with 3,4-dihydroxybenzoic acid as acid moiety; Iwanaga et al. 2010a). The phenylpropanoid pathway starting with L-phenylalanine gives rise to the hydroxycinnamic acid part and L-tyrosine probably is the precursor for the

alcoholic acceptor moiety, namely piscidic and fukiic acid (see below).

Nomenclature of fukinolic acid and cимicifugic acids and related compounds

From a chemical point of view, cимicifugic acids and fukinolic acid are classified as hydroxycinnamic acid esters (although cимicifugic acid H as a benzoic acid ester is an exception). They are composed of a glycolated hydroxyphenyllactic acid moiety, namely fukiic acid ((3,4-dihydroxybenzyl) tartaric acid) or piscidic acid ((4-hydroxybenzyl) tartaric acid), or benzyl tartaric acid (for cимicifugic acid M), and a hydroxycinnamic or benzoic acid (cимicifugic acid H) moiety (Fig. 1). Piscidic and fukiic acid have the same stereochemistry, possess the same side chain and only differ by number of hydroxyl groups at the aromatic ring (Yoshihara et al. 1974).

Fukinolic acid has been first isolated from *Petasites japonicus* and its structure has been elucidated as an

ester of caffeic acid and fukiic acid (Sakamura et al. 1973). Similar esters isolated from *Actaea* species have been named cимicifugic acids. Their “old” names cимicifugic acids A to N were given in the order of their detection (Gödecke et al. 2009).

A structure-based nomenclature has been established by Gödecke et al. (2009) taking the structural properties of cимicifugic acids into account (Fig. 1). Consequently, the cимicifugic acids (CA) were named with two letters to clarify the condensation products as follows: piscidic acid (P) or fukiic acid (K) connected with cinnamic acid (C), 4-coumaric acid (P), 4-methoxycinnamic acid (M), caffeic acid (K), ferulic acid (F), isoferulic acid (I), 3,4-dimethoxycinnamic acid (D) and sinapic acid (S). The abbreviation “C”, however, was used for caffeic acid (instead of cinnamic acid) by Qiu et al. (2014) leading to the name CA KC for fukinolic acid instead of CA KK as proposed by Gödecke et al. (2009). Besides the structures and the nomenclature, Fig. 1 also includes some chemical and physical properties of these hydroxycinnamic acid esters.

R1	R2	...acid	R3	R4	R5	...acid	first compound name	systematic name		molecular mass	$\lambda_{\max}$ [nm]	log ϵ
OH	OH	fukiic (K)	OH	OH	H	caffeic	fukinolic acid	CA KK	$C_{20}H_{18}O_{11}$	434.35	332	4.26
OH	OH	fukiic (K)	OCH ₃	OH	H	ferulic	cимicifugic acid A	CA KF	$C_{21}H_{20}O_{11}$	448.376	328	4.28
OH	OH	fukiic (K)	OH	OCH ₃	H	isoferulic	cимicifugic acid B	CA KI	$C_{21}H_{20}O_{11}$	448.376	313	4.27
OH	OH	fukiic (K)	H	OH	H	4-coumaric	cимicifugic acid C	CA KP	$C_{20}H_{18}O_{10}$	418.35	313	4.27
H	OH	piscidic (P)	OH	OH	H	caffeic	cимicifugic acid D	CA PK	$C_{20}H_{18}O_{10}$	418.35	332	4.25
H	OH	piscidic (P)	OCH ₃	OH	H	ferulic	cимicifugic acid E	CA PF	$C_{21}H_{20}O_{10}$	432.377	325	4.16
H	OH	piscidic (P)	OH	OCH ₃	H	isoferulic	cимicifugic acid F	CA PI	$C_{21}H_{20}O_{10}$	432.377	326	4.25
OH	OH	fukiic (K)	OCH ₃	OCH ₃	H	trans-3,4-dimethoxycinnamic	cимicifugic acid G	CA KD	$C_{22}H_{22}O_{11}$	462.403	328	3.17
OH	OH	fukiic (K)	3,4-dihydroxybenzoic acid			3,4-dihydroxybenzoic	cимicifugic acid H	-	$C_{18}H_{16}O_{10}$	392.313	324	4.2
OH	OH	fukiic (K)	OCH ₃	OH	OCH ₃	sinapic	cимicifugic acid I	CA KS	$C_{22}H_{22}O_{12}$	478.402	331	4.1
H	OH	piscidic (P)	OCH ₃	OH	OCH ₃	sinapic	cимicifugic acid J	CA PS	$C_{22}H_{22}O_{11}$	462.403	329	4.04
H	OH	piscidic (P)	H	OH	H	4-coumaric	cимicifugic acid K	CA PP	$C_{20}H_{18}O_9$	402.351	316	4.4
H	OH	piscidic (P)	OCH ₃	OCH ₃	H	trans-3,4-dimethoxycinnamic	cимicifugic acid L	CA PD	$C_{22}H_{22}O_{10}$	446.404		
H	H	benzyl-tartaric	OH	OH	H	caffeic	cимicifugic acid M	-	$C_{20}H_{16}O_9$	402.351		
OH	OH	fukiic (K)	OCH ₃	OCH ₃	H	cis-3,4-dimethoxycinnamic	cимicifugic acid N	[CA KDcis]	$C_{22}H_{22}O_{11}$	462.403	325	4.24

Fig. 1 Nomenclature of cимicifugic acids and fukinolic acid according to Gödecke et al. (2009) modified after Werner and Petersen (2019). $\lambda_{\max}$ and log ϵ values (methanol) are taken from Yannai (2012)

In addition, shomasides A and B have been isolated from *A. dahurica*/*A. heracleifolia*. They are galactosides of cimicifugic acids A and E, respectively. Shomaside C from the same plant species is a glucoside of cimicifugic acid E. In all cases, the sugar moieties are attached to the 4-OH-group of the ferulic acid part (Iwanaga et al. 2010a; Yim et al. 2012a).

Biosynthesis of fukinolic and cimicifugic acids

Fukinolic acid and most cimicifugic acids are esters composed of a benzyltartaric acid moiety and a hydroxycinnamic acid moiety (Fig. 1). The formation of fukinolic acid has been investigated by Hasa and Tazaki (2004). Five isotope-labeled compounds have been fed to axenically cultivated plants of *Petasites japonicus*: [1,2- $^{13}\text{C}_2$] and [2- ^{13}C] sodium acetate, [2- ^{13}C] malonic acid, [3- ^{13}C] L-phenylalanine and [1- ^{13}C] L-tyrosine. Based on these ^{13}C -labeling experiments, L-tyrosine and acetate were incorporated into the benzyltartaric acid moiety and L-phenylalanine into the caffeic acid moiety of fukinolic acid (Fig. 2). According to these ^{13}C -labeling experiments the synthesis of fukiic acid is based on 4-hydroxyphenylpyruvic acid or—as we suggest—4-hydroxyphenyllactic acid derived from L-tyrosine while the glycol side-chain is derived from acetate. Thus, Hasa and Tazaki (2004) proposed the introduction of an acetyl unit either from acetic acid or from acetyl-CoA. This, however, leaves the source of the OH-group needed for esterification open. Whether the biosynthesis takes place in the whole plant or in specific plant organs has not been reported.

The caffeic acid moiety of fukinolic acid is proposed to be synthesized by the conversion of [3- ^{13}C] L-phenylalanine into caffeoyl-CoA by the well-known phenylpropanoid pathway. The esterification of the caffeic acid moiety from caffeoyl-CoA and fukiic acid finally leads to fukinolic acid. Alternatively, fukiic acid could be coupled to 4-coumaric acid with 3-hydroxylation following the esterification.

Whereas the cinnamic acid moiety of cimicifugic acids is quite variable, the benzyltartaric acid moiety is—to our present knowledge—restricted to piscidic and fukiic acid (Fig. 1) with exception of cimicifugic acid M where the benzyltartaric acid part carries no hydroxylation at the aromatic ring (Iwanaga et al. 2010b). To our best knowledge, there is currently no

published information on neither the site of synthesis, nor the transport of these compounds, nor their subcellular localization. However, undifferentiated callus and suspension cultures of *Petasites japonicus* as well as *Actaea racemosa* accumulate fukinolic and cimicifugic acids to some extent (own unpublished results), thus specialized organs may not be essential.

The biosynthesis of cimicifugic acids has not been investigated on enzyme and gene levels until recently and still requires further investigation. Our group identified a hydroxycinnamoyltransferase from *Actaea racemosa* (Werner and Petersen 2019). This enzyme, namely hydroxycinnamoyl-CoA:piscidic acid hydroxycinnamoyltransferase (ArHPT1) or cimicifugic acid synthase (CAS), transfers a hydroxycinnamic acid moiety from hydroxycinnamoyl-CoA to piscidic acid (Fig. 3). Four hydroxycinnamoyl-CoA thioesters have been accepted by CAS. The highest affinity has been measured for *p*-coumaroyl-CoA with a K_m of $6.8 \mu\text{M} \pm 2.3 \mu\text{M}$. In contrast, the lowest substrate affinity has been detected for feruloyl-CoA (K_m $51.4 \mu\text{M} \pm 11.8 \mu\text{M}$). Caffeoyl-CoA (K_m $16.3 \mu\text{M} \pm 4.6 \mu\text{M}$) and sinapoyl-CoA (K_m $10.5 \mu\text{M} \pm 2.7 \mu\text{M}$) are ranged in between. The products resulting from the catalytic activity of CAS are cimicifugic acids as follows: CA PK (with caffeoyl-CoA), CA PF (with feruloyl-CoA), CA PS (with sinapoyl-CoA) and CA PP (with *p*-coumaroyl-CoA). As CAS is a soluble enzyme localization of this part of the biosynthesis in the cytosol can be assumed.

Occurrence and biological functions of fukiic and piscidic acids

Whereas various hydroxycinnamic acid esters as e.g. chlorogenic acid or rosmarinic acid are wide-spread in the plant kingdom, hydroxycinnamic acid esters with benzyltartaric acids, namely fukiic and piscidic acid, are less widely distributed. Examples for the occurrence of piscidic and fukiic acid as free acids are given in Table 1. Surprisingly, fukiic and piscidic acids occur in a number of genera of various families, without fukinolic acid or cimicifugic acids being detected at the same time, since fukinolic acid and cimicifugic acids, however, have to date only been detected in a few genera (Table 2).

Piscidic acid has been found in species of the monocotyledonous plant families Amaryllidaceae,

Fig. 2 Biosynthetic pathway of fukinolic acid as proposed by Hasa and Tazaki (2004). The grey symbols represent the ¹³C labeled positions of the fed substrates (light grey box) and their precursor metabolites

Asparagaceae, Dioscoreaceae and Xanthorrhoeaceae as well as in the eudicot plant families Cactaceae, Euphorbiaceae, Fabaceae and Quillajaceae. Piscidic acid was reported to be an active compound of *Dioscorea nipponica* used to treat chronic bronchitis (Toshima et al. 1999). Moreover, piscidic acid is involved in the uptake of phosphate by roots of *Cajanus cajan* (Ishikawa et al. 2002).

Fukiic acid, on the other hand, is less widely distributed and has been isolated from species of the Asteraceae, Fabaceae and Myrtaceae. It was reported to be a main compound in the darkening process of *Petasites japonicus* tissues after cutting (Sakamura et al. 1973). Furthermore, synthetic racemic fukiic acid showed in vitro activities against HIV-1 integrase (IC₅₀ = 0.25 μM) but failed to be active in antiviral assays with MT-4 cells (Queffelec et al. 2008).

Occurrence of fukinolic acid and cimicifugic acids

To our current knowledge, the occurrence of fukinolic acid and cimicifugic acids is mainly restricted to *Actaea* (*Cimicifuga*) species (Ranunculaceae) and to *Petasites japonicus* (Asteraceae) (Table 2). Apart from these two genera, cimicifugic acid D has been described to occur in *Platycodon grandiflorum* (Campanulaceae; Wang et al. 2017a), cimicifugic acids B and E in a peach flower extract (*Prunus persica*, Rosaceae; Kwak et al. 2018) and an unspecified cimicifugic acid in *Desmodium styracifolium* (Fabaceae; Zhao et al. 2007).

Since the initial isolation of fukinolic acid from leaves of *Petasites japonicus* (Sakamura et al. 1969) this compound has also been described in almost all *Actaea* species (see Table 2 and literature cited therein). Interestingly, the closely related cimicifugic

Fig. 3 Enzymatic formation of cимицифугици acids by cимицифугици acid synthase (CAS) from *Actaea racemosa*. CAS catalyzes the transfer of a hydroxycinnamic acid moiety from hydroxycinnamoyl-CoA thioesters (left grey box) to piscidic acid. The variety of the resulting cимицифугици acid products (grey box on the right-hand side) is determined by the four accepted

substrates: caffeoyl-CoA (K_m $16.3 \mu\text{M} \pm 4.6 \mu\text{M}$) (CA PK), feruloyl-CoA (K_m $51.4 \mu\text{M} \pm 11.8 \mu\text{M}$) (CA PF), sinapoyl-CoA (K_m $10.5 \mu\text{M} \pm 2.7 \mu\text{M}$) (CA PS), *p*-coumaroyl-CoA (K_m $6.8 \mu\text{M} \pm 2.3 \mu\text{M}$) (CA PP) (data from Werner and Petersen 2019)

acids except cимицифугици acid D (Lee et al. 2019) have not been found in *Petasites japonicus* or other *Petasites* species. Furthermore, there are no reports about the occurrence of any fukinolic or cимицифугици acids from other *Petasites* species apart from *P. japonicus*. *Cimicifuga* rhizomes used in traditional Chinese medicine are composed of the species *C. simplex*, *C. dahurica*, *C. heracleifolia* and *C. foetida* (Kusano 2001; Yim et al. 2012b) whereas *Cimicifugae* rhizoma described in the European Pharmacopoeia only contains *Cimicifuga* (= *Actaea*) *racemosa*.

Besides free cимицифугици acids, Takahira et al. (1998a) isolated cимицифугици acids A, B, E and F as 1-methylester from *A. simplex* underground parts. Cимицифугици acid E and F 1-methylesters were also found in *A. dahurica* by Qin et al. (2017). Dehydrocимицифугици acids A and B were found by Li et al. (2003) in *A. racemosa*. Iwanaga et al. (2010a, b) furthermore detected glucosides and galactosides of cимицифугици acids in “*Cimicifugae* rhizoma” composed of *A. heracleifolia* and *A. dahurica*, namely shomaside A and B being cимицифугици acid A and E galactosides and shomaside C being cимицифугици acid E glucoside.

Unfortunately, quantitative data on the contents of fukinolic and cимицифугици acids in plant material are scarce. Investigations were made to optimize the extraction process of phenolic acids from *A. racemosa* (Mukhopadhyay et al. 2006). The influence of solvent, amount of extraction steps, temperature, pressure and particle size on the total yield of phenols of *A. racemosa* has been analyzed. A solvent mixture of methanol:H₂O in a ratio of 60:40 (v/v) was declared to be most suitable ($19.35 \text{ mg/GAE g}^{-1}$) (GAE = gallic acid equivalents). Furthermore, 92% of the phenols were isolated in the first two extraction steps. The yield of the first cycle was around $17.90 \text{ mg/GAE g}^{-1}$ (87% of the total phenols), followed by $1.81 \text{ mg/GAE g}^{-1}$ (8.8% of the total phenols) in the second cycle and 4.2% in the third one ($0.86 \text{ mg/GAE g}^{-1}$). The optimum extraction temperature was described with 90°C ($25.29 \text{ mg/GAE g}^{-1}$) and the ideal range of particle size is given between 0.25 and 0.425 mm. Unfortunately, this publication only gives insight into the total content of hydroxycinnamic acids and does not focus on the amount of their esterified derivatives.

Nuntanakorn et al. (2007) analyzed dried roots/rhizomes of four different American *Actaea* species with respect to their hydroxycinnamic acids as well as fukinolic/cимицифугици acids. *A. rubra* displayed the

Table 1 Examples for the occurrence of piscidic and fukiic acids in different plant species

Compound	Plant family	Species	References
Piscidic acid	Amaryllidaceae	<i>Galanthus nivalis</i>	Krogh (1969)
		<i>Hippeastrum solandriflorum</i>	Carvalho et al. (2015)
		<i>Narcissus poeticus</i>	Okumura et al. (1955)
	Asparagaceae	<i>Agave americana</i>	Nordal and Ogner (1964), Krogh (1967)
	Cactaceae	<i>Opuntia ficus-indica</i>	Nordal et al. (1966a), Teles et al. (1994), Missaoui et al. (2020)
		<i>Coryphantha macromeris</i>	Cabañas García et al. (2020)
	Dioscoreaceae	<i>Dioscorea nipponica</i>	He et al. (1980), Ho et al. (1980)
	Euphorbiaceae	<i>Euphorbium resinifera</i>	Krogh (1967)
	Fabaceae	<i>Piscidia erythrina</i>	Freer and Clover (1901), Bridge et al. (1948), Moore and Eng (1956), Nordal et al. (1966b), Heller and Tamm (1975)
		<i>Cajanus cajan</i>	Ae et al. (1990), Otani et al. (1996), Ishikawa et al. (2002)
		<i>Sophora tonkinensis</i>	Ding and Chen (2008)
		<i>Sophora alopecuroides</i>	Wang and Ma (2009), Wang et al. (2020)
		<i>Sophora flavescens</i>	Zhang et al. (2013)
		<i>Retama sphaerocarpa</i>	Touati et al. (2017)
		<i>Aspalathus pendula</i>	Stander et al. (2017)
		<i>Vicia faba</i>	Valente et al. (2018)
		<i>Albizia anthelmintica</i>	Sobeh et al. (2020)
		<i>Cyclopia spec.</i>	Stander et al. (2019)
		<i>Retama raetam</i>	Saada et al. (2018)
	Quillajaceae	<i>Quillaja saponaria</i>	Maier et al. (2015)
	Xanthorrhoeaceae	<i>Aloe zebrina</i>	Krogh (1969)
Fukiic acid	Asteraceae	<i>Petasites japonicus</i>	Sakamura et al. (1969), Sakamura et al. (1973)
	Fabaceae	<i>Piscidia erythrina</i>	Heller and Tamm (1975)
		<i>Vicia faba</i>	Abu-Reidah et al. (2017)
	Myrtaceae	<i>Myrtus communis</i>	Díaz de Cerio et al. (2018)

highest content of total phenolics with 2.92% of the dry weight, followed by *A. podocarpa* (1.71%) whereas *A. racemosa* and *A. pachypoda* contained only 0.62% and 0.36%, respectively. Fukinolic acid was the main ester in *A. racemosa* (0.18%) and *A. pachycarpa* (0.21%), followed by cimicifugic acid B in *A. racemosa* (0.16%) and cimicifugic acid A (0.12%) in *A. pachycarpa*. The main compound in *A. podocarpa* was cimicifugic acid A (0.85%) closely followed by cimicifugic acid E (0.81%). *A. rubra* contained 1.11% cimicifugic acid B, 0.77% fukinolic acid and 0.69% cimicifugic acid A. Yu et al. (2019) optimized the extraction protocol for *A. dahurica* in comparison to the protocol proposed by the European Pharmacopoeia and yielded appr. 0.01% cimicifugic acid D, 0.03% cimicifugic acid B, 0.07% cimicifugic acid E and 0.10%

cimicifugic acid F related to dry powdered plant material; the yields were increased by 31–57% compared to the standard extraction protocol. The drying process also has a strong impact on the contents of extractable phenolic acids. As determined by Qin et al. (2017) drying at higher temperatures (90–110 °C) resulted in higher yields. Amounts isolated from *A. dahurica* (dried at 110 °C) ranged at 0.03%, 0.11%, 0.04%, 0.08% and 0.07% of the dry material for cimicifugic acids A, B, D, E and F, respectively. The extraction method as well as storage influenced the amounts of fukinolic acid and cimicifugic acids A and B. The primary extract contained 2.60%, 0.86% and 1.47% of these compounds which turned out to be stable for 9 weeks at room temperature (Jiang et al. 2008a).

Table 2 Occurrence of fukinolic acid and cimicifugic acids in *Actaea* (*Cimicifuga*) species, *Petasites japonicus*, *Platycodon grandiflorum* and *Prunus persica*; FA fukinolic acid, CA cimicifugic acid

Species	CA															References
	F	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
	A	K	K	K	P	P	P	K		K	P	P	P			
	K	F	I	P	K	F	I	D		S	S	P	P	D		Gödecke et al. (2009)
<i>A. racemosa</i>																Kruse et al. (1999)
																Löser et al. (2000)
																Li et al. (2003)
																Fabricant et al. (2005)
																Stromeier et al. (2005)
																Nuntanakorn et al. (2006)
																Nuntanakorn et al. (2007)
																Gödecke et al. (2009)
<i>A. simplex</i>		m	m			m	m									Fabricant et al. (2005)
																Takahira et al. (1998b)
																Takahira et al. (1998a)
<i>A. japonica</i>																Kusano (2001)
																Iwanaga et al. (2010b)
<i>A. rubra</i>																Kusano (2001)
<i>A. pachypoda</i>																Iwanaga et al. (2010b)
<i>A. podocarpa</i>																Nuntanakorn et al. (2007)
<i>A. dahurica</i> + <i>A. heracleifolia</i>																Nuntanakorn et al. (2007)
<i>A. heracleifolia</i>																Iwanaga et al. (2010a)
<i>A. foetida</i>																Yim et al. (2012a)
<i>A. acerina</i>																Kusano (2001)
<i>A. dahurica</i>																
<i>Petasites japonicus</i>																Yu et al. (2019)
																Qin et al. (2017)
<i>Platycodon grandiflorum</i>																Sakamura et al. (1969, 1973)
<i>Prunus persica</i>																Lee et al. (2019)
																Wang et al. (2017a)
																Kwak et al. (2018)

m the respective cimicifugic acids were isolated as 1-methylester

The different relative amounts of single fukinolic acid and cimicifugic acids has often been used to discriminate between different *Actaea* species. Geng et al. (2019) investigated quality control of roots/rhizomes material to distinguish between five *Actaea* species. The analysis was established by the comparison of HCAA (hydroxycinnamic acid amides) and HCAE (hydroxycinnamic acid esters) on the basis of UV and MRM (mass spectrometry with multiple reaction monitoring) profiles. *A. dahurica* was characterized by high amounts of isoferulic acid, cimicifugic acid B and cimicifugic acid F. *A. foetida* also exhibited high amounts of cimicifugic acid B and F, whereas the amount of isoferulic acid, cimicifugic acid A, cimicifugic acid E and fukinolic acid was less intense. The spectrum of *A. pachypoda* showed strong

peaks of fukinolic acid and cimicifugic acid A. Additionally, *A. pachypoda* is characterized by the clear appearance of cimicifugic acid E and ferulic acid. In contrast *A. podocarpa* exhibited high amounts of cimicifugic acid A followed by cimicifugic acid E. *A. racemosa* showed high amounts of fukinolic acid, cimicifugic acid A and B. In comparison, the intensity of cimicifugic acid E and F, isoferulic acid and ferulic acid was low. In conclusion, the differentiation of *Actaea* species has been more specific by mass spectrometry (HCAA-analysis) than by UV (HCAE-analysis).

He et al. (2006) postulated to use the difference of cimicifugic acid levels to identify roots/rhizomes of ten *Actaea* species. The analysis by HPLC and the different absorption maxima are hints for the

identification of *Actaea* species. All three investigated acids, namely cimicifugic acid A (326–329 nm), B (326–329 nm) and fukinolic acid (330 nm) were present in *A. racemosa*, *A. dahurica* and *A. japonica*. In contrast, *A. binternat*, *A. foetida* and *A. heracleifolia* contained none of the hydroxycinnamic acid esters. A combination of the presence of cimicifugic acid A and fukinolic acid was detected in *A. rubifolia*, *A. acerina* and *A. simplex*. Strikingly, *A. americana* showed none of these compounds.

Further investigations on the characterization of caffeic acid derivatives by LC/MS/MS were performed by Li et al. (2003). A gradient of water with 0.05% acetic acid and acetonitrile with 0.05% acetic acid has been used with different conditions at a constant flow rate of 1.6 ml/min. The retention times of cimicifugic acid A and B were 21.9 min and 22.7 min. The detection by MS/MS showed a peak with m/z 447 (cimicifugic acid A/B) and m/z 253 (loss of (iso-)ferulic acid). Fukinolic acid eluted at 16.7 min and was characterized by a mass of m/z 433. The identification of fukinolic acid and cimicifugic acids A/B was done using standards, whereas cimicifugic acids E and F were characterized by the comparison of fragmentation patterns.

Bittner et al. (2016) tested 208 samples of roots and rhizomes of *Actaea* species, most of them belonging to *A. racemosa* (157 samples). Further 46 samples came from other *Actaea* species, namely *A. cimicifuga* (13), *A. cordifolia* (10), *A. simplex* (11), *A. dahurica* (3), *A. heracleifolia* (3), unknown *Actaea* species (6) and additionally five common black cohosh samples. Fukinolic acid and five cimicifugic acids A–F were analyzed by RP-PDA-HPLC. The dry and ground plant material (400 mg) was repetitively extracted in an ultrasonic bath with 5 ml 70% methanol. After three extraction steps > 99% of the phenolic acids were extracted and this was considered a quantitative extraction. The relative amounts of various fukinolic and cimicifugic acids (determined as caffeic acid equivalents) were used to identify different *Actaea* species and discriminate between them. Table 3 shows that the relative amounts differ from species to species and might be useful to identify various *Actaea* species and adulterations of commercial drugs.

The extraction of hydroxycinnamic acid esters from *A. racemosa* roots and rhizomes has further been investigated by Burdette et al. (2002). A two-step extraction protocol was performed, resulting in a

methanolic extract (A, 1250 g) and a methanol/H₂O (6:4) extract (B, 112 g). The cimicifugic acid containing extract (B) was further purified by successive HPLC steps. Calculated from the methanol extract, the yields were 0.001% (12.5 mg), 0.0008% (10 mg), 0.005% (62.5 mg) and 0.0008% (10 mg) for fukinolic acid, cimicifugic acid A, B and F.

Callus cultures of *Actaea racemosa* have been established for in vitro plant regeneration (Lata et al. 2002). Callus induction was best using MS medium (Murashige and Skoog 1962) supplemented with 1.0 mM naphthalene acetic acid (NAA) and 0.5 mM thidiazuron (TDZ). Changing the hormone composition to 2.0 or 4.0 μ M TDZ led to shoot regeneration and the shoots could be rooted on media containing different auxins at 2.5 and 5.0 μ M. Addition of activated charcoal was essential for successful rooting. The regenerated plant material, however, was not analyzed for hydroxycinnamic acid esters.

Biological activities of fukinolic acid and cimicifugic acids

Cimicifugic acids and fukinolic acid are generally regarded as minor compounds with respect to the therapeutic use of the respective plants (*Actaea* and *Petasites* species) and their extracts. Mostly, the bioactivity assays aim at evaluating the effects of triterpenoids for *Actaea* extracts or sesquiterpenes for *Petasites* extracts. For the genus *Cimicifuga/Actaea* this has been recently reviewed by Guo et al. (2017) and Salari et al. (2021). A review concerning the use of *Petasites* species as medicinal plants has been published by Aydin et al. (2013). Thus, although there is a variety of effective specialized compounds present in *Actaea* (*Cimicifuga*) and *Petasites* species, the aim of this review is to focus on the hydroxycinnamic acid esters. The species named in the following are named according to the respective literature, explaining the mixed usage of the genus names *Actaea* and *Cimicifuga*. The biological activities of fukinolic acid and cimicifugic acids are described in more detail in the following and are summarized in Table 4.

Anti-inflammatory activities

Neutrophil elastase (EC 3.4.21.37) is an important enzyme participating in inflammatory reactions. It is

Table 3 Relative mean amounts of different fukinolic and cimicifugic acids in six different *Actaea* species

Species						
<i>Actaea racemosa</i>	FA	CA B	CA A	CA E	CA F	CA D
<i>Actaea cimicifuga</i>	CA A	FA	CA E	CA D	CA B	
<i>Actaea cordifolia</i>	FA	CA A	CA D	CA E	CA B	CA F
<i>Actaea dahurica</i>	CA F	CA B	CA D	CA E	FA	CA A
<i>Actaea heracleifolia</i>	CA F	CA B	CA E	CA D	CA A	FA
<i>Actaea simplex</i>	FA	CA D	CA A	CA E	CA B	CA F

FA fukinolic acid, CA cimicifugic acid (data from Bittner et al. 2016)

active in the destruction of the basement membrane and usually shows elevated plasma levels during active inflammation (Löser et al. 2000). Fukinolic acid and cimicifugic acids have been isolated from *C. racemosa* rhizomes. Löser et al. (2000) described the dose-dependent inactivation of a human leukocyte elastase by fukinolic acid, consequently possessing anti-inflammatory effects. Furthermore, the esterification seemed to make fukinolic acid ($IC_{50} = 0.23 \mu M$) a stronger inhibitor of the active site of neutrophil elastase than caffeic acid ($IC_{50} = 93 \mu M$; Melzig et al. 1999). The authors suggested that this might be due to the better interaction of the ester with the more lipophilic active site of the enzyme. Additionally, cimicifugic acids A ($IC_{50} = 2.2 \mu M$), B ($IC_{50} = 11.4 \mu M$), E ($IC_{50} > 50 \mu M$) and F ($IC_{50} = 18 \mu M$) successfully interacted with this enzyme, although cimicifugic acid E turned out to be a weak inhibitor (Löser et al. 2000). Even weaker was the selective peptidic elastase inhibitor elastatinal with an IC_{50} of $1150 \mu M$ which was used as positive control. Docking simulation was used to determine the possible binding site of fukinolic acid to human neutrophil elastase, but the site could not be determined exactly (Steinbrecher et al. 2006). Previously, elastase inhibition combined with docking studies revealed a caffeic acid moiety coupled to a lipophilic residue as prerequisite for good binding to human leukocyte elastase (Siedle et al. 2003).

Wang et al. (2017b) established a system using mice with *Pseudomonas aeruginosa*-caused pneumonia to evaluate anti-inflammatory effects of components of Cimicifugae rhizoma extracts. The whole extract exerted a dose-dependent anti-inflammatory effect. TNF- α -induced activation of NF- κ B could be reduced by more than 50% (compared to appr. 80% for dexacortol as positive control) by components of the

extract, namely caffeic acid, ferulic acid/isoferulic acid, fukinolic acid and cimicifugic acids C/D, A/B and E.

Cimicifugic acid D was identified as another anti-inflammatory ingredient in *P. japonicus* (Lee et al. 2019). Lipopolysaccharide (LPS)-stimulated macrophages were analyzed for their production of the inflammatory mediators NO and prostaglandin E_2 (PGE_2). Additionally, iNOS (inducible nitric oxide synthase) and cyclooxygenase-2 (COX-2; prostaglandin synthase) were investigated on the basis of their expression levels. Cimicifugic acid D showed anti-inflammatory effects inhibiting the synthesis of nitric oxide (NO; $IC_{50} = 12 \mu M$) and PGE_2 ($IC_{50} = 43 \mu M$) in LPS-induced macrophages, whereas caffeic acid and fukinolic acid were inactive ($IC_{50} > 50 \mu M$) in this assay (caffeic acid: $IC_{50} > 50 \mu M$ for NO, $IC_{50} = 45.7 \mu M$ for PGE_2). Molecular docking analyses revealed a strong affinity of cimicifugic acid D for iNOS and COX-2, explaining the inhibition of NO and PGE_2 synthesis. The expression levels for both proteins, however, were not increased.

Antioxidant activities

During metabolic processes, a series of reactive oxygen species (ROS) is formed in vivo having toxic properties on various metabolic processes including DNA integrity. In cells, a variety of counteracting mechanisms are present including antioxidant compounds, repair and exchange mechanisms. The bioactivity of several hydroxycinnamic acid esters isolated from *A. racemosa* rhizomes/roots has been elucidated. Fukinolic acid and cimicifugic acid A, B and F were found to act as antioxidants in a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical assay (Burdette et al. 2002). In conclusion, these antioxidant agents

Table 4 Summary of biological activities of fukinolic acid and cimicifugic acids with effective concentrations and information on the applied test system

Compound	Biological effect	Further information	Inhibitory concentration	References
Fukinolic acid	Anti-inflammatory	Inhibition of neutrophil elastase (human leukocyte elastase)	IC ₅₀ = 0.23 µM	Löser et al. (2000)
		Not active (IC ₅₀ > 50 µM) towards NO-formation	IC ₅₀ > 50 µM	Lee et al. (2019)
		Not active (IC ₅₀ > 50 µM) towards synthesis of PGE ₂	IC ₅₀ > 50 µM	
		Suppression of TNF-α-induced NF-κB activation	No value given	Wang et al. (2017b)
	Antioxidant	Inhibition of lipopolysaccharide-stimulated NO production	IC ₅₀ = 0.30 mM	Watanabe et al. (2007)
		Reduction of intracellular iNOS (inducible NO synthase)	IC ₅₀ = 0.3–0.6 mM	
		Reduction of iNOS mRNA level	No value given	
		NO radical scavenging activity	IC ₅₀ ≥ 20 µM	
		O ₂ ^{•−} scavenging activity	IC ₅₀ = 0.83 µM	
		OH [•] radical scavenging activity	IC ₅₀ = 1.01 mM	
		DPPH radical scavenging activity	IC ₅₀ = 20.2 µM	
			IC ₅₀ = 23 µM	Nuntanakorn et al. (2006)
			IC ₅₀ = 12.9 µM	Nuntanakorn et al. (2007)
			IC ₅₀ = 14.1 µM	Burdette et al. (2002)
		20% inhibition of menadione-induced DNA strand breakage (MDA-MB-231 breast cancer cell line)	At 0.46 µM	
	Antiviral	Antiviral activities (human enterovirus EV-A71)	IC ₅₀ = 9.9 nM	Ma et al. (2019)
	Cytotoxicity and anti-tumor	No cytotoxicity at 115.1 nM (human virus-infected rhabdomyosarcoma cell line)	115.1 nM	
		No detection of cytotoxic effects in S30 cells	At 0.46 µM	Burdette et al. (2002)
		Anti-cancer efficacy on MDA-MB-453 breast cancer cells	IC ₅₀ = 0.21 µM	Jiang et al. 2008b
	Inhibition of Cyt. P450	inhibition of CYP 1A2, 2D6, 2C9, 3A4	IC ₅₀ = 1.8–7.1 µM*	Huang et al. (2010)
	Estrogenic activity	126.3% increase of proliferation in estrogen-dependent MCF-7 cell line	At 50 nM	Kruse et al. (1999)
	Hyaluronidase	Strong (< 200 µM) inhibition of hyaluronidase	IC ₅₀ = 144 µM	Iwanaga et al. (2010a, b)
	Vasoactivity	Vasorelaxation on norepinephrine-treated rat aortic strips	At 300 µM	Noguchi et al. (1998)
	Promotion of wound-healing	51% inhibition of collagenase	With 100 µg (appr. 0.23 µM**)	Kusano et al. (2001)
	seed germination	inhibition of α-amylase	IC ₅₀ = 40 µM	Kusano et al. (1998)
		Inhibition of carboxypeptidase A	IC ₅₀ = 173 µM	

Table 4 continued

Compound	Biological effect	Further information	Inhibitory concentration	References
Cimicifugic acid A	Miscellaneous effects	suppression of type I allergic reactions	IC ₅₀ = 4.8 µM	Shimoda et al. (2006)
	Anti-inflammatory	Inhibition of neutrophil elastase (human leukocyte elastase)	IC ₅₀ = 2.2 µM	Löser et al. (2000)
		Suppression of TNF-α-induced NF-κB activation	No value given	Wang et al. (2017b)
	Antioxidant	DPPH radical scavenging activity	IC ₅₀ = 27.7 µM	Burdette et al. (2002)
			IC ₅₀ = 12 µM	Nuntanakorn et al. (2006)
			IC ₅₀ = 21.9 µM	Nuntanakorn et al. (2007)
	Antiviral	Antiviral activities (human enterovirus EV-A71)	IC ₅₀ = 47.5 nM	Ma et al. (2019)
		inhibition of EV-A71 replication	IC ₅₀ = 223.0 nM	
	Cytotoxicity and anti-tumor	Influence of critical apoptosis marker PARP	IC ₅₀ = 15.38 µM	Yim et al. (2012b)
		No detection of cytotoxic effects in S30 cells	At 0.45 µM	Burdette et al. (2002)
		Anti-cancer efficacy on MDA-MB-453 breast cancer cells	IC ₅₀ = 0.17 µM	Jiang et al. 2008b
		cleavage of apoptosis marker PARP	At 20 µM	Yim et al. (2012b)
	Inhibition of Cyt. P450	Inhibition of CYP 1A2, 2D6, 2C9, 3A4	IC ₅₀ = 7.2–9.7 µM*	Huang et al. (2010)
	Hyaluronidase	Strong (< 200 µM) inhibition of hyaluronidase	IC ₅₀ = 112 µM	Iwanaga et al. (2010a, b)
	Promotion of wound-healing	47% inhibition of collagenase	With 100 µg (appr. 0.23 µM**)	Kusano et al. (2001)
Cimicifugic acid B	Seed germination	Inhibition of α-amylase	IC ₅₀ = 24.1 µM	Kusano et al. (1998)
		Inhibition of carboxypeptidase A	IC ₅₀ = 198 µM	
	Miscellaneous effects	Influence on serotonin 5HT ₇ receptor	IC ₅₀ = 120 µM	Farnsworth et al. (2008)
	Anti-inflammatory	Inhibition of neutrophil elastase (human leukocyte elastase)	IC ₅₀ = 11.4 µM	Löser et al. (2000)
		Suppression of TNF-α-induced NF-κB activation	No value given	Wang et al. (2017b)
	Antioxidant	DPPH radical scavenging activity	IC ₅₀ = 22.9 µM	Burdette et al. (2002)
			IC ₅₀ = 21 µM	Nuntanakorn et al. (2006)
			IC ₅₀ = 23.1 µM	Nuntanakorn et al. (2007)
	Antiviral	ATP-docking results against dengue virus components	–143.5 kJ/mol	Powers and Setzer (2016)
		RNA-docking results against dengue virus components	–117.8 kJ/mol	
	Cytotoxicity and anti-tumor	Influence of critical apoptosis marker PARP	IC ₅₀ = 12.42 µM	Yim et al. (2012b)
		No detection of cytotoxic effects in S30 cells	At 0.45 µM	Burdette et al. (2002)
		Anti-cancer efficacy on MDA-MB-453 breast cancer cells	IC ₅₀ = 0.37 µM	Jiang et al. 2008b

Table 4 continued

Compound	Biological effect	Further information	Inhibitory concentration	References
Cimicifugic acid C		Cleavage of apoptosis marker PARP	20 μ M	Yim et al. (2012b)
	Inhibition of Cyt. P450	Inhibition of CYP 1A2, 2D6, 2C9, 3A4	IC ₅₀ = 7.35–12.6 μ M*	Huang et al. (2010)
	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 82 μ M	Iwanaga et al. (2010a, b)
	Promotion of wound-healing	64% inhibition of collagenase	With 100 μ g (appr. 0.23 μ M**)	Kusano et al. (2001)
	Miscellaneous effects	Influence on serotonin 5HT ₇ receptor	IC ₅₀ = 2.67 μ M	Farnsworth et al. (2008)
	Anti-inflammatory	Suppression of TNF- α -induced NF- κ B activation	No value given	Wang et al. (2017b)
	Hyaluronidase	Inhibition of hyaluronidase	IC ₅₀ = 251 μ M	Iwanaga et al. (2010a, b)
	Vasoactivity	Vasocontraction on norepinephrine-treated rat aortic strips	At 300 μ M	Noguchi et al. (1998)
	Wound-healing promotion	47% inhibition of collagenase	With 100 μ g (appr. 0.23 μ M**)	Kusano et al. (2001)
	Anti-inflammatory	Inhibition of NO-formation	IC ₅₀ = 12 μ M	Lee et al. (2019)
Cimicifugic acid D		Inhibition of PGE ₂ -synthesis	IC ₅₀ = 43 μ M	
		Influence on NO-formation and PGE ₂ production is explained by a strong affinity of cimicifugic acid D towards iNOS and COX-2 in docking studies	–log (FBE) = –10 kcal/mol (iNOS)	
			–log (FBE) = –8.3 kcal/mol (COX2)	
		Suppression of TNF- α -induced NF- κ B activation	No value given	Wang et al. (2017b)
	Hyaluronidase	strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 153 μ M	Iwanaga et al. (2010a, b)
	Vasoactivity	Vasorelaxation on norepinephrine-treated rat aortic strips	At 300 μ M	Noguchi et al. (1998)
	Promotion of wound-healing	20% inhibition of collagenase	With 100 μ g (appr. 0.23 μ M**)	Kusano et al. (2001)
	Anti-inflammatory	Inhibition of neutrophil elastase (human leukocyte elastase)	IC ₅₀ > 50 μ M	Löser et al. (2000)
		Suppression of TNF- α -induced NF- κ B activation	No value given	Wang et al. (2017b)
	Antioxidant	DPPH radical scavenging activity	IC ₅₀ = 65.5 μ M	Nuntanakorn et al. (2007)
Cimicifugic acid E	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 120 μ M	Iwanaga et al. (2010a, b)
	Promotion of wound-healing	26% inhibition of collagenase	With 100 μ g (appr. 0.23 μ M**)	Kusano et al. (2001)
	Anti-inflammatory	Inhibition of neutrophil elastase (human leukocyte elastase)	IC ₅₀ = 18 μ M	Löser et al. (2000)
	Antioxidant	DPPH radical scavenging activity	IC ₅₀ = 20.5 μ M	Burdette et al. (2002)

Table 4 continued

Compound	Biological effect	Further information	Inhibitory concentration	References
Cimicifugic acid G	Cytotoxicity	No detection of cytotoxic effects in S30 cells	IC ₅₀ = 151.5 μ M At 0.46 μ M	Nuntanakorn et al. (2007) Burdette et al. (2002)
	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 92 μ M	Iwanaga et al. (2010a, b)
	Promotion of wound-healing	37% inhibition of collagenase	With 100 μ g (appr. 0.23 μ M**)	Kusano et al. (2001)
	Antioxidant	DPPH radical scavenging activity	IC ₅₀ = 37 μ M	Nuntanakorn et al. (2006)
	Antiviral	ATP-docking results against dengue virus components	−141.7 kJ/mol	Powers and Setzer (2016)
		RNA-docking results against dengue virus components	−122.6 kJ/mol	
	Cytotoxicity	32% decrease of MCF-7 cell proliferation	At 30 μ M	Nuntanakorn et al. (2006)
	Estrogenic activity	1.14-fold increase of MCF-7 cell proliferation	At 10 μ M	
Cimicifugic acid H	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 138 μ M	Iwanaga et al. (2010a, b)
		Inhibition of hyaluronidase	IC ₅₀ = 525 μ M	
Cimicifugic acid I	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 143 μ M	Iwanaga et al. (2010a, b)
Cimicifugic acid J	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 193 μ M	Iwanaga et al. (2010a, b)
		Antiviral activities (human enterovirus EV-A71)	IC ₅₀ = 59.9 nM	
Cimicifugic acid K	Cytotoxicity	Inhibition of EV-A71 replication	IC ₅₀ = 151.4 nM	Iwanaga et al. (2010a, b)
	Hyaluronidase	Inhibition of hyaluronidase	IC ₅₀ = 255 μ M	
Cimicifugic acid L	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 102 μ M	Iwanaga et al. (2010a, b)
Cimicifugic acid M	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 173 μ M	Iwanaga et al. (2010a, b)
Cimicifugic acid N	Hyaluronidase	Strong (< 200 μ M) inhibition of hyaluronidase	IC ₅₀ = 120 μ M	Iwanaga et al. (2010a, b)

*The range of IC₅₀ value for different CYP isoforms; **Data as given in the reference; FBE = free binding energy

scavenge ROS, the inhibitory concentrations detected here were IC₅₀ = 14.1 μ M for fukinolic acid and IC₅₀ = 27.7 μ M (A), IC₅₀ = 22.9 μ M (B) and IC₅₀ = 20.5 μ M (F) for cimicifugic acids. In addition, fukinolic acid at 200 μ g/ml (0.46 μ M) inhibited menadione-induced DNA strand breakage in a breast cancer cell line (MDA-MB-231) by approximately 20% while caffeic acid was considerably more effective (appr. 70% inhibition at 100 μ g/ml = 0.56 μ M).

At the same concentration (200 μ g/ml = 0.45–0.46 μ M) none of the above-mentioned compounds showed signs of cytotoxicity.

A further DPPH free-radical assay elucidated the antioxidant property of cimicifugic acid G along with cimicifugic acids A, B and fukinolic acid (Nuntanakorn et al. 2006). Fukinolic acid and cimicifugic acids from rhizomes and roots of *C. racemosa* possessed the ability to scavenge radicals. The IC₅₀

values were at 12 μM (cimicifugic acid A), 21 μM (cimicifugic acid B), 37 μM (cimicifugic acid G) and 23 μM (fukinolic acid) compared to gallic acid ($\text{IC}_{50} = 29.9 \mu\text{M}$) which was tested as positive control. The same group tested further compounds from *Actaea* species in DPPH free-radical assays and found IC_{50} values of 12.9 μM (fukinolic acid), 21.9 μM (cimicifugic acid A), 23.1 μM (B), 65.5 μM (E) and 151.5 μM (F) compared to ascorbic acid ($\text{IC}_{50} = 105.5 \mu\text{M}$) as positive control. Piscidic acid derivatives (with only one OH-group at the aromatic ring of the benzytartaric acid derivative) thus turned out to be less active (Nuntanakorn et al. 2007).

Watanabe et al. (2007) tested the scavenging activity of fukinolic acid (FA; isolated from *P. japonicus*) for superoxide anions (O_2^-), hydroxyl radicals ($\text{OH}^\bullet$), NO radicals and DPPH radicals in comparison to chlorogenic acid (CGA), gallic acid (GA) and epigallocatechin gallate (EGCG). In all assays, FA proved to be active, but with varying relation to the other natural products. O_2^- scavenging followed the order $\text{ECGC} > \text{GA} > \text{FA} > \text{CGA}$, while $\text{OH}^\bullet$ scavenging was best by ECGC followed by $\text{CGA} > \text{FA} > \text{GA}$. NO and DPPH scavenging were in the sequence $\text{ECGC} = \text{GA} > \text{FA} > \text{CGA}$ and $\text{ECGC} > \text{GA} > \text{FA} > \text{CGA}$, respectively. IC_{50} values were determined for fukinolic acid at 0.83 μM (for O_2^-), 1.01 mM ($\text{OH}^\bullet$), $> 20 \mu\text{M}$ (NO radicals) and 20.2 μM (DPPH radicals). Furthermore, the lipopolysaccharide-stimulated NO production was measured in an immortalized mouse cell line. Here FA ($\text{IC}_{50} = 0.30 \text{ mM}$) showed a slightly better activity than CGA ($\text{IC}_{50} = 0.55 \text{ mM}$). The concentration of iNOS was inhibited by FA ($\text{IC}_{50} = 0.3\text{--}0.6 \text{ mM}$) while CGA was not active in this concentration range. The levels of iNOS mRNA were also more strongly reduced by FA than by CGA.

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay was used to evaluate the activity of fukinolic acid in extracts from leaves and roots of *P. japonicus* in comparison to Trolox; the leaf extract showed slightly higher activity (Kim et al. 2012).

A flower bud extract of *P. japonicus* was tested for its antioxidant compounds by Hiemori-Kondo and Nii (2020). The most active compound in the DPPH-radical scavenging assay was fukinolic acid (FA) followed by different caffeoylquinic acid derivatives which showed one third of the activity of FA or less.

Estrogenic activities

The estrogenic activity of fukinolic acid (at a dose range of 5×10^{-7} to $5 \times 10^{-9} \text{ M}$) has been tested with MCF-7 breast cancer cells (Kruse et al. 1999). Fukinolic acid had been isolated from *C. racemosa* rhizomes before. It gave rise to an increase in cell proliferation of 126.3% at a concentration of 50 nM in comparison to 120% for estrogen (0.1 nM). Also, cimicifugic acid G isolated from *A. racemosa* has been classified as estrogen-effective substance by stimulating the cell proliferation of MCF-7 cells (Nuntanakorn et al. 2006). In comparison to untreated cells, cimicifugic acid G induced a 1.14-fold stimulation of cell proliferation at 10 μM . Enterolactone and estradiol were used as positive controls and showed cell growth stimulation of 1.84-fold at 16.8 μM and 3.6-fold at 3.7 nM, respectively. No estrogenic activity was, however, found by Stromeier et al. (2005) and Farnsworth et al. (2008).

Antiviral effects

An extract of rhizomes of *C. heracleifolia* was analyzed for its antiviral activity against human enterovirus EV-A71 and the total extract as well as isolated fractions containing fukinolic acid ($\text{IC}_{50} = 4.3 \mu\text{g/ml} = 9.9 \text{ nM}$), cimicifugic acid A ($\text{IC}_{50} = 21.3 \mu\text{g/ml} = 47.5 \text{ nM}$) and J ($\text{IC}_{50} = 27.7 \mu\text{g/ml} = 59.9 \text{ nM}$) were identified as the active compounds inhibiting virus replication (Ma et al. 2019). The cytotoxicity of these compounds was at 115.1 nM for fukinolic acid and 223.0 nM and 151.4 nM for cimicifugic acids A and J, respectively. The EV-A71 virus and other related enteroviruses cause hand-foot-and-mouth disease and in severe cases neurological disorders. Since there is currently no efficient drug and no reliable treatment of EV-A71, cimicifugic acids might be a promising therapy (Ma et al. 2019). Cimicifugic acids B and I turned out to be inactive. The authors deduced structure–activity relationships from their results. Thus, a caffeic acid substitution in the hydroxycinnamic acid part (as in fukinolic acid) is favorable and methylation of one of the OH-groups reduced the antiviral activity. Additionally, assays testing the activity of fukinolic acid against the viral 3C peptidase showed no activity and thus virus replication is inhibited by a different mechanism.

Computational docking studies were performed with several proteins from Dengue virus. Here, cimicifugic acids B and G showed very good docking results towards the ATP-binding site of the viral helicase (Powers and Setzer 2016). The docking energy displayed -143.5 kJ/mol for cimicifugic acid B and -141.7 kJ/mol for cimicifugic acid G. In comparison, the affinity towards the RNA site was lower with -117.8 kJ/mol (cimicifugic acid B) and -122.6 kJ/mol (cimicifugic acid G).

Cytotoxicity and anti-cancer activities

Although a number of investigations has been made to clarify the cytotoxic potential of fukinolic acid and cimicifugic acids, the results are not homogenous. Hydroxycinnamic acid esters investigated by Burdette et al. (2002) did not show any cytotoxic effects at 200 µg/ml (= 0.45–0.46 µM) in human breast cancer S30 cells. Fukinolic/cimicifugic acids analyzed by Huang et al. (2010) did not damage liver carcinoma Hep-G2 cells in concentrations up to 50 µg/ml (~ 0.12 µM). However, a concentration of 100 µg/ml (~ 0.23 µM) resulted in cell mortality of 89–100%.

Nuntanakorn et al. (2006) did not detect a cytotoxic effect of cimicifugic acid G at 10 µM in MCF-7 breast cancer cells, in contrast, cell proliferation was stimulated, but concentrations of 30 µM caused cytotoxicity of 32%. Furthermore, Ma et al. (2019) reported cimicifugic acids and fukinolic acid to possess cytotoxic activity at concentrations between 115.1 and 223.0 nM.

A different breast cancer cell line (MDA-MB-453) was used to evaluate the anti-cancer efficiencies of fukinolic acid and cimicifugic acids A and B with IC₅₀ values of 90 µg/ml (0.21 µM), 74 µg/ml (0.17 µM) and 165 µg/ml (0.37 µM), respectively (Jiang et al. 2008b).

Yim et al. (2012b) investigated the cytotoxic activities of the butanol extract of *C. heracleifolia* as well as of isolated cimicifugic acids A, B, E and F in a human colon cancer cell line (HCT116). Furthermore, the hydroxycinnamic acid esters were analyzed for their influence on the expression of a critical apoptosis marker PARP (poly(ADP ribose) polymerase), an enzyme involved in the repair of DNA single-strand breaks. Cimicifugic acid A (IC₅₀ = 15.38 µM) and B (IC₅₀ = 12.42 µM) showed a dose-dependent

(2.5–40 µM) anti-proliferative effect on HCT116 cells, whereas cimicifugic acids E and F and piscidic acid did not show cytotoxic effects. In Western blot analysis, cimicifugic acids A and B induced a visible increase of cleaved PARP at 20 µM and 40 µM. The authors related the investigated activities to the fukiic acid part of the molecule, since piscidic acid derivatives were inactive.

Inhibition of collagenases

Collagens are ubiquitous in animals. They represent a family of fibrous structural proteins in the extracellular matrix of tendons, ligaments, bones, cartilage and connective tissues (Shoulders and Raines 2009). Collagenases or matrix metalloproteinases (MMP) are involved in wound healing and high levels of MMPs are related to delayed healing (Tardáguila-García et al. 2019). Thus, inhibition of collagenases/MMPs would be beneficial to promote wound healing.

Kusano et al. (2001) reported an effect of cimicifugic acids A–F and fukinolic acid on collagenolytic enzymes. All tested acids inhibited collagenases, but the effect of fukinolic acid and cimicifugic acids A–C (47–64% collagenase inhibition at 100 µg per assay corresponding to 0.22–0.24 µM as given by the authors) was significantly stronger than the effect of cimicifugic acids D–F (20–37%) compared to an inhibition of 51% by rosmarinic acid (at 0.28 µM). This inhibition was dose-dependent. The collagenolytic activity of trypsin was inhibited as well. Consequently, the presence of a second hydroxyl group on the aromatic ring as in fukiic acid (the alcoholic part of cimicifugic acids A–C and fukinolic acid) enhances the collagenolytic activity. In conclusion, extracts from *Cimicifuga* rhizomes might be used for therapeutic aspects due to the prevention of collagen degradation in inflammatory processes.

Inhibition of cytochrome P450 enzymes and hepatotoxicity

Cytochrome P450 enzymes (CYP) are ubiquitous in living organisms, participating in detoxification, drug metabolism as well as biosynthetic reactions (Nair et al. 2016). In order to investigate the observed hepatotoxic activity in women treated with extracts of *C. racemosa*, the effect of the triterpenes and the hydroxycinnamic acid esters were differentiated

Table 5 IC₅₀ values for hyaluronidase inhibition of cimicifugic acid-related compounds (fukinolic acid = FA) from *Actaea* spec. against rosmarinic acid (RA) as control (values taken from Iwanaga et al. 2010a, b)

	RA	FA	Cimicifugic acid														Shomaside		
Com- pound			A	B	C	D	E	F	G	H	I	J	K	L	M	N	A	B	C
IC ₅₀ [μM]	545	144	112	82	251	153	120	92	138	525	143	193	255	102	173	120	573	430	663

Compounds showing better hyaluronidase inhibition than RA are shaded grey

(Huang et al. 2010). Fukinolic acid and cimicifugic acids A and B were examined for their inhibitory effect towards the CYP enzymes 1A2, 2D6, 2C9 and 3A4 and displayed IC₅₀ values of 1.8–7.2 μM (fukinolic acid), 7.2–9.7 μM (cimicifugic acid A) and 7.35–12.6 μM (cimicifugic acid B), respectively. Selective inhibitors of the four tested CYPs showed IC₅₀ values in the sub-micromolar range. CYP-inhibition by the triterpene glycosides, on the other hand, was only weak (IC₅₀ = 25–100 μM). At the same time, the growth of Hep-G2 cells (a human liver cancer cell line) was not inhibited by *C. racemosa* extracts up to concentrations of 50 μg/ml but strong cytotoxicity was shown at concentrations exceeding 100 μg/ml (89–100%).

Hyaluronidase inhibition

Hyaluronidases are used medicinally to degrade hyaluronic acid which is a component of the complex polymer of the extracellular matrix resulting in an increased diffusion and resorption (Buhren et al. 2016). Fukinolic acid, cimicifugic acids A–N as well as shomasides were tested for their hyaluronidase inhibitory activities against rosmarinic acid (RA) as control (Iwanaga et al. 2010a, b). Many of them showed lower IC₅₀ values than RA for hyaluronidase inhibition (Table 5).

Vasoactive effects

Fukinolic acid and cimicifugic acids A–E were tested for vasoactivity with rat aortic strips pre-contracted with norepinephrine (Noguchi et al. 1998). Interestingly, cimicifugic acid C (0.3 mM) induced minimal contraction, whereas fukinolic acid and cimicifugic acid D at the same concentration acted as a relaxant. The contrasting effects might be explained by the

differing substitution patterns in the hydroxycinnamic acid part, namely the caffeoyl group in the relaxing fukinolic acid and cimicifugic acid D and the 4-coumaroyl group in the contractive cimicifugic acid C. Cimicifugic acids A, B and E showed no vasoactive effects. Further experiments suggested that the relaxing effect could be explained by a decreased influx of extracellular Ca²⁺ through receptor-operated Ca-channels.

Miscellaneous effects

On the way to explain the effect of black cohosh extracts against hot flushes in menopausal women, an inhibitory effect of cimicifugic acids A and B on the serotonin 5HT₇ receptor was observed with IC₅₀ values of 120 μM and 2.67 μM (Farnsworth et al. 2008).

Shimoda et al. (2006) investigated the effect of an extract from aerial parts of *P. japonicus* on mast cell degranulation and found that fukinolic acid was most potent with an IC₅₀ value of 4.8 μM. The compound could therefore be useful to suppress type I allergic reactions for example during hay fever.

Influence on seed germination

Besides a number of other factors, the germination of seeds is dependent on α-amylase, an enzyme hydrolyzing the storage carbohydrate starch into metabolizable sugars enabling the growth of the seeds (Damaris et al. 2019). In a study on seed germination, the influence of fukinolic acid and cimicifugic acid A on seeds of two monocotyledonous and two dicotyledonous plants have been observed (Kusano et al. 1998). The grade of inhibition of germination and seedling growth (determined as root length) varied from plant species to plant species. Root length was

reduced to an average of 35% by fukinolic acid and cimicifugic acid A and 18% by rosmarinic acid at a concentration of 0.3 mM. Both compounds presumably inhibited seed germination and root growth due to the interaction with α -amylase and carboxypeptidase A. The latter enzyme is an exopeptidase cleaving aliphatic and aromatic L-amino acids at the C-terminus of a peptide (Barber and Fisher 1972). As both enzymes are participating in a variety of metabolic processes, further investigations on the hydroxycinnamic acid esters might be promising. The mean inhibitory concentrations (IC₅₀) of fukinolic acid were at 40 μ M for α -amylase and 173 μ M for carboxypeptidase A. IC₅₀ values of cimicifugic acid A were 24.1 μ M for α -amylase and 198 μ M for carboxypeptidase A. The positive control for α -amylase acarbose displayed an IC₅₀ of 92 μ M and the positive control for carboxypeptidase A 1,10-phenanthroline an IC₅₀ of 421 μ M (Kusano et al. 1998). Therefore, both hydroxycinnamic acid esters were more potent than the acknowledged inhibitors for the respective enzymes. Cimicifugic and fukinolic acids thus might have allelopathic effects in the plants' environment.

Conclusion

Plants of the genera *Actaea* and *Petasites* growing in Europe, Eastern Asia and North America have been used in traditional medicine for the therapy of a multitude of diseases. In Eastern Asia, especially China, various *Actaea* (*Cimicifuga*) species are used in traditional Chinese medicine as well as as functional food ingredient. Here, the content of isoferulic acid indicates the quality of Cimicifugae rhizoma, consisting of *A. foetida*, *A. heracleifolia* and *A. dahurica* (Shen et al. 2013; Guo et al. 2017). In North America and Europe, *Actaea* (*Cimicifuga*) *racemosa* rhizome extracts are administered against premenstrual and postmenopausal afflictions mainly due to the observed estrogen-like effects of its triterpene lactone glycosides (e.g. actein and deoxyactein) (Borrelli et al. 2003; He et al. 2006).

Besides being part of the diet in Japan, despite the pyrrolizidine alkaloids present in this plant, *P. japonicus* has been used against asthma and allergic reactions (see review by Aydin et al. 2013). Petasin and related sesquiterpenoids are well known from

other *Petasites* species (especially *P. hybridus*) due to their spasmolytic activity (Bachmann 2017).

Since the main pharmacological activities of *Actaea* (*Cimicifuga*) and *Petasites* extracts are caused by other natural compounds, the possible positive or negative effects of the hydroxycinnamic acid esters, namely the fukinolic and cimicifugic acids have been neglected. The quantities in which the hydroxycinnamic acid esters occur in the respective plants or plant organs and the extracts prepared thereof have, however, to be taken into account in order to assess their applicability as purified compounds and the possible side effects when using whole extracts. Calculating with data from Kruse (2000) the main hydroxycinnamic acid ester in rhizomes of *A. racemosa* is fukinolic acid with about 4–5 mg per g dry drug (0.4–0.5% of the drug dry weight). Other cimicifugic acids are considerably less abundant. The data by Nuntanakorn et al. (2007) also indicate contents at or less than 1% in several *Actaea* species. Similarly, contents of about or less than 0.1% of the dry weight have been determined by Niu et al. (2019) in various *Actaea* species. Since the amounts of the hydroxycinnamic acids in plant material is rather restricted and only few plant species are known to produce these fukinolic and cimicifugic acids, biological activities requiring higher concentrations of these compounds do not appear to be promising to be exploited in near future. All the reported biological activities were gained from in vitro studies. In vivo studies specializing on the hydroxycinnamic acids of *Actaea* and *Petasites* species are lacking.

Much effort has been directed to evaluate the pharmacological effects of the extracts and the active compounds. Although the chemical composition of the active compounds has been elucidated and many biological activities have been characterized, there are still gaps in our knowledge of fukinolic and cimicifugic acids and their activities. The substitution pattern at the aromatic rings of the acids seems to determine anti-inflammatory (Löser et al. 2000), antioxidant (Nuntanakorn et al. 2007), antiviral (Ma et al. 2019), cytotoxic (Yim et al. 2012b) and vasoactive activities (Noguchi et al. 1998). However, it remains unclear, if every effect is relatable to structural differences on fukinolic and cimicifugic acids. Furthermore, not every medicinal aspect has been investigated in vivo. Probably the results might differ from those observed in vitro. Strikingly, the

mechanism of action of cimicifugic acids and fukinolic acid has not been described yet and information on the bioavailability is lacking.

The extract from the rhizomes of *Actaea (Cimicifuga) racemosa* is commonly used for the relief of pre- and postmenopausal symptoms and the effect is related to its estrogen-like effects of triterpene lactone glycosides and hydroxycinnamic acid esters (Kruse et al. 1999; Borrelli et al. 2003; He et al. 2006). On the other hand, increased cell proliferation of MCF-7 breast cancer cells has been observed with fukinolic acid and cimicifugic acid G. Cimicifugic acids A and B have, however, been shown to have anti-tumor effects in a different breast cancer cell line (Kruse et al. 1999; Jiang et al. 2008b). Since the probability to suffer from breast cancer is increasing with the age of women, the use of *Actaea (Cimicifuga) racemosa* extracts for postmenopausal symptoms should probably be regarded more critically. Furthermore, cell viability assays have been performed in independent studies. Fukinolic and cimicifugic acids showed cytotoxicity in vivo even in the sub-micromolar range in different cancer cell-lines, but data regarding cytotoxic effects in non-cancer cell lines are lacking. Further investigations on the pharmacological efficiency of these hydroxycinnamic acid esters are needed for possible clinical applications.

Structurally related compounds such as rosmarinic acid (the ester of caffeic acid and 3,4-dihydroxyphenyllactic acid) or chlorogenic acid (caffeoyl-5-quinic acid) are far better investigated for their pharmacological activities, probably due to their better availability. Moreover, these compounds occur widely throughout the plant kingdom. The multiple pharmacological activities of chlorogenic acid have recently e.g. been summarized by Miao and Xiang (2020) and those of rosmarinic acid by Amoah et al. (2016). This information may be used as a blueprint for further investigations of fukinolic and cimicifugic acids, although the structural differences in the non-caffeic acid part of the molecules might have a strong impact on their physiological behaviour.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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