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Review

Characterization of diarylheptanoids: An emerging class of bioactive natural products

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ABSTRACT

Diarylheptanoids are a class of secondary plant metabolites with a wide variety of bioactivity. Research on their phytochemistry and phytoanalysis is rapidly growing and the number of identified structures bearing the aryl-C_7-aryl skeleton is at present approaching 500. Historically, the yellow pigment curcumin has been characterized as the first diarylheptanoid and the extensive research on naturally occurring analogues is still ongoing. In this review, studies dealing with the characterization of linear and cyclic derivatives are discussed from the phytoanalytical point of view. Isolation, fractionation and purification strategies from natural sources along with their chromatographic behavior and structural characteristics are discussed. The role of various techniques used for the extraction (such as Soxhlet extraction, sonication, maceration/percolation, microwave-assisted extraction, supercritical carbon dioxide extraction); isolation (liquid–liquid extraction, column chromatographic techniques, preparative thin-layer and high-performance liquid chromatography, centrifugal partition chromatography, counter-current chromatography); separation (thin-layer chromatography, high-performance liquid chromatography, gas chromatography, capillary electrophoresis) and structural characterization (UV/Vis spectroscopy, infrared spectroscopy, X-ray crystallography, mass spectrometry, nuclear magnetic resonance spectroscopy and circular dichroism spectroscopy) are critically reviewed.

Contents

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1. Introduction

Diarylheptanoids, characterized by a 1,7-diphenylheptane skeleton, constitute a group of natural products gaining emerging interest over the last few decades due to their remarkable biological activities. The more than 400 diarylheptanoids that have been identified so far [1] can be divided into linear or macrocyclic compounds. In diarylether- and biaryl-type constituents the aromatic rings are connected to form [7.1]-*meta,para*- or [7.0]-*meta,meta*-cyclophanes, respectively (Fig. 1) [1–4].

The aromatic rings of linear diarylheptanoids are often hydroxylated or methoxylated. C-4′ and C-4″ hydroxyl groups can be acetylated or glycosylated, compounds with unsubstituted aromatic rings are scarce. The aliphatic C_7 chain is either saturated or can have up to three double bonds. Further possibility is the presence of carbonyl groups at C-3 and/or C-5 (Table 1). The C-5 can be substituted by a hydroxyl group that may be free or engaged in another function: methyl, acetyl, sulfate or glycosyl groups can be attached. Diarylheptanoids can occur as mono-, di- or triglycosides. The sugar moieties may be further substituted by phenolcarboxylic acids [1,2,4,5].

According to Lv and She [1,2] (Table 1), linear diarylheptanoids can be divided into several classes. There are compounds, where a 1,5- or a 3,6-oxy bridge (a pyran or a furan ring) is formed within the C_7 chain (Fig. 2) [6]. Diarylheptanoids can also possess flavonoid (e.g. chalcone, flavanone) moieties at C-5 or C-7 (Fig. 2.) [7]. Dimeric compounds with antiproliferative activity were also isolated from *Alpinia* species, additionally, a chalcone moiety can also be attached to the dimeric diarylheptanoid skeleton [8,9]. The monomeric units can be linked through a pyridine moiety, too (Fig. 2) [10]. Diarylheptanoids bearing a special structure such as 1,3- and 1,5-diarylheptanoids comprise the last class (Table 1) [2,11]. Compounds conjugated to an ellagitannin [12] or a gallotannin unit [13] as well as those with a monoterpene [10] or a sesquiterpene moiety [14] have also been reported. Linear diarylheptanoids are abundant in plants of the genera *Curcuma*, *Zingiber*, *Alpinia* (Zingiberaceae), *Alnus* and *Betula* (Betulaceae) [1–3].

Cyclic diarylheptanoids are distributed in *Myrica* (Myricaceae), *Acer* (Aceraceae), *Garuga* (Burseraceae), *Corylus*, *Betula*, *Carpinus* (Betulaceae), and *Juglans* (Juglandaceae) species. The heptane chain of diarylether-type and biaryl-type cyclic diarylheptanoids can be saturated or unsaturated, and carbonyl or hydroxyl groups may also be attached. The aromatic rings can be hydroxylated or methoxylated at certain carbon atoms, *O*-glycosylation can also occur (Figs. 1 and 2) [1–4,15]. A special compound with an ether bond between the side chain and the aryl group has also been isolated (Figs. 1 and 2) [16].

Diarylheptanoids have been reported by numerous studies to possess diverse bioactivities including anti-inflammatory [17], pro-apoptotic [18], anti-influenza [19], anti-emetic [20], and estrogenic [21] actions, being their anti-cancer activity that basically has caught the interest of researchers. *In vitro* and *in vivo* cytotoxicity against human cancer cell lines of diarylheptanoids has been shown [22,23]. *In vivo* citotoxicity of oregonin (Fig. 2) has been proved in the B16 murine melanoma model [24]. Although a paper doubting the biological activity and therapeutic utility of curcumin (Fig. 2) – due to its unstable, reactive and non-bioavailable properties – has recently been published [25], the interest towards curcuminoids and diarylheptanoids is still undiminished.

2. Extraction and isolation of diarylheptanoids

2.1. Extraction

There are two prevalent strategies for the extraction of diarylheptanoids. The first method involves the extraction carried out under reflux, in a Soxhlet apparatus, by sonication, or by maceration/percolation with organic solvents such as chloroform, dichloromethane, ethyl acetate, methanol/ethanol, aqueous methanol/ethanol. Although a certain loss may occur, this method allows simple extraction of diarylheptanoids. The initial step is usually followed by subsequent liquid–liquid extractions with hexane, chloroform, ethyl acetate, ethanol, or *n*-butanol [26–31]. The alternative option for diarylheptanoid extraction employs sequential solvent extraction with hexane, chloroform, or ethyl acetate, followed by methanol, ethanol or *n*-butanol [32–34]. A selection of the methods applied in diarylheptanoid extraction and isolation is presented in Table 2.

Extraction techniques aiming to reduce the need for solvents, the extraction time and the costs of producing plant extracts have been developed. In supercritical (and subcritical) fluid extraction (SFE) carbon dioxide is used as the main solvent, thus the consumption of organic solvents can be minimized. With the addition of organic modifiers, higher yields of polar target compounds and a broader range of the extracted constituents can be achieved [48].

Supercritical carbon dioxide extraction [49] and subcritical water extraction [50] have been used to obtain diarylheptanoids from plant materials. Turmeric (*Curcuma longa*) rhizomes were extracted with supercritical carbon dioxide as well as with supercritical carbon dioxide and ethanol as co-solvent. The addition of ethanol increased the extraction yield of curcuminoids and decreased the solvent consumption. Curcumin is insoluble in water, poorly soluble in hydrocarbon solvents, and soluble in alcohols. Ethanol promoted the solubilization of the polar substances (e.g. diarylheptanoids) present in the rhizomes [51]. Supercritical fluid extraction (SFE) coupled to supercritical fluid chromatography (SFC) has been applied for the analysis of *C. longa* rhizomes. The absence of curcumin in the extracts obtained by pure carbon dioxide was confirmed. The addition of methanol enabled the extraction of the curcuminoids, more than 90% recovery for curcumin was

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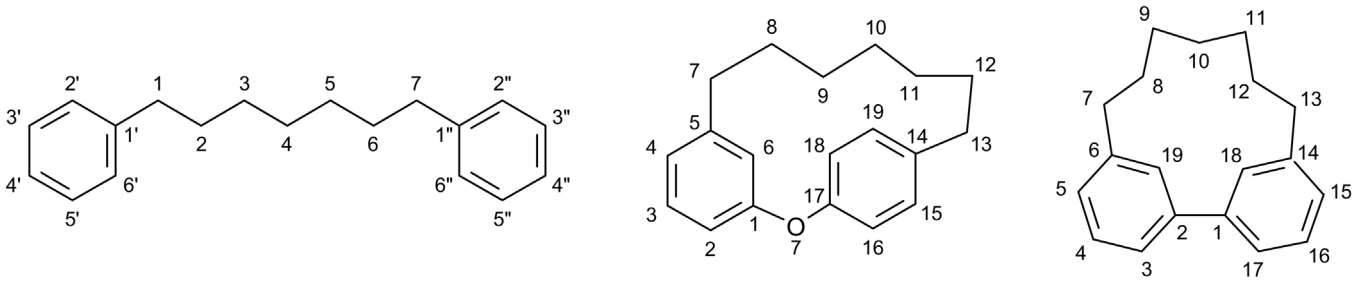

Fig. 1. The chemical structures of linear, diarylether-type and biaryl-type cyclic diarylheptanoids.

Table 1
Structural characteristics of naturally occurring diarylheptanoids according to the classification of Lv and She [2].

Type	Subtype	Skeleton	Structural characteristics
linear diarylheptanoids	I	heptane chain with or without olefinic bonds, carbonyl groups and/or a kavain side chain	saturated aliphatic chain hydrocarbon one to three–olefinic bonds one carbonyl and/or several olefinic bonds two carbonyls and/or several olefinic bonds one kavain side chain
	II	pyran or furan ring in the carbon chain skeleton	1,5-oxy bridge 3,6-oxy bridge
	III	a flavonoid moiety attached to the carbon chain skeleton	a chalcone group a flavanone group a flavonol group an isoflavanone group
	IV	dimeric linear diarylheptanoids (diarylheptanoid units linked through one or two epoxy-rings, or two diarylheptanoids with one chalcone moiety, or diarylheptanoid units linked by a pyridine moiety or through the phenyl groups)	
	V	unusual structures (1,3-, 1,5- and 1,7-diarylheptanoids, with varying degrees of unsaturation, oxygenation and in one case a fused C-10 terpene moiety; diarylheptanoid glycoside with an ellagitannin moiety)	
cyclic diarylheptanoids	VI	*meta*,*para*-cyclophanes	saturated aliphatic chain hydrocarbon one olefinic bond one to two carbonyls one carbonyl and one olefinic bond
	VII	*meta*,*meta*-cyclophanes	saturated aliphatic chain hydrocarbon one to two double bonds one to two carbonyl groups one carbonyl and one double bond unusual structures ether bond between the side-chain and the aryl group or a *p*-benzoquinonyl moiety

achieved. Higher proportions of methanol, however, would make it impossible to reach the supercritical state, thus causing the loss of the advantages using SFE [52].

A representative thin-layer (TLC) chromatogram of *Alnus glutinosa* bark SFE extracts obtained with carbon dioxide and different proportions of ethanol can be seen in Fig. 3. The increase in ethanol concentration enhanced the extraction yield, as indicated by the various color intensities of the oregonin and hirsutenone (Fig. 2) zones on the TLC plate.

Further extraction techniques, to obtain diarylheptanoid rich-extracts, include pressurized liquid extraction (PLE), accelerated solvent extraction (ASE), fluidized bed extraction (FBE), and the use of ionic liquids. Hu et al. developed a pressurized liquid extraction (PLE) method with 70% aqueous bioethanol as the solvent to obtain diarylheptanoids from *Zingiber officinale*. In this technique, liquid solvents at elevated temperatures and pressures are used, and as a result, reduced time and solvent volume is required to yield constituents from plants. Additionally, the extracts from PLE showed a different composition compared to Soxhlet extraction, the recovery of diarylheptanoids was significantly improved [53]. Common hazel (*Corylus avellana*) stems and leaves were extracted by a solid/liquid extraction method, using a solvent system accelerated with static pressurization (Zippertex technology) [54].

Carbamate ionic liquids possess low toxicity and volatility. Additionally, organic compounds show high solubility in carbamate ionic liquids, therefore these liquids have a potential to be used as solvents to overcome low solubility of natural products (e.g. curcumin) in water. Extraction of curcumin from turmeric using a carbamate ionic liquid was investigated, *N*,*N*-dipropylammonium *N*′,*N*′-dipropylcarbamate was used [55]. Extraction of curcuminoids from turmeric was examined using several bromide ionic liquids [56].

Wakte et al. isolated curcumin from the rhizomes of *C. longa* by Soxhlet, microwave-, ultrasound- and supercritical carbon dioxide assisted extraction techniques. The extraction efficiency of the methods was evaluated in terms of curcumin yields and extraction time. High extraction recovery for curcumin in the shortest extraction time was obtained with microwave-assisted extraction [57]. Diverse extraction methods, such as hydrodistillation, low pressure solvent extraction, Soxhlet extraction, and SFE using carbon dioxide with different co-solvents for the extraction of curcuminoids have been compared by Li et al. [58]. Soxhlet extraction, fluidized

Table 2
Isolation strategies of diarylheptanoids from various plant sources.

Sample, extraction	Fractionation	Further purification	Isolated diarylheptanoids	Ref.
Acer nikoense stem bark	partitioned in EtOAc/H_2O	EtOAc-soluble fraction: silica gel CC, further separated by silica gel CC, and prep. HPLC	diarylether-type cyclic compounds: acerogenins A and I, (*R*)-acerogenin B, 9-oxoacerogenin A, acerosides I, III, VI, and B1	[35]
hexane; the defatted residue extracted with MeOH	the aqueous layer extracted with BuOH	BuOH-soluble fraction: Diaion HP-20 CC, further chromatographed on ODS (and silica gel CC), then prep. HPLC	biaryl-type cyclic compound: 9-*O*-glucosyl-acerogenin K	
			linear compounds: aceroside VIII, acerogenin G, (−)-centrolobol	
Alnus glutinosa bark	silica gel 60 CC	prep. HPLC-DAD, C18 column	linear glycosides: rubranosides A-B, aceroside VII, platyphylloside, platyphyllonol-5-*O*-xyloside, alnusides A-B, oregonin, hirsutanonol-5-*O*-glucoside, 1,7-diphenylheptan-5-*O*-glucosyl-3-one, 5-hydroxy-1,7-diphenylheptan-3-*O*-xyloside, 1,7-diphenylheptan-5-*O*-dimethoxycinnamoylglucosyl-3-one	[36]
$CHCl_3$-MeOH (1:1, v/v), at r.t., with ultrasonic bath in the last hour	(CH_2Cl_2-MeOH gradient, 100:0-60:40, v/v)	0.1% HCOOH-ACN gradient	linear aglycones: hirsutanonol, hirsutenone	
Alnus japonica stem bark	silica gel VLC	50% *n*-hexane-$CHCl_3$ fraction: repeated silica gel CC (*n*-hexane-EtOAc gradient)	linear diarylheptanoids: hirsutanonol, 5-methylhirsutanonol, platyphyllenone, hirsutenone	[37,38]
70% EtOH	(eluted with 50% *n*-hexane-$CHCl_3$ and EtOAc, separately)	EtOAc fraction: VLC ($CHCl_3$-MeOH gradient), subfractions: silica gel CC ($CHCl_3$-MeOH gradients) or C18 CC (MeOH-H_2O), purified by prep. HPLC	cyclic biaryl-type compounds: myricanone, (+)-*S*-myricanol, myricanone 5-*O*-glucoside, (+)-*S*-myricanol-5-*O*-glucoside, alnuheptanoid B	
Alnus formosana leaves	liquid–liquid partitioning with BuOH	*n*-BuOH soluble fraction: Sephadex LH-20 CC (MeOH) to give subfractions, which were subjected to HPLC-SPE-NMR (400 MHz) (ACN-H_2O gradients)	linear glycosides: rubranosides A-B, alnusides A-B, aceroside VII, oregonin, 2′″-*O*-cinnamoyloregonin, 2′″-*O*-*p*-coumaroyloregonin, 2′″-*O*-benzoyloregonin + 4 compds.	[17]
MeOH			linear aglycones: hirsutenone centrolobol, rubranol, hirsutanonol + 5 compds.; methoxy- and butyloxy derivatives	
Alnus hirsuta f. *sibirica* leaves	partitioned successively with $CHCl_3$, EtOAc and *n*-BuOH	EtOAc and *n*-BuOH extract: silica gel CC ($CHCl_3$-MeOH-H_2O gradients)	linear glycosides: aceroside VII, oregonin, platyphyllonol-5-*O*-xyloside + 5 mono- and diglycosides	[26]
80% MeOH by ultrasonication at r.t.		fractions from silica gel CC subjected to (semi)prep. HPLC (C18 column, ACN-H_2O and MeOH-H_2O) and Sephadex LH-20 CC (MeOH)	linear aglycones: (+)-hannokinol, hirsutanonol, 5-hydroxy-3-platyphyllone, hirsutenone + 3 compds. with saturated C_7 chain and 4 compds. with unsaturated C_7 chain	
Alpinia blepharocalyx seeds	extracted successively with hexane and ether	ether extract: silica gel CC ($CHCl_3$-MeOH gradient)	(dimeric) linear compounds with a chalcone moiety: calyxins A-B, F, H, epicalyxin B, 6-hydroxycalyxin F; blepharocalyxins A-B	[9]
95% EtOH		fractions: silica gel CC ($CHCl_3$-MeOH gradient), further subjected to Sephadex LH-20 CC, followed by prep. TLC; epimeric mixtures separated by chiral NP-HPLC	linear compounds with a flavanone moiety: calyxins C-E, epicalyxins C-D	
Alpinia officinarum rhizomes	partitioned with PE, $CHCl_3$, EtOAc and *n*-BuOH	$CHCl_3$ extract: silica gel CC (PE-EtOAc gradient), fractions subjected to silica gel CC (PE-EtOAc)	dimer diarylheptanoid with a pyridine ring: officinaruminane A	[10]
95% EtOH at r.t., three times		subfractions: repeated silica gel CC (PE-EtOAc), purified by prep. TLC (PE-Me_2CO or PE-EtOAc) or MPLC (silica gel, $CHCl_3$-MeOH or PE-Me_2CO)	diarylheptane monoterpene: officinaruminane B	
			1,7-diphenyl-4-hepten-3-ones: 3 compds.	
			5-hydroxylated or 5-acetoxylated linear aglycones: 3 compds.	
			a diarylheptanoid with a furan ring	
A. officinarum rhizomes	partitioned with EtOAc	*n*-hexane extract: Sephadex LH-20 CC ($CHCl_3$-MeOH), then by prep. HPLC (C18, MeOHH_2O)	diarylheptanoids with a 3,6-furan ring: alpinoid D + 1 compd.	[39]

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Table 2 (*Continued*)

Sample, extraction	Fractionation	Further purification	Isolated diarylheptanoids	Ref.
MeOH	the EtOAc-soluble extract was again partitioned between *n*-hexane and MeOH-H_2O	MeOH-H_2O extract: Sephadex LH-20 CC (MeOH), then Sephadex LH-20 CC ($CHCl_3$-MeOH), followed by silica gel CC (n-hexane-EtOAc), and prep. HPLC (C18, MeOH-H_2O)	linear compounds with unsaturated C_7 chain alpinoid E + 5 compd.	
		or subjected to silica gel CC (n-hexane-EtOAc gradient), and prep. HPLC (C18, MeOH-H_2O)	hydroxylated or methoxylated linear compounds with saturated C_7 chain: 10 compds.	
Betula platyphylla inner bark	residue was extracted with Et_2O	Et_2O extract: silica gel CC ($CHCl_3$-MeOH and hexane-EtOAc) and Sephadex LH 20 (MeOH)	linear glycosides: acerosides VII-VIII	[40]
MeOH under reflux		Et_2O insoluble compounds: silica gel CC ($CHCl_3$-MeOH) and Sephadex LH 20 (MeOH-H_2O)	1,7-diphenyl-3-hepten-5-one and a 2-hydroxy derivative	
		a mixture of aceroside VII and betuloside was purified by droplet CCC using $CHCl_3$-MeOH-H_2O (4:4:3, v/v)	cyclic diarylheptanoids: acerogenin E, 3,5′-dihydroxy-4′-methoxy-3′,4”-oxo-1,7-diphenyl-1-heptene	
Corylus avellana leaves	MeOH extract fractionated on Sephadex LH-20 CC (MeOH)	fractions purified by semiprep. HPLC (C18 column, MeOH–H_2O)	diarylether-type cyclic diarylheptanoids:	[41]
n-hexane, $CHCl_3$, MeOH, at r.t.			with 1 or 2 unsaturated bonds in the C_7 chain: giffonins A–H	
			biaryl-type cyclic diarylheptanoid diglycoside: giffonin I	
Curcuma kwangsiensis rhizomes	concentrated extract suspended in water and passed through a HP-20 macroporous resin column (EtOH-H_2O)	silica gel CC (cyclohexane, then $CHCl_3$-MeOH gradient)	diarylheptanoids with a tetrahydropyran ring (1,5-epoxy derivatives): 3 compds.	[42]
refluxed with 95% EtOH, twice		fractions from silica gel CC: ODS CC (MeOH-H_2O)	3,5-dihydroxy-1,7-diphenylheptanes: 4 compds.	
		fractions from ODS CC: Sephadex LH-20 CC (MeOH)	3-acetoxy-5-hydroxy-1,7-diphenylheptanes: 4 compds.	
		fractions from Sephadex LH-20 CC: prep. HPLC (C18, MeOH-H_2O)	3,5-diacetoxy-1,7-diphenylheptanes: 3 compds.	
Curcuma kwangsiensis rhizomes	partitioned successively with cyclohexane, EtOAc, and *n*-BuOH	EtOAc extract: silica gel CC, subfractions chromatographed on Sephadex LH-20 CC, then ODS CC or prep. TLC, finally HPLC (C18); enantiomeric mixtures separated by chiral RP-HPLC (ACN-H_2O)	3-hydroxylated or 3-acetoxylated linear aglycones with saturated and unsaturated C_7 chain: 12 compds.	[43]
70% EtOH			linear aglycones with saturated and unsaturated C_7 chain: 6 compds.	
Curcuma longa dried rhizomes	EtOAc extract subjected to silica gel CC (CH_2Cl_2-MeOH)	repeated MCI gel CC (MeOH-H_2O), silica gel CC (PE-EtOAc, then CH_2Cl_2-MeOH), and Sephadex LH-20 CC (MeOH-H_2O)	diarylheptanoids with a sesquiterpene moiety: bisabolocurcumin ether derivatives	[14]
PE and EtOAc			1,5-diphenylpenta-1,4-dien-3-one	
			1,7-diphenylhepta-1,4,6-trien-3-one	
Hedychium coronarium rhizomes 95% EtOH	partitioned in $CHCl_3$ and *n*-BuOH	BuOH fraction: Sephadex LH-20 CC (MeOH)	diarylheptanoids with a tetrahydropyran ring: hedycoropyrans A–C	[8]
		fractions: CPC ($CHCl_3$-MeOH-H_2O or $CHCl_3$-MeOH-H_2O-*n*-PrOH; normal followed by reverse mode)	with a tetrahydrofuran ring: hedycorofurans A–D	
		subfractions: silica gel CC, Sephadex LH-20 CC, semiprep. C18 HPLC		
Myrica rubra bark	extract partitioned with *n*-hexane, EtOAc, and *n*-BuOH	EtOAc extract: Toyopearl HW-40C CC (MeOH-H_2O and H_2O-Me_2CO), then YMC-gel ODS CC (MeOH-H_2O), finally Sephadex LH-20 CC (EtOH)	biaryl-type cyclic diarylheptanoids	[44]
macerated with aqueous EtOH	precipitate separated by C18 SPE	*n*-BuOH extract: Toyopearl HW-40C CC (MeOH-H_2O and H_2O-Me_2CO), then C18 CC (MeOH-H_2O)	myricanol, myricanone, actinidione, myricanone	
			5-*O*-(6′-*O*-galloyl)-glucoside, (*R*)- and (*S*)-myricanol 5-*O*-(6′-*O*-galloyl)-glucosides	
		elute from C18 SPE: prep. NP-TLC	myricanol 11-sulfate, juglanin B 11-sulfate	
M. rubra bark	partitioned in *n*-BuOH-H_2O	*n*-BuOH soluble fraction subjected to normal and reversed-phase silica gel CC, and prep. HPLC	biaryl-type cyclic diarylheptanoids: myricanane A-B	[45]
			5-*O*-arabinosyl-glucosides; myricanone and its 5-*O*-glucoside, neomyricanone-5-*O*-glucoside, myricanol and its 11-*O*-glucoside, (+)-*S*-myricanol-5-*O*-glucoside	

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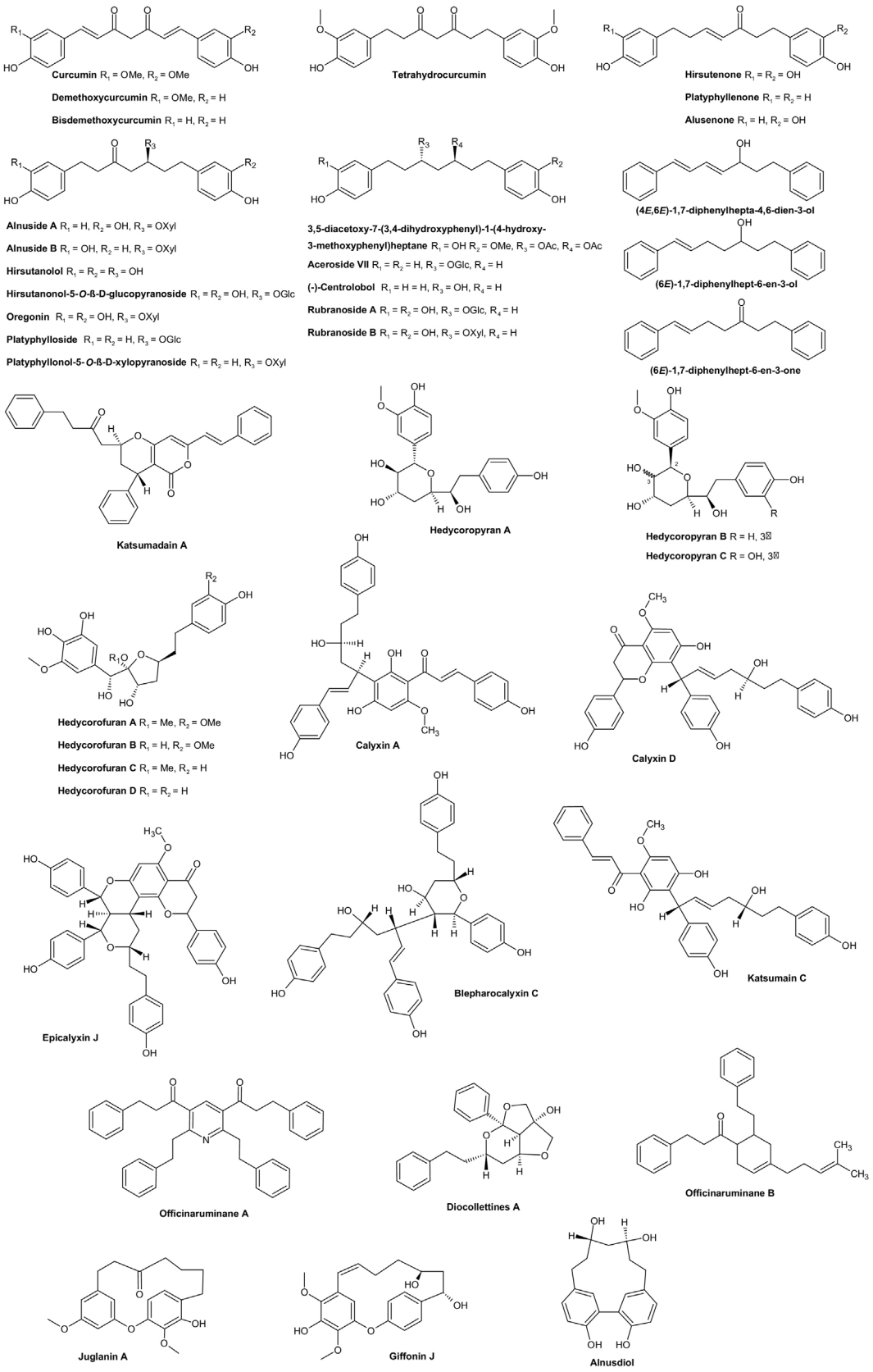

Fig. 2. Structures of selected diarylheptanoids discussed in the review.

Table 2 (*Continued*)

Sample, extraction	Fractionation	Further purification	Isolated diarylheptanoids	Ref.
MeOH				
Pyrostria major leaves EtOAc	subjected to silica gel CC (CH_2Cl_2-MeOH gradient)	fractions were purified by prep. HPLC (C18 column; MeOH-0.1% HCOOH in H_2O gradient)	3-*O*-D-glucosides of 5-formyl-3-hydroxy-1,7-diphenylheptanes and 3,5-dihydroxy-1,7-diphenylhepanes	[46]
Renealmia alpinia rhizomes, *Renealmia cernua* leaves	partitioned between H_2O-$CHCl_3$ and H_2O-EtOAc	$CHCl_3$ phase: silica gel CC (PE-EtOAC gradient, then EtOAc-MeOH), then silica gel MPLC (PE-EtOAc or $CHCl_3$-MeOH gradient)	linear aglycones with saturated and unsaturated C_7 chain: renecernin, hirsutanonol, renealpin, hirsutenone, platyphyllenone, alusenone, tetrahydrobisdemethoxycurcumin	[47]
MeOH at r.t.		EtOAc phase: Sephadex LH-20 CC (MeOH), then silica gel MPLC ($CHCl_3$-MeOH), and cyclic MPLC		
Zingiber officinale rhizomes	alcoholic residue was partitioned with PE, EtOAc, and *n*-BuOH	EtOAc extract: silica gel CC (PE-EtOAc gradient), repeated silica gel CC and Sephadex LH-20 CC ($CHCl_3$-MeOH or benzene-Me_2CO gradients), then prep. TLC	linear methoxylated aglycones with saturated and unsaturated C_7 chain: hexahydrocurcumin, gingerenones A, C + 5 compds.	[30]
soaked in 95% EtOH at r.t.		*n*-BuOH extract: silica gel CC ($CHCl_3$-MeOH gradient), repeated silica gel CC ($CHCl_3$-MeOH-H_2O or $CHCl_3$-MeOH-AcOH gradients), or Sephadex LH-20 CC (MeOH)	a linear diacetoxylated aglycone	
			1,5-epoxy-diarylheptanoids: 4 compds. sulfated diarylheptanoids: shogasulfonic acid A + 2 compds.	

Abbreviations: ACN: acetonitrile, MeOH: methanol, BuOH: butanol, $CHCl_3$: chloroform, EtOAc: ethyl acetate, H_2O: water, HCOOH: formic acid, Me_2CO: acetone, PE: petroleum ether, CC: column chromatography, MPLC: middle pressure liquid chromatography, HPLC: high-performance liquid chromatography, TLC: thin-layer chromatography, RP: reversed-phase, NP: normal-phase, r.t.: room temperature.

bed extraction, and accelerated solvent extraction methods were applied using organic solvents of different polarity (hexane, ethyl acetate, and ethanol) to obtain diarylheptanoid-rich extracts from *Alnus incana* and *A. glutinosa*, the Soxhlet method possessed the highest extraction efficiency for diarylheptanoids [59].

2.2. Isolation

The extracted diarylheptanoids can be fractionated using open column chromatography (CC), vacuum liquid chromatography (VLC) [34,60,61], middle pressure liquid chromatography (MPLC) [62,63], flash chromatography [64,65], preparative thin-layer chromatography (TLC) [66], or preparative high-performance liquid chromatography (HPLC) (Table 2) [67]. Cyclic MPLC has also been employed [47]. The most common procedures apply normal-phase chromatography on silica gel, gel filtration on Sephadex LH-20, and reversed-phase chromatography using C18-bonded silica columns or Diaion HP-20 resin [8,68–71]. Solvents including various mixtures of hexane, chloroform, ethyl acetate, and methanol are used. 2,3-Digalloyl oregonin was isolated from the 80% acetone extract of *Alnus sibirica* leaves by column chromatography using Amberlite XAD-2, Sephadex LH-20, MCI-gel, CHP 20P and ODS-B gel with an MPLC system [13]. Diarylheptanoid sulfates were isolated by the combination of size exclusion and reversed-phase chromatography [44,72] or by a sequential application of both together with normal-phase chromatography [73]. The $AgNO_3$ column chromatography of the petroleum ether extract of *Alpinia officinarum* yielded the highly lipophilic diarylheptanoid 1,7-diphenylhept-3-ene-5-one [74]. Commercially available curcumin consists of a mixture of the three main curcuminoids, where curcumin is the major compound, while demethoxycurcumin and bisdemethoxycurcumin (Fig. 2.) are present only as minor constituents. In a recent study, a purification process to obtain the three curcuminoids as pure compounds has been reported. Repeated crystallizations followed by silica gel CC of the remaining mother liquors yielded the pure compounds with high recoveries. However, the authors claimed that purified curcumin rapidly degraded, while demethoxycurcumin and bisdemethoxycurcumin were found to be more stable, and their presence in the mixture enhanced the stability of curcumin [75].

The isolation of the compounds frequently requires several subsequent or repeated applications of chromatographic techniques [76]. However, it is also possible to obtain the compounds just in a few fractionation steps. Masullo et al. isolated hydroxylated diarylether-type and biaryl-type macrocyclic diarylheptanoids (giffonins J-P, Fig. 2) from the methanol extract of *C. avellana leaves*. The extract was fractionated on a Sephadex LH-20 column, and the fractions were further purified by semipreparative HPLC on a C18 column [77]. Novaković et al. extracted the bark of *A. glutinosa* with a chloroform-methanol (1:1, v/v) mixture and fractionated the crude extract on a silica gel column with different gradient elution systems. Similar fractions from the silica gel CC were combined after TLC analysis and further fractionated by semi-preparative HPLC on a C18 column into pure compounds [78]. During the isolation of diarylheptanoid glycosides with cinnamoyl moieties attached to the glucose residues, acid-catalyzed and photochemical *cis-trans* isomerization of the double bond in the cinnamic acids occurred [79,80]. Purification of these compounds by HPLC using pure water instead of 0.025% formic acid as the aqueous eluent yielded pure constituents [36].

Diarylheptanoids possessing a diarylheptanoid and a chalcone moiety were isolated from *Alpinia blephyarocalyx* and *Alpinia katsumadai* as epimeric mixtures, e.g. calyxins B-C, F, H, and katsumain C, together with their epimers epicalyxins B-C, F, H, and 7-epikatsumain C, respectively (Fig. 2). These stereoisomers could be separated by preparative HPLC on a chiral column [81–83]. Dong et al. isolated an enantiomeric mixture of diarylheptanoids with a tetrahydropyran skeleton from *Dioscorea villosa*. (1*S*,3*S*,5*S*)- and (1*R*,3*R*,5*R*)-1,7-bis(4-hydroxyphenyl)-1,5-epoxy-3-hydroxyheptanes were again separated by normal-phase HPLC on a chiral column [84].

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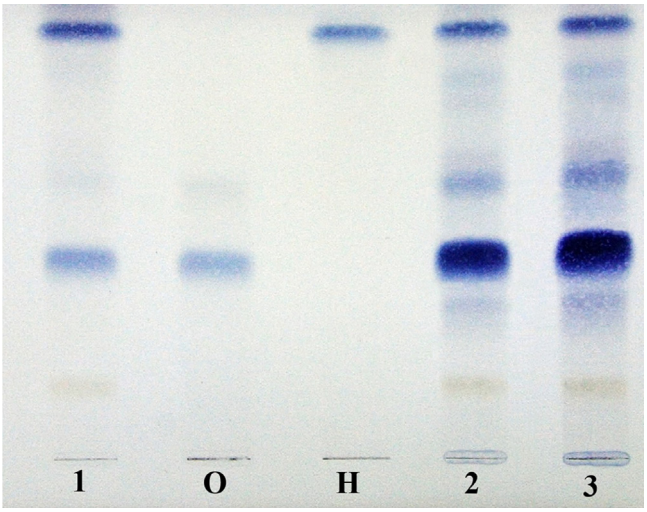

Fig. 3. TLC chromatogram of *A. glutinosa* SFE extracts. 1: *A. glutinosa* 10% EtOH SFE extract, O: oregonin standard solution, H: hirsutenone standard solution, 2: *A. glutinosa* 15% EtOH SFE extract, 3: *A. glutinosa* 20% EtOH SFE extract. Stationary phase: silica gel 60 F_{254} 10 × 10 cm, mobile phase: chloroform:methanol:formic acid (75:25:2, v/v), post-chromatographic derivatization: vanillin–sulfuric acid reagent (0.5 g vanillin in 100 ml sulfuric acid-ethanol, 40:10, v/v), heating at 100–105 °C. SFE extracts: dried and milled black alder bark samples (0.6 g) were extracted for one hour, at 40 °C and 20 MPa with 10%, 15%, and 20% ethanol, in a Jasco system (CO_2 pump with a cooling system, pump, mixer, extraction vessels, column thermostat, back-pressure regulator).

2.3. Further isolation techniques

Diarylheptanoids with estrogenic activity from the seeds of *Amomum longiligulare* were separated by high-speed counter-current chromatography (HSCCC) and purified by HPLC [85].

Wu et al. obtained six diarylheptanoids from the petroleum ether fraction of *A. officinarum* rhizome methanol extract by counter-current chromatography (CCC) using a three-phase solvent system consisting of *n*-hexane, ethyl acetate, acetonitrile, and water, while preparative C18 HPLC was used for further purification [86]. The diarylheptanoid 1,7-diphenyl-4,6-heptadien-3-one, together with five flavonoids was isolated from *A. katsumadai* by microwave-assisted extraction (MAE) followed by HSCCC. The two-phase solvent system was composed of hexane–ethyl acetate–methanol–water [87].

The methanol extract of *Betula platyphylla* inner bark was subjected to silica gel CC (eluted with chloroform-methanol and hexane-ethyl acetate mixtures) and Sephadex LH-20 CC to obtain a mixture of the arylbutanoid glucoside betuloside and the linear diarylheptanoid glucoside aceroside VII (Fig. 2). The mixture was subjected to droplet CCC to obtain the pure compounds [40]. HSCCC was used for preparative scale separation of the active constituents from the ethanol extract of *Alnus japonica* stems. HSCCC separation of the ethyl acetate fraction from the crude extract yielded hirsutanonol-5-*O*-β-D-glucopyranoside, 3-deoxohirsutanonol-5-*O*-β-D-glucopyranoside, and hirsutenone with recoveries of 95.4%, 97.0%, and 93.7% (purities: 94.7%, 90.5%, and 98.6%), respectively [88].

Oregonin was isolated from the methanol extract of *Alnus formosana* leaves that was subjected to liquid–liquid partitioning. The *n*-butanol soluble fraction was separated on a Sephadex LH-20 column to provide an oregonin-rich fraction that was further purified by CPC to yield pure oregonin [89]. Abedini et al. also aimed to optimize the isolation of oregonin from the methanol extract of *A. glutinosa* bark. Fractionation of the crude extract was performed by CPC using a three-phase solvent system resulting in a recovery of 72% (850 mg) for oregonin at purity higher than 94% after a single injection [90].

Hedycoropyrans A–C containing a tetrahydropyran moiety and hedycorofurans A–D (Fig. 2) comprising a tetrahydrofuran moiety were isolated from the ethanol extract of *Hedychium coronarium* rhizomes. The *n*-butanol soluble fraction was separated on a Sephadex LH-20 column, the fractions were subjected to CPC using two-phase solvent systems. Further separation of the CPC subfractions using silica gel and Sephadex LH-20 CC and semi-preparative HPLC (C18) yielded pure compounds [6].

3. Structure elucidation of diarylheptanoids

3.1. UV/Vis spectrophotometry

All diarylheptanoids exhibit absorption in the range of 250–290 nm. Curcumin presents a further broad absorption band in the visible region with a maximum in the 410–430 nm range. Curcumin exhibits keto-enol tautomerism, where the enol form is characterized by the formation of a strong intramolecular hydrogen bond. Additionally, curcumin is ready to form intermolecular H-bonds in solution. Thus, the intense absorption band in the visible region appears due to the extended conjugated system of the aromatic rings, the unsaturated bonds in the C_7-chain, and the intramolecular H-bonding of the enol function [91–94]. The UV/Vis absorption spectrum of curcumin in acetonitrile can be seen in Fig. 4.

UV/Vis spectrophotometry is commonly used for the quantification of total curcuminoid content of medicinal plant extracts, formulations, food, etc. [95,96]. The European Pharmacopoeia 9th edition publishes two official herbal drug monographs, Turmeric rhizome and Javanese turmeric (Curcumae longae rhizoma and Curcumae zantorrhizae rhizoma), which require the quantitative determination of total dicinnamoyl methane derivatives (expressed as curcumin) using a spectrophotometric method for the quality control of the drugs. Absorption of ethanol extracts prepared by a reflux method is measured at 425 nm [97,98]. These spectrophotometric methods are, however, not suitable to quantify the individual curcuminoids.

Ahmed et al. elucidated the encapsulation of curcumin preparing different lipid-based nanoemulsions and conventional emulsions. Additionally, the bioaccessibility of curcumin was investigated, when released in an *in vitro* model simulating small intestine digestion conditions. The concentration of curcumin in lipid phases was determined by spectrophotometry [99].

3.2. Near-infrared (NIR) spectroscopy

Tanaka et al. developed a quantitation method for the determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in turmeric samples, using NIR spectroscopy and multivariate statistics. Characteristic absorptions of curcuminoids around 1620–1740 nm could be assigned to C—H stretching vibrations, while substitution of the benzene ring – reducing the symmetry of the molecule – generated additional vibrational bands in the region of 2100–2550 nm. The prediction of the composition in the pulverized turmeric samples by partial least-squares regression (PLS-R) analysis showed high correlation with the results from HPLC quantitation [100]. Kasemsumram et al. reported an application of moving window partial least squares regression (MWPLSR) to determine the total curcuminoid content in rhizomes of *C. longa* by NIR spectroscopy [101].

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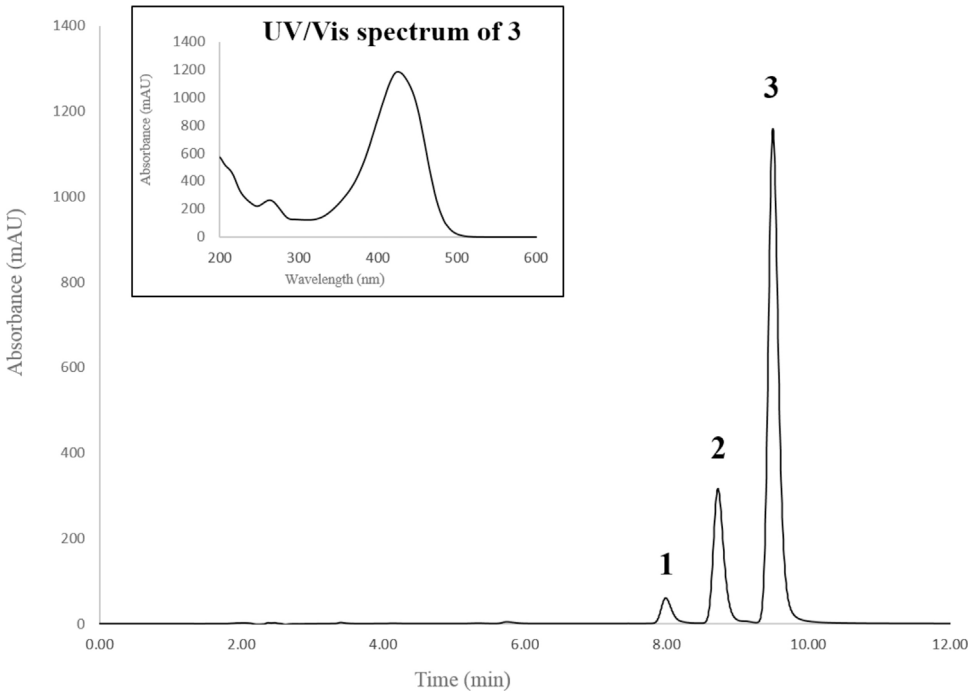

Fig. 4. HPLC-DAD chromatogram of bisdemethoxycurcumin (1), demethoxycurcumin (2), and curcumin (3). Inset: UV/Vis spectrum of curcumin (3). Stationary phase: Zorbax SB-C18 (150 × 3.0 mm i.d., 3.5 μm particle size) column, mobile phase: 0.1% (v/v) formic acid in water (eluent A) and acetonitrile (eluent B), in a linear gradient system (0 min: 50% B, 15 min: 65% B, 16 min: 100% B), flow rate: 0.3 ml/min, column temperature: 30 °C, detection wavelength: 420 nm. The chromatographic separation was performed on an Agilent 1100 HPLC system (degasser, binary gradient pump, autosampler, column thermostat, diode array detector).

3.3. X-ray crystallography

X-ray crystallographic analysis of diarylheptanoids is commonly applied to investigate the absolute structure of the compounds. The structure of the plane-chiral compound, (*R*)-(+)-galeon from *Myrica gale* var. *tomentosa* was determined by direct X-ray crystallographic analysis of the corresponding *p*-bromobenzoate derivative [102]. The absolute configuration of the biaryl-type diarylheptanoid (−)-alnusdiol (Fig. 2) and the relative configuration of the diarylether-type compound (−)-maximowicziol A, isolated from the heartwood of *Betula maximowicziana*, were proposed according to X-ray and CD analyses [76]. The absolute configuration of diocollettines A (Fig. 2), a diarylheptanoid derivative from *Dioscorea collettii* with an unusual skeleton where 6/5/5 fused rings are attached to the heptane chain, was also assigned, according to single-crystal X-ray diffraction and experimental electronic circular dichroism (ECD) data. Triclinic crystals of diocollettines A, formed after crystallization in cyclohexane/dichloromethane, were studied by X-ray crystallography [103]. Powder X-ray diffraction was used for the characterization of purified curcumin, demethoxycurcumin, and bisdemethoxycurcumin solid phases [75].

3.4. Circular dichroism (CD) spectroscopy

Curcumin is an achiral molecule [91], however, several diarylheptanoids bear a stereogenic center on the C_7 moiety (e.g. giffonins A–I), while giffonins J–P are characterized by at least two stereogenic centers in the alkyl chain [104]. There are also derivatives lacking an asymmetric carbon atom but possessing planar or axial chirality, e.g. (*R*)-(+)-galeon [102,105]. Tedarene B isolated from the marine sponge *Tedania ignis* shows central, axial, and planar chirality in the same molecule [106].

The stereochemistry of hedycoropyrans A–C and hedycorofurans A–D, isolated from *H. coronarium* rhizomes was evaluated by ECD spectroscopic analyses [6]. The sulfonated linear diarylheptanoid C-1 epimers, kaempsulfonic acids A and B were obtained from the rhizomes of *Kaempferia galanga*. ECD spectroscopic data were compared with those from the computational calculation method. Additionally, molybdenum(II) acetate induced circular dichroism (ICD) was also applied for the establishment of their absolute configurations [72]. The relative configurations of the biaryl-type giffonins T and U from *C. avellana* could be assigned by a combined quantum mechanical/NMR approach, the orientations of the hydroxyl groups on the heptyl chain, however, could not be determined. The absolute configurations were finally assigned, according to comparison of calculated and experimental ECD spectra [104]. The absolute configurations of some diarylheptanoids could be determined based on the data from the CD exciton chirality analyses of the corresponding 3,5-bis-*p*-bromobenzoyl and 3,5-bis-*p*-dimethylaminobenzoyl derivatives [46,107].

3.5. Nuclear magnetic resonance (NMR) spectroscopy

The NMR characteristics of naturally occurring linear and cyclic diarylheptanoids characterized until 2012 have been reviewed by Lv and She [1,2]. They provided a comprehensive collection of 409 distinct structures along with the available ^{13}C NMR data of the aglycones. Due to the plethora of core structures, a wide selection of NMR solvents was applied for sample preparation. Irrespective of the core type (linear or cyclic) altogether $2 \cdot 6 = 12$ (diaryl) + 7 (heptanoid) = 19 carbon atoms build up the diarylheptane skeleton. The structure identification, however, starts with the evaluation of the ^1H NMR spectrum which provides several entry points into the structure identification. Careful inspections of the aromatic region furnish the number and pattern of substitutions on the aromatic rings (usually ABX or AA'XX' systems) and also provide the number of unsaturation (sp^2 carbon-bound hydrogens) in the heptane chain (if any). Counting the aliphatic resonances in the heptane chain also suggests the type of substitution such as hydroxylation or the presence of keto group(s) as well. The number of methoxy

substitution can also be evident from the ^{1}H NMR spectrum, similarly to the presence of any glycosylation. The ^{1}H-^{1}H connectivities and the individual resonances of each spin system are usually mapped by COSY and TOCSY experiments, while these separated spin systems are attached to each other using the direct ^{1}H-^{13}C and long-range ^{1}H-^{13}C spin–spin couplings. Some representative examples presenting straightforward structure elucidation of linear diarylheptanoids can be found in references [32,43,46,108,109], while those of the cyclic derivatives are reported in [34,76,104,110].

The relative stereochemistry of linear diarylheptanoids (especially of those bearing other moieties) [111] and that of the cyclic ones [77] are usually established through NOESY/ROESY data. Masullo et al. have also deduced the relative configurations of giffonins by comparing experimental and predicted ^{1}H and ^{13}C values [77]. Further structural confirmations (site of glycosylation, absolute configuration of chiral centers) require chemical transformation of the compounds and subsequent NMR data collection on the modified structure. The removal of the sugar moieties is usually completed by acid hydrolysis, while unknown stereochemistry is resolved by the derivatization of diarylheptanoids by Mosher's acid.

The bioactivity-guided approaches are emerging in the field of natural product discovery [112], in which NMR can also facilitate the identification of the bioactive compounds by pattern recognition strategy. Abedini et al. [90] reported the use of ^{13}C NMR dereplication method for the unambiguous identification of oregonin, hirsutanonol, and alusenon 1a in the methanol extract of *A. glutinosa* bark. ^{1}H NMR-guided fractionation was also successfully applied for the identification of new linear diarylheptanoid structures produced by *D. villosa*. The ^{1}H iterative Full Spin Analysis (HiFSA) highlighted the characteristic doublets (AA'XX' system) of the *para*-disubstituted aromatic rings allowing the identification of several pyran-containing diarylheptanoid isomers [84].

Further advantage of NMR spectroscopy resides in its application as a detector in hyphenated techniques. With the use of HPLC-SPE-NMR, a pre-screening of the plant extract is possible thereby facilitating the identification of minor and new compounds in complex plant matrices. Yi-Chun et al. successfully applied this setup for the identification of ten diarylheptanoids in the *n*-butanol fraction of *A. formosana* leaves [17].

As NMR can also provide dynamic data on flexible molecules as well, solution conformation of curcumin was also determined by the NMR analysis of molecular flexibility in solution (NAMFIS) method. ^{1}H-^{1}H distances derived from quantitative NOE measurements and ^{3}J coupling-derived dihedral angle constraints helped to identify individual contributing conformers to the solution distribution of curcumin [113].

4. Separation of diarylheptanoids

4.1. Thin-layer chromatography (TLC)

Tønnesen et al. analyzed the applicability of different TLC systems for the estimation of curcuminoid content in turmeric samples. Separation of the three curcuminoids could be achieved using normal-phase silica gel TLC plates and a mobile phase with a mixture of chloroform-ethanol. Tailing of the spots, irreversible adsorption of the samples, and degradation of the colored compounds was observed. Chromatographic separation of curcumin, demethoxycurcumin, and bisdemethoxycurcumin on silica gel TLC plates can be unsatisfactory, because of the intense tailing, which occurs due to the enolic and phenolic moieties in the compounds. Addition of acid to the mobile phase reduced the tailing without impairing the separation. To overcome the difficulties regarding TLC separation and quantitation of curcuminoids, alternative methods applying modified stationary phases were also developed. With an amino-bonded stationary phase curcumin showed improved stability and decreased adsorption to the stationary phase at the application spot [114], while Rasmussen et al. reported the separation of curcuminoids using dihydrogen phosphate impregnated silica gel TLC plates [115].

The monographs of the European Pharmacopoeia require the identification of the official herbal drugs Curcumae longae rhizoma and Curcumae zanthorrhizae rhizoma by the detection of curcuminoids using TLC. The extracts of the drugs are separated on silica gel F254 plates with a mobile phase of acetic acid-toluene (20:80, v/v). Curcuminoids are detected without derivatization at 365 nm as green fluorescent zones. After post-chromatographic derivatization with anisaldehyde solution and heating at 100–105 °C, they can be visualized as reddish, brown and yellow zones at 365 nm [97,98].

A high-performance thin-layer chromatographic (HPLTC) method – providing reduced analysis time and more compact zones as compared to TLC – was developed for the simultaneous quantitation of curcuminoids in *C. longa* and *Curcuma amada* samples. The separation was achieved on silica gel 60 F_{254} HPLTC plates eluted with a mobile phase of chloroform-methanol. UV detection at 366 nm was applied, since it afforded better resolution compared to that at 430 nm [116]. Pathania et al. improved a quantitative HPTLC method using a stationary phase with spherical silica particles providing better resolution, reproducibility, and dense bands, as compared to other plates [117]. Another HPTLC method with densitometric detection at 430 nm was developed and validated for the analysis of curcumin as a bulk drug and in formulations. In this method, 10 min saturation of the chamber with the mobile phase could also improve resolution and resulted in a sharp zone for curcumin. The limits of detection and quantitation were 8 ng/spot and 25 ng/spot, respectively [118]. Similar results were reported by Paramasivam et al. for the densitometric analysis of the three curcuminoids. The limit of quantitation was estimated to be 0.1 μg/spot, the linearity of the method was analyzed in the range of 1–20 μg/spot for the three compounds [119].

A validated HPTLC method was developed for the quality control of *Alnus nepalensis* and the quantification of the linear diarylheptanoid aglycones platyphyllenone, alusenone, hirsutenone, and hirsutanonol, as marker compounds (Fig. 2). The quantitation was carried out by densitometry at 610 nm after derivatization with vanillin–sulfuric acid reagent [120]. As a follow up to this work, the diarylheptanoid glycosides platyphylloside, oregonin, and hirsutanonol-5-*O*-β-D-glucopyranoside (Fig. 2) were quantified in *A. nepalensis* by a validated HPTLC method. The linearity of the method was proved in the range of 333–3330 ng/spot [121].

As a representative example, the TLC separation of diarylheptanoids from *A. glutinosa* SFE extracts is shown in Fig. 3. The linear diarylheptanoid glycosides and aglycones appeared in visible light as blue zones, the presence of oregonin and hirsutenone was proved by comparison of their retention factors with those of standard compounds.

4.2. High-performance liquid chromatography (HPLC) with UV detection

High-performance liquid chromatography and recently ultrahigh-pressure liquid chromatography (UHPLC) are the most relevant separation methods in diarylheptanoid analysis. Chromatographic separation is usually performed in the reversed-phase mode, on C18-bonded silica columns. Both isocratic and gradient elution systems are applied, with water containing acetate or formate buffer, and methanol or acetonitrile as the most common eluents. The addition of acid to the aqueous eluent can prevent the formation of diarylheptanoid phenolate (or enolate) ions, therefore the resolution of diarylheptanoids can be improved

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Table 3
HPLC methods for the analysis of diarylheptanoids in different sample matrices.

Detected compounds	Sample	Stationary phase temperature	Mobile phase (v/v), flow rate	Detection	Ref.
C, DMC, BDMC validated (ICH)	*Curcuma longa*	Waters BEH Shield RP C18 2.1 × 100 mm, 1.7 μm 30 °C	0.05% phosphoric acid in H_2O-ACN 34:66 0.4 ml/min	420 nm	[124]
C, DMC, BDMC	standard solutions, turmeric MeOH extracts	Discovery1 HS C18, 3 μm, 150 × 2.1 mm 40 °C	A: 5 mM $HCOONH_4$ and 0.1% HCOOH in H_2O, B: ACN 0 min: 5% B, 2 min: 5% B, 57 min: 100% B, 60 min: 100% B, 65 min: 5% B, 75 min: 5% B; 0.25 ml/min	FTICR QIT MS (−) and (+)-ESI MS	[125]
C, DMC, BDMC quantification	SFE extracts of *Curcuma*	Shimadzu Hypersil ODS-C18 250 × 4.5 mm ambient temperature	CH_3COOH with the pH adjusted to 2.88, prepared in the proportion of 55:45 with ACN and H_2O 1 ml/min	425 nm	[51]
curcumin quantification	*C. longa* ethanol extract	Lichrosphere RP C18 250 × 4 mm	MeOH–IPA–H_2O—CH_3COOH 20:27:48:5 0.5 ml/min	420 nm	[96]
curcumin quantification validated (AOAC International)	food, dietary supplements containing *C. longa*	Kinetex C18 2.1 × 30 mm, 2.6 μm 55 °C	A: 0.1% HCOOH in H_2O, B: 0.1% HCOOH in ACN 0 min: 28% B, 1 min: 28% B, 2 min: 30% B, 4 min: 30% B, 4.1 min: 50% B, 6.0 min: 50% B; 1.4 ml/min	425 nm	[126]
C, DMC, BDMC quantitation validated	food samples (tablets, candies, tea)	Senshu Pak PEGASIL ODS C18, 2 × 150 mm, 5 μm	A: 0.01% CH_3COOH in H_2O, B: ACN 0 min: 45% B, 15 min: 95% B; 0.2 ml/min	ESI–MS/MS SIM	[127]
curcumin degradation kinetics	standard solutions	Waters C18 150 × 3.9 mm, 5 μm and J.T. Baker C18 150 × 3.9 mm, 5 μm	MeOH–1% CH_3COOH in H_2O 35:65 and THF–H_2O 40:60, 1% citric acid, pH 3.0 1.0 ml/min	280 nm	[128]
C, THC quantitation, validated	rat plasma	Phenomenex Luna C18 250 × 4.6 mm	ACN—H_2O 70:30, with 0.005% CH_3COOH 0.2 ml/min	(−)-ESI MS/MS MRM	[129]
curcumin, glucuronide and sulfate conjugates quantification	human plasma	μBondapak C18 250 × 4.6 mm, 10 μm	A: 0.1% CH_3COOH–65% MeOH–35% H_2O, B: MeOH 0 min: 100% of A, 15 min: 0% A; 2.0 ml/min	420 nm	[130]
DPH-4,6-dien-3ol DPH-6-en-3-ol	nanoemulsions of *Curcuma comosa* hexane extract	Thermo Hypersil silica 250 × 4.6 mm, 5 μm 25 °C	*n*-hexane–CH_2Cl_2 35:65 1.5 ml/min	302 or 250 nm	[131]
DPH-6-en-3-one DPH-6-en-3-ol DPH-4,6-dien-3-ol quantitation validated	plasma, brain, liver, kidney, ovary, uterus samples	Thermo Hypersil silica 250 × 4 mm, 5 μm 25 ± 1 °C	*n*-hexane–CH_2Cl_2 40:60 1.5 ml/min	302 or 250 nm	[132]
THC, galanganol C	*Zingiber mioga* and *Zingiber officinale* MeOH extract	Acquity BEH C18 100 × 2.1 mm, 1.7 μm	ACN—H_2O containing 0.1% HCOOH 0 min: 5% A, 10 min: 100% A, 12 min: 5% A 0.3 ml/min	(−) and (+) QTOF MS	[133]
acetoxy diarylheptanoids	*Z. officinale* rhizome, root, leaf, shoot	Discovery HS C18 150 × 2.1 mm, 3 μm 40 °C	A: 5 mM $HCOONH_4$ and 0.1% HCOOH in H_2O, B: ACN 0 min: 5% B, 2 min: 5% B, 57 min: 100% B, 60 min: 100% B, 65 min: 100% B; 0.25 ml/min	(−) and (+)-ESI MS IT MS/MS	[134,135]
10 diarylheptanoids quantitation (DAD)	steamed *Z. officinale* rhizome	Zorbax Extend-C18 4.6 × 150 mm, 5 μm 25 °C	A: H_2O, B: ACN 0 min: 20% B, min: 90% B, min: 100% B, 100% B 1 ml/min	280 nm (+)-TOF MS	[136]
7 diarylheptanoids	MeOH extracts of *Renealmia alpinia* *Renealmia cernua*	Hypersil BDS C18 250 × 4.6 mm, 5 μm	A: 15 mM *ortho*-phosphoric acid and 1.5 mM tetrabutylammonium hydroxide in H_2O, B: MeOH 0 min: 20% B, 17 min: 90% B, 20 min: 100% B, 28 min: 100% B; 1.0 ml/min	230 nm	[47]
hexahydrocurcumin, yakuchinone A quantification	*Alpinia officinarum* leaves MeOH extract	Kinetex XB-C18 2.1 × 50 mm, 2.6 μm 40 °C	A: 0.1% HCOOH in H_2O, B: MeOH 0 min: 0% B, 0.01 min: 2% B, 1.01 min: 2% B, 1.02 min: 35% B, 4.02 min: 35% B, 15.02 min: 90% B, 15.03 min: 2% B, 20.02 min: 2% B; 0.3 ml/min	(+)-ESI MS/MS	[137]

Table 3 (*Continued*)

Detected compounds	Sample	Stationary phase temperature	Mobile phase (v/v), flow rate	Detection	Ref.
23 diarylheptanoids	SFE extracts of *A. officinarum*	Acquity BEH C18, 1.7 μm, 2.1 × 50 mm 25 °C	A: 0.1% HCOOH in H_2O, B: ACN 0 min: 22% B, 6 min: 36% B, 11.5 min: 42% B, 14.5 min: 50% B, 18 min: 80%B, 21 min: 80% B, 25 min: 100% B 0.25 ml/min	Q-TOF (+)-ESI MS/MS	[49]
yakuchinones A and B, oxyphyllacinol quantification	*Alpinia oxyphylla* capsular fruits	Phenomenex Synergi Fusion-RP 2.0 × 50 mm 40 °C	A: 0.1% HCOOH in H_2O, B: 0.1% HCOOH in MeOH 0 min: 0% B, 1 min: 0% B, 1.01 min: 80% B, 3.51 min: 100% B, 3.52 min: 0% B, 6.02 min: 0% B	(+)-ESI MS/MS MRM	[138]
aurogenin G, alusenone platyphyllenone	*Alnus nepalensis* extracts	Waters Spherisorb ODS-2, 250 × 4.6 mm, 10 μm	MeOH—H_2O containing 0.1% TFA 60:40 1 ml/min	224 nm	[139]
oregonin quantification	*Alnus rubra* leaves	Luna C18 150 × 4.6 mm 5 μm	ACN—H_2O 25:75 0.6 ml/min	280 nm	[140]
oregonin quantification	*A. glutinosa*	Kinetex C8 4.6 × 100 mm, 2.6 μm	A: H_2O with 0.025% TFA, B: ACN 0 min: 20% B, 5 min: 40%, 5.5 min: 100% B, 7.5 min: 100% B; 1.5 ml/min	210/254/280/366 nm	[90]
platyphylloside	*Betula pubescens, Betula pendula*	Nova-Pak C18 Radial-Pak 8 × 100 mm	A: 0.01 M pH 2.8 sodium phosphate in H_2O, B: ACN 0 min: 5% B, 30 min: 20% B, 40 min: 60% B, 42 min: 80% B; 1.5 ml/min	280 nm	[141]
aceroside VIII, platyphylloside quantification	standardized extract of *B. platyphylla* bark	Waters Xterra RP18 5 μm, 4.6 × 150 mm 0 min: 80% B, 60 min: 20% B 0.3 ml/min	A: ACN, B: H_2O with 0.03% HCOOH	280 nm (−)-ESI MS	[122]
juglanin A, juglanin B, rhoiptelol, regiolone quantification, validated	*Juglans regia* green husks MeOH extracts	Sinochrom ODS-AP C18 250 × 4.6 mm, 5 μm 25 °C	A: ACN, B: 2% CH_3COOH in H_2O 0 min: 80% B, 15 min: 30% B, 20 min: 30% B, 21 min: 80% B, 25 min: 80% B; 1.0 ml/min	280/259 nm	[142]
carpinontriol B quantification	hazelnut shells MeOH extract	Nucleodur 100-5 C18 150 × 4.6 mm, 5 μm	A: H_2O, B: MeOH 0 min: 5% B, 3 min: 5% B, 7 min: 30% B, 17 min: 30% B, 35 min: 50% B, 50 min: 100% B; 0.8 ml/min	230 nm	[143]
asadanin quantification	*Corylus avellana* kernels	Kinetex Luna Phenyl-Hexyl 1.7 μm, 100 × 2.1 mm 45 °C	A: 1% HCOOH in H_2O, B: 1% HCOOH in ACN 0 min: 5% B, 1 min: 5%, 3 min: 30% B, 12 min: 70% B, 13 min: 100% B, 13.5 min: 100%, 14.5 min: 5% B, 19.5 min: 5% B; 0.4 ml/min	MS/MS, TOF MS MRM	[63]
oregonin, hirsutenone quantitation validated (ICH)	*C. avellana* and *Corylus maxima* leaves and bark	Zorbax SB C18 150 × 3.0 mm, 3.5 μm 25 °C	A: 0.2% CH_3COOH: B: MeOH 0 min: 30% B, 20 min: 100% B, 22 min: 100% B, 23 min: 30% B, 26 min: 30% B; 0.3 ml/min	280/340 nm ESI-QQQ MRM	[144,145]
giffonins Q-S quantification validated (EMA)	*C. avellana* flowers	Atlantis T3 5 mm RP C18 150 × 2.1 mm	A: 0.1% HCOOH, B: ACN 0 min: 5% B, 5 min: 5% B, 50 min: 100% B, 55 min: 100% B; 0.2 ml/min	ESI –MS, MRM	[146]

Abbreviations: $HCOONH_4$: ammonium formate, HCOOH: formic acid, CH_3COOH: acetic acid, TFA: trifluoroacetic acid, H_2O: water, CH_2Cl_2: dichloromethane, ACN: acetonitrile, MeOH: methanol, C: curcumin, DMC: demethoxycurcumin, BDMC: bisdemethoxycurcumin, THC: tetrahydrocurcumin, DPH-6-en-3-one: (6*E*)-1,7-diphenylhept-6-en-3-one, DPH-4,6-dien-3-ol: (4*E*,6*E*)-1,7-diphenylhepta-4,6-dien-3-ol, DPH-6-en-3-ol: (6*E*)-1,7-diphenylhept-6-en-3-ol, DAD: diode array detection, ESI: electrospray ionization, IT: ion trap, TOF: time-of-flight, MS/MS: tandem mass spectrometry, SIM: selected ion monitoring, MRM: multiple reaction monitoring.

[122]. HPLC analysis is usually performed at ambient temperature, however, higher temperatures are also commonly used to reduce analysis time and improve the repeatability of retention times [123]. UV/Vis detection is a prevalent method in HPLC analyses of diarylheptanoids; (for the spectral characteristics see Section 3.1). Selected examples for the application of HPLC coupled to different detection techniques in diarylheptanoid analyses are listed in Table 3.

4.2.1. HPLC of the diarylheptanoids in Zingiberaceae species

The literature on HPLC analysis of diarylheptanoids is dominated by the papers on *Curcuma* and *Zingiber* species. Plants belonging to these genera contain typically linear diarylheptanoid aglycones, i.e.

conjugates with sugars do not occur. The polarity of the compounds is determined by the saturation and substitution of the C_7 chain as well as by the functional groups of the aromatic rings.

Numerous papers focus on the chromatographic separation of curcumin and its demethoxy derivatives. It is known that the highly reactive diketone group of curcuminoids can form intra- and intermolecular hydrogen bonds. It has been assumed that curcuminoids bind irreversibly to the accessible, non-derivatized silanol groups on the silica surface of the stationary phase in C18 columns, thus causing loss of reproducibility [147]. Other authors claimed when citing this work, that due to the very labile characteristics of curcuminoids, C18 columns are preferred for their HPLC analysis [148]. Tønnesen and Karlsen applied a modified amino-bonded column as stationary phase and ethanol as the mobile phase which provided baseline separation of the three curcuminoids [149]. Rouseff stated that the common reversed-phase solvents, water-methanol and water-acetonitrile mixtures did not provide satisfactory resolution on a C18 column, furthermore, tailing did occur, when analyzing curcuminoid pigments in turmeric. Separation of the compounds was obtained by a water-tetrahydrofuran mixture, though the elution order of the three constituents was reversed by the organic modifier to 1. curcumin, 2. demethoxycurcumin, and 3. bisdemethoxycurcumin [150], as compared to the elution order of the compounds obtained with a water-acetonitrile solvent system (Fig. 4.).

It has to be mentioned, that residual silanol groups are eliminated in end-capped C18 stationary phases, thus peak tailing effect is reduced. Nevertheless, another crucial condition is the acidity of the eluents applied. A validated C18 HPLC method was developed for the analysis of curcuminoids in turmeric rhizome extracts and in order to study the effect of different ionization suppressing agents such as citric acid, acetic acid, *ortho*-phosphoric acid, and trifluoroacetic acid on resolution and peak symmetry. Good peak shape and resolution could only be achieved with trifluoroacetic acid due to the partial deactivation of the stationary phase by the highly electronegative fluorine atoms [151]. A reversed-phase UHPLC method with an analysis time of 2 min was developed by Cheng et al. for the simultaneous quantification of curcuminoids in *C. longa.* The authors suggested that the use of a column with an embedded carbamate group incorporated into the bonded phase prevented peak tailing of the analytes. The elution order of the curcuminoids, however, was reversed on the C18 column embedded with carbamate groups compared to the conventional C18 column (Fig. 4). Acetic acid, phosphoric acid, and trifluoroacetic acid all gave good resolution and symmetry factor. Increase of the column temperature up to 35 °C improved the resolution and the symmetry of the peaks [124]. The characteristic HPLC-DAD chromatogram of curcumin, demethoxycurcumin, and bisdemethoxycurcumin can be seen in Fig. 4.

HPLC methods applying a C18 column and isocratic elution have been employed in the quantification of curcuminoids. Bos et al. validated a method to determine curcumin, demethoxycurcumin, and bisdemethoxycurcumin contents in rhizomes of *Curcuma* species. The chromatographic separation was performed using isocratic elution with a methanol-0.1% TFA in water-ACN mixture as mobile phase. Baseline separation of the analytes was achieved in a run time of 10 min [152]. Heath et al. developed and validated an isocratic HPLC method to quantify curcumin in plasma and urine samples [153]. Curcumin is reported to have anti-amyloidogenic activity, however, its efficacy is limited due to its low bioavailability. In order to improve the ability of curcumin to cross the blood-brain barrier, it was encapsulated in polylactide-co-glycolic-acid nanoparticles. The drug loading capacity and encapsulation efficiency was determined [154]. HPLC with isocratic elution is also used in solubility and stability studies of curcumin preparations [155]. Pan et al. investigated the pharmacokinetic properties of curcumin after i.p. administration to mice using isocratic, reversed-phase HPLC assays [156].

Reversed-phase HPLC and UHPLC methods employing C18 columns and gradient solvent systems are universally applied for the determination of curcuminoid contents in diverse *Curcuma* samples [126,148,157]. The pharmacokinetic parameters of curcumin in healthy human volunteers were investigated after a single oral dose of 10 or 12 g. Free curcumin, its glucuronide, and sulfate conjugates were analyzed in serum samples using a C18 HPLC method with a linear gradient. The LOD of the method was 50 ng/ml, however, free curcumin was detected in only one subject [130].

For the phytochemical characterization of medicinal plant extracts that comprise numerous diarylheptanoids with diverse physical/chemical properties (e.g. glycosides – aglycones), yet contain several structural analogues at the same time, reversed-phase HPLC methods with (multi-step) gradient elution systems and long elution times are needed [47,60,158]. Wu et al. optimized a C18 HPLC method with gradient elution to achieve complete separation of *A. officinarum* diarylheptanoids in 60 min [86].

The HPLC separation of less polar diarylheptanoids has been performed using normal-phase methods. Estrogenic effect of *Curcuma comosa* and its two major components, (4*E*,6*E*)-1,7-diphenylhepta-4,6-dien-3-ol and (6*E*)-1,7-diphenylhept-6-en-3-ol (Fig. 2.) was reported. To improve the poor solubility, the plant extract and the two diarylheptanoids were formulated into nanoemulsions. A validated, normal-phase HPLC method was used to gain information on the entrapments, drug loading ratios and *in situ* intestinal absorption rates of diarylheptanoid-nanonemulsions [131]. Su et al. studied the pharmacokinetic profile and organ distribution of the phytoestrogenic compounds from *C. comosa* hexane extract in rats after i.v. or p.o. administration. The quantities of the analytes were measured by a validated, normal-phase HPLC method [132].

4.2.2. HPLC study of polyhydroxylated diarylheptanoids

Plant species belonging to the Betulaceae (and Juglandacee) family contain typically linear or macrocyclic diarylheptanoids conjugated with saccharide moieties [159,160]. The polarity of the compounds may be further increased when the constituents are hydroxylated [41,77].

Qualitative and quantitative phytoanalytical studies by RP-HPLC were performed on inner bark of *Betula pendula* and *Betula pubescens*. Platyphylloside was quantified and found to be present in high amounts in *B. pendula* and at low levels in *B. pubescens* [141]. Santamour and Lundgren investigated about 70 taxa of the genus *Betula*. The tests included RP-HPLC analyses of inner bark extracts on a C18 column with a multi-step gradient in 42 min. Determination of platyphylloside contents helped the identification of the diverse species and verification of interspecific hybrids [161]. The contents of oregonin in *A. glutinosa* extracts have been determined using isocratic [140,162] and also gradient [59] C18 HPLC methods. Kinetic analyses on aqueous stability of hirsutenone were carried out by a reversed-phase HPLC method [163]. An analogous method was applied to determine hirsutenone concentrations when inclusion complexation with β-cyclodextrin was performed [164]. Biaryl-type (juglanin B) and diarylether-type (juglanin A, rhoiptelol) macrocyclic diarylheptanoids (Fig. 2) in methanol extracts from the green husks of *Juglans regia* have been determined simultaneously by a validated HPLC-DAD method. The separation was achieved on a C18 column with gradient elution. Acetonitrile was chosen as the organic eluent, since it gave better resolution, peak shape and stable baseline compared with methanol, while 2% (v/v) acetic acid in water as the aqueous eluent suppressed the peak tailing and good separation could be achieved [142].

Abedini et al. obtained fractions by CPC from the methanol extract of *A. glutinosa* bark and analyzed the diarylheptanoid com-

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position of those with antimicrobial activity by HPLC. The authors used, contrary to the routine methods, a C8 core-shell column as the stationary phase [90]. In our previous work, the contribution of the individual phenolic compounds including linear diarylheptanoids to the total antioxidant activity of *C. avellana*, *Corylus colurna*, and *Corylus maxima* extracts was studied by an HPLC method coupled off-line to the DPPH radical scavenging assay [165].

4.3. Further LC detection methods

4.3.1. HPLC with fluorescence detection (HPLC-FLD)

Curcuminoids exhibit strong intrinsic fluorescence [166]. The emission spectrum of curcumin is a broad band. The fluorescence maximum is detected around 520–525 nm in acetonitrile, while in H-bond donors (e.g. alcohols) greater red shifts (λ_{Fl} = 550–565) are exhibited [92,93]. A fluorometric method was developed to determine curcumin in yogurt and mustard samples. Dynamic ranges of the methods covered 4 orders of magnitude concentrations (0.27–1500 ng/ml), a detection limit as low as 0.08 ng/ml was achieved [167]. Tønnesen and Karlsen applied their HPLC method with a fluorescence detection mode to determine the content of individual curcuminoids in *Curcuma* samples. The fluorimetric excitation maximum was at 420 nm, while the emission at 470 nm resulting in a very low detection limit. Even 20 pg bisdemethoxycurcumin could be detected [149].

4.3.2. HPLC with evaporative light scattering detection (HPLC-ELSD)

The diarylheptanoids from the seeds of *A. longigilugare* with estrogenic activity were isolated by HSCCC and preparative HPLC. The contents of the estrogenic diarylheptanoids were determined by HPLC, with a low temperature evaporative light scattering detector in *Amomum* samples of different origin [85]. For the evaluation of cytotoxic compounds from *C. avellana* stems and leaves, an HPLC–MS method with a photodiode array detector and an evaporative light-scattering detector was employed [54].

4.3.3. HPLC with electrochemical detection (HPLC-ECD)

1,7-Diphenylhept-4-en-3-one and its derivatives with hydroxyl substitution on one aromatic ring, as well as diarylheptanoid compounds with a 4″-hydroxy-3″-methoxyphenyl ring from the rhizomes of *A. officinarum* have been separated by a C18 HPLC method and quantified using electrochemical detection (ECD) in the oxidative mode. The HPLC-ECD method showed higher sensitivity than the UV detection for the compounds that could undergo redox reactions. These include compounds possessing electroactive functional groups, such as the hydroxyl or methoxy groups of an aromatic ring [168].

4.4. Liquid chromatography-mass spectrometry (LC–MS)

4.4.1. Ionization interfaces, mass analyzers

Mass spectrometric (MS) detection coupled to HPLC is the method of choice for the chromatographic separation, qualitative and quantitative phytochemical characterization of plant extracts containing diarylheptanoids.

Atmospheric pressure ionization techniques, such as electrospray ionization (ESI) [122,129,169–171], with both negative [134] and positive [138,172] ion modes have been almost exclusively applied in the MS evaluation of diarylheptanoids. They can be ionized readily and provide simple MS/MS spectra in negative ionization mode, however, the sensitivity of (−)-ESI is low in comparison with (+)-ESI. On the other hand, complex TIC chromatograms and stronger background noise are produced –and adduct ions are also often formed in the positive mode. Further ionization techniques applied for the analysis of curcuminoids are thermospray MS and particle beam MS with electron ionization source [173].

The mass analyzers used in the characterization of diarylheptanoids are time-of-flight (TOF) MS, which enables accurate mass analysis [50,53,63,136], triple-quadrupole (QQQ) MS [75,146,174] that provides additional structural information by the observation of the fragmentation pattern of the target analytes, while quadrupole-time-of-flight (Q-TOF) technique offers exact mass measurement of both precursor and fragment ions [49,175,176]. High-resolution ion trap MS analyzer was also applied in the characterization of diarylheptanoid fragmentation in *Z. officinale* [135].

4.4.2. Fragmentation behavior of diarylheptanoids

Unambiguous identification of diarylheptanoids is not possible using LC–MS exclusively, however, structural information can be gained by evaluating the fragmentation of the molecular ions in (high-resolution) tandem or multiple-stage MS analyses. Linear diarylheptanoids can be classified according to differences in the heptane skeletons. Homologs belonging to the same class differ by substitution patterns on the aromatic rings. The fragmentation behavior is diagnostic for diarylheptanoids belonging to different classes of homologous series. As an example, the (−)-ESI and (+)-ESI fragmentation of 1,6-heptadiene-3,5-diones comprising curcuminoids is described, according to the results of Jiang et al. The fragmentation behavior of the three major curcuminoids in quadrupole ion trap MS with positive and negative ESI mode, as well as by sustained off-resonance irradiation (SORI) experiments in a Fourier transform ion cyclotron resonance (FTICR) MS was characterized. Both ion trap and ICR instruments provided the same fragment ions. Diagnostic fragments were identified and the fragmentation schemes were proposed for the three curcuminoids [125,177]. Curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) possess a common structural moiety of 3- and 5-oxo groups on the 1,6-heptadiene skeleton. In the negative mode, precursor ions at *m*/*z* 307, 337, and 367 (ions A) were detected and were assigned to $[M\text{-}H]^-$ of BDMC, DMC, and C, respectively (Fig. 5). In the MS/MS spectra of the $[M–H]^-$ ions the base peaks (labelled as B) were the ions *m*/*z* 187, 187/217, 217. The presence of one or two methoxy groups on the aromatic rings resulted in the mass shifts by 30 Da with reference to the *m*/*z* 187 product ion of BDMC. There were two alternative routes, that could lead to the loss of a neutral moiety and as a result, to the formation of product ion B. Rearrangement of B and loss of a CO_2 resulted in formation of ions C (*m*/*z* 143, 143/173, 173). Product ions D (*m*/*z* 119, 119/149, 149) could be formed from both the diketone and the keto-enol form of A (Fig. 6). The authors produced monoacetate derivatives of all the compounds, and additionally the diacetate of curcumin. The fragmentations of these compounds with protected phenolic hydroxyl groups were analyzed in direct infusion (−)-ESI–MS/MS and LC-(−)-ESI–MS/MS experiments, in order to elucidate, whether the enolic hydroxyl groups were accessible for deprotonation, and thus formed the $[M\text{-}H]^-$ ions. It was suggested, that both the phenolate and the enolate molecular ions could be detected in (−)-ESI of the curcuminoids. The formation of the phenolate $[M\text{-}H]^-$ ion was favored in the presence of two *p*-hydroxyl groups on the aromatic rings, whereas when both *p*-hydroxyl groups were substituted, only the enolate $[M\text{-}H]^-$ ion was formed.

The fragmentations of curcuminoids in (+)-ESI–MS/MS were different from the patterns observed for (−)-ESI–MS/MS (Fig. 7). The protonated molecular ions $[M+H]^+$ for BDMC, DMC, and C were detected at *m*/*z* 309, 339, and 369 (ions E), respectively. It was proposed, that the ionization in positive mode occurred on the C_7 chain instead of on the aromatic rings. The diagnostic product ions, all originating from the protonated precursor ion $[M+H]^+$ were detected for all three curcuminoids. The rearrangement of

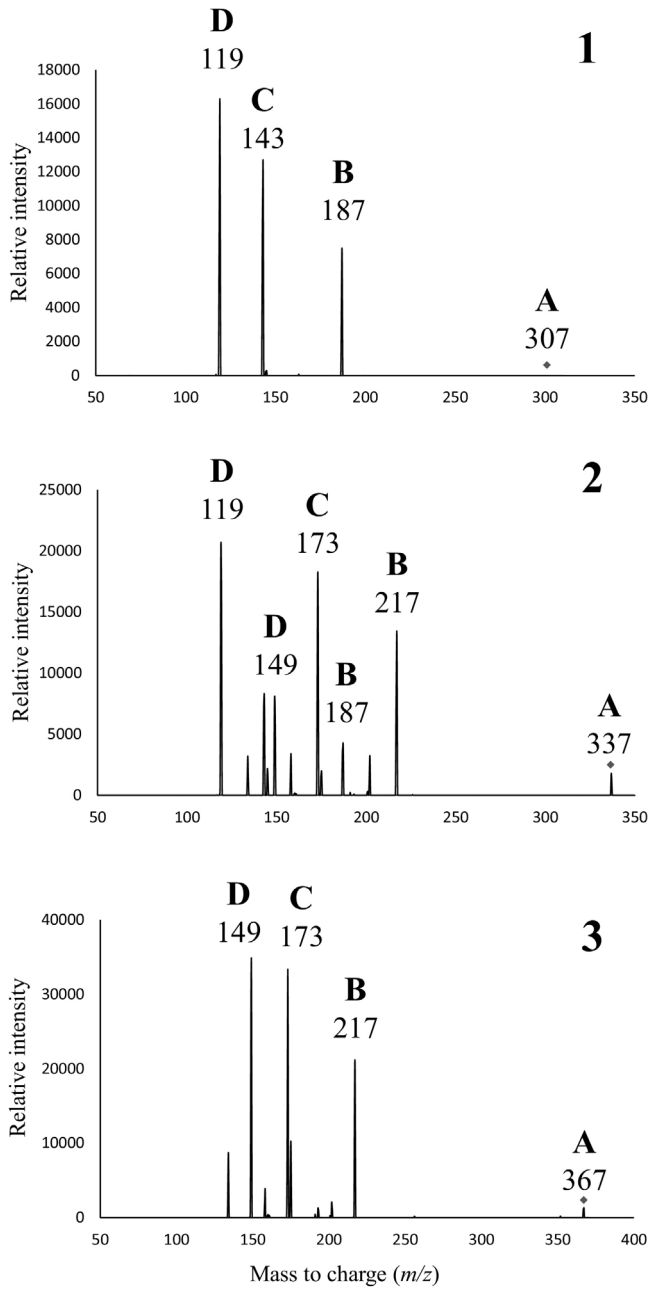

Fig. 5. The (−)-ESI mass spectra of bisdemethoxycurcumin (1), demethoxycurcumin (2), and curcumin (3). The alphabetical notation of the major diagnostic fragment ions refers to the (−)-ESI fragmentation scheme of the compounds shown in Fig. 6. ESI–MS conditions were as follows: Agilent 6410 Triple Quadrupole mass spectrometer with an electrospray ion source in negative ionization mode, temperature: 350 °C, nebulizer pressure: 40 psi (N_2), drying gas flow rate: 9 l/min (N_2), capillary voltage: 4000 V, fragmentor voltage: 120 V, collision energy: 15 eV, high purity nitrogen was used as collision gas.

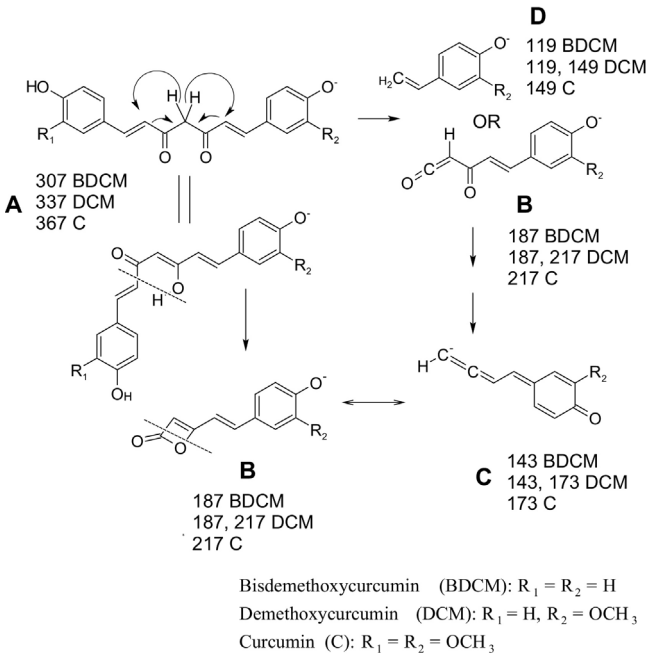

Fig. 6. The (−)-ESI fragmentation scheme of bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and curcumin (C) depicting the major diagnostic fragment ions, according to Jiang et al. [125,177]. The alphabetical notation of the major fragment ions refers to the (−)-ESI mass spectra of the compounds shown in Fig. 5.

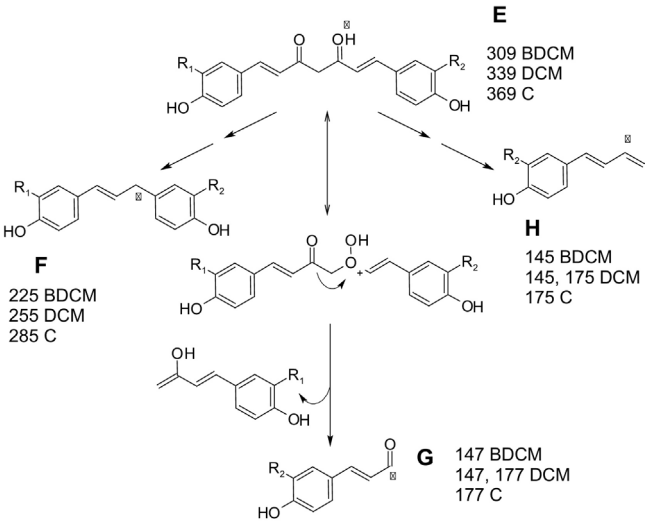

Fig. 7. The (+)-ESI fragmentation scheme of curcuminoids (BDMC: bisdemethoxycurcumin, DMC: demethoxycurcumin, C: curcumin) showing the major fragment ions [125,177].

the protonated molecular ions E and the loss of a 1-hydroxy-3-ketocyclobutene moiety yielded product ions F (*m/z* 225, 255, and 285). Ions G (*m/z* 147, 147/177, 177) were generated by a 3,4-bond cleavage of E and a neutral loss of a 1-aryl-3-hydroxy-1,3-butadiene moiety. Ions H (*m/z* 145, 145/175, 175) were produced by an oxo migration in the diketone form of E and a neutral loss of a 3-arylcarboxyprop-2-ene moiety [125,177].

The (−)-ESI mass spectra, the (−)-ESI, and (+)-ESI fragmentation schemes of curcumin, demethoxycurcumin, and bisdemethoxycurcumin depicting the major diagnostic fragment ions are shown in Figs. 5–7, respectively.

Neutral losses generated in the fragmentation of diarylheptanoids provide certain structural information. Loss of 18 Da resulting in the product ion $[M+H\text{-}H_2O]^+$ in the positive ionization mode suggests the presence of a hydroxyl group on the heptane chain instead of on the aromatic rings. When the corresponding ion $[M+H-2H_2O]^+$ with lower abundance is also produced, it is proposed that an oxo group exists on the heptane skeleton rather than a second hydroxyl group, and the product ion is formed by a rearrangement and elimination of H_2O from the ion $[H+H\text{-}H_2O]^+$. The presence of $[M+H-2H_2O]^+$ with higher relative intensity than

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that of $[M+H-H_2O]^+$ suggests the presence of a of 3,5-dihydroxy moiety on the alkyl chain instead of on the aromatic rings. The elimination of 16 Da points to the presence of a hydroxyl group on the aromatic rings, while the loss of 60 Da suggests the presence of an acetoxy group. Diarylheptanoids containing 3,5-diacetoxy moieties were described for *Z. officinale*. The product ions $[M+H-60]^+$ and $[M+H-120]^+$ were commonly detected in their mass spectra, due to the successive loss of two molecules of acetic acid [135,175].

The composition of the saccharide moieties can be assumed by observing neutral losses produced in negative ESI ionization mode during collision-induced dissociation of diarylheptanoids. In case the compounds are glycosylated through a hydroxyl group on the C_7 chain, the neutral loss of 180 Da refers to a hexose residue, while that of 150 Da points to a pentose moiety [144,145]. Nevertheless, when the sugar residues are attached to a hydroxyl group on the aromatic rings (e.g. in the cyclic biaryl-type giffonin I), the loss of 162 Da corresponds to a hexose, while that of 132 Da refers to a pentose moiety [146].

The literature regarding ESI fragmentation of diarylheptanoids covers plant extracts, foodstuff as well as a TCM formula. Chromatographic and MS/MS fragmentation characteristics were collected from *B. pendula* and *Betula papyrifera* samples [178,179]. Phenolics including linear diarylheptanoid glycosides and aglycones with *invitro* antioxidant activity were analyzed by LC-DAD-ESI-TOF and LC-ESI-QQQ-MS in *C. colurna* leaves, bark, catkins and involucres [180]. A multiple-component tablet containing standardized extracts of three medicinal plants was also studied [175]. Chemical composition of fresh, dried, and steamed ginger has been evaluated and compared using TOF-MS [136]. Ma et al. performed the metabolic profiling of greenhouse-grown and *in vitro* micropropagation derived *C. longa* and *Z. officinale* plants [134,169]. Table 4 contains the characteristic molecular ions and fragment ions of several diarylheptanoids obtained by diverse MS techniques.

LC–MS has also been used in stability and pharmacokinetic analyses of diarylheptanoids. Phase 1 metabolism of demethoxycurcumin was investigated by LC/MS^n [171], while Burapan et al. reported recently on new colonic metabolites of curcumin, demethoxycurcumin, and bisdemethoxycurcumin produced by the human intestinal bacterium *Blautia* sp. [181]. The diarylheptanoids yakuchinones A-B and oxyphyllacinol, as well as their reduced and monoglucuronide metabolites have been investigated by LC–MS/MS in SRM mode in rat plasma after p.o. administration of *Alpinia oxyphylla* extract. [182]. Autoxidation and horseradish peroxidase-catalyzed oxidation of curcumin and curcumin-glucuronide have been investigated by ESI–MS [170,183].

4.4.3. Quantitative MS characterization of diarylheptanoids

Tandem mass spectrometry in selected ion monitoring (SIM) or multiple-reaction monitoring (MRM) modes is frequently applied for the quantification of diarylheptanoids. A quantitative LC–MS method employing an ESI interface was developed to determine the curcuminoid content of food samples, e.g. tablets, candies, and tea. The highest signal intensity for curcuminoids was obtained with a fragmentor voltage of 120 V and 0.01% acetic acid in water as aqueous mobile phase. The sample preparation of foodstuff with low curcuminoid content comprised a solid-phase extraction (SPE) pretreatment process, the curcuminoids retained on the C8 cartridges were eluted with ethanol [127]. In our previous work, a validated LC-ESI–MS/MS method was developed using the MRM mode to determine hirsutenone and oregonin contents of *C. avellana* and *C. maxima* leaf and bark samples. The linearity of the method for hirsutenone was evaluated in the range of 0.01–50 μg/ml. Concentrations of 0.002 μg/ml and 0.008 μg/ml were estimated as LOD and LOQ values, respectively [144,145]. The content of the cyclic diarylheptanoid alnusone in *C. avellana* male flowers has also been quantified by ESI-QQQ-MS in MRM mode. The method possessed a linear dynamic range between 0.0015-0.020 μg/ml [146]. Asadanin, a bitter tasting cyclic diarylheptanoid has recently been isolated from hazelnut kernels. Sensory analysis of hazelnut samples, followed by LC–MS/MS quantitation of asadanin showed evidence for the box bug (*Gonocerus acuteangulatus*) infection as the major inductor of asadanin production in hazelnut kernels and, in consequence, as the reason for bitter off-taste development [63]. LC-ESI–MS/MS was applied for the quantification of yakuchinone A together with hexahydrocurcumin in *A. officinarum* leaves and rhizomes. The LOD was 0.05 ng/ml for yakuchinone A. The linearity of the method was evaluated in the range of 1–100 ng/ml and 2–2000 ng/ml for yakuchinone A and hexahydrocurcumin, respectively [137]. Liu et al. developed a validated MS/MS method to determine curcumin and its metabolite tetrahydrocurcumin (Fig. 2) in rat plasma. The method had an LLOQ of 0.5 ng/ml, which corresponded to 2.5 pg for the 5 μl injection volume [129].

4.5. Less common separation techniques

4.5.1. Gas chromatography (GC)

GC offers high resolution and low detection limit, though it has some limitations in diarylheptanoid analysis, due to the thermal degradability and non-volatile character of the compounds. Jolad et al. analyzed the MS fragmentation patterns of diarylheptanoids in *Z. officinale* extracts by GC–MS. They detected gingerinone A and tetrahydrocurcumin as well as 3-hydroxy-5-acetoxyheptanes, 3,5-diacetoxy-heptanes, and diarylheptanoids containing a 5-methoxy and a 3-keto group. However, the partial or total thermal decomposition of the compounds occurred [184,185]. Derivatization of the target analytes is intended to eliminate this disadvantage. Harvey detected ginger constituents – diarylheptanoids (hexahydrocurcumin, demethylhexahydrocurcumin), gingerols, and shogaols – as trimethylsilyl derivatives by GC–MS [186]. Phenolic compounds including diarylheptanoids in the sapwood of *B. pendula* were quantified by GC equipped with a flame ionization detector and an HP-5 capillary column, after derivatization with a silylating mixture [187].

4.5.2. Capillary electrophoresis (CE)

Lechtenberg et al. separated and quantified the three curcuminoids in *Curcuma domestica* and *Curcuma xanthorrhiza* using a capillary zone electrophoresis (CZE) method in an analysis time of less than 5 min [188]. Yuan et al. also developed a validated CZE method with UV detection for the quantitation of curcuminoids. The compounds could be separated within 7 min. The method was applied to determine curcuminoids in urine samples from rats administered curcumin [189]. An optimized CZE method with UV detection was developed for the simultaneous determination of the macrocyclic diarylheptanoids rhoiptelol and juglanins A-C in green walnut husks [190]. Oregonin content in *Alnus* samples and biological matrices was also quantified using a fast CZE method with UV detection. The optimized conditions allowed as short analysis time as 6 min. Oregonin contents were examined in spiked human serum samples as well as in pig urine and dialysate samples after intragastrical administration of an oregonin-rich fraction from the leaves of *A. formosana* [89]. Watanabe et al. developed a rapid micellar electrokinetic chromatography (MEKC) method using a high molecular mass surfactant for the separation and determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in turmeric samples. The method was compared to an isocratic HPLC separation, the detection limits for curcuminoids were 0.02 μg/ml (by HPLC) and 0.1 μg/ml (by MEKC) [191]. A microemulsion electrokinetic chromatography (MEEKC) method was developed for the separation and quantitative analysis of curcuminoids in turmeric

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Table 4
Mass spectrometric features (analysis) of diarylheptanoids.

Compound	Negative ionization, mass analyzer	Molecular ion $[M-H]^-$ (m/z)	Fragment ions (m/z)	Ref.
Hirsutenone	ESI QQQ	327	289, 254, 239, 205, 121	[144,145,180]
1,7-Bis-(4-hydroxyphenyl)-4,6-heptadien-3-one	ESI QQQ	293	236, 211, 191, 163, 135	[180]
Centrolobol hexoside	ESI ion trap	461	299, 177, 121	[179]
Centrolobol hexosyl-pentoside	ESI ion trap	593	461, 299, 177, 121	[179]
Centrolobol hexosyl-pentosyl-pentoside	ESI ion trap	725	593, 461, 299	[179]
Aceroside VII	ESI QTOF	461	n.d.	[178]
Aceroside VIII	ESI QTOF	593	n.d.	[178]
Platyphyllonol-pentoside	ESI QQQ	445	295, 189, 83	[145]
Platyphylloside	ESI ion trap ESI QTOF	475	295	[178,179]
(5*S*)-5-hydroxy-1,7-bis-(4-hydroxyphenyl)-3-heptanone 5-*O*-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside	ESI QTOF	607	475	[178]
5-Hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)-heptan-3-one	ESI QQQ	329	179, 165, 149, 121	[144,145]
1-(4-Hydroxyphenyl)-7-(3,4-dihydroxyphenyl)-heptan-3-one-5-*O*-pentoside	ESI QQQ	461	311, 189, 121	[145]
Oregonin	ESI QQQ	477	417, 335, 327, 205, 125	[144,145,180]
Hirsutanonol-hexoside	ESI QQQ	507	327, 205	[144,145]
5-Hydroxy-1,7-bis-(4-hydroxyphenyl)-hept-4-en-3-one-hexoside	ESI QQQ	473	293, 89	[144,145]
Bisdemethoxycurcumin	ion trap ESI MS/MS	307	187, 143	[125,177]
Demethoxycurcumin	ion trap ESI MS/MS	337	217, 187, 173	[125,177]
Curcumin	ion trap ESI MS/MS	367	217, 173	[125,177]
Dihydrobisdemethoxycurcumin	ion trap ESI MS/MS	309	203, 189, 119	[177]
Dihydrodemethoxycurcumin	ion trap ESI MS/MS	339	337, 219, 189, 149, 119	[177]
Dihydrocurcumin	ion trap ESI MS/MS	369	233, 219, 175, 149, 135	[177]
Asadanin	ESI TOF	343	343, 283, 270, 211, 193	[63]
Alnusone	ESI QQQ	293	251, 83	[146]
Giffonin I	ESI QQQ	587	293	[146]

Compound	Positive ionization, mass analyzer	Molecular ion (m/z)	Fragment ions (m/z)	Ref.
5-Hydroxy-1,7-bisphenyl-3-heptanone	ESI Q-TOF	283	265, 247, 149, 133, 131, 117, 105, 91	[49]
1-Phenyl-7-(4-hydroxy-3-methoxylphenyl)-4-en-3-heptanone	ESI Q-TOF	311	293, 163, 161, 137, 133, 105	[49]
1-Phenyl-7-(4-hydroxyphenyl)-4-en-3-heptanone	ESI Q-TOF	281	263, 161, 143, 133, 107, 105	[49]
5-Hydroxy-7-(4-hydroxyphenyl)-1-(4-hydroxy-3-methoxylphenyl)-3-heptanone	ESI Q-TOF	345	327, 309, 179, 177, 163, 149, 137, 107	[49]
5-Hydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone	ESI Q-TOF	361	343, 324, 177, 137	[49]
5-Hydroxy-l,7-bis(4-hydroxy-3-methoxyphenyl)-3-heptanone	ESI TOF	375 $[M+H]^+$	357 $[M+H-H_2O]^+$, 177, 163, 145, 137	[49]
5-Hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone	ESI Q-TOF	413 $[M+Na]^+$ 391 $[M+H]^+$	373 $[M+H-H_2O]^+$, 355 $[M+H-2\,H_2O]^+$ 243, 223, 197, 193, 179, 177, 153, 137	[49,136,175]
3,5-Dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane	ESI TOF	399 $[M+Na]^+$	359 $[M+H-H_2O]^+$, 341 $[M+H-2\,H_2O]^+$ 163, 137	[136]
3,5-Dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-heptane	ESI TOF	429 $[M+Na]^+$	389 $[M+H-H_2O]^+$, 371 $[M+H-2\,H_2O]^+$ 191, 167	[136]
3-Acetoxy-5-hydroxy-1,7-bis(3,4-dihydroxyphenyl)-heptane	ESI Q-TOF	413 $[M+Na]^+$ 391 $[M+H]^+$	373 $[M+H-H_2O]^+$, 355 $[M+H-2\,H_2O]^+$ 313 $[M+H-H_2O\text{-}AcOH]^+$ 243, 193, 179, 149, 131	[136,175]
3-Acetoxy-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptane	ESI TOF	405 $[M+H]^+$	387 $[M+H-H_2O]^+$, 345 $[M+H\text{-}AcOH]^+$ 207	[175]

Table 4 (*Continued*)

Compound	Positive ionization, mass analyzer	Molecular ion (*m*/*z*)	Fragment ions (*m*/*z*)	Ref.
3-Acetoxy-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxy-5-methoxyphenyl)heptane	ESI TOF	457 $[M+Na]^+$	417 $[M+H\text{-}H_2O]^+$, 375 $[M+H\text{-}AcOH]^+$ 357 $[M+H\text{-}H_2O\text{-}AcOH]^+$ 230, 179, 155, 137	[135,136]
3-Acetoxy-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)heptane	ESI TOF	471 $[M+Na]^+$	431 $[M+H\text{-}H_2O]^+$	[135,136]
		467 $[M+NH_4]^+$	371 $[M+H\text{-}H_2O\text{-}AcOH]^+$, 193, 163	
3,5-Diacetoxy-7-(4-dihydroxy-3-methoxyphenyl)-1-(3,4-dihydroxy-5-methoxyphenyl)-heptane	ESI TOF	499 $[M+Na]^+$	417 $[M+H\text{-}AcOH]^+$,	[136,175]
		495 $[M+NH_4]^+$	357 $[M+H\text{-}2\ AcOH]^+$, 217, 179, 163	
3,5-Diacetoxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-heptane	ESI TOF	513 $[M+Na]^+$	431 $[M+H\text{-}AcOH]^+$	[136,175]
		509 $[M+NH_4]^+$	371 $[M+H\text{-}2\ AcOH]^+$, 217, 193, 167, 137	
Dihydro-bisdemethoxycurcumin	ESI ion trap	311	225, 147, 119	[177]
Dihydrodemethoxycurcumin	ESI ion trap	341	323, 245, 177, 147	[177]
Dihydrocurcumin	ESI ion trap	371	353, 285, 245, 177, 145	[177]
Bisdemethoxycurcumin	iontrap ESI–MS/MS	309	239, 225, 215, 189, 147	[125,177]
Demethoxycurcumin	iontrap ESI–MS/MS	339	269, 255, 245, 177, 175, 147	[125,177]
Curcumin	iontrap ESI–MS/MS	369	299, 285, 245, 177, 175	[125,177]
7-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadien-3-one	ESI iontrap	325	219, 107	[177]
1,7-Bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadien-3-one	ESI iontrap	355	219, 177, 137	[177]
1-(4-Hydroxy-3-methoxyphenyl)-7-(4-hydroxy-3,5-dimethoxyphenyl)-4,6-heptadien-3-one	ESI iontrap	385	367, 249, 189, 137	[177]

capsules and powdered turmeric used as coloring additive in food [192].

4.5.3. Supercritical fluid chromatography (SFC)

Supercritical fluid chromatography coupled online to SFE has been applied for the analysis of *C. longa* rhizomes. SFC separation of curcumin was carried out using silica, diol-silica, ODS-silica, and polystyrene-divinylbenzene (PS-DVB) copolymer columns, carbon dioxide as eluent, and methanol as organic modifier. Curcumin was strongly retained on the silica and diol-silica columns, while it was rapidly eluted from the ODS-silica column. Satisfactory separation of curcuminoids was achieved with the PS-DVB copolymer column [52].

5. Conclusions

Diarylheptanoid-containing herbal drugs are not only official in Pharmacopoeias but also present in significant amount in dietary supplements. Due to their beneficial biological effects such as anti-inflammatory, estrogenic, cytotoxic, anti-amyloidogenic, and anti-emetic activity, the discovery of new natural diarylheptanoid sources and their phytochemical characterization is of primary importance. The orthogonal analytical toolkit discussed in our review demonstrated that the combination of various separation techniques in conjunction with state-of-the-art spectrometric and spectroscopic methods can overcome the isolation and structural characterization challenges posed by these compounds. However, more efforts are needed in expanding environment-friendly and economic large-scale isolation and purification procedures. The chemical instability and the low bioavailability of diarylheptanoids also necessitate technological advances, in order to improve their drug-like properties. Recent results in the discovery of new diarylheptanoids clearly project that further unique chemical entities will be identified, especially those of cyclic derivatives. The identification of species/genera specific structures and minor representatives will aid and advance the authentication of herbal products and the quality control of dietary supplements.

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