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Acorus Linnaeus: A review of traditional uses, phytochemistry and neuropharmacology

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Xiao-Lin Feng,[‡a] Yang Yu,[‡a] Da-Peng Qin,[a] Hao Gao,[*a] and Xin-Sheng Yao[*a]

***Acorus* Linnaeus is a genus of perennial herbs distributed from the northern temperate to the subtropical regions, and has been widely used as traditional folk medicine in China and India since ancient times. Phytochemical studies have shown the presence of numerous beneficial compounds, such as phenylpropanoids, lignans, sesquiterpenoids, alkaloids and others. Neuropharmacological studies have revealed that the *Acorus* rhizome extract and its constituents, particularly α- and β-asarone, possess anticonvulsant, antiepileptic, neuroprotective, memory enhancing, and sedative properties. This review summarises the traditional uses, phytochemistry, and neuropharmacological activities of *Acorus* Linnaeus.**

Introduction

In 1998, the Angiosperm Phylogeny Group (APG) revised the classification of Acoraceae as a monotypic family.[1-2] However, *Acorus* has historically been considered a member of the Araceae family, although the family Acoraceae was already established in 1820.[2-3] Several significant morphological, anatomical and embryological characteristics in addition to DNA evidence has supported the view that *Acorus* is not closely related to the Araceae at all.[3-4] However, the phylogenetic relationships among the species of *Acorus*, which are usually divided into 2 to 5 species, remain unclear.[3-5]

According to the traditional use and recent studies on *Acorus*, in this review, we follow the systematic classification established by H. Li.[5] The Genus *Acorus* contains four species, *A. calamus* L., *A. tatarinowii* Schott, *A. gramineus* Soland and *A. rumphianus* S.Y.Hu, which are widely distributed in eastern and southern Asia. With the exception of *A. Rumphianus*, the species have historically been used in traditional medicine, particularly for the treatment of central nervous system (CNS) diseases in several ancient Asian countries.[5] The rhizomes of *A. tatarinowii* have been recorded in the Chinese Pharmacopoeia since 1985. However, the rhizomes of *A. calamus* are thought to be adulterants of the rhizomes of *A. tatarinowii* in the modern Chinese herbal medicine market.

The well-known traditional herbal use of *Acorus* has inspired numerous studies on their rich constituents and pharmacological activities. This comprehensive review on *Acorus* summarises the traditional herbal use and the phytochemicals that have currently been identified. Furthermore, a preliminary comparison between *Acorus* treatment of CNS diseases in traditional medicine and in pharmacological studies is presented.

Traditional uses

Primarily because of their rich content of essential oil, the rhizomes of the *Acorus* species have traditionally been used to treat CNS disorders, respiratory system diseases, gastrointestinal disorders and other diseases. The roots and leaves have not been as widely used because of their low oil content.[3]

A. calamus, which is commonly known as "sweet flag" or ''calamus'', is native to Central Asia and Eastern Europe.[6] The ancient peoples of China used *A. calamus* as an emetic in dyspepsia and as a sedative, nerve tonic, antimicrobial agent, and expectorant.[5] In the Ayurvedic system of medicine, the rhizomes of *A. calamus* are thought to possess aromatic, stimulant, bitter tonic, emetic, expectorant, emmenagogue, aphrodisiac, laxative, diuretic, antispasmodic, carminative, and anthelmintic properties. *A. calamus* has been used for the treatment of numerous diseases, such as mental ailments, including epilepsy, schizophrenia, and memory disorders; chronic diarrhoea and dysentery; bronchial catarrh; intermittent fevers; and asthma, among others.[8-9] Additionally, *A. calamus* has been widely used in traditional folk medicine in America and Indonesia for gastrointestinal disorders, such as colic pain, diarrhoea and the radix in the therapy of diabetes.[3]

A. tatarinowii (Shi-chang-pu in Chinese), recorded in "Shen Nong's Herbal Classic", is a famous traditional Chinese medicine (TCM) for treating CNS diseases. In "Shen Nong's Herbal Classic", *A. tatarinowii* belongs to the superior medicinal herbs because of its ability to prolong life. The dried rhizomes of *A. tatarinowii* have been conventionally prescribed by traditional Chinese doctors to cure difficult diseases, such as epilepsy, amnesia, apoplexy and dementia, or in combination with other medicinal herbs for the improvement of learning and memory.[5, 10]

A. gramineus, which is also known as "Japanese sweet flag", has historically been used for the treatment of cognitive decline, bronchial catarrh, stomach ache, edema and as an insecticide.[5, 11] *A.*

rumphianus has not been used to treat diseases because of its rare distribution.

Phytochemistry

Over the past fifteen years, studies on bioactive phytochemicals from *Acorus* species have significantly increased, and an increasing number of bioactive constituents has been discovered and reported. The major active constituents identified were α- and β-asarone (**1-2**) (Fig. 1), to which most of the bioactivities of the *Acorus* species were attributed.[12]

1. Phenylpropanoids

The most notable constituents of *Acorus* oil are α- and β-asarone (**1-2**). Mazza[13] has investigated the essential oil constituents of two varieties of *A. calamus* L. The primarily volatile constituents were detected as hydrocarbons, carbonyl compounds, alcohols and phenols using gas chromatography-mass spectrometry. The European essential oil substantially differed from that of the Indian variety, which was characterised by a higher β-asarone (**2**) content (77.68%).

The compounds α- and β-asarone (**1-2**) have been reported to exhibit a wide spectrum of biological activities. α-Asarone (**1**) showed remarkable hypolipidemic activity,[14, 15] neuroprotective effect,[16] antimicrobial and insecticidal activities.[17] β-Asarone (**2**) has been found to improve cognitive function,[18] inhibit adipogenesis[19] and exhibit positive effects on epilepsy.[20] Asarone tablets (α-asarone, **1**) have been clinically used as a bronchial asthma and bronchitis prescription drug in China. Unfortunately, toxic and genotoxic studies of α- and β-asarone (**1-2**) have indicated that these compounds may pose a risk to human health, including embryotoxicity and maternal toxicity in rats, hepatotoxicity in rat-cultivated hepatocytes, and *in vivo* and *in vitro* genotoxic damage in mammalian cells.[21] The contrasting effects of α-asarone (**1**), *i.e.*, its efficient therapeutic potential and toxicity, prompted us to identify analogues that exhibit a potent pharmacological effect but with low toxicity. To date, 15 asarone analogues (**3-17**) (Fig. 1), through hydroxylation, carbonylation and epoxidation in the C3 segment of asarone, have been isolated from *Acorus* L.[22-27] Among these analogues, γ-asarone (**3**) has been isolated and identified as an isomer of α- and β-asarone (**1-2**); however, detailed biological studies have not been performed on this compound.[22] (*Z*)-3-(2,4,5-trimethoxyphenyl)acrylaldehyde (**4**), 1-(2,4,5-trimethoxyphenyl) propan-2-one (**6**), 1-(2,4,5-trimethoxyphenyl)propan-1-one (**7**) and 1-(2,4,5-trimethoxyphenyl) propan-1,2-dione (**9**) exhibited weak activity or induce an increase in cAMP levels at concentrations of 50 μM ($P < 0.05$) in N1E-115 neuroblastoma cells.[23] Therefore, future studies to investigate the pharmacological toxicity and properties of compounds that are structurally analogous to asarones are necessary.

In addition to the asarone analogues, methyl eugenol derivatives are also common types of phenylpropanoids in *Acorus* L., including (*E*)-methyl isoeugenol (**18**),[27] (*Z*)-methyl isoeugenol (**19**),[27] methyl eugenol (**20**),[13] and methyl eugenol analogues (**21-24**).[23-25] Methyl eugenol (**20**) is a common component of spices and is directly added to food as a flavouring substance.[28]

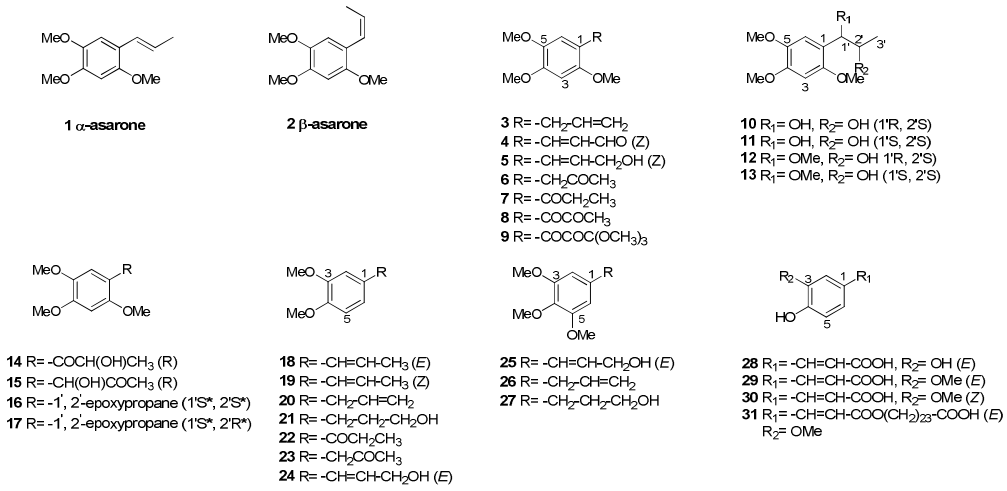

Fig.1 The structures of phenylpropanoids (**1-31**).

Additional phenylpropanoids: 3′,4′,5′-trimethoxycinnamyl alcohol (**25**),[25] 1-(3,4,5-trimethoxyphenyl)-2-propene (**26**),[13] 3-(3,4,5-trimethoxyphenyl)propan-1-ol (**27**),[24] caffeic acid (**28**),[29] ferulic acid (**29**),[29] (*Z*)-coniferyl alcohol (**30**)[24] and *trans*-24-feruloyloxy-tetracosanoic acid (**31**)[30] were also obtained (Fig. 1). Among these phenylpropanoids, (*Z*)-coniferyl alcohol (**30**) significantly inhibited nitric oxide (NO) levels (IC_{50} = 14.08 μM) in lipopolysaccharide (LPS)-stimulated BV-2 cells. Caffeic acid (**28**) tablets are used to treat or prevent bleeding during surgery, obstetrics and gynaecology, or general medicine (facilitating haemostasis). Ferulic acid (**29**) has been proposed as a potential treatment for numerous chronic diseases, such as Alzheimer's disease, cancer, and cardiovascular diseases, among others; however, the clinical efficacy of ferulic acid (**29**) requires additional documentation.[31]

2. Lignans

Two 7,7′-monoepoxy lignans, (+)-veraguensin (**32**) and galgravin (**34**) (Fig. 2),[32] have been isolated from *A. tatarinowii*. Kim *et al.*[11] have investigated the bioactive lignans from the rhizomes of *A. gramineus*. Ganschisandrin (**33**), ligraminol A (**35**), 5-methoxygalbelgin (**36**) and ligraminol B (**37**) (Fig. 2), were evaluated for inhibition of NO production in an activated murine

microglial cell line. Compounds **35** and **37** moderately inhibited NO production, with IC_{50} values of 21.51 and 22.96 μM, respectively.

Eleven 8-*O*-4′-neolignans have been identified from *A. gramineus*: (1R,2S)-rel-1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-hydroxypropyl)-2″,6″-dimethoxyphenoxy]-1,3-propanediol(**38**), ligraminol E (**39**), (7R,7R)-4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (**40**), (7S,8S)- 4,7,9,9′-tetrahydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan (**41**), 7S,8R-*erythro*-4,7,9,9'-tetrahydroxy-3,3'-dimethoxy-8-*O*-4'-neolignanl (**42**), ligraminol D (**43**), (-)-(7R,8R)-virolin (**44**), (7R,8R)-polysyphorin (**45**), surinamensinols A (**46**) and B (**47**), and ligraminol C (**48**) (Fig. 2).[11, 25] Because the MeOH extract of *A. gramineus* rhizomes exhibited significant anti-inflammatory and cytotoxic activities, Kim *et al.*[11, 25] have investigated the anti-inflammatory effects and cytotoxic activities of these 8-*O*-4′-neolignans. Ligraminol D (**43**) and surinamensinols A (**46**) and B (**47**) significantly inhibited NO levels in LPS-stimulated BV-2 cells, with IC_{50} values of 18.41, 17.91 and 8.17 μM, respectively. Surinamensinols A (**46**) and B (**47**) exhibited moderate anti-proliferative activities against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines, with IC_{50} values ranging from 4.17 to 26.18 μM. In particular, surinamensinols A (**46**) and B (**47**) exhibited potent cytotoxicity against the A549 cell line, with IC_{50} values of 4.17 and 5.41 μM, respectively. Additionally, ligraminol D (**43**) exhibited weak inhibitory activity against the proliferation of the evaluated cell lines, with an IC_{50} value of 9.54 μM.

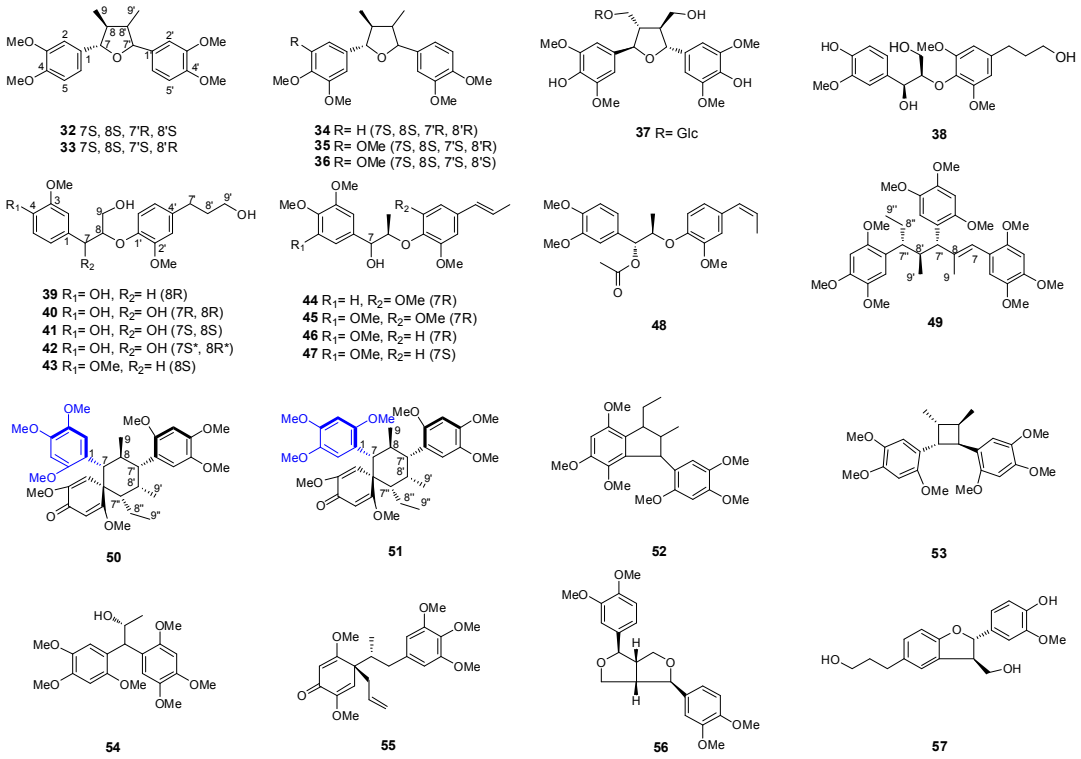

Fig. 2 The structures of lignans (**35-57**).

The tatanans (**49-51**) are members of novel sesquinlignans with the unprecedented carbon skeleton that is characterised by a unique C8–C7' linkage pattern. Structurally, tatanans B and C (**50-51**) are atropisomers with a hindered rotation around the C-1–C-7 bond and possess an unprecedented spiro[5.5]undecane skeleton with two benzyl moieties attached to C-7 and C-7' (Fig. 2, the position of hindered rotation was highlighted by blue colour).[33] The possible biosynthesis pathway of the tatanans from *A. Tatarinowii* may derive from three α- or β-asarone (**1** or **2**) through a complex enzymatic reaction. Tatanans A, B and C (**49-51**) (Fig. 2) exhibited potent and selective *in vitro* glucokinase-activating activities but were inactive against dipeptidyl-peptidase-4, α-glycosidase, the Na^+-glucose cotransporter, and aldose reductase.[33] Recently, Qing *et al.*[34] have reported the total synthesis of tatanan A (**49**) in 13 steps utilising a series of sequential [3,3]-sigmatropic rearrangements in addition to a concise enantioselective total synthesis of the more complex atropisomeric tatanans B and C (**50-51**). In contrast to the previous report, these authors utilised pure recombinant human glucokinase and demonstrated that tatanans do not function as allosteric activators of glucokinase. Therefore, future *in vitro* and *in vivo* studies are necessary to confirm these conflicting results.

In addition to these three types of lignans, additional lignan types, such as compounds **52-57**,[23-25, 32, 35-36] have also been reported (Fig. 2). Magnosalin (**53**) has been reported to inhibit NO levels in LPS-stimulated BV-2 cells, with an IC_{50} value of 18.73 μM.[24]

3. Sesquiterpenoids

Acorus plants are a rich source of various sesquiterpenoids, which are represented by the acoranes. Acorane-type sesquiterpenoids with the characteristic spiro[4.5]decane skeleton

are biosynthesised from *cis, trans*-farnesyl pyrophosphate *via* the bisabolane cation.[37] Since the isolation of acorone (**58**) and isoacorone (**59**) from the essential oil of *A. calamus*. in 1948,[38] 18 acorane derivatives (**58-75**)[26, 39-41] have been isolated from the *Acorus* species (Fig. 3). Pharmacological investigation of acoranes has indicated that 1-hydroxyepiacorone (**61**) exhibited potent anti-germination activity.[26] *In vitro* assays have demonstrated that calamusin D (**62**) (10 μM) exhibited weak hepatoprotective activities against *N*-acetyl-*p*-aminophenol-induced HepG2 cell damage. Moreover, (-)-acorenone (**63**) exhibited weak Gamma-aminobutyric acid type A receptors ($GABA_A$) modulating properties (241% ± 23.1%; EC_{50} = 34.0 ± 6.7 μM).

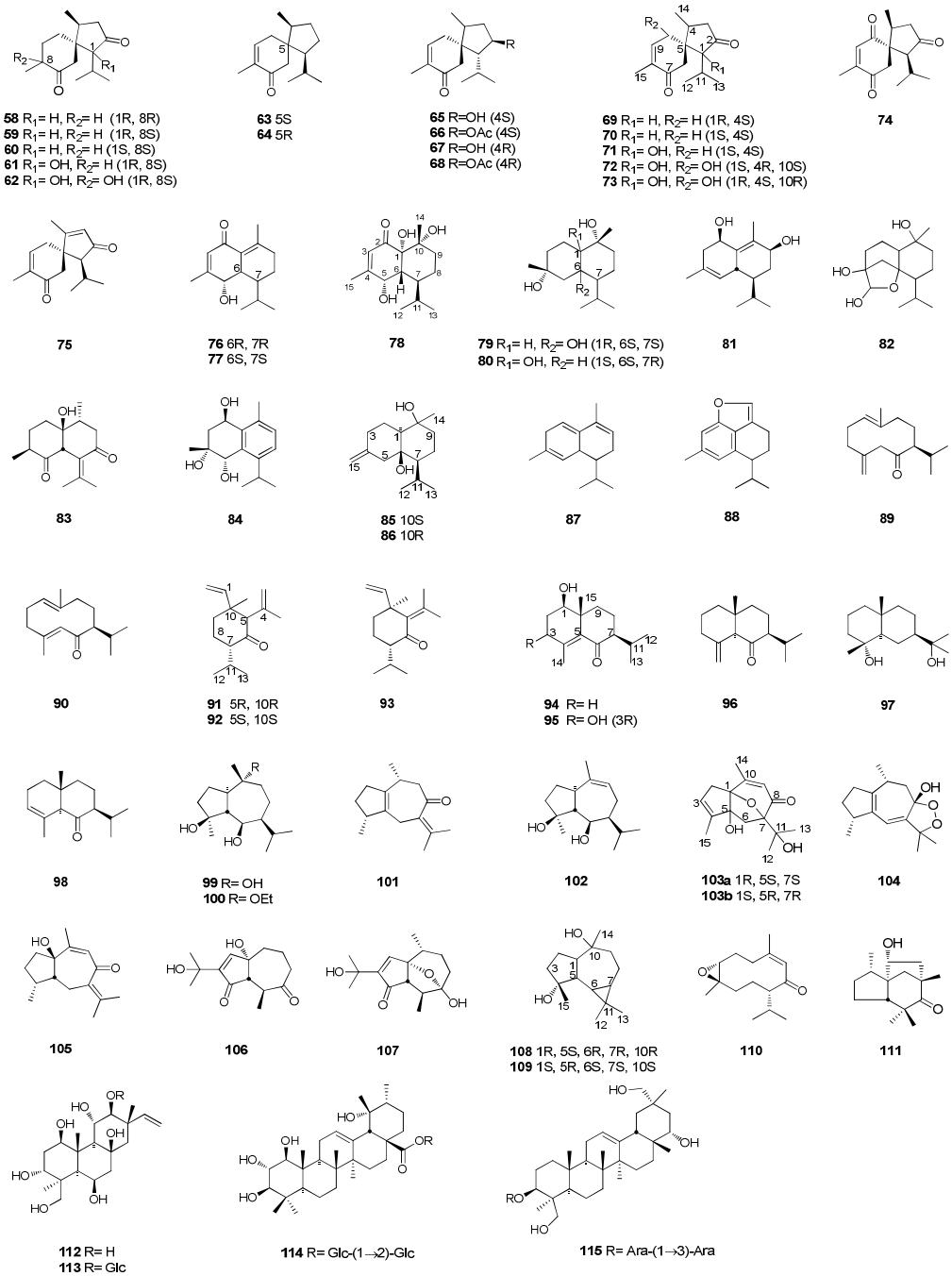

Fig. 3 The structures of sesquiterpenoids, diterpenoids and triterpenoids (**58**-**115**).

An increasing number of studies have reported the isolation and structure elucidation of sesquiterpenoids. Cadinanes represent a second major type of sesquiterpenoids from the *Acorus* species. Tatarinowins A (**76**), B (**77**) and C (**78**) have been isolated from *A. tatarinowii*.[23, 41-42] Chemical investigation of the rhizomes of *A. calamus* has led to the isolation of 10 cadinanes: 1,4a,6(2H)-naphthalenetriol (**79**),[30] 1,6,8a(1H)-naphthalenetriol (**80**),[30] 1,7-naphthalenediol (**81**),[30] benghalensitriol (**82**),[30] calamusin G (**83**),[40]

calamusin H (**84**),[40] calamendiol (**85**),[30] isocalamenediol (**86**),[26] (-)-cadala-1,4,9-triene (**87**)[43] and acorafuran (**88**) (Fig. 3).[44]

Four types of sesquiterpenoids rearranged from the germacryl cation, *i.e.*, germacranes (**89-90**),[40, 45] elemanes (**91-93**),[40, 46] eudesmanes (**92-98**),[26, 30, 47] and guaianes (**99-105**) (Fig. 3),[30, 40-42, 48] have been identified. Moreover, studies have also identified **106-111** (Fig. 3).[49]

4. Diterpenoids and triterpenoids

Chemical investigation of *A. tatarinowii* has led to the isolation of the isopimarane diterpenes (tatarol (**112**) and its glycoside, tataroside (**113**) (Fig. 3)).[50] Two triterpenoid saponins, *i.e.*, 1β,2α,3β,19α-tetrahydroxyurs-12-en-28-oicacid-28-*O*-{-β-D-glucopyranosyl(1→2)}-β-D-galactopyranoside (**114**) and 3β,22α,24,29-tetrahydroxyolean-12-en-3-*O*-{-β-D-arabinosyl(1→3)}-β-D-arabinopyranoside (**115**) (Fig. 3), have been isolated from the extract of *A. calamus*.[51]

5. Amides and alkaloids

In 1997, Wang *et al.*[52] were the first to isolate two amides from *A. tatarinowii*, tataramide A (**116**) and tataramide B (**117**). Additional studies on the alkaloid constituents of this plant led to the isolation of five alkaloids, acortatarin A (**118**), acortatarin B (**119**), tatarinine A (**120**), tatarine A (**121**) and 1H-pyrrole-1-butanoic acid (**122**).[42, 53] Among these alkaloids, acortatarins A-B (**118-119**) are two novel spiroalkaloids with an unusual morpholine motif. The most interesting of these compounds is acortatarin A (**118**), which significantly inhibited reactive oxygen species production in high glucose-stimulated mesangial cells in a dose- and time-dependent manner. Furthermore, acortatarin A (**118**) inhibited the high glucose-induced extracellular matrix production *via* the inhibition of NADPH oxidase activation, suggesting that acortatarin A (**118**) represents a new therapeutic candidate for diabetic nephropathy.[54] For future biological studies of acortatarins, Sudhakar and co-workers have developed a synthetic strategy using readily available D-sugars as the starting material. This convergent total synthesis has revealed the revision of the absolute configuration of acortatarin A (**118**) and the structural revision of acortatarin B (**119**) (see Fig. 4).[55]

6. Miscellanous

Selcuk *et al.*[56] were the first to investigate the constituents of the leaves of *A. calamus* and identified apigenin 7-*O*-β-D-glucoside (**123**) and apigenin (**124**). An additional eight flavonoids (**125-132**) have been identified from the rhizomes of the *Acorus* species,[25, 57-62] including two flavonol glycosides (**126**, **129**)[57-58] and a flavone C-glycoside (**130**).[60] Apigenin (**124**), which is abundantly present in common fruits and vegetables, has been demonstrated to possess substantial anti-oxidant, anti-inflammatory and anti-carcinogenic properties.[63] A clinical trial to verify the hypothesis that dietary supplementation with bioflavonoids will diminish the recurrence rate of colonic neoplasia will begin in May 2015.[64]

Lee *et al.*[65] have identified three new quinone derivatives (**133-135**) from *A. gramineus*, which exhibited significant anti-inflammatory effects *via* the reduction of NO levels in LPS-stimulated BV-2 cells.

Eleven sterols (**136-146**) have been identified in *Acorus* L., most of which are stigmasterol, daucosterol and sitosterol derivatives.[30, 32, 66] Rai *et al.*[67] have isolated and characterised a xanthone glycoside (**147**) from the rhizomes of *A. calamus*. Yang *et al.*[68] have investigated the decoction of the rhizomes of *A. tatarinowii*. Four compounds were identified as 2,5-dimethoxybenzoquinone (**148**), benzoic acid (**149**) and furfuraldehydes (**150-151**). Detailed information on these compounds is summarised in Table 1.

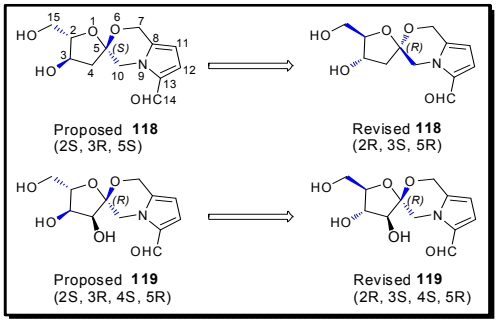

Fig. 4 The revised absolute configuration of acortatarin A (**118**) and acortatarin B (**119**).

Table 1 Flavonoids, quinones, sterols and other constituents isolated from *Acorus* species

NO.	Chemical name	Part	Source plant	Ref.
123	apigenin 7-*O*-β-D-glucoside	leaves	*A. calamus*	[56]
124	apigenin	leaves	*A. calamus*	[56]
125	norizalpinin	rhizomes	*A. calamus*	[57]
126	galangin-3-*O*-β-D-glucopyranosyl-7-*O*-β-L-rhamnopyranoside	roots	*A. calamus*	[58]
127	3-*O*-methylkaempferol	rhizomes	*A. gramineus*	[25]
128	noranhydroicaritin	rhizomes	*A. tatarinowii*	[59]
129	luteolin-8-*C*-β-D-glucopyranoside	roots	*A. calamus*	[60]
130	luteolin 6,8-*C*-diglucoside	–	*A. calamus*	[61]
131	5,4'-dihydroxy-7,8-dimethoxyflavone	rhizomes	*A. calamus*	[62]
132	5-hydroxy-7,8,3',4'-tetramethoxyflavone	rhizomes	*A. calamus*	[63]
133	(7'R,8R,8'S)-7'-(2',4',5'-trimethoxyphenyl)-4,7α,8-trimethoxy-8,8'-dimethyl-2,5-quinone	rhizomes	*A. tatarinowii*	[65]

134	7'-(2',4',5'-trimethoxyphenyl)-4-methoxy-8,8'-dimethyl-2,5-quinone	rhizomes	*A. tatarinowii*	[65]
135	1-*cis*-propenyl-1S,6R-epoxy-4-methoxy-2,5-quinone	rhizomes	*A. tatarinowii*	[65]
136	β-stigmasterol	rhizomes	*A. tatarinowii*	[32]
137	β-sitosterol	rhizomes	*A. calamus*	[66]
138	7β-hydroxy-β-sitosterol	rhizomes	*A. calamus*	[66]
139	7α-hydroxy-β-sitosterol	rhizomes	*A. calamus*	[66]
140	daucosterin	rhizomes	*A. calamus*	[66]
141	stigmasta-5,25-diene	rhizomes	*A. calamus*	[30]
142	4'-*O*-docosanoyl-3-*O*-β-D-glucosyl-sitosterol	rhizomes	*A. calamus*	[66]
143	stigmast-4-ene-6β-ol-3-one	rhizomes	*A. calamus*	[66]
144	6β-hydroxystigmasta-4,22-diene-3-one	rhizomes	*A. calamus*	[66]
145	stigmast-5-en-3β-ol-7-one	rhizomes	*A. calamus*	[30]
146	stigmasta-5,22-dien-3β-ol-7-one	rhizomes	*A. calamus*	[30]
147	4,5,8-trimethoxy-xanthone-2-*O*-β-D-glucopyranosyl(1→2)-*O*-β-D-galactopyranoside	rhizomes	*A. calamus*	[67]
148	2,5-dimethoxybenzoquinone	rhizomes	*A. tatarinowii*	[68]
149	4-hydroxy-3-methoxybenzoic acid	rhizomes	*A. tatarinowii*	[68]
150	5-hydroxymethyl-2-furaldehyde	rhizomes	*A. tatarinowii*	[68]
151	2-furancarboxaldehyde,5,5′[oxybis (methylene)]	rhizomes	*A. tatarinowii*	[68]

Neuropharmacology

The *Acorus* species exhibits significant CNS actions, such as anticonvulsant, neuroprotective, memory enhancing and sedative properties, which validates its use to treat certain CNS diseases in the Ayurvedic, Chinese and other medicinal systems (Table 2).

1. Anticonvulsant

The anticonvulsant activity of *Acorus* extracts have been studied *in vivo*, validating the traditional use of this herb as an anticonvulsant and antiepileptic. The decoction and volatile oil from the rhizomes of *A. tatarinowii* were extracted by traditional decocting and supercritical CO_2 fluid extraction methods. Both the decoction extract and the volatile oil can prevent convulsions and convulsion-related GABAergic neuronal damage in the brain in the prolonged pentylenetetrazol (PTZ) kindling model. The volatile oil exhibited less efficacy for PTZ-induced convulsions.[10] To compare the anticonvulsant activity, the raw and classically processed rhizomes of *A. calamus* were screened against the maximal electroshock seizure model to evaluate the influence of the classical purification procedure on the pharmacological action of *A. calamus*. The raw and classically processed samples exhibited significant anticonvulsant activity by decreasing the duration of the tonic extensor phase.[69] *A. calamus* has also been demonstrated to possess the ability to prevent the development of $FeCl_3$-induced epileptogenesis by modulating antioxidant enzymes; this finding suggests the potential of *A. calamus* for development as an effective antiepileptic drug.[70] Huang *et al.*[71] have characterised the action of α-asarone (**1**) on the excitability of rat hippocampal neurons in culture and on the epileptic activity induced by PTZ or kainate injection *in vivo*. Under the whole-cell configuration, α-asarone (**1**) induced inward currents in a dose-dependent manner, with an EC_{50} value of 248 ± 33 μM. These results suggested that α-asarone (**1**) inhibited the activity of hippocampal neurons and produced an antiepileptic effect in the CNS by enhancing tonic GABAergic inhibition. Additional studies on α-asarone (**1**) in various animal seizure models have suggested that α-asarone (**1**) exhibits a favourable antiepileptic activity.[72-73] In a clinical trial, Pan *et al.*[74] found that α-asarone (**1**) (30-90 mg, *p.o.*, tid) to be effective in 59% of 32 episodes of grand mal epilepsy. And in the control group, 8 of 15 epileptic patients were controlled by treating with phenytoin (0.1 g, *p.o.*, bid-tid). There was no significant difference between these two groups. Importantly, the advantage of fewer adverse effects and larger safety dosage range suggested that α-asarone (**1**) may be the drug of choice for these particular grand mal epilepsy patients.

2. Neuroprotection

A potential neuroprotective activity of the ethanol:water (1:1) extract of the rhizomes of *A. calamus* has been reported using a middle cerebral artery occlusion-induced ischaemia model. Ischaemic rats treated with *A. calamus* exhibited significant improvement in neurobehavioural performance, increased reduced glutathione levels and SOD activity in both the cortex and the corpus striatum, and an improved neurological function score.[75] In the study evaluating the effects of the essential oil (EO) from *A. gramineus*, EO inhibited the glutamate-induced excitotoxicity in a dose-dependent manner, with an IC_{50} value of 0.241 mg/mL. EO exerted a more potent neuroprotection against the toxicity induced by NMDA (IC_{50} = 0.139 mg/mL). Receptor-ligand binding studies have revealed that EO dramatically inhibited the specific binding of a use-dependent NMDA receptor-ion channel blocker [^{3}H]MK-801, indicating an NMDA receptor antagonist-like action.[76] The effects of the water extracts of six medicinal herbs on the cytotoxic action of amyloid-β1-40 (Aβ1-40) have been evaluated in PC-12 cells, and only the *A. gramineus* extract significantly decreased Aβ1-40-induced cell death. Furthermore, eugenol and β-asarone (**2**) were isolated and identified as the major active constituents. Purified eugenol and β-asarone (**2**) protected PC-12 cells from the toxic effect of Aβ1-40.[77] A randomised controlled animal study has indicated that *A. gramineus* and α-asarone (**1**) increased Bcl-2 expression, decreased Bax expression, and reduced the number of apoptotic hippocampal neurons during PTZ-induced epileptic seizures in immature rats.[78]

3. Memory enhancement

To investigate whether *A. gramineus* (AG) influenced cerebral ischaemia-induced neuronal and cognitive impairments, Lee *et al.*[79] have examined the effect of AG on ischaemia-induced cell death in the striatum, cortex and hippocampus and on learning and memory-impaired rats in the Morris water maze and radial eight-arm maze.

AG exhibited a protective effect against ischaemia-induced neuronal loss and learning and memory damage. Geng *et al.*[18] have investigated the effects of β-asarone (**2**) on cognitive function and neuronal apoptosis in rats subjected to Aβ injection in the hippocampus and have studied its mechanism of action. Oral administration of β-asarone (**2**) (12.5, 25, or 50 mg/kg for 28 d) ameliorated the Aβ (1-42)-induced cognitive impairment and reversed the increase in apoptosis in the hippocampus. β-Asarone (**2**) attenuated the Aβ (1-42)-induced neuronal apoptosis in the hippocampus *via* reversal of the down-regulation of Bcl-2 and Bcl-w, caspase 3 activation, and c-Jun N-terminal kinase phosphorylation. Moreover, the essential oil extracted from the rhizomes of AG improved cognitive function in aged animals, possibly by increasing the relative levels of norepinephrine, dopamine and serotonin and by decreasing the activity of acetylcholinesterase (AChE) in the cerebra.[80] Because cognitive performance and memory are related to acetylcholine levels, several studies have illustrated that the AChE inhibitory effect of the genus *Acorus* may account for its traditional use. The methanol extract of *A. calamus* exhibited significant AChE inhibition at a concentration of 200 mg/mL.[81] The *in vitro* AChE inhibitory effect of the ethanol extract, the essential oil of the rhizomes of *A. calamus*, and its major constituents have been evaluated using Ellman's method. The IC_{50} values obtained for the ethanol extract, the essential oil, β-asarone (**2**) and α-asarone (**1**) were 182.31 ± 16.78 μg/mL, 10.67 ± 0.81 μg/mL, 3.33 ± 0.02 μM and 46.38 ± 2.69 μM, respectively.[82]

Table 2 Comparison of application on CNS of traditional use and modern pharmacological activity

Traditional use	Pharmacological activity	Plant*	Parts/constituent	Assay/ Study	Result/ Activity	Ref.
Epilepsy	Anticonvulsant Antiepileptic	AT	Decocting extract	PTZ kindling model	Prevent convulsion-related GABAergic neuron damage	[10]
			Volatile oil	PTZ kindling model	Less effective	
		AC	Rhizomes	Maximal Electro Shock seizure model	Decreased the duration of tonic extensor phase	[69]
		AC	Rhizomes	$FeCl_3$-induced epileptogenesis	decreased	[70]
			α-Asarone (**1**)	Induced inward currents	EC_{50} = 248 ± 33 μM	[71]
				PTZ or kainate injection model	Prolonged the latency to clonic and tonic seizures	
					Reduced the mortality	
			α-Asarone (**1**)	Mice and rats seizure models	Effective anticonvulsant activity	[72], [73]
			α-Asarone (**1**)	Epileptic patients	Effective rate: 59%	[74]
Memory disorders	Neuroprotection	AC	Ethanol:water (1:1) extract	Increased in lipid peroxidation	Cortex, 157%; corpus striatum, 58%	[75]
				Decreased in glutathione levels	Cortex, 59%; corpus striatum, 34%	
				Decreased superoxide dismutase (SOD) activity	Cortex, 64%; corpus striatum, 32%	
				Rota-Rod performance and grid walking test	Significant improvement in neurobehavioural performance	
		AG	Essential oil	Inhibited glutamate-induced excitotoxicity	IC_{50} = 0.241 mg/mL	[76]
				Inhibited toxicity induced by NMDA	IC_{50} = 0.139 mg/mL	
		AG	Rhizomes	Cytotoxic action of Aβ (1-40)	Decreased Aβ (1-40)-induced cell death	[77]
			β-Asarone (**2**)	Basal Ca^{2+} intake	Weak inhibited	
			Eugenol	Aβ-induced Ca^{2+} intake	10 μM (50% response)	
			α-Asarone (**1**)	Pentylenet etrazol induced epileptic seizures in immature rats	Reduced the number of apoptotic hippocampal neurons	[78]
Cognitive decline, Amnesia, Dementia, Memory disorders	Memory enhancement	AG	Methanol extract	Ischemia-induced cell death	Reduced cell death in the hippocampal CA1 area	[79]
				Morris water maze test	Significant improvement in escape latency to find the platform	
				Radial eight-arm maze test	Improvement of the number of choice errors	
		AG	Essential oil	Step-down passive avoidance test and Y maze	Improved the latency and number of errors	[80]
				Levels of norepinephrine, dopamine and serotonin	Increased	
				Levels of acetylcholinesterase	Decreased	
			β-Asarone (**2**)	Aβ hippocampus injection rats	Ameliorated Aβ (1-42)-induced cognitive impairment and reversed the increase of apoptosis in the hippocampus	[18]
		AC	Methanol extract	*In vitro* AChE assay	IC_{50} = 200 mg/mL	[81]
		AC	Ethanol extract	*In vitro* AChE assay	IC_{50} = 182.31 ± 16.78 mg/mL	[82]
			Essential oil		IC_{50} = 10.67 ± 0.81 mg/mL	
			β-Asarone (**2**)		IC_{50} = 3.33 ± 0.02 μM	
			α-Asarone (**1**)		IC_{50} = 46.38 ± 2.69μM	
Sedation, Analgesia, Schizophrenia	Sedative	AC	Ethanol extract	Norepinephrine level	Cerebral cortex, increased; midbrain and cerebellum, decreased	[83]
				Serotonin level	Cerebral cortex, increased; midbrain, decreased	
				Dopamine level	Caudate nucleus and midbrain, increased; cerebellum, decreased	
			β-Asarone (**2**)	Locomotor analysis	Significant decrease in the total number of crossings	[7]
				Body temperature	Hypothermic	

	[^{3}H]-CP 55940 binding assay	Exert a direct agonistic activity on CB1 receptors

* AT (*A. tatarinowii*); AC (*A. calamus*); AG (*A. gramineus*).

4. Sedative

The ethanol extract of *A. calamus* exerts its depressive action by altering the electrical activity and differentially altering monoamine levels in different regions of the brain.[83] Zanoli *et al.*[7] have found that β-asarone (**2**) exerted sedative and hypothermic, but not analgesic effects. When administered with the cannabinomimetic drug WIN 55-212-2, β-asarone (**2**) potentiated certain typical behavioural activities induced by cannabinoids in animals. Binding assays, which were performed on cortical synaptic membrane preparations using a specific cannabinoid radioligand ([^{3}H]CP-55, 940), indicated that β-asarone (**2**) does not exert a direct agonistic activity on CB1 receptors. Therefore, β-asarone (**2**) cannot be considered a pure cannabinomimetic agent, although it may act as an allosteric modulatory agent.

Conclusion

As reviewed herein, chemical investigation of the *Acorus* species has revealed rich secondary metabolites, whereas only asarones have demonstrated significant effects on different biological properties, particularly anticonvulsant, neuroprotective, and memory enhancing effects. Future studies are necessary to identify additional constituents that exhibit potent pharmacological effects at low doses. Nevertheless, the differences in the chemical compositions among the *Acorus* species remain unknown, which presents challenges in validating their different traditional uses. The elucidation of the chemical compositions of *Acorus* species will expand the medicinal resources of *Acorus* and will significantly protect their native plant resources.

Traditional uses of the *Acorus* species were, in most cases, supported by pharmacological studies, particularly for the treatment of CNS diseases. Although beneficial effects on the treatment of convulsive and epileptic diseases have been demonstrated in *in vitro* and *in vivo* models, more clinical trials still be required. The potential for neuroprotective and cognitive and memory improvement activities has suggested that *Acorus* represents a promising treatment for dementia or other diseases with cognitive decline, such as Alzheimer's disease. To properly evaluate the results of these studies, the *Acorus* species are assumed to act as a 'delivering servant' or with a 'Kaiqiao' effect in TCM formulas for the treatment of CNS diseases and are capable of increasing the uptake of active compounds in the brain.[84, 85] Although studies have suggested that *Acorus* can increase the permeability of the blood-brain barrier[86] and facilitate the uptake of ginsenosides Rg1, Re and Rb1 in the brain following oral administration of Kai-Xin-San preparations,[87] additional studies are necessary to elucidate their precise mechanism in the treatment of CNS diseases. Despite the important and varied phytochemical and neuropharmacological studies available, clinical trials are necessary to confirm the use of this species in medical practice.

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Notes and references

[a]*Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, P. R. China. E-mail: tyaoxs@gmail.com; tghao@jnu.edu.cn; Tel (Fax): +86-20-85221559.*

‡These authors have contributed equally to this work.

[1] The Angiosperm phylogeny Group, *Ann Missowri Bot Gard*, 1998, **85**, 531-553.
[2] The Angiosperm phylogeny Group, *Bot J Linn Soc*, 2009, **161**, 105-121.
[3] Flora of China Editorial Committee, Acoraceae through Cyperaceae, Flora of China, Science Press: Beijing, 2010, pp. 1-2.
[4] Y. Y. Chen, D. Z. Li, H. Wang, *Acta Botanica Yunnanica*, 2002, **24**, 699-706.
[5] H. Li, Araceae, Flora Repubulicae Popularis Sinicae, Science Press: Beijing, 1979, **vol. 13**, ch. 2, pp. 7-8.
[6] H. Gilani Anwar Ul, J. Shah Abdul, M. Ahmad, F. Shaheen, *Phytother Res*, 2006, **20**, 1080-1084.
[7] P. Zanoli, R. Avallone, M. Baraldi, *Phytother Res*, 1998, **12**, 114-116.
[8] P. K. Mukherjee, V. Kumar, M. Mal, P. J. Houghton, *Pharm Biol*, 2007, **45**, 651-666.
[9] K. R. Kirtikar, B. D. Basu, Indian Medicinal Plants, M/s Bishen Sing, Mahendra Pal Sing Publishers: Dehradun, 1987, **Vol. IV**, pp. 1229-1230.
[10] W. P. Liao, L. Chen, Y. H. Yi, W. W. Sun, M. M. Gao, S. Tao, S. Q. Yang, *Epilepsia*, 2005, **46**, 21-24.
[11] K. H. Kim, H. K. Kim, S. U. Choi, E. Moon, S. Y. Kim. K. R. Lee, *J Nat Prod*, 2011, **74**, 2187-2192.
[12] X. Y. Yang, F. K. Chen, *Shenyang Yaoke Daxue Xuebao*, 1999, **16**, 71-78.
[13] G. Mazza, *J Chromatogr*, 1985, **328**, 179-194.
[14] C. Gomez, G. Chamorro, M. A. Chavez, G. Martinez, M. Salazar, N. Pages, *Plantes Medicinales et Phytotherapie*, 1987, **21**, 279-284.
[15] L. Rodríguez-Páez, M. Juárez-Sanchez, J. Antúnez-Solís, I. Baeza, C. Wong, *Phytomedicine*, 2003, **10**, 397-404.
[16] I. D. Limón, L. Mendieta, A. Díaz, G. Chamorro, B. Espinosa, E. Zenteno, J. Guevara, *Neurosci Lett*, 2009, **453**, 98-103.
[17] R. A. Momin, M. G. Nair, *J Agric Food Chem*, 2002, **50**, 4475-4478.
[18] Y. T. Geng, C. C. Li, J. C. Liu, G. H. Xing, L. Zhou, M. X. Dong, X. Y. Li, Y. C. Niu, *Biol Pharm Bull*, 2010, **33**, 836-843.
[19] M. H. Lee, Y. Y. Chen, J. W. Tsai, S. C Wang, T. Watanabe, Y. C. Tsai, *Food Chem*, 2011, **126**, 1-7.

[20] Y. Q. Fang, R. M. Fang, G. L. Fang, Y. Jiang, S. Y. Fu, *Zhongguo Zhongyao Zazhi*, 2008, **33**, 534-536.
[21] M. Cassani-Galindo, E. Madrigal-Bujaidar, G. Chamorro, F. Díaz, J. Tamariz, J. J. Espinosa-Aguirre, *Toxicol in Vitro*, 2005, **19**, 547-552.
[22] A. K. Sinha, R. Acharya, B. P. Joshi, *J Nat Prod*, 2002, **65**, 764-765.
[23] X. G. Tong, G. S. Wu, C. G. Huang, Q. Lu, Y. H. Wang, C. L. Long, H. R. Luo, H. J. Zhu, Y. X. Cheng, *J Nat Prod*, 2010, **73**, 1160-1163.
[24] K. H. Kim, E. Moon, H. K. Kim, J. Y. Oh, S. Y. Kim, S. U. Choi, K. R. Lee, *Bloorg Med Chem Lett*, 2012, **22**, 6155-6159.
[25] C. H. Park, K. H. Kim, I. K. Lee, S. Y. Lee, S. U. Choi, J. H. Lee, K. R. Lee, *Arch Pharm Res*, 2011, **34**, 1289-1296.
[26] K. Nawamaki, M. Kuroyanagi, *Phytochemistry*, 1996, **43**, 1175-1182.
[27] M. Della Greca, P. Monaco, L. Previtera, G. Aliotta, G. Pinto, A. Pollio, *Phytochemistry*, 1989, **28**, 2319-2321.
[28] R. L. Smith, T. B. Adams, J. Doull, V. J. Feron, J. I. Goodman, L. J. Marnett, P. S. Portoghese, W. J. Waddell, B. M. Wagner, A. E. Rogers, J. Caldwell, I. G. Sipes, *Food Chem Toxicol*, 2002, **40**, 851-870.
[29] Y. Dong, R. B. Shi, B. Liu, *Zhongguo Yaoye*, 2008, **17**, 18-19.
[30] W. W. Dong, D. J. Yang, R. H. Lu, *Planta Med*, 2010, **76**, 454-457.
[31] C. Mancuso, R. Santangelo, *Food Chem Toxicol*, 2014, **65**, 185-195.
[32] Y. Dong, R. B. Shi, B. Liu, *Beijing Zhongyiyao Daxue Xuebao*, 2007, **30**, 61-63.
[33] G. Ni, Z. F. Shen, Y. Lu, Y. H. Wang, Y. B. Tang, R. Y. Chen, Z. Y. Hao, D. Q. Yu, *J Org Chem*, 2011,**76**, 2056-2061.
[34] Q. Xiao, J. J. Jackson, J. M. Bowler, A. Basak, B. G. Miller, A. Zakarian, *Nat Chem*, 2013, **5**, 410-416.
[35] D. B. Saxena, *Phytochemistry (Elsevier)*, 1986, **25**, 553-555.
[36] T. D. Nguyen, T. H. Nguyen, D. T. Pham, W. C. Taylor, *Tap Chi Hoa Hoc*, 2007, **45**, 356-362.
[37] Y. Hashidoko, S. Tahara, J. Mizutani, *Phytochemistry*, 1991, **30**, 3729-3739.
[38] F. Sorm, V. Herout, V. Terpenes, *Collect Czech Chem C*, 1948, **13**, 177-205.
[39] J. Vrkoc, V. Herout, F. Sorm, *Collect Czech Chem C*, 1961, **26**, 3183-3185.
[40] Z. Y. Hao, D. Liang, H. Luo, Y. F. Liu, G. Ni, Q. J. Zhang, L. Li, Y. K. Si, H. Sun, R. Y. Chen, D. Q. Yu, *J Nat Prod*, 2012, **75**, 1083-1089.
[41] X. L. Feng, Y. Yu, H. Gao, Z. Q. Mu, X. R. Cheng, W. X. Zhou, X. S. Yao, *RSC Adv*, 2014, **4**, 42071-42077.
[42] X. G. Tong, B. Qiu, G. F. Luo, X. F. Zhang, Y. X. Cheng, *J Asian Nat Prod Res*, 2010, **12**, 438-442.
[43] M. Rohr, P. Naegeli, *Phytochemistry (Elsevier)*, 1979, **18**, 328-329.
[44] A. V. Tkachev, A. M. Gur'ev, M. S. Yusubov, *Chem Nat Compd+*, 2006, **42**, 696-698.
[45] M. Niwa, A. Nishiyama, M. Iguchi, S. Yamamura, *Bull Chem Soc Jpn*, 1975, 48, 2930-2934.
[46] H. Minato, R. Fujioka, K. Takeda, *Chem Pharm Bull*, 1971, **19**, 638-640.
[47] M. Niwa, A. Nishiyama, M. Iguchi, S. Yamamura, *Chem Lett*, 1972, **9**, 823-826.
[48] M. Rohr, P. Naegeli, J. J. Daly, *Phytochemistry (Elsevier)*, 1979, **18**, 279-281.
[49] H. Ueda, C. Katayama, J. Tanaka, *Bull Chem Soc Jpn*, 1980, **53**, 1263-1266.
[50] M. F. Wang, A. N. Lao, H. C. Wang, *Chinese Chem Lett*, 1997, **8**, 37-38.
[51] R. Rai, I. R. Siddiqui, J. Singh, *Indian J Chem B*, 1998, **37B**, 473-476.
[52] M. F. Wang, A. N. Lao, H. C. Wang, *Chinese Chem Lett*, 1997, **8**, 35-36.
[53] X. G. Tong, L. L. Zhou, Y. H. Wang, C. F. Xia, Y. Wang, M. Liang, F. F. Hou, Y. X. Cheng, *Org Lett*, 2010, **12**, 1844-1847.
[54] Z. F. Zhao, L. L. Zhou, X. Chen, Y. X. Cheng, F. F. Hou, J. Nie, *Chin Med J*, 2013, **126**, 1230-1235.
[55] G. Sudhakar, V. D. Kadam, S. Bayya, G. Pranitha, B. Jagadeesh, *Org Lett*, 2011, **13**, 5452-5455.
[56] S. S. Selcuk, M. Bahar, A. H. Mericli, *Acta Pharmaceutica Sciencia*, 2009, **51**, 83-85.
[57] A. Patra, A. K. Mitra, *Indian J Chem B*, 1979, **17B**, 412-414.
[58] V. K. Saxena, P. Saxena, *Journal of the Institution of Chemists (India)*, 2005, **77**, 40-43.
[59] H. Tao, E. Y. Zhu, Z. T. Wang, *Zhongguo Tianran Yaowu*, 2006, **4**, 159-160.
[60] V. K. Saxena, P. Saxena, *Journal of the Institution of Chemists (India)*, 2009, **81**, 39-43.
[61] E. G. El'yashevich, G. A. Drozd, K. E. Koreshchuk, N. I. Bezuglaya, V. I. Manych, L. I. Shestak, *Khim Prir Soedin+*, 1974, **10**, 94.
[62] C. Q. Xiao, L. J. Weng, X. Y. Zhang, X. Zhao, C. X. Zhou, *China Tradit Herb Drug*, 2008, **39**, 1463-1465.
[63] S. Shukla, S. Gupta, *Pharm Res*, 2010, **27**, 962-978.
[64] ClinicalTrials.gov, http://clinicaltrials.gov/ct2/show/NCT00609310?term=Apigenin&rank=1, (accessed Nov 2014).
[65] S. Y. Lee, E. J. Moon, S. Y. Kim, S. U. Choi, K. R. Lee, *Biosci Biotechnol Biochem*, 2013, **77**, 276-280.
[66] W. W. Dong, W. Jiao, M. C. Deng, C. B. Yang, J. M. Yue, R. H. Lu, *J Chin Chem Soc-Taip*, 2008, **55**, 1277-1279.
[67] R. Rai, A. Gupta, I. R. Siddiqui, J. Singh, *Indian J Chem B*, 1999, **38B**, 1143-1144.
[68] X. Y. Yang, F. C. Chen, L. J. Wu, *China Tradit Herb Drug*, 1998, **29**, 730-731.
[69] S. D. Bhat, B. K. Ashok, R. N. Acharya, B. Ravishankar, *Ayu*, 2012, **33**, 119-122.
[70] R. Hazra, K. Ray, D. Guha, *Hum Exp Toxicol*, 2007, **26**, 947-953.
[71] C. Huang, W. G. Li, X. B. Zhang, L. Wang, T. L. Xu, D. Z. Wu, Y. Li, *Neuropharmacology*, 2013, **65**, 1-11.
[72] J. K. Miao, Q. X. Chen, X. M. Wu, C. Li, X. P. Zhang, *Int J Pharmacol*, 2012, **8**, 567-571.
[73] Q. X. Chen, J. K. Miao, C. Li, X. W. Li, X. M. Wu, X. P. Zhang, *Biol Pharm Bull*, 2013, **36**, 23-30.
[75] P. K. Shukla, V. K. Khanna, M. M. Ali, R. Maurya, M. Y. Khan, R. C. Srimal, *Hum Exp Toxicol*, 2006, **25**, 187-194.

[76] J. Cho, J. Y. Kong, D. Y. Jeong, K. D. Lee, D. U. Lee, B. S. Kang, *Life Sci*, 2001, **68**, 1567-1573.
[77] Y. Irie, W. M. Keung, *Brain Res*, 2003, **963**, 282-289.
[78] L. B. Yang, S. L. Li, Y. Z. Huang, J. M. Liang, Y. H. Wang, *NRR*, 2008, **3**, 19-24.
[79] B. Lee, Y. K. Choi, H. Kim, S. Y. Kim, D. H. Hahm, H. J. Lee, I. Shim, *Life Sci*, 2003, **74**, 435-450.
[80] H. Zhang, T. Han, C. H. Yu, K. Rahman, L. P. Qin, C. Peng, *J Pharm Pharmacol*, 2007, **59**, 301-309.
[81] M. H. Oh, P. J. Houghton, W. K. Whang, J. H. Cho, *Phytomedicine*, 2004, **11**, 544-548.
[82] P. K. Mukherjee, V. Kumar, M. Mal, P. J. Houghton, *Planta Med*, 2007, **73**, 283-285.
[83] R. Hazra, D. Guha, *Biogenic Amines*, 2003, **17**, 161-169.
[84] L. F. Zhu, Y. Fang, *J Math Med*, 1998, **11**, 46-47.
[85] Z. Y. Hu, Y. Huang, G. Liu, F. Liu, W. X. Zhou, Y. X. Zhang, *Pharmacol Clin Chin Mater Clin Med*, 2012, **28**, 252-256.
[86] Y. Hu, M. Yuang, P. Liu, L. H. Mu, H. Wang, *Chin J Chin Mater Med*, 2009, **34**, 349-351.
[87] W. Wang, Q. P. Liao, L. H. Quan, C. Y. Liu, Q. Chang, X. M. Liu, Y. H. Liao, *J Ethnopharmacol*, 2010, **131**, 313-320.

