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A review on the phytopharmacological studies of the genus *Polygala*

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ABSTRACT

Ethnopharmacological relevance: The genus *Polygala*, the most representative genus of the Polygalaceae family, comprises more than 600 species from all over the world of which around 40 are distributed in China, some of them, being used in the Traditional Chinese Medicine system.

Aim of the review: We intend to discuss the current knowledge about the traditional uses, and the newest phytochemical and pharmacological achievements with tentative elucidation of the mechanism of action on the genus *Polygala* covering the period 2013-2019 to provide a scientific support to the traditional uses, and to critically analyze the reported studies to obtain new insights for further researches.

Materials and methods: The data were systematically collected from the scientific electronic data bases including SciFinder, Scopus, Elsevier, PubMed and Google Scholar.

Results: This literature overview reported several traditional uses of different species of *Polygala*, mainly against wounds, inflammation, cardiovascular and central nervous system disorders. *P. altomontana*, *P. caudata*, *P. flavescens*, *P. glomerata*, *P. japonica*, *P. molluginifolia*, *P. sibirica*, *P. tenuifolia* are the main species which have been studied in the last few years. Phytochemical studies showed that they contain triterpene saponins, triterpenes, terpenoids, xanthenes, flavonoids, coumarins, oligosaccharide esters, styryl benzoic compounds, benzophenones, and polysaccharides. Pharmacological *in vitro* and *in vivo* studies and proposal of the mechanisms of action indicated that pure constituents and extracts of *Polygala* ssp exhibited significant anti-inflammatory, neuroprotective, antiischemic, antidepressant, sedative, analgesic, antiatherosclerosis, antitumor and enzyme inhibitory properties.

Conclusion: This review on traditional uses and phytopharmacological potential of the genus *Polygala* revealed updated insights which can be explored for further mechanism-based pharmacological activities and structure/activity relationships studies and a better comprehension of the development of Chinese medicine preparations. However some pharmacological studies showed several gaps such as incomplete methodologies and ambiguous findings. More high scientific quality preclinical studies with pharmacokinetic considerations will be required in the future to assess the traditional uses of some species of this genus. This might lead to efficacy and safety issues in clinical trials and to potential medicinal applications.

Key words: *Polygala, traditional uses, bioactive natural products, pharmacological studies*

Abbreviations:

A β , amyloid β ; ACh acetyl choline; AChE, acetylcholinesterase; AD, Alzheimer Disease; AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Bcl-2, B-cell lymphoma-2; BDNF, brain-derived neurotrophic factor; BW, body weight; COX-2, cyclooxygenase-2; CUMS chronic unpredictable-mild stress; GABA, gamma-aminobutyric acid; D-Gal-N, D-galactosamine; DPPH, 2,2-diphenyl-1-picrylhydrazyle; ERK1/2 extracellular signal-regulated kinase; GPX, glutathione peroxidase; GSH, reduce glutathione; HO-1, heme-oxygenase-1; iNOS, inducible nitrite oxide synthase; I- κ B α , nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; Il-1 β , interleukine-1 β ; Il-6, interleukine-6; LD₅₀, lethal dose 50%; LDH, lactate deshydrogenase; LPS, lipopolysaccharide; MDA, malondialdehyde; MPO, myeloperoxidase; NO, nitrite oxide; MAPK, Mitogen activated protein kinase; MDA, malondialdehyde; MDR, multi-factorial drug-resistant; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MMP matrix metalloproteinase; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; MWM, Morris-water maze; NF- κ B, nuclear factor-kappa B; NA, noradrenaline; NLRP-3, nod like receptor-3; NO, nitrite oxide; Nrf2, nuclear factor erythroid-2-related factor 2; NT-3, neurotrophin-3; (OGD), oxygen/glucose deprivation; PG E2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; PP 2A, protein phosphatase 2A; RANKL, receptor activator of NF- κ B ligand; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin; TGN, tenuigenin; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid-1; TNF- α , tumor necrosis factor alpha; XO, xanthine oxidase.

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1 Introduction

Polygalaceae, with some ca 1000 species and a limited number of 12 to 20 genera is divided into three tribes, Xanthophylleae, Polygaleae, and Moutabeae. *Polygala* is the most representative genus of the Polygalaceae family comprising ca 50 % of the species of the family. This genus including trees, shrubs, and herbs consists of more than 600 species with accepted Latin names (The Plant List, 2013). They are widely distributed throughout the world except New Zealand, Polynesia, and Arctic zones. Around 39 species are distributed in China. The name *Polygala* was given by the ancients to plants whose galactagogue and galactogen properties were emphasized (Delaude, 1992). Some species are used in local and traditional medicine as expectorant, anti-inflammatory and for treating disturbance of the central nervous system (CNS). The plants of this genus are well known to produce triterpene saponins, having mainly presenegenin as aglycone and the chemotaxonomic importance is often highlighted (Lacaille-Dubois and Mitaine-Offer, 2005, Klein Junior et al. 2012, Lacaille-Dubois et al., 2013). Several other classes of specialized metabolites such as xanthenes, oligosaccharides, lignans and other phenolic compounds have also been reported. The main objective of this review is to analyze the use of *Polygala* species in different traditional medicine systems and analyze the newest phytochemical and pharmacological assessments since our last review dated from 2013, in which we only focused on the triterpene saponins from a chemical and pharmacological point of view (Lacaille-Dubois et al., 2013), and to highlight the gaps in the scientific studies for future research opportunities.

The main species which have been reported in the literature during the period 2013-2019 are *P. altomontana*, *P. boliviensis*, *P. caudata*, *P. flavescens*, *P. glomerata*, *P. japonica*, *P. molluginifolia*, *P. sibirica*, and *P. tenuifolia*.

2. Traditional uses

The reports on the traditional uses of Polygalaceae are relatively scarce and we found references on the following species, which are summarized in Table 1. The roots of *Polygala tenuifolia* Willd (*Polygala radix*, Chinese name “Yuan-Zhi”) (Synonyms: *P. sibirica* var. *angustifolia* Lebed, *P. sibirica* var. *tenuifolia* Backer & Moore, *P. tenuifolia* var. *uncinata* Harv (www.theplantlist.org/) have been extensively used as a traditional Chinese medicine to improve memory and cognitive function in China for more than 2000 years. This drug is

officially listed in the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015) and in some countries in the North of China, the species is used to the conception of a drug against cough and as a stimulant (Delaude, 1992). The rhizome of *P. tenuifolia* Willd has been used in traditional Chinese medicine for treatment of inflammation, learning and memory deficits, dementia, neurasthenia and cancer (Yoo et al., 2014). *Polygala* root (Japanese name “Onji”) is also described in the 17th edition of the Japanese Pharmacopoeia (The Japanese Pharmacopoeia Seventeen Edition,” 2016). It is an important drug commonly incorporated in Kampo prescriptions which are used as anti-neurosis agent, tonic, and sedative agent (Uchikura et al., 2018). *Polygala* root has also been used as an expectorant, a substitute of the root of *P. senega* L. or *P. senega* L. var. *latifolia* Torrey et Gray. Many phytochemical and pharmacological studies have been carried out on *P. tenuifolia* in the contrast with the following species. *Polygala acicularis* Oliv. is known under the African name of “kienga” in the region of Kinshasa in Zaire and is used in the treatment of infected wounds. A similar usage was mentioned in the region of Brazzaville (Bouquet, 1969). *P. arenaria* Willd in the region of Kisantu in Zaire, is reported to be used against smallpox and bioactive saponins were isolated and characterized (Mitaine-Offer et al., 2005). A leave extract of *P. bakeriana* Chod , is used in Burundi as wormer (Petit, 1958) and contains saponins with presenegenin. *P. cyparissias* A. St. -Hil. & Moq (synonym: *P. cyparissias* var. *corisoides* (A. St. -Hil.) Chodat s used in folk medicine as anesthetic (Klein Junior et al., 2012). *P. glomerata* Lour., a herb growing mainly in southern China, has long been used in local medicine for treatment of acute tonsillitis, myelitis, hepatitis, pharyngitis, pulmonary tuberculosis, and whooping cough (Jiangsu New Medical College. Dictionary of traditional Chinese medicine. Shanghai, 1997). *P. japonica* HOUTT, a synonym of the accepted name *P. sibirica* L. (www.theplantlist) is an annual herb which is widely distributed in China, Japan, Philippine, and Vietnam. It has been used in traditional medicine in Vietnam in the treatment of bronchitis, amnesia, kidney injury, and as expectorant, anti-inflammatory, antibacterial, sedative , and antidepressant agents in the south of China. (Editorial board of china herbal of state administration of traditional Chinese Medicine, 1999). Furthermore, it was reported to be used in several inflammatory disorders such as acute tonsillitis, pharyngitis, myelitis and nephritis (Quang et al., 2018). *P. sabulosa* A.W. Benn., popularly known as "timutu-pinheirinho," has been used in Brazilian local medicine for the treatment of bowel and kidney disorders and as an expectorant (Nascimento et al., 2015). *P. senega* L. var. *latifolia* Torrey et Gray has been used traditionally by the North Eastern Americans as expectorant, antitussive, diuretic, anti-inflammatory, against the snake bites, and was the

object of several old scientific works (Delaude, 1992, Lacaille-Dubois et al., 2013). *P. sibirica* L. var. *megalopha* Fr., a native medicinal plant of Yunnan province (China) has long been used in local medicine for the treatment of fever, arthralgia and viper bites (Huang et al., 2015).

3. Phytochemistry

A series of previously undescribed compounds (**1-47**, Figs. 1-7), some of them of biological interest together with several known ones have been isolated in recent phytochemical investigations from around 10 species of the genus *Polygala*. They are presented in Figs1-7, together with some known ones which have been studied from a pharmacological point of view. They belong to the phytochemical classes of triterpene saponins, triterpenes, terpenoids, xanthenes, flavonoids, coumarins, styryl-pyrones, benzophenones, oligosaccharide esters, polysaccharides, along with a few other various derivatives (steroid, polysaccharide, macrolide). Bioguided fractionation based on the existing traditional knowledge were lacking in most of the reviewed literature. The procedure of extraction, and purification of the secondary metabolites was achieved according to the classical methodologies described in the different articles such as obtention of a crude extract which is purified by several solid/liquid chromatographic procedures using solvents of increasing polarity. The structure elucidation is achieved by extensive 1D and 2D NMR and MS spectroscopic analyses, and sometimes by X-ray diffraction. More recently, a rapid, sensitive and robust method for the characterization of multiple components in *P. tenuifolia* roots by ultra-high performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (UPLC/Q-TOF MS) was developed. By verifying elemental compositions and MS/MS fragmentations ions, along with their chromatographic retention times as well as retrieving reference literature, 35 compounds (mainly xanthenes, oligosaccharide esters, saponins) from *Radix Polygalae* were tentatively characterized by comparing the in-house formula database (Liu et al., 2017).

3.1 Triterpene saponins and triterpenes

Saponins is the most frequently occurring class of secondary metabolites in *Polygala* genus (Lacaille-Dubois et al., 2013). Around 10 previously undescribed triterpene saponins

were isolated during the period covered by this review (Fig. 1). An oleanane-type saponin, 2 β ,3 β -dihydroxy-29-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-olean-12-en-28-oic acid named polyjaposide (**1**) was isolated from the MeOH extract of the dried roots of *P. japonica* Houtt together with the known tenuifolin, polygalasaponin XXVIII and XLVII, and fallaxsaponin A (Quang et al., 2018). From the aerial parts of *P. sibirica*, five triterpenoid saponins, named sibiricasaponins A-E were isolated and characterized together with nine known ones (Song et al., 2013). They were elucidated as 3 β ,19 α -dihydroxyurso-12-ene-23,28-dioic acid 3-*O*- β -D-glucuronopyranoside (**2**), pomolic acid 3-*O*-(3-*O*-sulfo)- α -L-arabinopyranoside (**3**), pomolic acid 3-*O*-(4-*O*-sulfo)- β -D-xylopyranoside (**4**), pomolic acid 3-*O*-(2-*O*-acetyl-3-*O*-sulfo)- α -L-arabinopyranoside (**5**), and 3-*O*- β -D-glucopyranosyl medicagenic acid 28-*O*- β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)-(4-*O*-acetyl)-[β -D-apiofuranosyl-(1-3)]- β -D-fucopyranosyl ester (**6**). The known saponins were identified as senegenin, tenuifolin, polygalasaponin XLVIII, XXVIII, XXXIII, XXXV, XXXVI, arilloside A, 3-*O*- β -D-glucopyranosylpresenegenin-28-*O*- β -D-xylopyranosyl-(1 \rightarrow 4)-[β -D-apiofuranosyl-(1 \rightarrow 3)]- α -L-rhamnopyranosyl-(1 \rightarrow 2)-(3,4-di-*O*-acetyl)- β -D-fucopyranosyl ester. It is interesting to note that beside the common aglycone presenegenin, and medicagenic acid, we encountered in this plant some ursane-type aglycones such as pomolic acid and 3 β ,19 α -dihydroxyurso-12-ene-23,28-dioic acid, which are rarely reported from plants of the *Polygala* species (Song et al., 2013). Three saponins were isolated from *P. flavescens* and elucidated a 3-*O*- β -D-glucopyranosyl medicagenic acid 28-*O*{ α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-apiofuranosyl-(1 \rightarrow 3)]-[4-*O*-acetyl]}- β -D-fucopyranosyl ester (**7**), 3-*O*- β -D-glucopyranosyl presenegenin 28-*O*-{ β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]-[4-*O*-acetyl]}- β -D-fucopyranosyl ester (**8**), and 3-*O*- β -D-glucopyranosyl medicagenic acid { β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)-[β -D-glucopyranosyl-(1 \rightarrow 3)]}- β -D-fucopyranosyl ester (**9**) (De Leo et al., 2017). From *P. tenuifolia*, two acylated triterpene glycosides were isolated and elucidated as 3 β -[(β -D-glucopyranosyl)oxy]-2 β ,27-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*- β -D-apiofuranosyl-(1 \rightarrow 3)-[β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)-3-*O*-(*E*)-3,4,5-trimethoxycinnamoyl- β -D-fucopyranosyl ester (**10**), and 3 β -[(β -D-glucopyranosyl)oxy]-2 β ,27-dihydroxyolean-12-ene-23,28-dioic acid 28-*O*- β -D-apiofuranosyl-(1 \rightarrow 3)-[β -D-galactopyranosyl-(1 \rightarrow 4)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-

rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-4-*O*-(*E*)-3,4-dimethoxycinnamoyl-β-D-fucopyranosyl ester (**11**), together with five known ones (Kuroda et al., 2014). The chemical investigation of *P. sibirica* led to the isolation of presenegenin and tenuifolin (Zhou et al., 2014).

It is interesting to note that the derivatives of presenegenin **8**, **10**, **11** which were reported during 2013-2019 share the same partial sequence 3-*O*-Glc-presenegenin 28-*O*-{Xyl-(1→4)-Rha-(1→2)-Fuc} ester, a common structural feature in the family which is considered as a potential chemotaxonomic marker of the Polygalaceae family (Lacaille-Dubois et al., 2013).

Senegenin (= tenuigenin (TGN), 12-(chloromethyl)-2,3-dihydro-27-norolean-13-ene-23,28-dioic acid, C₃₀H₄₅ClO₃, MW 537) (Fig. 1) is a triterpene obtained after ethanolic hydrochloride hydrolysis of tenuifolin extracted from the roots of *Polygala senega* and other *Polygala* species (Delaude, 1992). Surprisingly, many publications considered tenuigenin as a major bioactive compound from the roots of *P. tenuifolia* and all the reliable pharmacological data were obtained with a commercial product from Chinese institutions without any further details. Polygalacic acid (Fig. 1) is a triterpene characterized as 2β,3β,16α,23-tetrahydroxy-Δ¹²-oleanen-28-oic acid which has been obtained after hydrolysis of triterpene saponins from *Polygala tenuifolia* (Guo et al., 2016).

3.2 Terpenoids

An original molecule was obtained from *P. flavescens* and characterized as 3β-hydroxy-5,6-epoxy-β-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**12**, Fig. 2) (De Leo et al., 2017). A megastigmane glycoside was isolated from *P. sibirica* L. var. *megalopha* Fr. and characterized as (6*R*,7*E*,9*R*)-9,13-dihydroxy-4,7-megastimadien-3-one-9-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**13**, Fig. 2) (Zhou et al., 2016). Tenuiside F, a megastigmane glycoside was isolated from *P. tenuifolia* and characterized as (3*S*,5*R*,6*S*,7*E*,9*R*)-7-megastigmene-3,6,9-triol 9-*O*-β-D-[2'-*O*-β-D-(6''-*O*-sinapoyl)-apiofuranosyl] glucopyranoside (**14**, Fig. 2) together with a known one, kiwionol (Shi et al., 2013).

3.3. Xanthenes

The species of *Polygala* are characterized by the accumulation of xanthenes as chemical markers, whose the main new representatives are presented in Fig. 3. Three original glomeraxanthenes A-C which are xanthonolignoid-C-glycosides with a *E*-dihydrobenzofuran fused xanthone skeleton and a 2-hydroxymethyl-5-hydroxyl-2-pentenoic acid moiety in the sugar chain were isolated from an ethanol extract of *P. glomerata* whole plant (**15-17**) (Li et al., 2014). A xanthone, 1,5-dihydroxy-2,3-methylenedioxyxanthone (**18**), was isolated from four species of *Polygala* (*P. campestris*, *P. densiracemosa*, *P. linoides* and *P. pulchella*) together with three known ones 1,5-dihydroxy-2,3-dimethoxyxanthone, 1,5-dihydroxy-3-methoxyxanthone, and 1,5-dihydroxy-6',6'dimethylpyrano[2',3':3,2]xanthone (Tizziani et al., 2018). These xanthenes shared the same substitution pattern 1,3,5, which occur frequently in the genus *Polygala*. Two xanthenes were isolated from the whole plant *P. sibirica* L. var. *megalopha*, and characterized as 3-hydroxy-1,2,6,7,8-pentamethoxyxanthone (**19**) and 6-*O*- β -D-glucopyranosyl-1,7-dimethoxyxanthone (**20**) together with 14 known ones (Zhou et al., 2018a). Another xanthone, named tenuiside A, was isolated from *P. tenuifolia* aerial parts and determined to be 4-*O*- β -D-glucopyranosyl-1,7-dihydroxyxanthone (**21**) together with 11 known xanthenes (Shi et al., 2013). Five known xanthenes were isolated from the roots of *P. sibirica* L., lancerin, 6-hydroxy-1, 2, 3, 7-tetramethoxyxanthone, 1, 3, 7- trihydroxy-2-methoxyxanthone, onjixanthone II, and 1, 2, 3, 6, 7-pentamethoxyxanthone (Zhou et al., 2014). Euxanthone (Fig. 3) (2,7-dihydroxyxanthone) is a xanthone derivative isolated from *Polygala caudata* (Lin et al., 2005) whose significant pharmacological effects have been reported so far.

3.4. Flavonoids

Four flavonol glycosides were isolated from *P. flavescens* (Fig. 4) and characterized as quercetin 3-*O*-(5-*O*-benzoyl)- β -D-apiofuranosyl-(1 \rightarrow 2)-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (**22**), quercetin 3-*O*-(5-*O*-sinapoyl)- β -D-apiofuranosyl-(1 \rightarrow 2)-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (**23**), quercetin 3-*O*-(5-*O*-feruloyl)- β -D-apiofuranosyl-(1 \rightarrow 2)-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (**24**), and quercetin 3-*O*-(5-*O*-*p*-coumaroyl)- β -D-apiofuranosyl-(1 \rightarrow 2)-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside (**25**), together with two known quercetin 3-*O*- β -D-apiofuranosyl-(1 \rightarrow 2)-*O*-[α -L-rhamnopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranoside, and rutin (De Leo et al., 2017). From *P. sibirica* L. var. *megalopha*, two flavonol glycosides (Fig. 4) with a rare hydroxymethyl function at C-6 were isolated and characterized as 3,3'-dihydroxy-6-

hydroxymethyl-5,7,4'-trimethoxyflavone-3-*O*- β -D-apiofuranosyl-(1 \rightarrow 2)- β -D-galactopyranoside (**26**, polygalin H), and 3-hydroxy-6-hydroxymethyl-5,7,4'-trimethoxyflavone-3-*O*- β -D-apiofuranosyl-(1 \rightarrow 2)- β -D-galactopyranoside (**27**, polygalin I), together with the known polygalin D (Huang et al., 2015). A pyranoisoflavone (Fig. 4) was isolated from the ethyl acetate fraction of *P. molluginifolia* A. St.-Hil (synonym= *P. areguensis* A.W. Benn., *P. orthiocarpa* Chodat) and characterized as 5,3',4'-trihydroxy-6'',6''-dimethylpyrano [2'', 3'':7,6] isoflavone (**28**) (ISO) (Nucci-Martins et al., 2016). A phytochemical investigation of *P. molluginifolia* (root, stems, leaves and flowers) resulted in the isolation of four pyranoisoflavones (Fig. 4) , of which 3'4'-dihydroxy-6'', 6'', 6''', 6'''-tetramethylbis(pyrano[2'',3'':5,6::2''',3''':7,8]isoflavone (**29**) was reported herein for the first time. The three other isoflavones, although they are known synthetic compounds are described here as new natural products, 3',4'-dimethoxy-5-hydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone (**30**), 3'-methoxy-5,4'-dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone (**31**), and **28** (Venzke et al., 2013).

3.5. Coumarins

An unusually substituted coumarin poligalen (**32**, Fig. 4) was isolated from the chloroform extract of the aerial parts of *P. boliviensis* A.W. Benn. (synonym: *P. weberbaueri* Chodat, www.theplantlist.org/). The structure elucidated by extensive 2D NMR experiments was confirmed by X-ray diffraction (Silva et al., 2016). A known coumarin, auraptene was isolated from *P. altmontana* Lüdtké, Bold. & Miotto. (Tizziani et al., 2018).

3.6. Styryl-pyrones

A new styryl-2 pyrone (Fig. 5) was isolated together with four known ones from the hydroalcoholic extract of the whole plant *P. altmontana* Lüdtké, Boldrini & Miotto and its structure was elucidated as 4-methoxy-6-(11,12-methylenedioxy-10-methoxystyryl)-2-pyrone (**33**) (Tizziani et al., 2018). This is the second species of *Polygala* to present the accumulation of styryl-2 pyrones.

3.7. Benzophenones

P. sibirica and *P. tenuifolia* were reported as sources of benzophenones (Fig. 5). A 2,6-dimethoxy-benzophenone-4-*O*- β -D-apiofuranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (**34**) was

isolated from *P. sibirica* L. var. *megalopha* Fr. (Zhou et al., 2016). Another new benzophenone was isolated from the roots of *P. sibirica* L. and characterized as 2, 4, 6, 4'-tetrahydroxy-3'-methoxybenzophenone-6-*O*- β -D-glucopyranoside (**35**, sibiriphenone A) (Zhou et al., 2014), whereas three benzophenones tenuisides B-D were isolated from *P. tenuifolia* aerial parts and characterized as 4-*O*- β -D-glucopyranosyl-2-hydroxy-6-methoxybenzophenone (**36**), 2-*O*- β -D-glucopyranosyl-4-hydroxy-6-methoxybenzophenone (**37**), 2-*O*- β -D-glucopyranosyl-4,4',6-trihydroxy-3'-methoxybenzophenone (**38**) (Shi et al., 2013).

3.8. Oligosaccharide esters

A phytochemical study of the roots of *P. japonica* resulted in the isolation of a phenyl propanoid glycoside, characterized as 1-*O*-cinnamoyl- β -D-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranosyl-(2 \rightarrow 1)- β -D-glucopyranoside (**39**, Fig. 6) named japonicaside A, and the known β -D-[(1-*O*-cinnamoyl)-(3-*O*-benzoyl)]-fructofuranosyl-(2 \rightarrow 1)- α -D-glucopyranosyl-(2 \rightarrow 1)-6-*O*-acetyl- β -D-glucopyranoside, tenuifoliside A (β -D-3-*O*-(3,4,5-trimethoxycinnamoyl)]-fructofuranosyl- α -D-[6-*O*-(*p*-hydroxybenzoyl)]-glucopyranoside) (Fig. 6) and tenuifoliside B (β -(3-*O*-sinapoyl)-fructofuranosyl- α -D-[6-*O*-(*p*-hydroxybenzoyl)]-glucopyranoside), β -D-[(3-*O*-(3,4,5-trimethoxycinnamoyl)]-fructofuranosyl- α -D-[6-*O*-(4-methoxybenzoyl)]-glucopyranoside, β -D-(3-*O*-sinapoyl)-fructofuranosyl- α -D-(6-*O*-sinapoyl)-glucopyranoside, and 6-acetyl-3,6'-diferuloylsucrose (Quang et al., 2018). Two oligosaccharide esters (Fig. 6) were isolated from *P. flavescens* and elucidated as β -D-(6-*O*-benzoyl)fructofuranosyl-(2 \rightarrow 1)-[β -D-glucopyranosyl-(1 \rightarrow 3)-6-*O*-acetyl- α -D-glucopyranoside (**40**) and its deacetylated structural analog (**41**) together with the known 3,6'-di-*O*-sinapoylsucrose and reiniose F (De Leo et al., 2017). Five saccharide esters were isolated from *P. sibirica* L., the 6-*O*-(*E*)-sinapoylpoligalitol named sibiricose A7 (**42**), and the known polygalatenosides A and C, the 3'-*O*-3,4,5-trimethoxycinnamoyl-6-*O*-4-methoxybenzoyl sucrose, and tenuifoliside C (Zhou et al., 2014) (Fig. 6). Furthermore two compounds, polygalaonjisides A (**43**) and B (**44**), were isolated from a methanol extract of *P. tenuifolia* and characterized as syringic acid 4-*O*-(2'-*O*- β -D-apiosyl)- β -D-glucoside and 2-*O*-(β -D-glucosyl)-3'-*O*-benzoylsucrose, respectively (Uchikura et al., 2018) whereas the known tenuifoliside A (Fig. 6) has been previously isolated as one of the phenyl propanoids from a water extract of the dried roots of *P. tenuifolia* (Kim et al., 2013).

3.9. Other secondary metabolites

A compound named poligapolide was isolated from the ethyl acetate extract of *P. tenuifolia* together with 9 known compounds and characterized as (9R)-(-)-9-peptandecanolide (**45**, Fig. 7) (Yoo et al., 2014). A steroid, α -spinasterol (5 α -stigmasta-7,22-dien-3 β -ol) (Fig. 7) was isolated from *P. altomontana*. (Tizziani et al., 2013), and from *P. campestris*, *P. densiracemosa*, *P. linoides* and *P. pulchella* (Tizziani et al., 2018). A polysaccharide was isolated and characterized from *P. tenuifolia*. NMR and GC/MS indicated that it possesses as repeating unit a backbone consisting of 1,4,6-linked- β -D-galactopyranose, 1,4-linked- β -D-glucopyranose and 1,4-linked- β -D-galactopyranose with non-reducing terminal 1-linked- α -D-glucopyranose attached to O-6 of 1,4,6-linked- β -D-galactopyranose in the ratio of 3.97:2.01:2.00:1.92 (**46**) (Yao et al., 2018). A coniferyl alcohol glycoside named tenuiside E was isolated from *P. tenuifolia* and characterized as (-)-(7R,8S)-7',Z-dehydroconiferyl alcohol 4-O- β -D-glucopyranoside (**47**) (Shi et al., 2013).

4. Pharmacology

We will report here the published pharmacological activities of around 10 *Polygala* species and their isolates. We also will try to evaluate critically them by highlighting some research gaps to justify further research in an area. The articles included the anti-inflammatory activity in *P. japonica*, *P. tenuifolia*, *P. boliviensis*, *P. molluginifolia*, *P. sabulosa*, neuroprotective activity in *P. glomerata*, *P. tenuifolia*, and *P. molluginifolia*, antiischemic effect in *P. sibirica*, antidepressant effect in *P. japonica*, *P. tenuifolia*, hypnotic-sedative effect in *P. tenuifolia*, analgesic effect in *P. molluginifolia*, antiatherosclerosis activity in *P. caudata*, cytotoxic, antitumor activity in *P. flavescens*, *P. densiracemosa*, *P. campestris*, *P. molluginifolia*, *P. caudata*, and xanthine-oxidase inhibitory activity in *P. sibirica* L. var. *megalopha*.

4.1. Anti-inflammatory activity

4.1.1. Cellular models

Several cellular models were used for *in vitro* tests to determine the anti-inflammatory effects and mechanism of action of some *Polygala* species crude extracts and pure compounds. The most relevant results are described below. The inflammation is considered to be a major risk factor in the pathogenesis of chronic diseases where the macrophages are important immune cells. The root of *P. tenuifolia* is well known for its use in the treatment of

neurasthenia, dementia, amnesia, and inflammation and the isolated phenyl propanoid glycoside, tenuifoliside A (Fig. 6) was investigated for its anti-inflammatory effects in RAW 264.7 and murine peritoneal macrophages which were stimulated by lipopolysaccharide (LPS) at 1µg/ml (Kim et al., 2013). The results showed that tenuifoliside A (extracted and isolated from *P. tenuifolia* Wild roots with a purity >98%) (5, 10, 20, 40 µM) inhibited the production of nitrite oxide (NO), inducible nitric oxide synthase (iNOS), prostaglandine E2 (PG E2) and cyclooxygenase (COX)-2 in a dose-dependent manner as measured by enzyme immunoassay in comparison with the control group LPS-treated macrophages. Furthermore, it suppresses the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β through inhibition of the transcription factors such as nuclear factor-kappaB (NF-κB) and mitogen-activated protein kinases (MAPKs) signaling pathways (Kim et al., 2013), which provide some explanation on the mechanism of anti-inflammatory effect. The presence of tenuifoliside A in the extracts of *P. tenuifolia* may explain the traditional use of *P. tenuifolia* for the treatment of inflammation. However more researches are needed to evaluate the anti-inflammatory activity of this compound in comparison with a positive control such as a commercial anti-inflammatory drug, and to validate such results in an animal model.

Polygalen (**32**), is a coumarin isolated from *P. boliviensis* and whose structure was unequivocally established and confirmed by X-ray analysis (Silva et al., 2016). In the concentration range (30-300 µM), it was shown to exhibit immunomodulatory effects, reducing the levels of the pro-inflammatory cytokines IL-6 and TNF after LPS stimulation (1µg/ml) in RAW 264.7 peritoneal macrophages as assessed by ELISA (p<0.05 compared with LPS/vehicle group), in a concentration-dependent manner without altering cell viability assessed by the MTT assay. All the experiments were carried out in comparison with dexamethasone (1 mM), a well-known anti-inflammatory drug, which was used as a positive control. However, it potentiates NF-κB activation in a concentration-dependent manner (Silva et al., 2016). This property distinguished polygalen from many other coumarins which reduce the expression of the cytokines mediated by NF-κB inhibition, an important transcription factor involved in the transcription of TNF and IL-6 in macrophages. Therefore, further pharmacological studies must be carried out to explore these contradictory results and to suggest potential targets of polygalen at this stage of research.

Tenuigenin (TGN) (purity >98 %), was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). It was also shown to

prevent IL-1 β -induced inflammation in human osteoarthritis chondrocytes (Wang et al., 2016). Osteoarthritis, a chronic degenerative joint disease is characterized by a progressive loss of articular cartilage and inflammation of synovium, and the chondrocytes, the resident cells in articular cartilage play an important role in cartilage degradation. When stimulated with IL-1 β , they lead to the production of inflammatory mediators such as NO and PG E2 and matrix metalloproteinases MMPs which can degrade the extracellular matrix of cartilage and play critical roles in the physiopathology of osteoarthritis. In the search for effective drugs to treat osteoarthritis, TGN was investigated for its anti-inflammatory effects and mechanism of action. The results showed that TGN at the concentration up to 8 μ g/ml had no cellular toxicity on chondrocytes. Thus, cells were treated with increasing concentrations of TGN (2, 4, 8 μ g/ml) for 24 h in the subsequent studies. It was found that TGN significantly suppressed IL-1 β (10 ng/ml) -induced NO, PG E2, MMP-1, MMP3, and MMP-13 production in chondrocytes in a dose-dependent manner ($p < 0.01$ vs. IL-1 β group). Furthermore, the anti-inflammatory mechanism of TGN is due to its ability to significantly inhibit the PI3K/AKT/NF- κ B signaling pathway, P13K and AKT being the upstream molecules of NF- κ B which demonstrated to play important roles in NF- κ B activation. These results of the targets and signaling pathways involved in this *in vitro* model should be analyzed with caution, since the experiments were conducted without a positive control and further *in vivo* testing is needed to validate such preliminary results.

In another study, polygalacic acid (obtained from *P. tenuifolia* Willd), which was purchased from Shangai Purone Biotechnology (purity > 97%) (Fig. 1) (50, 100 μ M) was found to significantly suppress the IL-1 β -induced COX-2, MMPs expression in rat chondrocytes in a dose-dependent manner ($p < 0.05$ vs. IL-1 β group), and the IL-1 β -induced activation of Wnt/b-catenin and the MAPK signal pathway. Furthermore, polygalacic acid treatment prevented the degeneration of cartilage in a rat model of osteoarthritis (Xu et al., 2018). However, the *in vivo* studies doesn't give enough informations on the design of experiments which are performed without a commercial antiosteoarthritis agent, without data of the bioavailability of TGN in the animals, and the concentration which reach the systemic blood flow. Therefore, the results of these studies on TGN and polygalacic acid are not enough documented to conclude that TGN and polygalacic acid may have a potential therapeutic value as anti-osteoarthritis agents.

Osteoclasts are multinuclear giant cells that have unique ability to degrade bone. The search for new medicines that modulate the formation and function of osteoclasts is a

potential approach for treating osteoclast-related bone diseases. RANKL (receptor activator of NF- κ B ligand), a member of the TNF receptor-ligand family, is the most crucial factor for osteoclasts differentiation both *in vitro* and *in vivo* and NF- κ B is the most important and essential signaling for both osteoclast differentiation and survival. TGN (purity >98%, obtained from the Guidechem (Shanghai, China)) was shown to significantly inhibit RANKL-induced osteoclastogenesis by downregulating NF- κ B activation in a dose-dependent manner (Yang et al., 2015). Consistent with the *in vitro* results, it was shown that TGN suppressed RANKL-induced bone loss in an animal model. However, this study was too preliminary, since only a limited number of mice (n=6) was used without comparison of the activity with a positive control. Further research works are needed before to conclude of the potential use of TGN in the treatment and alleviation of osteoclast-related disease such as osteoporosis.

4.2.2. Mouse model

The *in vivo* models of pleurisy, peritonitis, pneumonia, liver and kidney injury were explored in order to establish the *in vivo* anti-inflammatory effect and the mechanism of action of some *Polygala* species crude extracts and pure compounds.

The anti-inflammatory activity of *P. molluginifolia*, a native plant from Rio Grande do Sul (southern Brazil) which is rich in flavonoids was investigated on a mouse model of carrageenan (1%, intrapleural route)-induced pleurisy which is a useful and reliable model for screening new anti-inflammatory drugs. The crude hydroalcoholic extract of the bark (50-200 mg/kg, i.p.), its derivate fractions including its aqueous (10-50 mg/kg, i.p.), ethyl acetate, (10-50 mg/kg, i.p.), hexane (25-100 mg/kg, i.p.) fractions and the pure isoflavone (ISO, **28**, Fig. 4) (1-5 mg/kg, i.p.) and rutin (2.5-10 mg/kg, i.p.) (Fig. 4), two compounds isolated from the most active fractions, showed important anti-inflammatory effects in comparison with dexamethasone (0.5 mg/kg) by inhibiting leukocyte migration and exudate concentrations (mostly $p < 0.01$), decreasing the activities of some pro-inflammatory enzymes (myeloperoxidase (MPO) and adenosine deaminase (ADA)), and the production of important mediators (NO, TNF- α , IL-1 β) in this *in vivo* model with the exception of rutin which was unable to reduce the NO levels (Arruda-Silva et al., 2014). In addition, ISO (5 mg/kg, i.p.) and rutin (10 mg/kg, i.p.) reduce the TNF- α and IL-1 β mRNA expression levels, and decreased the phosphorylation of the p65 NF- κ B subunit (% of inhibition: 33.7 ± 5.0 for ISO and 50.1 ± 5.2 , for rutin), similarly to the effect observed with dexamethasone (0.5 mg/kg, i.p.). These results showed that *P. molluginifolia* has a significant anti-inflammatory action

and this effect is due at least in part to the presence of ISO and rutin in large amounts, which affect the NF- κ B pathway (Arruda-Silva et al., 2014). This work represents a robust pharmacological study in which many parameters have been taken into account in order to rely the anti-inflammatory effect to isolated compounds. However, additional studies on isolated cells are needed to define the exact key point of the NF- κ B cascade on which these isolated compounds act. A similar study was conducted on *P. sabulosa* to test the anti-inflammatory effects of the crude extract (50 mg/kg), the acetonic fraction (25 mg/kg) and the main compound 7-prenyloxi-6-methoxycoumarin (1 mg/kg) (Fig. 4) by using the same mouse inflammation model in comparison with dexamethasone (1 mg/kg). The results showed that they reduced inflammation in the pleural cavity and lungs. This effect was evidenced by significant reduction on the same inflammatory parameters evaluated, except the inability of the crude extract to inhibit exudate formation. The results obtained with the pure 7-prenyloxi-6-methoxycoumarin (1 mg/kg, i.p.) suggested that this important anti-inflammatory effect was at least in part due to its ability to inhibit the NF- κ B and p38 MAPK pathways activation which represent the main intracellular triggers of extensive inflammatory cascades (Nascimento et al., 2015). Furthermore, the hydroalcoholic extract of *P. sabulosa* (HEPs, 3-300 mg/kg, intragastric gavage, i.g.) was shown to possess significant anti-inflammatory effects in another mouse model of LPS (0.02 μ g/kg)-induced peritonitis, which might be mediated through inhibition of TLR-4 signaling and pro-inflammatory cytokines (IL-1 β , 100 mg/kg, TNF- α , 100-300 mg/kg). The steroid isolated from *P. sabulosa*, α -spinasterol (0.001-10 mg/kg, ip or i.g.) (Fig. 7) significantly reduced inflammatory cell infiltration in LPS-injected mice in comparison with dexamethasone used as a positive control (0.5 mg/kg, i.p.) and might be linked at least in part to the anti-inflammatory effects of *P. sabulosa* (Borges et al., 2014). This hypothesis has to be validated because HEPs are a complex mixture of other constituents (i.e., coumarin, styrylpyrones, β -sitosterol), any of which may be responsible for its properties. Thus, additional studies needed to be addressed with other constituents present in *Polygala sabulosa*.

In a study of Yu et al., 2017, TGN (Fig. 1) was shown to protect against *S. aureus*-induced pneumonia in mice. TGN (2, 4, 8 mg/kg) significantly attenuated *S. aureus*-induced lung histopathological changes, MPO activity in lung tissues, and inflammatory cytokines production (TNF- α , IL-1 β) in the bronchoalveolar lavage fluid (BALF) in a dose-dependent manner ($p < 0.01$ vs. *S. aureus* group) without a positive control. It was shown that the anti-inflammatory activity of TGN was related to the inhibition of iNOS and COX-2 expression

via downregulation of the MAPK and NF- κ B, and up-regulation of the nuclear factor erythroid-2-related factor 2/heme-oxygenase-1 (Nrf2/HO-1) signaling pathways (Lv et al., 2016). Nrf2, an important transcription factor, has been reported to play a significant role in protecting cells in response to oxidative stress. Nrf2 exhibited antioxidant effect by inducing phase II detoxifying enzymes particularly the HO-1 (Jia et al., 2017). Therefore, activating Nrf2 could inhibit the inflammatory response. TGN also protected against LPS/D-Gal-N (D-galactosamine)-induced acute liver injury in mice by suppressing inflammatory and oxidative responses (Jia et al., 2017). Namely, administered at 2, 4, 8 mg/kg, TGN was found to inhibit important markers of liver injury, the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels together with TNF- α and IL-1 β production in a dose-dependent manner ($p < 0.01$ vs. LPS/Gal-N group). It also inhibited LPS/Gal-N-induced Apoptosis Signal-regulating Kinase 1 (ASK1) expression, NF- κ B and MAPKs activation and upregulated the expression of Nrf2 and HO-1 as assessed by Western blot analysis. In addition, histological changes in the liver tissues induced by LPS/GalN and LPS/GalN-induced malondialdehyde (MDA) production were attenuated by TGN suggesting that TGN inhibited LPS/GalN liver injury by inhibiting inflammatory and oxidative response (Jia et al., 2017). This study was carried out on many biochemical parameters evaluated by reliable techniques, but before to conclude that TGN might be a potential therapeutic agent for the treatment of liver injury, a comparison with a positive control is needed together with consideration of pharmacokinetics properties. Similarly, TGN was shown to significantly inhibit the inflammatory response, ameliorating kidney tissue damage, alleviating sepsis-induced acute kidney injury (AKI) in mice by reducing pathological changes and significantly decreasing the levels of blood urea nitrogen, serum creatinine and kidney coefficient. TGN (5, 20, 50 mg/kg) significantly inhibits the production of inflammatory cytokines considered as a reliable marker of sepsis (TNF- α and IL-6) in a dose-dependent manner ($p < 0.05$, or $p < 0/01$), and mediators (NO and PG E2) ($p < 0.05$) (Xu et al., 2017) and significantly inhibits the activation of the NF- κ B signaling pathway.

4.2. Activity on the Central Nervous System

4.2.1. Anti-neuroinflammatory effect

Neuroinflammation plays an important role in the development of neurodegenerative diseases such as Parkinson's and Alzheimer diseases. Microglia, the resident brain macrophages are the key mediators of neuroinflammatory responses. Activation of brain microglial cells during the inflammatory process results in the release of proinflammatory mediators (NO and PG E2) and cytokines (TNF- α , IL-1 β , IL-6, IL-12), which in case of excessive secretion may cause several neuroinflammatory and neurodegenerative diseases (Ransohoff 2016). Therefore suppression of mediator and cytokine production in LPS-stimulated microglial cells might be an important target for the treatment of such diseases.

Two phenylpropanoid glycosides, tenuifoliside B (Fig. 6) and β -D-[3-*O*-(3,4,5-trimethoxycinnamoyl)]-fructofuranosyl- α -D-[6-*O*-(4-methoxybenzoyl)]-glucopyranoside isolated from *Polygala japonica* Houtt were tested for their anti-inflammatory effects in an *in vitro* model of LPS-stimulated BV2 microglial cells (Quang et al., 2018). Both compounds were shown to be not cytotoxic at concentrations of 20 and 40 μ M, respectively as determined by the MTT assay, and to inhibit NO and PGE2 production in a dose-dependent manner (IC₅₀ ranging from 11.7 to 22.5 μ M) ($p < 0.01$ compared with the group treated with LPS only), using Griess reaction and ELISA kit, respectively. These effects were confirmed by the decrease in iNOS and COX-2 expression levels, using Western blot analysis. Furthermore, they significantly suppressed the release of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-12 mRNA expression) in LPS-stimulated BV2 cells, tenuifoliside B being more active with an IC₅₀ ranging from 5.9 to 9.5 μ M.

A triterpene saponin, polygalasaponin-F (PS-F, Fig. 1, 3-*O*- β -D-glucopyranosyl bayogenin 28-*O*-[β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl] ester) extracted from *P. japonica* (Li et al., 2006) was shown to decrease the release of the inflammatory cytokine TNF- α in LPS-stimulated BV2 cells which were pretreated with/without inhibitors (Yan et al., 2015). The mechanism of action involved the inhibition of the toll-like receptor (TLR)-4, phosphatidylinositol 3-kinase (PI3K) or I- κ B α phosphorylation, which completely prevents the release of TNF- α induced by LPS without affecting cell viability. In addition PS-F exhibited a similar trend regarding TNF- α release, AKT phosphorylation and NF- κ B translocation. These results suggest that PS-F reduces neuroinflammatory cytokine release through the regulation of the TLR4-PI3K/AKT-NF- κ B signaling pathway (Yan et al., 2015).

Another *in vitro* study reported the inhibitory effect of the compounds isolated from *P. tenuifolia* aerial parts against LPS-induced NO production in BV-2 microglia cells and the

most active compound was the known 1,7-dihydroxy-4-methoxyxanthone (Fig. 3) with an IC_{50} of 7.4 μ M), comparable to that of the positive control indomethacin (IC_{50} 7.1 μ M), whereas the four other xanthone derivatives showed moderate activity (IC_{50} in the range of 16.2-38.5 μ M) (Shi et al., 2013). Therefore, this xanthone might be a potential natural source of NO inhibitors used in the treatment of neurodegenerative disorders. A preliminary structure/activity relationship of xanthones for NO inhibition has shown that the 7-OH group seems to have a significant contribution for the inhibitory activity and when the 7-OH group is replaced by a 7-OCH₃ group, the inhibition decreased obviously (Shi et al., 2013).

The glomexanthones A-C isolated from *P. glomerata* (**15-17**, Fig. 3) were tested for their neuroprotective effect on L-glutamic acid-induced cellular damage in human neuroblastoma SK-N-SH cells, including the decrease of cell viability expressed as a percentage of the control groups, by using the MTT assay with resveratrol, as a positive control. At 10 μ M they increased the cell viability on L-glutamic acid (30 mM) treated group, showing a moderate neuroprotective effect ($p < 0.05$ for **15**, $p < 0.01$ for **16** and **17** vs. model). The data presented here presented an interest from a phytochemical point of view, since the three compounds **15-17** possess a 2-hydroxymethyl-5-hydroxy-2 pentenoic acid moiety, which is rare in natural products, but the impact of the biological data are limited since the assay has been carried out at only one dose of compound, and no followed by further studies on the mechanism of action. (Li et al., 2014).

Wang et al., 2017a showed that TGN (Fig. 1) (1, 2, 4 μ M) strongly inhibited LPS-induced inflammatory mediator (TNF- α , IL-1 β , IL-6, and PGE2) production in an in vitro model of LPS-stimulated microglial cells as measured by ELISA. This response was shown to be dependent of the activation of Nrf2/ HO-1 signaling pathway. In another study to elucidate the mechanism of action, TGN has been shown to provide neuroprotection in neurodegenerative disorders in vitro by downregulating the release of NO, MMP-9, and pro-inflammatory cytokines TNF- α , IL-1 β from LPS-activated microglia in a dose-dependent manner (Lu et al., 2017). It also directly protects the neurons through neuritogenesis, antiapoptosis, and antioxidant effects (Lu et al., 2017).

In all these *in vitro* assays on microglia cells, no positive control was used except the indomethacin in the study of 1,7-dihydroxy-4-methoxyxanthone and the resveratrol in the study of glomexanthones. Furthermore, these *in vitro* studies should be completed by *in vivo* experiments to avoid false positive results derived from interaction of the compounds/extracts

with the milieu, before any conclusion can be drawn on the pharmacological potential of the compounds of *Polygala* spp as candidates for the treatment of neuro-inflammatory disorders.

4.2.2. Neuroprotective effects

4.2.2.1. Neurodegenerative diseases

TGN (Fig.1) was shown to exert neuroprotection in the progression of Parkinson Disease (PD) which is characterized by a progressive loss of dopaminergic neurons in substantia nigra and depletion of dopamine in the striatum (Fan et al., 2017). In an *in vivo* PD mouse model based on the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 20 mg/kg, i.p.), TGN (25, 50 mg/kg) was shown to increase striatal dopaminergic levels and improved MPTP-induced motor deficits by using openfield test and rotarod test. TGN significantly improved the degeneration of dopaminergic neurons and inhibited NLRP3 inflammasome activation in substantia nigra in this PD model. Furthermore, to elucidate the mechanism of action, TGN was shown to reduce ROS production and suppress NLRP3 inflammasome activation, subsequent caspase-1 cleavage and $\text{IL-1}\beta$ secretion in BV2 microglia cells. These results revealed the anti-inflammatory role of TGN, partly through the inhibition of NLRP3 inflammasome activation in microglia and suggest promising further studies on TGN as a potential candidate for PD therapy (Fan et al., 2017).

Neuroplasticity is the essential process in brain physiological functions such as learning and memory formation and TGN has been shown to potentiate the basic synaptic transmission, the potential mechanism of action remains unclear (Wei et al., 2015). The mechanism of neuroprotection or nootropic effects of TGN was examined by reliable techniques using *in vitro* field potential electrophysiology and whole-cell patch clamp techniques in acute hippocampal slices from rats (Wei et al., 2015). The results of this study showed that TGN enhances the basic synaptic transmission by regulating presynaptic intracellular calcium. These findings will contribute to explain the mechanism of TGN as a nootropic product or neuroprotective drug in the future.

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by a progressive decline in cognitive functions and is associated with pathological aggregations of the amyloid β ($\text{A}\beta$) peptide and neurofibrillary tangles of aggregated hyperphosphorylated tau proteins in the brain. Numerous pharmacological studies have demonstrated that oxidative stress is involved in AD-type neurodegeneration with cognitive impairments (Huang et al., 2018). The increase of the ROS can lead to mitochondrial

dysfunction, tau hyperphosphorylation, and A β overexpression. TGN has been used to improve memory and cognitive function in Traditional Chinese Medicines for centuries. Its possible *in vivo* protective effect against learning and memory impairments, oxidative stress, and tau protein phosphorylation was tested on intracerebroventricular -streptozotocin (ICV-STZ, 3 mg/kg)-induced cognitive decline in a rat model of sporadic AD. This animal model has pathological characteristics comparable to those of human patients.

Daily treatment with TGN (purity >98%) (2, 4, 8 mg/kg, intragastric route) for 28 days significantly improved the memory-related behaviors assessed by the Morris-water maze (MWM) test in a dose-dependent manner and markedly reduced changes in malondialdehyde (MDA) and 4-hydroxy-2-nonenal adducts, significantly inhibited STZ-induced reduction in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in the hippocampus. Furthermore, it reduced the hyperphosphorylation of tau proteins induced by STZ. This study showed that TGN can ameliorate the cognitive dysfunction, oxidative stress and tau hyperphosphorylation in this *in vivo* model and suggested that it might be a useful agent for the treatment of AD (Huang et al., 2018). Further studies are required to compare the activity with a positive control, and to address some pharmacokinetic data, before concluding on the anti-AD potential of TGN on the basis of these assays.

It is well documented that the selective inhibition of central acetylcholinesterase (AChE) might increase acetylcholine (ACh) neurotransmission, resulting in improvements in cognitive function. The isoflavones from *P. molluginifolia* were tested for their ability to inhibit AChE and for their antioxidant effects. The new 3',4'-dihydroxy-6'',6'',6'''-tetramethylbis(pyrano[2'',3'':5,6::2'',3'':7,8] isoflavone (**29**) and a known dimethylpyranoisoflavone (5,3',4'-trihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone, Fig. 4) showed the best results with IC₅₀ of 68 and 84 μ M, respectively, compared with Reminyl® (IC₅₀ 32 μ M) (Venzke et al., 2013). Both compounds possess OH groups at C3' and C4' in the B ring of the isoflavone, which suggests that these groups may enhance the AChE inhibition. The addition of a second dimethylpyran ring fused to the A ring also increased the inhibition of AChE, as observed when comparing both compounds. These two isoflavones were shown to possess antioxidant activity similar to ascorbic acid as a positive control in the *in vitro* DPPH free-radical scavenging assay. However, this preliminary study based on a chemical reaction, doesn't allow relevant pharmacological conclusions on the potential *in vivo* antioxidant activity. As a consequence of neuroinflammation, oxidants are released and oxidative stress occurs in the brain, which are observed in the development of

AD. Since Reactive Oxygen Species (ROS) are controlled by intracellular antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, further biological studies on these targets and pharmacological *in vivo* investigations are required before suggesting the potential therapeutic interest of this phytochemical class in the treatment of neurodegenerative diseases.

4.2.2.2. Cognitive impairments

TGN was shown to significantly attenuate LPS-induced deficit in memory function in the Morris-water maze and passive avoidance tests which are two classical behavioral tests on mice ($p < 0.01$, vs. LPS group) (Wang et al., 2017a). The animals were randomly divided into five groups of 12 animals: control group, LPS group, LPS+TGN (5 mg/kg), LPS+TGN (10 mg/kg), LPS+TGN (20 mg/kg). LPS was dissolved in sterile saline and injected intraperitoneally (0.25 mg/kg). TGN was orally administered for 3 weeks prior to LPS injection and the mice were examined three days after LPS treatment. The profiles for spatial learning, such as escape distance (cm), escape latency (sec), and step-through latency (sec) changed significantly ($p < 0.01$ vs LPS group). After the behavioral tests, the mice were killed and biochemical analysis were undertaken on the cortex and hippocampus. The expression of TNF- α , IL-1 β , IL-6, Nrf2, and HO-1 were detected by Western blot analysis. The results showed that TGN dose-dependently inhibited the LPS-induced inflammatory cytokines TNF α and IL-1 β by activating Nrf2 signaling pathways, confirming the previous *in vitro* study. The design of this study is very well analyzed and may suggest in the future more deeply analysis of long-term experiments.

Estrogen deficiency is associated with cognitive impairments and may result in the synaptic morphological changes in the hippocampus and prefrontal cortex (Cai et al., 2013). The hormone replacement therapy has proven to be effective in preventing and reversing the memory and learning deficiencies. However, the conventional estrogenic treatment remains a drawback and might increase the risk of breast cancer and venous thromboembolism. In an *in vivo* study of Cai et al., 2013, which used two classical behavioral tests, the in step-through passive avoidance and Y-maze tests in ovariectomy-induced amnesia model in mice, the effects of TGN (4 mg/kg, orally) were assessed on the learning and memory abilities at different periods (4, 8, and 12 weeks) after surgery in comparison with estradiol benzoate (20 μ g/day, subcutaneously) (Cai et al., 2013). Furthermore, the number of NO synthase (NOS)

positive neurons and the synaptic measurement of hippocampal CA-1 area were examined. The results showed that oral treatment of TGN at 4 mg/kg could reduce the loss of NOS positive neurons and prevent the synaptic morphological changes induced by ovariectomy. These results suggest that TGN can ameliorate learning and memory impairments in ovariectomized mice and might be useful in the treatment cognitive dysfunction caused by surgical menopause. To support such a conclusion, further researches are required to evaluate the activity with at least two doses and to examine the mechanism of TGN ameliorating cognitive deficiency.

An *in vivo* study of Guo et al., 2016 examined the effect of polygalacic acid (purity 98 %) (Fig. 1) (3, 6, 12 mg/kg, orally) on scopolamine (Scop, 1 mg/kg, i.p.)-induced memory impairment in mice and the potential mechanisms involved. Scopolamine was used to promote amnesia in the animals, because it has been shown to impair memory by blocking the muscarinic cholinergic receptors in the brain. Furthermore, scopolamine significantly increases AChE activity and decreased the ACh level. Memory-related behaviors were assessed using the classical MWM assay. Cholinergic and neuroinflammatory activities were measured in the brain tissue, together with the markers of oxidative stress, in comparison with galanthamine as positive control (2 mg/kg). The results showed that polygalacic acid significantly reversed the scopolamine-induced effects. It facilitated central cholinergic activity by significantly inhibiting AChE activity and elevating acetylcholine and choline acetyl transferase (ChAT) levels in the brain. Polygalacic acid significantly reduced the activities of the pro-inflammatory cytokine IL-1 β and enhanced the activity of the anti-inflammatory cytokine IL-10. In addition, it significantly increased the activities of SOD, and decreased the content of MDA and GSH. Therefore, these results suggest that this triterpene may exert significant neuroprotective effects on cognitive impairment, at least in part by increasing cholinergic neurotransmission and decreasing neuroinflammation and oxidative stress (Guo et al., 2016). The design of the study has been carefully explained. The doses have been scientifically selected and were based on the conversion from clinical adult dose of *Radix Polygalae* and the results were compared with negative and positive controls.

4.2.2.3. Brain ischemia

Ischemic strokes occur when blood supply to part of the brain is decreased, resulting in glucose and oxygen deficiency and eventually brain damage. Stroke-related neurologic deficits affect language, cognition, and motor functions, which severely affects patients' quality of life. Therefore the search for natural compounds having less side effects than the thrombolytic agents is required. They are very few reported studies in the genus *Polygala* on

this activity. The saponins of *P. sibirica* were tested for several *in vitro* assays related to neural system activities, including the anti-ischemic effect on oxygen/glucose deprivation (OGD) model on rat pheochromocytoma (PC12) cells, which is an *in vitro* model for stroke, which mimic ischemic-like conditions, and shows similarities with the *in vivo* models of brain ischemia. In this preliminary study, only tenuifolin (Fig. 1) showed a weak anti-ischemic effect ($EC_{50} = 46.7 \mu\text{M}$) in this OGD assay, whereas the other compounds didn't show any significant activities (Song et al., 2013). This study consisting in a cell viability assay without positive or negative controls, without giving the tested doses, doesn't have pharmacological relevance, and was presented here as a complementary study of the chemical part of *P. sibirica*.

4.2.3. Antidepressant activity

Depression is a leading cause of the disability and mortality in our society. It is characterized by anorexia, hypothermia, pessimistic and a series of depressive behaviors (Palazidou, 2012). Due to side effects of the available antidepressant drugs, and the non-response of patients to current treatments, there is a great need to find new antidepressant agents for treatment of depression. The following *in vivo* studies aim to investigate the antidepressant activity in mice by using valuable behavioral tests associated with depression including the classical the tail suspension test and the forced swimming test relative to controls and to evaluate the possible mechanism of action.

The aqueous extract of *P. japonica* was tested at 30 mg/kg, in comparison with the positive control fluoxetine (2 mg/kg) which is a selective hydroxy-tryptamine (5-HT) reuptake inhibitor and clinically effective antidepressant. The results showed that the extract alleviates in a significant manner depression-like behaviors in a model of depression induced by chronic restraints and normalizes the deficit in hippocampal neurogenesis induced by chronic stress. It was demonstrated that the increase of neurogenesis was related to the antiapoptotic effect of the extract on the newborn neurons by activation of Bcl-2 and phospho-Erk1/2 (extracellular signal-regulated kinase) (Zhou et al., 2018b). These results suggested that *P. japonica* may protect from depression by counteracting reduced hippocampal neurogenesis induced by chronic stress. The constituents of this extract were analyzed by UPLC-QTOFMS (positive ion mode) and comparison with m/z data from the literature revealed the presence of 14 compounds including mainly xanthenes, flavonoids and triterpenes, two of them, 7-hydroxy-1,3-dimethoxyxanthone and quercetin being the main

components (Zhou et al., 2018b). Although this study explored carefully the effects of an aqueous extract of *P. japonica* at only one dose of 30 mg/kg on depression-like behaviors against a positive and negative control, with reliable statistics, further works are needed to evaluate the role of impaired hippocampal neurogenesis as a contributory factor for depression. Furthermore, additional doses of the extract should be tested in order to see a possible dose-dependent effect and a complementary work on the main isolated compounds might help to correlate the observed effect to well characterized molecules.

P. tenuifolia which is known in traditional Asian medicine to exert a variety of neuropsychiatric effects. Its 70 % ethanolic extract was tested for antidepressant-like effects in mice in several models of behavioral despair and chronic stress at various doses (0.1, 1, 10 mg/kg) and time scales of oral administration in order to elucidate the possible mechanism of action. Results showed that the extract appeared to exert its effects by modulating glutaminergic synapses in critical brain circuits of depression (Shin et al., 2014). It was shown that the anti-depressant-like effect of *P.tenuifolia* was associated with phosphorylation of GluR1 AMPA receptor subunit (glutamate receptor 1 α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) but not mTOR (mammalian target of rapamycin) in the hippocampus. *Polygala* radix may possibly substitute for other rapid-onset antidepressants like ketamine that are associated with addiction risk and unacceptable side effects. This reliable study of good scientific quality from a pharmacological point of view should be completed by an additional phytochemical study in order to give more robustness to the LC chromatogram giving poor information on the constituents which might be responsible of the effects.

A study of Li et al., 2017, reported that a treatment of mice with senegenin (purity >98 %) (= tenuigenin, Fig. 1) (4 and 8 mg/kg) remarkably ameliorated chronic unpredictable mild stress (CUMS)-induced behavioral abnormalities in mice, such as improving locomotor activity, decreasing immobility time in tail suspension test and forced swimming test, and increasing sucrose intake in sucrose preference test, in comparison with fluoxetine (20 mg/kg) used as a positive control which is a selective 5-hydroxytryptamine (5-HT) reuptake inhibitor and clinically effective antidepressant. Furthermore, senegenin improved protein levels of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) expression and down-regulated $\text{IL-1}\beta$, $\text{TNF-}\alpha$ secretion possibly through inhibiting the activation of NF- κ B regulating NLRP3 inflammatory pathway (Li et al., 2017). These results demonstrate the important role of senegenin in treatment of CUMS-induced depression in mice. The study using a diversity of relevant depression-like behavioral tests is carefully described using two

doses of test material and comparison with a negative and positive control and has tentatively elucidated the mechanism of action.

4.2.4. Hypnotic-sedative activity

Polygalasaponins extracted from the roots of *P. tenuifolia* possess anxiolytic and sedative-hypnotic activity and tenuifolin (Fig. 1) was the main constituent of this extract. Cao et al., 2016 investigated the hypnotic effect and possible mechanism of tenuifolin at 20, 40, 80 mg/kg in freely moving mice in comparison with the positive control diazepam (6 mg/kg). The results have shown that tenuifolin at 40 and 80 mg/kg (orally) showed a significant sleep-enhancing activity in mice increasing total sleep by 42.1% and 42.5% ($P < 0.001$), respectively, and concomitantly decreasing wakefulness in comparison with the vehicle control. This effect seems to be mediated, at least in part, by the activation of GABAergic systems and/or by inhibition of noradrenergic systems (Cao et al., 2016). Since this study is conducted with high dose levels of tenuifolin in comparison with the positive control, it can be followed by bioavailability and pharmacokinetics studies to know the metabolites which are responsible of the activity. However, the currently available information may bring a scientific contribution to the use of *Polygla tenuifolia* as a sedative drug in local medicine.

4.2.5. Analgesic activity

The *in vivo* antinociceptive effect of the hydroalcoholic extract of *P. molluginifolia* (10-1000 mg/kg intragastrically) and the isolated 5,3',4' -trihydroxy-6'',6''-dimethylpyrano [2'',3'':7,6]isoflavone (**28**) (ISO, 10-100 mg/kg, orally) was investigated in behavioral animal models of acute pain, such as formalin and plantar incision-induced postoperative pain in mice, together with the mechanism underlying this effect (Nucci-Martins et al., 2016). They elicit a potent dose-dependent inhibition of the nociceptive behavioral response in animal models of pain without causing sedation or locomotor dysfunction. This antinociceptive effect may be mediated, at least in part, by the activation of endogenous opioid receptors and/or by the inhibition of transient receptor potential ankyrin 1 (TRPA1), and transient receptor potential vanilloid-1 (TRPV1) channels. Finally the antinociception was reversed by the preadministration of naloxone (1 mg/kg, i.p.). Before to conclude that this study brings a scientific evidence for the use of *P. molluginifolia* in the search for natural analgesic

phytomedicines, additional experiments are required with a positive control such as a commercial analgesic drug, and with pharmacokinetics studies.

4.3. Activity on the cardiovascular system

Atherosclerosis (AS) related to the chronic inflammation of the arterial vessel walls plays a key role in the pathophysiology of cardiovascular diseases such as myocardial infarction and stroke (Benjamin et al., 2018). Among the factors contributing to atherosclerosis, we can find the excessive oxidative stress, lipoprotein modification, immune reactivity and inflammation which finally lead to endothelial dysfunction, an initial event in atherogenesis. The (ox-LDL)-induced cell injury constitutes the major trigger of endothelial dysfunction. Euxanthone (Fig. 3) isolated from *P. caudata* was shown to protect human umbilical vein endothelial cells (HUVECs) from ox-LDL (100 µg/ml)-induced cytotoxicity, apoptosis, and DNA fragmentation in a dose-dependent manner ($p < 0.01$), when used at 5, 10 µM, for 6h before ox-LDL exposure (Li et al., 2019). Furthermore, it significantly protected the HUVECs against ox-LDL-induced oxidative damage by restoring the antioxidant system (SOD, CAT, GSH-Px, MDA) and decreasing ROS levels. The compound inhibits inflammation in HUVECs treated with ox-LDL, in a dose-dependent manner, reducing the levels of MPC-1, IL-1 β and TNF- α , through Nrf2 activation via the MAPK pathway. Although this study has carefully analyzed in details many parameters, further *in vivo* studies in comparison with an antiatherosclerotic agent should be carried out before promoting euxanthone as a promising agent against AS.

4.4. Cancer-related activities

We report here recent advances in the search for potential chemotherapeutic agents from *Polygala* species. *In vitro* activity (cytotoxicity or growth inhibitory activity, or enzymatic inhibition) as well as *in vivo* antitumor activity on transplanted tumors in animals are useful test systems for the discovery of potential chemotherapeutic agents.

The human isoform 5 of the enzyme lactate dehydrogenase (hLDH5) which is overexpressed in many types of invasive tumors, catalyzes the final step of glycolysis, the reduction of pyruvate to lactate, thus playing a key role in cancer cell metabolism (De Leo et al., 2017). Therefore the search for natural hLDH5 inhibitors represents an attractive goal to find new effective anticancer drugs. The isolates of *P. flavescens* ssp. *flavescens* were tested for their *in vitro* LDH inhibitory activity, and 3,6'-di-*O*-sinapoylsucrose (Fig. 6) showed an

IC₅₀ value of $90.4 \pm 4.4 \mu\text{M}$, activity which was comparable or slight better than the positive control galloflavin (IC₅₀ $102.4 \pm 15 \mu\text{M}$) (De Leo et al., 2017). Modeling studies were carried out to suggest the putative mode of interaction of this molecule in the active site of enzyme. These results encourage further studies to verify the *in vivo* antitumor potential of the isolates of *P. flavescens*.

A study of Tizziani et al., 2017 reported a screening for cytotoxic activity against 6 tumor cell lines including lymphoma HL-60 (human promyelocytic leukemia), Jurkat (human immortalized line of T-lymphocyte) and THP-1 (human monocytic cell line derived from an acute monocytic leukemia patient) cell lines and carcinoma [MCF-7, MDA-MB-231 (human breast carcinoma) and HCT-116 (human colorectal carcinoma)] cell lines in comparison with non-cancerous Vero cells (derived from the kidney of an African green monkey) of 10 species of crude hydroalcoholic extracts of *Polygala* genus growing in Brazil by using the classic MTT cell viability assay. Control groups include treatment with 0.5 % DMSO (negative control) and etoposide (10 μM , positive control). All extracts were tested in triplicate in two independent experiments. Only the extracts of *P. densiracemosa*, *P. campestris* and *P. molluginifolia* (50 $\mu\text{g/ml}$) inhibited the tumor cell viability by more than 50% and presented low inhibition of proliferation for non-cancer cells. The best results were obtained for the *P. molluginifolia* extract at 50 $\mu\text{g/ml}$, with inhibition of the cell viability of HL-60 (83%), Jurkat (78%), MDA-MB-231 (75%), MCF-7 (70%) and HCT-116 (64%), however this species also inhibited the viability of the Vero cells (32%). A selectivity for Jurkat (66% inhibition of cell viability) and HL-60 (78% inhibition of cell viability) cells was observed with *P. campestris*, whereas *P. densiracemosa* showed selectivity only for HL-60 cells (66% inhibition of cell viability) with low toxicity against non-tumor cells (11% and, 12 % inhibition, respectively). This showed that these *Polygala* ssp are promising sources for further studies with several doses of extracts in order to assess an IC₅₀ value, with other anticancer drugs as positive controls and with purified isolated compounds. Although this *in vitro* study presented some gaps, using extracts at one dose, the authors performed the experiments with great care and emphasized the absence of interference with the medium, which is relatively rare in most *in vitro* assays which are described in the literature.

Euxanthone (2,7-dihydroxyxanthone, Fig. 3) extracted from *P. caudata* (Lin et al., 2005) was tested at 2.5, 5, 10, and 20 μM for cancer cell viability. It was shown to significantly inhibit the growth and survival of CRC cell lines as indicated by CCK-8 assay (HT-29, HCT 116, SW480, SW 630, LOVO) in a dose and time-dependent manner with IC₅₀

ranging from 20.38 ± 5.23 to $61.35 \mu\text{M}$ at 24h and from 13.66 ± 2.96 to $36.92 \pm 4.06 \mu\text{M}$ at 48h, depending of the cell type, whereas it did not affect the viability of the human colon NCM460 epithelial cell line (IC_{50} $206.25 \pm 21.9 \mu\text{M}$, 24h, $139.36 \pm 18.2 \mu\text{M}$, 48h). SW480 and HT-29 were the most sensitive to this drug and were chosen for further experiments. Euxanthone was shown to significantly promote apoptosis in the CRC cells as detected by the tunnel and annexin V assays through the activation of the caspase-dependent apoptotic pathway and the involvement of mitochondrial intrinsic apoptosis pathway. In this complete and reliable study, the effect of euxanthone was also analyzed on the metastatic and invasive abilities of CRC cells showing that at both 2.5 and 5 μM doses, it significantly reduced the migratory capacity of CRC cells, while their invasive activity was also reduced in a dose-dependent manner (Wang et al., 2018). Furthermore, euxanthone also effectively suppressed tumor growth *in vivo* at 20 and 40 mg/kg/d and pulmonary metastasis in a CRC xenograft murine model by targeting cancerous inhibitor of protein phosphatase 2A (CIP2A)/PP2A signaling (Wang et al., 2018). This robust *in vitro* and *in vivo* study indicating that euxanthone exerts a potent anticancer effect on CRC cells precisising the mechanism of action, used only a negative control to express the results. A comparison with a positive control such as a commercial anticancer drug used in CRC, would be to consider in order to confirm these first results.

A study of Kuete et al., 2016 evaluated the cytotoxicity of seven phenolics compounds including euxanthone, occuring in African medicinal plants against a panel of 9 cancer cell lines including drug-sensitive and multifactorial drug-resistant (MDR) phenotypes, by using the resazurin reduction assay with doxorubicin (DXR) as a positive control and DMSO dissolving the samples as negative control. Each assay was done at least two times with 6 replicates each. To compare tumor cells with normal cells, HepG2 liver cancer cells and AML12 normal hepatocytes were used. Even if euxanthone was not sufficiently cytotoxic ($\text{IC}_{50} > 10 \mu\text{M}$ in all the tested cells) in comparison with the other tested compounds, it displayed collateral sensitivity of resistant phenotypes as compared to their respective sensitive counterparts. As example, cytotoxic effects against the drug sensitive leukemia CCRF-CEM cells (IC_{50} $34.23 \mu\text{M}$), and its multidrug-resistant CEM/ADR5000 subline (IC_{50} $49.34 \mu\text{M}$) were observed, and euxanthone displayed selective cytotoxic effects (IC_{50} HepG2 cells: $14.98 \mu\text{M}$, IC_{50} AML12 cells: $>175.44 \mu\text{M}$, selectivity index >11.71). Therefore, this molecule which is studied here for the first time towards MDR cancer cells with complete and

reliable experimental data, deserves more investigation to develop cytotoxic drugs against MDR cancers (Kuete et al., 2016).

Continuing to investigate the activity of euxanthone, Zhu et al., 2018 examined its role in ovarian cancer using an *in vitro* model (SKOV3 and OVCAR3 human ovarian cancer cell lines) and an *in vivo* xenograft model. Euxanthone (10 and 20 μM for 24 or 48 h) decreased cell viability in a dose and time dependent-manner in both cell lines as assessed by a cell counting Kit (CCK)-8 assay, and blocked the cell cycle progression at G2/M phase as measured by flow cytometry. It induced apoptotic cell death in a caspase-dependent manner as measured by flow cytometry and TUNEL assay and led to the accumulation of autophagosomes as evaluated by Western blotting. Investigation of the mechanism of action revealed that euxanthone induced apoptosis and autophagy by modulating pSTAT3/Bcl-2 signaling. The *in vivo* studies by using a xenograft model have shown the selective antitumor effect of euxanthone (40, and 80 mg/kg/d, i.p., for 24 days). These *in vitro* and *in vivo* experimental data supported further investigations in comparison with a positive control before to consider euxanthone as a potential chemotherapeutic agent in the treatment of ovarian cancer.

One polysaccharide extracted from the roots of *P. tenuifolia* (PTP, **46**, Fig. 7), tested *in vitro* at three concentration (0.5, 1 and 2 mg/ml) for 48 h on human ovarian carcinoma OVCAR-3 cells showed a significant inhibitory effect on the cell growth and colony formation ($p < 0.01$, when compared to control cells) (Zhang et al., 2015). As underlying mechanism of action, this effect was found at least in part mediated by downregulation of Bmi-1 and telomerase activity, suggesting the potential of PTP as a new chemotherapeutic candidate against human ovarian cancer. The prolongation of this work was conducted to investigate the antitumor effect *in vivo* against SKOV3 xenograft tumor growth in BALB/c mice (the tumor size in mm^3 and tumor weight in g and tumor growth inhibition rate) versus a negative control of phosphate buffer saline (PBS) as well as the mechanism of action. (Yao et al., 2018). The tumor growth was suppressed in mice after two weeks of polysaccharide administration (10, 20, and 40 mg/kg, oral gavage five days per week for two consecutive weeks), with an inhibition rate of 26.30, 31.29, and 50.31 % , respectively compared to the negative control, and the reduced tumor growth was due in part to induction of cell apoptosis of tumor tissues, as detected by the TUNEL assay ($p < 0.05$, $p < 0.01$). Furthermore, the serum levels of vascular endothelial growth factor (VEGF), one of the most important angiogenic factors regulating tumor-induced angiogenesis and epidermal growth factor receptor (EGFR) were significantly decreased in mice treated with the three doses of PTP in comparison with

the level in tumor tissues of untreated mice ($p < 0.05$, < 0.01). Also protein and mRNA levels of angiogenic factors were significantly downregulated (Yao et al., 2018). Further investigations with a positive control are required before ascertaining that PTP could be useful in the treatment of patients with ovarian cancer.

The phosphatidylinositol-3kinase/protein kinase B (PI3Akt/Akt) cell survival pathway is particularly activated by several key human pathogenic viruses such as HIV-1. This activation involves specific viral proteins, among them Tat (transactivator of transcription) of HIV-1. The transfection of HIV-1 tat into CHME5 cells (a human microglial cell line) and human macrophages elevated their cytoprotective phenotypes against cellular stresses. This pathway is also highly activated in many cancer cells. Therefore, (PI3/Akt) inhibitors have been extensively searched as potential anti-cancer and anti-HIV-1 dementia agents. In the search for PI3K/Akt inhibitors from *P. tenuifolia*, Yoo et al., 2014 isolated 9 compounds from the ethyl acetate fraction of *P. tenuifolia* which potently showed anti-cytoprotective effect by employing HIV-1 Tat-induced cytoprotective phenotype of CHME5 cells. One of them (9R)-(-)-9-peptandecanolide, named poligapolide (**45**, Fig. 7), (5, 10, 20 μM , 48h) showed the most potent activity in a dose-dependent manner, which is comparable to the positive control miltefosine (a commercial Akt-inhibitor). As a mechanism of action, it was shown that poligapolide exerted its effect by inhibiting Akt phosphorylation in the PI3K/Akt pathway. This phytopharmacological on an original compound using bioassays with a positive and negative control, several doses, and analysis of mechanism of action, deserves further *in vivo* investigations to promote this new compound as a PI3/Akt inhibitor.

Most of the cancer-related studies reported here were carried out *in vitro* on various cell lines, except three studies on isolated compounds from *P. caudata* (euxanthone) and *P. tenuifolia* (polysaccharide, and polygapolide) which were conducted in an animal model. Therefore, further preclinical and clinical researches may promote these plant species as potential sources of anticancer agents

4.5. Xanthine oxidase inhibitory effect

Xanthine oxidase is considered to be an important biological source of superoxide radicals. These and ROS contribute to the oxidative stress on the organism and are involved in many pathological processes, among them inflammation, atherosclerosis, cancer, and aging. The enzyme xanthine oxidase catalyzes the oxidation of xanthine to uric acid, which was measured according to the increasing absorbance at 290 nm. Inhibition of xanthine oxidase

results in a decreased production of uric acid, which may be beneficial in the treatment of gout. A megastigmane glycoside (**13**, Fig. 2) isolated from *P. sibirica* L. var. *megalopha* was found to inhibit the xanthine oxidase in a concentration-dependent manner with an IC_{50} of 6.48 μ M, exhibiting a similar effect as the positive control allopurinol (IC_{50} 4.34 μ M) (Zhou et al., 2016). Two new flavonol glycosides polygalin H (**26**), and I (**27**) with a rare hydroxymethyl at C-6 (Fig. 4) isolated from this species were found to be strong inhibitors of xanthine oxidase with an IC_{50} of 9.48 and 8.31 μ M, respectively, which deserves further *in vivo* studies (Huang et al., 2015).

4.6. Other pharmacological activities

A study of Wang et al., 2017b investigated whether the aqueous extract of *P. tenuifolia* (PTE) possesses anti-obesity activity and how this activity affects liver gene expression and gut microbiota. The study was carried out on 3T3-L1 adipocytes and a high-fat diet-induced obese mouse model to determine the effects of PTE on lipid accumulation. Liver gene expression and gut microbiota profiles following PTE treatment were conducted to elucidate possible mechanisms. The authors reported that PTE (500 μ g/ml during four days) exhibited strong activity without causing cytotoxicity, reducing lipid accumulation in the 3T3-L1 cells through reducing lipid formation and serum triglyceride levels without producing hepatotoxicity or nephrotoxicity and by increasing lipase activity. To investigate the effects of PTE *in vivo*, the mice were fed with a High Fat Diet (HFD) for 4 weeks to induce obesity. After treatment with PTE (250 mg/kg, daily for five weeks), the increased body weight, the serum elevated triglyceride content and liver steatosis in the high-fat diet-induced obese mice were each reduced, without altering food intake. They also proved that the anti-obesity effect of PTE is probably caused by the modulation of hepatic gene expression and gut microbiota composition in mice (Wang et al., 2017b). This study was performed with only one dose of PTE and with only a negative control. Before concluding that PTE can be possible candidate to treat obesity, this work should be continued by analyzing several doses of PTE, by the comparison with a positive control (such as metformine or orlistat which are clinically used anti-obesity drugs), by the isolation and characterization of the compounds which might be responsible of the observed effects, and finally by additional toxicological and clinical studies.

5. Toxicity

The toxicological aspects of some *Polygala* species have been neglected due to their long history of use for various purposes. In a study of Shin et al., 2014, the preclinical safety of the alcoholic extracts of the roots of *P. tenuifolia* was assessed using an acute and subchronic oral toxicity test in Sprague-Dawley rats and beagle dogs. The study was conducted with 6 animals of each sex for the acute toxicity assay and 40 animals for the subchronic assay, in comparison with the control group without treatment. The extract was administered at 2g/kg for the acute toxicity test and at 360, 600, 1000 mg/kg for the subchronic toxicity test. In the rats, only one female treated by 2g/kg died. No mortalities were also observed in the dogs. Although vomiting, or hemorrhage was found in some dogs, there were no serious abnormalities. In the subchronic toxicity assay in the rats, two animals died in the female group at 1.0 g/kg/d and there also were no adverse effects on the body weight, hematological and biochemical findings. This study demonstrated that the root extract of *P. tenuifolia* was not toxic in rats and beagle dogs (Shin et al., 2014).

6. Conclusion

The goal of this review is to critically summarize the knowledge on the genus *Polygala* from a phytochemical and pharmacological point of view since our previous review in which we concentrated our attention on bioactive triterpene saponins (Lacaille-Dubois et al., 2013) and the review of Klein Junior et al., 2012, which focussed on the genus *Polygala* as a good source of pharmacologically active compounds.

About fifty previously unreported compounds have been isolated and characterized by extensive spectroscopic methods from around 10 species of *Polygala* genus, including mainly saponins, triterpenes, terpenoids, xanthenes, flavonoids, isoflavones, coumarins, benzophenones, styryl-pyrones, oligosaccharide esters, and a polysaccharide, some of them being of biological interest. In the literature review, most compounds were isolated without bioguided fractionation. Of the 10 species which have been used in Traditional Asian and African Medicine for inflammation, dementia, amnesia, neurasthenia and cancer, three of them have been largely studied from a phytopharmacological point of view, *P. tenuifolia*, *P. japonica*, and *P. sibirica*, resulting in the tentative elucidation of the mechanism of action of extracts and isolates bringing a scientific evidence linked to the traditional uses. Most of the performed pharmacological studies were carried out by using *in vitro* and some *in vivo* bioassays. Finally, one can conclude that the root of *P. tenuifolia* (*Polygalae radix*) is the most important plant which has been attracting increased interest in recent years owing to the

pharmacological effects of its constituents including effects related to the central nervous system such as anti-neuroinflammatory, neuroprotective, antidepressant, sedative-hypnotic activities, together with the anti-inflammatory effects, the cytotoxic, and antitumor activities. Tenuigenin, a triterpene has been largely studied for its neuroprotective, antidepressant, and anti-inflammatory effects. Other biologically active metabolites from *P. tenuifolia* were reported such as tenuifolin, a sedative triterpene saponin, polygalacic acid, a neuroprotective triterpene, tenuifoliside A, an anti-inflammatory oligosaccharide ester, and the antitumor polysaccharide and polygalolide, which could scientifically support the use of this plant in Traditional Chinese Medicine. The studies on *P. japonica* which are more limited led to the isolation of two oligosaccharide esters and polygalasaponin F which might be therapeutic candidates for the treatment of neuroinflammatory disorders and the studies on *P. sibirica* concerned the neuroprotection and cytotoxic effects on tumor cells. Finally, Polygala species which are not used in Traditional Medicine such as *P. boliviensis*, *P. caudata*, *P. flavescens*, *P. glomerata*, and *P. molluginifolia*, represented interesting sources of novel bioactive compounds in the field of inflammation, neuroprotection, pain, and cancer related activities.

All these results analyzed in this review were based on modern phytochemical and pharmacological methods. They have shown that the *Polygala* genus is of substantial importance from a phytochemical point of view with the isolation of original secondary metabolites and some interesting chemotaxonomic conclusions. However, after a critically evaluation of the publications, we highlighted some current gaps in the literature from a pharmacological point of view, which needed to be taken into account for further research on the genus *Poygala*. Firstly, the pharmacological studies on crude extracts (alcoholic or aqueous extracts) should be followed by the bio-guided isolation and characterization of the active constituents, and in-deep investigations of the mechanism of action. Secondly, some pharmacological studies were performed by using only *in vitro* bioassays, which might provide false positive results by interaction of the tested material with the milieu. Thirdly, when the *in vivo* assays were carried out, sometimes with a limited number of animals, a limited number of doses, the toxicological and pharmacokinetics studies were scarce. Fourthly, the lack of comparison with a standard positive control and sometimes negative control is frequently observed in both *in vitro* and *in vivo* studies, which limited the relevance of the results.

Since the currently available pharmacological *in vitro* and *in vivo* studies are incomplete to justify the traditional uses of some species of Polygala, it is recommended to perform in the future in-deep preclinical studies to provide better scientific knowledge of

these plants of interest. This might lead to efficacy and safety issues in clinical trials and to potential medicinal applications

Author's contributions

MALD designed the study, collected the chemical and pharmacological literature data and wrote the manuscript. ACOMO and CD reviewed the literature on the traditional uses of *Polygala* species. All authors approved the final version of the manuscript.

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Legends of Figures 1-7

Fig. 1 Structure of saponins and triterpenes

Fig. 2 Structure of terpenoids

Fig. 3 Structure of xanthones

Fig. 4 Structure of flavonoids and coumarins

Fig. 5 Structure of styryl-pyrones and benzophenones

Fig. 6 Structure of oligosaccharide esters

Fig. 7 Structure of other metabolites

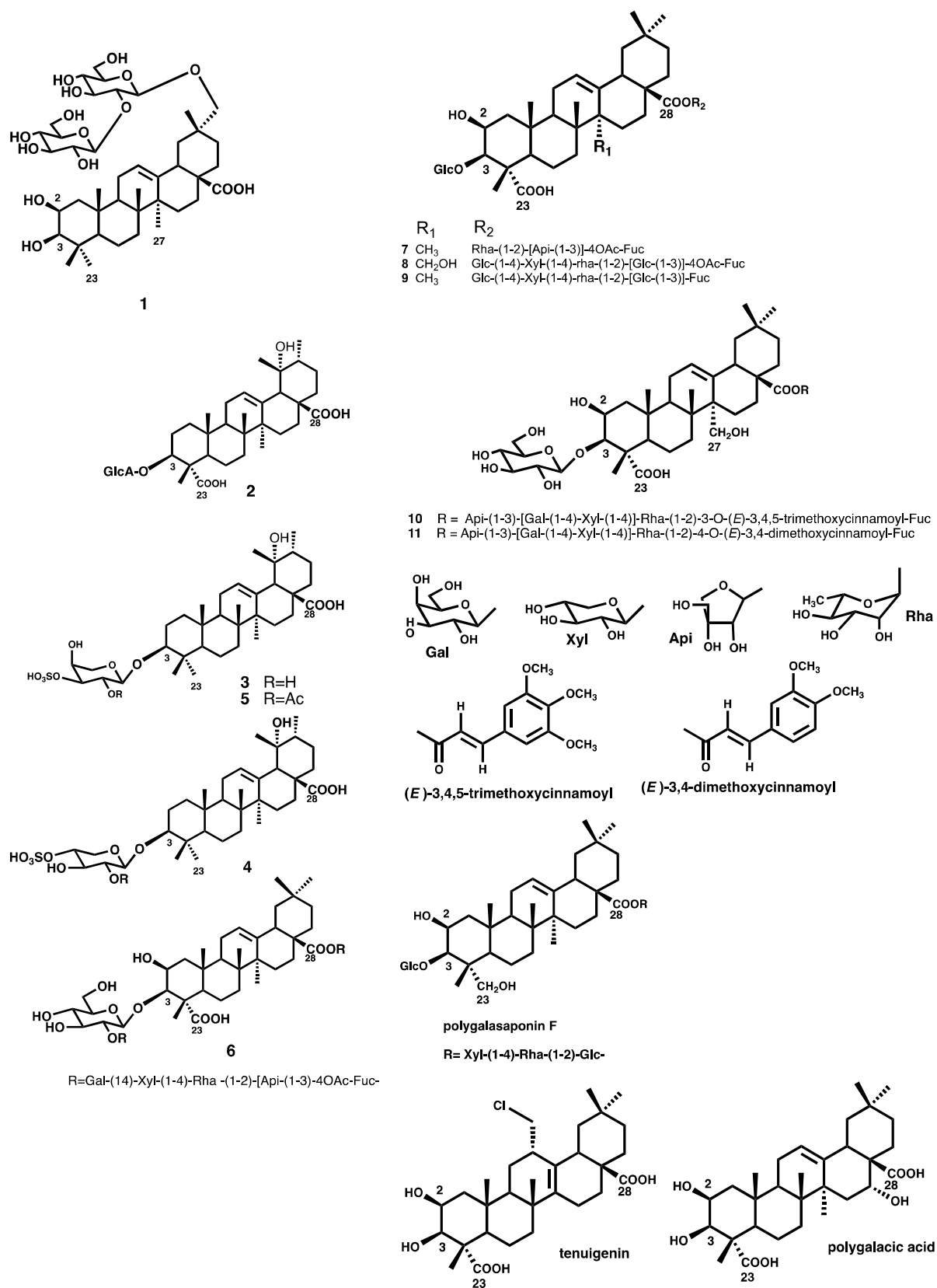


Fig. 1. Structure of saponins and triterpenes

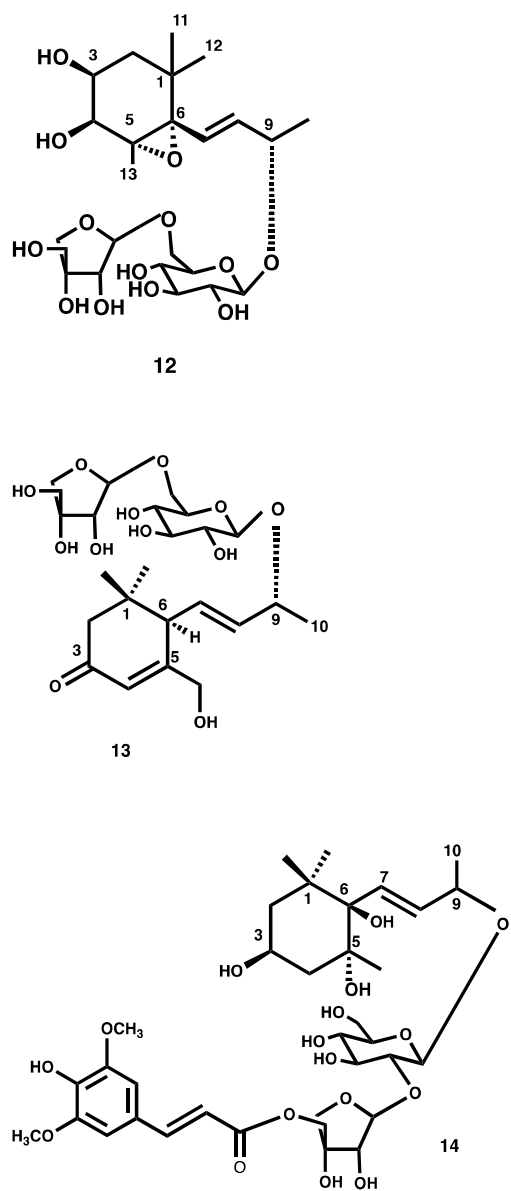


Fig. 2 Structure of terpenoids

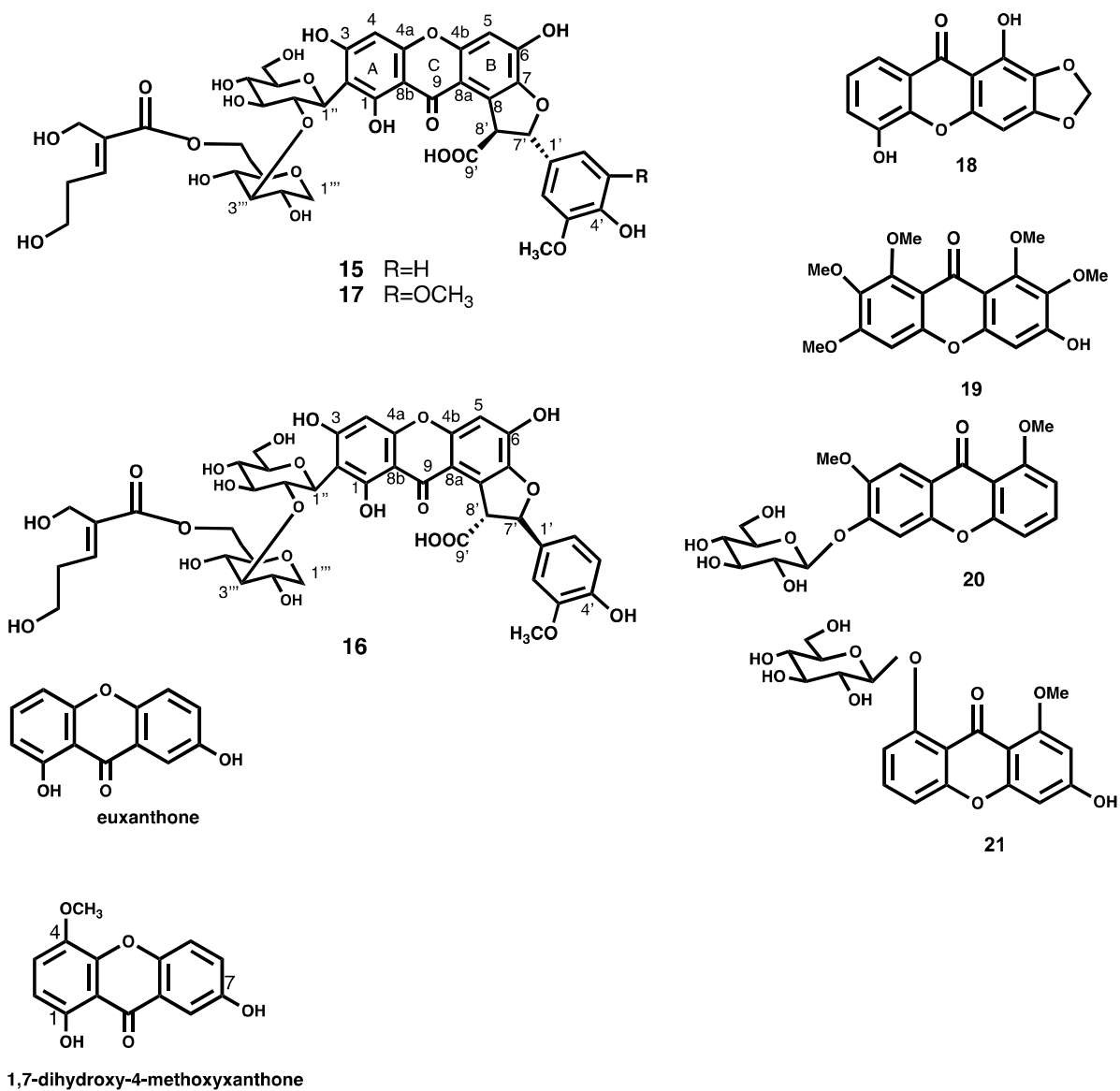


Fig. 3. Structure of xanthones

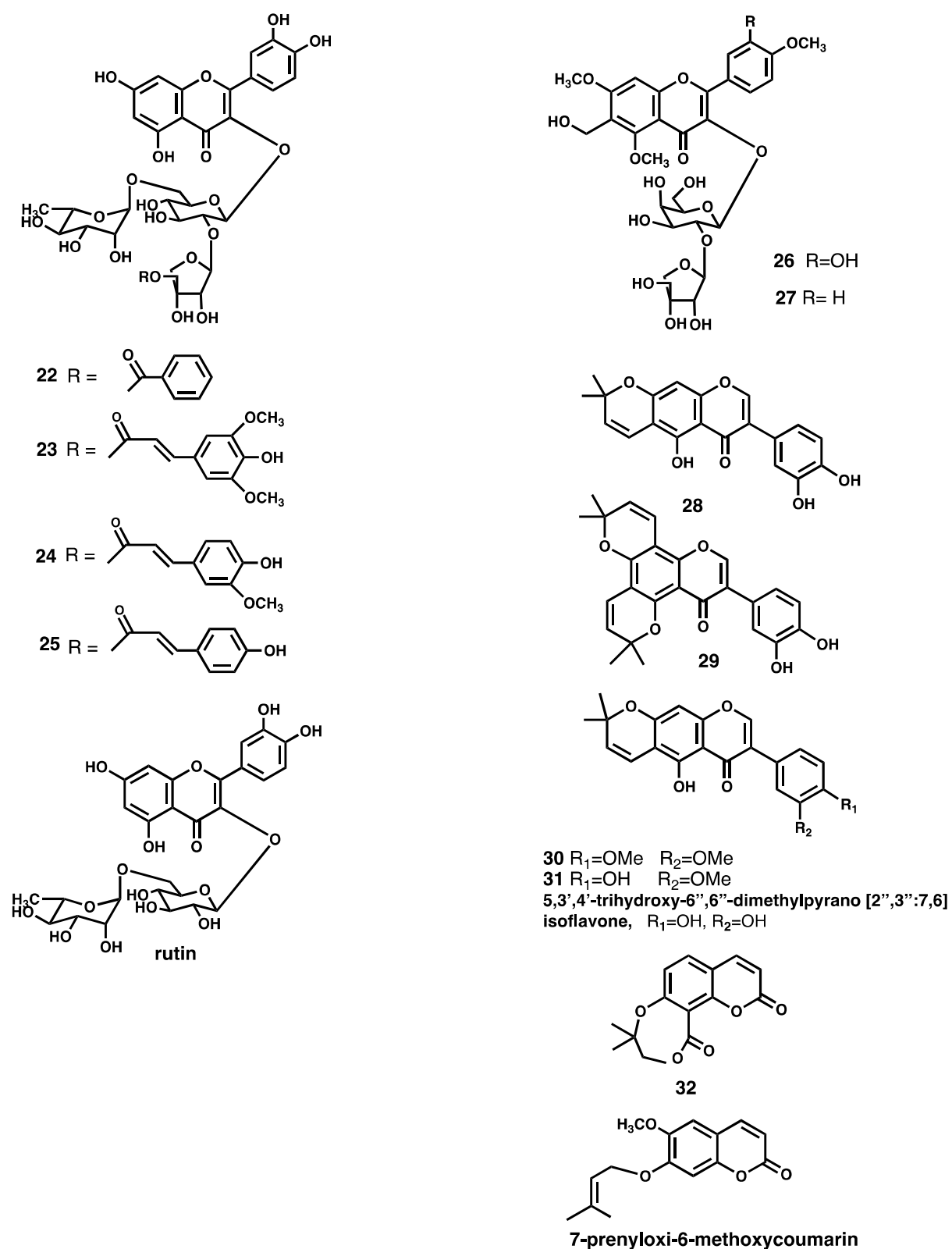
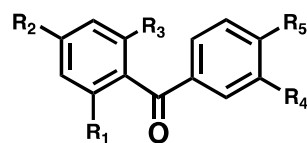
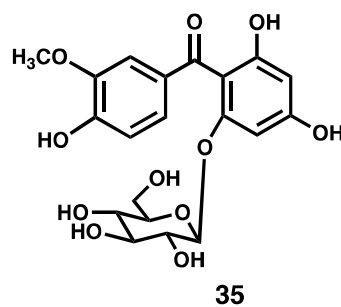
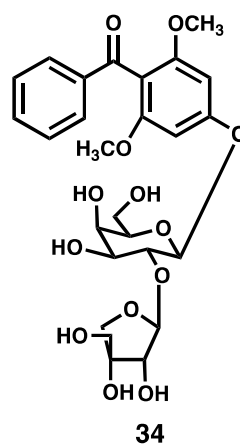
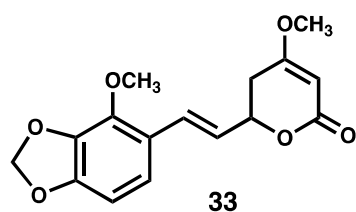


Fig. 4. Structure of flavonoids, coumarins



	R1	R2	R3	R4	R5
36	OH	Glc	OCH ₃	H	H
37	Glc	OH	OCH ₃	H	H
38	Glc	OH	OH	OCH ₃	H

Fig.5 Structure of styryl-pyrones, and benzophenones

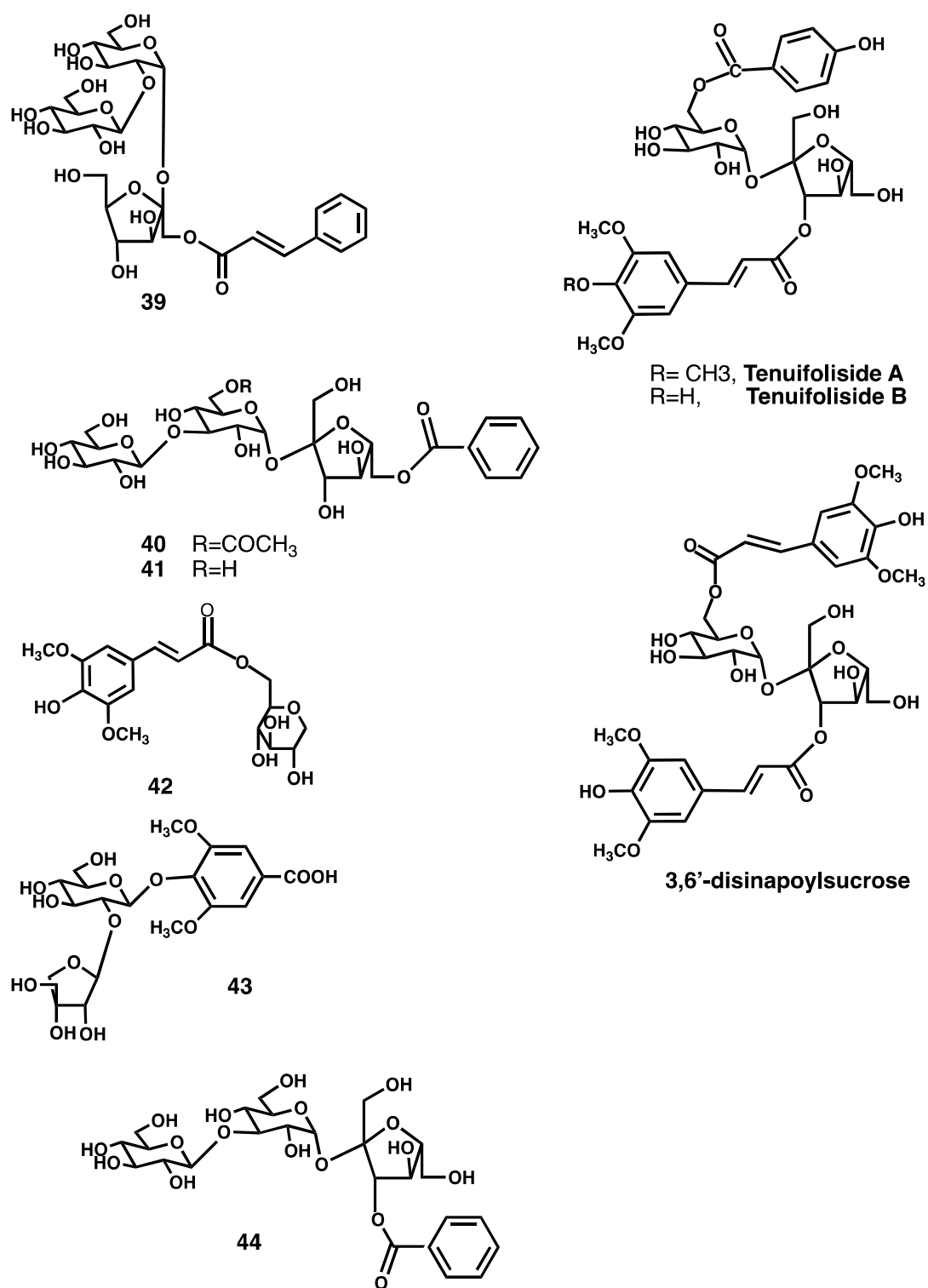
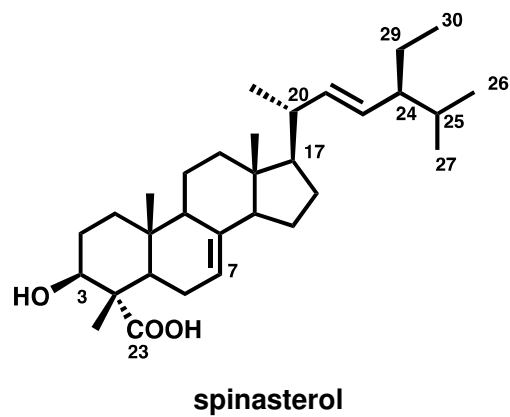
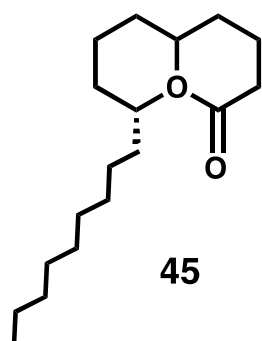


Fig. 6 Structure of oligosaccharide esters



Linkage pattern	Molar ratio
1-linked Glcp	1.92
1,4,6-linked Galp	3.97
1,4-linked Galp	2.01
1,4-linked Glcp	2.00

Backbone of the polysaccharide **46**

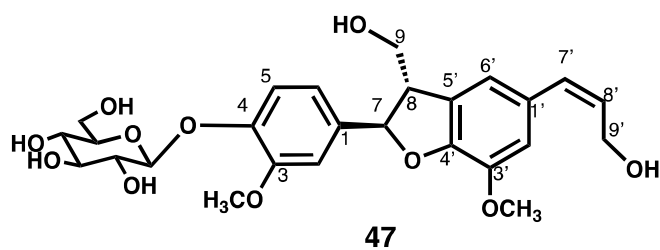


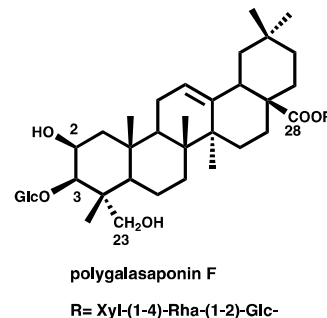
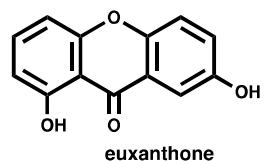
Fig. 7 Structure of other metabolites

Table 1 Traditional uses of some *Polygala* species

<i>Polygala</i> species	Plant part used	Traditional uses	Country	References
<i>P. acicularis</i> Oliv.	Whole plant	-infected wounds	Democratic Republic of Congo	Bouquet, 1969
<i>P. arenaria</i> Willd.	Whole plant	-smallpox	Tropical Africa	Delaude, 1992
<i>P. bakeriana</i> Chodat	Leaves	-wormer	Burundi	Petit, 1958
<i>P. caudata</i> Rehd et Wils	whole plant	-expectorant, sedative	China	Lin et al., 2005
<i>P. cyparissias</i> A. St. -Hil. & Moq	whole plant	-anesthetic of topical use, treatment of inflammation, asthma, allergy	Brazil	Klein Junior et al., 2012 Nascimento et al. 2015
<i>P. glomerata</i> Lour.	whole plant	-treatment of acute tonsillitis, myelitis, hepatitis, pharyngitis, pulmonary tuberculosis and whooping cough	Southern China	Jiangsu New Medical College. Dictionary of traditional Chinese medicine. Shanghai, 1997.
<i>P. japonica</i> Houtt	roots	-treatment of bronchitis, amnesia, aphrodisiac, good effects on the kidney -expectorant, anti-inflammatory, antibacterial, sedative, antidepressant	Vietnam South of China	Chi, 2012 Editorial board of China Herbal of State Administration of Traditional Chinese Medicine, 1999, Quang et al., 2018.
<i>P. sabulosa</i> A.W. Benn.	whole plant	-treatment of bowel, kidney and lung disorders, expectorant	Brazil	Marques, 1996. Nascimento et al., 2015
<i>P. senega</i> var. <i>latifolia</i> Torr &	roots	-expectorant, antitussive, diuretic, treatment of inflammation, snake bites, and CNS	North Eastern United States of America	Delaude, 1992

Gray		diseases.		
<i>P. sibirica</i> L.	whole plant	-tonic, sedative, expectorant	China	Chinese Pharmacopoeia (2010 edition)
<i>P. sibirica</i> L. var. <i>megalopha</i> Fr.	whole plant	-treatment of fever, inflammation, arthralgia and viper bites	China	Huang et al., 2015
<i>P. tenuifolia</i> Willd	roots	-tonic, expectorant, stimulant to treat bronchial asthma, chronic bronchitis and cough -sedative, -treatment of depression, insomnia, memory and cognitive function failure, heart palpitations with anxiety, disorientation, mental disorders	China	Chinese Pharmacopoeia commission, 2015 Delaude, 1992 Klein Junior et al., 2012
	rhizoma	-treatment of inflammation, learning and memory deficit, neurasthenia, cancer	China	Yoo et al., 2014
	roots	-anti-neurosis, tonic and sedative	Japan Kinshasa, Kisantu (Republic democratic of Congo, RDC) Burundi	Uchikura et al., 2018 Delaude, 1992 Bouquet, 1969 Petit, 1958 Wu et al., 2012
	roots	-treatment of amnesia and anxiety	Korea	Choi et al., 2011
	leaves	-tonic for the kidneys	RDC, Burundi	Delaude, 1992; Jiangshu New Medical College.
	aerial parts	-expectorant, sedative, and anti-inflammatory	China	Dictionary of Traditional Chinese Medicine. Shanghai, 1997

GRAPHICAL ABSTRACT



Secondary metabolites

triterpene saponins, triterpenes, terpenoids
xanthenes, flavonoids, coumarins
styryl-pyrones, benzophenones, oligosaccharide esters
steroids, polysaccharide

Pharmacological activities

anti-inflammatory, neuroprotective, antidepressant
analgesic, anti-atherosclerosis, cytotoxic, antitumor
xanthine-oxidase inhibitory effect, antiobesity,
prevention of osteoporosis

Traditional uses

tonic, expectorant, stimulant to treat bronchial asthma, chronic bronchitis and cough,
sedative, treatment of depression, insomnia, memory and cognitive function failure,
heart palpitations with anxiety, disorientation, mental disorders