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Structural diversity and biological activities of secondary metabolites isolated from the genus *Selaginella*

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Abstract The genus *Selaginella* belongs to the family Selaginellaceae, which is well known for its unique structural classes of natural products and their wide range of biological effects. This review provides a comprehensive list of unique secondary metabolites isolated from the genus *Selaginella* and also provides insight into their important biological activities. For the benefit of the readers, this review is divided into two main sections. The first section elaborates on the natural products isolated from *Selaginella*, with the emphasis on compounds exclusive to this genus, while the second section provides an in-depth discussion of a number of different pharmacological activities that the compounds and *Selaginella* extracts exhibited.

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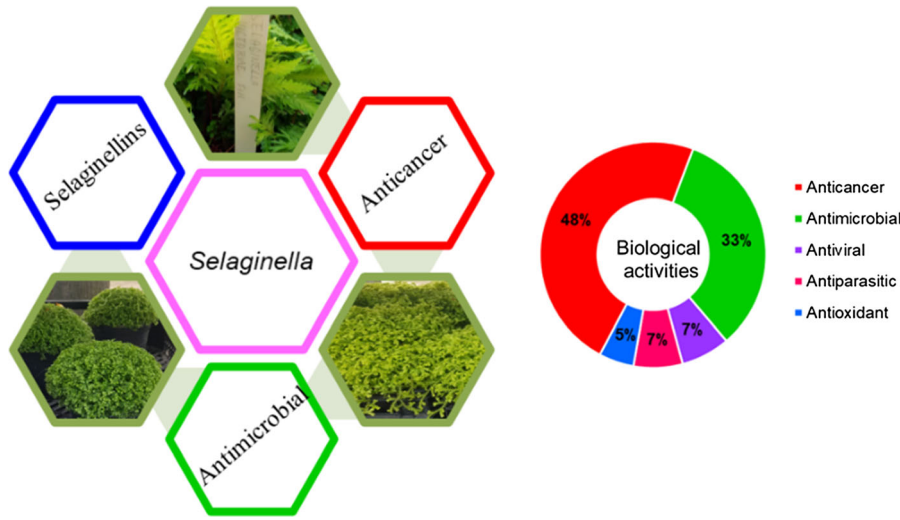
Graphic abstract



Keywords *Selaginella* · Selaginellins · Selaginpulvilins · Flavonoids · Anticancer activity · Antimicrobial activity

Introduction

Selaginella is the only surviving genus in the family Selaginellaceae, and contains approximately 700 species (Yang et al. 2012; Bieski et al. 2015; Nguyen et al. 2015a, b; Woo et al. 2019). Also known as spike moss, it is globally distributed, mostly in tropical and sub-tropical regions (Nguyen et al. 2015a, b). Numerous *Selaginella* species are used in traditional medicines for the treatment of various diseases including inflammation, dysmenorrhea, chronic hepatitis and hyperglycemia (Nguyen et al. 2015a, b; Le et al. 2017; Zhang et al. 2017). About 50 of them are found in China and of these, 20 species have been reported in Chinese folk medicines (Yang et al. 2012). *S. tamariscina* (Beauv.) and *S. pulvinata* are two species reported in the Chinese Pharmacopoeia as Juanbai and are used as traditional herbs (Zhang et al. 2020). *S. tamariscina* (Beauv) and *S. pulvinata* Maxim (Hook. et Grev.) have been used in Traditional Chinese Medicine for the treatment of amenorrhea, dysmenorrhea, metrorrhagia, abdominal lumps in women and used in folk medicine as an anticancer, chronic hepatitis, anti-inflammatory, and antidiabetic agent and also to improve blood circulation (Liu et al. 2014; He et al. 2019). This genus has been extensively studied for the discovery of bioactive secondary metabolites, and a variety of molecules unique to *Selaginella* have been reported (Yang et al. 2012; Woo et al. 2019). Natural products specific to *Selaginella* include selaginellins and selaginellin analogues, which are pigments with a unique para-quinone methide and alkynylphenol carbon skeleton (Yang et al. 2012; Liu et al. 2018a, b), and selaginpulvilins, which possesses an unprecedented 9,9-diphenyl-1-(phenylethynyl)-9H-fluorene skeleton (Liu et al. 2014). The colour of these compounds changes from red to pink (pH 1.0 to 1.5) and red to purple (pH 7.5 to 8.0) which is attributed to their unique structural features and tautomerization (Zhang et al. 2007; Kim et al. 2015). Other well-known structural classes, such as flavonoids and lignans, have also been commonly isolated from *Selaginella*.

For the purpose of this review, the chemical structures of compounds that have only been isolated from the genus *Selaginella* for the first time are shown here. Structures of the other well-known compounds are shown in the Supplementary Information. This review has been divided into two main sections: Firstly, natural products isolated from *Selaginella* and secondly, biological activities of pure compounds or extracts from *Selaginella*. The first section has been subdivided into different structural classes, which include: selaginellins, selaginpulvilins, biflavonoids,

flavonoids, diterpenes, alkaloids, lignans and neolignans and saponins. Together with the unique secondary metabolites, the biological activities of these compounds are also briefly discussed in this section. The pharmacological activities of compounds or extracts obtained from *Selaginella* are detailed in the following section. This section has also been further subdivided into various biological activities, including anticancer, antimicrobial, neuroprotective, and immunomodulation activities. Due to a large number of publications relating to anticancer activities, an in-depth overview in this area has been provided. To make the biological activity data concise for our readers, we have summarized this information in various tables. The main goal of this review is to provide a comprehensive analysis of the structural diversity, phytochemistry, and pharmacological activities of the unique secondary metabolites isolated from the genus *Selaginella*.

Natural products isolated from the genus *Selaginella*

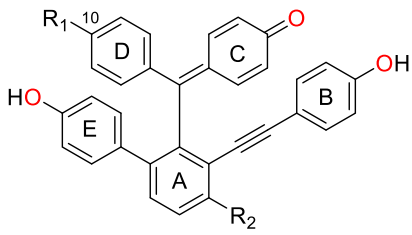
The structures of all the compounds mentioned below were determined by NMR and MS data analyses, while the absolute configuration was determined by experimental and/or calculated ECD analysis. In cases where crystals were obtained, single-crystal X-ray diffraction data analysis was used to confirm the absolute configuration. Due to the expansion in the chemical and biological investigation of *Selaginella*, an increasing number of new compounds are isolated and reported frequently, which in some instances has caused "coincidental simultaneous assignment" (Ramabharathi and Schuehly 2014) of the same trivial names to distinct compounds by independent groups. The compounds in question are specifically selaginellins P, Q, T, and U and selaginpulvilins E and J. Therefore, to avoid further confusion, each of these compounds and their biological activities will be discussed separately.

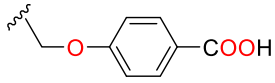
Selaginellins

The first member of this group, selaginellin (**1**) (Fig. 1), was identified from the acetone extract of *Selaginella sinensis* after chromatographic purification (Zhang et al. 2007). The methoxy derivative (**1a**), a racemate of compound **1**, was synthesized, crystallized and X-ray crystallographic analysis was utilized for its structure determination (Zhang et al. 2007). After the isolation of selaginellin, a number of selaginellin analogues were isolated, characterized and screened for their biological potential across a range of assays as discussed below.

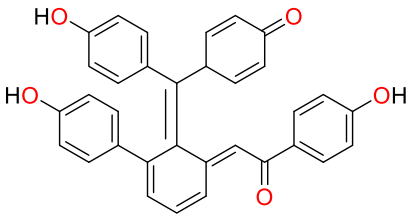
Two unique pigments, namely selaginellins A (**2**) and B (**3**), together with selaginellin, were isolated from the Chinese herb, *S. tamariscina* (Cheng et al. 2008). The ability of compounds **1–3** to inhibit soluble epoxide hydrolase (sEH) was evaluated for their potential use in the treatment of cardiovascular diseases (Kim et al. 2015), exhibiting low IC_{50} values of 4.2 ± 0.2, 3.1 ± 0.1, 8.2 ± 2.2 μM respectively. This indicates that these compounds can be developed as potential inhibitors (Kim et al. 2015). Selaginellin C (**4**) was isolated as a brown powder along with selaginellin and selaginellin A from *S. pulvinata* (Hook. et Grev.) Maxim (Tan et al. 2009). The biological activity of compound **4** was not reported in the original article. Three new selaginellin derivatives, selaginellin D–F (**5–7**), were isolated from the ethyl acetate extract of *S. pulvinata* (Hook. et Grev.) Maxim (Cao et al. 2010a, b). Selaginellin E was obtained as an inseparable mixture with the ratio of 1:0.95 (**6a**:**6b**) and was believed to be a chromatographic artefact as this compound was not detected in the original extract. The structural elucidation of selaginellin F (**7**) proved rather challenging due to the limited amount isolated and an overlap of NMR signals, however following extensive NMR analysis in different deuterated solvents, the structure was confirmed to be (R,S)-4-[(40-hydroxy-2-[(4-hydroxylphenyl)(4-oxocyclo-hexa-2,5-dienylidene)methyl]-3-[(4-hydroxy-phenyl)eth-ynyl)biphenyl-4-yl]methoxy]benzoic acid (Cao et al. 2010a, b).

Selaginellin G (**8**) and H (**9**) isolated from *S. pulvinata*, exhibited good antifungal activity against *C. albicans* with an IC_{50} value of 5.3 μg/mL (Cao et al. 2010a, b). Selaginellins I–J (**10–11**) (Xu et al. 2011a, b) and K–L (**12–13**) (Xu et al. 2011a, b) were isolated from an ethanolic extract of *S. tamariscina* (Xu et al. 2011a, b). The biological activities of these compounds were not reported (Xu et al. 2011a, b). Two new selaginellin derivatives, selaginellins M (**14**) and N (**15**), together with the known compounds, selaginellin (**1**), selaginellins A (**2**) and C (**4**) were



	R_1	R_2
1	H	CH_2OH
1a	H	CH_2OCH_3
2	H	H
3	H	CH_3
4	OH	$CH(OH)CH_2OH$
5	OCH_2CH_3	CH_2OH
6	OH	$CH(OCH_3)_2$
7	OH	
14	OCH_3	CH_2OH
15	OCH_3	H
16	OH	OCH
18	OCH_2CH_3	H
19	OCH_2CH_3	CHO
20	OCH_2CH_3	CH_3

6a R = $CH(OCH_3)_2$
6b R = CHO



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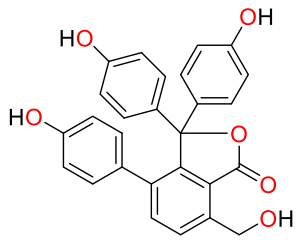

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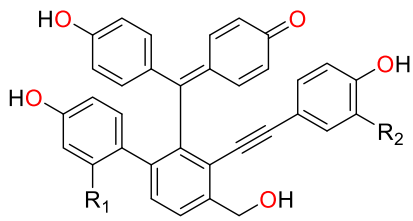
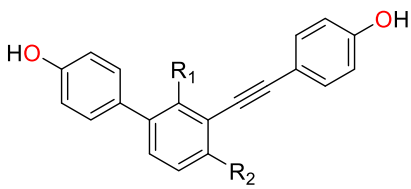


	R_1	R_2
10	OH	H
11	H	OH



	R_1	R_2
12	CHO	OH
13	CH_3	OH
21	OH	CH_3
22	OH	H

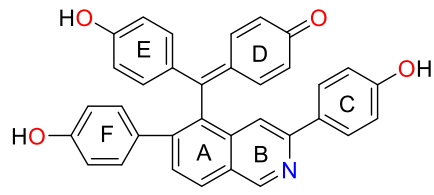


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Fig. 1 Chemical structures of compounds **1**–**22**

purified from the 50% ethanolic extract of *S. tamariscina* (Zhang et al. 2012). The cytotoxic activities of compounds **14** and **15** together with selaginellin and selaginellin A were tested in vitro against U251 (human glioma), HeLa (human cervical carcinoma), and MCF-7 (human breast cancer) cells. Compound **14** exhibited inhibitory activity against all the cell lines tested, while compound **15** was only active against the MCF-7 cell line (Zhang et al. 2012).

During a phytochemical investigation, one new selaginellin derivative, named selaginellin O (**16**) and three known selaginellins; selaginellin (**1**), selaginellin A (**2**), and selaginellin M (**14**), were isolated from *S. tamariscina* (Beauv.) Spring (Yang et al. 2012). Selaginellin O (**16**), together with selaginellin M (**14**) and selaginellin, were screened for their cytotoxic activities against HeLa cells. All the compounds exhibited moderate cytotoxic activity, with selaginellin O (**16**) exhibiting the most significant inhibitory activity. Its IC_{50} value of 26.4 μM suggests that the formyl group at C-10 might be important for its activity (Yang et al. 2012). A novel selaginellin-type isoquinoline alkaloid, selaginisoquinoline A (**17**), together with three ethoxy selaginellins P–R (**18–20**) were reported from the ethanolic extract of *S. pulvinata* (Cao et al. 2015). Compound **17** was isolated as a racemic mixture. To obtain biological data, compounds **17–20** were screened for cytotoxicity against human alveolar basal epithelial, gastric and hepatocellular adenocarcinoma cells (BGC-823, SMMC-7721 and A549). All these compounds exhibited moderate cytotoxic activity. Furthermore, the compounds exhibited good anti-fungal activities against *C. albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum*, and *T. mentagrophytes*, with IC_{50} values of 4–32 μg/mL (Cao et al. 2015). Another article published in the same year (2015) reported the isolation and characterization of two new selaginellin derivatives from the Chinese herb *S. tamariscina* (Beauv.) which was coincidentally also named selaginellins P (**21**) and Q (**22**) (Xu et al. 2015). However, as can be noted from the chemical structures, these compounds are very distinct from **18** and **19**, which were also given the trivial names selaginellin P and Q. No biological data was reported for compounds **21** and **22**. Selaginellin S (**23**) (Fig. 2) was isolated from *S. moellendorffii* Hieron (Zhu et al. 2016). When applied to a human hepatoblastoma cell line (HepG2.2.15), selaginellin S inhibited the production of hepatitis B surface antigen (HbsAg) (IC_{50} 0.026 μg/mL) and another hepatitis B virus (HBV) protein HBeAg (IC_{50} 0.032 μg/mL) after 48 h. Selaginellin S also significantly reduced the intracellular and released HBV-DNA concentrations (Zhu et al. 2016).

To avoid confusion, it is important to mention that three independent articles were published on the isolation of new natural products that were simultaneously given the trivial names selaginellin T and two of them selaginellin U. Selaginellin T (**24**) and a novel example of naturally occurring triarylbenzophenone analogue, selagibenzophenone A (**25**), were isolated from the Chinese plant *S. pulvinata* (Liu et al. 2018a, b). The isolated compounds were evaluated for phosphodiesterase-4 (PDE4) inhibitory effects, and the results showed that compound **25** possessed good inhibitory activity, with an IC_{50} value of 1.04 ± 0.07 μM, while compound **24** exhibited moderate activity, with an IC_{50} value of 9.35 ± 0.60 μM (Liu et al. 2018a, b).

Furthermore, in 2017, Woo et al. described the isolation of four new selaginellins, T–W (**26–29**) from *S. tamariscina* (Beauv (Le et al. 2017). Selaginellin T (**26**), structurally different from **24**, exhibited moderate inhibitory activity on protein-tyrosine phosphatase 1B (PTP1B) enzyme, with an IC_{50} value of 57.9 μM, while selaginellins U–V (**27–29**) exhibited significantly better activity, with IC_{50} values ranging from 4.8–15.9 μM, further suggesting that this class of compounds has the potential to inhibit the activity of the PTP1B enzyme (Le et al. 2017). Together with eleven known selaginellin derivatives (selaginpulvilin A–E, selaginellin, selaginellin A, B, G, M and O), two new lactone-containing selaginellins were also coincidentally given the trivial names selaginellins T and U (**30**) which were isolated from *S. tamariscina* (Zhu et al. 2017). Interestingly, selaginellin T from this report was structurally the same as compound **24**, while selaginellin U (**30**) was very distinct from compound **27**. Selaginellin U (**30**), which possessed a rare unsaturated δ-lactone ring attached to ring A, was a new structural type among selaginellins. Due to complications during structure elucidation, biomimetic semi-synthesis was carried out from the trimethyl ether of selaginellin not only to determine the structure of selaginellin U (**30**), but also to establish a plausible biogenetic pathway for both compounds (Zhu et al. 2017). Compound **30** was fully

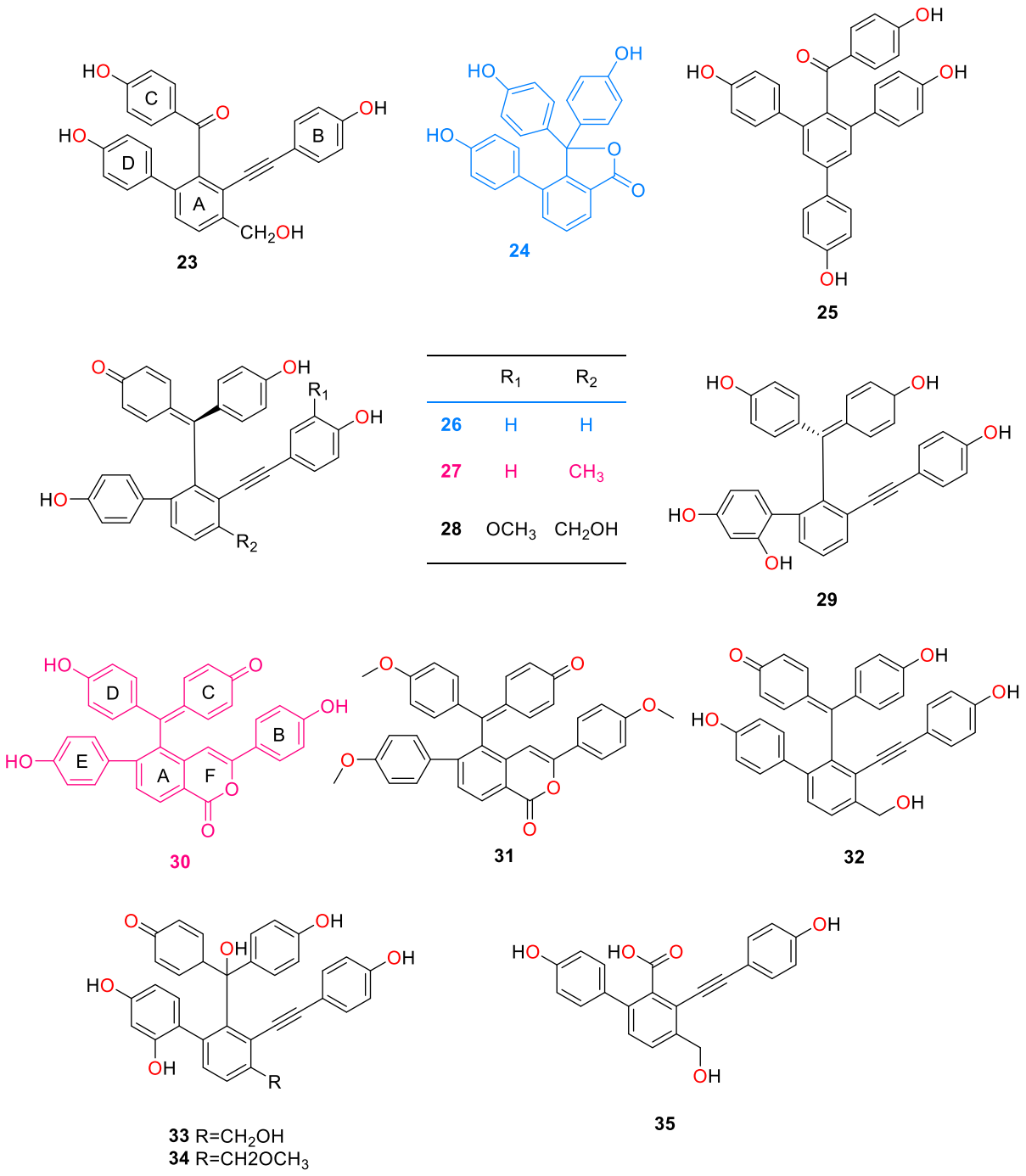


Fig. 2 Chemical structures of compounds **23**–**35**

methylated using MeI/K_2CO_3 to yield **31** and by comparing the NMR data, the structure of **30** was established.

Three new selaginellin derivatives, selariscinin A–C (**32–34**), together with the known compounds, selaginellin and 10-methoxylated selaginellin M, were

isolated from the methanolic extract of *S. tamariscina* while searching for new antidiabetic agents (Nguyen et al. 2015a, b). These compounds exhibited a significant stimulatory effect on glucose uptake when tested using 2-NBDG (a fluorescent tracer used for monitoring glucose uptake into living cells) in 3T3-L1 adipocyte cells at 5 μM. The inhibitory effect on the PTP1B enzyme of these compounds was also examined. Compound **33** exhibited a comparable inhibitory effect (IC_{50} value of 4.6 ± 0.1 μM) to the positive control, ursolic acid (IC_{50} value of 3.5 ± 0.1 μM). The authors suggested that these analogues might have the potential to be developed as an insulin mimetic for antidiabetic agents (Nguyen et al. 2015a, b).

Bioassay-guided isolation of methanol extract of *S. tamariscina* that exhibited a stimulatory effect on glucose uptake in 3T3-L1 adipocyte cells led to the isolation of two new compounds, selariscinin D (**35**) and E (**36**, is a bioflavonoid, Fig. 5), along with five previously reported bioflavonoids (Nguyen et al. 2015a, b). The stimulatory effect of all the isolated compounds were further evaluated on glucose uptake using 2-NBDG in 3T3-L1 adipocyte cells, where the results indicated that compound **35** significantly stimulated 2-NBDG uptake by a 1.24 ± 0.05 fold induction as compared to the DMSO control (Nguyen et al. 2015a, b). Diselaginellins A (**37**) and B (**38**) (Fig. 3), an ether-linked dimeric selaginellin derivative, together with selaginellin and selaginellin B, were isolated from the ethanol extract of *S. pulvinata* (Cao et al. 2017). Diselaginellin B was found to be moderately cytotoxic with an IC_{50} value of 9.0 μM and antimetastatic towards SMMC-7721cell line (Cao et al. 2017). Selaginones A (**39**) and B (**40**) represent the first example of aryl-substituted anthraquinone derivatives isolated from *S. tamariscina* (Beauv.) Spring (Liu et al. 2018a, b). Selagibenzophenone B (**41**), a triarylbenzophenone, was also isolated during this study (Liu et al. 2018a, b). Selaginones A, B and selagibenzophenone B were evaluated for their cytotoxic activity against human hepatocellular adenocarcinoma cell lines (HCC, SMMC-7721 and MHCC97-H). Selagibenzophenone B exhibited moderate cytotoxicity with the IC_{50} values of 39.8 and 51.5 μM, respectively while selaginones A and B were not active (Liu et al. 2018a, b).

Selaginpulvilins

Selaginpulvilins (Fig. 4) are a class of natural products unique to the genus *Selaginella* which possess an unprecedented 9,9-diphenyl-1-(phenylethynyl)-9H-fluorene skeleton (Liu et al. 2014). To date, approximately 20 selaginpulvilins have been isolated (Liu et al. 2014; Huang et al. 2017; Yao et al. 2017a, b; Zhang et al. 2017; Woo et al. 2019). First four members of the selaginpulvilin series, selaginpulvilins A–D (**42–45**), together with four known selaginellins (selaginellin, selaginellin A, N and H) were isolated from *S. pulvinata*. Spectroscopic analysis was utilized to elucidate the structures of the new compounds while single-crystal X-ray diffraction was used to confirm the structure and absolute configuration of selaginpulvilin A (Liu et al. 2014). All the compounds were evaluated for their ability to inhibit phosphodiesterase-4 (PDE4D2) and exhibited noteworthy activity, with IC_{50} values ranging from 0.11 to 5.13 μM. Rolipram was used as a positive control, with an IC_{50} value of 0.54 μM. Selaginpulvilin B (**43**) exhibited the strongest activity, which was fivefold higher than the positive control. The unique skeleton of selaginpulvilins makes them a potential candidate for the development of PDE4 inhibitors (Liu et al. 2014). Selaginpulvilins E–J (**46–51**) were isolated during a large-scale chemical investigation from *S. pulvinata* (Zhang et al. 2017). Selaginpulvilin E possessed a unique 6-(4-hydroxyphenyl)-2H-pyran-2-one moiety (Zhang et al. 2017). Selaginpulvilins E–J were also evaluated for their ability to inhibit PDE4D2 and exhibited significant inhibitory activities, with IC_{50} values ranging from 0.22 to 1.38 μM (Zhang et al. 2017). Total synthesis of selaginpulvilins A–J (**42–51**) was also reported in this study (Zhang et al. 2017).

The issue of trivial names appeared yet again with regards to the structures of selaginpulvilin E and J (Yao et al. 2017a, b; Zhang et al. 2017). Zhang and co-workers described six new analogues of selaginpulvilins (E–J) (**46–51**) (Zhang et al. 2017), while in a simultaneous publication, Yao and co-workers reported a new derivative that was also named selaginpulvilin E (**52**) along with selagintamarlin A (**53**) (Yao et al. 2017a, b). However, selaginpulvin E (**52**) from Yao's study was structurally identical to compound **51**, which was given the trivial name selaginpulvin J (Zhang et al. 2017). Compound **53** was described as a novel selaginellin derivative with a

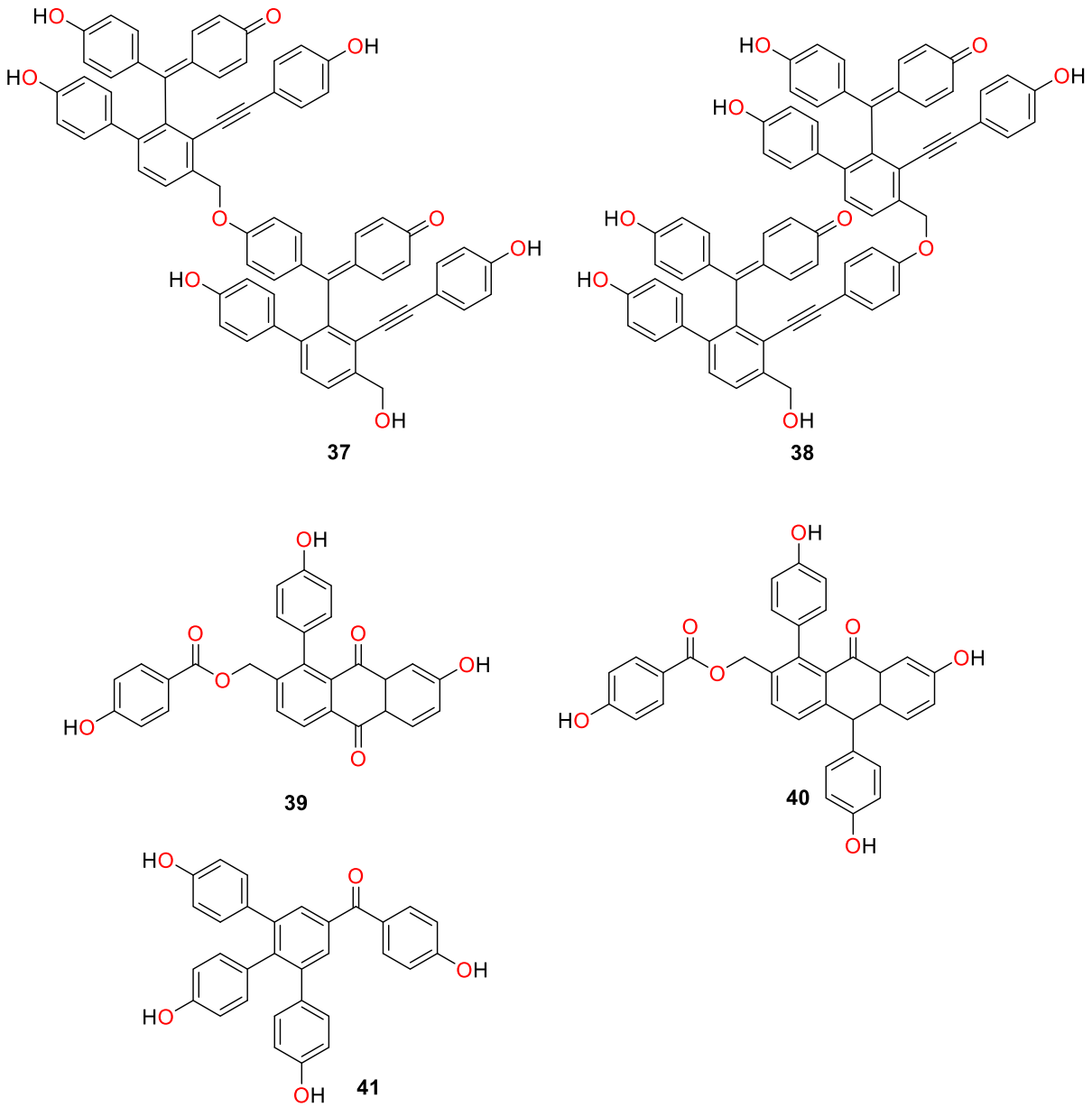
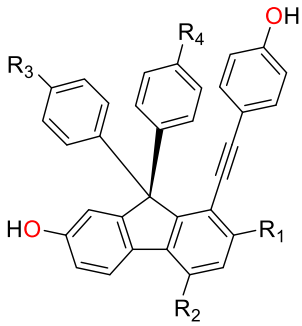
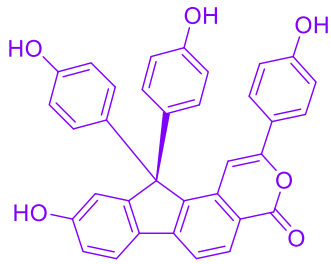


Fig. 3 Chemical structures of compounds **37**–**41**

unique 1H-2-benzopyran unit. Along with selaginpulvilin E (**52**) (again structurally different but given the same sequential trivial name as compound **46**), eight known selaginellin (selaginpulvilins A–D, selaginellin A, B, E and O) analogues were also isolated from *S. tamariscina* (Yao et al. 2017a, b). A plausible biosynthetic pathway for selagintamarlin A was also proposed with selaginellin O being the most likely biosynthetic precursor. Selaginellin O would undergo oxidation and hydration followed by ring closure to form a pyran ring, thus giving selagintamarlin A. All the isolates were tested for their inhibitory activity against PDE4D2 and exhibited potent inhibitory activity (IC_{50} values of 40–1680 nM), with selagintamarlin A exhibiting the strongest activity (IC_{50} value of 40 nM), which was 20-fold higher than the positive





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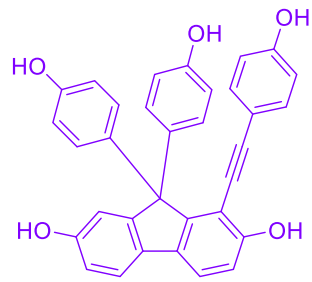

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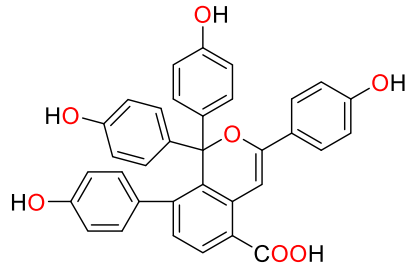
	R_1	R_2	R_3	R_4
42	CH_2OH	H	OH	OH
43	CHO	H	OH	OH
44	CH_3	H	OH	OH
45	H	H	OH	OH
47	COOH	H	OH	OH
48	CH_2OH	OH	OH	OH
49	$CH(OCH_3)_2$	H	OH	OH
50	H	OH	OH	OH
51	OH	H	OH	OH
54	CHO	H	OCH_3	OCH_3
55	H	H	OCH_3	OCH_3
56	CH_3	H	OCH_3	OCH_3
57	CH_2OCH_3	H	OCH_3	OCH_3
58	CH_2OH	H	OCH_3	OCH_3
59	CH_2OCH_3	H	OH	OH
60	CH_2OCH_3	OH	OH	OH
61	CH_3	H	OCH_3	OH
62	CH_2OH	H	OCH_3	OH
63	CH_2OCH_3	H	OH	OCH_3



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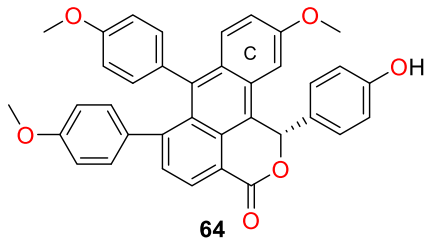

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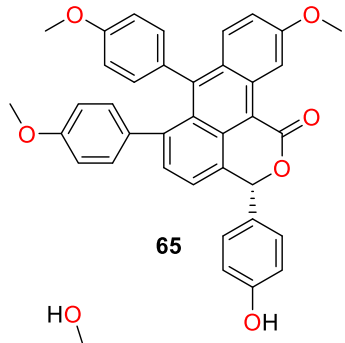

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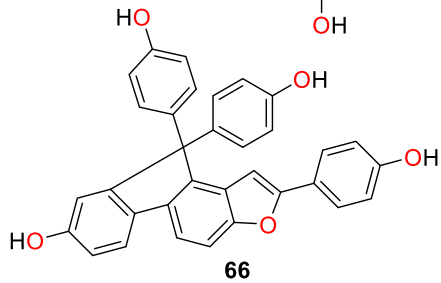

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Fig. 4 Chemical structures of compounds **42**–**66**

control, rolipram (IC_{50} = 850 nM) (Yao et al. 2017a, b).

Selaginpulvilins K (**54**) and L (**55**), fluorene derivative, were isolated from the ethanolic extract of *S. pulvinata* (Yao et al. 2017a, b). Compounds **54** and **55** exhibited high inhibitory activity against PDE4D2 with IC_{50} values of 11 and 90 nM, respectively. To identify the binding site, selaginpulvilin K (**54**) was crystallized with the catalytic domain of PDE4D2 and the crystal structure of the complex was determined by the X-ray diffraction method. The co-crystal structure identified an unusual binding mode and showed that selaginpulvilin K bound weakly to the active site and also provided **54** with the ability to interact in multi-sub pockets in the catalytic domain, resulting in high inhibitory potency (Yao et al. 2017a, b). MS/MS molecular networking technique was utilized to prioritize unknown selaginellin analogues from extracts and fractions of *S. tamariscina*. This molecular networking technique led to the isolation of eight new diarylfluorene derivatives: selaginpulvilins M–T (**56–63**) (Woo et al. 2019). Two unusual 1H,3H-dibenzo[de,h]isochromene derivatives were also isolated, selariscins A (**64**) and B (**65**). Their PDE4 inhibitory activities were studied, together with the binding modes of the analogues. Of the compounds tested, **56–64** exhibited an inhibitory effect, with IC_{50} values ranging from 2.8 to 33.8 μM, while the positive control, rolipram, exhibited an IC_{50} of 0.2 μM. Molecular docking studies were also conducted to determine the interactions between the selaginellin derivatives and PDE4D2 (Woo et al. 2019). A selaginellin analogue designated Isoselagintamarlin A (**66**), which possessed a rare benzofuran unit, was isolated from a 70% ethanol extract of *S. tamariscina*. Isoselagintamarlin A was also biomimetically synthesized via sequential oxidations and the intramolecular cyclization of selaginpulvilin A (Zhu et al. 2019).

The structural classes mentioned below were reported for the first time from the genus *Selaginella* however, they are not unique to *Selaginella*. These are secondary metabolites commonly found in the plant kingdom.

Biflavonoids

A new compound with 3′,8″-linked biflavonoids, (2″S)-2″,3″-dihydroamentoflavone-4′-methyl ether (**67**) (Zheng et al. 2011), together with six known compounds ((2S)-2,3 dihydroamentoflavone-4′-methyl ether, flavone-4′-methyl ether, (2S,2″S)-tetrahydroamentoflavone, (2S,2″S)-2,3,2″,3″-tetrahydroamento(2S)-2,3-dihydroamentoflavone, (2″S)-2″,3″-dihydroamentoflavone (**68**) and amentoflavone) (Fig. 5) were isolated from the ethanolic extract of *S. uncinata* (Desv.) Spring. All the compounds isolated were evaluated for their protective effect against anoxia by anoxic pheochromocytoma (PC12) cells assay. Compound **68**, tested at 45 μM, exhibited the most potent protective effect, with a promoted survival rate of 19.35 ± 1.53% compared to the positive control, baicalin 21.53 ± 1.17% (Zheng et al. 2011). From the ethanolic extract of *S. moellendorffii* Hieron, three new flavones, 5-carboxymethyl-4′,7-dihydroxyflavone (**69**), its ethyl ester (**70**), and butyl ester (**71**) were isolated (Cao et al. 2010a, b). Isolated natural products were evaluated for their anti-HBV activity using a HepG2.215 cell line transfected with the HBV genome. The ethyl ester (**70**) and butyl ester (**71**) exhibited anti-HBV activity at non-toxic concentrations, with SI values of ≥ 2 for HBsAg and 2 for HBeAg (Cao et al. 2010a, b).

A phytochemical investigation of an ethanolic extract of *S. uncinata* that exhibited a potent anti-anoxic effect in the anoxic PC12 cell assay resulted in the isolation of four new biflavonoids (**72–75**) (Zheng et al. 2008). The structures of the new compounds and their absolute configurations were determined with the aid of NMR and CD spectroscopy. Compound **75** exhibited a potent anti-anoxic effect in the anoxic PC12 cell assay (Zheng et al. 2008). From the methanolic extract of *S. doederleinii* Hieron, a new compound, 2,2″,3,3″-tetrahydrorobustaflavone 7,4′,7″-trimethyl ether (**76**) and previously reported robustaflavone 7,4′,7″-trimethyl ether (**77**) were isolated. Compound **77** was reported from the family Sellaginellaceae for the first time. Both the compounds were evaluated for their cytotoxicity against human colorectal and lung carcinoma, and erythroleukemia cell line (HCT116, NCI-H358 and K562) and exhibited moderate cytotoxicity towards the human cancer cell lines (Lee et al. 2008a, b). The EC_{50} values were 19.1 and 15.6 (HCT116), 23.5 and 20.1 (NCI-H358),

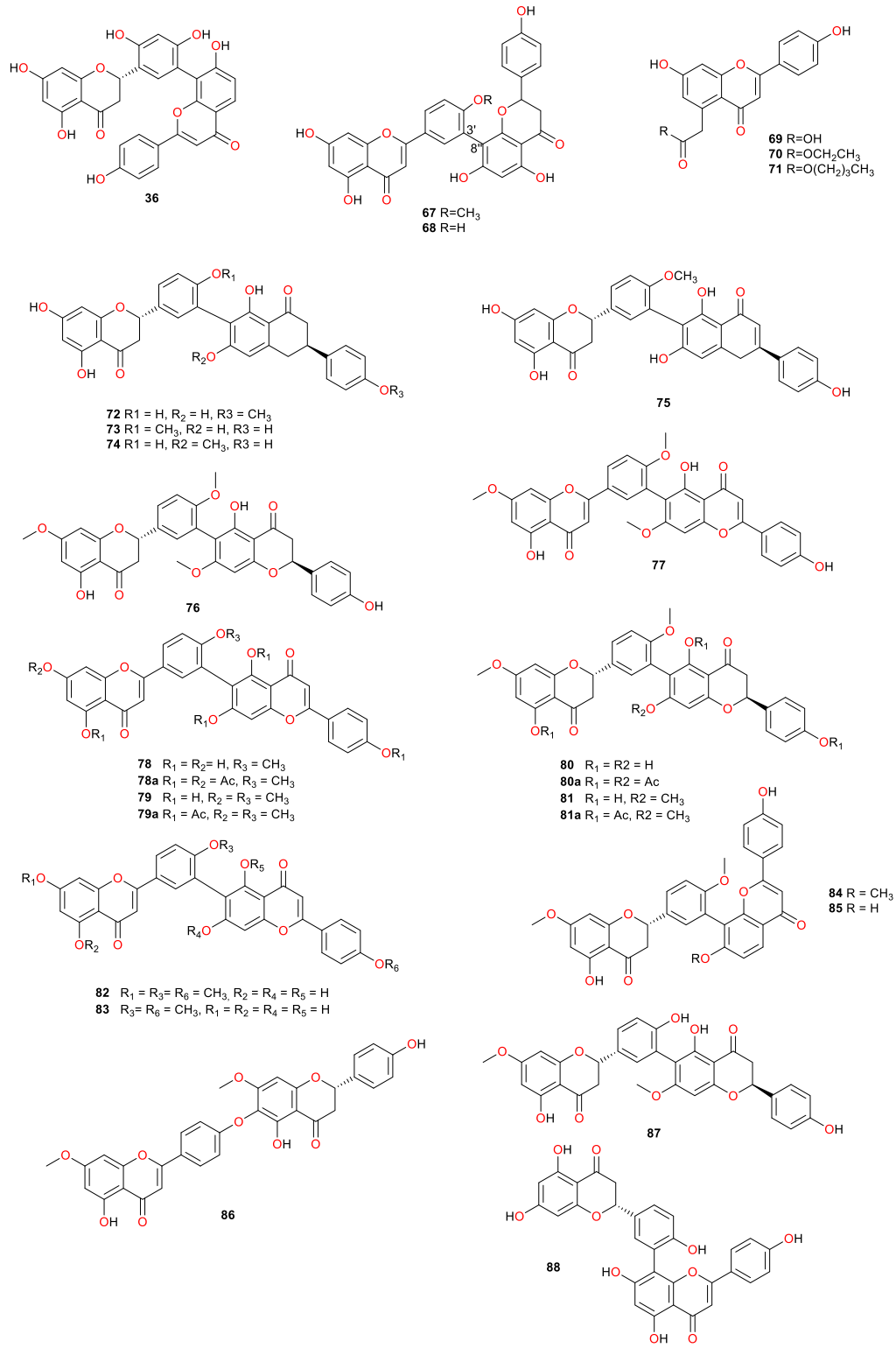

Fig. 5 Chemical structures of compounds **36**, **67**–**88**

and 28.8 and 22.5 μM for compounds **76** and **77**, respectively. A number of biflavonoids and sterols have been reported from *S. delicatula* (Lin et al. 2000). For this reason, the whole plant was re-examined for potential new bioactive compounds. The phytochemical investigation of this plant led to the isolation of four new biflavonoids (**78–81**) from the ethanolic extract of *S. delicatula* (Lin et al. 2000). The isolated compounds were evaluated for their cytotoxicity against a number of human tumour cell lines, which included adenocarcinoma of the cervix, lung, B cells, erythroleukemic cell line, and kidney (Raji, Calu-1, K562, HeLa, Vero, and Wish). Compounds **78** and **80** significantly inhibited Raji (77, IC_{50} = 34.2 ± 5.7 and 79, IC_{50} = 17.9 ± 2.4 μM) and Calu-1 (77, IC_{50} = 42.6 ± 2.4 and 79, IC_{50} = 15.8 ± 2.1 μM) cell growth (Lin et al. 2000).

In search for new cytotoxic compounds, more than 1,000 Formosan plants were screened for cytotoxicity and *S. delicatula* exhibited promising cytotoxic results. Due to this, it was selected for further chemical investigation (Chen et al. 2005). The phytochemical examination of the chloroform and n-butanol fractions of *S. delicatula* led to the isolation of five new biflavonoids, 7,4′,4‴-trimethyl ether (**82**), robustaflavone 4′,4‴-dimethyl ether (**83**), 2,3-dihydroamentoflavone 7,4′,7″-trimethyl ether (**84**), 2,3-dihydroamentoflavone 7,4′-dimethyl ether (**85**), and 2″,3″-dihydroisocryptomerin 7-methyl ether (**86**). The cytotoxicity of the compounds were evaluated against human colon adenocarcinomic and mouse leukemic (P-388 and HT-29) cell lines (Chen et al. 2005). Compounds **83** and **85** exhibited significant cytotoxicity, with ED_{50} values of 1.44 and 2.30 μg/mL against a P-388 cell line while compound **83** also exhibited significant cytotoxicity against HT-29 cell line with the ED_{50} value of 1.59 μg/mL (Chen et al. 2005).

A new compound, uncinatabiflavone C 7-methyl ether (**87**) and a compound with new configuration, (2R) 2, 3-dihydroamentoflavone (**88**) was isolated from the ethanolic extract of *S. uncinata* (Desv.). These compounds exhibited inhibitory activities against protein tyrosine phosphatase 1B (PTP1B) in an enzyme assay with IC_{50} values ranging from 4.6 to 16.1 μM (Xu et al. 2019).

Flavonoids

In search for novel bioactive secondary metabolites from *S. doederleinii*, an extensive phytochemical investigation was undertaken (Zou et al. 2017a, b). This chemical profiling resulted in the isolation of eight new flavonoids (Fig. 6), selagintriflavonoids A–H (**89–96**) which had a unique trimeric skeleton (Zou et al. 2017a, b). Selagintriflavonoids A–C included three naringenin units while selagintriflavonoids D–H contained apigenin and two naringenin units. The new compounds were evaluated for their β-secretase (BACE1) inhibitory properties and displayed BACE1 inhibition with the IC_{50} values ranging from 0.75 to 46.99 μM. Selagintriflavonoid A, which exhibited the most significant inhibitory effect with the IC_{50} of 0.75 μM, was identified as a potential compound for the treatment of Alzheimer's disease (Zou et al. 2017a, b). Chromatographic purification of a 75% ethanolic extract of the herb *S. doederleinii* yielded six new flavonoids (**97–103**) (Zou et al. 2017a, b). The new compounds had an aryl substitution at C-3′ position of naringenin or apigenin skeleton with compounds **97** and **103** possessing R configurations (Zou et al. 2017a, b). Compounds **101–103** also exhibited moderate cytotoxicity against human alveolar and lung adenocarcinoma, and an erythroleukemia cell line (NCI-H460, A549, and K562) with IC_{50} values ranging from 8.17 to 18.66 μM (Zou et al. 2017a, b).

In an effort to search for novel bioactive secondary metabolites, the plant *S. moellendorffii* Hieron was analysed. The 70% ethanolic extract of *S. moellendorffii* Hieron was subjected to chromatographic purification, which resulted in the isolation of five new carboxymethyl flavonoids (Fig. 7). Using spectroscopic methods and electronic circular dichroism (ECD), the structures and absolute configurations were confirmed to be: 5-carboxymethyl-3′,4′, 7-trihydroxyflavone (**104**), (2S)-5-carboxymethyl-3′,4′, chiral isomers, 7-trihydroxyflavonone (**105a**), (2R)-5-carboxymethyl-3′,4′, 7-trihydroxyflavonone (**105b**), (2S)-5-carboxymethyl-4′, 7-dihydroxyflavonone (**106**), 5- carbomethoxymethyl-4′, 7-dihydroxyflavone (**107**), and a new chromone named 5-carboxymethyl-7-hydroxychromone (**108**) (Zou et al. 2016a, b). The carboxymethyl subunit was positioned on C-5 in all the isolated compounds, which had previously not been reported for the genus *Selaginella* (Zou et al.

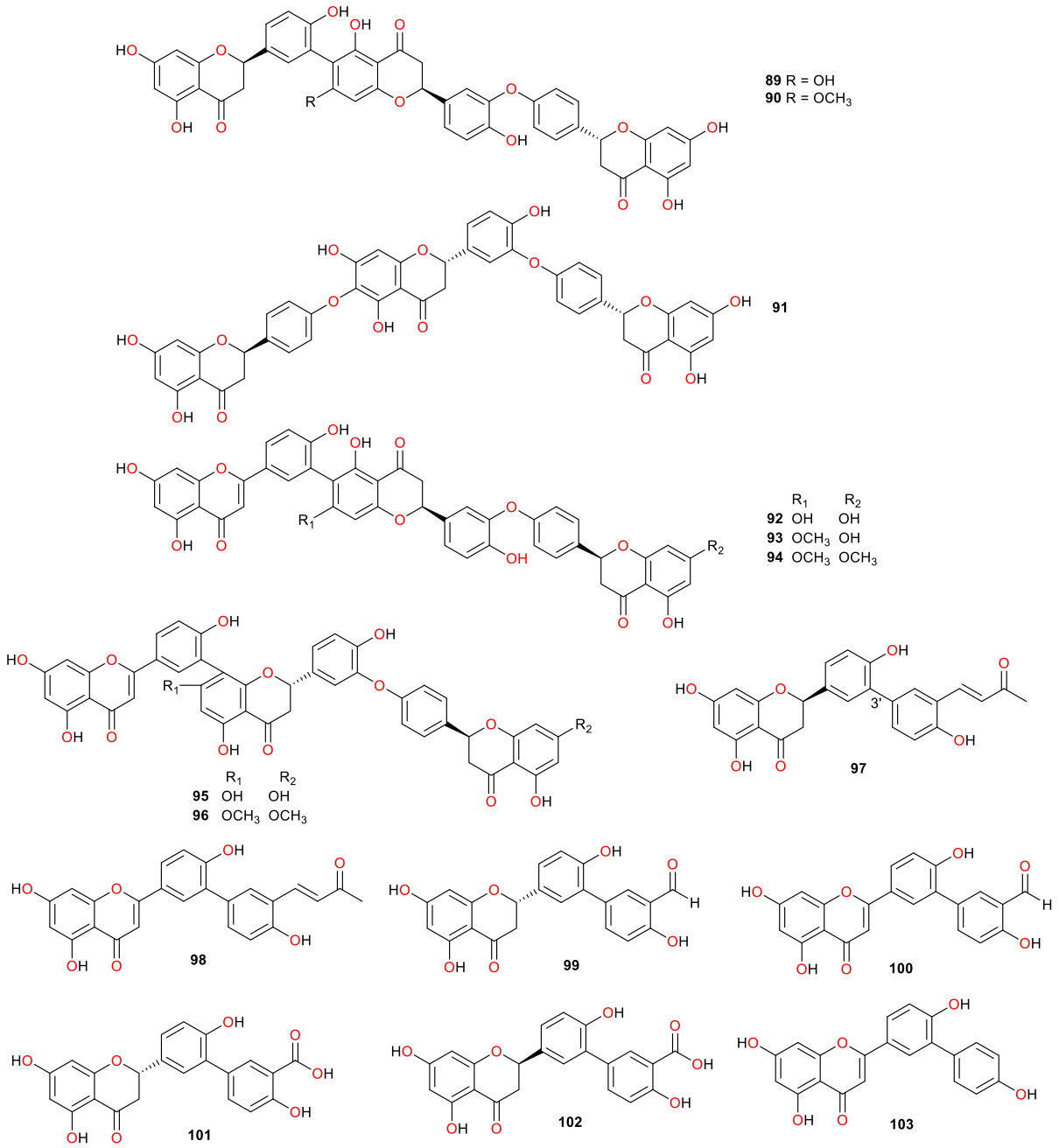

Fig. 6 Chemical structures of compounds **89–103**

2016a, b). The antibacterial effect of all the isolates were also evaluated against a range of Gram-negative and Gram-positive bacterial strains. **106** and **107** exhibited significant broad-spectrum antimicrobial activities against *E. coli*, with an MIC value of 25 µg/mL, while compound **108** was most active against Gram-positive bacteria, with an MIC value of 12.5 µg/mL against *S. aureus* and *S. pneumoniae* (Zou et al. 2016a, b). As part of the continuous search for new bioactive molecules from the genus *Selaginella*, *S. uncinata* was phytochemically investigated. Two new flavonoids, uncinataflavones A (**109**) and B (**110**),

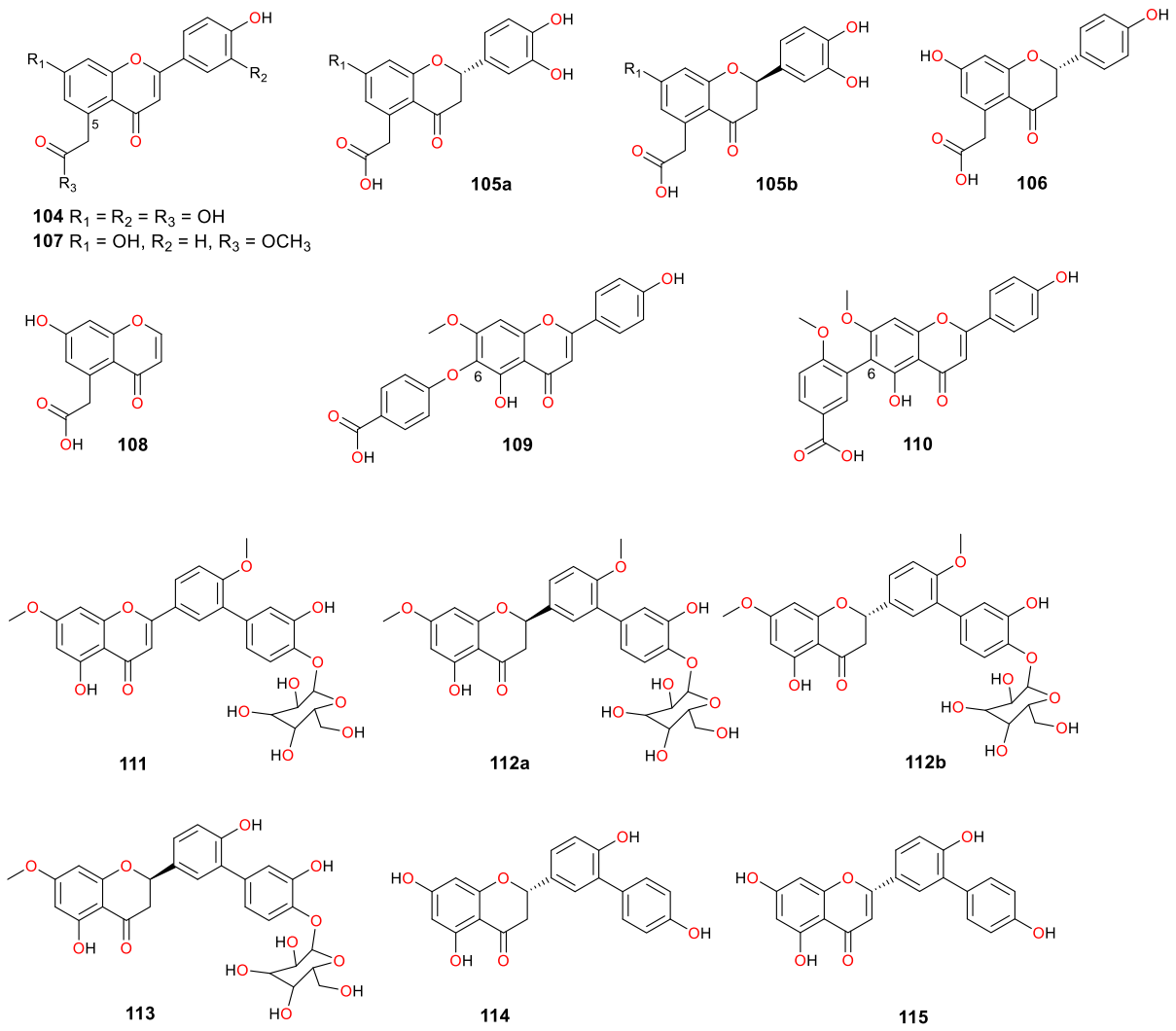

Fig. 7 Chemical structures of compounds **104–115**

were isolated from 75% ethanolic extract after chromatographic purification (Zou et al. 2016a, b). Compounds **109** and **110** were apigenin derivatives with aryl substituents at the C-6 position. Biological activities for the new compounds were not reported (Zou et al. 2016a, b). The phytochemical studies of *S. involven* yielded six novel apigenin derivatives, involvenflavones A–F, with 3′-aryl substitute (Long et al. 2015). Involvenflavones A–F (**111–115**) were also analysed for their protective effect against the injury of human umbilical vein endothelial cell (HUVECs) caused by high amounts of glucose. In the MTT viability test, all the compounds were shown to have potent protective effect in vitro at 3 μM (Long et al. 2015). The biosynthetic pathways for these compounds were proposed to be derived from the coupling reaction between apigenin and phenols, with a radical mechanism (Long et al. 2015).

Diterpenoids

The large-scale chromatographic separation and purification of the methanolic extract of *S. moellendorffii* and subsequent NMR and ECD analysis of pure compounds resulted in the identification of modified

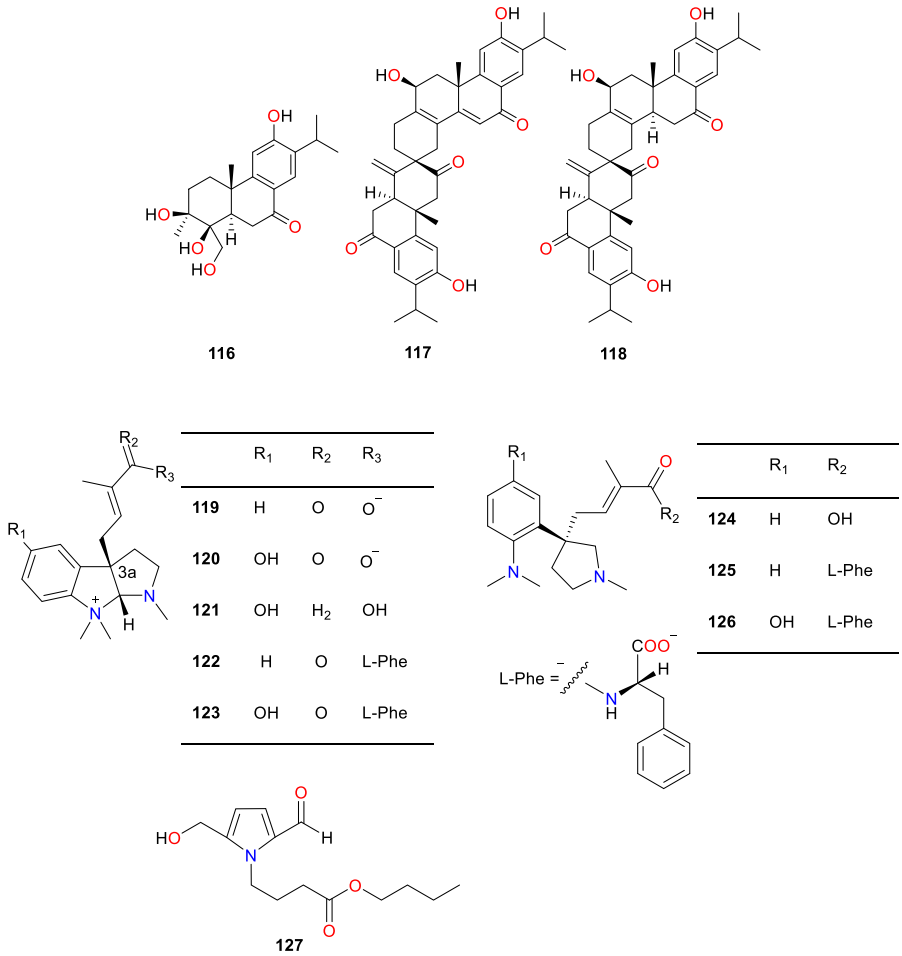

Fig. 8 Chemical structures of compounds **116–127**

abietene diterpenoid (3S,4S,5R,10S)-18(4 → 3)-abeo-3,4,12,18-tetrahydroxy-8,11,13-abietatrien-7-one (**116**), and two novel dimers, selaginedorffones A (**117**) and B (**118**) (Ke et al. 2018) (Fig. 8). Compounds **117** and **118** possessed a new cyclohexene moiety that is believed to be biosynthesized from two modified abietene diterpenoid units. The new compounds were evaluated for their growth inhibitory properties against HL-60 human leukaemia, A-549 human lung-cancer, SW480 human rectal-cancer, SMMC-7721 human liver-cancer, and MCF-7 human breast-cancer cell lines. Compounds **116** and **117** did not exhibit any activities against the tested cell lines. Compound **118** was cytotoxic towards the MCF-7 cells with an IC_{50} value of 9.0 μM, while it did not exhibit any significant activity against the other tested cell lines (Ke et al. 2018).

Alkaloids

Following a positive reaction to Dragendorff's reagent, eight new pyrrolidinoindoline alkaloids (**119–126**) were purified and characterized from the methanol extract of *S. moellendorfii* (Wang et al. 2009). These unique alkaloids (Fig. 8), possessed a 3-carboxybut-2-enyl side chain at the 3a position and two N8-methyl groups. Compounds **119**, **122**, and **124** were evaluated for antibacterial, cytotoxic, and acetylcholinesterase inhibitory activities, but did not exhibit any significant activities at 200 μg/mL (Wang et al.

2009). A new pyrrole alkaloid (**127**) was isolated from the butanolic extract of *S. delicatula* together with two new adenine analogues, delicatulines A and B. The compounds were evaluated for their inhibitory activities on HBV surface antigen and HBV DNA in HepAD38 cells and were found to possess only weak or no inhibitory activity (Yao et al. 2019a, b).

Lignans and neolignans

Selamoellenin A (**128**), isolated from *S. moellendorffii* Hieron, exhibited a protective effect even at low doses against high glucose concentration-induced injury of HUVECs at 0.01 nM concentrations (Zeng et al. 2017). Further phytochemical studies of the ethanolic extract of Chinese ethnic medicinal plant *S. moellendorffii* led to the isolation of three novel lignans, selamoellenins B–D (**129–131**) together with 11 known compounds (Zhu et al. 2018) (Fig. 9). The natural products were evaluated for their in vitro antitumor activities against four human cancer cell lines (hepatocellular, colon, gastric and urinary bladder carcinoma; MGC-803, A549, HepG2, T24) by the MTT viability assay using 5-fluorouracil (5-Fu) as positive control. The new compounds **130** and **131** decreased the T24 viability with IC_{50} values of 22.53 and 25.85 μM, respectively, while compound **129** was less cytotoxic, with an IC_{50} of 45.43 μM (Zhu et al. 2018). Three new compounds named pictalignan A–C (**132–134**) and three known derivatives, syringaresinol (**135**), 3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligan-4′,9,9′-triol (**136**), 4,9-dihydroxy-4′,7-epoxy-8′,9′-dinor-8,5′-neolignan7′-oic acid (**137**) were isolated from a 75% aqueous ethanol extract of *S. picta*, (Cheng et al. 2018). Compounds **132–134** exhibited moderate activity against HT-22 cells injured by L-glutamate treatment (Cheng et al. 2018). Compound **132** exhibited a better protective effect in the range of 10–15 μM than the positive control, dimethyl fumarate (Cheng et al. 2018).

The pyrrolidinoindoline alkaloid selaginellic acid, which was previously isolated from the whole plant *S. moellendorffii*, exhibited antiplatelet activity. This interesting bioactivity led Long and co-workers to further investigate this plant (Zhuo et al. 2016). Chromatographic purification of the methanolic extract of *S. moellendorffii* resulted in the isolation of two new neolignans, selaginellol (**138**) and selaginellol 4′-O-β-D-glucopyranoside (**139**) (Zhuo et al. 2016). Compounds **138** and **139** were evaluated for platelet aggregation activity induced by ADP or collagen, but did not show any significant activity (Zhuo et al. 2016). Tamariscinols U–W (**140–142**) which belong to the dihydrobenzofuran-type norneolignans were isolated from the ethanolic extract of *S. tamariscina*. These compounds were tested for their inhibitory effects against the mushroom tyrosinase. Tamarisconol U (IC_{50} = 5.75 μM) was three times more active than the positive control, kojic acid (He et al. 2019). Seven new neolignans, sinensiols A–G (**143–149**) together with two known compounds (**150–151**), were isolated from the whole plant of *S. sinensis* (Wang et al. 2007; Chen et al. 2019a, b). The planar structures of these compounds were determined by the analysis of spectroscopic data, and the absolute configuration was established by a comparison of the experimental CD data. Sinensiols A–D are dimers that are sesquilignans. All the isolated compounds were evaluated for their cytotoxicity against A549 and HepG2 human cancer cell lines, however, no growth inhibition of the cancer cells was observed (Wang et al. 2007; Chen et al. 2019a, b).

Saponins and sterols

The butanolic soluble fraction of the ethanolic extract of *S. uncinata* exhibited significant anti-anoxic activity. After solvent–solvent partitioning and chromatographic purification, two new steroidal saponin derivatives (Fig. 10), (3β,7β,12β,25R)spirost-5-ene-3,7,12-triol-3-O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl-(1 → 4)]-O-β-D-glucopyranoside (**152**) and (2α,3β,12β, 25R)-spirost-5-ene-2,3,12-triol-3O-α-L-rhamnopyranosyl-(1 → 2)-O-[α-L-rhamnopyranosyl(1 → 4)]-O-β-D-glucopyranoside (**153**) were isolated. This was the first report of steroidal saponins isolated from *S. uncinate* (Zheng et al. 2013). The anoxic PC12 cell assay was utilized to determine the protective effect against anoxia of compounds **152** and **153**. The tested compounds exhibited a potent protective effect (Zheng et al. 2013). The ethanolic extract of *S. tamariscina* yielded three new sterols, 3β,16α-dihydroxy-5α,17β-cholestan-21-carboxylic acid (**154**), 3 β-acetoxy-16α-hydroxy-5α,17β-cholestan-21carboxylic acid (**155**) and 3β-(3-hydroxybutyroxy)-16α-hydroxy5a,17β-

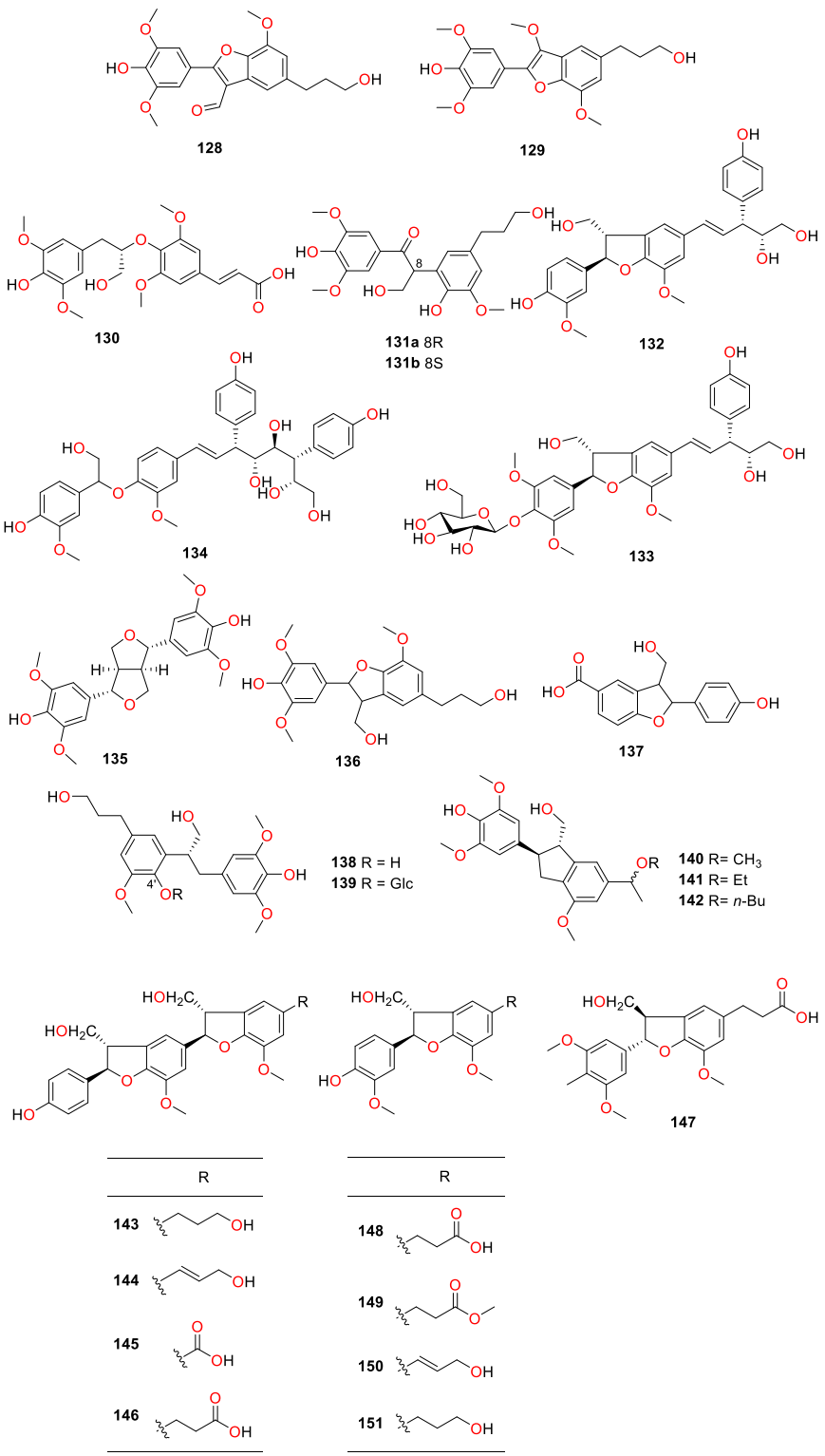

Fig. 9 Chemical structures of compounds **128–151**

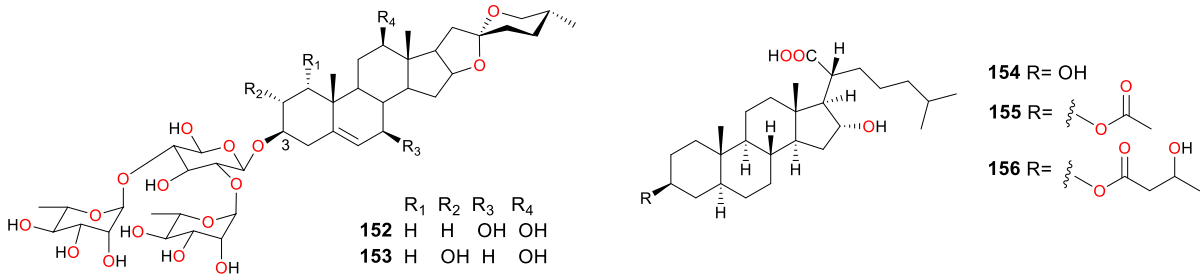

Fig. 10 Chemical structures of compounds **152–156**

cholestan-21-carboxylic acid (**156**) (Fig. 10). Compounds **154–157** were tested for their antiproliferative effects in human leukemia HL-60 cells. All three sterols exhibited weak cell growth inhibition compared to 5-fluorouracil (Gao et al. 2007).

Biological activities

The pharmacological profile of compounds isolated from various species of *Selaginella* has been summarised in Tables 1, 2, 3, 4 and 5 below. It is worthwhile to mention here that most publications only reported the biological activity of extracts, however, a number of research groups also reported the biological activities of the pure compounds. The disadvantage of such an approach is the uncertainty in the active compound responsible for the manifested activity, on the other hand it could better mimic potential synergic effect of the various compounds in the same plant. Each table provides details, comprised of the plant species, whether a pure compound or extract were tested, the test subject and the relevant activities.

Anticancer activity

The toxicity of extracts or pure compounds isolated from the *Selaginella* species was tested against up to 40 different cell lines (Table 1). To date, of approximately 700 species of the *Selaginella* genus (Bieski et al. 2015), only nine were characterized for cytotoxicity activity to the best of our knowledge. These nine species include *S. convolute*, *S. delicatula*, *S. doederleinii*, *S. labordei*, *S. moellendorffii*, *S. picta*, *S. pulvinata*, *S. tamariscina*, and *S. uncinata*. Usually water, ethanol, ethyl acetate, chloroform and hexane were used as extracting solvents; although the essential oil was prepared and tested as well. Half of the published papers deal with crude extracts and the rest reported the isolation and testing of pure compounds, which can further lead to determining the mechanism of anticancer activity at the molecular level. Unfortunately, in 97 percent of published papers, the mode of action could be simple toxicity rather than anticancer activity, as most of the publications only dealt cell lines derived from carcinomas and did not use nonmalignant cell lines as controls. Likewise, none of the current publications compared the *Selaginella* extracts compounds with some of the drugs commercially used for the treatment of selected cancers. Therefore, such results can hardly prove anticancer activity, because the simple cytotoxicity of the compounds is not excluded. When a control cell line was involved, it was usually represented by human dermal fibroblasts (HDF), mouse neuronal (HT-22) or monkey kidney (Vero) cells. However, both the cancer cell line and the nonmalignant cell line should be preferably of human origin and of the same tissue origin to ensure that the selective anticancer effect is not caused by species or tissue differences in sensitivity (Calderon-Montano et al. 2014). Ideally, at particular concentration within the therapeutic window, the control cells should continue to grow and proliferate, while the cancer cells are inhibited. However, the data for such a conclusion is frequently missing, except in a paper by Wang et al. (Wang et al. 2015a, b), which showed that the ethyl acetate extract of *Selaginella doederleinii* was 2–4 times more toxic for e.g. cervical, hepatocellular and prostatic adenocarcinoma than for African green monkey kidney cells.

Table 1 Anticancer activity of *Selaginella* extracts and pure compounds

Species	Extract/compound	Cell line	Activity (IC_{50})	References
S. moellendorffii	5-Carboxymethyl-4′,7-dihydroxyflavone ethyl ester (**70**)	A549	μM: 1.0 (**157**), 5.4 (**159**)	Cao et al. (2010a, b)
		BGC-823	μM: 6.3 (**70**), 1.0 (**160**)	
	Ginkgetin (**157**)	BEL-7402	μM: 5.0 (**157**), 6.1 (**158**), 6.8 (**159**), 1.4 (**160**)	
	Isoginkgetin (**158**)			
	Robustaflavone 4′-methyl ether (**159**)			
	Hinokiflavone (**160**)			
	Selaginedorffone B (**118**)	MCF-7	9.0 μM	Ke et al. (2018)
	Compound **161**	HT-29	9.6 nM	Zhoua et al. (2016)
S. pulvinata	Diselaginellin B (**38**)	SMMC-7721	9 μM	Cao et al. (2017)
S. tamariscina	Ethanol	HONE-1	200 μg/mL decrease the viability after 48 h by 20%	Yang et al. (2007), Hsin et al. (2013), Yang et al. (2013a, b) and Yang et al. (2013a, b)
		U2OS	antimetastatic effects	
		Saos-2	inhibits the invasiveness of human oral squamous-cell carcinoma	
		HOS	~ 34 μg/mL	
		HSC-3	NA	
		LLC	NA	
	Selaginellin M (**14**)	U251	μg/mL: 15 (**14**), 25 (**15**), 33 (**1**), 27 (**2**), 19 (**162**), 30 (b), 76 (**164**)	Zhang et al. (2012)
	Selaginellin N (**15**)			
	Selaginellin (**1**)	HeLa	μg/mL: 17 (**14**), 23 (**15**), 38 (**1**), 23 (**2**), 28 (**4**), 10 (**162**), 19 (**162**);52 (**163**), 48 (**164**)	
	Selaginellin A (**2**)			
	Selaginellin C (**4**)	MCF-7	μg/mL: 24 (**14**), 34 (**15**), 90 (**1**), 49 (**2**), 33 (**4**), 30 (**162**), 39 (**160**), 56 (**164**)	
	Neocryptomerin (**162**)			
	Hinokiflavone (**160**)			
	Pulvinatabiflavone (**163**)			
	7″-O-methylamentoflavone (**164**)			
	Amentoflavone (**165**)	MCF-7	~ 125 μM	Lee et al. (2011) and Pei et al. (2012)
		SiHa	~ 80 μM	
		CaSki	~ 40 μM	
	Compound **170**	HL-60	μM: 28.8 (**170**), 77.3 (**171**), 35.4 (**172**)	Gao et al. (2007)
	Compound **171**			
	Compound **172**			
	Water	HL-60	~ 800 μg/mL	Ahn et al. (2006)
	Sumaflavone (**166**)	HDF	Protection against UV irradiation—0.78 μM (**166**), 1.8 μM (**165**)	Lee et al. (2008a, b)
	Amentoflavone (**165**)			
	Ethyl acetate	HT-29	3 μg/mL	Li et al. (2014a, b)
		HeLa	3 μg/mL	
		Bel-7402	8 μg/mL	
	Selagibenzophenone B (**41**)	SMMC-7721	40 μM	Liu et al. (2018a, b)
		MHCC97-H	52 μM	

Table 1 continued

Species	Extract/compound	Cell line	Activity (IC_{50})	References
S. doederleinii	Ethyl acetate	A549	53 μg/mL or 52 μg/mL	Wang et al. (2015a, b) and Gang et al. (2017)
		7721	66 μg/mL	
		HeLa	38 μg/ml or 76 μg/mL	
		Eca-109	62 μg/mL	
		DU145	71 μg/mL	
		HepG2	66 μg/mL	
		PC12	> 150 μg/mL	
		Vero	> 150 μg/mL	Wang et al. (2015a, b)
	Extract	TW03	Inhibition of the cells growth by arresting the cell cycle at S phase	Jing et al. (2009)
	Total biflavonoids	A549	121 μg/mL	Li et al. (2017)
		7721	132 μg/mL	
	Amentoflavone (**165**)	A549	μg/mL: 36 (**165**), 42 (**168**), 19 (**169**)	Li et al. (2014a, b)
	Robustaflavone (**167**)	PC-9	μg/mL: 6 (**165**), 37 (**167**), 8 (**168**), 50 (**169**), 7 (e), 9 (**171**)	
	Compound **169**			
	Heveaflavone (**170**)	HL-60	μg/mL: 46 (**165**), 48 (**167**), 46 (**170**), 49 (**174**)	
	Compound **171**			
		K562	μg/mL: 5 (**165**), 39 (**168**)	
		CNE2	μg/mL: 17 (**165**), 43 (**167**), 16 (**170**)	
	Ethanol	CNE1	0.6 g/mL inhibits 96% viability	Lian et al. (2013) and Yao et al. (2017a, b)
		C666-1	0.6 g/mL inhibits 97% viability	
		LLC	36 μg/mL	
		B16	96 μg/mL	
	Essential oil	A549	47 μg/mL	Wang et al. (2015a, b)
		7721	34 μg/mL	
S. picta	Pictalignan A (**132**)	HT-22	protective effect against the injury of cells induced by L-Glutamate	Cheng et al. (2018)
	Pictalignan B (**1133**)			
	Pistalignan C (**134**)			
S. delicatula	Compound **83**	P-388	μM: 1.4 (**83**), 3.5 (**84**), 2.3 (α-tocopheryl quinone)	Chen et al. (2005)
	Compound **84**			
	α-Tocopheryl quinone	HT-29	μM: 1.6 (**83**), 5.3 (**84**), 6.6 (α-tocopheryl quinone)	
	Compound **85**	Raji	13 μM inhibits 34 (a), 18 (**85**) % of cells	Lin et al. (2000)
		Calu-1	13 μM inhibits 43 (a), 16 (**85**) % of cells	
S. labordei	Ethyl acetate	HT-29	5 μg/mL	Li et al. (2014a, b)
		HeLa	2 μg/mL	
		Bel-7402	1 μg/mL	
S. convoluta	Hexane (H)	HCT-116	50 mg/mL inhibits 20 (C) % of cells	Macedo et al. (2018)
	Chloroform (C)	OVCAR-8	50 mg/mL inhibits 17 (C) % of cells	
	Ethyl acetate (EA)	SF-295	50 mg/mL inhibits 24 (H), 62 (C), 19 (EA) % of cells	
S. uncinata	Ethyl acetate	HT-29	34 μg/mL	Li et al. (2014a, b)
		HeLa	15 μg/mL	
		Bel-7402	20 μg/mL	

Table 2 Antimicrobial activity of *Selaginella* extracts and pure compounds

Species	Extract/compound	Microorganism	MIC (mg/mL)*	References
S. involvens	Ethanol extract	*Propionibacterium acnes*	0.25	Hwang et al. (2012)
	Petroleum ether (PE)	*Escherichia coli*	IZ (mm, 10 mg/spot): 12 (PE), 8 (B), 11 (M), 10 (W)	
	Benzene (B)	*Pseudomonas sp.*	IZ (mm, 10 mg/spot): 10 (PE), 7 (B), 7 (M), 10 (W)	
	Methanol (M)			
	Water (W)			
S. bryopteris	Acetone extract	*Escherichia coli*	12.5	Verma et al. (2015)
		Klebsiella pneumoniae	6.3	
		Enterococcus faecalis	25	
		Staphylococcus aureus	6.3	
		Candida albicans	6.3	
		Candida krusei	3.1	
		Candida tropicalis	6.3	
	β-Sitosterol (**172**)	*Escherichia coli*	0.3 (**173**), 0.6 (**174**)	
	Heveaflavone (**170**)	*Klebsiella pneumoniae*	0.3 (**173**)	
	Vanillic acid (**173**)			
	β-Sitosterol β-D-glucoside (**174**)	*Enterococcus faecalis*	NA	
		Staphylococcus aureus	0.3 (**173**)	
		Candida albicans	0.3 (**172**), 0.6 (**170**), 0.3 (**173**), 0.3 (**174**)	
		Candida krusei	0.3 (**172**), 0.6 (**170**), 0.3 (**173**), 0.6 (**174**)	
		Candida tropicalis	0.3 (**173**), 0.3 (**174**)	
S. inaequalifolia	Petroleum ether (PE)	*Escherichia coli*	IZ (mm, 10 mg/spot): 11 (PE), 11 (B), 11 (M), 11 (W)	Duraiswamy et al. (2010)
	Benzene (B)	*Pseudomonas sp.*	IZ (mm, 10 mg/spot): 9 (PE), 8 (B), 7 (M), 8 (W)	
	Methanol (M)			
	Water (W)			
S. convulata	Ethanol extract:	*Bacillus cereus*	3.12 (H), 1.56 (C), 0.78 (E)	Macedo et al. (2018)
	Hexane (H) fraction	*Enterococcus faecalis*	3.12 (H), 3.12 (C), 0.39 (E)	
	Chloroform (C) fraction	*Escherichia coli*	1.56 (H), 1.56 (C), 0.39 (E)	
	Ethyl acetate (EA) fraction	*Klebsiella pneumoniae*	1.56 (H), 0.78 (C), 1.56 (E)	
		Salmonella enterica	1.56 (H), 1.56 (C), 0.78 (E)	
		Serratia marcescens	3.12 (H), 1.56 (C), 0.39 (E)	
		Shigella flexneri	3.12 (H), 1.56 (C), 0.78 (E)	
		Staphylococcus aureus	6.25 (H), 3.12 (C), 1.56 (E)	
	Water extract	*Acinetobacter sp.*	2.6	Gang et al. (2017)
		Klebsiella sp.	12.5	
	Ethanol extract	*Escherichia coli*	1.3	
		Enterobacter sp.	0.8	
		Acinetobacter sp.	1.3	
		Klebsiella sp.	1.0	
S. doederleinii	Ethanol extract:	*Bacillus subtilis*	IZ (mm, 12 mg/well): 12 (PE), 9 (D), 9 (E), 8 (M), 10 (W)	Gang et al. (2017)
	Petroleum ether (PE) Fraction	*Staphylococcus aureus*	IZ (mm, 12 mg/well): 13 (PE), 8 (DE), 7 (E), 8 (M), 12 (W)	
	Diethyl ether (DE) fraction	*Escherichia coli*	IZ (mm, 12 mg/well): 14 (PE), 9 (DE), 8 (E), 12 (M), 11 (W)	
	Ethyl acetate (EA) fraction	*Pseudomonas sp.*	IZ (mm, 12 mg/well): 12 (PE), 8 (DE), 8 (E), 9 (M), 10 (W)	
	Methanol (M) fraction			
	Water (W) fraction			

Table 2 continued

Species	Extract/compound	Microorganism	MIC (mg/mL)*	References
S. pallescens	Methanol/chloroform (1:1) extract	*Escherichia coli*	None up to 1 mg/disc	Rojas et al. (1999)
		Salmonella typhi		
		Shigella sp.		
		Vibrio cholera		
S. pulvinata	Selaginellin D (**5**)	*Staphylococcus aures*	IC_{50} (μg/mL): 4.9 (**1**), 1.2 (**2**), 1.2 (**3**)	Cao et al. (2010a, b)
	Selaginellin (**1**)		MIC (μg/mL): 9.6 (**1**), 4.6 (**2**), 2.6 (**3**)	
	Selaginellin A (**2**)	*Candida albicans*	IC_{50} (μg/mL): 5.3 (**5**), < 3.1 (**1**)	
	Selaginellin B (**3**)			
S. uncinata	Hexane (H) extract	*Candida albicans*	0.63 (H), 2.50 (C), 1.25 (EA), 0.63 (M)	Sit et al. (2017)
	Chloroform (C) extract	*Candida parapsilosis*	0.63 (H), 0.63 (C), 0.16 (EA), 1.25 (E), 0.63 (M), 1.25 (W)	
	Ethyl acetate (EA) extract	*Candida krusei*	0.16 (H), 0.16 (C), 0.16 (EA), 0.31 (E), 0.63 (M), 0.63 (W)	
	Ethanol (E) extract	*Cryptococcus neoformans*	0.16 (H), 0.31 (C), 0.31 (EA), 1.25 (E), 0.63 (M)	
	Methanol (M) extract	*Trichophyton mentagrophytes*	0.04 (H), 0.04 (C), 0.08 (EA), 1.25 (E), 0.63 (M)	
	Water (W) extract	*Bacillus cereus*	0.16 (H), 0.63 (C), 0.63 (EA)	
		Staphylococcus aureus	0.16 (H), 0.63 (C), 1.25 (EA)	
		Acinetobacter baumannii	2.50 (H), 1.25 (C), 1.25 (EA)	
		Escherichia coli	1.25 (C), 2.50 (EA)	
		Klebsiella pneumoniae	0.31 (H), 0.63 (C), 0.63 (EA)	
		Pseudomonas aeruginosa	1.25 (H), 1.25 (C), 0.63 (EA), 0.63 (E)	
S. lepidophylla	Methanol extract	*Helicobacter pylori*	MIC_{50}: 0.2–0.4 mg/mL (depending on the strain)	Robles-Zepeda et al. (2011)
S. tamariscina	Amentoflavone (**165**)	*Enterococcus faecium*	8 μg/mL	Hwang et al. (2013)
		Staphylococcus aureus	4 μg/mL	
		Streptococcus mutans	32 μg/mL	
		Escherichia coli O - 157	8 μg/mL	
		Escherichia coli	16 μg/mL	
		Pseudomonas aeruginosa	8 μg/mL	
		Candida albicans	Mitochondrial dysfunction leading to apoptosis	Hwang et al. (2012)
	Isocryptomerin (**175**)	*Saccharomyces cerevisiae*	18.1 μM	Lee et al. (2009a, b)
		Trichosporon beigelii	18.1 μM	
		Candida albicans	18.1 μM	
		Bacillus subtilis	20 μg/mL	Lee et al. (2009a, b)
		MRSA	10 μg/mL	
		Escherichia coli O - 157	20 μg/mL	

Table 2 continued

Species	Extract/compound	Microorganism	MIC (mg/mL)*	References
S. moellendorffii	Compound **104**	*Staphylococcus aureus*	0.2 (**104**), 0.1 (**106**), 0.2 (**107**), 0.01 (**107**), 0.2 (**176**),	Zou et al. (2016a, b)
	Compounds **105a** and **105b**	*Streptococcus pneumoniae*	0.1 (**104**), 0.1 (**105a** and **105b**), 0.03 (**106**), 0.1 (**107**), 0.01 (**107**), 0.1 (**176**), 0.1 (**177**)	
	Compound **106**	*Streptococcus β-hemolytic*	0.1 (**104**), 0.03 (**106**), 0.2 (**107**), 0.03 (**107**), 0.1 (**176**)	
	Compound **107**	*Escherichia coli*	0.03 (**106**), 0.03 (**107**), 0.1 (**107**), 0.1 (**176**)	
	Compound **108**	*Helicobacter pylori*	0.01 (**106**), 0.05 (**107**)	
	Compound **176**			
	Compound **177**			
	Essential oil	*Staphylococcus aureus*	40	Wang et al. (2018)
		Corynebacterium pyogenes	5	
		Pseudomonas aeruginosa	40	
S. helvetica	Essential oil	*Diplococcus pneumoniae*	20	Wang et al. (2018)
		Corynebacterium pyogenes	10	
		Escherichia coli	40	
		Pseudomonas aeruginosa	40	
S. delicatula	Essential oil	*Diplococcus pneumoniae*	40	Wang et al. (2018)
		Corynebacterium pyogenes	10	
		Escherichia coli	20	
		Pseudomonas aeruginosa	20	
		Proteus vulgaris	40	

IZ Inhibition zone, *NA* Not active

*Unless otherwise stated

The toxicity of *Selaginella* extracts was previously reported for hepatocellular, alveolar, skin, nasopharyngeal, prostatic, and oesophageal squamous cells, cervical, bone, and oral squamous cells, Lewis lung carcinoma and leukaemia cells with the IC_{50} ranging from 3 mg/L (ethyl acetate extract, cervical and colon adenocarcinoma) (Li et al. 2014a, b) to 800 mg/L (water extract, leukaemia) (Ahn et al. 2006). In addition, the *Selaginella* ethyl acetate extract was not toxic for the monkey kidney cells at up to 150 mg/L (Wang et al. 2015a, b). However, the crude extracts usually contain many biologically active compounds that could act additionally, synergistically or antagonistically.

Pure compounds isolated from *Selaginella* that were tested for cancer cell line toxicity included the following compounds: (3R)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one; 2,3-dihydroamentoflavone 7,4′-dimethyl ether; 2″,3″-dihydro-3′,3‴-biapigenin; 2″,3″dihydrorobustaflavone 7,4′, -dimethyl ether; 3′,3‴-binaringenin; 3β-(3-hydroxybutyroxy)-16α-hydroxy-5α,17β-cholestan-21-carboxylic acid; 3β,16α-dihydroxy-5α,17β-cholestan-21-carboxylic acid; 3β-acetoxy-16α-hydroxy-5α,17β-cholestan-21-carboxylic acid; 5-carboxymethyl-4′,7-dihydroxyflavone ethyl ester; 7,4′,7″,4‴-tetra-O-methyl-amentoflavone; 7″-O-methylamentoflavone; amentoflavone; diselaginellin B (**37**); ginkgetin; heveaflavone; hinokiflavone; isoginkgetin; neocryptomerin; pictalignan A–C (**128**–**130**); pulvinatabiflavone; robustaflavone; robustaflavone 4′,4‴-dimethyl ether; robustaflavone 4′-methyl ether; selaginbenzophenone B (**41**); selaginedorffone B (**115**); selaginellins (A (**2**), C (**4**), M (1**4**), N (**15**));

Table 3 Antiviral activity of *Selaginella* extracts and pure compounds

Species	Extract/compound	Virus		Activity (CPE = Cytopathic effect reduction, IC_{50} = concentration of the sample required to inhibit virus-induced CPE by 50%), NA = no activity	References
S. uncinata	Chloroform	*Togaviridae*	Chikungunya virus	CPE 34% at 80 μg/ml	Sit et al. (2017)
	Uncinoside A (**178**) Uncinoside B (**179**)	*Pneumoviridae*	Human orthopneumovirus (human respiratory syncytial virus)	IC_{50} 6.9 μg/ml (**178**); 1.3 μg/ml (**179**); 5.5 μg/ml (**180**)	Ma et al. (2003)
	Amentoflavone (**180**)	*Paramyxoviridae*	Human parainfluenza virus 3	IC_{50} 13.8 μg/ml (**178**); 20.8 μg/ml (**179**)	
		Orthomyxoviridae	Influenza A virus (H1N1)	NA (**178**); NA (**179**)	
S. moellendorffii	Ethyl acetate (EA) Total flavonoid (TF) Amentoflavone (AF)	*Picornaviridae*	Enterovirus B (coxsackie virus B3)	IC_{50} 16 μg/ml (EA); 19 μg/ml (TF); 25 μg/ml (AF)	Yin et al. (2014)
	Compound **69** Compound **70** Compound **71**	*Hepadnaviridae*	Hepatitis B virus	IC_{50} = ability to inhibit the secretion of HBV surface antigen > 0.98 mg/ml (**68**, toxic concentration); 0.17 mg/ml (**69**); 0.46 mg/ml (**70**)	Cao et al. (2010a, b)

sumaflavone; and α-tocopheryl quinone. (3R)-5,6,7-trihydroxy-3-isopropyl-3-methylisochroman-1-one seems to be the most active compound, with an IC_{50} value for human colon adenocarcinoma of 10 nM. This isochroman is followed by ginkgetin, hinokiflavone and robustaflavone 4′,4‴-dimethyl ether with IC_{50} values for human colon, alveolar basal epithelial cells, gastric adenocarcinoma and mouse leukaemia of around 1 μM.

Several papers described the isolation of the same compounds from different *Selaginella* species and reported comparable anticancer activities. Amentoflavone isolated from *S. tamariscina* and *S. doederleinii* exhibited IC_{50} in the range from 5 to 125 μM for human breast, cervical, alveolar, lung, nasopharyngeal carcinoma and leukaemia cells; in addition, at several times lower concentration (1.8 μM), it protected human dermal fibroblasts against UV irradiation. Hinokiflavone isolated from *S. tamariscina* and *S. moellendorffii* was active against human gastric, hepatocellular, cervical, breast carcinoma and glioma, with IC_{50} ranging from 1 μM to 39 μM. Finally, robustaflavone 4′-methyl ether isolated from both *S. moellendorffii* and *S. delicatula* exhibited activity against human alveolar and lung carcinoma, with IC_{50} ranging from 5 to approx. 50 μM.

Mechanisms of the anticancer activity

Most scientific publications aimed at determining the mechanism of activity of different types of *Selaginella* extracts. Since an extract is a mixture of many secondary metabolites, the synergistic, antagonistic and additive effects of such complex mixtures will also influence the biological activities. Only few authors tested the effects and mechanisms of action of pure substances. As shown in the previous chapter, *Selaginella* species are a rich source of unique compounds and only a limited number of these

Table 4 Antiparasitic activity of *Selaginella* extracts and compounds

Species	Extract/compound	Parasite	IC_{50} μg/ml, NA = no activity	References
S. vogelii	Methanol	*Plasmodium falciparum*	32.2 (toxic concentration)	Teinkela et al. (2018)
		Trypanosoma brucei	2.4	
S. sellowii	Polar hydroethanolic extract (PHE) Hydroethanolic (HE) Amentoflavone (**165**) Robustaflavone (**159**)	*Leishmania amazonensis*	100% suppression of the parasite load in the infection site and draining lymph nodes at an intralesional dose of 50 mg/kg/day × 5 (PHE); IC_{50} = 20.2 (HE); 0.1 (**165**); 2.8 (**159**)	Rizk et al. (2014) and Queiroz et al. (2016)
S. bryopteris	Fractions of ethanolic extract: Toluene (T) Ethyl acetate (EA) Butanol (B) Amentoflavone (**165**) Hinokiflavone (**160**) Compound **180** Compound **181**	*Plasmodium falciparum*	4.6 (T); 1.0 (EA); > 5 (B); > 9.3 (**165**); 2.3 (**160**); 4.5 (**180**); 9.0 (**181**)	
		Leishmania donovani	13.0 (T); 9.3 (EA); > 30 (B); > 55.6 (**165**); 2.9 (**160**); 1.6 (**180**); 4.2 (**181**)	
		Trypanosoma brucei rhodesiense	24.1 (T); 12.4 (EA); 28.5 (B)	
		Trypanosoma cruzi	> 30 (T); 20.5 (EA); > 30 (B)	

compounds have had their mechanism of action in living cells determined. For the crude *Selaginella* extracts, two main mechanisms were predicted. The first one is based on p53-mediated apoptosis, the second one on metalloproteinase inhibition.

The anticancer activity of *Selaginella* crude extracts is usually attributed to decreasing the bcl-2/bax mRNA level ratio (Ahn et al. 2006; Jing et al. 2009; Wang et al. 2015a, b). Bax (bcl-2 associated X protein) promotes cell death through permeabilization of the mitochondrial outer membrane in response to different cellular stresses (Khodapasand et al. 2015). This means that it functions as an apoptotic activator, which results in the release of cytochrome C and other pro-apoptotic factors from the mitochondria and activation of caspases (esp. caspase-3 Ahn et al. 2006; Li et al. 2014a, b; Wang et al. 2015a, b) and caspase-9 (Sui et al. 2016)). Bax is involved in p53-mediated apoptosis, as its expression is upregulated by the tumour suppressor protein p53. The p53 protein is a transcription factor that regulates not only bax, but also many other downstream target genes (Wang et al. 2015a, b). In contrast to Bax, Bcl-2 (derived from B-cell lymphoma where the protein was first discovered) prevents apoptosis by inhibiting the activity of Bax (Khodapasand et al. 2015). The ratio of bcl-2/bax mRNA level is therefore crucial for triggering the programmed death of cancer cells or inhibiting tumour growth by arresting the cell cycle at the S phase (Jing et al. 2009; Sui et al. 2016). Nowadays, many drugs activating bax are in clinical use for the treatment of cancer (Liu et al. 2016). A decrease in the gene expression of cyclooxygenase (COX-2), lipoxygenase (5-LOX, 12-LOX), 5-lipoxigenase-activating protein (FLAP) and survivin was demonstrated to accompany the decrease in bcl-2/bax mRNA level ratio (Wang et al. 2015a, b). The inhibition of COX, LOX and FLAP blocks the formation of prostaglandins and leukotrienes, and thus limits the inflammatory process (Martel-Pelletier et al. 2003) and prevents necrosis

Table 5 Antioxidant activity of *Selaginella* extracts and compounds

Species	Extract/compound	Method used	IC_{50} (μg/ml)/percentage of antioxidant activity (% AA)	References
S. convoluta	Crude ethanol extract fractions:	DPPH free-radical scavenging activity assay	IC_{50}: 289.5 (H); 107.0 (C); 47.2 (EA)	Macedo et al. (2018)
	Hexane (H)			
	Chloroform (C)	ß-carotene bleaching assay	% AA: 58.99 (H); 44.99 (C); 58.91 (EA)	
	Ethyl acetate (EA)			
S. doederleinii	Ionic liquid-assisted extraction, various parameters (IL)	DPPH free-radical scavenging activity assay	IC_{50}: 50–90 (IL); 60–140 (VO); 60–120 (FE)	Wang et al. (2015a, b) and Gang et al. (2017)
	Volatile oil (VO)	ABTS free-radical scavenging activity assay	IC_{50}: 65–180 (VO)	
	Fractions of ethanol extract (FE)			

(Wallach and Kovalenko 2014). The role of survivin lies in the inhibition of caspase. Its inhibition leads to no negative regulation of apoptosis. Metalloproteinases (MMPs) are involved in the breakdown of extracellular matrix in normal physiological processes; however, their increased expression was demonstrated in several types of tumours (Zucker et al. 1995; Morini et al. 2000; Groblewska et al. 2012). Tissue endogenous natural inhibitors of metalloproteinases (TIMPs; among them TIMP-1 and TIMP-2 were best characterised) form complexes with MMPs and inhibit cancer dissemination (Groblewska et al. 2012). Several authors demonstrated that *Selaginella* extracts inhibit MMP-1, (Lee et al. 2008a, b) MMP-2 and MMP-9 (Hsin et al. 2013) enzyme activity as well as their expression (Hsin et al. 2013; Yang et al. 2013a, b) and increase both TIMP-1 (Yang et al. 2007) and TIMP-2 expression (Hsin et al. 2013) through Erk1/2 (extracellular signal-regulated kinase) and a p38-dependent pathway (Hsin et al. 2013). Yang et al. demonstrated that the regulation of MMP-2 and MMP-9 expression is based on the regulation of their promoters (Yang et al. 2013a, b). The same authors further showed that *Selaginella* extract inhibits the phosphorylation of p38 and Akt. (Hsin et al. 2013). Similarly to MMPs, urokinase plasminogen activator (u-PA) expression was decreased in cancer cells as well; meanwhile, plasminogen activator inhibitor-1 (PAI-1), was increased (Yang et al. 2007).

Among the biflavonoids occurring in *Selaginella* species, sumaflavone and amentoflavone exhibited significant MMP-1 inhibitory activity. The IC_{50} values were approximately 10 × lower than the IC_{50} of retinoic acid (10 μM), which served as a positive control (Lee et al. 2011). Moreover, the cells treated with amentoflavone exhibited a series of cellular alterations related to apoptosis (Lee et al. 2011; Pei et al. 2012) and signs of mitochondrial dysfunctions—the reduction of mitochondrial inner-membrane potential, the release of cytochrome c from mitochondria, and activation of caspase 3 (Pei et al. 2012) and caspase 9 (Lee et al. 2011). Amentaflavone decreased the expression of Bcl-2, but elevated the apoptotic factor Bax (Lee et al. 2011). Similarly to amentoflavone, the apoptosis was induced by diselaginellin B (Cao et al. 2017). The findings that selaginellin (**1**) and selaginellin M (**14**) inhibited cytochromes P450 (CYP) with IC_{50} values of around 1 μM for CYP2C8 followed by other less inhibited cytochromes (CYP2C9 and CYP2J2) (Zanger and Schwab 2013) confirm the concept that extracts are mixtures of substances with complex effects. As the cytochromes P450 are the major source of variability in drug pharmacokinetics, their inhibition significantly slows down the biotransformation of most substances, including various bioactive compounds from *Selaginella* extract (Zanger and Schwab 2013).

Some recent publications demonstrated specific anticancer activity of *Selaginella* extracts. It was shown that hinokiflavone obtained from *Selaginella tamariscina* (Beauv.) triggers apoptosis and may inhibit the migration of breast cancer cells in Balb/c nude mice (Huang et al. 2020). Extract from *S. tamariscina* was reported to downregulate the growth of human lung cancer cells both in vitro and in vivo by

specifically inhibiting aldo–keto reductase (Jung et al. 2017). Amentoflavone was identified as the compound responsible for this activity. Delicaflavone isolated from *Selaginella doederleinii* Hieron triggered apoptosis via a mitochondrial pathway and the inhibition of MAPK signalling in HeLa cells (Yao et al. 2019a, b), and inhibited several kinases involved in signalling pathways of colorectal cancer cells (Yao et al. 2020). An oral proliposomes formulation was proposed to overcome the solubility-related limitation for the oral delivery of a biflavonoids extract from *S. doederleinii.* (Chen et al. 2019a, b) An alternative might be amorphous solid dispersion, which exhibited acceptable pharmacokinetics in rats (Chen et al. 2020).

Tackling drug resistance

Multidrug resistance (MDR) is a major challenge for the 21th century in cancer chemotherapy (Chambers et al. 2019). Drug efflux pumps and detoxification enzymes, such as aldo–keto reductases (AKRs), are typical proteins involved in the resistance of tumours (Jung et al. 2017). Overcoming such resistance with natural compounds, especially flavonoids, has been previously reported (Viktorova et al. 2019). Many flavonoids commonly consumed in the daily diet or dietary supplements are present in many evolutionary distinct plant species, including *Selaginella*. Therefore, Jung et al. (Jung et al. 2017) focused their attention on the modulation of MDR by *S. tamariscina* ethanol extract and amentoflavone, which is the dominant flavonoid in this species. Both the extract and flavone were able to inhibit human aldo–keto reductase AKR1B10 in a dose-dependent manner, with IC_{50} equal to 8 mg/L and 2 μM, respectively. In addition, the authors confirmed the anti-proliferative activity of lung cancer cells overexpressing AKR1B10 when cultivated with the extract or amentoflavone combined with doxorubicin. This observation leads to the conclusion that the extract and amentoflavone synergistically increase the doxorubicin anti-proliferative effect in human lung cancer cells overproducing AKR1B10. *S. tamariscina* ethanol extract also significantly inhibited lung tumour growth in mice (Zhu et al. 2017).

Animal testing

As the in vitro demonstration of cellular apoptosis is inaccurate for predicting the real antitumor activity of the compounds or extracts in vivo, *Selaginella* extracts were administered to mice (Yang et al. 2007; Le et al. 2012; Wang et al. 2015a, b) and rats (Chen et al. 2018). The results showed considerable antitumor activity of *Selaginella* extracts without toxicity for non-cancer cells (up to 10 g/kg) (Le et al. 2012). The oral administration of extract did not prevent Lewis lung carcinoma formation; however, it produced a significant inhibition of tumour growth (Yang et al. 2007; Le et al. 2012). The doses of 50 and 150 mg/kg/day of total biflavonoids extract inhibited the mouse tumour growth by 40 and 54%, respectively (Yao et al. 2017a, b). Moreover, the immune response of the mouse (TNF-α and IFN-γ production) was significantly enhanced (Yao et al. 2017a, b). Similarly, Sui et al. observed alveolar tumour growth inhibition accompanied by reduced expression of antigen Ki67 and reduced microvascular density (Sui et al. 2016). A decrease in Ki67 activity leads to an inhibition of ribosomal RNA synthesis (Rahmanzadeh et al. 2007) and microvascular density correlating with the aggressiveness of several cancers (Iakovlev et al. 2012). Lewis lung carcinoma metastases of rats treated with *Selaginella* extract (3 g/kg/day) decreased by 79% in 30 days without any apparent signs of toxicity (Yang et al. 2007).

As pharmacokinetics is one of the major parameters affecting the success of drugs, Chen et al. administered five biflavonoids (amentoflavone, robustaflavone, 2″,3″-dihydro-3′,3″-biapigenin, 3′,3″-binaringenin and delicaflavone) orally or intravenously in rats. After intravenous administration, the plasma concentration of all biflavonoids rapidly decreased and cleared out within 12 h. The distribution of 3′,3‴-binaringenin, delicaflavone and 2″,3″-dihydro-3′,3‴-biapigenin between plasma and the rest of the body after oral administration (Vd) were greater than those of amentoflavone and robustaflavone, indicative of a relatively greater tissue distribution. For the oral administration, the total biflavonoids were detected at a much lower plasma level (Chen et al. 2018). The mean oral bioavailability (F) was highest for delicaflavone (2.5%), followed by 2″,3″-dihydro-3′,3‴-biapigenin (1%). The lowest bioavailability was observed for amentoflavone (0.3%) (Chen et al.

2018). Unfortunately biflavonoids, being members of the flavonoid group, share the group's low bioavailability which limits their effects on health (Thilakarathna and Rupasinghe 2013). Many compounds are known for their biological activities in vitro; however, because of their poor solubility, rapid metabolism, or a combination of both, they are usually poorly bioavailable upon oral administration. In such cases, several mechanisms of drug-delivery systems could be used to increase drug penetration into the target place of action. One of them is nano-engineering which was successfully used for the delivery of flavonoids isolated from *S. bryopteris* (Bhargava et al. 2017). Both in vitro and in vivo studies with nano-encapsulated flavonoids exhibited increased mitoprotective activity in a dose-dependent manner when exposed to a carcinogen, and an inhibitory effect on tumour growth in mice (Bhargava et al. 2017).

Antimicrobial activity

To date, only 18 reports exist on the antimicrobial activity of *Selaginella* sp. Moreover, in comparison to anticancer properties, the antimicrobial activity of *Selaginella* species is not significant, and a small number of papers on this topic originate from only a few research groups. Moreover, some of the currently published papers lack the use of appropriate controls (e.g. evaluation of the antimicrobial activity of the pure solvents which were used for plant extraction). Considering the number of known *Selaginella* species, only some have been determined as antimicrobial. As with its other activities, the antimicrobial activity of *Selaginella* is mostly tested using crude extracts of plants. Moreover, essential oils prepared from several *Selaginella* species were also tested for their antimicrobial activity (Wang et al. 2018). *Selaginella bryopteris* extract was also used as a reducing agent for silver nanoparticle synthesis. This green synthesis produced antimicrobial particles; however, their activity was caused by the silver rather than by the plant extract, as published by the authors (Baskaran et al. 2018).

Selaginella extracts were found to be active against both bacteria and yeasts with no selectivity for Gram-positive or negative bacteria. The sensitivity of the particular strain used rather than the extraction solvent used is a major factor affecting antimicrobial activity results (Table 2). The minimal concentrations inhibiting visible growth (MIC) of bacteria are in the range of 0.4–6.3 mg/mL, and the same concentrations for the yeast lie in the range of 0.04–2.5 mg/mL. Non-polar extracts exhibited slightly better activity than the polar extracts. Joo et al. also reported on antimicrobial activity against the causative agent of acnes—*Propionibacterium acnes* (Joo et al. 2008). The MIC inhibiting the growth of these anaerobic bacteria was relatively low compared to the previously mentioned aerobic microorganisms (MIC = 0.25 mg/mL).

Amentoflavone, which is commonly isolated from *Selaginella*, exhibited antimicrobial activity besides anticancer activity. Despite the concentrations of amentoflavone inhibiting half of cancer cells (IC_{50}) being relatively low (tens of μg/mL), (Lee et al. 2011; Pei et al. 2012; Li et al. 2014a, b) the effective concentrations inhibiting the visible growth of bacteria (MIC) are about ten times lower (Hwang et al. 2012). The same shift in effective doses inhibiting bacterial and cancer cells is evident for selaginellin (**1**) (Cao et al. 2010a, b; Zhang et al. 2012). Both amentoflavone and isocryptomerin inhibited human pathogenic bacteria (Lee et al. 2009a, b; Hwang et al. 2013) and drug-resistant strains (e.g. 10 μg/mL of isocryptomerin inhibited methicillin-resistant *S. aureus* (Lee et al. 2009a, b)). Moreover, Hwang et al. reported a synergistic effect of both amentoflavone (Hwang et al. 2013) and isocryptomerin (Lee et al. 2009a, b) when applied in binary drug therapy together with some clinically used antibiotics (ampicillin, cefotaxime and chloramphenicol). This synergistic effect could eliminate the multidrug resistance phenotype of bacteria.

The mechanism of the activity of amentoflavone on *Candida albicans* and cancer cells may be the same, as both are eukaryotic cells. Amentoflavone was shown to cause disruption of the mitochondrial functions in a number of studies (Lee et al. 2011; Hwang et al. 2012; Pei et al. 2012). Extract of *S. tamariscina* was reported to eliminate four strains of *Microcystis aeruginosa* that form cyanobacterial bloom (Lee et al. 2020). Amentoflavone was identified as the major active compound in the extract responsible for this activity. Fractions of *S. convolute* activated the nociceptive peripheral pathway, resulting in an antinociceptive effect (de Sa et al. 2012; Oliveira-Macedo et al. 2019).

Neuroprotective activity

Oxidative stress is an imbalance between the concentration of reactive oxygen (synthesised as by-product of metabolism) and the way in which the organism can destroy this reactive species. A high concentration of these reactive species causes many different diseases (for example Parkinson's disease, atherosclerosis, and heart attack). The rather poor antioxidant potential of *Selaginella* species is mentioned above; therefore, some neuroprotective activity could be suggested. However, to date, there have only been a few articles about the neuroprotective activity of *Selaginella*.

Experiments with mice treated with ethanol extract from *S. convulata* support the theory of the traditional use of this plant as an analgesic agent. In addition, the extract did not affect motor coordination (de Sa et al. 2012). Extract from the same plant was used for the preparation of ZnO nanoparticles. Both the extract and the nanoparticles possessed muscle relaxant activity (Xu et al. 2020). Moreover, the aqueous extract of *S. delicatula* was tested on a *Drosophila* model mimicking Parkinson's disease symptoms. The extract offered concentration-dependent protection against rotenone-induced lethality, ROS production and protein carbonyl and hydroperoxide levels. Simultaneously, the extract restored the activity of antioxidant enzymes (superoxide dismutase, glutathione reductase), glutathione-S-transferase and membrane-bound enzymes (NADH-cytochrome c reductase and succinate dehydrogenase), suggesting its propensity to protect mitochondrial integrity. Finally, the extract normalized the activity levels of acetylcholinesterase and dopamine depletion. These findings suggest that the extract has concentration-dependent neuroprotective potencies (Girish 2012) and reduces mitochondrial dysfunction (Chandran and Muralidhara 2013).

Immunomodulation activity

Inflammation is a complex biological response of an organism to the presence of foreign agents. This protective reaction includes the immune system and blood circulation. The function of this reaction is to eliminate damage, remove foreign agents and initiate the tissue repair process. Many substances can affect the immune system and thereby regulate inflammatory processes via many mechanisms. Several *Selaginella* extracts previously demonstrated the ability to modulate the immune system. The oral administration of *S. bryopteris* methanol extract at doses of up to 2 g/kg did not exhibit marked toxicity in in vivo models, and local treatment resulted in a reduction in the redness and swelling of inflammation, which was also achieved by the aqueous extract of *S. moellendorffii* (Zhao et al. 2017). This study also revealed the mechanism of its anti-inflammatory effect, which is the reduction of nitrate oxide (NO) accumulation (an important mediator of the inflammatory process, production inducible by nitric oxide synthesis) and inhibition of anti-inflammatory cytokine production in the tissue. Indomethacin (a reference anti-inflammatory drug) has a similar mechanism (Paswan et al. 2017). Selagin, originating from *S. participens*, exhibited thymus growth-stimulatory activity in adult mice; moreover, it protected immunocompromised mice against *Aspergillus fumigatus* infection, although without any direct antifungal activity in vitro (Gayathri et al. 2011). The ethanol extract of *S. tamariscina* was able to inhibit the production of inflammatory mediators and pro-inflammatory cytokines in a dose-dependent manner. Amentaflavone, one of the main active substances of *Selaginella*, inhibited the expression of nitrate oxide synthase by NF-κB deactivation (Woo et al. 2005). At the same time, the extract showed potential for free radical neutralization and prevented their formation (Won et al. 2018).

Asthma is a chronic inflammatory disease characterised by inflammation, bronchial hyperactivity and airway obstruction. Flavonoids from *S. uncinata* weakened airway hyper-reactivity in ovalbumin-induced asthma in rats. T-cell-associated cytokine levels as well as IgE and IgA were decreased, while IFN-γ was elevated in treated rats (Yu et al. 2017). The effect of *Selaginella* extracts on asthma was also tested in vitro. Phosphodiesterase-4 (PDE4) catalyses the hydrolysis of secondary messengers (cyclic adenosine monophosphate and guanosine monophosphate) and is associated with respiratory diseases. There are many inhibitors of this enzyme, but only roflumilast is approved for the treatment of chronic obstructive pulmonary disease. Selaginpulvilins B (**43**) and C (**44**) proved to be potent inhibitors of this enzyme. Their IC_{50} values were 0.11 ± 0.02 and 0.18 0.02 μM respectively, and thus were more effective than roflumilast (IC_{50} 0.54 ± 0.04 μM) (Liu et al. 2014). Nanotechnology is emerging in the field of medicinal

plant research. This is mainly because this technology offers solutions in the field of transport or stability of the active ingredients. The oral administration of silver nanoparticles synthetized in the presence of *S. myosurus* extract significantly reduced paw edema in a mouse model (Kedi et al. 2018).

A recent paper indicated that robustaflavone-4′-dimethyl ether isolated from *S. uncinata* downregulates the activation of neutrophils by inhibiting the cytokine receptor FLT3-mediatied AKT and MAPK pathways (Wu et al. 2020). The nanoparticles prepared by using *Selaginella tamariscina* Carbonisata extracts were shown to activate the fibrinogen system, resulting in haemostatic effects in rat and mouse tail amputation and scratch models (Zhao et al. 2020). Similarly, silver nanoparticles prepared by green technology using *S. bryopteris* extract exhibited anticoagulant and antiplatelet properties together with antimicrobial effects (Dakshayani et al. 2019).

Other activities

The number of papers describing antiviral (Table 3) and antiparasitic (Table 4) activity of the *Selaginella* species is even lower. Whole extracts were mainly used, although a paper described the activity of unique chemical entities (Kunert et al. 2008; Cao et al. 2010a, b). Promising extracts sometimes exhibited significant toxicity, which probably limits their future use. As the methodology is broad, it is difficult to compare the results or distinguish between a selective effect against given pathogen from a general protective effect of the extracts on the cells in the test. Antioxidant activity (Table 5) of the *Selaginella* species has only minor potential for two reasons. One is that the total antioxidant activity is relatively low compared to the controls, second, the main compound (if identified) is usually a common (bi)flavonoid, which could be extracted from many other plant species or may be present in the daily diet. Biflavonoids isolated from *S. uncinata* were suggested to be natural antidiabetic agents, as they acted as potent inhibitors of protein tyrosine phosphatase 1B, causing insulin resistance by interfering with the intracellular insulin signalling pathway (Dwi 2011).

Conclusions

We sought to provide a comprehensive review summarising current knowledge about the chemical composition and biological activities of *Selaginella* species. The Selaginellaceae family has been shown to provide a remarkably rich panel of compounds potentially useful in medical applications including flavonoids, lignans, terpenes and a large group of unique compounds named selaginellins. Notably, numerous novel compounds were also identified from various *Selaginella* extracts. In the course of this review, the "coincidental simultaneous assignment" of the same trivial name to different structures was

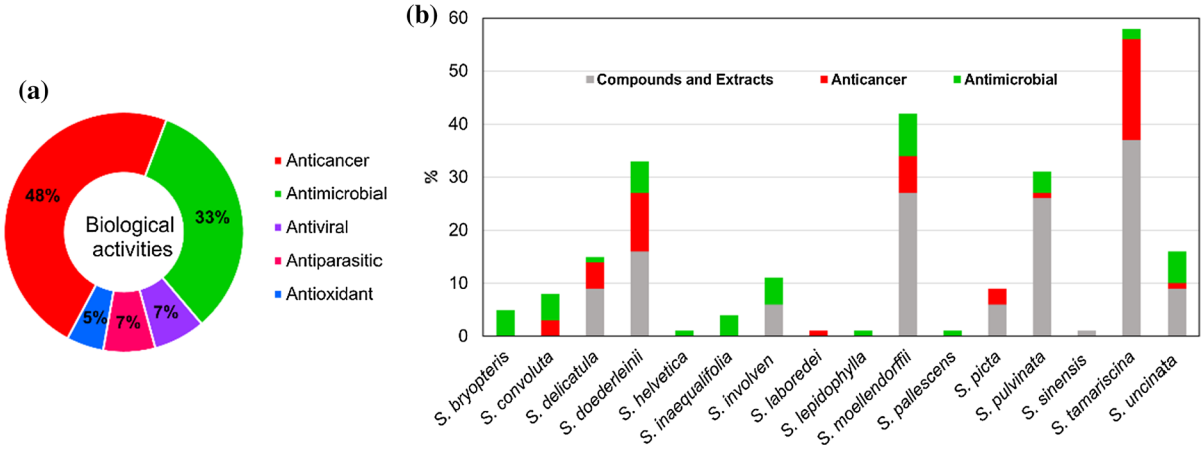

Fig. 11 Distribution of publications describing different biological activities (**a**) and *Selaginella* sp. as producers of new compounds and their anticancer and antimicrobial activities (**b**)

also uncovered. As suggested by Ramabharathi and Schuehly (2014), it is also our belief that appropriate rules should be adhered to when giving a trivial name to natural products.

The diverse structural classes of compounds isolated from the genus *Selaginella* exhibited a wide range of interesting biological activities as indicated in various sections above. Analysis of the biological activities (Fig. 11a) revealed that almost half the publications explored the cytotoxic effects of the extracts or pure compounds obtained from *Selaginella*. This was closely followed by their antimicrobial activities. Further analysis of *Selaginella* as producers of new compounds together with their anticancer and antimicrobial activity identified four main *Selaginella* species (*S. tamariscina*, *S. moellendorffii*, *S. pulvinata*, and *S. doederleinii*) as sources of new compounds with a high number of anticancer or antimicrobial activities (Fig. 11b.). As most of the potentially active compounds are present at very low concentrations in the extracts, further fractionation and purification of the crude extracts may provide positive results and avoid concentration limits and, in some cases, the antagonistic effect of the compound in complex mixtures. Due to the interesting pharmacological effects of the extracts and secondary metabolites, further research in this field is highly desirable.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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