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Review Article

Arylnaphthalene lignans with a focus *Linum* species: a review on phytochemical, biotechnological and pharmacological potential

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Abstract

Lignans are a large group of dimeric phenylpropanoids with a long and distinguished history of medicinal use in the ancient cultures of many peoples. The two main groups, -aryltetralin and arylnaphthalene lignans, are leading compounds with important pharmacological properties and a wide range of biological activities. While the first group is well studied mainly for the production of podophyllotoxin, for arylnaphthalene lignans, the data on the availability of a sustainable resource for their production is are still insufficient. The *Linum* genus, comprising approximately 180 species, is notable for its arylnaphthalene lignans production like justicidin B and isojusticidin B. The pharmacological potential of arylnaphthalene lignans includes cytotoxic, antiviral, anti-inflammatory and antiprotozoal effects. The review highlights the use of biotechnology by *in vitro* cultures for optimising lignan production. Structural elucidation of novel lignans underscores the ongoing diversity and potential discoveries in this botanical domain, providing an important additional information of arylnaphthalene lignans.

Keywords

Linum, arylnaphthalene lignans, *in vitro* production, hairy roots, justicidin B

Introduction

Lignans are a large group of dimeric phenylpropanoids, defined as *β*-*β*'-dimers of phenylpropane (C_6C_3) units. This widespread group of natural products has a long and distinguished history of medicinal use in the ancient cultures of many peoples. The two main groups, aryltetralin and arylnaphthalene lignans, are leading compounds with important pharmacological properties and a wide range of biological activities. While the first group is well studied mainly to produce podophyllotoxin, for arylnaphthalene lignans, the data on the availability of a sustainable resource for their production are still insufficient. Arylnaphthalene lignans are distinguished not only by different and unique chemical structures, but also by a wide range of functionalities that present significant potential for use in the pharmaceutical industry. The diversity of lignans makes them promising prodrugs, offering opportunities for the development of new pharmaceutical agents (Teponno et al. 2016).

Arylnaphthalene lignans are still poorly-studied bioactive compounds that are found restrictedly in nature

regarding the scientific publications. The plants which have been identified as a source of arylnaphthalene lignans belong to different families, such as Linaceae (namely the blue-flowered members from Sect Linum, section Adenolinum and section Syllinum (Schmidt et al. 2010; Bolsheva et al. 2022), as well as Phyllanthaceae, Acanthaceae, Rutaceae and Burseraceae. *Cleistanthus* species is one of the natural sources of arylnaphthalene lignans. These trees are usually used for house construction (Laha et al. 2018). The juice of the bark of *Cleistanthus collinus* (Roxb.) Benth.ex Hook.f. is applied externally in snakebite (Prusti and Behera 2007) while leaves' decoction of *C. sumatranus* (Miq.) Muell. is used against haematuria (Li and Xing 2016). *Phyllanthus amarus* Schum. and Thonn. is a key plant in the Indian Ayurvedic system of medicine to treat hepatitis and various problems related to the stomach, kidney, genitourinary system, liver and spleen, as well as genital infections (Unander 1998; Kumar 2020). Several species of Phyllanthus are used in traditional treatment of various health problems (Table 1) and are known to contain a variety of arylnaphthalene lignans. *Justicia adhatoda* L. is very important plant for the Meitei community of Manipur, India for food and to cure many ailments like cough, fever, asthma and dysentery, to cure wounds, inflammatory swellings and rheumatic joints, bronchitis, cough, fever, headache, respiratory ailments and skeleto-muscular problems etc. (Anon 2013; Joshi et al. 2021). In the Philippines, *Justicia gendarussa* Burm.f is used in dislocation of bones and rheumatism (Belgica et al. 2021) (Table 1). Haplophyllum species are used in Iraq, as a salve for wounds (Al-Snafi 2018), as well as *H. tuberculatum* Forssk. (A. Juss.), in Tunisia (Hamrouni et al. 2023), *H. acutifolium* (DC.) G. Don in the Iran (Ghorbani 2005) and *H. padicellatum* Bunge ex Boiss. (Bibi et al. 2014) are used to treat dermal wounds, inflammation, piles and skin diseases (Table 1). *Protium unifoliolatum* Engl. is also part of the plant sources rich in arylnaphthalene lignans which is traditionally used by Yanomami Indians in Brazil to treat congestion and respiratory infections (Milliken and Albert 1997), while the bark of *P. nodulosum* Swart. is applied by the native people of Peru as anti-abortive agent (Jovel et al. 1996).

The taxonomic genus *Linum*, a constituent of the Linaceae family, covers a diverse range of approximately 180 species worldwide. Traditional taxonomy has classified *Linum* into five sections (McDill et al. 2009). Beyond its taxonomic differences, *Linum* has attracted considerable attention for its secondary metabolites, with a predominant focus on lignans. Notably, literature extensively features compounds of the aryltetralin class, such as 6-methoxypodophyllotoxin, derived from various *Linum* species (Alfieri et al. 2021). Data about the traditional use of *Linum* species are insufficient. The most popular plant is flax, *L. usitatissimum* used as a source of fibre and oil (Uğurlu and Dönmez 2012; Bussmann et al. 2016) which has been known since Neolithic time (Zohari 1986). In addition, *L. usitatissimum* is used as a treatment of diabetes type II, namely the leaves of this plant in Pakistan (Zain-ul-Abidin et al. 2018) or the seeds in Morocco (Skalli et al. 2019). Crushed or powdered seeds of *L. usitatissimum* are used to cure pain or treat wounds and the oil from the plant is used for body aches in Iran (Moghanloo et al. 2019). *L. album* Boiss is used to treat gastritis in the Fars Province of Iran (Delfan et al. 2015). *L. pubescens* is applied in skin disorders and prostate disorders in Palestina (Ali-Shtayeh et al. 2000). The seeds of *L. perenne* are used as a cure for urinary tract infections, toothache and high blood pressure in Pakistan (Ali and Qaiser 2009) (Table 1). An overview of the genus reveals that arylnaphthalene lignans, mostly justicidin B and isojusticidin B, are occasionally accumulated in certain *Linum* species, particularly within the *Linum* section (Fig. 1).

This suggests a widespread distribution of arylnaphthalene lignans within this taxonomic subdivision. In contrast, aryltetralin lignans are frequently identified in members of the *Syllinum* section (Konuklugil et al. 2007).

This comprehensive study aims to provide an informed overview of arylnaphthalene lignans within the *Linum* species, shedding light on their distribution and significance within the genus.

Methods of data acquisition

The conducted extensive literature review was by using Scopus, Web of Knowledge, as well as PubMed. The search spanned from 1989 to the present, incorporating diverse combinations of keywords such as "arylnaphthalene lignans", "genus *Linum*", "secondary metabolites", "biotechnology" and "tissue cultures". A thorough examination of more than 140 articles was undertaken to curate this review.

Biological activities

Arylnaphthalene lignans, known for their diverse biological properties, are promising lead compounds in the search for novel therapeutics. Their multifaceted attributes underscore their potential for innovative drug development (Hemmati and Seradj 2016). The activities listed below demonstrate the multifaceted nature of lignans as complex bioactive compounds that exhibit a wide range of biological effects without lacking in specificity.

Cytotoxic activity

Justicidin B exhibited the strongest growth inhibitory effect IC_{50} = 0.20 μg/ml on HeLa cells, with no detectable cytotoxicity observed within the first 28 hours. Another test against cell types L-6 cells and PBMCs justicidin B showed IC_{50} = 3.30 μg/ml (Gertsch et al. 2003). MTT test was carried out comparing justicidin B and etoposide cytotoxicity, which were tested amongst three leukemic cell lines LAMA-84, K-562 and SKW-3 and showed that justicidin B at higher concentrations can inhibit the proliferation of malignant cells in the same manner as etoposide used as

Table 1. Plant sources of arylnaphthalene lignans and arylnaphthalene lignan lactones* with data from ethnobotanical studies.

Species	Compounds	Ethnobotanical and ethnopharmacological data
***Linum* L. Linaceae**		
L. altaicum (Ledeb. ex Juz.)	justicidin B, isojusticidin B (Schmidt et al. 2010)	N. A.
L. austriacum L. Incl. *L. a.* subsp. *glaucescens* (Boiss.) which is a synonym of *L. glaucum.* (Boiss. & Noë)	justicidin B (Schmidt et al. 2010), isojusticidin B, justicidin B (Mascheretti et al. 2021; Mohagheghzadeh et al. 2002)	N. A.
L. leonii (F. W. Schultz)	justicidin B, isojusticidin B (Schmidt et al. 2010)	N. A.
L. lewisii. L. (Pursh)	isojusticidin B secoisolariciresinol 7-*O*-*β*-D-apiofuranosyl-diphyllin (tuberculatin) 7-*O*-*β*-D-xylofuranosyl-(1→5)-*O*-*β*-D-xylofuranosyl-diphyllin 7-*O*-*β*-D-xylofuranosyl-(1→5)-*O*-*β*-D-xylofuranosyl-(1→5)-*O*-*β*-D-glucosyl-diphyllin (Schmidt et al. 2010; Dougué Kentsop et al. 2022)	N. A.
L. narbonense L.	justicidin B (Ionkova et al. 2013)	N. A.
L. perenne L.	justicidin B; isojusticidin B; diphyllin glycosides (Schmidt et al. 2010; Tóth et al. 2023)	Seeds are ground and fried. Then a paste is prepared which is used in urinary tract infections. Seeds are chewed and kept for few minutes in between the jaws for toothache. It is also used for high blood pressure in various preparations. (Ali and Qaiser 2009).
***Cleistanthus* Hook.f. ex Planch.* Phyllanthaceae**		
C. collinus (Roxb.) Benth. ex Hook	N. A.	Snakebite (Prusti and Behera 2007), haematuria (Li and Xing 2016).
C. sumatranus (Miq.) Muell.		
***Phyllanthus* L.* Phyllanthaceae**		
P. oligospermus Hayata	phyllanthusmins A-C, justicidin-A, diphyllin and haplomyrtin, methyl vanillate, vanillin, 3,4-dimethoxybenzoic acid, methyl ferulate (Wu and Wu 2006), phyllanthusmins D-E, phyllanthusmins A-C,	N. A.
Glochidion poilanei (Beille) R.W.Bouman (synonym *P. poilanei* (Beille)	cleistanthin B (Ren et al. 2014, Park et al. 2021) N. A.	Hepatitis (Unander 1998), problems related to stomach, genitourinary system, liver and spleen (Kumar 2020).
P. amarus Schum. and Thonn.	N. A.	Hepatitis, vitiligo, anti-septic, anti-inflammatory, diabetes, wounds, gonorrhea, rheumatic fever (Kumar and Chaturvedi 2010).
***Justicia* L.* Acanthaceae**		
J. adhatoda L.	carinatone, diphyllin, justicidin A, taiwanin E, tuberculatin (Susplugas et al. 2005, Park et al. 2021)	Cough, fever, asthma and dysentery (Anon 2013).
J. gendarussa Burm.f. Acanthaceae	N. A.	Wounds, inflammatory (Joshi et al. 2021) swellings, rheumatic joints, dislocated bones (Belgica et al. 2021).
***Haplophyllum* A. Juss.* Rutaceae**		
H. acutifolium (DC.) G. Don	diphyllin, justicidin A (Park et al. 2021)	Wounds (Al-Snafi 2018; Ghorbani 2005), wounds and inflammation (Hamrouni et al. 2023), wounds, piles, skin diseases (Bibi et al. 2014).
H. tuberculatum Forssk. (A. Juss.)	N. A.	
H. padicellatum Bunge ex Boiss.	N. A.	
H. suaveolens	haploborin, arabelline (Ivanova et al. 2001)	
***Protium* Engl.* Burseraceae**		
P. unifoliolatum Engl.	5-methoxyjusticidin A (Siani et al. 1998)	Respiratory infections (Milliken and Albert 1997), Anti-abortive (Jovel et al. 1996).
P. nodulosum Swart.	N. A.	

N. A. No data available.

a reference (Vasilev et al. 2006). Cytotoxic potential of diphyllin and justicidin B was evaluated on human tumour cell lines (Jurkat, PC-3, HepG2, Colon205) and normal peripheral blood mononuclear cells (PBMCs). None of the two compounds exhibited cytotoxic effects (Rao et al. 2007). There are results demonstrating that justicidin B exerts potent cytotoxic and pro-apoptotic effects against acute myeloid leukaemia HL-60 cells, initiating apoptosis within 12–24 hours through intrinsic mitochondrial cell death-signalling pathways (Momekov et al. 2014). Other studies evaluate the cytotoxic effects of Justicidin B, an arylnaphthalene lignan, on human leukaemia K562 cells. It is shown that justicidin B inhibits K562 cell viability in a dose-dependent manner, with an average IC_{50} 45.40 mM after 48 hours of treatment. Treatment with justicidin B leads to a dose-dependent decrease in superoxide dismutase (SOD) activity in K562 cells, indicating an alteration in redox system homeostasis (Luo et al. 2014). Justicidin B demonstrated potent cytotoxic and pro-apoptotic effects against MDA-MB-231 and MCF-7 breast cancer cell lines (Momekov et al. 2011). Justicidin B exhibits significant cytotoxicity against lymphoma cell lines, particularly the multiple myeloma RPMI-8226 cells, which display heightened sensitivity compared to the approved anti-lymphoma drug, etoposide. Mechanistic insights reveal that justicidin B induces apoptosis through NF-kB

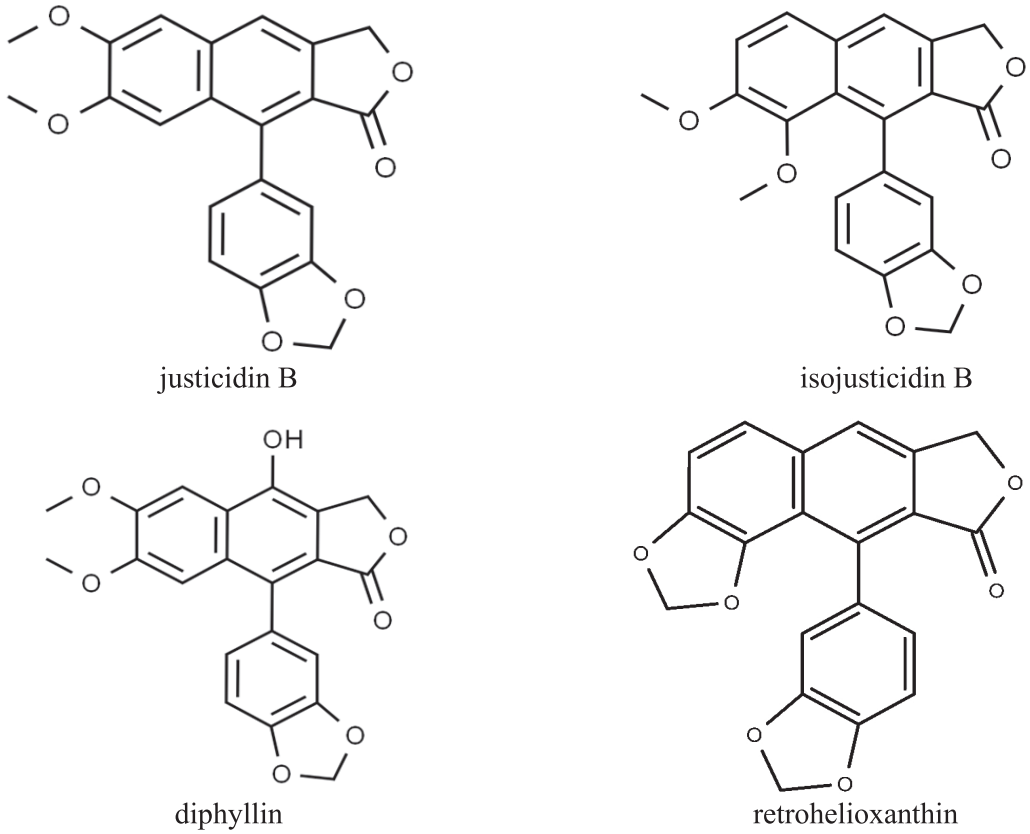

Figure 1. Notable arylnaphthalene lignans, isolated from *Linum* species.

inhibition and caspase activation in susceptible cell lines, highlighting its potential as a therapeutic intervention, especially in non-Hodgkin's lymphomas and multiple myeloma, while demonstrating minimal toxicity in in vivo assessments (Ilieva et al. 2014). The cytotoxicity of diphyllin was tested to LoVo cell line (human colon tumour line) and the IC_{50} = 7.55 ± 0.75 μg/ml (Innocenti et al. 2002). In another study, cytotoxic activity of diphyllin and justicidin B was tested on a BGC-823 cell line, where diphyllin was used as positive control. Justicidin B exhibited greater activity, suggested by the variations in polarity (Jin et al. 2014). Tuberculatin demonstrated heightened cytotoxic effects against several cell lines in comparison to diphyllin. Furthermore, tuberculatin exhibited potent stimulation of the tumour necrosis factor-receptor (TNF-R) generation in lipopolysaccharide (LPS)-stimulated mouse macrophage-like RAW 264.7 cells. (Day et al. 2002).

The potent inhibitor of bone resorption, justicidin B, is being used as a lead compound for new antirheumatic drugs. Justicidin B may have significant clinical utility as a lead compound in the treatment of bone cancer and osteoclastogenesis due to its cytotoxic and bone resorption inhibitory properties (Ilieva et al. 2014).

Antiviral activity

The antiviral efficacy of lignans encompasses a multifaceted array of mechanisms, such as their ability to inhibit reverse transcriptase. At a concentration of 33 μg/ml, justicidin B exhibits a level of inhibition of 8% against anti-HIV-1 RT activity (Chang et al. 1995). The antiviral activity of arylnaphthalene lignans, specifically justicidin B and diphyllin, was investigated through experimentation on virus-infected cells. These lignans exhibited efficacy in significantly decreasing the number of Sindbis virus plaque and, at some point, murine cytomegalovirus (MCMV) (MacRae et al. 1989). A recent study shows that diphyllin and justicidin B exhibited notable efficacy against SARS-CoV-2 at a concentration of 12.5 μM. This highlights their potential as promising candidates for further exploration as antiviral agents (Tóth et al. 2023). Justicidin B and diphyllin display a minimum inhibitory concentration (MIC) ≥ 0.06 μg/ml and 0.25 μg/ml, respectively, against vesicular stomatitis virus C (VSV) (Park et al. 2021). Unfortunately, justicidin B did not demonstrate significant antiviral activity against human cytomegalovirus (Cow et al. 2000).

Anti-inflammatory activity

Justicidin B has exhibited promising potential in another domain, as an anti-inflammatory agent. Since bone destruction is the final stage of rheumatoid arthritis, scientists have used justicidin B, a powerful inhibitor of bone resorption, as a starting point for developing new drugs that can help prevent this process (Apers et al. 2003). Justicidin B is tested as an autoimmune disease-modifying antirheumatic drug for treatment of rheumatoid arthritis. The inhibitory effect evaluated by Raisz's method of the bone resorption of 25 μg/ml solution shows 47%

^{45}Ca release (Baba et al. 1999). Justicidin B and diphyllin exhibit IC_{50} = 12.5 and 50 μM, respectively, for inhibiting NO production from activated peritoneal macrophages. These concentrations also demonstrated a similar inhibition pattern for cytokine production (TNF-alpha and IL-12). Moreover, at a concentration of 100 μM, both justicidin B and diphyllin show 50% inhibition of NO production from peritoneal macrophages pre-activated with LPS/IFN- for 24 hours (Rao et al. 2007).

Antifungal activity

Justicidin B exhibited significant activity against *Fusarium oxysporum*, with an inhibition rate of 67% observed at both MIC and minimum fungicidal concentration (MFC), determined at 8.00 μg/ml (Windayani et al. 2015). Furthermore, Justicidin B shown strong suppression MIC ≥ 1 μg/ml and ≥ 4.00 μg/ml, respectively, against strains of *A. fumigatus and Candida albicans* (Gertsch et al. 2003). Justicidin B is only half as potent as miconazole or amphotericin B against *Aspergillus fumigatus*. Notably, *Cryptococcus neoformans* and *Blastoschizomyces capitatus* have shown resistance to justicidin B, increasing the need for exploring alternative treatments (Gertsch et al. 2003). Justicidin B exhibits robust antifungal activity against *Aspergillus niger* and *Aspergillus flavus* compared to matairesinol (Panagouleas et al. 2003).

Antiprotozoal activity

Justicidin B was tested for antiprotozoal activity. It has shown strong activity against *Trypanosoma brucei rhodesiense* with IC_{50} = 0.20 μg/ml, moderate against *Trypanosoma cruzi* IC_{50} = 2.60 μg/ml, but weak against *Plasmodium falciparum* (IC_{50} ≥ 5.00 μg/ml) (Gertsch et al. 2003).

Antibacterial activity

In literature, there is contradictory information regarding the antibacterial activity of arylnaphthalene lignans. Based on El-Gendy et al. study, the MIC for justicidin B against bacterial species, such as *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *B. subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *M. smegmatis* and *Corynebacterium xerosis* was 2.50, 0.50, 0.20, 2.00, 1.00, 0.50, 5.50 and 7.00 μg/ml, respectively (Apers et al. 2003). However, B. Gertsch et al. reported an absence in the bacterial activity of justicidin B against *B. cereus*, *S. aureus*, *P. aeruginosa* and *E. coli.* (Gertsch et al. 2003).

Antiparasitic activity

The arylnaphthalene lignan which is found to exhibit antiparasitic activity is justicidin B. Justicidin B demonstrated potent activity against the trypomastigote form of *T. brucei rhodesiense*, with an IC_{50} = 0.20 μg/ml, (melarsoprol was used as the positive control IC_{50} = 0.003 μg/ml, moderate activity against *T. cruzi* IC_{50} = 0.20 μg/ml and weak activity as antimalarial because of IC_{50} > 5 μg/ml in comparison to chloroquine IC_{50} = 0.12 μg/ml (Gertsch et al. 2003).

Antiplatelet activity

Justicidin B and diphyllin were tested for their antiplatelet activity. Both compounds did not exhibit higher potency than indomethacin. Although justicidin B IC_{50} = 8.0 ± 1.2 μM is confirmed to have greater antiplatelet activity than that of aspirin IC_{50} = 20.3 ± 2.1 μM (Chang et al. 1995). Justicidin B did not show on adrenaline-induced aggregation in human platelet-rich plasma IC_{50} = 104.8 ± 25.3 μM. This discrepancy may be attributed to the potentially higher binding capacity for plasma (Wu et al. 2007).

In vitro biotechnological methods of production

As previously mentioned, arylnaphthalene lignans exhibit significant efficacy across various domains. Nevertheless, their extraction from field-grown plants is associated with notably diminished yields. A way to overcome these limitations lies in the utilisation of *in vitro* cultures, a strategy that holds promise for enhancing production efficiency. The main advantage of *in vitro* cultivation, compared with traditional plant cultivation, is the possibility of acquiring the desired molecules under controlled conditions, independently of climatic variations and soil characteristics throughout the plant's growth cycle. In literature, there are data for some types of *in vitro* cultures in section *Linum*, for example, *L. austriacum* (Mohagheghzadeh et al. 2002), *L. altaicum* (Konuklugil et al. 2007), *L. narbonense* (Ionkova et al. 2013), *L. leonii* (Schmidt et al. 2007), *L. glaucum* (Mohagheghzadeh et al. 2009), *L. lewisii* (Dougué Kentsop et al. 2022) and *L. perenne* (Jullian-Pawlicki et al. 2015). For *L. lewisii*, a hairy root culture has been developed with the aim to advance the production (Dougué Kentsop et al. 2022), using different type of elicitors in two types of root tissue - hairy root and adventitious root. The yield of justicidin B in the two types of root tissue and with the most effective elicitors methyl jasmonate (MeJA) and coronative (COR) is shown in Table 2. For *L. lewisii*, *L. altaicum* and *L. austriacum ssp. Euxium*, other *in vitro* cultures that were established are suspension, although, the accumulation of compounds of interest is insufficient (Konuklugil et al. 2007). The hairy root cultures of *L. perenne* represent another source of accumulation of arylnaphthalene lignans and a possible route for identifying new compounds, not yet characterised. The studies included identification efforts aimed at determining the exact structures of justicidin and isojusticidin. Significantly, comparable identification data for diphyllin glycosides are lacking and there is an absence of specific information regarding the yield obtained of this research (Jullian-Pawlicki et al. 2015). Another

example for *in vitro* cultures which produce justicidin B are the hairy roots and cell suspension cultures of *L. perenne* cv. Himmelszelt (Hemmati et al. 2007). In a native plant of *L. perenne*, three arylnaphthalene lignans were isolated not only justicidin B and isojusticin B as mentioned above, but also retrohelioxanthin (Fig. 1) and four lignans of the aryldihydronaphthalene type (Schmidt et al. 2007). This expands the range of recognised compounds, contributing to a more comprehensive understanding of the constituents derived from *L. perenne.* In addition, novel arylnaphthalene lignans, named linadiacin A and B, were discovered and identified in the underground parts of *L. austriacum* and *L. perenne*. These previously undescribed compounds contribute to the expanding knowledge of the chemical constituents found in these plant species (Tóth et al. 2023). Mohagheghzadeh et al. (2009) conducted a concurrent assessment of three *Linum* species, namely *L. tenuifolium*, *L. bienne* and *L. glaucum*, regarding the accumulation of arylnaphthalene lignans. The examination encompassed both callus and shoot formation. Notably, successful establishment of *in vitro* cultures was achieved for all three *Linum* species under consideration. However, it is noteworthy that, amongst these, only *L. glaucum* exhibited the identification of a singular arylnaphthalene lignan - justicidin B (Mohagheghzadeh et al. 2009). *L. narbonense* was subject of experiments using alternative propagation methods specifically aimed at increasing the yield of justicidin B. Four different cell culture types, including callus, suspension and two hairy root variants, were systematically developed both in 300 ml flasks, as well as in 2 litre bioreactors. The goal of this effort was to recognise the optimal methodology that would result in the highest production of justicidin B. Table 2 provides accurate amounts for the four-week cultivation period and the potential for using this *Linum* species for justicidin B production (Ionkova et al. 2013). Cell cultures, including callus and hairy roots, have been established for *L. leonii*. This innovative approach holds considerable promise as an alternative and efficient means of justicidin B production using the unique advantages presented by these cultured entities (Vasilev et al. 2006). Diverse *in vitro* cell cultures, including calluses, cell suspension in two different types of media, normal roots and hairy roots, have been generated from *L. austriacum*, reflecting a considerable interest in optimising the production of justicidin B. This comprehensive investigation has facilitated the identification and quantification not only of justicidin B, but also of isojusticidin B, thereby advancing understanding of the complete lignan profile within the context of *L. austriacum* cell cultures (Mohagheghzadeh et al. 2002). In the pursuit of enhanced justicidin B production, the pivotal role of elicitors becomes apparent, with MeJA and COR identified as compounds of particular significance (Mascheretti et al. 2021). The outcomes of the study reveal noteworthy improvements in the lignan content, with COR-treated hairy roots exhibiting a 1.5-fold increase, while callus and adventitious roots demonstrated a two-fold augmentation in lignan content. Additionally, hairy roots samples, subjected to MeJA elicitation, displayed apparent increase. These findings underscore the potential of elicitation strategies

Table 2. Arylnaphthalene lignans from *in vitro* cultures of *Linum* species.

***Linum* species**	**Compounds**	**Type of *in vitro* culture [Yield]**
L. lewisii	justicidin B	CSC [0.16–0.30% DW] (Konuklugil et al. 2007), ARC [N. A.], HRC [N. A.] (Dougué Kentsop et al. 2022)
	isojusticidin B, secoisolariciresinol, 7-*O*-*β*-D-apiofuranosyl-diphyllin (tuberculatin), 7-*O*-*β*-D-xylofuranosyl-(1→5)-*O*-*β*-D-xylofuranosyl-diphyllin, 7-*O*-*β*-D-xylofuranosyl-(1→5)-*O*-*β*-D-xylofuranosyl-(1→5)-*O*-*β*-D-glucosyl-diphyllin	CSC [N. A.] (Konuklugil et al. 2007), ARC [N. A.], HRC [N. A.] (Dougué Kentsop et al. 2022)
L. perenne	justicidin B, isojusticidin B, diphyllin glycosides	CSC, HRC [N. A.] (Jullian-Pawlicki et al. 2015)
L. glaucum	justicidin B	CC, shoots [N. A.] (Mohagheghzadeh et al. 2009)
L. altaicum	justicidin B, isojusticidin B	CSC [0.92–0.96% DW] (Konuklugil et al. 2007)
L. narbonense	justicidin B	CC [1.57 mg/g DW], CSC [0.09 mg/g DW], HRC in flasks [7.78 mg/g DW], HRC in bioreactor [7.89 mg/g DW] (Ionkova et al. 2013)
L. leonii	justicidin B, isojusticidin B	CC [2.01 mg/g DW], HRC [10.80 mg/g DW], CC HRC [N. A.] (Vasilev et al. 2006)
L. austriacum	justicidin B	CC [2.90 mg/g DW], CSC [1.80 mg/g DW], CSC without coconut water [6.70 mg/g DW], NRC [12.50 mg/g DW] (Mohagheghzadeh et al. 2002), ARC [3.94 mg/g DW], COR [15.74 mg/g DW], HRC [16.90 mg/g DW (4 weeks); 4.89 mg/g DW (3 weeks)], MeJa [14.71 mg/g DW], HRC in bioreactor [21.30 mg/g DW] (Mascheretti et al. 2021)
	isojusticidin B	CC [0.4 mg/g DW], CSC [0.4 mg/g DW], CSC without coconut water [1.3 mg/g DW], NRC [7.4 mg/g DW], HRC [2.5 mg/g DW] (Mohagheghzadeh et al. 2002)
L. austriacum ssp. *euxinum*	isojusticidin B	CSC [0.50–0.96% DW] (Konuklugil et al. 2007)

CC – callus culture; CSC - cell suspension culture; NRC - normal roots culture; HRC - hairy root culture; ARC – adventitious roots culture; Ctrl – control; COR – coronatine; MeJa – methyl jasmonate; N. A. No data available.

to significantly influence lignans yield in *L. austriacum* cell cultures, contributing valuable insights for the advancement of justicidin B production methodologies.

Methods of analysis and identification of arylnaphthalene lignans

In the extraction process for *L. lewisii*, 80% (v/v) ethanol was used and the subsequent analysis involved Thin Layer Chromatography (TLC) along with High-Performance Liquid Chromatography (HPLC) and purification procedures described by Mascheretti et al. (2021). Structural characterisation of the lignans was accomplished through Nuclear Magnetic Resonance (NMR) (Dougué Kentsop et al. 2022). Beyond the previously identified justicidin B and isojusticidin B, *L. lewisii* revealed the presence of 7-*O*-D-apiofuranosyl-diphyllin (tuberculatin) and, notably, two novel glycosylated diphyllin compounds were elucidated: 7-*O*-D-xylofuranosyl-(1→5)-*O*-D-xylofuranosyldiphyllin and 7-*O*-D-xylofuranosyl-(1→5)-*O*-D-xylofuranosyl-(1→5)-*O*-D-glucosyl diphyllin (Konuklugil et al. 2007). Similarly, in *L. altaicum*, TLC and HPLC methods were employed to confirm the presence of arylnaphthalene lignans. Structural confirmation of justicidin B and isojusticidin B was achieved through ^{1}H-NMR and Gas Chromatography-Mass Spectrometry (GC-MS) (Konuklugil et al. 2007). For *L. perenne*, purification involved semi-preparative HPLC and the High-Resolution Mass Spectrometry (HR-MS) spectra of purified lignans were acquired using a QToF instrument. Electrospray ionisation (ESI)-MS/MS was employed for structural elucidation, revealing the presence of various arylnaphthalene lignans, including two previously unknown compounds: diphyllin-3-pentose and diphyllin-2-hexose. The exact structure of these compounds remains undetermined, warranting further investigation. In the case of *L. perenne* cv. Himmelszelt, NMR analysis was utilised for structural identification, revealing the presence of justicidin B, diphyllin and a novel diphyllin glycoside - diphyllin-7-(2-*β*-xylopyranosid)-*β*-apiofuranoside, known as majidine (Hemmati et al. 2007). Analysis of *L. glaucum* involved HPLC, confirming the presence of justicidin B, while *L. altaicum* underwent extraction and purification using methanol (MeOH), with HPLC used for both structural identification and quantitative analysis (Mohagheghzadeh et al. 2009). For *L. leonii*, preparative TLC was employed for isolation, followed by analysis through GS-MS and NMR. Further experiments are deemed necessary to distinguish between justicidin B and isojusticidin B (Schmidt et al. 2007). *L. austriacum*, extensively studied within the *Linum* genus, was initially described for the presence of arylnaphthalene lignans in 2002, identifying justicidin B and isojusticidin B (Mohagheghzadeh et al. 2002). Subsequent analysis in 2021 revealed another unidentified molecule, consistent across *in vitro* cultures. Preliminary identification was conducted through HPLC, with ^{13}C NMR analysis utilised due to the structural similarities. Quantitative analysis was also performed using HPLC. The complexity of *L. austriacum* and the continual discovery of novel lignan structures underscore the need for ongoing research in this genus (Mascheretti et al. 2021).

Conclusions

The observed cytotoxic and apoptotic effects of justicidin B on K562 cells highlight its potential therapeutic relevance in leukaemia treatment, emphasising its distinct impact on NFkB expression, aligning with established antileukaemic and pro-apoptotic activities. Despite the identification of numerous lignans from *in vitro* cultures, the lack of precise structural determination imposes further investigation to enhance our understanding. This underscores the need for continued research efforts to elucidate the exact structures of these lignans, contributing to a more comprehensive knowledge base. Considering the potential of *L. leonii* hairy roots as an alternative production system for justicidin B, the optimization of these roots holds significance. Such optimisation not only offers a promising avenue for increased justicidin B production, but also serves as a potential model for the development of novel therapeutic agents, emphasising the relevance of ongoing research in this domain.

Despite significant advances in the isolation and characterisation of many arylnaphthalene lignans over the past decade, considerable work remains to be done to explore the full potential of this class of secondary metabolites. Given that these compounds are often produced in limited quantities by plants, the development of *in vitro* culture production methods to facilitate further bioactivity studies is a major challenge for future research. Thus, the genus *Linum* could be considered as a potential source of pharmacologically important metabolites, as well as responsive to *in vitro* biotechnology approaches which could increase the yield and variety of arylnaphthalene derivatives. Solving this challenge will be essential to discovering the vast resources of bioactive compounds contained in lignans.

As a strong inhibitor of bone resorption and a cytotoxic agent, this arylnaphthalene lignan can be used as a molecule to create agents to combat bone metastasis. For this reason, the search for new sources of arylnaphthalene-type lignans (justicidins) is of considerable medical interest. A sustainable biotechnological supply of this valuable lignan would be a highly efficient, economically feasible and, most importantly, socially significant alternative for human health.

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