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REVIEW

Lignan Enhancement: An Updated Review on the Significance of Lignan and Its Improved Production in Crop Plants

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ABSTRACT

Lignans are a prominent group of phenolic compounds ubiquitously present in the plant kingdom, playing a critical role in both plant physiology and human health. Structurally they are characterized by the dimerization of two phenylpropane units to attain diverse chemical configurations that contribute to their wide range of biological activities. In plants, lignans function primarily as defense molecules, protecting against pathogens, herbivores, and environmental stressors. These compounds also participate in plant growth regulation and lignification processes. From a nutritional and medicinal perspective, lignans are valued for their significant health benefits. They act as phytoestrogens, which can modulate estrogen receptors in humans, thereby offering protective effects against hormone-related diseases such as breast cancer and menopausal symptoms. Additionally, lignans possess potent antioxidant and anti-inflammatory properties, contributing to cardiovascular health and cancer prevention. This review aims to provide a comprehensive overview of the importance of lignans in plants, highlighting their chemical diversity, health-promoting properties and stress adaptation. By synthesizing recent research findings, this review underscores the significance of lignans in enhancing plant resilience under stress and human health. It explores their potential applications in the food and pharmaceutical industries. Besides, it will also focus on the widely used method for enhancing the production of these high-value secondary metabolites in plant cell cultures to meet their requirement in pharmaceutical industries.

KEYWORDS

Abiotic stress; antioxidants; biotic stress; cancer; elicitor; lignan

List of Abbreviations

AG	Astragalosides
AHP	Anhydropodorhizol
ALA	α-linolenic acid
ANHSEC	Anhydrosecoisolariciresinol
BA	Betulinic acid
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene

CA	Cinnamic acid
CAD	Cinnamyl alcohol dehydrogenase
CAT	Catalase
CCR	Cinnamoyl-CoA reductase
CHD	Coronary heart disease
DCG	Dehydrodiconiferyl alcohol glucoside
DOP	Deoxypodophyllotoxin
END	Enterodiol
ENL	Enterolactone
GGCG	Guaiacylglycerol-β-coniferyl alcohol ether glucoside
HPH	Hypophyllanthin
HSQC	Heteronuclear Single Quantum Coherence
LARI	Lariciresinol
LDG	Lariciresinol diglucoside
MAT	Matairesinol
MED	Medioresinol
MeJa	Methyl jasmonate
MPTOX	Methoxypodophyllotoxin
NAA	Naphthaleneacetic acid
NH	Niranthin
OA	Oleanolic acid
PA	Phenylalanine
PAL	Phenylalanine ammonio-lyase
PCBER	Phenylcoumaran benzylic ether reductase
PDDG	Pinoresinol di-β-d-glucoside
PDG	Pinoresinol-β-d-glucoside
PH	Phyllanthin
PINO	Pinoresinol
PT	Phyltetralin
PTOX	Podophyllotoxin
ROS	Reactive oxygen species
SDG	Secoisolariciresinol diglucoside
SECO	Secoisolariciresinol
Ses	Sesamin
SOD	Superoxide dismutase
Syr	Syringaresinol
UA	Ursolic acid
WIKS	Wikstromol

1 Introduction

Lignans are secondary metabolites formed through the oxidative coupling of phenylpropanoid units [1]. These naturally occurring compounds are primarily found in plants, often as glycosides or oligomers [2]. Lignans are widespread across various plant species and possess several pharmacological properties, including antiviral, antimicrobial, antioxidant, antifungal, antifeeding, and insecticidal activities [2,3]. In plants, lignans serve a critical function in protecting against pests and pathogens while also supporting the plant's overall growth and development. Over the years, hundreds of lignans have been identified in

numerous plant species, and some of these compounds are converted by gut microbiota into enterolignans-such as enterolactone and enterodiol-which exhibit various beneficial biological effects, including antitumor, anti-estrogenic, and antioxidant activities [4].

Early research focused mainly on two lignans, secoisolariciresinol (SECO) and matairesinol (MAT), which were known to be metabolized into enterolignans. However, recent studies have expanded this understanding to include secoisolariciresinol diglucoside (SDG), the most prevalent lignan found in linseed. The gut microbiota also converts SDG into enterodiol and enterolactone. Asia, Europe, and the Mediterranean cultivate linseed, a herb particularly rich in SDG [5,6]. SDG is essentially the glycosylated form of SECO [7]. The pharmacological properties and commercial use of linseed are due to its high α-linolenic acid (ALA) content and a range of bioactive compounds, including lignan [8]. Lignans are also present in other foods, including grains, seeds, fruits, vegetables, coffee, wine, and tea. New enterolignan precursors such as arctigenin, lariciresinol (LARI), 7-hydroxy-matairesinol (MAT), syringaresinol, and pinoresinol (PINO) have also been recently discovered [9].

The concentration and bioactivity of lignans are influenced by several factors, including plant species, environmental conditions, and the stage of ripening. Environmental elements such as soil composition, rainfall, UV exposure, pathogenic infections, air pollution, mechanical injury, and temperature extremes can all impact the polyphenol content in plants. Additionally, cultivation methods such as greenhouse, field, organic, or biological practices can also affect lignan production. Ripening stages significantly influence the concentration of phenolic compounds and anthocyanins. Beyond their role in plant defense, lignans contribute to abiotic stress tolerance and improve the nutritional quality of plant-based foods, highlighting their importance in both plant physiology and pharmacology [10].

Given the growing global population and the increasing need for crops that are both resilient to environmental stress and capable of improving human health, enhancing lignan production has become a priority [11]. However, extracting lignans can be challenging due to the complexity and variability in the structures and polarities of phenolic compounds. To improve extraction efficiency, methods that increase surface area, such as reducing sample particle size, are often employed. Since lignan concentrations in plants are typically low, which may not meet the demand in pharmacological applications, strategies like biotic and abiotic elicitation, as well as genetic engineering, are being explored to enhance lignan biosynthesis in plants [7,12,13].

This review aims to summarize recent progress in understanding the sources, types, and significance of lignans in crop plants. We will discuss the biological roles of lignans in plant growth under stress and their potential therapeutic applications in human health. Additionally, we will evaluate current biotechnological approaches to increase lignan production and explore future research directions aimed at optimizing lignan content. Ultimately, this review seeks to highlight the potential of enhancing lignan production as a strategy for improving agricultural sustainability, crop resilience, and human nutrition.

2 Structure, Synthesis, Sources, and Type of Lignans

2.1 Structure and Synthesis

Plant lignans are diphenolic compound groups made up of coniferyl alcohol precursors. Based on the cyclization pattern and oxygen incorporation, lignans are classified into dibenzylbutyrolactone, dibenzocyclooctadiene, dibenzylbutane, dibenzylbutyrolactol, aryltetralin, arylnaphthalene, furofuran, and furan (Fig. 1a–g). Synthesizing these lignans results in several sub-classes, among which artigenin, sesamin, enterolactone, medioresinol, SECO, MAT, LARI, and PINO are major lignans exhibiting various pharmacological properties [14,15]. A complete detail of the synthesis can be found in the review by Dar et al. [16].

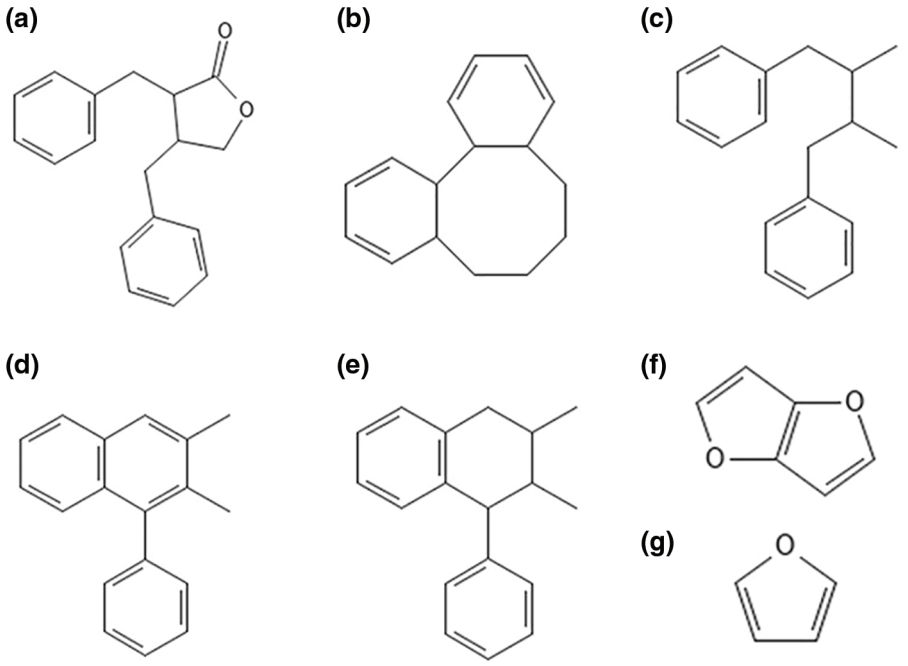

Figure 1: Structure of different types of lignans (a) dibenzylbutyrolactone, (b) dibenzocyclooctadiene, (c) dibenzylbutane, (d) aryltetralin, (e) arylnaphthalene, (f) furofuran, (g) furan

2.2 Types and Sources

Being a natural product, lignans are widely available in several species of plants. Motyka et al. [17] studied the podophyllotoxin (PTOX) belonging to the aryltetralin lactone lignan. Other lignans exhibiting similarity in structure with human estrogens were used as the cancer-preventing substance [18]. Several food sources of lignan are oilseeds and nuts, fruits, non-alcoholic beverages, alcoholic beverages, vegetables, and cereals, which are shown in Fig. 2. Gupta et al. [19] studied the presence of PTOX in the *Podophyllum hexandrum* Royle, which is a Himalayan native plant. They found a rich PTOX content in their rhizomes and roots. But owing to their overuse, they have been declared as endangered species. Various trials have been carried out for a constant supply of high-yielding clones of PTOX.

PTOX accumulates in various plant species belonging to Linaceae, Cupressaceae, Lamiaceae, and Podophyllaceae [20].

Anjum et al. [9] studied the lignan accumulation in *Linum* species and found that the commercial *Linum usitatissimum*, a fiber-rich species, accumulated the PTOX. But they are in small quantities and not suitable for production on a large scale. Several other studies on the plant culture were carried out with *Linum nodiflorum*, *Linum flavum*, and *Linum album* for lignan accumulation. PTOX accumulation was observed mainly in *L. album*, and the accumulation of 6-methoxypodophyllotoxin (6MPTOX) was observed in *L. nodiflorum* and *L. flavum* species. To optimize the cell culture further, the addition of phytohormones was carried out in the medium of growth. Experiments revealed that the Murashige & Skoog medium, when supplemented with a calculated amount of naphthaleneacetic acid and sucrose, was an optimal growth medium for Linum suspensions. The accumulation rate of PTOX in these media is found to be lesser than that of *P. hexandrum* root or rhizomes. However, the biomass generation of *Linum* is comparatively higher than the *Podophyllum* species. The cell culture of *L. nodiflorum* resulted in a higher accumulation of 6MPTOX than that of the *P. peltatum* roots and rhizomes. Various studies were carried out using various elicitors for inducing a defense reaction that increases the lignan accumulation [21].

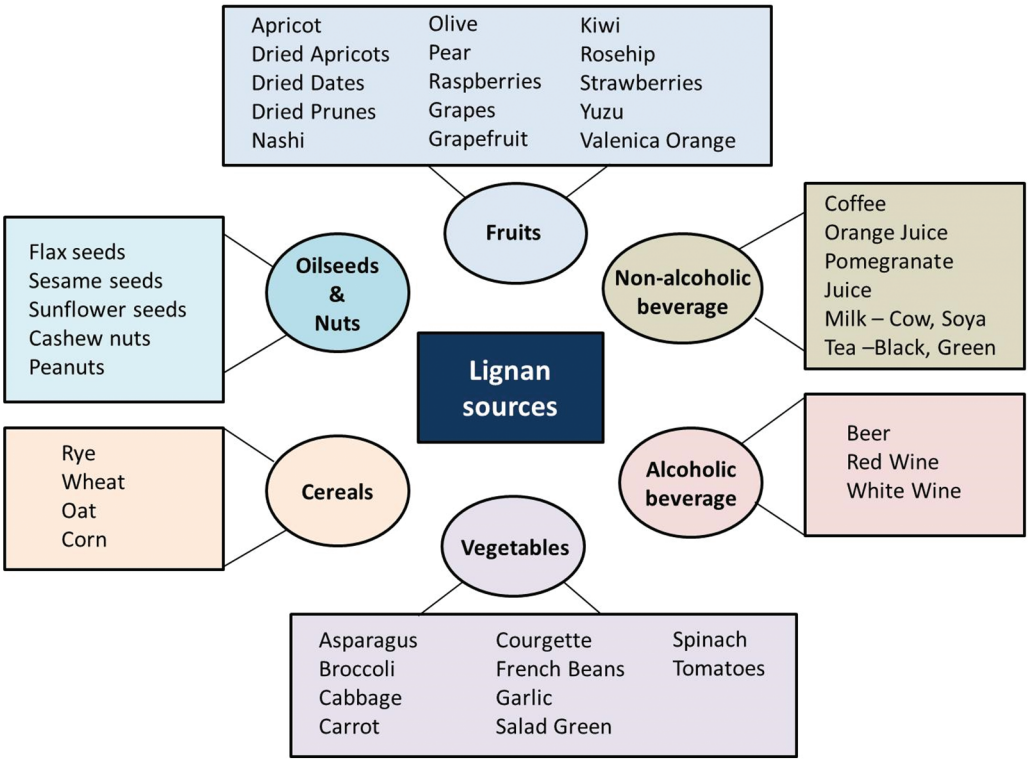

Figure 2: Schematic representation of various sources of lignan. The major sources of lignan are fruits, cereals, vegetables, oilseeds, nuts, and alcoholic, and non-alcoholic beverages

Doussot et al. [22] investigated lignans such as deoxypodophyllotoxin (DOP) and 6MPTOX contents in plant species *L. flavum*, *Juniperus*, and *Callitris*. In addition to these lignans, new types of lignans such as MAT, PTOX, PTOX glucoside, SECO glucoside, and yatein were also found to be present in *Callitris* and *Junipers*. Koulman et al. [23] proposed a fast procedure for the extraction of lignans present in *Anthriscus sylvestris*. In their method, they separated lignans such as DOP, anhydropodorhizol (AHP), and yatein in large concentration variations from the species *Anthriscus sylvestris* growing at several geographical locations.

Podophyllum peltatum L., commonly known as Himalayan Mayapple, is a major source of PTOX. Tashackori et al. [24] reported the presence of PTOX in *Podophyllum peltatum* L. and enhanced its production using a fungal elicitor. Szopa et al. [25] observed lignan accumulation in *Schisandra chinensis* through high-performance liquid chromatography (HPLC). They found that Schisandrol A and B were present in different concentrations in the fruits and leaves of *Schisandra chinensis*. Nikule et al. [26] identified polyphenolic lignans in *Phyllanthus tenellus* using HPLC analysis. They detected phyllanthin (PH), niranthin (NH), phyltetralin (PT), and hypophyllanthin (HPH) in varying concentrations. The lignan profile of *Linum tauricum* and the influence of AM bacterial strain were studied by Ionkova et al. [27], who observed the accumulation of 4-demethyl-6-methoxypodophylotoxin (4DMMPTOX) and 6MPTOX through hairy root culture of the species.

Kancharla et al. [28] investigated the lignan content accumulation in S*esamum indicum*, where the major lignans were sesamolin and sesamin. Other lignans, such as sesaminol, PINO, sesamol, LARI, MAT, piperitol, episesamin, and samin, were found in lower concentrations with some of them formed by transformation during the processing of oil and seeds. Andargie et al. [29] studied the production of

lignans in *Daphne odora* cell culture and callus. The lignans such as LARI, PINO, SECO, MAT, and wikstromol (WIKS) were produced through cell suspension culture. The different types of lignan are reported in various crops, fruits, nuts, and vegetables [7]: however, the level of lignan ingestion and its bioavailability, depend on the type of diet consumed, which can be variable.

3 Lignan and Human Health (Food and Pharmaceutical Importance)

3.1 Lignan in the Food Industry

The increasing prevalence of diseases linked to unhealthy lifestyles has driven a growing demand for healthy foods and nutritional supplements, raising awareness of the significant benefits of lignans. The global lignan market is projected to surpass $90 million by 2026 [30]. Currently, efforts are underway to determine lignan content based on specific genotypes for biofortification breeding and the development of functional foods [31]. In recent years, new plant varieties have been bred to enhance lignan content.

There is increasing interest in lignan-containing materials within the food industry, with primary sources including oilseeds, cereals, legumes, vegetables, and fruits [7] (Fig. 2). Among oilseeds, sesame seeds (*Sesamum indicum* L.), which contain sesaminol have the highest lignan content after flaxseed (*Linum usitatissimum* L.), while cashew nuts (*Anacardium occidentale L.*) also provide a relatively rich source of lignans. In cereals, lignans are predominantly found in the outer layers of the grain, with significantly lower concentrations in the endosperm. Some of the crops richest in lignans include rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa L.*), and triticale (×Triticosecale), while lower concentrations are found in other grains [2]. Lignans can improve the nutritional value of foods such as cereals, breads, and dairy products. Flaxseed, a potent source of lignan, is frequently incorporated into products to promote cardiac health [5]. Lignan bioavailability varies on its method of consumption. A study involving healthy adults who consumed whole, crushed, and ground flaxseed (0.3 g/kg body weight) for 10 days revealed that whole flaxseed significantly increased the bioavailability of enterolignans by 28% and crushed flaxseed by 43% compared to control [32]. Sprouting flaxseed enhanced both lignan production and mineral content [33]. Lignan mitigates the oxidation of lipids and oils, thereby prolonging the shelf life of items such as sauces, baked goods, and munchies [34]. A significant milestone has been achieved in the development of solin flax (edible oil) through mutagenesis, resulting in a point mutation in the *LuFAD3A* and *LuFAD3B* genes, which encode microsomal desaturases. This advancement has broadened the applications of linseed oil for edible purposes [35].

3.2 Lignan and Pharmaceutical Importance

3.2.1 Lignan in the Prevention of Cancer

Lignans offer notable health benefits to humans, particularly in the treatment of chronic diseases such as cancer. Due to their phenolic structure, lignans possess strong antioxidant properties, making them highly effective in combating breast and prostate cancers. Teodor et al. [36] reviewed the biological effects of lignans derived from medicinal plants in humans. They were involved in the prevention of cancer and have antioxidant, anticarcinogenic, antimutagenic, and anti-estrogenic effects.

The role of lignans in human health is mainly in reducing the risk of colon cancer, breast cancer, prostate cancer, endometrial cancer, and cardiovascular diseases. The gut flora present in the human digestive system converts plant lignans into enterolignans, which are proactive against chronic diseases and reduce the risk of tumors [37]. Podophyllotoxin is one of the most notable lignans, with its semi-synthetic derivatives showing significant pharmacological activities, including antineoplastic properties. Derivatives such as etoposide and teniposide are used to treat testicular cancers and acute lymphoblastic leukemia, respectively [38]. Lignans are being investigated for their potential function in the prevention and treatment of hormone-dependent malignancies, with a particular emphasis on breast cancer, because of their estrogen-modulating effects [4]. Lignans, particularly those made from flaxseeds, have demonstrated potential in inhibiting the

proliferation of prostate cancer cells by regulating hormone metabolism and generating anti-inflammatory effects [39].

3.2.2 Lignan in Cardiovascular Disease Treatment

Lignans are known to reduce the risk of cardiovascular diseases. Taulabi et al. [40] showed the influence of flaxseed lignan in lowering blood pressure and reducing cardiovascular disease risks. The high content of alpha-linolenic acid and omega-3 fatty acids helps lower total cholesterol levels and supports weight loss. Research suggests that consuming flaxseed powder for 12 weeks can result in reductions in blood pressure, total cholesterol, and body mass index (BMI). Furthermore, higher doses of flaxseed and prolonged consumption may be even more effective in reducing certain cardiovascular risk factors. Nutraceuticals: Lignan-rich supplements are marketed based on their potential to reduce the risk of cardiovascular diseases and hormone-related diseases (e.g., breast cancer and prostate cancer) [41].

Neolignans and lignans can reduce cholesterol levels in plasma, lowering the risk of developing cardiovascular disease [42] and preventing hypertension [43]. A higher long-term intake of lignans has been linked to a notably reduced risk of total coronary heart disease (CHD) in both men and women. There may be synergistic effects between lignan and fiber consumption in reducing CHD risk, potentially by boosting the production of enterolignans [44].

3.2.3 Lignan and Diabetes

Researchers also identified lignans as antidiabetic compounds, with SDG from flax seeds being the most prominent antidiabetic lignan [45]. Furthermore, studies have shown that regular consumption of products enriched with SDG lowers C-reactive protein levels. Dietary lignan intake, evaluated through a 7-day diet record, was linked to reduced HbA1c levels (with percentage changes ranging from −0.92% to 1.50%), along with decreased C-reactive protein levels and improved lipid profiles. Thus, long-term lignan consumption was associated with a reduced risk of type 2 diabetes, especially in individuals with obesity and premenopausal women [46]. Lignans may exhibit advantageous effects in the management of type 2 diabetes by enhancing insulin sensitivity and reducing blood glucose levels [47].

3.2.4 Other Miscellaneous Roles of Lignan

Lignans are recognized as phytoestrogens due to their structural similarity to steroids, exhibiting both estrogenic and antiestrogenic properties. Research has indicated that these phytoestrogens can alleviate menopausal symptoms, such as osteoporosis, climacteric symptoms, and estrogen-dependent cancers in postmenopausal women [48]. Enterolignans, which are metabolites of lignans, can bind to estrogen receptors alpha and beta, which are present in various tissues. Although lignans have been reported to weakly inhibit aromatase, also known as estrogen synthetases or synthases, which are enzymes crucial for estrogen biosynthesis [49], they may also inhibit 5α-reductase enzymes and stimulate the production of sex hormone-binding globulin [50]. Some lignans, like LARI, MAT, SECO, PINO, and nortrachelogenin, are excellent at fighting free radicals, which makes them useful for both prevention and treatment [51]. Lignans have the potential to promote neuroprotection by crossing the blood-brain barrier and exhibiting antioxidant and anti-inflammatory responses. This enables the creation of lignan-based remedies for neurodegenerative diseases such as Parkinson's and Alzheimer's [52,53]. Lignans have demonstrated antiviral properties in numerous studies, which has sparked interest in their potential as a source of infection-fighting compounds [54]. Lignans have the potential to function as potent antioxidants, effectively neutralizing destructive free radicals that can cause cellular damage. This property renders them functional in the development of neuroprotective, anti-inflammatory, and anti-aging medications [4]. The Fig. 3 provides a quick overview of lignan's role in disease, infection, obesity, and stress.

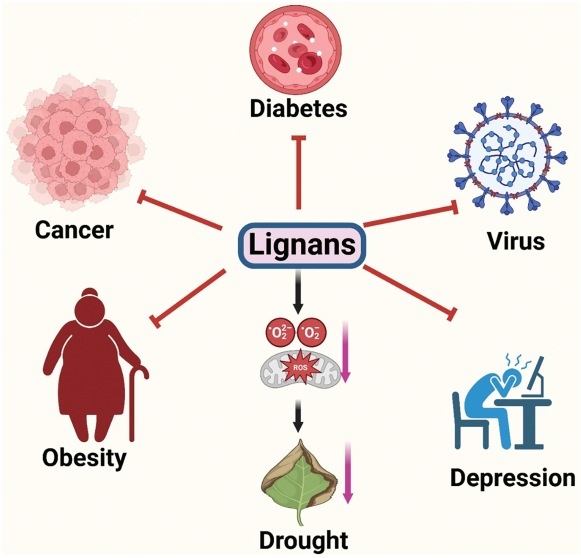

Figure 3: Lignan and its impact on plants and humans. The above figure shows the deleterious impact of lignan deficiency on human mental and physical health and in plants in the excessive ROS generation and visible stress symptoms

4 Lignan and Abiotic/Biotic Stress Tolerance

Lignans are a potent weapon that plants use to withstand stress [1]. SECO, PINO, MAT, sesamin (Ses), syringaresinol (Syr), and LARI are the seven major types of lignans, which possess antibacterial and antifungal properties [4]. Plants, when confronted with stress, adapt to it by altering the gene expression of proteins that regulate the biosynthesis of metabolites, which are critical for interactions between the plant and its environment. Polyphenols represent a key category of specialized metabolites that are essential for many physiological processes in a plant's life cycle, particularly in stress responses. Adverse environmental conditions such as drought, extreme temperatures, salinity, heavy metal exposure, and ultraviolet radiation trigger the phenylpropanoid biosynthetic pathway, leading to the accumulation of diverse phenolic compounds [55]. Plants cope with stress either through mechanical or chemical defense. The first line of defense is the mechanical defense, and lignan can contribute to the plant's defense by being incorporated into cell walls, making them slightly more resistant to degradation and reinforcing the plant's structural barriers against pathogens and herbivores. The xylem tissue of linseed contains lignan, which makes the stem stiff and strong and lowers the permeability of the cell wall to prevent pathogens from entering [56].

The other mode of defense includes the chemical defense in which secondary metabolites play an essential role. In response to environmental stress signals, plants can resist stress by modulating the accumulation of secondary metabolites [57]. Plant phenolics, or polyphenols, are widely distributed secondary metabolites that are derivatives of the shikimate, phenylpropanoid, and pentose pathways [58]. The precursor of the shikimate pathway, shikimic acid is generated via erythrose 4-phosphate (pentose phosphate pathway) combined with phosphoenol pyruvate (glycolytic pathway). Phenylalanine obtained from the shikimate/phenylpropanoid pathway leads to synthesizing other phenylpropanoid metabolites [59]. Several other metabolites, such as flavonoids, lignans, lignins, tannins, etc., are formed through this pathway [58,60]. Therefore, the phenylpropanoid pathway serves as a boundary of carbon metabolic flux of primary metabolites into secondary metabolites [61].

Lignan synthesized via the phenylpropanoid pathway is structurally analogous to lignin, a molecule that fortifies cell walls [62]. Many lignans are naturally harmful to pathogens. They can damage the membranes of invading microorganisms or interfere with their metabolic processes to provide a direct antimicrobial effect [63]. Some lignans serve as precursors of signaling molecules essential for immunological responses. They can modulate the concentrations of reactive oxygen species and other defense-related hormones, optimizing the immune response to avert excessive damage to plant tissues [64]. Lignans play a crucial role in plant immunity by increasing structural integrity and biochemical resistance [65].

Initially, *in vitro* studies demonstrated the potential of the lignans Ses, asarin, and PINO to combat insect stress [66]. Researchers found that SDG can raise the levels of catalase (CAT) and superoxide dismutase (SOD), which are involved in free radical scavenging [4]. Lignans are reported to have an important role in helping plants cope with biotic and abiotic stresses during growth and development [67]. The antioxidant, antiviral, antibacterial, and antifungal properties of lignans are responsible for their plant defense mechanisms [68]. Wan et al. [69] revealed that lignan possesses antifungal properties in sesame tissue extracts. Similarly, Li et al. [70] reported the insecticidal properties of furofuran lignans derived from the Himalayan shrub lopseed (*Phryma leptostachya* L.). Lignans function as antioxidants that can scavenge ROS, which typically accumulate excessively under stressful conditions [21]. Drought stress may alter the concentration of lignans in the primary sesame (*Sesamum indicum* L.). More drought-tolerant genotypes exhibited elevated levels of sesamin and sesamolin [71]. Phenolic compounds have been shown to play a beneficial role in protecting living organisms from various oxidative stressors. They are crucial for maintaining redox homeostasis and offer potential strategies for improving plant resistance to stress [72]. Lipid peroxidation is a damaging oxidative process that alters membrane structures, thereby affecting their biological functions. Lignans can protect membranes from this damage by scavenging free radicals [73].

It has been shown that increasing LARI biosynthesis in *Isatis indigotica*, especially in tetraploids compared to diploids, helped roots grow and made the plants better able to handle salt and drought stress. A key enzyme in lignan biosynthesis, 4-coumarate A ligase 3 (Ii4CL3), is activated by the transcription factor IiWRKY34. Drought stress may change the level of lignans in the main sesame, depending on genotypes. More drought-tolerant genotypes exhibited higher levels of sesamin and/or sesamolin [74].

Dirigent (DIR) proteins have been shown to mediate the biosynthesis of lignans and lignin in plants under stress, indicating their role in adaptive plant responses [75]. The expression of *IiWRKY34* is positively associated with LARI accumulation and the enhanced stress resistance observed in these plants [76]. Analysis of the expression patterns of selected DIR genes in flax revealed the involvement of specific DIRs in the formation of (−)-PINO in seed coats and (+)-PINO in vegetative tissues, as well as in response to hormonal or fungal elicitor stress [76]. Lignan biosynthesis is also stimulated by stress hormones, such as jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) [21].

The expression of genes involved in the phenylpropanoid pathway, which is essential for the synthesis of lignans, is frequently altered by abiotic stress [77]. Phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL) are among the critical enzymes in this pathway that are upregulated in response to stress [78]. This ultimately results in a higher production of lignan precursors, such as coniferyl alcohol, which is subsequently converted into lignans through the phenylpropanoid pathway [79]. Lignans play protective roles in plants, particularly during abiotic stress. Two flax varieties, Flanders and Astella, were compared for their salt tolerance ability based on lignan accumulation by Debnath et al. [80]. It was observed that Flander exhibited a greater up-regulation of antioxidants and osmoprotective mechanisms, indicating its superior ability to scavenge ROS and protect cells compared to Astella. Additionally, the two-tailed qPCR analysis revealed a more pronounced upregulation of three miRNAs associated with lignan biosynthesis: *miR168a*, *miR399g*, and *miR828a*.

Phenylcoumaran benzylic ether reductase (*PtPCBER*) is a member of the PINO-LARI, isoflavone, phenylcoumaran benzylic ether reductase (PIP) family, which plays a role in the lignan biosynthetic pathway. Two soluble enzymes, pinoresinol-lariciresinol reductase (PLR) and PCBER, catalyze the reduction of benzylic ether in the 8–8′ and 8–5′ linked lignans. PCBER facilitates the formation of various lignans through NAD(P)H-dependent 7-O-4 reduction of precursor compounds [79,81]. Finally, lignans undergo glycosylation modification and are stored as lignan glycosides in plant tissues [82,83]. Overexpression of *PtPCBER* enhanced lignan production, which in turn scavenged ROS generated by salt stress in poplar. This reduction in ROS alleviated oxidative damage due to high salt stress, resulting in increased salt tolerance in transgenic poplar plants [84].

Lignan structure makes them effective antioxidants, capable of scavenging harmful ROS that typically accumulate under stress conditions. For instance, the flaxseed lignan SDG and its mammalian metabolites, ED and EL, exhibit antioxidant activity without prooxidant effects by reducing lipid peroxidation and decreasing deoxyribose oxidation and DNA strand breakage [85,86]. Suja et al. [87] screened the antioxidant activities of isolated and purified compounds from sesame cake, including sesamol, sesamin, sesamolin, sesaminol diglucoside, and sesaminol triglucoside. Using the β-carotene-bleaching assay and the inhibition of linoleic acid peroxidation by the thiocyanate method, they found varying degrees of antioxidative activity among the lignans, with free-form lignans generally showing greater antioxidant power than glucosides. Lignans from the root bark of fringe tree (*Chionanthus virginicus* L.), such as phillyrin, pinoresinol-β-d-glucoside (PDG), and pinoresinol di-β-d-glucoside (PDDG), demonstrated significant antioxidant activity in comparison to standard antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol and its water-soluble analog trolox in a series of *in vitro* tests [88]. In these assays, it was observed that lignans with an oxygen-free benzylic position exhibited higher radical-scavenging activity [89]. The antioxidant activity of lignans is a fundamental aspect of their positive role in plant responses and tolerance to abiotic stress. Additionally, their participation in lignification and cell-wall synthesis contributes to stress responses. For example, drought stress can alter lignan levels in sesame (*Sesamum indicum* L.), with more drought-tolerant genotypes showing higher levels of sesamin and/or sesamolin [71].

Experiments on metal stress have shown that the Cd-tolerant CB671 genotype of oilseed rape (*Brassica napus*) accumulates lignans and cell-wall saccharides. This suggests that cell-wall priming may play a role in its tolerance. In contrast, the Cd-sensitive genotype (ZD622) accumulates phenolics, particularly from the upstream subclasses of flavonoids. The observed accumulation of lignans and cell-wall saccharides in the Cd-tolerant CB671 genotype may indicate an enhanced antioxidant capacity within the cell wall, which likely helps mitigate the stress caused by elevated cadmium (Cd) levels [90].

Beyond their role in responding to abiotic stress, lignans are crucial for plant defense against pathogens. They achieve this by inhibiting microbe-derived degradative enzymes like cellulases, polygalacturonases, glucosidases, and laccases. Additionally, lignans can act as insecticides by disrupting the endocrine systems of insects [77].

Biotic and abiotic stress conditions are often associated with elevated levels of stress hormones such as SA. Research has shown that applying these stress hormones externally can boost lignan biosynthesis. For instance, adding 50 μM sSA to flax (*Linum usitatissimum* L.) cell cultures resulted in a two-to four-fold increase in the production of lignans like SDG and lariciresinol diglucoside (LDG), as well as neolignans such as dehydrodiconiferyl alcohol glucoside (DCG) and diacylglycerol-β-coniferyl alcohol ether glucoside (GGCG) compared to controls [91]. Additionally, methyl jasmonate was found to stimulate lignan biosynthesis in *Isatis indigotica* hairy root cultures, enhancing both gene expression and metabolite accumulation of coniferin, LARI, SECO, and PINO [92]. Utilizing stress hormones to elicit

phenylpropanoid biosynthesis is increasingly becoming a favored biotechnological approach for producing pharmacologically valuable polyphenols, including biologically active lignans [21].

Hamade et al. [93], through NMR and LC-MS-based metabolomics studies, observed the role of lignan in flax osmotic stress tolerance. Their observations suggested that the mechanism of osmotic stress response of the transgenic line (deficient in lignan synthesis) is somehow different from that of the wildtype, leading to the hypothesis that balanced lignan content may be important for proper stress response.

5 Lignan Content Determination

The demand for lignan sources, as well as methods for their identification, extraction, purification, and analysis, has been steadily increasing in recent years. Among the various extraction and identification methods, mass spectrometry and liquid and gas chromatography were the most common. Charlet et al. [94] presented an HPLC-based quantification procedure for SECO in flaxseed. They used the procedure for the purification of anhydrosecoisolariciresinol (ANHSEC) from the hydrolyzed extract of flaxseed. They carried out a time study for the optimum acid hydrolysis condition evaluation. This method was used in the extract of various organs of plants from roots to fruits for the distribution study of lignans.

The SECO lignan contents in flaxseed were measured through gas chromatography. Before performing hydrolysis, they performed ethanol lignan extraction from both trimmed and untrimmed flaxseeds and observed a lower lignan concentration yield than that of the direct hydrolysis method. Also, through analysis, they observed similar lignan content both in trimmed and untrimmed flaxseeds. They achieved an efficient recovery by reducing the steps of preparation and resources [95]. Bhatnagar et al. [96] presented a fast lignan evaluation method using the ultraviolet absorption-based spectrophotometric procedure for the lignan content determination in sesame oil. Since the extraction of sesamol and sesamin using HPLC needs solvents and columns, they claimed that the developed method is fast and less expensive in sesame oil lignan determination. The experiment results showed that the UV method has the same correlation coefficient in lignan determination and a slightly higher value of lignan contents compared to that of the HPLC method.

A new approach based on Heteronuclear Single Quantum Coherence (HSQC) NMR spectrometry was adopted by Xiao et al. [97] to determine the lignan contents of *Sambucus williamsii*. Those lignans were bioactive containing carbon signals from oxybenzyl. They compared the resonance signal peak of oxybenzyl carbon with PINO. The experimental results showed that the NMR spectrometry method was reliable in the quantization of complex botanical mixtures with better accuracy. They also calculated the amount of lignan to be approximately 10%–15% of the total extract. Various experimentation methods for the quantitative analysis of lignan content have been researched till now using different species rich in lignan content.

HPLC is frequently used to quantify and separate lignans. HPLC can detect the interaction between the stationary phase of the chromatograph and specific lignan compounds [98]. Liquid chromatography-mass spectrometry (LC-MS) is employed to quantify and identify lignan metabolites [99]. Nuclear Magnetic Resonance (NMR) Spectroscopy provides precise structural information on lignan molecules [93]. Fourier-transform infrared (FTIR) spectroscopy helps in identifying the functional groups present in lignans [100]. Solvent extraction is a prevalent technique that uses solvents such as methanol or ethanol to isolate lignans from plant sources. Supercritical Fluid Extraction (SFE) is a more sophisticated method used for the extraction of lignans [98]. Fig. 4 provides a view of the various methods used for lignan content extraction.

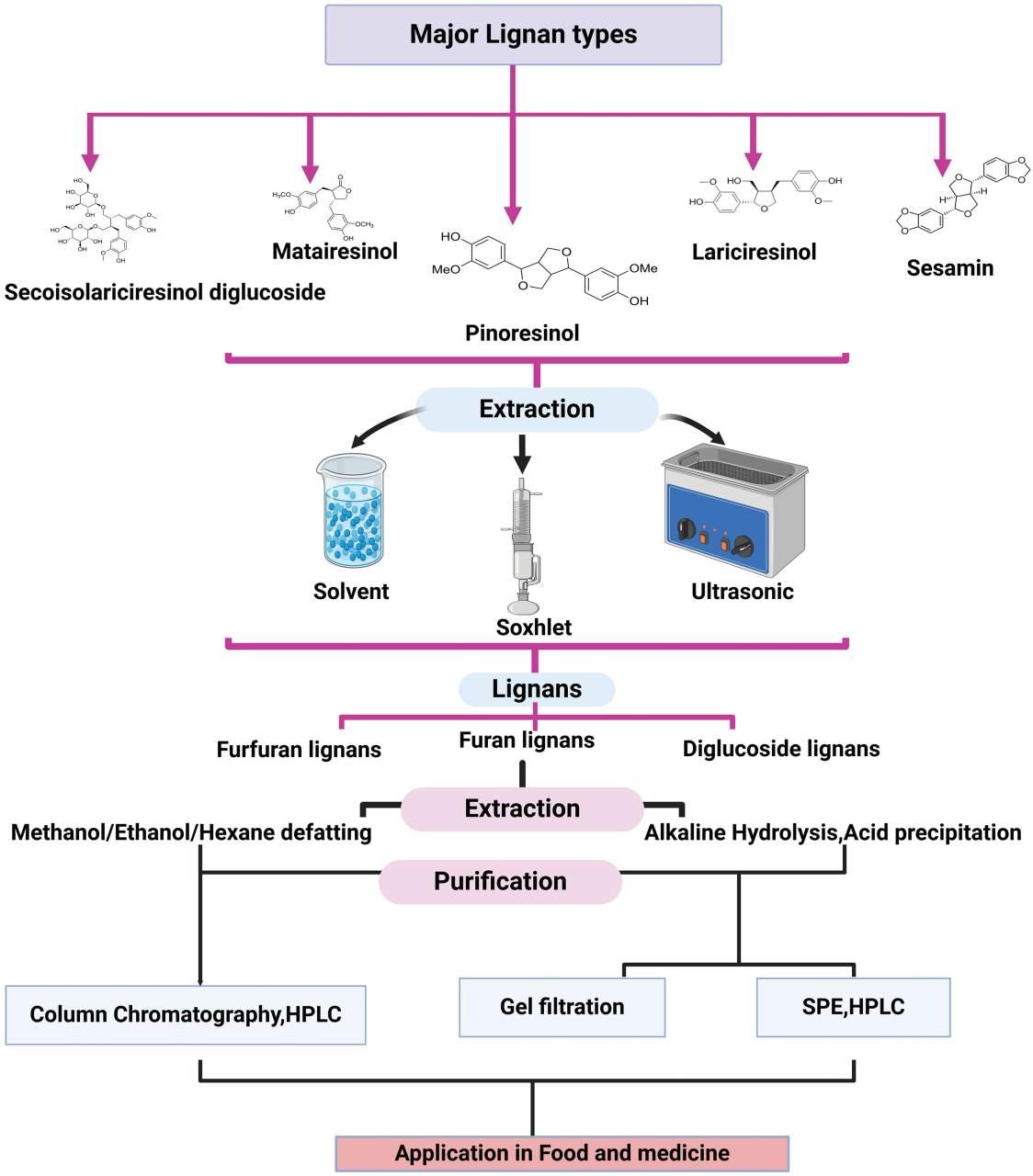

Figure 4: Major lignan type with their extraction, purification and determination method

6 Lignan Enhancement

6.1 Lignan Production Enhancement through Biotechnological Methods

Lignan production from plants is declining due to factors such as suboptimal cultivation practices, high extraction costs, low lignan content, and long generation cycles. Additionally, factors like species type, plant maturity, age, genetic composition, environmental conditions, and physiological factors all limit the lignan content. To address this, lignan production needs to be enhanced through efficient and sustainable methods. In this regard, various studies have focused on biosynthetic approaches and metabolic strategies to boost lignan yields. Therefore, biotechnological methods for producing lignan have garnered significant

attention in recent years. Among several methods available for lignan enhancement, the most efficient, reliable, and commonly used include *in vitro* suspension cultures, using microbial elicitors, and genetic engineering methods [7,9,101]. In this review, the impact of these three methods on lignan accumulation in various plant species is presented.

6.1.1 In Vitro Suspension Culture Method for Lignan Enhancement

In vitro, or micropropagation, is a method of plant multiplication asexually in an aseptic condition to enhance the lignan contents in the plant species that are vital commercially. Initially, the tissue culture method of *Linum* species was introduced, and since then various species were involved in this process. Yildiz et al. [102] studied the explant's source effects on greenhouse and *in vitro*-influenced *Linum usitatissimum*. Through experiments, they analyzed the explants producing the shoot, the shoot number, and the overall number of shoots for each sample. They observed better regeneration of shoots by hypocotyl explants, and the seedlings grown *in vitro* were more suitable than the ones that were grown through the greenhouse method.

Samadi et al. [103] presented the hairy root culture method in *Linum mucronatum* species using *Agrobacterium rhizogenes* stains for lignan production. They also used liquid culture media based on Murashige-Skoog for the hairy root establishment. They observed the successful hairy root production of transgenic nature. Highly transforming hypocotyl segment-type explants were the reason for the production of hairy roots, and these roots provided a suitable platform for the optimization and production of aryltetralin lignans like PTOX and its derivatives. Similarly, *in vitro* solid cultures (microshoots and callus), cultivation periods (10, 20, and 30 days), and different concentrations of plant growth regulators-BA, IBA, and GA_3 (ranging from 0 to 3 mg/L) in Murashige and Skoog medium were tested for the presence of several lignans, including dibenzocyclooctadiene lignans (schisandrin, gomisin G, schisantherin A and B, deoxyschisandrin, and schisandrin C), dibenzylbutane lignans (hernicine B), aryltetralin lignans (wulignan A1 and A2, epiwulignan A1, enshicine, epienshicine, and dimethylwulignan A1), as well as triterpenoids (kadsuric acid and schisanhenric acid). Their presence was confirmed using UHPLC–MS/MS analysis. The total amounts of secondary metabolites in the extracts from *in vitro* cultures were found to be 13.0, 7.0, and 1.4 times higher than those in leaf extracts, which were analyzed for comparison. This is the first report on the biosynthetic potential of cells from *Schisandra henryi in vitro* cultures [104]. In *Linum usitatissimum*, a high level of lignan accumulation was recorded through root-derived calli. Lignans such as SDG, LARI diglucoside (LDG), dehydrodiconiferyl alcohol glucoside (DCG), and guaiacylglycerol-β-coniferyl alcohol ether glucoside (GGCG) were observed in the culture (100). Both *in vitro* cultures of flax (both callus and adventitious root) were studied. However, it was the adventitious root culture that efficiently produced a higher amount of lignans (at day 40) and neolignans (at day 30) than the callus culture of flax [9].

Cell culture of *L. persicum* was done to study lignan production and evaluation for anti-carcinogenic activity on the MCF7 cell line. Lignan contents were studied in cells, callus, plantlets, capsules, leaves, stems, and roots using the HPLC technique. It was observed that the lignan PTOX was highest in capsules and pinoresinol in leaf and root organs. The shoot extract of the *in-vitro* plantlets has greater cytotoxic effect on breast cancer cells (IC50 = 5 μg/mL) [105]. Hepatoprotective and anticancer polyphenolic lignans, phyllanthin, hypophyllanthin, niranthin, and phyltetralin were observed both in natural plants and *in vitro* cultures of *Phyllanthus tenellu*s Roxb. In a culture medium incorporated with 1.0 mg/L of 6-benzylaminopurine (BAP), there was maximum shoot regeneration, while indole-3-acetic acid (IAA) supplementation at 2 mg/L was superior for induction of rooting in *in-vitro* raised shoots. Such plantlets showed 100% survival under field conditions. The content of lignan was higher in callus grown on Murashige and Skoog (MS) medium supplemented with 2.0 mg/L Naphthaleneacetic acid (NAA). These concentrations of nutrients and variations in hormones (auxin and cytokinin) can be used

to develop protocols for mass propagation of lignan and biotechnological approaches for the improvement of *P. tenellus* [26].

Khan et al. [56] produced potent lignans and antioxidant secondary metabolites in linseed through *in vitro* micropropagation. They used hypocotyl explants, supplemented them with thidiazuron at 0.5 mg/L concentration with kinetin at 0.5 mg/L in the basal growth media, and observed 86.87% shoot induction frequency and higher shoot organogenesis in 4 weeks. Thidiazuron, with kinetin, activated the antioxidant system (total phenol, flavonoid, DPPH free radical scavenging, phenylalanine ammonia-lyase activity, SOD expression). It also enhanced the pharmacologically active lignans such as SECO, SDG, and ANHSEC diglucoside in the regenerated shoots. This method can be used by scaling it up for commercial lignan production to meet the requirement.

Scarlet flax (*Linum grandiflorum* L.) *in vitro* culture was done to obtain lignans and neolignans for antioxidant and anti-inflammatory applications. The hypocotyl and cotyledon explants were supplemented with different concentrations of α-naphthalene acetic acid (NAA) and thidiazuron either alone or in combinations. NAA (1.0 mg/l) was better in inducing callus formation in hypocotyl explants than thidiazuron alone. The impact of NAA was higher compared to both the NAA and thidiazuron combination, although lesser than thidiazuron alone. This shows that there was no synergy between NAA and thidiazuron. The biomass of NAA-treated explants was higher, but in combination with thidiazuron, it further increased, showing a synergy between the two PGRs, particularly for cotyledon-derived explants. Lignans like SECO, LARI, and neolignan (dehydrodiconiferyl alcohol (DCA)) naturally accumulate in their glycoside forms. The study focuses on enhanced biomass accumulation and efficient production of (neo) lignans in *L. grandiflorum* callus cultures [106].

Lignan-like phyllanthin and hypophyllanthin were observed in the shoot culture of *P. amarus* Schum. & Thonn. by direct organogenesis. Various plant growth regulators (PGRs) were used in different combinations and their impact on lignan was observed (PGRs: kinetin(Kin), 6-benzylaminopurine (BAP), 2-isopentenyladenine (2iP), 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea, thidiazuron, auxin, andindole-3-butyric acid (IBA)). Lignan production was maximum in the shoot culture grown on Murashige and Skoog's (MS) medium supplemented with Kin 0.25 mg/L. Both phyllanthin and hypophyllanthin increased, showing the potential for large-scale cultivation of *P. amarus* shoots for medicinally important lignan production [107].

In 2024, Silva et al. [108] observed that biomass production and PTOX content were measured in the root cultures of *Hyptis suaveolens* (L.) Poit grown in liquid medium supplemented with different concentrations of indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), vitamins, and myo-inositol. High-performance liquid chromatography with diode-array detection (HPLC-DAD) was used for PTOX quantitation. MS medium supplemented with 1 mg/L IBA and 0.5 mg/L NAA was maximally effective in enhancing PTOX concentration in the roots and also root dry weight. Vitamin and myo-inositol supplementation also enhanced PTOX content.

In another study for enhancing lignan production, Dougué Kentsop et al. [101] observed adventitious (ARL) and hairy roots (HRL) grown under *in vitro* conditions in *Linum lewisi. L lewis* synthesizes arylnaphthalene lignans such as justicidin B and also has phenol content and antioxidant activity. Four other lignans were also observed through NMR spectroscopy in the root extracts. The ARL and HRL cultures showed a higher justicidin B production compared to other *Linum* species cultures, and the production was further enhanced by elicitation with MeJa. Overall, the growth performance of HRL lines was better than ARL lines, and HRL6 showed higher justicidin B content than ARL1 at 3, 4, and 5 weeks ($p \leq 0.05$). HPLC analysis on methanolic extracts of HRL6 and ARL1 revealed the presence of other molecules besides justicidin B. It is worth noting that *in-vitro* culture enhances lignan production to a much higher extent than plants growing under natural conditions and in this regard, various PGRs and

other chemicals play an essential role. Accumulation of lignan was observed using suspension culture of seedlings of *Linum album* after *the* addition of DOP. DOP was found to induce the production of PTOX and 6MPTOX lignans [109]. A similar experiment was done on *Linum album* through the accumulation of feeding experiments with hairy root culture after the addition of coniferaldehyde. Lignans LARI, PINO, and PTOX accumulation were enhanced by 14.8, 8.7, and 1.5 times, respectively, with coniferaldehyde [110]. In *Linum flavum*, lignan (5MPTOX) production in hairy root culture was improved by 1.5% to 3.5% on dry weight compared to untransformed tissue [111]. In *Phyllanthus amarus* shoot culture, the application of PGRs affected shoot development and the accumulation of biologically active lignans through direct organogenesis. The total lignan content, comprising phyllanthin and hypophyllanthin, was approximately 8–10 mg/g DW, comparable to or exceeding the levels found in plant material harvested from natural environments [107]. The *in vitro* method is further used for lignan enhancement by the elicitation method, which is discussed in the next section.

6.1.2 Elicitation Methods for Lignan Enhancement Using in Vitro Culture Methods

Elicitors are foreign molecules that stimulate the production of secondary metabolites by triggering stress responses or defense mechanisms in living cells. Depending on their nature, elicitors are classified into biotic and abiotic categories. Biotic elicitors include substances like yeast or fungal extracts, chitosan, cellulase, and chitin, while abiotic elicitors are physical or chemical stressors that induce similar responses. (Fig. 5). The physical stressors include UV light, temperature, pH, etc., and the chemical stressors include heavy metal ions like silver (Ag), copper (Cu), cadmium (Cd), Nickel (Ni), etc. In addition, the third component of abiotic elicitors includes signaling compounds such as methyl jasmonate (MeJa) and SA. It has been evident from various research that the use of elicitors improves lignan production.

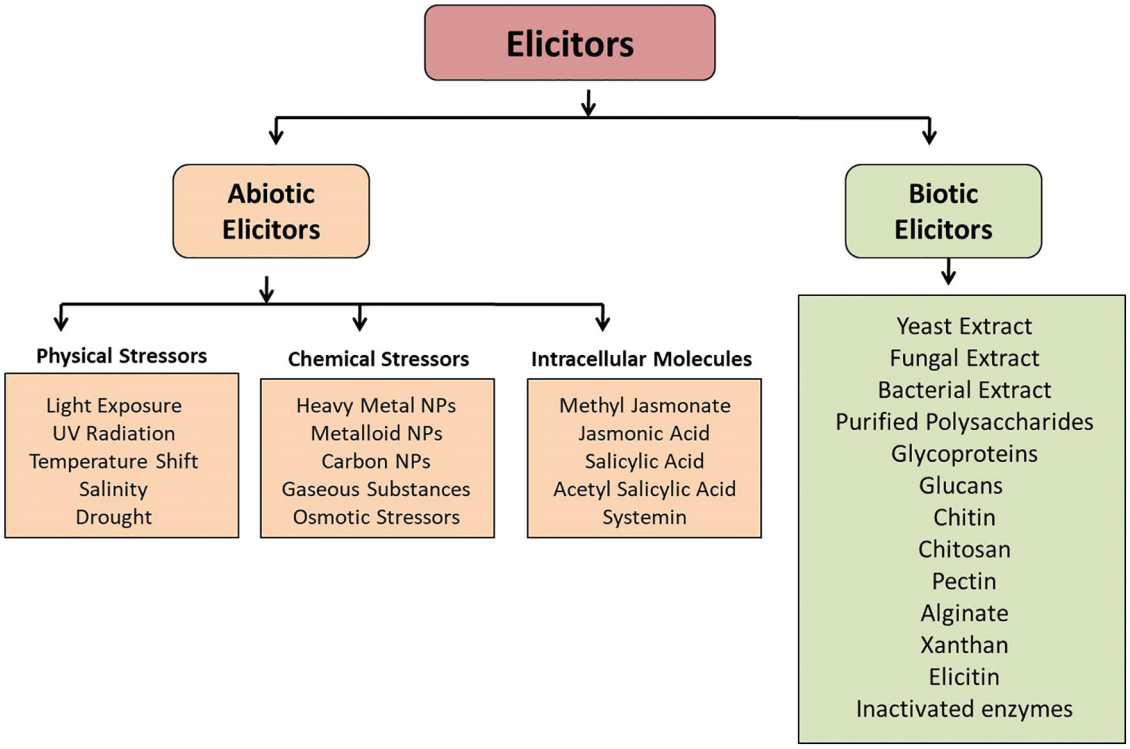

Figure 5: Schematic representation of different types of elicitors. The major classes of elicitors are abiotic elicitors (physical, chemical, and intracellular) and biotic elicitors

Biotic Elicitation

Li et al. [112] studied the effect of yeast extract as a biotic elicitor on lignan production in *Salvia castaneta*. They recorded the accumulation of tanshinone and plant growth in root cultures. Experiment results showed a 1.8–1.99 times increase in tanshinone contents by using yeast extract in comparison with silver ion and MeJa, respectively. Yeast extracts also enhanced the dry mass and cryptotanshinone content. Through gene expression study it was evident that the yeast extracts heightened the expression levels of isopentenyl isomerase. Zhao et al. [113] presented a study using yeast polysaccharides as an elicitor for the stimulation of secondary metabolites in the root culture of *Fagopyrum tataricum*. They recorded a 3.2-times enhancement in flavonoid yield compared to the control culture and enhanced the flavonoid accumulation. Ming et al. [114] reported the influence of mycelium extract and the fraction of polysaccharide produced from *Trichoderma atroviride* on the secondary metabolites of the root culture of *Salvia miltiorrhiza*. They performed an HPLC analysis procedure and observed that steady growth in hairy roots was recorded in the growth period, and it was rapid in treated groups. Also, the enhancement effect of both mycelium and polysaccharides on the production of tanshinones was similar, and a slight increase was recorded when treated with polysaccharides for an exposure period greater than 18 days. Szopa et al. [12], through their study, reported that elicitors like chitosan (200 mg/L), yeast extract (3000 mg/L), ethephon (25 µM/L), and methyl jasmonate (50 µM/L) affect lignan production in microshoot cultures of both female (F) and male (M) *Schisandra rubriflora* Rehd. et Wils. Elicitors were given at 10 days of culture, and samples were collected after 1, 2, 4, 6, and 8 days post-elicitation. UHPLC–MS/MS analysis revealed the presence of dibenzocyclooctadiene, aryltetralin, dibenzylbutane, and tetrahydrofuran lignans and neolignans in the biomass extracts. All elicitors enhanced lignan production. The male cultures in bioreactors exhibited the highest total lignan content in their biomass extract, with MeJa yielding 153.20 mg/100 g DW. Among the bioreactors and agitated culture, it was the agitated culture that showed 3.29 times and bioreactors 1.13 times higher lignan than in non-elicited cultures. Tashackori et al. [24] found that the elicitation by the root endophytic fungus *Piriformospora indica* produced H_2O_2 molecules in the hairy roots of *Linum album*, resulting in alterations in physiological, biochemical, and molecular responses. These changes included adjustments in the antioxidant mechanisms and the synthesis of lignans. Analyzing the metabolic responses to this elicitation reveals that both enzymatic and non-enzymatic defense mechanisms play crucial roles in the adaptation of *L. album* hairy roots to the influence of *P. indica*, besides enhancing lignan production.

Fungal elicitor *Fusarium graminearum* extract was used to study lignan PTOX and MPTOX enhancement in *Linum album*. Its extract resulted in the highest increases of PTOX, reaching 190 µg g^{-1} DW, and LARI, at 260 µg g^{-1} DW, which were two-fold and three-fold higher than the untreated control, respectively. In contrast, *Trichoderma viride* extract boosted the accumulation of MPTOX, rather than PTOX, up to 160 µg g^{-1} DW, representing a 2.4-fold increase compared to the control. The enhancement of lignan production by these fungal elicitors was associated with increased expression of several key genes involved in their biosynthesis, including phenylalanine ammonia-lyase, cinnamoyl-CoA reductase, cinnamyl-alcohol dehydrogenase, and PINO-LARI reductase [115].

Gononcharuk et al. [116] studied the effect of yeast extract as an elicitor at various concentrations (200–1000 mg/L) on the accumulation of phenolic compounds in *Linum grandiflorum* Desf. cells cultured *in vitro*. It was observed that both the total phenol content and the levels of phenylpropanoids increased significantly in the cell culture, particularly at higher YE concentrations (500 and 1000 mg/L). Such an increase in the content of phenylpropanoids and the expression of genes involved in the phenylpropanoid pathway following exogenous treatment of *in vitro* plant cultures with biotic elicitors, such as polysaccharides, suggest, a regulatory role for phenylpropanoids, which are characterized by their strong radical-scavenging ability, in maintaining redox homeostasis in plant cells. The finding of elicitation by biotic elicitors is summarized in Table 1.

Table 1: Study of lignan enhancement using biotic elicitors

Elicitor	Plants species	Biotic elicitors	Effect on lignan	Reference
Arbuscular mycorrhiza-like fungus *Piriformospora indica*	*Linium album*	Fungal elicitors were added.	Multi-fold enhancement in the Production of PTOX in plant cell cultures.	[117]
Piriformospora indica	*Linium album*	Autoclaved and filter-sterilized culture filtrate of *P. indica* was added with the hairy root culture *L. album*.	3.8 times increase in PTOX concentration, and 4.4 times increase in MPTOX concentration.	[118]
Chitosan	*Linum usitatissimum*	Chitosan with various concentrations was added to the *L. usitassimum* cell cultures, and biomass accumulation of lignan and neo lignan was evaluated.	7.3-fold enhancement was achieved for LDG, 3.5-fold in DCG, and 2-fold in SDG.	[119]
Chitosan	*Scrophularia striata Boiss*	Cell growth and viability, lignan accumulation, and exposure time were investigated.	Up to a 1.9-fold increase in the gene expression of PAL was achieved in reduced exposure time.	[120]
Yeast extract	*Azadirachta indica*	Investigated the influence of yeast extract on cell growth and secondary metabolites synthesis through gene expression analysis.	Highest accumulation of secondary metabolic compound was observed after 2 days of exposure to 245 mg/L of yeast extract.	[121]
Chitosan, yeast extract, ethephon, methyl jasmonate	*Schisandra rubriflora*	The UHPLC–MS/MS method was employed to qualitatively and quantitatively analyze 24 compounds from lignans, as well as neolignans, in biomass extracts.	3.29 and 1.13-fold increase in the lignan content.	[12]

Abiotic Elicitation

Gai et al. [122] studied the use of ultraviolet (UV) radiation as an elicitor in *Astragalus membranaceu* (AM) HRC for the enhancement of Astragalosides (AG) production. They observed that maximum production of AG was recorded in the AM of 32-day-old exposed to UV radiation. This production was 1.32 times higher than that of the non-treated control production. Zhang et al. [123] evaluated the impact of silver nanoparticles as an elicitor in *Artemisia annua* for the enhancement of artemisinin production. They recorded a 3.9 times increase in the production of artemisinin in the 20-day root culture of *Artemisia annua*. An investigation on the impact of KCl and $CaCl_2$ with applied salt stress as an elicitation method on the hyoscyamine content of various Datura species was performed by Harfi et al. [124]. KCl with a concentration of 2 g/L was applied to *Datura tatula* for 10 h contact time and on

Datura stramonium, innoxia for a 24 h contact time. The results were compared with the application of $CaCl_2$ on both the species for 10 and 24 h elicitation time. They observed a maximum hyoscyamine content production in *D. tatula* by using $CaCl_2$ over an elicitation period of 24 h.

The use of signal molecules such as MeJa and SA on the secondary metabolite accumulation in *Rhinacanthus nasutus* was investigated by Cheruvathur et al. [125]. They carried out a time study on the production of rhinacanthin without elicitor for 4 weeks and with the addition of MeJa and SA for a culture period of 6 weeks in MS medium. They observed the highest accumulation of RC content with the addition of MeJa.

Similar enhanced lignan production was observed by Sykłowska-Baranek et al. [126], who studied the potential for accumulating six lignans in two lines of Taxus x media hairy roots. Hairy root cultures from the KT and ATMA lines were treated with various precursors, including coniferyl alcohol (CA at 1, 10, or 100 μM), l-phenylalanine (100 μM), and methyl jasmonate (100 μM). Additionally, two-phase *in vitro* cultures utilizing perfluorodecalin (PFD) as a gas carrier and *in situ* extractant were employed. It was noted that the two hairy root lines exhibited different profiles in lignan production. While in the untreated controls, KT roots did not produce SECO or LARI lignan, ATMA roots lacked MAT. Treatment with CA at 1 or 10 μM in ATMA roots led to the production of both LARI and SECO, whereas only LARI was detected at 100 μM CA. Elicitation with 1 μM CA and MeJa together resulted in the highest yields of hydroxymatairesinol aglyca and lariciresinol glucosides, measuring 37.88 and 3.19 μg/g DW, respectively. It was the combined treatment of 1 μM CA, PHEN, and MeJa that significantly stimulated lignan production, particularly in cultures supplemented with PFD-aerated or degassed conditions. In ATMA roots, these conditions were optimal for MAT reaching levels of 199.86 and 160.25 μg/g DW in PFD-aerated and PFD-degassed cultures, respectively. For KT roots, only hydroxymatairesinol and coniferin/CA levels increased, with maximum yields of 59.29 and 134.60 μg/g DW in PFD-aerated and PFD-degassed conditions, respectively.

To make lignan extraction more cost-effective, chitosan can be used, which is a low-cost, toxic non-toxic effective elicitor for lignan production. Jiao et al. [127] observed that chitosan stimulated the production of lignan 6MPTOX in the *Linum album*. This production was mediated by the signaling of second messengers, including cytosolic free Ca^{2+}, hydrogen peroxide (H_2O_2), and nitric oxide (NO), by activating plasma-membrane Ca^{2+} channels and NADPH oxidase, as well as nitrate reductase/nitric oxide synthase-like enzymes. Inhibitors like EGTA and W-7 reduced the induction of key enzymes involved in lignan biosynthesis, indicating the role of secondary messengers in lignan synthesis. Additionally, chitosan increased SA levels, which in turn stimulated 6MPTOX production by affecting phenylalanine ammonia-lyase (PAL) and pinoresinol-lariciresinol reductase (PLR) activity. Real-time PCR results suggested that mitogen-activated protein kinase 6 (MAPK6) function downstream of these second messengers and SA signaling pathways, potentially integrating signals that modulate lignan biosynthesis. Overall, the findings highlight the regulatory role of Ca^{2+}/CaM, H_2O_2, and NO in lignan biosynthesis, with SA and MAPK6 serving as key mediators in this process [128].

Sagharyan et al. [129] reported that MeJa-induced lignan productions except for LARI and the highest amounts of PTOX and 6MPTOX, approximately 4 and 5 times higher, were analyzed at 50 μM MeJa concentration, which was 4 and 5 times that of the control, respectively. MeJa was found to induce oxidative changes by redirecting free sugars and amino acids toward the production of lignans in *L. album* cells.

Andi et al. [130] performed their experiment on *V. vinifera*, which is known for its rich content of stilbene compounds, particularly resveratrol, which are thought to offer dietary protection against cardiovascular diseases and certain cancers. Two light conditions (135.1 μmol $s^{-1}m^{-2}$ radiation *vs*. dark) and varying concentrations of phenylalanine (Phe; 0, 0.1, 0.5, and 1 mM) and methyl jasmonate (MeJa;

0 and 25 μM) were tested, both individually and in combination. It was reported that dark-grown cultures accumulated significantly higher levels of total stilbenes (resveratrol + piceid) compared to those exposed to high light. Notably, the combinations of dark with 1 mM Phe and dark with 25 μM MeJa resulted in increased levels of total phenolics, flavonoids, and stilbenes.

Compounds like SA and MeJa were found to enhance the production of secondary metabolites. Benavide et al. [131] cultivated the hairy roots of *Phyllanthus acuminatus* in 250 mL flasks under a photoperiod of 16 h of light and 8 h of darkness for four weeks, using diffused light. Elicitors were tested at concentrations of 50 and 200 μM. Non-targeted analysis of the different treatments was performed using high-resolution mass spectrometry (HR-MS). The identified metabolites were categorized into phenylpropanoids, phenols, and mucic acids, and a statistical analysis of their relative concentrations was conducted. Compound intensities were assessed using heat maps to visualize the over-expression of metabolites for each elicitation. Dendrograms were used to group compounds exhibiting similar responses across the various treatments. SA at 50 μM concentration shows higher production of secondary metabolites. Karimzadeh et al. [132] found that using nanoparticles could act as another method of elicitation for lignan enhancement. They found that the highest activity of CAD (cinnamyl alcohol dehydrogenase) occurred at a concentration of 60 mg/L of nano-ZnO at various time points. Additionally, a concentration of 150 mg/L of nano-TiO_2 also enhanced CAD activity. The maximum level of total phenols was found to be 150 mg/L of nano-TiO_2. Various concentrations of nano-TiO_2 consistently increased total lignan levels at all intervals. The greatest amounts of total phenols and lignans were recorded at 30 and 60 mg/L of ZnO. This study highlighted the differing effects of nanoparticles on enzyme activity and secondary metabolite production in flax plant cell suspension cultures, influenced by both the concentration and type of nanoparticles used.

Anjum et al. [9] reported that the adventitious root culture in flax caused more efficient production of lignans (at day 40) and neolignans (at day 30) compared to callus culture. HPLC analysis showed that the accumulations of SDG, 5.5 mg g^{-1} DW, and dehydrodiconiferyl alcohol glucoside (DCG, 21.6 mg/g DW) were twice as high, while diacylglycerol-β-coniferyl alcohol ether glucoside (GGCG, 4.9 mg/g DW) and lariciresinol glucoside (LDG, 11.9 mg/g DW) were 1.5 times higher in adventitious root culture than in callus culture. Additionally, the highest total phenolic production (119.01 mg/L) was recorded in adventitious root culture at day 40, showing an antioxidant-free radical scavenging activity of 91.01%. Thus, it could be suggested that adventitious root culture is a promising candidate for scaling up industrial production of these pharmacologically and nutritionally valuable metabolites. Various other studies are also reported in Table 2.

Table 2: Study on lignan enhancement using abiotic elicitors

Elicitor	Plants species	Study performed	Effect	Reference
Methyl jasmonate	*Podophyllum hexandrum*	Evaluated the impact of the biotic elicitor on the accumulation of lignan and carried out the gene analysis to explore the stability of the genes.	ROS production was observed to be increasing, which induces lignan accumulation. The upregulation of biosynthetic pathway genes was also recorded and was not affected by ROS induced with MeJa.	[133]
ZnO	*Linum usitatissimum*	The capability of nano elicitor in the lignan and	Increased accumulation of lignans and neolignans was	[134]

(Continued)

Table 2 (continued)

Elicitor	Plants species	Study performed	Effect	Reference
		neolignan accumulation enhancement was studied at different periods of cell cultures.	achieved at repeated elicitation at periodic intervals.	
Photoperiod regimes	*Linum usitatissimum*	Accumulation of lignans and neo-lignans using abiotic elicitor strategies with UV-C radiation was studied.	Up to a 2.25-fold increase in lignan and neo-lignan accumulation was achieved using the photoperiod along with UV radiations.	[135]
ZnO	*Linum usitatissimum*	The consequences of nano elicitor on stem and seedling derived callus of flax were studied through HPLC analysis.	Enhanced production of lignans and neolignans was observed.	[136]
Methyl jasmonate	*Linum flavum*	Accumulation patterns of PTOX-related lignan compounds through hairy root culture with the addition of strains of *Agrobacterium rhizogenes* and investigated the effects of adding MeJa and feeding with ferulic acid.	ATCC 15834 strain enhanced the lignan accumulation. The highest accumulation of aryltetralin lignan was observed during the addition of MeJa and ferulic acid feeding.	[137]
Methyl jasmonate	*Linum thracicum*	Investigated the effect of elicitation technique on the lignan production through HPLC analysis.	MeJa elicitation enhanced the accumulation of MPTOX by 2.3 times with the Li-20 of eight-day old.	[138]
Methyl jasmonate	*Isatis indigotica*	Evaluated the response of li049 to the treatment of MeJa, SA, and ABA.	Higher lignan production was achieved by li049 expression.	[139]
Ag^{+}	*Linum album*	Investigated the lignan production enhancement by the abiotic elicitor.	Presumptive inhibition of the production of ethylene was observed.	[140]
ZnO	*Momordica charantia*	Studied the effect of nano elicitors on biochemical and physiological properties and compared with chitosan and MeJa elicitation.	An increased number of flavonoids was observed which increases the lignan accumulation.	[141]
SiO_2 NPs	*Dracocephalum kotschyi Boiss*	Investigated the effect of SiO_2 NP elicitor on growth, lignan accumulation, and	13, 13.42, and 10-times increase in the three anticancer lignan production was observed.	[142]

(Continued)

Table 2 (continued)

Elicitor	Plants species	Study performed	Effect	Reference
		phenolic compound contents at 24 and 48 h exposure time.		
Zinc sulphate	*Pepper calluses*	The influence of $ZnSO_4$ with different concentrations exposed for the periods of 24, 48, and 72 h on the plant protein and phenolic compound contents was investigated.	An increase in phenolic compounds was observed at an exposure of 72 h period.	[143]
Copper oxide NPs	*Gymnema sylvestre*	Investigated the effects of CuO NPs with different concentrations over 48 h of exposure on phenolic compounds.	An increase in phenolic compounds was observed.	[144]
Ag	*Linum usitatissimum*	Studied the influence of nano elicitors on the biochemical properties and biological activities on different species.	Enhanced production of lignans and neolignans was observed.	[145]
Methyl jasmonate	*Glycine max*	Studied the impact of MeJa on isoflavone production through gene expression analysis.	10-fold enhanced isoflavone production was observed on the 9th day after the application of MeJa.	[146]
Methyl jasmonate	*Linum album*	Varying concentrations of MeJa (0, 50, 100, 150, and 200 μM) influence the accumulation of lignans by affecting the contents of free sugars and amino acids.	4 and 5-fold increase in the PTOX and 6MPTOX content compared with the control.	[129]
ZnO	*Tanacetum parthenium*	Investigated the impact of nano elicitor on the yeild, synthesis of secondary metabolities and Zn absorption.	Enhanced production of lignan contents was observed.	[147]
Methyl jasmonate	*Linum lewisi*	ARL and HRL were obtained and characterized using NMR spectroscopy in terms of their phenol content, antioxidant activity, and justicidin B production.	Justicidin B levels doubled in ARL and increased by 2.5-fold in HRL, while phenol content and antioxidant activity also doubled.	[101]

6.1.3 Genetic Engineering Methods for Lignan Enhancement

Genetic engineering is identified as a promising factor for the evolution of genetic pathways during regulatory functional limitations. In the metabolic pathway elucidation and enzyme identification, this approach of capturing and analyzing the DNA, proteins, metabolites, and mRNA of the targeted cell constitutes a potential tool. Several kinds of research have been triggered after the increased accumulation of cinamoil derivatives reported by Howles et al. [148] in the plant species of transgenic tobacco. Various strategies in genetic engineering have been evolved to enhance lignan production and plant growth. Elevated lignan levels in a transgenic wheat line have been successfully attained by genetically engineering the overexpression of the *PLR* gene [149].

Chang et al. [150] investigated the phenylalanine ammonia-lyase and hydroxycinnamoyl transferase overexpression impact on the chlorogenic acid accumulation in transgenic tobacco. The impact on the phenolic acid and rutin generation was also carried out. They observed a 3-fold increased accumulation of chlorogenic acid due to overexpression and a 12-fold enhancement in rutin level in the plant species. Kim et al. [151] presented a genetic engineering method for the biosynthesis of lignan in the suspension cell culture of *Forsythia koreana*. The transgenic overexpression of RNA interference was generated by them, and they observed the impact on the production of PINO. They observed a MAT loss and enhanced PINO accumulation compared with that of the non-transformant. Further, they reported the production of sesamin through the transgenic co-expression of CYP81Q1 enzymes in sesame seeds.

In a study by Zhang et al. [152], lignan biosynthesis was studied in the hairy roots of *Isatis indigotica* by using MeJa as an elicitor. They investigated the metabolic and transcriptional changes, identifying potential key catalytic steps and regulatory genes involved in lignan biosynthesis. The process was categorized into three phases: signal response, transcriptional activation of metabolic pathways, and metabolite accumulation. A total of 17 hub genes were identified through co-expression network analysis, with 4CL3 selected for validation. RNA interference of *4CL3* led to a significant decrease in lignan production, indicating its crucial role in the biosynthesis pathway, specifically through the esterification of caffeic acid. Overall, the study offers new insights into lignan biosynthesis and highlights targets for metabolic engineering in *I. indigoti*ca.

Amani et al. [153] showed that elicitation on *Ficus carica* hairy roots, using MeJa and cell extract from the fungus *Piriformospora indica* enhanced the total phenolic and flavonoid content, antioxidant activity (assessed using DPPH and FRAP assays), gene expression related to phenolic and flavonoid biosynthesis, and HPLC analysis of key phenolic compounds was evaluated. MeJa treatment induced high expression levels of chalcone synthase (CHS), flavonoid 3′-hydroxylase (F3′H), and transcription factors MYB3 and bHLH. In contrast, *P. indica* elicitation resulted in the highest expression levels of phenylalanine ammonia-lyase (PAL) and UDP-glucose flavonoid 3-O-glucosyltransferase (UFGT). Furthermore, *P. indica* treatment led to significant increases in the content of several phenolic compounds, including gallic acid, caffeic acid, rutin, quercetin, and apigenin. The enhancement in gene expression paves the way for further enhancement of different secondary metabolites through the use of elicitors.

To ensure a reliable supply of natural products, new production strategies have been developed, including gene transfer into heterologous organisms and metabolic engineering [154]. Perrin et al. [155] used the cofactor boosting approach to enhance the precursor of lignan PTOX in yeast. The biosynthesis of PTOX occurs through a three-step process: first, tyrosine and phenylalanine are deaminated to produce p-coumaric acid (pCA), which is then hydroxylated and O-methylated to yield caffeic acid (CaA) and ferulic acid (FA), respectively. Each of these enzymatic steps, catalyzed by heterologous enzymes, requires cofactors such as NADPH, FAD (H_2), and SAM, whose availability has been optimized through engineered synthesis and recycling methods. This approach not only streamlines the production of essential cofactors but also significantly boosts the overall yield of PTOX precursors in yeast, paving the way for more efficient biosynthesis of valuable natural products. Chen et al. [156] highlighted the crucial role of managing the supply of cofactors utilized by heterologously expressed enzymes. Their integrated

approach, which involved synthesizing and recycling three distinct cofactors, as well as enzyme overexpression, subcellular pathway reorganization, and the degradation of inhibitory by-products, led to a significant increase in the synthesis of CaA and FA. This strategy not only facilitates the high production of PTOX and other lignan-derived compounds but, when combined with traditional optimization methods, is likely to enhance the synthesis of many other natural products that require cofactors shortly.

Environmental conditions can impact the biosynthesis of secondary metabolites by altering gene expression through mechanisms involving miRNAs and transcription factors. The phenylpropanoid pathway is involved in secondary metabolite, formation, and miRNAs are known to participate in phenylpropanoid biosynthesis by targeting transcription factors. Thus, miRNA-based regulation of phenylpropanoid biosynthesis is extremely important [157].

The transcriptional activity of the key gene involved in lignan synthesis, pinoresinol-lariciresinol reductase (PLR), was examined using RT-PCR and promoter-reporter transgenesis during flax seed development. The *LuPLR* gene was found to be expressed in the seed coat, indicating its role in the production of the PLR enzyme in mature seeds, which supports its participation in SDG synthesis. To further investigate the function of the PLR enzyme in lignan biosynthesis, an RNAi approach utilizing hairpin dsRNA structures was employed for functional analysis of the *LuPLR1* gene. Transgenic plants with silenced *LuPLR1* genes showed a failure to accumulate SDG, and HPLC analysis revealed a significant reduction in SDG levels in these transgenic seeds [158]. They said that miRNAs can regulate lignan biosynthesis, and therefore they can be targeted to increase lignan production through the phenylpropanoid pathway.

The glycosylation of lignan is achieved by uridine glycosyltransferases (UGTs). It has been shown by Chen et al. [159] that the *UGT71B5s* are involved in lignan glycosylation in *Isatis indigotic*a. Several other studies on the genetic engineering method of lignan enhancement of different plant species are given in Table 3.

Table 3: Study on lignan enhancement using genetic engineering techniques

Gene	Plants species	Study performed	Effect on lignan	Reference
Phenylalanine ammonia-lyase (PAL), cinnamoylCoa reductase (CCR), cinnamyl-alcohol dehydrogenase (CAD), and pinoresinol-lariciresinol reductase (PLR).	*Linum album*	Through the subculture of *L. album*, stable tetrapoloids were produced and the autopolyploidy effects on the phytochemical and morphological characteristics were measured.	1.39- and 1.23-fold enhancement in the production of PTOX in the leaves and stems was achieved.	[160]
LuPLR1 gene	*Linum usitatissimum*	Ca^{2+} fluorescence imaging, gene experiment, lignan quantification, and RT-qPCR analysis were performed to analyze the inference of Ca^{2+} signaling.	Raise in Ca^{2+} intracellular concentration and lignan accumulation was observed.	[161]
MYB Gene—*AmMYB308*, *AmMYB330*	*Transgenic Tobacco*	Studied the limiting effects of *MYB* gene overexpression on the metabolism and biosynthesis of lignan.	Observed a 17% reduction in lignan accumulation in the vascular tissue of transgenic plants.	[162]
Enhanced Response to *ABA 1 (ERA1)* gene	*Linum usitatissimum*	Gene expression analysis on *LuERA1* was carried out *in silico*.	Resulted in a positive impact on lignan synthesis was observed. The accumulation of lignan was increased through reduced expression level of *LuPLR1*.	[163]

(Continued)

Table 3 (continued)

Gene	Plants species	Study performed	Effect on lignan	Reference
CYP81Q1 gene	*Forsythia*	Transgenic *Forsythia* plants were created that express the *CYP81Q1* gene heterologous. Using high-performance liquid chromatography (HPLC) and LC-mass spectrometry, sesamin and its intermediate piperitol in the leaves of two distinct transgenic lines of *F. intermedia* and *F. koreana*.	Co-expressing CYP81Q1 and *S. indicum* oxidoreductase genes in transgenic *Forsythia* plants is a potential future strategy to enhance CYP81Q1 activity for increased sesamin production. Furthermore, red light exposure has been shown to elevate sesamin content in transgenic *Forsythia* cells.	[164]
WRKY11 gene	*Lilium regale*	A WRKY transcription factor, WRKY11, was isolated from *L. regale* and its role during the interaction between *L. regale* and *F. oxysporum* was characterized using UHPLC-MS/MS.	LrWRKY11 is a key regulatory factor in Fusarium wilt resistance in *L. regale*. It interacts with SA/JA signaling pathways and upregulates LrDIR1 expression, enhancing lignin and lignan accumulation in response to *F. oxysporum* infection.	[75]
PlDIR1 gene	*Phryma leptostachya*	Identification of *PlDIR* genes and studying their functions in lignan biosynthesis by performing a transcriptome-wide analysis and characterizing the catalytic activity of the PlDIR1 protein.	15 *DIR* genes in *Phryma leptostachya*, highlighting the role of *PlDIR1* in lignan biosynthesis, particularly in the formation of (±)-pinoresinol, thus enhancing the understanding of lignan metabolic pathways.	[165]

6.2 Breeding Approach for Lignan Production

The presence of lignans, such as sesamin and sesamol, enhances the pharmaceutical value of sesame oil. Although wild sesame (*Sesamum mulayanum*) has lower oil yields, it contains significantly higher lignan levels. To improve the oil profile of cultivated Indian sesame (*S. indicum*), interspecific hybridization was performed between *S. indicum* and *S. mulayanum*. The selected F6 recombinant lines exhibited high oil content and a superior lignan profile, along with distinct phenotypic traits. Sesamin synthase, a crucial enzyme in the lignan biosynthetic pathway, was studied during seed development in both parent species using semi-quantitative and qRT-PCR analysis. The recombinant lines with elevated sesamin content showed increased expression of the sesamin synthase gene, which may serve as a potential candidate for allele-based molecular marker development in future sesame breeding programs [166]. The development of molecular markers has accelerated the process of flax molecular breeding and has improved yield and quality. Presently, simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers in the whole genome have been developed for flax. Development and application of novel InDel (insertion/deletion) markers in flax (*Linum usitatissimum* L.) through whole-genome re-sequencing could be used for flax germplasm identification, genetic diversity analysis, and molecular marker-assisted breeding [29].

Additionally, breeding programs may use the identification of *Linum spp*. Cultivars with significant lignan content to introduce and enrich genes encoding pathway enzymes and produce cultivars with higher lignan content. There is genetic variability that can be used for breeding, as evidenced by the significant variations in lignan content between accessions and cultivars. The heredity of the primary lignan concentration in sesame seeds must be understood in order to develop sesame as a functional diet. There is little information on how lignan content is inherited. There have been reports of breeding initiatives in India to increase the lignan content of sesame [167]. The crossing of a parent with high lignan content with a low content tester produced offspring with lower lignan content than parents. Efforts should be made to cross two high-lignan-content parents to observe a potential benefit. Breeding

sesame for lignan content is still in its infancy, and systematic efforts to target lignans have only recently begun. The development of sesame cultivars with higher lignan levels presents a valuable opportunity for farmers to tap into diverse industries, as highlighted by Khuimphukhieo et al. [168]. The heritability findings from the RIL population studied by Wu et al. [169] further support the notion that lignan-rich sesame lines can be effectively selected from appropriate parental crosses. This genetic insight allows for targeted breeding strategies that can enhance the nutritional and commercial value of sesame seeds.

Xiao et al. [74] reported that *IiW.RKY34* expression, which is involved in lignan biosynthesis, activated *Ii4CL3*, a crucial rate-limiting enzyme in lignan biosynthesis. The expression of *IiWRKY34* enhanced the polyploidy vigor of *I. indigotica* and its lignan content.

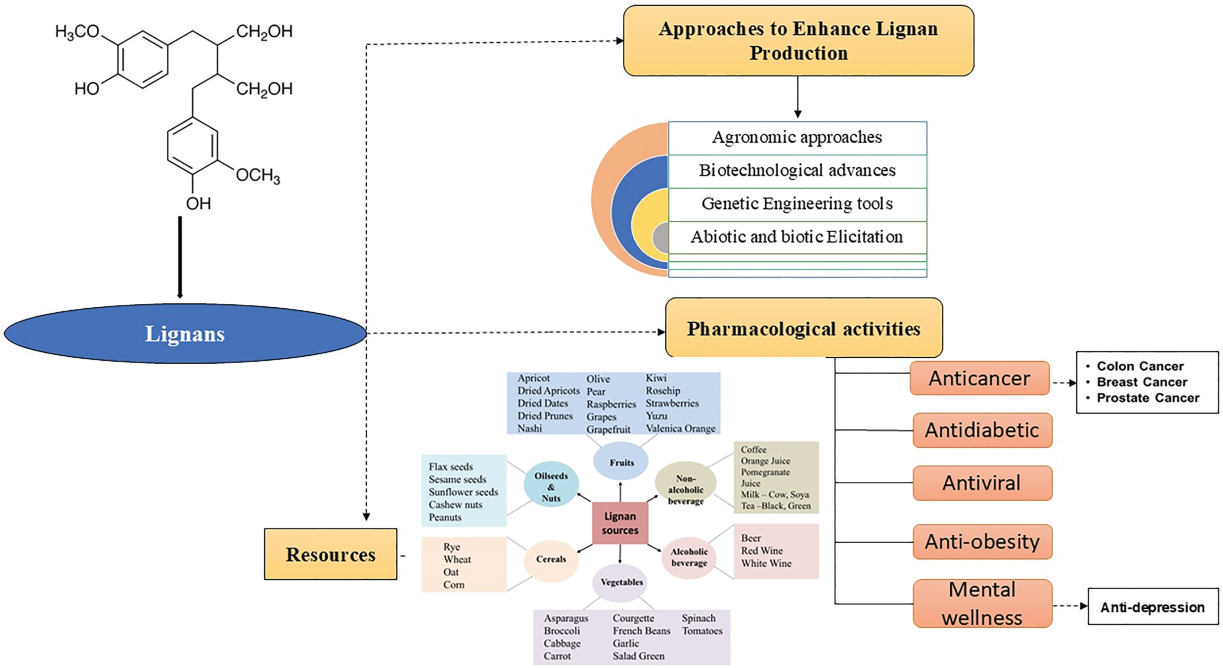

Figure 6: Different sources and elicitation methods for lignan production and their impact on humans

7 Conclusion

This review has examined the significant progress made in lignan research, emphasizing the enhanced production of lignans in crop plants. The findings illustrate the critical roles that lignans play in plant defense and their promising health benefits for humans, particularly in relation to cancer prevention and cardiovascular health. By employing innovative biotechnological approaches, such as genetic engineering and metabolic pathway optimization, researchers have opened new avenues for increasing lignan content. Looking ahead, the trend of enhancing lignan production offers promising opportunities for both agriculture and health. Lignan-rich foods not only serve as health supplements but also possess disease-fighting properties, while in plants, lignans play a role in stress tolerance. Future research should focus on optimizing production methods and investigating the ecological impact of lignan-enhanced crops. This will help ensure that these advancements support both agricultural sustainability and public health in a balanced and beneficial way.

We have presented a comprehensive analysis of various biotechnological approaches for enhancing lignan production in different plant species, with a focus on *in vitro* culture techniques, elicitor application, plant breeding, and genetic engineering. *In vitro* culture has been identified as a highly effective method for lignan production, particularly in Linum species, providing a viable alternative to rare plant species. This approach has enabled the successful production of lignans such as PTOX, MPTOX, dPTOX, SDG, and sesamin from Linum species, as well as from *Sesamin indicum* and *Phyllanthus amarus*. The use of biotic elicitors (e.g., yeast extract, NAA) and abiotic elicitors (e.g., UV, temperature, pH, and chemical agents such as Ag, Al_2O_3, SiO_2, TiO_2, ZnO, and MeJa) has been shown to significantly enhance lignan accumulation. Through the manipulation of metabolic pathways and the analysis of DNA, proteins, metabolites, and mRNA in target cells, substantial increases in the production of lignans could be achieved. Future research should focus on the discovery of novel enzymes involved in lignan biosynthesis and the development of methods to increase the production of lignan to exploit its antioxidant, anti-inflammatory, and anticancer properties. Fig. 6 summarizes the major elicitors for lignan production and the importance of lignan, which is already discussed in the text.

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