

# Isolation and Bioactivities of Furfuran Type Lignan Compounds from Edible Plants

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**Abstract:** Lignans constitute a group of phytochemicals, which are produced by oxidative dimerization of two phenylpropanoid units. Furfuran type lignans such as secoisolariciresinol, matairesinol, lariciresinol or pinoresinol are widely distributed in edible plants, and most of those dietary lignans are metabolized by the gut microflora to enterolactone and enterodiol, also known as enterolignans, traditionally classified as phytoestrogens. The rich sources of lignans are flaxseed, sesame seeds, cereal products, and *Brassica* vegetables. There is a growing interest in biological functions of lignans from edible plants, since a higher intake of edible plants containing lignans is known to reduce the incidence of certain chronic diseases. This review deals with the isolation and preparation of furfuran type lignans from edible plants, and their bioactivities such as anticancer, antioxidant, cardiovascularprotective, neuroprotective, and anti-inflammatory activities, so that recent informations about bioactive lignans from edible plants may be available for the development of potential functional food agents. In this article, patents based information is also discussed.

**Keywords:** Lignan, edible plant, isolation, bioactivities, furfuran.

## 1. INTRODUCTION

Lignans are phenolic compounds widely distributed in plants [1-7].

Lignans are a class of secondary plant metabolites, which are produced from shikimic acid via the phenylpropanoid pathway, produced by oxidative dimerization of two phenylpropanoid units [1,2]. A larger part of lignans, present in different parts of plants, are found to be present as glycosidic conjugates of plants. Recent progresses in extraction method and structural elucidation contributed to accurate quantification of broad spectrum of lignans in edible plant sources [3,4,6,8]. Those techniques have been employed for a large scale preparation of lignans. Of edible plants, the most common dietary sources of mammalian lignan precursors are unrefined grain products. The highest concentrations of lignan have been found in flaxseed, followed by unrefined grain products, particularly rye and barley. Lignans are also found in the *Prunus* fruits or the leaves of edible plants such as *Petasites japonicus* [9,10]. The type of lignan differs according to the species and the amounts of lignans vary in different parts of the plants [3, 4, 6]. Representative furfuran type lignans from edible plants, such as matairesinol, secoisolariciresinol, lariciresinol and pinoresinol, are known to be converted by gut microflora to mammalian lignans, enterolactone or enterodiol [7,11,12], which show more beneficial effects on human health, compared to lignan precursors Fig. (1). Previous studies proposed that lignan- rich diet had benefits in decreasing the risk of breast or prostate cancer, and the risk of cardiovascular disease [13,14]. Further, they showed some antioxidant action and anti-inflammatory action. In addition,

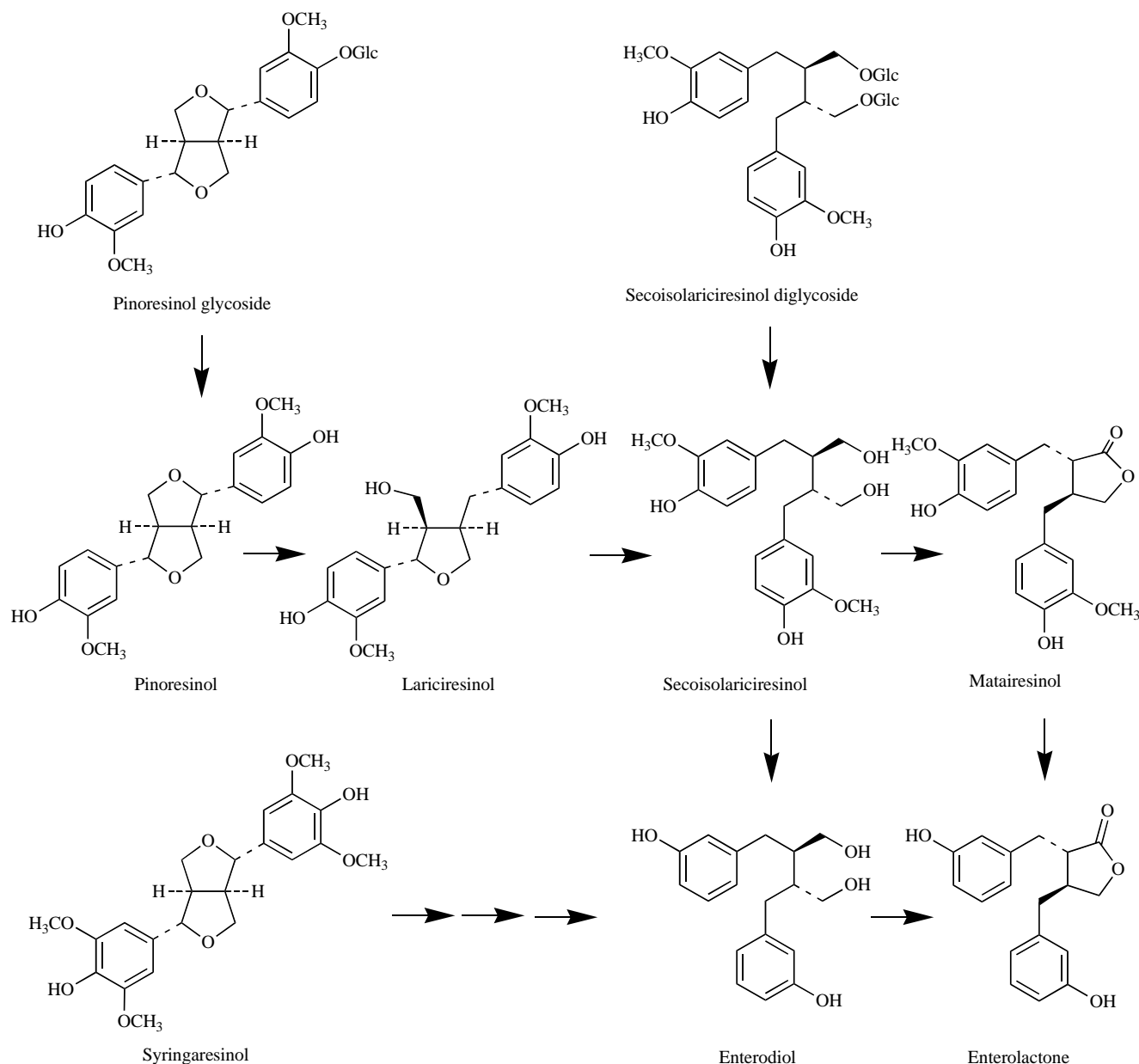
lignans might be of use in preventing oxidative stress in tissues such as brain or liver. However, the lignans containing phenolic hydroxyl groups are targets of phase II metabolic reactions, conjugation with glucuronic acid, sulfate or glutathione, which result in the decrease of pharmacological activity of lignans. Taken together, the biological efficacy of lignan diets may depend on the amount or type of lignans present in the plants. The aim of this review is to provide a list of furfuran type lignans originating from edible plants, and discuss about the preparation of these lignans and their bioactivities.

## 2. EXTRACTION AND PREPARATION

### 2-1. Separation of Lignans

Solvent extraction is a traditional method for extracting lignans from plant sources. However, other less polar components present in most plant tissues may interfere with the subsequent separation of lignan if a polar solvent is used. Therefore, the sequential solvent extraction is recommended for efficient separation of lignan compounds. For this purpose, lipophilic compounds are removed with non-polar organic solvents such as hexane or dichloromethane [15,16]. Meanwhile, the hydrophilic constituents including the lignans are extracted with polar solvents such as acetone, methanol or ethanol. In some cases, the addition of polar solvents such as water to the sample may increase the recovery of more polar compounds such as lignan glycosides [17]. Meanwhile, some lignans of low or medium polarity can be efficiently extracted with more non-polar solvent. Direct extraction with a hot polar solvent, appropriate for lignans of a low polarity, has also been used for extraction of some plant lignans [18-21]. However, the subsequent clean-up step to separate the lignans from the polar extract is to be considered in respect to conveniency and recovery. A recently-introduced method for extraction of plant lignans is the accelerated solvent extraction, which is carried out at

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**Fig. (1).** Proposed metabolism of furfuran type lignans, modified from a previous report [7] Glc, glucosyl group.

higher temperature and pressure and under inert nitrogen atmosphere. This method may enable fast and convenient extraction using relatively small amounts of solvent [16,22,23]. Lignans in some plant materials require special pretreatments before extraction. Polar lignans such as secoisolariciresinol diglycoside (SDG), present in the plant as ester-linked oligomers or polymers [24,25], seem to be readily soluble in aqueous methanol or ethanol. Nonetheless, the subsequent alkaline hydrolysis is required to release free secoisolariciresinol. Furthermore, in case of glycosides, additional hydrolysis steps, enzymatic or non-enzymatic, are used for the release of free aglycone [24,26]. Separately, several analytical procedures, including the combinations of enzymatic, acidic and alkaline hydrolysis, before or after extraction, have been developed to simplify the subsequent chromatographic analysis of extracts containing lignan glycosides.

## 2-2. Preparative Isolation of Lignans

Preparative enrichment and separation of individual lignans in pure form is demanded for the preparation and characterization of unknown lignans or the development of functional foods. The recent finding that knotwoods from some tree species contain large amounts of free aglycone lignans has provided the opportunity to isolate pure lignans in gram quantity [22,23,27]. Some lignan isolated in a large scale from spruce was used as a starting material for making other lignans [27,28]. The basic principle for isolating lignans in a large scale consists of the freeze-drying and grinding, the removal of lipophilic components using hexane extraction, and the extraction of hydrophilic portion with an acetone-water mixture or with ethanol. Pure lignans can then be obtained by flash chromatography on normal-phase silica columns and / or by solvent crystallization [15]. Although

flash chromatography is a convenient method for preparative isolation of selected lignans from raw extracts, the conditions of separation and purification need to be optimized. For the preparation of lignan-containing food, the employment of polar solvents such as ethanol or ethyl acetate is to be considered for the dietary safety.

In addition, various chromatographies such as open-column chromatography, medium-performance liquid chromatography (MPLC), semi-preparative HPLC, and preparative TLC have also been used to isolate lignans [19,26,29,30]. The choice of column, either normal-phase or reverse-phase, for preparative separation of lignans appears to be important in terms of yield. However, experience has shown that silica gel chromatography in some cases actually gives better separation than reverse phase chromatography [23,28]. Although the solvent of elution can vary according to the type of lignan, the solvent for final elution, suitable for the development of functional food, is to be considered.

### 2-3. Quantitative Analysis

As pure reference lignan standards have been available, the quantitative analyses have been advanced. Whereas the simple way to determine lignans is in aglycone form, some kinds of hydrolysis methods are necessary in order to release ether or ester-linked lignans [4,24]. Simply, after removal of non-edible parts from the fruits and vegetables, the solid foods were either directly chopped or cut into smaller pieces prior to freezing in liquid N<sub>2</sub>. After addition of the internal standards, the samples were consecutively submitted to alkaline and enzymatic hydrolysis with  $\beta$ -glucuronidase (*Helix pomatia*) to release the lignan aglycones. Then, the samples were extracted with diethyl ether, and subjected to liquid chromatography-tandem mass spectrometry analyses. Separately [6], the hydrolyzed extracts were purified by C<sub>18</sub> column separation and DEAE-Sephadex chromatography, and then the final samples were derivatized using silylation to be subsequently injected into the chromatographic system. Recent data indicate that the available databases largely underestimate the amount of enterolignan precursors in foods. In this respect, the quantitative analyses of lignans present in plants are to be assessed in consideration of the extraction yield of total lignans, free or bound forms.

## 3. REPRESENTATIVE EDIBLE PLANTS AS SOURCES OF LIGNANS

### 3-1. Flax

Lignans occur in a diversity of food sources such as nuts, grains, seeds, fruits and vegetables [4]. Flaxseed is potentially the richest source of phytoestrogens including lignans. The primary lignan found in flaxseed is 2,3-bis (3-methoxy-4-hydroxybenzyl) butane-1,4-diol (secoisolariciresinol) which is stored as the conjugate secoisolariciresinol diglucoside (SDG) in its native state in the plant [4]. Flax seed contains higher levels of this lignan than any other plant food [4,15]. One of the major problems associated with flax foods is the toxicity associated with cyanogenic glycosides, present in flaxseed, which may cause goitrogenic problems. Therefore, cyanogenic glycosides are to be removed during lignan extraction [31]. Defatting of flax meal with hexane is known to produce an enrichment of all individual cyanogenic glycosides [30]. Lignans have

originally been known to exist as components of a soluble ester-linked complex. Therefore, sodium and barium methoxides have been used for methanolysis to release lignans free of other compounds [32]. However, these processes suffer from drawbacks in that they can not be scaled up for commercialization [33,34]. For a large scale preparation of flax lignans, materials such as flax, flaxseed or defatted flax are extracted with alcohol at a suitable temperature to minimize growth of microbes and facilitate extraction of the lignan complex from plant material [4]. The slurry containing lignan complex was subjected to filtration or centrifugation, and the resulting aqueous portion is then dried to obtain a powder. Ultrafiltration is useful to remove cyanogenic sugars and other undesirable compounds. In further purification, the concentrated lignan sample is subjected to liquid-chromatography with methacrylic polymer resin, which is eluted with ethanolic water. The treatment of the lignan fraction with 0.1% sodium hydroxide (pH 7.6), followed by acid treatment (pH 3.5), gave rise to the bottom precipitate, which contains lignans devoid of cyanogenic glycosides.

There have been reports on the phytochemical benefits of flaxseed lignans. Feeding with purified lignan at 5% flaxseed diet levels significantly reduced colon and mammary carcinogenesis in animals [35]. Additionally, flaxseed supplementation might have a beneficial effect on prostate cancer biology [36]. Separately, it was reported that flaxseed lignans prevented the development of Type I and Type II diabetes [37]. Especially SDG, a major lignan in flax, was reported to delay the onset of type I and II diabetes in an *in vivo* rat model due to its antioxidant action [38,39]. In addition, flaxseed lignans acted as a hypotensive agent with ability to lower blood pressure [40], provided benefits against Lupus Nephritis [41], and reduced the development of hypercholesterolemic atherosclerosis in animals due to a lipid-lowering action [42], along with the potential antioxidant activity [43,44]. Separately, the expressed bioactivity of secoisolariciresinol diglucoside was suggested to be related mainly to the conversion of secoisolariciresinol diglucoside to enterolignan *in vivo* system. An epidemiological study indicated that high serum ENL level was related to a reduction in the mortality caused by cardiovascular disorders [45]. Related studies suggested that ENL displayed a higher biological activity than its plant precursors [44]. The biological activation of secoisolariciresinol diglucoside to enterolignans is a prerequisite for the release from the plant matrix after ingestion and bioactivation through gut microbial action [46-48]. In flax seed, secoisolariciresinol diglucoside is present as a macromolecular structure bound by hydroxymethyl glutaric acid [49]. Deglycosylation of secoisolariciresinol diglucoside can be performed in the small intestine either by enzymatic activity present in the brush border of the gut mucosa or by bacterial enzymatic activity [50]. Taken together, the possible factors responsible for the bioactivation of lignans in the large intestine are diet, transit time, intestinal redox state, and the type and activity of the colonic microflora [51].

### 3-2. Sesame

In consideration of total lignan content, the sesame seeds still remain the most lignan-rich species among foodstuffs.

Noteworthy, the content of sesame lignans including the lipophilic sesamin type lignans [4] is higher than that in linseeds. Sesamin, one of the principal lignan compounds in sesame, is contained in sesame seeds in amounts of 0.1-0.5%. In contrast, episesamin, a stereoisomer of sesamin, does not naturally occur in sesame seeds. During purification steps, sesamin undergoes epimerization to give episesamin as a by-product, and sesamins refined from sesame oil are known to contain sesamin and episesamin in proportions of nearly 1:1 by weight ratio [52]. Lipid-soluble lignans such as sesamin and sesamolin were isolated from sesame using a reverse-phase column. Similarly, sesaminol glucosides were isolated from the same source [53]. For isolation of lignans from defatted oil seed, ground and defatted oil seeds were extracted with methanol. Then, methanol extracts were further fractionated using a sequential solvent elution on silica gel column. The lignan-rich fraction was further purified by preparative high-performance liquid chromatography (HPLC) system. A procedure for the large scale extraction from oil seeds employs the extraction of roasted and defatted oil seeds with hot water, and subsequent separation of lignans on Diaion HP 20 open column using 60% ethanol. For quantitative determination of lignans in sesame seeds, a two-step method applying the combination of methanolysis and enzymatic hydrolysis was employed to generate aglycone form. However, the accurate determination of lignans in sesame seeds was interfered probably due to a varying degree of esterification in different sesame seed samples [4]. For the refinement of sesamin and episesamin, a reverse-phase HPLC with Develosil ODS-UG-5 column, eluted by acetonitrile gradient was utilized [54]. A variety of bioactivities were expressed by refined sesamins [55]. They improved hepatic function [56], alleviated alcoholism and symptoms of withdrawal [57], showed anti-inflammatory and infection-protective action [58], and inhibited  $\Delta 5$ -unsaturation enzymes [59,60]. In addition, sesame oil containing sesamin revealed the actions of suppressing migraine [61] and sesamin had an autonomic nerve system-modulating activity [62]. In comparison, there was no significant difference between sesamin and episesamin in terms of activity for inhibiting fatty acid synthases [63]. Taken together, the mixture of sesame lignans might be utilized for the preparation of functional foods as well as pharmaceutical preparations.

### 3-3. Rye and Barley

Recent studies have shown that lignans are present in cereal foods; they account for no less than 5% of the weight in rye and wheat bran, which has the highest lignan content of all cereals. Especially, 7-hydroxymatairesinol, sensitive to destructive extraction, is the dominant lignan in wheat, whereas syringaresinol is dominant in rye [64]. Dry cereal sample was extracted with polar solvent at high temperature, and subjected to enzymatic hydrolysis in the presence of digesting enzymes, which hydrolyze glycoside forms of lignans in cereal brans. For the increase of recovery yield, the acidic or alkali hydrolysis, preceded by enzymatic hydrolysis, was necessary. For the determination of lignan content in sample, GC-MS [65] or LC/MS [4] has been employed. The combination of alkaline and acid extractions gives the highest yield of secoisolariciresinol and hydroxymatairesinol, concentrated in the bran layer of the

grain of rye and wheat [4], since these lignans seem to occur more in esterified forms in cereals than other lignans. Generally, there was a strong association between the intake level of plant lignans and the circulating level of enterolignan [66]. Noteworthy, the bioavailability of secoisolariciresinol and matairesinol was much higher than the mean bioavailability of lariciresinol, pinioresinol and syringaresinol. Thus, conversion of mammalian lignan precursors to enterolactone seems to depend on the amount of total lignans as well as the type of lignans present in cereal grains.

### 3-4. Adlay

An activity-guided fractionation [67] was used to identify the antioxidative components from hulls of adlay (*Coix lachryma-jobi* L.). In the sequential solvent fractionation, the butanol-soluble fraction exhibited the greatest capacity to scavenge 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radicals. This may be in contrast to the highest antioxidant activity of ethyl acetate fraction among butterbur leaves extracts [68]. One lignan, responsible for antioxidant action of butanol extract of seed hulls, was identified to be syringaresinol, which showed a strong DPPH radical scavenging action with EC<sub>50</sub> value of approximately 59  $\mu$ M, and the other lignan, 4-ketopinioresinol, showed a weaker inhibition.

### 3-5. Prunus

Prunes are the dried fruits of some cultivars of *Prunus domestica* L. (Rosaceae), originating from the Caucasus region in western Asia, recognized as a healthy food (9). In addition, the fibers from *Prunus amygdala* [69] are used for the preparation of lignans. Pitted prune fruits were homogenized with 90% aqueous ethanol [9] to produce an extract, which was partitioned between hexane and water. The water-soluble fraction was further separated by Diaion HP-20, and the MeOH eluate was rechromatographed on Sephadex LH-20 gel and ODS columns. Subsequent rechromatography of the lignan fraction over Sephadex LH-20 on silica gel columns afforded two antioxidant lignans, pinioresinol monoglucopyranoside and 3-(beta-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2R,3S)-dihydrobenzofuran, which possessed oxygen radical absorbance capacity (ORAC) values of 1.09 and 2.33 micromol of Trolox equiv/micromol, respectively [9].

### 3-6. Butterbur

The leaves of *Petasites japonicus* Maxim., a perennial plant widely grown in Japan and Korea, have been used as an edible vegetable. Recent studies showed that petasinophenol [70] and flavonoid glycosides [71], isolated from *P. japonicus*, inhibited eukaryotic DNA polymerase rhamda and DNA polymerase alpha, respectively. In the course of screening antioxidant components, the ethyl acetate fraction and butanol fraction of leaves of *P. japonicus* extracts demonstrated a remarkable antioxidative action in DPPH radical scavenging assay [72]. Neuro-protective bio-activity-guided fractionation indicates that, the neuro-protective compound, purified using reverse phase column and silica gel column, is a new lignan, named petasignolide A [73]. The amount of petasignolide A, isolated from leaves of *Petasites japonicus*, is estimated to

be 0.0143 mg per g dry leaves. Further, the lignan compound was treated with naringinase or  $\beta$ -D-glucosidase to obtain an aglycone compound, 9-hydroxypinoresinol [74], and a sugar derivative, identical to that of authentic D-glucose. In DPPH radical scavenging assay, 9-hydroxypinoresinol was at least twice more potent than petasignolide A. In animal experiment, the butanol fraction showed the greatest neuroprotective action against seizure and mortality caused by kainic acid. Especially, the prevention by butanol extract against kainic acid induced cytotoxicity in C1 and CA3 domains of hippocampus was confirmed [72]. In further study, the neuroprotective action was found to be mainly due to petasignolide A, present in butanol fraction. This was further confirmed by oral administration of petasignolide A, which delayed the onset time of seizure and increased the survivability [72-74]. Moreover, 9-hydroxypinoresinol, the hydrolysis product of petasignolide A, was more potent than petasignolide A in neuroprotection [74]. Consistent with the above finding, 9-hydroxypinoresinol and petasignolide A appeared to alleviate oxidative stress-related biochemical parameters in brain tissue as well as liver tissue. Thus, the extract from butterbur leaves is suggested to be neuroprotective against oxidative stress in brain tissue [75].

### 3-7. Other Foods

Dietary lignans are present at considerable concentrations in fiber-rich foods, e.g., whole grain products [3,76]. Interestingly, after ingestion, most dietary lignans undergo an extensive transformation by the gut microflora, leading to the formation of enterolignans. The estimation of secoisolariciresinol and matairesinol amount in foods [77-79] provided a basis for the estimation of lignan intake [80-82]. However, as databases including values for secoisolariciresinol, matairesinol, lariciresinol, and syringaresinol have been collected from extensive studies, the assessment of lignan intake are to be modified. For this purpose, liquid chromatography-tandem mass spectrometry has been used to quantify lariciresinol, pinoresinol, secoisolariciresinol and matairesinol [3]. The richest source of lignans was flaxseed (approximately 301 mg/100 g), which contained mainly secoisolariciresinol. Also, the lignan concentrations in sesame seeds (approximately 29 mg/100 g, mainly pinoresinol and lariciresinol) were relatively high. For grain products, the lignan concentration ranged from 7 to 764  $\mu$ g/100 g. The total lignan content of cereals species can be in the following order ; rye > wheat > oat > spelt wheat > Japanese rice > wild rice > buckwheat > barley > amaranth > corn > millet > red rice > brown rice . However, the lignan content in wheat exceeds that in rye when the alkaline-extractable portion of 7-hydroxymatairesinol is considered [4]. Also, *Brassica* vegetables contained unexpectedly high levels of lignans (185-2321  $\mu$ g/100 g), mainly pinoresinol and lariciresinol. In most cases the amount of lariciresinol and pinoresinol was greater than that of secoisolariciresinol and matairesinol. However, the alkaline extraction, followed by acid extraction, resulted in an increased yield of most of the lignans, indicating that lignans could be linked to other compounds. Thus, the combination of alkaline and acid extraction gives the highest yield in case of almost all other cereal species. Although the difference of lignan content may be at least partly due to difference in analytical methods, it is also possible that the content of some lignans may vary

within the same species depending on factors such as genetic factors or growth conditions.

## 4. GENERAL BIOACTIVITIES

There have been reports that furfuran lignans from edible plants possess a variety of bioactivities as demonstrated in Table 1.

### 4-1. Antiatherosclerosis Effect

Lignan complex from flax seed was suggested to be beneficial in preventing atherosclerosis, and reducing risk factors for coronary artery disease and stroke [82]. Concerned with this, there are epidemiological studies on the associations between enterolignan concentrations in biological fluids or the intake of plant lignans and chronic disease risk. In case-control studies, there was an inverse associations of serum lignans with cardiovascular diseases in Finnish studies [83,84]. The lignan secoisolariciresinol diglucoside from flaxseed has been shown to be effective in decreasing serum cholesterol and reducing the extent of atherosclerosis in the hypercholesterolemic rabbit [85]. Additionally, flaxseed lignan secoisolariciresinol diglucoside (SDG) was suggested to prevent and alleviate hypercholesterolaemic atherosclerosis by inducing adiponectin mRNA expression and showing beneficial effects on lipid metabolism in diet-induced obesity in mice [86]. A very recent study indicates that enterolactone induces HO-1 in HUVEC in a time- and concentration-dependent manner presumably via the transcription factor Nrf2, which may contribute to its vasculoprotective effects [87]. Nonetheless, it was reported that flax lignan complex failed to produce regression of atherosclerosis [88]; the inhibitory effect of flax lignan on atherosclerosis acceleration was suggested to be ascribed to the removal of cholesterol in the diet.

### 4-2. Antitumor Activity

Metastasis is a major cause of morbidity and mortality in breast cancer, and the invasion of tumor cells may play a crucial role in the metastatic process. While some lignans inhibited the invasion of a breast cancer cell line (MDA-MB-231) [89], secoisolariciresinol and its metabolite enterodiol induced a significant decrease in cell invasion at relatively high concentrations [90]. Meanwhile, the mammalian lignans enterodiol and enterolactone were observed to show an inhibitory effect on breast and colon carcinoma [91]. Matairesinol also expressed a potent cytotoxic effect on human promyelocytic leukemia HL-60 cells [92], and in mechanistic analysis, matairesinol-induced cell death was suggested to be associated with DNA damage and apoptosis. In an animal experiment, hydroxymatairesinol showed an antitumor activity by expressing a statistically significant inhibitory effect on tumor growth in 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary cancer [5,93]. Anticarcinogenic effects of dietary hydroxymatairesinol were also evident when administration started after DMBA induction. Hydroxymatairesinol was relatively non-toxic when given to animals as well as healthy male volunteers. Moreover, hydroxymatairesinol had no estrogenic or anti-estrogenic activity at 50 mg per kg weight in immature rats. Separately, it has been suggested that PLC 1 plays a key role in the proliferation and progression

**Table 1. Summary of Furfuran Type Lignans Demonstrating Biological Activities**

Compound	Plant Source	Bioactivity	Ref.
Secoisolariciresinol	Flax, sesame	Antioxidant	[44]
	Cereal grain	Anticancer	[13,90]
Secoisolariciresinol diglucoside	Flax, sesame	Antioxidant activity	[44]
	Cereal grain	Anticancer, Antiapoptotic	[13,88]
		Antiathersclerosis	[85,86]
		Diabetes	[38,39]
		Hypotensive	[40]
Matairesinol	Sesame, flax	Antioxidant activity	[13]
Hydroxymatairesinol	Cereal grain	Anticancer	[92]
		Anticancer	[93]
Lariciresinol	Sesame, flax, vegetable	Anticancer	[13]
		Anti-inflammatory	[97,98,100]
Pinoresinol	Sesame, flax, cereal grain vegetable	Anticancer	[13]
		anti-inflammatory	[97, 98,101]
Pinoresinol glucoside	Prunes	Antioxidant activity	[9]
		Anti-inflammatory	[98,100]
Syringaresinol	Adlay, Sesame	Antioxidant	[67,104]
	Cereal grain	Anti-inflammatory	
4-Ketopinoresinol	Adlay	Antioxidant activity	[67]
Sesamin	Sesame	Antioxidant	[103,104,108]
		Antiproliferative effect	[95]
		Hepatoprotective	[58]
		Anti-inflammatory	[62]
Sesamolin	Sesame	Antioxidant	[108]
Petalsignolide A	Petasites	Antioxidant, neuroprotective action	[73,76]
9-Hydroxypinoresinol		Neuroprotective action	[74]

of human cancer [94]. Noteworthy, sesamin [95] exhibited a dose-dependent inhibition of PLC $\gamma$ 1 activity. Presumably in support of this, the IC<sub>50</sub> values of sesamin in the inhibition of cancer cell lines such as A549, MCF-7 and HCT-15 were close to IC<sub>50</sub> values of seamin in the inhibition of PLC $\gamma$ 1. Thus, it is supposed that inhibition of PLC $\gamma$ 1 may be one of mechanisms responsible for antiproliferative effect on the human cancer cells. However, antitumor activity of flax lignan is to be evidenced extensively in clinical tests.

#### 4-3. Anti-inflammatory Action

Carrageenan-induced rat paw oedema is a widely used model to investigate the pathophysiology of an acute local inflammation [96]. Recently, the carrageenan-induced rat paw oedema formation has been frequently employed in the screening of anti-inflammatory agents. Lariciresinol and isolariciresinol expressed their anti-inflammatory activities by significantly inhibiting carrageenan-induced hind paw edema in mice [97]. These were supported by their potent *in*

*vitro* inhibitory effect on the production of TNF- $\alpha$ , a pro-inflammatory cytokine [98]. In addition, lariciresinol glycoside, pinoresinol, pinoresinol glycoside and syringaresinol glycoside also showed anti-inflammatory effects [98]. Macrophages and lymphocytes, playing an important role in host immune responses, are proliferated and activated by inflammatory signal compounds, such as lipopolysaccharide (LPS). As a result, they secrete pro-inflammatory mediators such as cytokines (TNF- $\alpha$ , ILs) and lipid mediators (prostaglandin E and leukotriene B), as well as reactive oxygen and nitrogen intermediates [99]. Isolariciresinol, lariciresinol glycoside, pinoresinol, pinoresinol glycoside and syringaresinol glycoside were found to significantly inhibit TNF- $\alpha$  production from mouse macrophages [100]. In addition, pinoresinol and syringaresinol glycoside showed significant suppressive effects on NO production triggered by LPS. However, lignan compounds seemed to interfere with biosynthetic pathway for TNF- $\alpha$  production, rather than NO formation, in activated macrophages. In related experiment to see the

effect of lignan on concanavalin A or interleukin-2 - induced lymphocyte proliferation, syringaresinol glycoside potently inhibited T lymphocyte proliferation induced by concanavalin A or interleukin-2. In addition, pinoresinol showed a significant inhibitory effect on cytokine production from LPS (or phytohemagglutinin)-stimulated human peripheral mononuclear cells [101].

#### 4-4. Antioxidant Action

Most of lignans containing hydroxyl group was suggested to exhibit antioxidant action according to the number or position of hydroxyl group. In Trolox-equivalent antioxidant activity (TEAC) and chemiluminescence (CL) assays, 8-hydroxypinoresinol glycoside and 8-hydroxypinoresinol showed high antioxidant properties [102]. The aglycone hydroxypinoresinol displayed more powerful antioxidant activity than pinoresinol. Likewise, aglycone 9-hydroxypinoresinol was more potent than its precursor, petasalignolide A [74]. Thus, the antioxidant action of pinoresinol derivatives depends on the number of hydroxyl group in the structure. The antioxidative function of sesamin on exercise-induced lipid peroxidation was observed in animals using strenuous physical exercise as a trigger for oxidative stress [103]. Further, sesamin, scavenging free radicals, exerted a strong protective effect against exercise-induced lipid peroxidation. Separately, syringaresinol and sesamin, isolated from Chinese propolis, were observed to inhibit lipid peroxidation in rat liver microsomes potently [104]. Consistent with this, sesamin exhibited an antioxidative effect on lipid and alcohol metabolism in the rat liver. Further, sesamin and sesaminol elevated -tocopherol concentration and decreased thiobarbituric acid-reactive substance (TBARS) level in the blood plasma and liver of rats. In a separate experiment [105], sesamin was more effective than sesamol in reducing serum and liver lipid levels while sesamol is stronger in increasing hepatic fatty acid oxidation.

#### 4-5. Neuroprotective Action

Some lignans, with antioxidant activity, were observed to express a neuroprotective action in excitotoxin-induced neurotoxicity in rat cortical or hypoxic neuronal cells [106-108]. Furthermore, the antioxidant action of lignans from edible plants was extended to their neuroprotective action in animal experiments. Oral administration of 9-hydroxypinoresinol and its glycoside, petasalignolide A, showed a protective effect on the seizure and mortality caused by kainic acid [73,74]. In addition, these lignans successfully prevented the loss of the GSH peroxidase activity and the lipid peroxidation in brain tissue, which was exposed to kainic acid, an excitotoxin. In comparison, 9-hydroxypinoresinol, a metabolite of petasalignolide A, was more effective than its precursor glycoside, petasalignolide A in preventing kainic acid-induced neurotoxicity [72-74]. Under the same condition, quercetin or pinoresinol, despite their antioxidant action, showed no significant effect on the seizure and mortality caused by kainic acid. Thus, petasalignolide A and its aglycone, 9-hydroxypinoresinol seems to have antioxidant activity in brain tissue, and thereby exert a neuroprotective effect. Thus, the extract containing 9-hydroxypinoresinol derivative may be usefully used in the prevention and treatment of neurodegenerative diseases. Taken

together, antioxidant action of lignans is supposed to be responsible for various bioactivities of lignans, since cellular oxidative stress is intimately linked to disease states such as carcinogenesis, inflammation, or atherosclerosis. However, other bioactivities of lignans are not necessarily related to the number of hydroxyl group, suggesting that the antioxidant action may not be necessarily required to the expression of various bioactivities.

### 5. CURRENT & FUTURE DEVELOPMENTS

This review covers preparation of lignans from edible plants and biological activities of furfuran type lignans. Recently-introduced methods for extraction of plant lignans, such as accelerated solvent extraction, preceded by enzymatic or non-enzymatic pretreatments, is used for the convenient preparation of lignans. For a large scale preparation of lignans from freeze-dried plants, extraction of hydrophilic portion with polar solvents at high temperature, followed by flash chromatography, is recommended. Representative furfuran type lignans were introduced in this review in respect to bioactivities. They were found to have antioxidant, anticancer, antiatherosclerosis, anti-inflammatory, and neuroprotective activities in animal experiments, although extensive studies to prove the clinical efficacy remains to be carried out. Antioxidant activity of lignans seems to depend on the number or position of hydroxyl group in the structure of lignans; hydroxypinoresinol displays more powerful antioxidant activity than pinoresinol glucoside or pinoresinol. In this respect, the metabolism of lignans to enterolignans *in vivo* may be implicated in the practical bioactivities of lignans. However, other bioactivities of lignans are not necessarily related to the number of hydroxyl group, suggesting that antioxidant action is not necessarily required for the expression of various bioactivities. Consistent with this, recent studies indicate that bioactivities of lignans may be expressed through various mechanisms in addition to antioxidant action. The lignan preparations can be provided preferably in crude lignan extract from edible plants, or in compositions of isolated lignans and additional antioxidant compounds. Also, the compositions can be in any form suitable for use in supplementing the diet as a functional food.

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