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High value valorization of lignin as environmental benign antimicrobial

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ABSTRACT

Lignin is a natural aromatic polymer of *p*-hydroxyphenylpropanoids with various biological activities. Noticeably, plants have made use of lignin as biocides to defend themselves from pathogen microbial invasions. Thus, the use of isolated lignin as environmentally benign antimicrobial is believed to be a promising high value approach for lignin valorization. On the other hand, as green and sustainable product of plant photosynthesis, lignin should be beneficial to reduce the carbon footprint of antimicrobial industry. There have been many reports that make use of lignin to prepare antimicrobials for different applications. However, lignin is highly heterogeneous polymers different in their monomers, linkages, molecular weight, and functional groups. The structure and property relationship, and the mechanism of action of lignin as antimicrobial remains ambiguous. To show light on these issues, we reviewed the publications on lignin chemistry, antimicrobial activity of lignin models and isolated lignin and associated mechanism of actions, approaches in synthesis of lignin with improved antimicrobial activity, and the applications of lignin as antimicrobial in different fields. Hopefully, this review will help and inspire researchers in the preparation of lignin antimicrobial for their applications.

1. Introduction

Lignin is one of the three major components of plant cell wall, composing of 10–40% of the dry mass of lignocellulosic biomass. It is conventionally released as waste from biomass refinery industries. Annual production of lignin worldwide from paper and pulp industry is about 70 million tons [1]. Currently, ninety-eight percent of industrial lignin is burnt for fuel while the other 2% is isolated and commercially utilized [1]. In respond to climate changes, the biomass refinery industrial is steadily increasing [2], which produces a large amount of lignin residues. It is estimated that global production of lignin will increase to 225 million tons per year as a byproduct of biofuel production by 2030 [3]. As a result, lignin valorization is receiving increasing interests [4].

Lignin is an aromatic heteropolymer, which has an important role in plant defense against pathogen invasion [5,6]. The major intermediates of monolignol and lignin biosynthesis are shown to be antimicrobial compounds against a wide range of yeasts and bacteria [7,8]. The deposition of lignin into plant cell wall is shown to spatially restrict, which can encompass bacteria in the extracellular space [9]. In addition, many reports have revealed that isolated lignin is an environmentally friendly and biocompatible bioactive polymer [10–12], which promotes the application of lignin as biological materials.

Therefore, there have been increasing interests to take advantage of the bioactivities of lignin for biological material production [13–15]. Many reviews have discussed the biomedical and biotechnological applications of lignin [15–17], such as delivery vehicles [18], bioimaging [19], tissue engineering [20], wound healing [21] et al. One of the initial driving forces of converting lignin into biomaterials is originated from its nontoxic antimicrobial feature. The intrinsic antimicrobial properties of lignin make it a desirable biomaterial because microbial pathogens

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constitute a grave threat to the health of humans. As such, lignin has been long explored as antimicrobial compounds. The use of lignin as antimicrobial compounds has at least three benefits. Firstly, providing biocompatible, and environmentally benign antimicrobials to address cytotoxicity and antimicrobial resistance issues of conventional antimicrobial formulas. Secondly, opening new windows for high value application of condensed lignin, which is believed to be the major challenge for lignin variolization. Thirdly, reducing the carbon footprint of antimicrobial industry.

There are some publications that have reviewed the antimicrobial properties of lignin [10,11,22–25]. Those publications are focused on the applications of lignin as antimicrobial materials in different fields [10,16,23,24]. However, lignin is a highly heterogeneous polymer. The structure of lignin may be altered by its plant sources [26–28], isolated methods and process parameters [29,30], post-treatments [31], and chemical modifications [14,32]. Though there are reviews discussing the influence of extraction methods on antimicrobial activities of lignin [22], and antibacterial lignin-based nanoparticles [16,25], there is not any reviews relating the antimicrobial properties of lignin to its chemistry structure. To shed light on the synthesis of lignin and lignin derived materials with tailored antimicrobial activity, we reviewed the relationship between chemical structure and antimicrobial activity of lignin, and the chemistry behind the technologies in tailoring antimicrobial properties of lignin. Also, we discussed the principles of lignin chemistry associated with its applications in a various of fields. This review provides the community with a base understanding of lignin chemistry to synthesize novel lignin derived antimicrobial materials.

2. Descriptors of lignin chemistry

Lignin is a large group of natural polyphenylpropanoids. The most salient characteristics of lignin is heterogeneity, which makes it impossible to describe lignin as simple as polyethylene described by $-(CH_2-CH_2)_n-$. Many previous reviews have tried to comprehensively discuss the chemical structure of lignin [33–39]. Herein, we chemically descript lignin with monomers, linkages, functional groups, and molecular weight as the parameters.

2.1. Lignin monomers

There are 35 monomers found to be naturally incorporated into lignin with coniferyl alcohol (G), sinapyl alcohol (S), 4-hydroxycinnamyl alcohol (H), and their conjugates being the most primary ones [40]. Novel monolignols continue to be discovered, such as tricin [41], caffeyl alcohol [42], piceatannol, monolignol benzoates [35,43].

Fortunately, a specific plant uses only a few of those monomers to constitute its lignin (Fig. 1A). Generally, lignin from softwood species is primarily constituted of G units with traces H units, although H units can be up to 30% in compression softwood [44]. Lignin of conventional hardwood species is constituted of G, and S units with trace H units. Specially, lignin of *Salicaceae and Araliaceae* family is constituted of G, S units and their *p*-hydroxybenzoates (*p*HB), primary of sinapyl *p*-hydroxybenzoate (S-*p*HB) [45–47]. The *p*HB level can be up to 8.6% of lignin of *Populus* species [48]. Besides, kenaf extensively uses 4-hydroxycinnmayl acetates as monomer in its lignin biosynthesis [49]. Studies of isolated

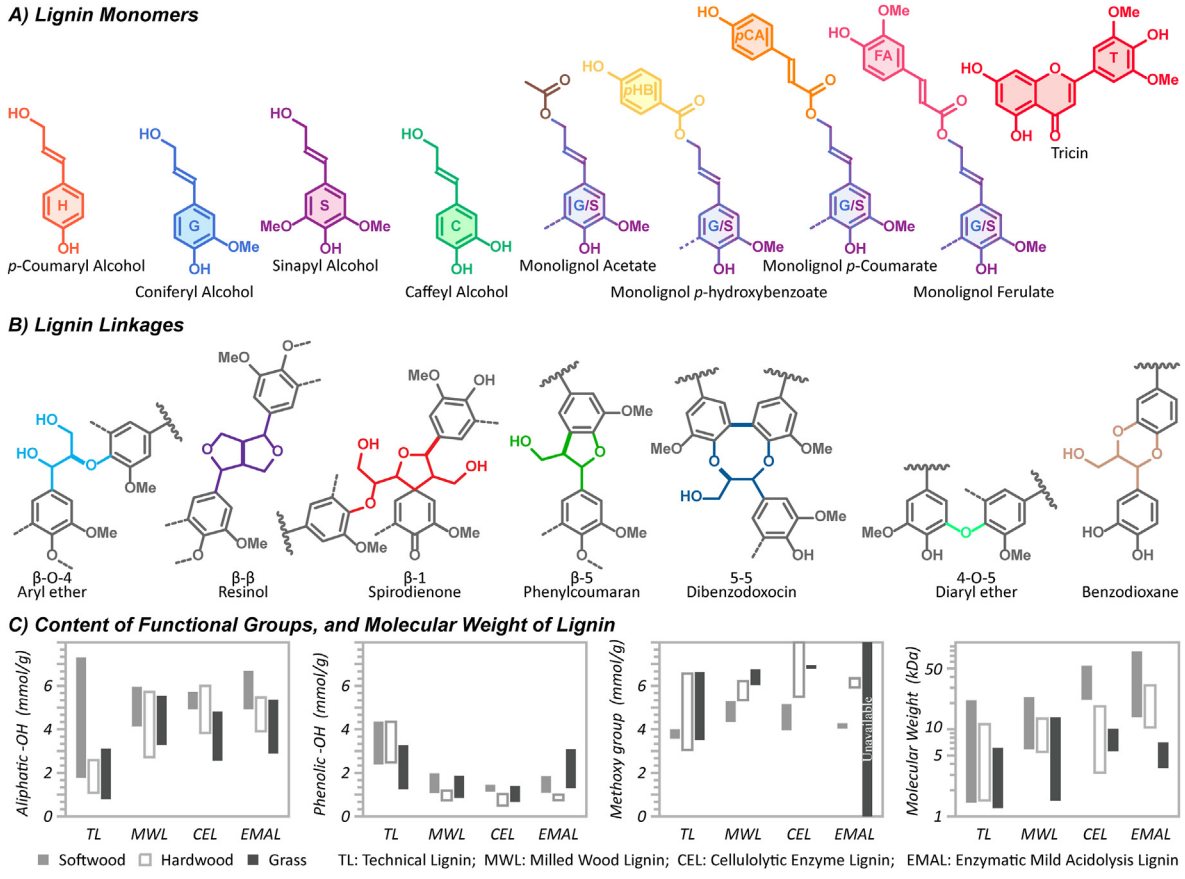

Fig. 1. (A) Conventional lignin monomers; (B) Well identified lignin linkages, (C) Distribution of functional groups and molecular weight (Mw) of lignin [57–76].
Notes: a. Throughout the whole text, in case of otherwise defined, it means the weight average molecular weight by molecular weight (Mw).

kenaf lignin suggests over 50% lignin acetylation, predominantly on S unit [50,51].

Lignin of monocots may compose of various monomers, including the G and S units, hydroxycinnamates, and tricin. Monolignol *p*-coumarate (*p*CA) and ferulate (FA) conjugates are the primary hydroxycinnamates which can be found in various monocot biomass, such as corn stover, wheat straw, rice straw, sugarcane bagasse, and switchgrass [33]. The *p*CA usually attaches to lignin polymer as pendent γ-functional groups, but not incorporate into the lignin polymer chains [33]. Tricin is also generally found as ending group of lignin from grass biomass [41]. Typically, the content of tricin can be up to ~5% in lignin of wheat straw [52,53].

Another interesting lignin monomer is caffeyl alcohol (the C unit). The C-lignin coexists with G/S lignin but independently deposits into plant seed coats [54]. Typically, lignin seed coats of vanilla compose of 100% C-lignin [42]. This C-lignin is a linear benzodioxane polymer which is highly acid-resistant and believed to be an ideal lignin for full biomass utilization [55,56].

The biosynthesis of lignin is known as highly plastic which is capable of making uses a wide range of hydroxyphenylpropanoids [77]. However, the other lignin monomers are usually minorities as lignin constitutions presented only in a special plant or in a specifical section of a plant, such as hydroxystilbene in Norway spruce bark [78]. Readers interested in these monomers are referred other reviews [35,40]. On the other hand, researchers show great passion in harnessing the plasticity of lignification to design lignins that are ready for biomass refinery, such as the Zip-lignin [79], and the curcumin lignin [80,81].

2.2. Lignin linkages

The polymerization of monolignols into lignin is known to follow a free radical coupling mechanism. There have been some excellent reviews on the free radical coupling of monolignols [34,82]. It is a pure chemical process, resulting in a couple of lignin linkages. Those well identified lignin linkages (Fig. 1B) include the β-O-4, β-β, β-5, β-1, 5-5, and 4-O-5 linkages [34]. All these linkages can be found from the G-lignin (lignin of softwood) with β-O-4 linkage being the predominant one, while only the β-O-4, β-β, and β-1 linkages can be found from S-lignin because of the substitution of aromatic C-5 position of S unit by methoxy groups [34]. However, plants of hardwood and monocot copolymerize both G and S units to constitute their lignin, which may involve the S unit into linkages of β-5, 5-5, and 4-O-5 because of cross coupling with the G unit. The C-lignin composes of 100% benzodioxane linkage, leading to a linear homopolymer [83].

These lignin linkages are generally found in native lignin, but extensively modified by biomass fractionation processes, such as the kraft pulping. Most biomass fractionation processes associate with extensive cleavage of β-O-4 linkage which results in condensed technical lignin, although a few are developed to produce protected lignin or native-like lignin [84]. The chemistry of technical lignin is particularly complex and lignin linkages remain largely unknown. Only approximately 45% of the kraft lignin structure can be assigned with 27% of which being native lignin linkages [85]. The linkages in isolated lignin will be further discussed in the following section of "Chemical structure of isolated lignin".

2.3. Functional groups of lignin

As the linkages in conventional technical lignin remain largely unknown, the functional groups in lignin are generally applied to characterize technical lignin. The most important functional groups in lignin include aliphatic hydroxy groups (-OH), phenolic –OH, methoxy groups and the aromatic ring. Depending on plant sources and isolation method, the content of functional groups in lignin can vary over a wide range (Fig. 1C). A survey of 17 lignin samples showed that the content of aliphatic –OH is 1.5–6 mmol/g, and the content of phenolic –OH is 1–4.5 mmol/g in lignin [86]. The content of methoxy groups in lignin is 3–8 mmol/g [70,87,88]. The presence of these functional groups opens windows to convert lignin into valuable advanced functional materials. For example, tough and adhesive hydrogels were synthesized from de-methylated lignin [89].

2.4. Molecular weight of lignin

In addition, molecular weight is another important characteristic of lignin (Notes: Throughout the article, weight average of molecular weight is default with kDa as the unit). The molecular weight of lignin is usually in the range of 1–50 kDa [60]. The molecular weight of lignin can be significantly altered by the isolation processes, the sources of plant species, and post refinery processes (Fig. 1C). There has been an excellent review [60] addressing the issues associated with molecular weight of lignin, including techniques for determination of molecular weight of lignin, impact of biomass sources and isolation method on molecular weight of lignin, and altering molecular weight of lignin by genetic regulation of lignin biosynthetic pathways.

3. Chemical structure of isolated lignin

Naturally, lignin is incorporated into plant cell wall together with cellulose and hemicellulose. Biomass fractionation is prior to make use of this abundant biomaterial. There have been many processes developed to fractionate biomass into pulp and isolated lignin. The isolated lignin is usually named according to the method of biomass fractionation, such as kraft lignin from the kraft pulping process. Although, there are more isolated lignins than we can cover, and we avoid discussing all the isolated lignins. Kraft lignin, soda lignin, lignosulfonate, and organosolv lignin represent the most important available lignins, we will review the chemical structure of these lignin. In addition, as biomass fractionation processes continue to emerge, we also discuss these emerging lignins separately.

3.1. Kraft lignins

The kraft pulping process utilizes sodium hydroxide and sodium sulfide solution to separate lignin from the pulp under elevated temperature (150–170 °C) [90]. It is estimated that 1 M NaOH aqueous solution at 170 °C has roughly the same deprotonating capabilities as $NaNH_2$ (pKa 35) at room temperature [90]. Under such cooking conditions, the chemical structure of lignin undergoes dramatic changes. The formation of a quinone methide by loss of an anion from the C_α position of a phenolate ion is the key step (Fig. 2A). Following, the quinone methide may undergo several reactions to give both lignin fragments and recondensed polymers. In the study by Lancefield et al. [85], it is revealed that native type lignin linkages account for 23–27% and kraft derived linkage for 17–18% of kraft lignin. A total of approximately 45% of the lignin structure can be assigned with the others remain unknown [85]. Even worse, in the study by Crestini et al. [91], the native lignin linkages of β-O-4, β-5, and β-β in softwood kraft lignin were only determined to be 3.19%, 0.83%, and 2.43%, respectively, by the-state-of-art quick quantitative HSQC method. Gellerstedt reviewed the chemical structure of softwood kraft lignin [92]. It is shown that Norway spruce kraft lignin comprises β-O-4, β-5, β-β, 5-5, 4-O-5 linkages of 8%, 3%, 2%, 18%, and 9%, respectively, which is noticeably different from those of milled wood lignin of 43%, 12%, 3%, 11%, and 4%, respectively [92]. However, the residual kraft lignin isolated by acidolysis of a *Picea mariana* kraft pulp of kappa no. 31.5 shows a β-O-4 content of 22.7% associated with β-β of 3.9%, but without detectable β-5, and β-1 linkages [93]. In case of hardwood kraft lignin, Mun et al. [94] showed that β-O-4 moieties were hardly detected from the kraft lignin associated with cooking condition of 21–23% active alkali (as Na_2O), 23% sulfidity, and pulping temperature of 160–165 °C. Similar results were also reported by Fernández-Costas et al. [95] that kraft process cleaves extensively the

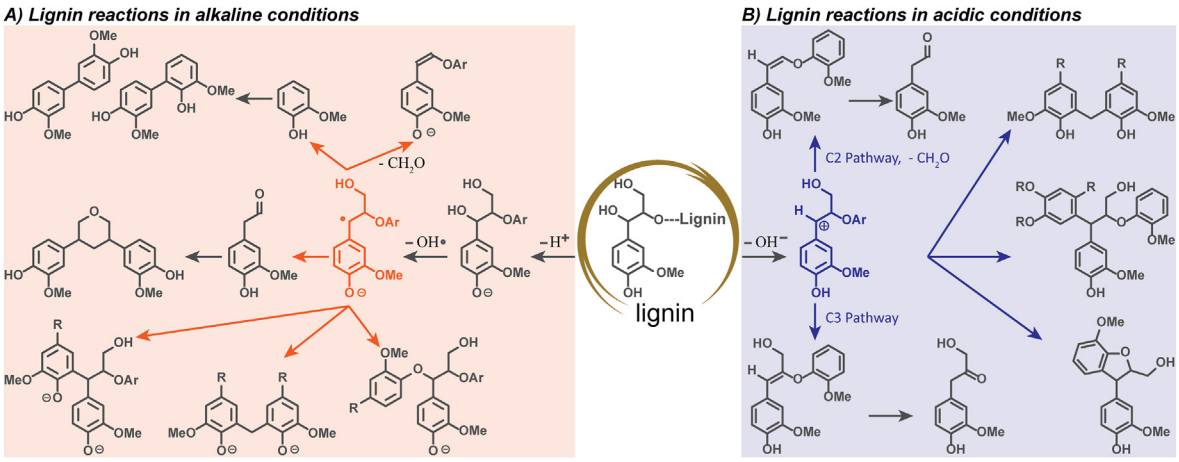

Fig. 2. Reaction of lignin during typical processes of isolation.

β-O-4 linkage of *Eucalyptus globulus* lignin but conserve the β-β linkage. Tsutsumi et al. [96] investigated the difference of reactivity between syringyl lignin and guaiacyl lignin in alkaline systems with β-O-4 model compounds, softwood and hardwood dioxane lignins, and softwood and hardwood meals. Their results indicate that β-O-4 linkages of syringyl lignin is cleaved much more easily than that of guaiacyl lignin which may explain the low β-O-4 content in hardwood kraft lignin [96].

In addition to the linkages, functional groups, and molecular weight of kraft lignin are also significantly modified when compared with those of native lignin. Gellerstedt [92] reviewed that compared with native lignin (MWL), kraft lignin comprise of less aliphatic –OH groups, but more phenolic –OH, and more aliphatic methylene groups. The reduction in aliphatic –OH, and increment in phenolic –OH is reasonable because of the well document reactions of elimination of terminal hydroxymethyl groups as formaldehyde, and the cleave of ether bonds of lignin, respectively [92]. The increment of aliphatic methylene groups is a bit mysterious which may be explained by several different mechanisms [92], including: 1st, Reduction reactions during pulping; 2nd, Formation of diarylmethane structures; 3rd, Radical coupling reactions between lignin and fatty acid radicals; 4th, Dihydroconiferyl alcohol also contribute to the total amount of aliphatic methylene groups in kraft lignin. In addition, the methoxy groups of kraft lignin is generally lower than those of native lignin which is due to the well documented demethylation reactions [91,92]. The demethylation is caused by nucleophilic attack of hydrosulphide anions to methoxy groups in lignin during kraft pulping. It is estimated that about 20% of the aromatic units are demethylated during kraft pulping [91]. In contrast to its heterogeneity in linkages, and functional groups, the molecular weight of kraft lignin seems less heterogeneous than native lignin. Different studies have come to similar results that kraft lignin has lower molecular weight and narrower polydispersity index than MWL [60,97,98]. This phenomenon is expected because the extensive cleavage of ether bonds of lignin during kraft pulping regardless of the recondensation of the depolymerized lignin fragment.

3.2. Soda lignin

Analog to kraft process, the soda process uses sodium hydroxide to separate lignin from the pulp under elevated temperature. Most the sulfide free reaction of lignin in the kraft process is thus expected in the soda process, which therefore afford lignin with similar structures in view of their linkages, functional groups, and molecular weight [99–101]. The difference between these two processes is that no sodium sulfide is applied with the soda process, which results in a relatively mild, and sulfide free pulping condition. A recently study by Zhao et al. [99] with dimeric β-O-4 model compounds showed that soda process is less effective in cleaving of the β-O-4 linkage of lignin, but more favors in elimination of terminal hydroxymethyl groups, and generation of arylglycerol structures because of the absence of sulfide ions. Therefore, the soda pulping process is mainly applied for herbaceous, and hardwood to a certain extent, whilst the kraft process is applied for wood, both softwood and hardwood. Noticeably, herbaceous plants make use of a wide range of monolignols to compose their lignins which are quite different from those of wood. The outstanding compounds of these monolignols are *p*-coumarate and ferulate. They are presented as ester of G/S units and polysaccharides in native lignin. These ester linked hydroxycinnamates can be released by alkaline treatment, and may be washed off from lignin depending on the recovery processes [102]. In addition, because of the high ash content of herbaceous plants, soda lignin may comprise of a large amount of ash as impurity. As was revealed by Domínguez-Robles et al. [103], soda lignin from wheat straw was composed of 64.9% klason lignin, and 29.9% ash.

3.3. Lignosulfonate

Lignosulfonate is a water soluble polymer that account for 90% of the total market of commercial lignin [104]. Typically, lignosulfonate is isolated from spent liquid of sulfite pulping processes. Alternatively, it can also be produced by chemical modification of isolated lignin, such as kraft lignin, with sulfites. Two terms, lignosulfonate and sulfonated lignin, are alternately used two name those sulfonate functionalized lignin. In a review by Aro and Fatehi [104], lignosulfonate is applied to identified those isolated from sulfite pulping processes, and sulfonated lignin is used to identified modified lignin with sulfites because of the significantly different methods to synthesize these lignins. However, because a majority of pulp mills have employed kraft process in replacement of sulfite process, sulfonated lignin is attracting increasing attention.

Regardless of their origin, a noticeable character of lignosulfonate is the presence of sulfonate groups. It is well documented that sulfite anion should attack the aromatic C_α and substitute the hydroxy group at C_α by a SN1 reaction during the sulfite pulping. Indeed, sulfite anion is likely able to substitute any free benzyl alcohols. In the study by Konduri and Fatehi [105], water soluble sulfonated lignin was synthesized by the reaction of hardwood kraft lignin with formaldehyde and sodium sulfite, which afforded functionalized lignin with sulfomethyl groups at the aromatic C_5 position. Lignin model compounds studies showed that formaldehyde could react with any phenol at the *ortho* position producing in hydroxymethyl substituted phenol that would further react with sulfite to afford *ortho*-sulfomethyl phenol [106]. It is therefore suggested that lignosulfonate should comprise of benzylsulfonate groups which tune the water solubility of lignosulfonate.

Depending on pH of the reaction, sulfonation of lignin may undergo in two different pathways. Under neutral or alkaline conditions, the formation of a quinone methide intermediate is the first step [107]. This mechanism is analogous to that of the kraft process. The quinone methide intermediate would be further substituted, decomposed, or recondensed. The substitution of quinone methide intermediate by sulfite anion at the Ar-C_α position is known to be a fast reaction [107]. The degree of substitution of sulfonate could reach 15 sulfonates per aromatic units in a few minutes [107]. In addition, β-O-4 dimeric model experiments showed that sulfite could further attack the sulfonated β-O-4 structure which resulted in cleavage of the β-O-4 linkage and produced phenylpropane-α, β-bissulfonate units [107]. In case of acidic sulfite processes, formation of benzylic cation is the key step [108], which is analogous to any acidic biomass fractionation processes. The substitution of benzylic cation is done by bisulfite anions. A certain amount of bisulphite ions must be present, otherwise, lignin would extensively condense [107,109]. On the other hand, higher degree of substitution of lignin by sulfonate can be attained in acid conditions than in neutral or alkaline conditions [107] because the generation of benzylic cation do not limit to phenolic compounds so that both phenolic and non-phenolic can be sulfonated [108].

3.4. Organosolv lignin

Organosolv pulping uses organic or aqueous-organic solvent systems to fractionate biomass into rich cellulose pulp, water-soluble hemicellulose stream, and organosolv lignin [110]. The organosolv process has advantages of clean fractionation, recovery of high-quality fractions over traditional alkaline and sulfite processes. Organosolv process may be operated under acidic, neutral, or alkaline conditions. Though alkaline organosolv process results in increased delignification, acidic organosolv process provides cellulose pulp with low degree of polymerization, shorter fiber lengths, and increased substrate porosity which facilitate hydrolysis of cellulose into glucose [111]. Along with the increasing interests in biofuel production from biomass, the acidic and neutral organosolv processes is dominant because of their efficiency regardless of that alkaline organosolv processes, such as soda-alcohol, were developed to provide cellulose pulp [110]. In addition, biomass release organic acids by autohydrolysis at elevated temperature. As a result, lignin undergoes acidolysis mechanism even under neutral organosolv processes, such as the neutral ALCELL pulping. Herein, reaction pathway of acidolysis of lignin will be discussed to understand the chemical structure of organosolv lignin.

Benzylic carbocation is the key intermediate of lignin reactions under acidic conditions (Fig. 2B). Proton cleaves aromatic C_α-O bonds to produce the benzylic carbocations whether it is from free phenolics-ending units of lignin, or substituted phenolics-internal units of lignin. However, Sturgeon et al. [112] revealed that the rate of acid-catalyzed β-O-4 cleavage in dimers with a free phenolic hydroxyl group is 2 orders of magnitude faster than in non-phenolic dimers. This result suggested that ending units of lignin are more ready to be converted into benzylic carbocation than internal units of lignin. Besides, solvents may also have a significant impact on the formation of benzylic carbocation. In the study by Jasiukaitytė-Grojzdek et al. [113], the conversion rate of the lignin model compound, benzyl phenyl ether, was the fastest in methanol, but much slower in ethanol, and the slowest in 75% ethanol/water. This phenomenon was explained by strong ability of methanol in stabilization of the carbocation intermediate, which is well known to accelerate the SN1 reactions [113]. The acid applied in organosolv fractionation can also impact the formation of carbocation intermediate from lignin. In the study by Imai et al. [114], kinetic analysis showed that the acidolysis of lignin dimeric model is a pseudo-first-order reaction. The reaction rate is much faster in the HCl, and HBr systems than in H_2SO_4 systems. Similar phenomenon was observed by Costa Lopes et al. [115] that ionic liquids with halides as counterion showed higher efficiency in the conversion of lignin dimeric models. The mechanism is explained by that halide anion binds the lignin intermediates during the dehydration step, stabilizing not only the transition state structure but also the dehydrated intermediate species [115].

Two reaction pathways, C3 pathway and C2 pathway, are well documented to the further decomposition of the benzylic carbocation intermediate (Fig. 2B), which are the major fragmental mechanism of lignin under acidic conditions. The C3 pathway involves in direct cleavage of enol ether structure, which is the product of deprotonation of the benzylic carbocation intermediate, and produces Hibbert Ketones. Whilst, the C2 pathway release formaldehyde from γ-position, then cleave the β-O-4 bond by hydrolysis, to yields C2 aldehydes. Model studies reveal that up to 50% products are produced by the C2 pathway. However, the monomers identified from acidolysis of lignin are exclusively composed of Hibbert ketones from the C3 pathway [116]. Deuss et al. [116] suggested that recondensation of aldehyde lignin fragments are attributed to the absence of C2 products upon acidolysis of lignin. The addition of diols into acidolysis of lignin can capture these aldehyde fragments by yielding acetals, which can be further converted into aromatic compounds in high yields by hydrogenation or decarbonylation [116]. The competition between C3 and C2 pathway may be alternated by different acids. Cox et al. [117] studied the catalytic degradation of lignin model compounds in acidic imidazolium ionic liquids. It is revealed that the conversion rate of lignin dimer is not related to the acidity of the applied ionic liquids, but corresponded to the hydrogen bond basicity of counter anions [117]. Anions with strong hydrogen bond basicity favor the stabilization of hydroxyl groups and prevent deprotonation through coordination, whilst the C2 pathway involves the deprotonation of γ-hydroxyl group to break off γ-carbon and yield formaldehyde [117]. As a result, the competition between C3 and C2 pathway can be turned by the hydrogen bond basicity of counter anions [117]. Anions of strong hydrogen bond basicity favor the C3 pathway while those of weak hydrogen bond basicity favor the C2 pathway.

Nevertheless, isolated organosolv lignin are usually extensively condensed with low monomers yield upon acidic catalytic depolymerizations [116]. This phenomenon is in part because of the cleavage of β-O-4 linkage during organosolv treatment, also largely be attributed to the condensation of lignin fragments. There are several condensations proposed, including the coupling of aromatics by formaldehyde released from the C2 pathway, the attack of benzylic carbocations by electron-rich aromatics, and the rearrangement of benzylic carbocations to form coumaran structures [39,118]. In case of the β-5 linkage, the α-O-4 bonding would be cleaved to yield a stilbene structure with the β-5 bonding intact, while the β-β structure is stable in organosolv treatment [119,120].

Regardless of these recondensations, organosolv lignin has noticeably lower molecular weight than those of native lignin [121], and also those of kraft lignin and lignosulfonate [122]. Hage et al. [121] showed that the weight average molecular weight of organosolv lignin and milled wood lignin isolated from *Miscanthus* were 4690, and 13,700, respectively, while Mimini et al. [122] showed that the weight average molecular weight of organosolv lignin, kraft lignin, and lignosulfonate were 1249, 3889, and 3994, respectively. Likewise, organosolv lignin also display a low content of aliphatic –OH groups, lower than those of native lignin, kraft lignin, and lignosulfonate [121,122]. On the other hand, organosolv lignin possess a high content of phenolic –OH groups, higher than those of native lignin, kraft lignin, and lignosulfonate [121,122]. All these characters of organosolv lignin are explained by the extensive cleavage of β-O-4 linkage, and the associated C2 pathway.

3.5. Emerging isolated lignin

Novel biomass fractionation technologies continue emerging which produce lignin with tailored structures (Fig. 3). These novel technologies include the γ-valerolactone (GVL) biomass fractionation [123], acidic lithium bromide (LiBr) pretreatment (ALB) [124], reductive catalytic fractionation [125] and the stabilization strategies [126]. As is generally

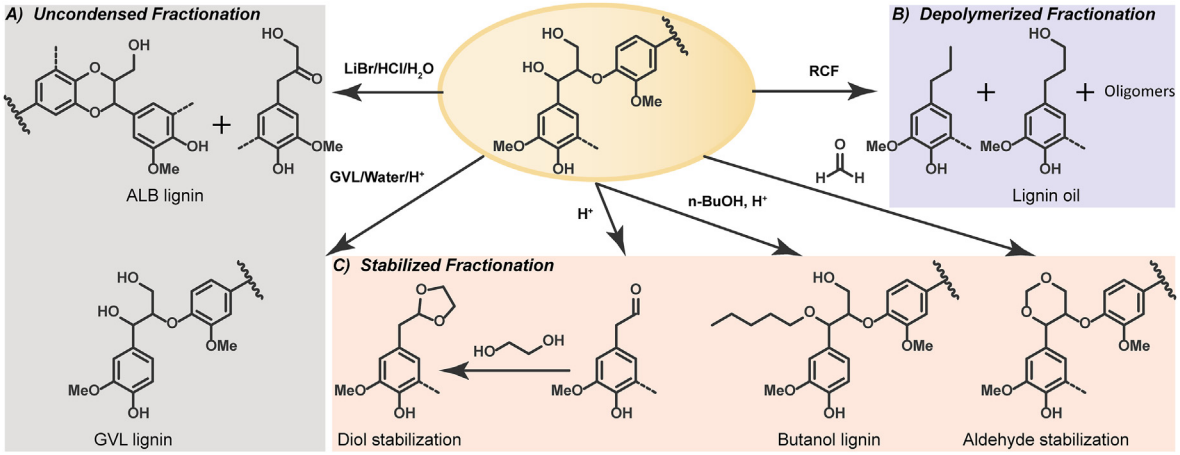

Fig. 3. Novel isolated lignins. A) The GVL and ALB processes depolymerize carbohydrates and resulted in uncondensed lignin [123,124]. B) Reductive catalytic fractionation generated depolymerized lignin oil [125]. C) Stabilized methods capture the reactive intermediates to inhibit the recondensation of lignin fragments [84, 126,131–133].

acknowledged, the chemical structure of lignin should be significantly impacted by the fractionation method. Though the GVL fractionation method is a typical organosolv process which usually leads to extensive cleavage of the ether bonds of lignin [127], it is capable of producing quite native-like lignin (Fig. 3A) by tuning the parameters of the process [123]. The GVL lignin possesses a chemical structure and properties significantly different to those of conventional kraft [128] and organosolv lignin, such as the alcell lignin [129]. By the ALB method (Fig. 3A), lignin of Hibbert's ketone and benzodioxane structure can be isolated [118]. The reductive catalytic fractionation depolymerizes lignin of 'virgin' biomass into an uncondensed, low-Mw lignin oil (Fig. 3B), comprising phenolic monomer and dimers up to 70%, by integrating catalysis into the oganosolv fractionation of biomass [130].

There are several reported stabilization strategies to isolate protected lignins (Fig. 3C), including mild alcohol fractionation, diol-based stabilization and diol trapping with aldehydes [84]. The mild alcohol fractionation is a typical organosolv pretreatment which pretreats biomass with high alcohol, typically *n*-butanol/water mixture, at temperature of 80 °C for 5 h [131]. This process produces α-alkoxylated lignin because of the incorporation of alcohol at the benzylic α-position of β-aryl ether units [131]. The diol-based stabilization showed to be a tunable process depending on the formula of solvent system applied [132]. Typically, while diols are the major composition of the solvent system, diols act as nucleophiles quenching the benzyl carbocation analog to the mild alcohol fractionation, which form etherified lignin with hydroxyl tail [134]. In the present of a primary solvent (≥50%), such as dimethyl carbonate, dioxane, the diols work as a stabilization agent to aldehyde fragment of acid catalytic depolymerization of lignin, which generates acetal-functionalized lignin oil comprising oligomers, dimers, and monomers [135].

Another interesting stabilization strategy is the aldehyde-assisted fractionation which adds aldehyde into the organo-solvent biomass fractionations and produces lignin with 1,3-dixoane structure in the side chain [133]. Rational selection of functional aldehyde allows for install specific functionality in the lignin. In the study by Dick et al. [136], the solubility properties of 25 lignins extracted with different aldehydes were studied. Water soluble lignins were extracted with carboxylate functional aldehydes while toluene soluble lignins were extracted with long chain fatty aldehydes [136]. Lignin with aldehyde in the backbone was extracted using a multifunctional aldehyde, namely terephthalic aldehyde, by Bertella and Luterbacher [137]. The introduction of aldehyde groups into lignin improves further modification of lignin by phenolation which generates lignin more reactive than kraft lignin or organosolv lignin to produce phenol formaldehyde resins [137].

4. Antibacterial activities of lignin

4.1. Antibacterial activity of lignin model compounds

Early in 1979, Zemek et al. [138] reported that antibacterial activity of guaiacyl and syringyl models of lignin against both gram-negative, *E. coli*, and gram-positive, *M. lichenifvrmis* bacteria, which concluded that the structure of the side chain of lignin played an important role in its antibacterial activity. Lignin derivatives with oxygen containing functional groups (-OH, –COOH, and –C═O) in the side chain are less effective, whereas those with double bond at the side chain are the most effective inhibitors against bacteria [138,139]. In another report by Barber et al. [7], the minimum inhibitory concentration (MIC) of the three typical lignin units, H, G, and S units, is at the level of ~5 mg/mL against various bacteria. Whereas, the corresponding γ-aldehyde compounds, *p*-coumaraldehyde, coniferaldehyde, and sinapaldehyde, are ten times more effective with MIC at the level of ~0.5 mg/mL [7]. These studies on lignin related model compounds have provided base knowledge on the origin of antibacterial activity of lignin in view of its chemical structure (Fig. 4).

Though those pioneering researches provide important evidences on understanding the antibacterial activities of lignin, they are limited to lignin related model compounds of low molecular weights (Fig. 4). It is reported that isoeugenol, a lignin related monomer, interacts reversibly with gram-negative bacterium's, *E. coli*, membranes through a non-disruptive detergent-like mechanism due to the hydrophobic nature of isoeugenol [140]. Another report shows that ferulic acid, usually found from lignin of herbaceous plants, lead to irreversible changes in membrane properties associated with leakage of essential intracellular constituents [141]. Nevertheless, the lignin macromolecule involves in quite different mechanisms of action as antibacterial material, including the reactive oxygen species (ROS) mechanism, and the adenosine triphosphate (ATP) depletion mechanism [142,143].

As a result, the lignin polymer has antibacterial property significantly different from its monomer models. In the report by Zemek et al. [138], it is shown that dimeric compounds are inhibitors roughly twice as effective as monolignols. Xie et al. [149] fractionated dehydrogenation polymer (DHP, a polymeric lignin model) of isoeugenol into four fractionations with different molecular weights and assayed their antimicrobial properties by diffusion method. It is shown that DHP with low molecular weight (Mw ≤ 621 g/mol) are effective inhibitor against both gram-positive and gram-negative bacteria, whereas DHP with high molecular weight (Mw ≥ 1211 g/mol) are not inhibitor against neither gram-positive nor gram-negative bacteria [149]. Night compounds,

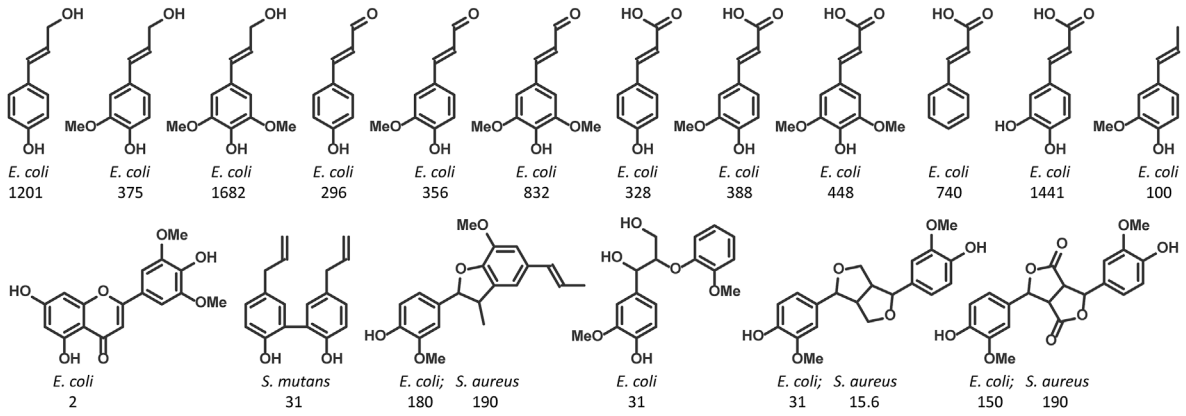

Fig. 4. The MIC value of lignin related model compounds, units: μg/mL [7,138,139,144–148].

including lignin dimer, trimer, tetramer and pentamer, were further isolated from the low molecular weight fractions [149]. Results of diffusion assay of the night compounds showed that dimeric compounds inhibited the growth of both *S. aureus* and *E. coli*, whereas trimeric, tetrameric, and pentameric compounds inhibited *S. aureus* only, but not against *E. coli* [149]. The β-5 linkage is believed to be the major contribution to the antimicrobial ability of lignin while the 5-5 linkage is believed to decrease the antimicrobial activities of lignin [149]. This result suggests that the antibacterial activity of lignin can noticeably differ from one to another according to its chemical structure.

4.2. Antibacterial activity of isolated lignin

It is explicit that all the monolignols, linkages, molecular weight and functional groups can impact the antibacterial activities of lignin, while the chemical structure of lignin can be significantly altered by its plant sources, method of isolation, processes of purification, and subsequent chemical modification. Current publications have extensively reported the impact of the extraction method on the antibacterial activities of lignin. Alzagameem et al. [150]. Extracted alcell lignins from spruce/-pine, beech, and miscanthus, and isolated kraft lignin from industrial black liquor. The antibacterial activity of these lignins was determined with diffusion assay which followed a trend in: alcell lignin of hardwood > kraft lignin of softwood > alcell lignin of softwood > alcell lignin of grass [150]. A preliminary review on the impact of extraction methods on antimicrobial activities of lignin can be found by Ndaba et al. [22].

However, there is not a general agreement on the antibacterial activities of lignin macromolecule. In the studies by Dong et al. [143], lignin extracted from residue of corn stover for ethanol production exhibited antimicrobial activities against gram-positive bacteria, *L. monocytogenes* and *S. aureu*, but not against gram-negative bacteria (*E. coli* and *S. enteritidis*). However, Núñez-Flores et al. [151] reported that protobind sulfur-free water-insoluble commercial lignin (pH ~4 in aqueous suspensions) showed no antimicrobial activity against the 26 microbial strains including gram-positive bacteria, and gram-negative bacteria. Also, the same group reported that lignosulphonates showed limit antimicrobial activity only against *D. hansenii* CECT 11364, and the gram-positives *S. aureus* CECT 240 and *B. thermosphacta* CECT 847, but showed no antimicrobial activity against most of the 26 microorganisms studied [152]. On the other hand, Wang et al. [153] showed that bamboo kraft lignin had a good antibacterial performance with MIC value of 1 mg/mL against *S. aureus*. Table 1 summarizes the reported antibacterial performance of different lignins. Overall, these raw isolated lignins show weak antibacterial activity with MIC at the level of mg/mL when compared with traditional antibiotics (MIC value at the level of tens μg/mL [154]), or synthetic antimicrobial polymer (MIC value in range of several to hundreds μg/mL [155]). On the other hand, it is also difficult to compare the activity of lignin across different reports, because 1st. Different bacteria are applied for tests in different reports. For example, some reports apply *S. aureus*, while some applied *L. innocua* for studies.

Table 1
Chemical structure, and antibacterial activity of isolated lignin. * β-O-4 is the content of β-O-4 linkage; Mw is the molecular weight of lignin; Al–OH is the content of aliphatic –OH groups; Ar-OH is the content of phenolic –OH groups.

Lignin	Chemical Structure*					Antimicrobial Activity	REF
	S/G	β-O-4	Mw kDa	Al–OH mmol/g	Ar-OH mmol/g		
Eucalyptus tetrahydrofurfuryl alcohol lignin	–	77.3%	2.93	~1.7	~2.4	**Inhibition Zone (mm)** *S. aureus*: 15; *E. coli*: 14	[156]
Bamboo kraft lignin	–	–	4.03	–	1.47	**MIC (mg/mL)** *S. aureus*: 1; *E. coli*: >4	[153]
corn stover bioethanol residue	–	–	–	–	–	**Susceptibility** *L. innocua*: >99%	[157]
Spruce alcell lignin	0	–	3.08	–	8.3	**Inhibition zone (mm)** *E. coli*: 13.3; *S. aureus*: 16.3	[158]
Spruce kraft lignin	0	–	7.19	–	7.9	**Inhibition zone(mm)** *E. coli*:17.7; *S. aureus*:19.2	[158]
Eucalyptus acell lignin	62:31	–	5.07	–	8.2	**Inhibition zone (mm)** *E. coli*:12.2; *S. aureus*:17.9	[158]
Eucalyptus kraft lignin	59:28	–	2.65	–	5.4	**Inhibition zone (mm)** *E. coli*:17.1; *S. aureus*:18.0	[158]
Sugarcane bagasse soda lignin	–	–	–	–	–	**MIC (mg/mL)** *S. epidermidis*: 4.096	[159]
Corn stover bioethanol residue	–	–	–	–	3.09	**MIC (mg/mL)** *S. aureus*: 1.25;	[143]

2nd. Different methods are applied for antibacterial activity assay, including inhibition zone assay, susceptibility, and MIC assay. Herein, we would like to suggest further reports on the antibacterial studies of isolated lignin to report at least the MIC value both against gram-negative, *E. coli,* and gram-positive, *S. aureus*, bacteria, so that the results from different reports can be compared to each other.

On the bright side, molecular weight, and phenolic hydroxy groups of lignin have been extensively reported and related to antibacterial property of lignin because of the feasible availability of gel permeation chromatography (GPC), ^{31}P NMR, and titration analytical methods. Lignin with low molecular weight and high phenolic hydroxy groups contents tends to be with increasing biological cytotoxicity, which is explained by the ROS mechanism associated with the polyphenol of lignin [160]. This structure and activity relation model has been applied to generate lignin fractions with enhanced antimicrobial activity. In the study by Xu et al. [156], lignin fractions with molecular weight increasing from 1890 to 7900 g/mol were prepared from organosolv lignin by solvent fractionation method using γ-valerolactone/water as solvent system. The inhibition zone of the lignin fractions against *S. aureus* increases from 10 mm to 17 mm along with the decreasing molecule weight, and increasing phenolic –OH groups [156]. However, the antibacterial property of lignin is poorly related to lignin's monomer composition, and the linkages, which is attributed to the limit availability of the analytical methods, such as quantitate HSQC, derivatization followed by reductive cleavage, and thioacidolysis, to determine the structural information of lignin.

4.3. Mechanism of action of lignin

Generally, the mechanism of action of antibacterial activity of lignin can be classed into two models [161]: 1st, Interaction with cell membrane resulting in the leakage of cellular contents and eventual cell death; 2nd, Binding with cytoplasmic components, i.e., protein, DNA, thereby altering the metabolic pathways. The membrane destabilizing model is widely accepted for the polymer-microbial interactions [162], while most small molecules work with the cytoplasmic compound binding model [161].

However, the mechanism of action of lignin against bacterial remains largely unknown. Polyphenolic compounds of lignin are usually ascribed as the cause leading bacterial cell death. In the study by Yang et al. [142], it is proposed that lignin polyphenols cause damage to bacterial cells by ROS. The ROS mechanism assumes that lignin polyphenols absorb ROS due to its high antioxidation behavior and induce oxidative stress when contacting with microorganisms [142]. The ROS is a well-known direct and efficient antibacterial mechanism that triggers multi mechanism of action by reactions with thiol groups of the bacterial cell [163].

According to the ROS mechanism, lignin with high activity of radical scavenger should possess high antibacterial activities. In the study by Dong et al. [143], lignins with different hydrophilic oxygen radical absorbance capacity were extracted from the residue of corn stover to ethanol production by altering the isolation parameters. The antibacterial activity of these extracted lignin shared similar tendency to its antioxidative activity [143]. However, the ROS mechanism is challenging by the fact that the antibacterial activities of lignin is not predicted by the content of phenolic hydroxyl group [130], while radical scavenging index is positively correlated to phenolic hydroxyl group content [164].

On the other hand, heterogeneity is the most prominent characteristic of lignin with molecular weight ranging from hundreds g/mol to hundred thousand g/mol [60]. It is thereby suggested that those lignin oligomers of low molecular weight can penetrate the cell membrane of bacteria and inhibit the synthesis of both ATP and ATPase [165] as most phenolic molecules do [166]. However, the studies on the inhibition of both ATP and ATPase synthesis are usually reported to dietary polyphenols but none is on the lignin oligomers [167].

Besides, lignins are generally modified either chemically or physically, which would significantly alter the mechanism of action of lignin antimicrobials [14]. A series of cationic lignin-based hyperbranched polymers were synthesized by Chee et al. [14] using 2-bromo-2-methylpropionyl lignin as macroinitiator by atom transfer radical polymerization. The cationic modified lignin is shown to inhibit microbes by the disruption of the outer membrane [168] as a typical pattern of cationic antimicrobial polymers [162]. In a study by Morena et al. [169], tellurium-lignin nanocomposites were synthesized by ultrasound assisted in situ reduction of tellurite to elemental tellurium. The tellurium-lignin nanocomposites showed enhanced ROS generation ability and thus possessed strong bactericidal activity against gram-negative bacteria reducing 99.9995% of these bacteria within 4 h [169].

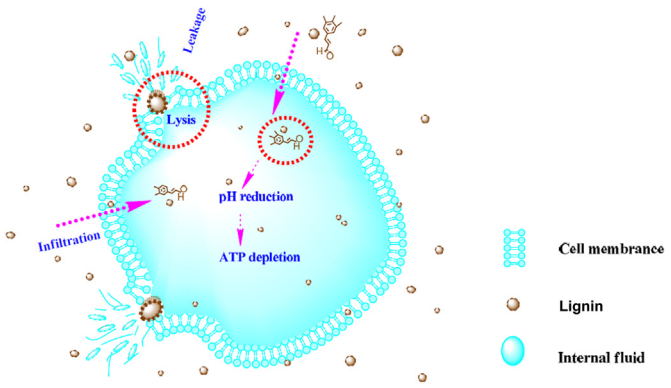

Fig. 5. Involved mechanisms for antibacterial behavior of lignin. Reprinted (adapted) with permission from Ref. [142]. Copyright © 2018, American Chemical Society.

Summarily, the ROS mechanism and inhibition of ATP and ATPase synthesis are the two proposed mechanisms of action of antimicrobial activities of lignin (Fig. 5). Although, these mechanisms are proposed extensively according to the studies on polyphenols which is a group of molecules analogous to lignin. Besides, the mechanism of action of lignin can be noticeably altered by the lignin modification technologies.

5. Antifungal activities of lignin

Different from bacteria which are unicellular prokaryote organisms lack of a nucleus and other membrane bound organelles, fungi can be either single-cell or very complex multicellular eukaryotic organisms. Early at 1968, Tehysheva et al. [138] had showed that lignin derivatives by alkaline oxidation treatment were fungicide effective. Zemek et al. [138,139] reported the antifungal properties of a series of various lignin model compounds. Ando et al. [175] quantitively determined the contribution of functional groups to the antifungal ability of lignin related aromatic monomers against the yeast *S. cerevisiae*. The contribution of different chemical groups to inhibitory value was reported as [175]: CH═CH: +3.0, CHO: +1.5, *p*-OH: +1.0, COOH: +0.5, m-OH: 0, OCHs: 1.0. Similarly, Almada et al. [176] reported that guaiacyl and hydroxyphenyl units possessed better antifungal properties than syringyl units, and the importance of the propyl chains.

In case of the antifungal activity of isolated lignin macromolecules, Gordobil et al. [158,177] determined the antifungal activity of alcell lignin, and kraft lignin isolated from both softwood and hardwood against *A. niger* by susceptibility test, which showed that kraft lignin generally presented higher inhibition potential than alcell lignin. However, Núñez-Flores et al. [151,152] reported that protobind sulfur-free water-insoluble commercial lignin (pH ~4 in aqueous suspensions), and lignosulphonates showed no antimicrobial activity against the studied yeast and molds. On the other hand, Melo et al. [170] extracted lignin from *C. pulcherrima* leaves by alkaline delignification method, which was an excellent antifungal material against *C. parapsilosis* (MIC,

Table 2
Chemical structure, and antifungal activity of isolated lignin.

Lignin	Chemical Structure*					Antifungal Activity	REF
	S/G	β-O-4	Mw kDa	Al–OH mmol/g	Ar-OH mmol/g		
Pulcherrima leaves soda lignin	–	–	2.50	–	0.72	**MIC_{50} (mg/mL)** *C. parapsilosis*: 0.031 *C. neoformans*: 0.015	[170]
Apple tree hydrolysis lignin	3:5	–	24.6	–	0.10	**Susceptibility** *S. cerevisiae*: 65%	[171]
Apple tree acetic acid lignin	3:4	–	7.20	–	7.2	**Susceptibility** *S. cerevisiae*: 50%	[171]
Apple tree alcell lignin	3:4	–	16.3	–	7.38	**Susceptibility** *S. cerevisiae*: 39%	[171]
Apple tree soda lignin	1:3	–	4.19	–	4.4	**Susceptibility** *S. cerevisiae*: 25%	[171]
Corn stover bioethanol residue	–	–	–	–	3.09	**MIC (mg/mL)** *C. lipolytica*: 3.75	[143]
UPM pine lignin	–	–	4.4–5.0	–	–	**MIC (mg/mL)** *C. jejuni*: 16; *S. tritici*: 32	[172]
UPM Beech lignin	–	–	0.8–1.5	–	–	**MIC (mg/mL)** *S. tritici*: 32	[172]
Kraft lignin	0	–	7.20	–	–	**Susceptibility** 73–87%	[158]
Organosolv lignin	0	–	5.79	–	–	**Susceptibility** 50–70%	[158]
Kraft lignin	–	–	6.00	–	–	**MIC (mg/mL)** *R . solani*: 0.20	[173]
Lignosulfonate	–	–	–	–	–	**MIC (mg/mL)** *Candida* spp.: 0.064–0.128	[174]

31.25 μg/mL), *C. guilliermondii* (MIC, 31.25 μg/mL) and *C. neoformans* (MIC, 15.62 μg/mL). Besides, the MIC value of commercial lignosulfonate against *Candida* spp. was found to be 64–128 μg/mL by Jha and Kumar [174], however, these MIC value of lignin was much higher than those of conventional antifungicides, such as echinocandins [178], in *Candida* spp. therapy. Obviously, the antifungal activity of lignin among these previous reports differs noticeably from one to another. A summary of previous reports on the antifungal activity of lignin can be found in Table 2. However, few information on the structure details of lignin can be found, and we are not able to relate the antifungal activity of lignin to its chemical structure. Nevertheless, Jha and Kumar [174] showed that antifungal activity of lignosulfonate can be predicted by computer aided in-silico drug design method using virtual screening, molecular docking, drug likeness, absorption, distribution, metabolism, excretion, and toxicity analysis, while bioavailability parameters are applied, including molecular volume, molecular weight, hydrogen bond acceptors, hydrogen bond donor, number of rotational bonds, octanol/water partition, topological polar surface area, and molar refractivity of lignin. This research highlights the potential that the antimicrobial activity of lignin may be modeled with drug design principles.

6. Antiviral activities of lignin

The COVID-19 pandemic has raised increasing interest in the development of antiviral medicines. Though lignin was indicated not possessing a significant antiviral activity against SARS-CoV-2 virus [181], it has been long discovered as effective anti-HIV and anti-influenza ingredient. At 1989, Suzuki et al. [179,194] had reported both inhibitory effect on HIV replication, and immunostimulant activities *in vitro* by solubilized lignins, including lignosulfonate. Qiu et al. [195] reported that lignosulfonic acid exhibited broad activity against HIV-1 isolates of diverse subtypes including two north America strains and a number of Chinese clinical isolates with EC50 ranging from 0.171 μg/mL to 6.323 μg/mL. In another study by Gordts et al. [190], lignosulfonic acid demonstrated inhibitory activity of HIV replication against a wide range of R5 and X4 HIV strains, and inhibited the transmission of HIV from persistently infected T cells to $CD4^+$ T cells. Generally, lignosulfonates show comparable anti-HIV activity with those of AZT, ddC, and sulfated polysaccharides [184]. In addition, general synergistic effects of lignin in combination with anti-HIV compounds, including 3′-azido-2′,3′-dideoxythymidine (AZT), nevirapine, PRO2000, maraviroc, tenofovir, and vitamin C, were demonstrated [190,195,196]. Riviere et al. [185] also reported the removal of viruses from contaminated water by cationic lignin. A summary of antiviral studies by lignin can be found from Table 3.

Multiple mechanisms of action may be involved in the inhibition of anti-HIV activity by lignosulfonate, one of which was explained by the entry inhibition of HIV, as those of tannins and polyanions such as dextran sulfate do [197]. This mechanism was testified by the fact that a lignosulfonate resistant mutant HIV-1 NL4.3, which acquired seven mutations in the HIV-1 envelope glycoproteins, showed cross-resistance with well-described HIV binding/fusion inhibitors, including feglymycin, enfuvirtide, PRO2000, and mAb b12 [190]. Lignosulfonate is believed to target envelope glycoproteins of virus because it is a highly, and negatively charged polymer, and lack of antiviral activity against non-enveloped virus such as the *Picornaviridae* Coxsackie type B4 and the *Reoviridae* Reovirus type 1 [190]. Multiple targets on viral gp120 as well as on host receptor CD4, and co-receptors CCR5/CXCR4 were identified as binding sites by lignosulfonate [195]. In another study by Mitsuhashi et al. [193], lignin was found to inhibit HIV-1 replication through suppression of HIV-1 transcription from LTR including activation via NF-κB. In addition, HSV infection was identified as a co-factor for increased HIV transmission in women, while lignosulfonate possessed synergistic antiviral activity against both HIV and HSV, which showed the potential of lignosulfonate in reducing crossing inflections by multiple viruses.

Carboxylated lignin fractions, lignin carbohydrate complexes, alkaline lignin, and synthetic lignin (DHP) are also reported to inhibit the growth of HIV virus. In a study by Nakashima et al. [197] carboxylated lignins were synthesized by enzymatic oxidative coupling of 4-hydroxy cinnamic acid, which were more effectively than natural lignin, and tannin-related materials in inhibiting proliferation of HIV. Anti-HIV activity of lignin carbohydrate complexes from different sources were well documented by Sakagami et al. [182], which quantitatively assessed the anti-HIV activity by SI. The highest anti-HIV activity was reported with

Table 3
Antiviral studies of isolated lignin. Notes: SI═CC50/EC50, where CC50 is the 50% cytotoxic concentration against mock-infected cells, and EC50 is the 50% effective concentration against virus-infected cells.

Lignin	Lignin Structure	Antiviral activity	Mechanism	REF
Water-soluble lignin compression	Unavialable	Inhibition against influenza, PEDV	Unavailable	[179]
Sugarcane bagasse organosolv lignin by glycerolysis	Analysis of Mw, β-O-4, log P, particle size, and ζ-potential	Inhibition against EMCV, TMEV, VSV, SINV, and NDV	Entry inhibitor with viral RNA intact	[180]
Functional lignin, soda, kraft, and organosolv lignin	Analysis of Mw by GPC, and –OH group by 31P NMR	Functional, and soda lignin inhibited HSV-2 (>99%)	Local generation of reactive oxygen species	[181]
Lignin carbohydrate complexes (LCC)	LCC from different plants	Highest SI = 311 again HIV from husk of *Theobroma cacao*	–	[182]
Beech LCC by methanol extraction after acidolysis	Analysis of Mw by GPC, –OH by P NMR, and linkages by Thioacidolysis and HSQC	IC50 against EMCV: 0.02 μg/mL; SI > 600	Carbohydrate moiety played a critical role in the inactivation of EMCV	[183]
Lignosulfonates	Determination of Mw, purity, and solubility	First-class anti-HIV agent with SI > 1210	High activity is along with high Mw, and solubility	[184]
Colloidal softwood kraft lignin particle	Analysis of particle size, and ζ-potential	Removal cowpea chlorotic mottle virus from water	Affinity of viruses to lignin by electrostatic, hydrophobic and π−π interactions	[185]
Organosolv lignin of sugarcane bagasse	Analysis of Mw by GPC, –OH by P NMR, and linkages by HSQC, thioacidolysis, and py-GCMS	Against EMCV: IC50: 0.08 μg/mL, SI > 2500	Entry inhibitor	[186]
Twenty-four structurally different lignosulfonates	Analysis of lignin origin, counter-ion, sulfur content, carboxylic acid percentage, and Mw	Anti-HIV activity of lignosulfonates is mainly dependent on their molecular weight	Entry inhibitors, and inhibit transmission across cells	[187]
Kraft lignin	–	Lignin protects shrimp against infection of yellow head disease	Lignin binds to envelope components of yellow head virus	[188]
Lignin extracted from mushroom culture, and alkaline lignin	–	SI > 200 against hepatitis C virus	Entry inhibitor	[189]
Lignosulfonate	–	Broad-spectrum anti-HSV activity with IC50 between 3.4 and 5.0 μg/mL	Entry inhibitor	[190]
Lignin carbohydrate protein complex from *Pimpinella anisum*	Analysis of lignin monomers, sugar components, and protein contents	Anti-HSV-1 (SI > 43), anti-HSV-2 (SI > 38), and anti-HCMV-1 (SI > 910)	–	[191]
DHP of caffeic acid, and ferulic acid	Analysis of Mw by GPC	Anti-HSV-1: IC50: 72–284 ng/mL	Entry inhibitor	[192]
Lignin fractions, and dimers	Analysis of Mw by GPC, and linkages by HSQC	Low Mw lignin and β-5 dimer showed superior anti-HIV activity	Inhibit HIV-1 by suppression of transcription from LTR	[193]

lignin carbohydrate complexes extracted from husk of *Theobroma* cacao with SI value of 311 [182,196], whereas, DHPs of phenylpropenoids with degree of polymerization of 23 was reported with SI value of 105. The anti-HIV of lignin carbohydrate complexes was lower than other popular anti-HIV agents, such as dextran sulfate (SI > 1130), curdlan sulfate (SI > 1748), AZT (SI = 6609), dideoxycytidine (SI = 2283), while lignosulfonate showed potent anti-HIV activity (SI > 1210) comparable with those of sulfated polysaccharides [184].

In addition, antiviral activity of different lignin against a wide range of viruses were reported, including influenza virus [198], HSV [199], rotavirus [200], enterovirus [200], human cytomegalovirus [191], measles virus [191], encephalomyocarditis virus (EMCV), Theiler's murine encephalomyelitis virus (TMEV), vesicular stomatitis virus (VSV), Sindbis virus (SINV), and Newcastle disease virus (NDV) [180]. These antiviral activities of lignin were usually attributed to interference with virus adsorption, rather than to inhibition of virus replication after adsorption [200]. In the study by Srisapoome et al. [188], it was also reported that kraft lignin efficiently inhibited yellow head virus infection in black tiger shrimp, which open an opportunity to develop practical method to control yellow head disease in crustacean farming with low-prices industrial waste.

An *in vivo* study of anti-herpes activities of lignin was performed by Zhang et al. [199] with HSV-1 skin lesion model in guinea pigs, and HSV-2 genital infection model in BALB/c mice. Lignin carbohydrate complex was extracted from *Prunella vulgaris* and formulated into a *Prunella* topical cream [199]. The 15% Prunella cream treatment significantly reduced skin lesions of HVS-1 infected guinea pigs, and increased survival rate and days-to-mortality of HSV-2 infected mice, which demonstrated the *in vivo* anti-HSV activity of the lignin carbohydrate complex, and suggested that the complex can be developed into an effective anti-herpes drug [199].

A clinical pilot study of lignin and ascorbic acid combination treatment of HSV-1 infection was performed by LOPEZ et al. [201]. Forty-eight patients of both genders with active lesions of HSV-1 were orally administered one mg of lignin-ascorbic acid tablet or solution three times per day for a month. No patients presented any side effects. Compared with previous episodes, reduction in the severity of symptoms, and reduction in the recurrence were reported by the majority of the patients, which indicated the possible applicability of lignin for prevention and treatment of HSV-1 infection.

There have been some studies trying to relate the antiviral activity of lignin to its structure. Suzuki et al. [179,194] related anti-HIV activity of lignin to its polyanionic nature, but not to the inherent chemical structures, and molecular weight of lignin, because both carboxylated, and sulfonated lignin showed anti-HIV activity. Nakashima et al. [197] showed the significance of lignin structure on its anti-HIV activity by a comparison of the anti-HIV activity of synthetic lignin, pine cone extract, alkali lignin, and lignosulfonate. Also, the anti-HIV activity of synthetic lignin could be noticeably altered by the synthetic methods, reaction parameter, and post treatments [197]. Similarly, Sakagami et al. [198] reported impact of synthetic methods on the anti-influenza activity of DHP, while Harada et al. [202] reported that the anti-influenza activity of both pine cone extract and commercial alkaline lignin was reduced by treatment with sodium chlorite, but not with sulfuric acid nor trifluoroacetic acid. Li et al. [183] reported that antiviral effects of the lignin carbohydrate complex extracted from beech wood by microwave acidolysis were significantly decreased by treatment with hemicellulose hydrolysis. Nevertheless, the polymeric structure of lignin is important because monolignols do not show any antiviral activity [198,203]. The important of lignin polymer in its antiviral activity was also proved by the fact that no noticeable effect on the anti-HIV activity of lignin was reported by the removal of polysaccharide residues by acid-catalyzed hydrolysis of lignin [182,204].

The impact of molecular weight of lignin on its antiviral activities was

reported by several studies, but associated quite different results. Oeyen et al. [187] reported that the broad antiviral activity of lignosulfonates was mainly dependent on their molecular weight by evaluating the inhibition of HIV and HSV transmission and replication in various cellular assays by 24 structurally different lignosulfonates. However, the study by Thakkar et al. [192] showed that the antiviral activity of synthetic lignin was independent on the chain length of DHP, because similar IC_{50} values were observed from DHP with molecular weight between 2.1 kDa and 13.2 kDa. In another study by Mitsuhashi et al. [193], both alkaline lignin and organosolv lignin were fractionated into low molecular weight fractions (<1 kDa) and high molecular weight fractions (>3 kDa). The low molecular weight fractions were more effective to inhibits HIV-1 gene expression. On the other hand, Raghuraman et al. [205] showed that lignosulfonate with molecular weight of 39.4 kDa showed IC_{50} values of 0.017 μM, and 0.32 μM against HSV-1, and HSV-2, respectively, much lower than those of lignosulfonate with molecular weight of 1.9 kDa.

The linkages of lignin can also have a significant impact on its antiviral activity. Raghuraman et al. [205] performed molecular modeling of six dimeric model of lignosulfonate, which demonstrated that selected lignosulfonate structures were likely to mimic certain heparan sulfate sequences. Li et al. [183] reported that native lignin, milled wood lignin, of beech wood exhibited no antiviral activity. In the study by Kimura et al. [186], lignin was isolated from sugarcane bagasse by microwave heating with aqueous glycerol containing 0.5% H_2SO_4. The isolated lignin without β-O-4 showed strong antiviral activity against encephalomyocarditis virus, while the antiviral activity of isolated lignin with β-O-4 linkage was very weak [186]. Mitsuhashi et al. [193] tested the anti-HIV activity of lignin dimeric model compounds of β-O-4, β-5, β-β, β-1, and 5-5 structures, which revealed that the compound containing β-5 bond had most potent inhibitory activity. These researches highlight the importance of lignin linkages on its antiviral activity.

Lastly, the antiviral activity of lignin may also be altered by the monomer constitutes, and functional groups of lignin. Nakashima et al. [197] reported that DHP synthesized from *p*-coumaric acid showed superior anti-HIV activity compared with caffeic acid, and ferulic acid. Also, the antiviral activity of DHP could be altered by post reduction or oxidation [197]. In the study by Boarino et al. [181], kraft lignin, organosolv lignin, and terephthalic aldehyde-protected lignin did not reveal any antiviral activity, whilst soda lignin, and acid catalyzed phenolated lignin showed strong antiviral activity. In the study by Riviere et al. [185], cationic particles coated by quaternary amine-modified softwood kraft lignin were also found to improve the binding interactions with viruses. These studies indicate the significance of functional groups to trigger the antiviral activity of lignin.

7. Improving the antibacterial/antifungal activities of lignin

Generally, the reported MIC value of isolated lignin against bacteria is at the level of several mg/mL. For example, the MIC of soda lignin from sugarcane bagasse against *S. epidermidis* is 4.096 mg/mL [98]. The MIC of commercial alkali lignin and lignin from residue of ethanol production against *S. aureus* is 2.5 mg/mL and 1.25 mg/mL, respectively [86]. However, such an antibacterial activity of lignin is weak when comparing to conventional industrial antibiotics, and antimicrobial polymers [206], such methylisothiazolinone, and 1,2-benzisothiazolin-3-one, which possess MIC values against *S. aureus* of 30–40 μg/mL [154]. Though lignin may perform a bit better as antifungal compounds, for example, the MIC value of lignosulfonate *Candida* spp. was 64–128 μg/mL. However, the antifungal activity of lignin remains weak when compared to traditional antifungal compounds, such as amphotericin B, voriconazole, and caspofungin, which typically possess MIC values against *Candida* spp. in range of 0.015–0.5 μg/mL [207]. Obviously, improvement of the antibacterial/antifungal performance of lignin is the key challenge to replace conventional antibiotics with lignin.

7.1. Fractionation of lignin

Fractionation of lignin has been extensively addressed to obtain lignin fractions with uniform properties (Fig. 6), which facilitates the rational valorization of lignin [208,209]. Remarkably, the structure and properties of lignin can be tuned by rational selection of the fractionation method and the operation parameters [210]. These parameters of lignin structure and properties include molecular weight, content of phenolic hydroxy groups, S/G ratios, content of methoxy groups, ROS scavenging ability, ζ-potential. Reducing the heterogeneity of lignin should facilitate its application as improved antibacterial materials.

Accordingly, the antibacterial activities of lignin fractions are significantly impacted by fractionation because of modification of its structures by the fractionation processes (Fig. 6). In a study by Wang et al. [153], aqueous ethanol (95%) soluble and insoluble fractions were extracted from bamboo kraft lignin. The soluble fraction showed low molecular weight (2518 Da) with high phenolic hydroxy groups content (1.94 mmol/g), while the insoluble fraction showed high molecular weight (5216) with low phenolic hydroxy content (0.99 mmol/g) by GPC and ^{31}P NMR studies [153]. Antibacterial behaviors of the lignin fractions were determined by diffusion method and MIC assay. The soluble fraction showed noticeably stronger antimicrobial activities with larger inhibition zone (16.72 mm against *S. aureus*) than those of bamboo kraft lignin (Inhibition zone: 12.86 mm), and the insoluble fraction (Inhibition zone: 9.69 mm) [153]. The improvement of antibacterial activities of the soluble fraction was attributed to the enhanced water solubility associated with high hydrophilic groups (COOH and phenolic OH) contents, which promoted the attraction of lignin molecules to bacteria [153].

In the studies by Xu et al. [156], four lignin fractions were prepared from organosolv lignin of eucalyptus by sequential precipitation from lignin in GVL/water solution (6/4 v/v). Molecular weight, phenolic OH content, antioxidant activity and antibacterial activity of the lignin fractions were determined by GPC, ^{31}P NMR, DPPH assay and diffusion method, which were closely relative to each other [156]. This results mean lignin fraction with low molecular weight possesses high phenolic OH content, strong antioxidant activity, and antibacterial activity, and *vice versa* [156]. Therefore, the lignin fraction with molecular weight of 1890 g/mol shows enhanced antibacterial activity than the raw lignin (molecular weight of 2930 g/mol) [156]. The mechanism of action of the lignin fraction against bacteria was proposed [156]. Lignin with phenolic hydroxyl group reduces the pH of cell membrane bacteria, which promotes the penetration of lignin fraction of low molecular weight into the cell and inhibits its growth [156].

In the study by Zheng et al. [160], kraft lignin of wheat straw was fractionated into three fractions by sequential ultrafiltration. The S/G ratio, β-O-4 content, phenolic OH content, molecular weight, and ROS scavenging ability of the lignin fractions were determined by HSQC, ^{31}P NMR, GPC studies and DPPH assay [160]. Their results showed that lignin fraction with low molecular weight and high phenolic OH content exhibited high cell toxicity, which was attributed to the fact that strong ROS scavenging activity of lignin activates cellular antioxidant system by over-expression of antioxidant genes, including HO-1, SOD-1, GCLC, and SOD-2 [160].

As usually, lignin fractions of low molecular weight are related to high phenolic content and strong antioxidant activities, and strong antibacterial activities thereof [153,156,160]. However, it is not a golden law since the mechanism on the enhanced antibacterial activity of lignin fraction is not clear [156,160]. Besides, there are also reports that no obvious difference on the antibacterial activities of lignin can be detected from lignin fractions with different molecular weight, and different S/G ratios [212,213]. It is therefore especially important to specify the sources of lignin, including the plant sources, isolation methods, and characterize the lignin and its fractions by determining the monomer constituents, linkages, molecular weight, and the functional groups.

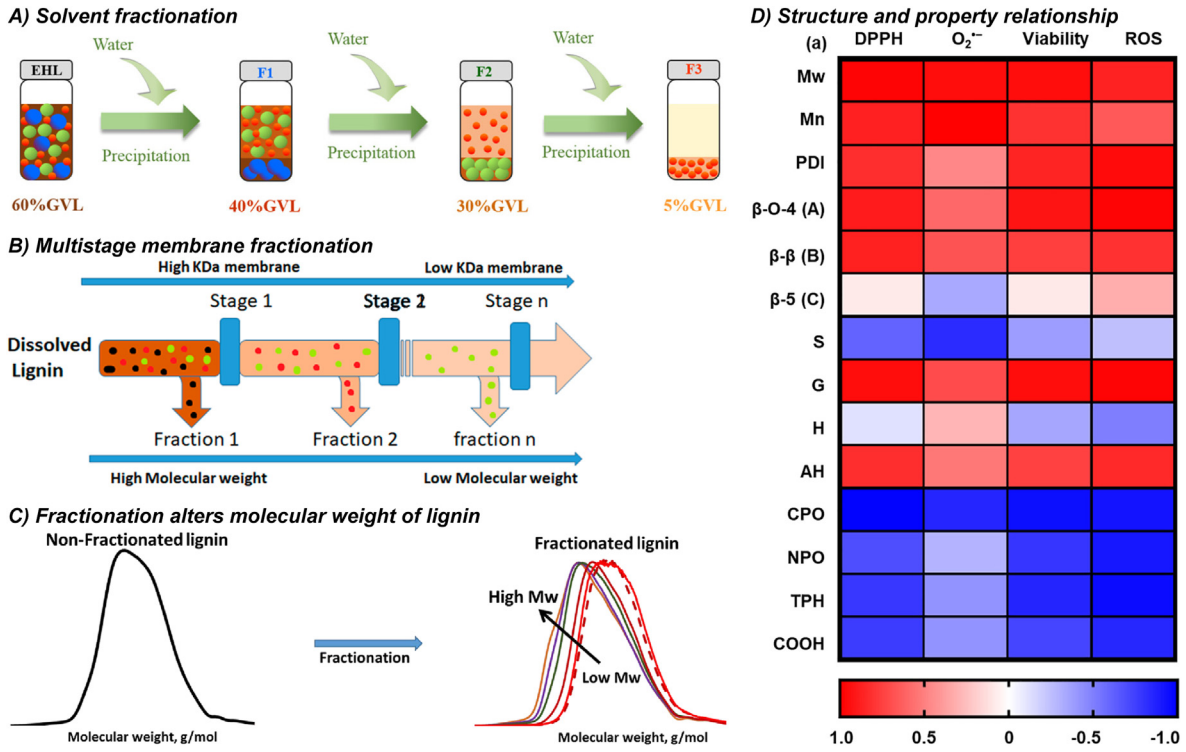

Fig. 6. Lignin fractionation processes, and structure and properties of lignin fractions. A) Solvent fractionation of lignin. Reprinted (adapted) with permission from Ref. [211]. Copyright © 2019, American Chemical Society. B) Membrane fractionation of lignin [210]. C) Molecular weight of raw lignin and lignin fractions [210]. Reprinted (adapted) with permission from Ref. [210]. Copyright © 2021, American Chemical Society. D) Heat map of Pearson R-values between the physicochemical properties and biological activities of lignin fractions [160].

7.2. Catalytic depolymerization

As aromatic polymers, lignin can be converted into phenolic oil upon catalytic depolymerization. On the other hand, creosote oil is one of the oldest historically used wood preservatives, composed of 85% polycyclic aromatic hydrocarbons, and 10–15% phenolic compounds [214]. Given the similar constituents between the creosote oil and the depolymerized lignin oil, researchers are using lignin oil as sustainable antimicrobials in replace of creosote oil.

Acid/based catalytic depolymerization, pyrolysis, hydrogenolysis, and catalytic oxidation are the representative processes that cleave lignin polymer into phenolic bio-oil. Acid/based catalyzed depolymerization of lignin is regarded as an indispensable technique that results in low-molecular-mass aromatics [215]. It is reported that acid catalyzed depolymerization of lignin at 200 °C in ethanol effectively improves its solubility and antioxidant activity by reducing the molecular weight of lignin [216]. In the study by Santos et al. [217], lignin oil was obtained from organosolv lignin by base-catalyzed depolymerization at 300 °C for 40 min. The lignin oil comprised various phenolic monomers by GC-MS, including phenol, *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, catechol, syringol, acetovanillone, 4-hydroxybenzoic acid et al., with catechol being most abundant compound [217]. The lignin oil was then formulated into a 1 wt% solution to the antifungal potential against *Trametes versicolor* fungus for wood protection purposes [217]. Durability of pine wood was increased with the treatment of lignin oil formulation [217]. Compared to the untreated samples, the wood treated with lignin oil reduced weight loss as high as 45.9% after exposure to the fungus *T. versicolor* for 48 weeks, which was classified as decay resistant [217].

Biocidal properties of bio-oil from pyrolysis of various biomass were reviewed by Mattos et al. [218], although a few are focused on the lignin oil. However, lignin generates aromatic oil by pyrolysis which is quite different from those of biomass oil comprising of both aromatic and hydrocarbons. Lignin oil by pyrolysis is the most toxic pesticide compared to the cellulose and hemicellulose oils [219]. In a study by Hossain et al. [220], lignin oil produced at 550 °C inhibited the growth of all microorganisms tested at 0.3 mg/mL. In the study by Kim et al. [221], wood block samples were treated with diluted bio-oil solutions prepared by fast pyrolysis of yellow poplar sawdust, and then subjected to hot water leaching treatment for 72 h. When the wood blocks were subjected to soil block tests using fungi *T. palustris* and *T. versicolor*, weight loss of the treated sample was 10-fold less compared to the control one [221]. Sixteen lignin derived phenolic compounds were identified from the pyrolysis oil by GC-MS analysis, including syringol, vanillin, vanillic acid, methoxy eugenol, syingaldehyde, and acetosyringone [221]. In the study by Lourençon et al. [222], lignin derived phenolic oil was produced by fast pyrolysis of residual wood fines in a pilot plant for further application as antifungal and hydrophobic agent for wood protection. The pinewood treated with the pyrolysis oil had decay resistance improved 4.5 times against *T. versicolor* and *G. trabeum* fungi [222]. These studies showed that pyrolysis of lignin provided phenolic bio-oil as promising sustainable preservatives.

Oxidation is another interesting lignin depolymerization process which generate highly functionalized, valuable monophenols [223]. Antimicrobial activity of ethyl acetate extracted bio-oil from oxidative depolymerized lignin with peracetic acid was tested against lactic acid bacteria and yeast [224]. Composition analysis revealed that the oxidative lignin oil mainly comprised of oligomeric lignin with limited monolignols [224]. Interestingly, the oxidative lignin oil inhibited the growth of commercial lactic acid bacteria (including antibiotic-resistant strains) at a concentration of 4 mg/mL but without any inhibition against an industrial yeast strain [224]. It is suggested that the lignin oil can be used as an antibiotic replacement with highly selective antimicrobial properties to facilitate fuel ethanol fermentation.

On the other hand, the presence of alkyl groups in the aromatic molecules enhances the antibacterial efficacy of phenolics while the oxidation method extensively converts the alkyl side chains of lignin into

benzylic hydroxy, aldehyde, and carboxyl groups [223]. Hydrogenolysis is such an interesting method that depolymerize lignin into alkyl phenolics selectively. Lignin oils were prepared from waste agricultural residues (corn stover, wheat straw, sugarcane bagasse) and energy crops (miscanthus, switchgrass) by reductive catalytic fractionation [225]. The MIC value of the lignin oil from miscanthus, switchgrass, and sugarcane bagasse against *S. aureus* was 2.5 mg/mL [225]. Structure and antimicrobial activity relations of the lignin oils were analyzed using principal component analysis method [225]. Four components, including methyl coumarate, propyl guaiacol, propyl syringol, and ethoxy phenol, had the strongest bacteriostatic influence associated with the lowest MIC [225]. The strong antimicrobial activity of these lignin monomers was attributed to the presence of long alkyl chains on syringol and guaiacol units [225].

In the study by Kalinoski et al. [165], lignin derived bio-oil was extracted using hexane, petroleum ether, chloroform, and ethyl acetate as solvents from the depolymerized corn stover lignin by catalytic transfer hydrogenolysis in supercritical ethanol with a Ru/C catalyst (Fig. 7). The lignin bio-oils inhibited all tested organisms at concentration 3 mg/mL, including gram-positive and gram-negative bacteria and yeast [165]. All the monomers, dimers, trimers, and larger oligomers were proved to be the driver and source for the antimicrobial activity of these lignin bio-oil [165]. Synergism between those compounds in the lignin bio-oil was proposed but without a clear mechanism of action of the antimicrobial properties [165]. Besides, the lignin bio-oils needed much higher concentrations to inhibit microbial growth when compared to traditional antibiotics which possess MIC values at the parts per million level. Therefore, the commercial applications of lignin oil might be limited to preservation of industrial products like furniture, coating, adhesive et al. but not suitable for medical or food related uses [165]. On the bright side, tuning lignin from an insoluble power state into an oil product will make it easier to be incorporated into a variety of products.

7.3. Chemical modification of lignin

Because of the presence of a variety of different functional groups, including hydroxy and aromatic groups, lignin is a polymer with high reactivity. Therefore, chemical modifications of lignin are conventionally applied to tune the properties of lignin. There are several excellent reviews summarizing the reaction associated with lignin functional groups and functionalization of the polymer with different technologies [14,32,226–228]. We will focus on the antimicrobial properties of the modified lignin but without extensive introduction of the lignin modification technologies.

To introduce additional antimicrobial properties into lignin, functional groups that contribute to antimicrobial activities of macromolecules are usually coupled to lignin (Fig. 8). Inspired by antimicrobial peptides and polyampholytes [233,234], Chen et al. [229] functionalized enzymatic hydrolysis lignin into amino-polyampholytes by Mannich reaction with three different cationic amino acids, namely arginine, lysine, and histidine (Fig. 8A). The antimicrobial activities of modified lignin were significantly improved with MIC value against *S. aureus* and *E. faecalis* of 10 mg/mL while that of the unmodified lignin was >20 mg/mL [229]. The mechanism was attributed to the enhancement of electrostatic and hydrophobic interactions between modified lignin (Fig. 8B) and microbes because of the introduction of the cationic amino acids, which caused bacteria cell death via membrane disruption [229]. Besides, the dead bacteria were found to be removed from the material by antifouling effect of anionic and cationic hydration layers because of the presence of both carboxylate and amino groups [229]. A similar antifouling mechanism was applied by Xu et al. [230], to synthesize lignin grafted poly(sulfobetaine methacrylate) zwitterionic polymeric hydrogel. The zwitterionic lignin hydrogel inhibited microbial growth of 94.8% against *E. coli* and 95.7% against *S. aureus* by susceptibility test [230].

Both the antimicrobial activities and mechanism can be tuned by altering the functional groups attached to modified lignin. In the study by Kaur et al. [231], the antimicrobial activities of lignin against both *Bacillus* sp. and *Klebsiella* sp. were improved via the introduction of epoxy by the reaction of lignin with epichlorohydrin in a sodium hydroxide solution (Fig. 8C). In the study by Ma et al. [232], trimethyl quaternary ammonium lignin (Fig. 8D) was synthesized and incorporated into polystyrene membrane. The resultant composite membrane inhibited the growth of *E. coli* by susceptibility test because of the binding of cationic trimethyl quaternary ammonium lignin to negatively charged cell surface of the microbe [232].

Remarkably, the backbone of lignin molecular chain might contribute significantly to the antimicrobial properties of chemically modified

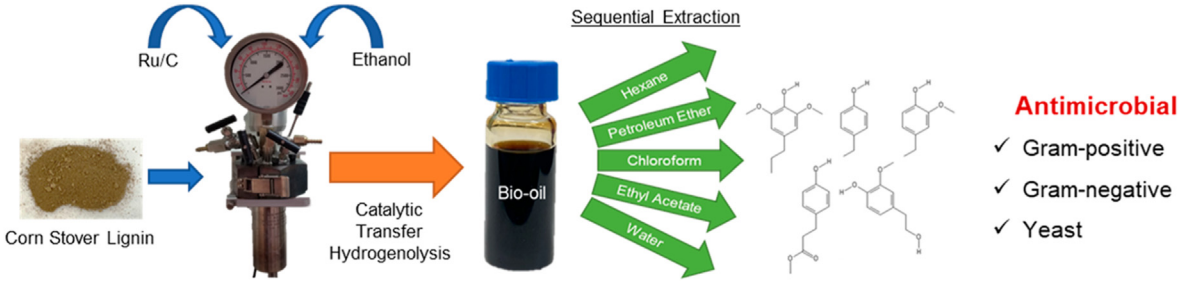

Fig. 7. Lignin oil from catalytic transfer hydrogenolysis of corn stover lignin fractions inhibit the growth of different microorganisms Reprinted (adapted) with permission from Ref. [165]. Copyright © 2020, American Chemical Society.

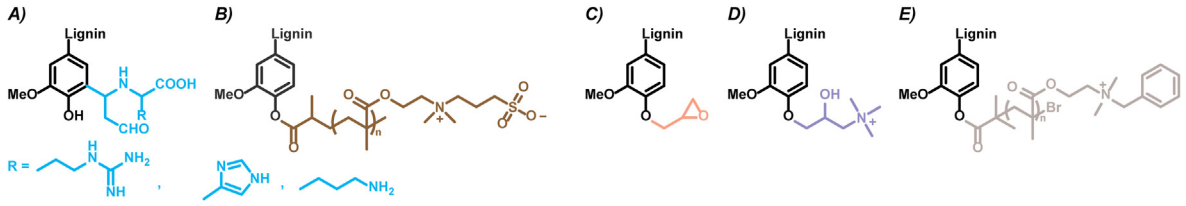

Fig. 8. Chemical modified lignin with antimicrobial activity. A) Amino acid-functionalized lignin [229]; B) Zwitterionic lignin grafted poly(sulfobetaine methacrylate) [230]; C) Epoxy lignin [231]; D) Lignin trimethyl quaternary ammonium salt [232]; E) Cationic lignin based hyperbranched polymers [168].

lignin. In a study by Chee et al. [168], the antimicrobial activities of a series of cationic lignin based hyperbranched polymers (Fig. 8E) were assayed. The results turned out that the antimicrobial activity was not related to substitution degree of lignin copolymers, though the lignin grafted copolymers could effectively inhibit a broad spectrum of bacteria with MIC value as low as 32 μg/mL [168]. Chee et al. [168] explained this phenomenon by the high heterogeneity of lignin, in addition to the functional groups, which could contribute significantly to the antimicrobial activity of modified lignin.

Although the antimicrobial activity of lignin has been noticeably enhanced by chemical modification. The approach also introduces cytotoxicity into modified lignin polymer. As was shown in the study by Chen et al. [229], the hemolytic activity to human red blood cell and cytotoxicity to human immortalized keratinocytes and HaCat increased with the modification of lignin with cationic amino acids, in consistent with the improvement in its antimicrobial activities. It is therefore that future studies on the chemical modification of lignin should base on both the cytotoxicity and antimicrobial activity to optimize the formula of the modified polymer.

7.4. Composites of lignin and antimicrobial compounds

It is conventional to combine two or more compounds to produce composite with desired properties in material science. This is also true when it comes to the subject of tuning the antimicrobial properties of lignin. By the introduction of another antimicrobial compound, it broadens the potential application of lignin as antimicrobial materials in a wide range of fields. These antimicrobials, applied to blend with lignin, include antimicrobial polymers, small molecules, and metallic antimicrobials. Because of the noticeable difference among these antimicrobial compounds, they are discussed separately.

7.4.1. Lignin and antimicrobial polymer alloys

Diverse polymeric composites have been developed for application in the fields of fibers, elastomers, plastic et al. by bending lignin with other natural or synthetic polymers [235]. The properties of resultant lignin derived composites have been dramatically improved [23]. However, most of those researches are pursuing environmentally friendly bulk composites, such thermoplastics, associated with bio-degradable and carbon neutral features [236]. Few have been focused on generating polymeric composites with improving antimicrobial properties by blending lignin with another polymer.

To the best our knowledge, chitosan is the only antimicrobial polymer used to combine with lignin to produce novel antimicrobial. In the study by Kim et al. [237], lignosulfonate-chitosan nanoparticles were prepared by mixing the lignosulfonate and chitosan solutions in the presence of a vegetal oil organic phase. Formation of the nanoparticles were driven by strong electrostatic interactions between the two oppositely charged polymers, which resulted in core-shell particles with high positive charged surface [237]. Interestingly, the lignosulfonate-chitosan nanoparticles (Fig. 9) presented improved antimicrobial activities against all the tested gram-negative and gram-positive bacteria without altering its cytotoxicity when compared with these of pure lignosulfonate or chitosan nanoparticles [237].

In the studies by Pandey et al. [238], chemically cross-linked lignosulfonate/chitosan composite was synthesized by Mannich reaction between lignosulfonate and chitosan (Fig. 9). The chemically cross-linked composite showed enhanced antimicrobial activity when compared with the ionic complex of lignosulfonate and chitosan [238]. The enhanced antimicrobial properties of this composite were attributed to the closed stack of the two polymers by covalent bonding which resulted in high positive surface charges and increased lactate dehydrogenase releasing from the bacterial cells by strong cell membrane disruption [238].

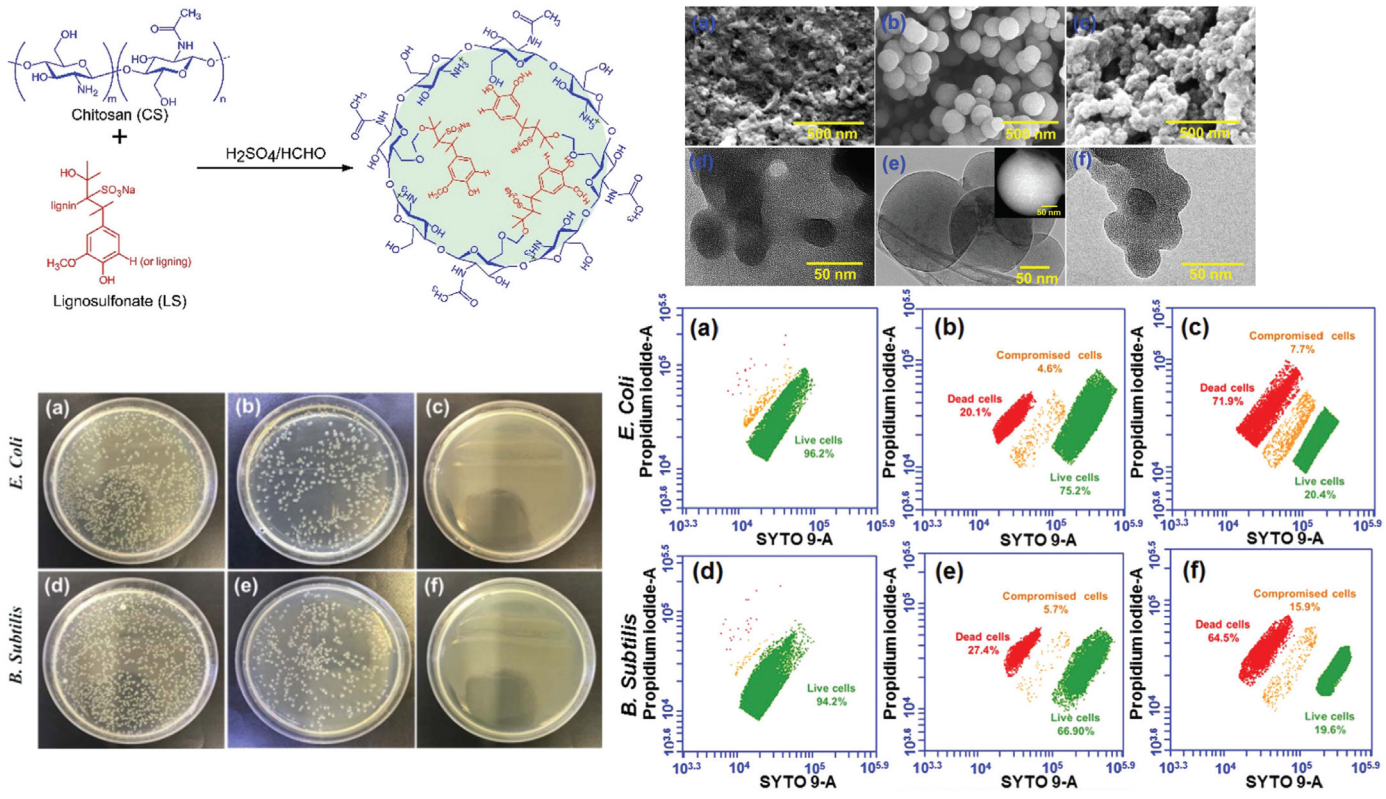

Fig. 9. Preparation of lignosulfonate and chitosan antimicrobial nanocomposite. A) Synthesis of lignin and chitosan nanoparticle; B) SEM image of lignin and chitosan nanoparticle; C) Antimicrobial assay of the nanoparticle by susceptibility test; D) Antimicrobial assay of the nanoparticle by flow cytometry [237].

Besides, the lignosulfonate and chitosan composite can form an antifouling thin layer because of the excellent the film formation ability of chitosan and the zwitterionic character associated with the presence of both amino and sulfonate groups [238]. Therefore, the composite can be used for surface protection by hindering bacterial attachment and preventing biofilm formation, and thus reducing bio-corrosion [239]. It is reported that the content of sulfur element, which is a major bio-corrosion product, reduced 31% after coating of carbon steel with the lignosulfonate chitosan composite [240]. In another study, it was reported that lignosulfonate chitosan composites exhibited a maximum of 84% carbon steel corrosion inhibition under dynamic flow conditions by electrochemical impedance analysis [241].

The introduce of another antimicrobial polymer into lignin can noticeably improve the antimicrobial performance of the resultant composite. However, these studies are limited to lignosulfonate. Lignosulfonate is a water-soluble anionic industrial lignin while a substantial portion of available industrial lignin is the water insoluble, such as kraft lignin (from wood) and soda lignin (from annual plant). Further researches are encouraged to use these water insoluble industrial lignins because of their abundance. Besides, novel antimicrobial polymers are encouraged to be blended with lignin, which should lead to novel antimicrobial composite with diverse application.

7.4.2. Lignin and molecular antimicrobial matrixes

Generally, lignin and molecular antimicrobial matrixes is known as drug carrier or drug delivery vehicle. Lignin antimicrobial drug carriers are different from those of lignin and antimicrobial polymer blending composites. Both lignin and antimicrobial polymer serve as major antimicrobial constituents in the polymer blending composites. However, lignin serves as carrier matrix in the case of lignin antimicrobial drug carriers. Many reviews have discussed several aspects of using lignin as drug carriers [242–247]. Herein, we only focus on formulations with antimicrobial activities. A list of such publications can be found in Table 4.

Improving the drug performances is the initial droving force to develop formulations using lignin as drug carriers. Al-Obaidy et al. [263] compared the antibiotic effect of free vancomycin with encapsulated vancomycin nanocarriers. The results showed that encapsulation of vancomycin into nanocarriers strongly enhanced the antibiotic action of vancomycin against three different proxy microorganisms, *C. reinhardtii*, *S. cerevisiae*, and *E. coli* [263]. The enhanced antibiotic action was attributed to the strong electrostatic interaction between the nanocarrier and negatively charged surface of microbial cell wall, which allowed the local accumulation of vancomycin on the cell wall [263].

Using lignin as the drug carrier takes advantages of the biocompatible, and environmental benign features of lignin. The *in vitro* cytotoxicity and hemotoxicity of lignin-based nanoparticles were assayed by Figueiredo et al. [264] using human umbilical vein cell line, human mammary carcinoma cell lines, human prostate cancer cell line, human colorectal adenocarcinoma cell line, human myeloid cell line, and human red blood cells. The lignin-based nanoparticles exhibited low cytotoxicity in different cell lines, and low *in vitro* hemolysis at concentrations up to 100 μg/mL [264]. In another study by Stine et al. [265], *in vivo* toxicity of lignin nanoparticles was assessed using embryonic zebrafish toxicity assays. Lignin nanoparticles were allowed for direct contacting with developing embryo by enzymatical removal of the chorion. The results revealed that lignin was nontoxicity to embryonic zebrafish at exposure concentration up to 640 mg/L [265]. Because of its nontoxicity, lignin is an ideal carrier for antimicrobial drugs with biological application.

In addition, lignin is biodegradable natural polymers under fungi attack. Taking advantage of this phenomenon, Fischer et al. [253] desired enzyme-responsive lignin nanocarriers for preventive and curative treatment of plant diseases by fungi infections. Pyraclostrobin, a fungicide, was loaded into the lignin nanocarrier by miniemulsion polymerization [253]. Pyraclostrobin was released and became active only in the presence of lignin-degrading fungi, such as grapevine trunk disease esca [253]. Furthermore, the drug did not leak from the lignin nanocarrier during storage [253]. The application of lignin as pathogen-specific enzymes responsive nanocarrier provides a novel plant diseases treatment technology that decreases untoward environmental effects from pollution of agrochemicals.

Lignin antimicrobial drug carriers have been developed into different forms, such as nanoparticle, hydrogel, and nano/micro capsules. The technologies are significantly different according to the synthesis of distinct types of carriers. To synthesize lignin and drug nanoparticles, solvent exchange by dialysis method is generally applied to load hydrophobic drug into lignin nanoparticles. In the study by Siddiqui et al. [250], irinotecan and lignin nanoparticles were prepared by dialysis of a dimethyl sulfoxide (1 mL) solution of the drug (1 mg) and lignin (10 mg) against deionized water. Irinotecan loaded lignin nanoparticles were irregular nanoparticles with particle size of approximately 160 nm, and drug content of approximately 13% [250]. The synthesis of lignin and

Table 4
Lignin-antimicrobial drug matrices: constituents, synthetic methods, and performance.

Carrier	Lignins	Drug loaded	Carrier synthesis	Results	REF
Nano-particle	Acetylated lignin	Porphyrin	Solvent exchange by dialysis	Preservation of porphyrin's photochemical properties	[248, 249]
	Kraft lignin	Irinotecan	Solvent exchange by dialysis	Decrease IC_{50} of irinotecan by 3 folds	[250]
	Acidolysis lignin	Essential oil	Solvent exchange by dialysis	Improve antimicrobial activity of essential oil	[251]
Hydrogel	Unknown	Curcumin	Cross-link by esterification	Improved therapy of microbial infection	[252]
Capsules	Kraft lignin methacrylate	Pyraclostrobin	Miniemulsion polymerization	Pathogen triggered drug release capsule for curative and preventive treatment plant diseases	[253]
	Lignin methacrylate	Azoxystrobin, Pyraclostrobin, Tebuconazole, Boscalid	Miniemulsion polymerization	Encapsulation reduces MIC value of the drugs	[254]
	Kraft lignin	Propiconazole	Sacrificial surfactant templates	Encapsulation driving forces: hydrophobic interactions, π−π stackings, hydrogen bond	[255]
	Kraft lignin	Propiconazole	Sacrificial surfactant templates	Enhanced antifungal performance for wood preservation	[256]
	Lignin nanoparticles	Essential Oil	Pickering emulsion	Disperse essential oil into water	[257]
	Lysine modified lignin	Curcumin	High internal phase emulsion	Increase chemical stability and bioavailability of curcumin	[258]
	Kraft lignin	Thymol	Emulsion	Stabilize thymol in water	[259]
	Lignin methacrylate	Plant Extracts	Self-assembly	Reduce the amount of bioactive compound used	[260]
	Lignosulfonate	Avermectin	Self-assembly	Antiphotolysis of the pesticide	[261]
	Cationic kraft lignin	*Trichoderma reesei*	Layer-by-layer encapsulation	*T. reesei* as both protective and curative treatments of grape esca	[262]

drug nanoparticle by solvent exchange is a feasible and straightforward approach by dissolving the materials followed by nanoprecipitation. However, the morphology of the carrier synthesized by this method is not controllable. Sphere nanoparticles was reported by Maldonado-Carmona [250], while irregular nanoparticles were reported by both Vettraino et al. [251] and Siddiqui et al. [250].

To synthesize drug loaded lignin hydrogels, lignin is chemically cross-linked with water soluble polymers to prepare a lignin hydrogel. Then, the drug is load onto the hydrogel by immersing the hydrogel into a solution of the drug. In the study by Larrañeta et al. [252], Gantrez™ S-97 was used as cross-linker to couple lignin and poly(ethylene glycol) via esterification reactions. The resultant lignin and poly(ethylene glycol) hydrogel was immersed into a 20 mg/L curcumin in acetone solution to load the drug [252]. The hydrophobic nature of lignin is attributed to the loading of the hydrophobic drug [252]. Different drug loaded hydrogels may be synthesized by this approach with different water soluble polymers and different drugs.

In addition, a variety of methods are applied to synthesize lignin and drug capsules, such as miniemulsion polymerization, surfactant templates, and self-assembly. The application of different synthesis approaches allows for encapsulation of different drugs with a variety of lignin sources. In the study by Machado et al. [254], four hydrophobic fungicides, namely azoxystrobin, pyraclostrobin, tebuconazole, and boscalid, were encapsulated individually by in situ miniemulsion polymerization of lignin methacrylate. The fungicide loaded lignin nano capsules showed an average particle diameter of ~200 nm with encapsulation efficiencies higher than 75% [254]. Interestingly, the nano capsules kept their effectiveness over a period of at least 4 years revealed by *in planta* studies [254].

In the studies by Ela et al. [255], propiconazole was loaded into double-shelled lignin nano capsules by sacrificial surfactant templates method. Different from the insoluble solid fungicides such as pyraclostrobin, propiconazole is a yellowish viscous liquid with solubility in water of 100 mg/L at 25 °C. The application of sacrificial surfactant templates allowed the synthesis of stable double shell nano capsules with unmodified kraft lignin [255]. Subsequently, propiconazole could be loaded into nano capsules in a water medium because of its modest solubility in water [255]. The mechanisms driving nano capsule formation was studied by analyzing the spatial distribution of the elements on the surface of the particles [255]. Intermolecular and intramolecular hydrogen bonding, and hydrophobic interactions, such as nucleation−-growth, π−π stackings of benzene rings, and plausibly ion−π stackings were determined to be the driving forces [255]. By biocidal test against *Gloeophyllum trabeum* in southern yellow pine wood, the double-shelled-propiconazole nano capsule system was an effective wood preservative comparable to the conventional chromated copper arsenate [256].

The diverse methods in synthesis of lignin and drug capsules make it highly dynamic in encapsulating different drugs. For example, plant extracts from *Rubia tinctorum, Silybum marianum, Equisetum arvense, and Urtica dioica* were encapsulated into chemically modified lignin by hydrogen bond driven self-assembly [260]. The encapsulation of plant extracts with lignin provided a natural origin for plant disease treatment. The antifungal efficiency of such natural antimicrobial nano carriers was demonstrated by *in vivo* assay of 20-year-old grapevines with clear grapevine trunk disease symptoms [260].

In another study by Peil et al. [262], antagonistic fungi, *Trichoderma reesei*, was encapsulated by layer-by-layer deposition of lignosulfonate and cationic lignin. The coating of lignin onto the surface of the spores of *Trichoderma reesei* kept the pores resting even when injected into a plant [262]. Interestingly, lignin is enzymatic depolymerized when the plant is infected with pathogenic fungi, which will release the free spores of *Trichoderma reesei* [262]. Then, the free spores germinate and kill the pathogenic fungi [262]. The application of lignin for encapsulation of spores provides a strategy that wraps up antagonistic fungi with plant constituent for plant disease protective and curative treatments.

As is discussed above, the lignin and drug matrixes make use of lignin as the carriers but not intend to improve the antimicrobial properties of the lignin itself. Nevertheless, this strategy does generate novel antimicrobial formulations with lignin being a major constituent. Most of the research efforts have been focused on the encapsulation technologies using lignin as the carrier and the controlled release of the loaded drug. Given that lignin itself is a bioactive material with enormous phenolic groups, it should be interesting to know whether the inherent bioactivities of lignin will impact the antimicrobial properties of the drug carrier of lignin.

7.4.3. Lignin and antimicrobial metal composite

Metals are the oldest and excellent antimicrobial with broad antimicrobial spectrum and multiple antimicrobial mechanism which is not compliance and increasing the likelihood of drug resistance of microorganisms [266]. Because of the presence of a variety of oxygen rich functional groups, lignin can serve as both stabilizer and reducing agents for metallic antimicrobials. On the other hand, the metallic antimicrobials are hazardous material with high cytotoxicity. The combination of metals with lignin takes advantages of the biocompatibility of lignin and high antimicrobial activity of metals. Such combinations allow to produce novel formulas with high antimicrobial activity and low cytotoxicity.

Like lignin and drug vehicles, the initial driving force of synthesis of lignin and metal formulations is to tackle with challenges of the application of metal as antimicrobial. These challenges include stabilizing the metallic materials, reducing hazardous reagents used for synthesis, and reducing cytotoxicity of the metallic antimicrobials. Nonetheless, lignin contributes usually a dominant portion in these lignin and metal complexes. Therefore, we will discuss the methods incorporating lignin with metals, and the antimicrobial performance of the resultant formulations. A list of these lignin and metal antimicrobial formulations can be found in Table 5. Hopefully, our efforts will show light on how lignin contributes to the composite antimicrobials of lignin and metals although lignin is not the primary antimicrobial ingredients.

Precious metals. Silver had been known to be strong and broad-spectrum antimicrobial against bacteria, viruses, and fungi with MIC value as low as 0.03 mM (3.24 μg/mL) in dark [284]. The oxygen rich functional groups allow lignin to serve as both stabilizer and reducing agents for elemental silver (Ag^0) antimicrobials. Tran et al. [267] synthesized lignin/Ag^0 hybrid nanoparticles by heating $AgNO_3$ and lignin aqueous solution at 100 °C. The lignin/Ag^0 hybrid nanoparticles inhibited the growth of both *E. coli* and *S. aures* at least for 72 h at 5 mg/mL [267]. Slavin et al. [268] synthesized lignin/Ag^0 sphere nanoparticles with particle size of ~20 nm by heating lignin and $AgNO_3$ aqueous solution at 60 °C for 3 days. The lignin/Ag^0 nanoparticles possessed MIC value of 10 μg/mL against a variety of multidrug resistant bacteria because it was able to enter bacterial cells through efflux pumps to inhibit the development of bacteria resistance [268].

In addition, the oxygen containing functional groups lead to negative charges of lignin when lignin molecules are dispersed into aqueous solution. The negative charges make lignin a good material for chelation and adsorption of silver ions. Richter et al. [285] embedded silver ions into lignin nanoparticles by infusing the lignin nanoparticles into an aqueous solution of $AgNO_3$ as silver source, and then coated the Ag^+ bound nanoparticle with a cationic polyelectrolyte. The binding of Ag^+ by lignin nanoparticles was modeled by a modified Langmuir adsorption isotherm with maximum weakly bound Ag^+ of 9.17 mg/g, and strongly bound Ag^+ of 1.4 mg/g, respectively [285]. The polyelectrolyte coated Ag^+ bound lignin nanoparticles showed high antimicrobial activity, which reduced 100% *P. aeruginosa* growth at 5 μg/mL Ag^+ equivalent [285]. Importantly, the lignin/Ag^+ nanoparticle would lose its antimicrobial activity gradually because of Ag^+ ion depletion [285]. These results suggest that lignin could be used to synthesize Ag^+ derived antimicrobials with low environmental impact when they are abandoned as waste.

Table 5
Lignin and metal composite antimicrobials: constituents, synthetic methods, and antimicrobial properties of the products.

Lignin	Metal state	Method	Final products	Antimicrobial properties	REF
Rice husk soda lignin	Ag^0	Lignin serves as reducing-capping agent	Lignin/Ag^0/SiO_2 nanoparticle	Inhibit bacterial growth >72 h	[267]
Alkali lignin	Ag^0	Lignin serves as reducing-capping agent	Lignin/Ag^0 nanoparticle	MIC of 10 μg/mL to multi-drug resistant bacteria	[268]
Softwood kraft lignin	Ag^+	Ion exchange between deprotonated lignin and Ag^+	Colloidal Ag/lignin particle	Inhibit 90% bacteria growth at 5 μg/mL Ag^+ equivalent with 24 h	[269]
Lignosulfonate	Ag^+	Chelation and redox adsorption of Ag^+ with polyaniline and lignosulfonate	Polyaniline/lignosulfonate/Ag^+ composite spheres	Inhibit >99.9% bacteria growth at 0.8 mg/mL within 24 h	[270]
Alkali lignin	$Ag(NH_3)_2OH$	Mixing lignin solution with silver ammonia complex	Ag/Lignin nanoparticle in polyacrylate hydrogel	Inhibit >97% bacteria growth within 24 h	[89]
Kraft lignin	Au^+/Au^0	Dispersing unbleached kraft pulp in an Au^+ solution followed by reduction	Nanoparticles loaded onto unbleached kraft pulp	Inhibit *S. aureus* growth at 0.13 wt% Au uptake	[271]
Industrial lignins	Au0	Lignin nanoparticle as both reducing and coating agent	Au^0@lignin nanoparticle	Inhibit *S. aureus* growth in 6 h under light	[272]
Kraft lignin	Au^0&Ag^0	Sequent growth of elemental metal on lignin nanoparticles	Au/Ag bimetallic lignin nanoparticle	Delay bacteria growth at least 36 h at 50 μg/mL	[273]
Kraft lignin	Au^0&Ag^0	Sequent growth of elemental metal on lignin nanoparticles	Functionalized Au/Ag bimetallic lignin nanoparticle	IC_{50} 0.25 μg/mL against the fungal strain *Candisa tropicalis*	[274]
Kraft lignin	Te^0	Sonicate treatment of a lignin and TeO_3^{2-} solution	Hybrid Te^0-lignin nanoparticle	>99.99% bacteria reduction at 2.39 μg/mL in 24 h	[169]
Kraft lignin	$Cu_4(OH)_6SO_4$	Mixing lignin, $CuSO_4$ and NaOH under stirring/ball mill	Hybrid organic−inorganic bulk materials	The lignin@Cu pesticide is better than commercial copper hydroxide by *in vivo* studies.	[275]
Alkali lignin	CuO	Lignin and CuO was sequently coated onto nanofiber of cellulose acetate	Lignin/Cu/Cellulose acetate nanofiber	Inhibition zone against bacteria ~2 mm	[276]
Kraft lignin	Cu_2O	Co-precipitation by adding $CuSO_4$ into a lignin sodium hydroxide solution	Nanocomposites	100% Bacteria reduction at 4 mg/mL within 30 min	[277]
Industrial lignins	Cu_2O	Co-precipitation by adding $CuSO_4$ into a lignin solution	Bulk material	MIC (mg/mL) *S. aureus*:0.78 *E. coli*: 12.5 *R. solani*: 0.2	[278]
Lignosulfonate	CuS	Dialysis of a solution of lignosulfonate, Cu^{2+}, and Na_2S	Nanoparticle	5.4-$\log_{10}$ CFU bacteria reduction under NIR radiation at 100 μg/mL within 5 min	[279]
Kraft lignin	ZnO	Co-precipitation from zinc acetate and lignin solution	Hybrid lignin-ZnO nanocomposite	Delay E. coli growth for 36 h at 500 μg/mL	[280]
Lignosulfonate	ZnO	Treatment of ZnO nanoparticle with lignin solution	Encapsulated ZnO nanoparticle by lignin	Inhibition zone against fungi >16 mm	[281]
Lignosulfonate	ZnO	Co-precipitation from Zn^{2+} and lignin solution	Paper coated with lignin/ZnO nanoparticle	MIC (μg/mL) *E. coli*: 125 *B. subtilis:* 62.5	[282]
Industrial lignins	TiO_2	Heating titanium and lignin solution	Coating of lignin-TiO2 nanocomposite	100% Inhibition against *E. coli* at 400 μg/mL	[283]

In another study by Lintinen et al. [269], lignin was deprotonated first by CH_3ONa and then used to bind Ag^+ by ion exchange. Because deprotonation resulted in phenolate lignin, the Ag^+ ions were strongly bound into deprotonated lignin at a high content, 5.8 wt%. The Ag^+ bound deprotonated lignin showed high antimicrobial activity with 90% inhibition of *E. coli* at 5 μg/mL Ag^+ equivalent, which was comparable to the free $AgNO_3$ solution at 5 μg/mL Ag^+ equivalent [269]. Interestingly, there was no need of cationic polyelectrolyte coating of the Ag^+ bound deprotonated lignin nanoparticles to achieve at high antimicrobial activity [269]. The reason was explained by the strong interaction between the Ag^+ ions and the phenolate lignin [269]. Therefore, the Ag^+ targeted at microbe specifically due to even more strong interaction between Ag^+ ions and biomolecules of microbes [269].

Though the lignin/Ag^+ complex showed good antimicrobial activity at a reasonable Ag^+ dosage, the amount of Ag^+ loading onto raw lignin is low [269,285]. Therefore, research efforts are deliberate to chemical modification of lignin to load a high content of Ag^+. Xiao et al. [286] functionalized lignin with both amino and disulfide groups. The functionalized lignin was able to trap Ag^+ as high as 1.34 g/g. Lv et al. [270] reported lignosulfonate and polyaniline composite spheres that adsorbed Ag^+ up to 2.16 g/g. These high Ag^+ loading complexes were reported with bactericidal rate of >99% against both *E. coli*, and *S. aureus*, but at hundreds μg/mL Ag^+ equivalent. Therefore, the impact of Ag^+ loading content on the antimicrobial performance of lignin and Ag^+ ion complex remains largely unknown. Besides, the impact of driving forces of the loading Ag^+ with lignin on the antimicrobial performance of the lignin/Ag^+ complex remains unclear neither.

In addition to silver, gold is the other noble metal that can be used as effective antimicrobial agent. Similar to the cases of silver, lignin can be used as both capping and reducing agent for gold ion. In the study by Johnston et al. [271], Au^{3+} ion was loaded onto unbleached kraft pulp by mixing the pulp and $AuCl_4^-$ in an aqueous solution. The content of loaded Au^{3+} ion could be tuned from 0.016 wt% to 1.6 wt% by adjusting the Au^{3+} to pulp ratio in the solution [271]. Subsequently, the Au^{3+} ion was converted into elemental gold nanoparticles by heating the Au^{3+} loaded pulp to 50–80 °C [271]. The Au^0 nanoparticle loaded unbleached kraft pulp exhibited effective antimicrobial properties with 0.13 wt% Au^0 [271]. In another study by Rocca et al. [272], lignin was coated onto Au^0 nanoparticles in a single one step using lignin as both the reducing and coating agent. The researchers mixed $HAuCl_4$ aqueous solution and lignin aqueous solution (pH = 12), and heated the mixtures to obtained lignin coated Au^0 nanoparticles. The nanoparticles showed bactericidal activity after 6 h of light exposure.

Tellurium, another precious metal, was also reduced to Te^0 nanoparticle by Morena et al. [169]. Like lignin coated Au^0 nanoparticle [272], Morena et al. [169] synthesized the lignin stabilized Te^0 nanoparticles by heating an alkaline solution of lignin and sodium tellurite at 60 °C under ultrasonic assistance. The lignin coated Te^0 nanoparticles possessed an MIC value as low as 1.2 μg/mL against *E. coli* [169]. The excellent antimicrobial activity of lignin coated Te^0 nanoparticles was

attributed to the generation of reactive oxygen species, which caused disruption of the cell envelope and oxidative damage to metabolic pathways, eventually leading to cell death [169].

Additionally, synergistic antioxidant and antimicrobial properties were incorporated into lignin, silver, and gold bimetallic nanocomplexes [273]. Chandna et al. [273] synthesized lignin bimetallic nanocomplexes using lignin as the sole source for reducing, capping, and stabilizing agent. The researchers synthesized lignin-Au^0 nanoparticles first by heating an alkaline aqueous solution of lignin and gold chloride at 55 °C for 6–8 h [273]. Subsequently, the lignin-Au^0 nanoparticles were coated with Ag^0 by addition of an aqueous $AgNO_3$ solution [273]. The lignin-Au^0-Ag^0 nanocomplexes showed an IC_{50} values of 0.24–0.27 μg/mL against three different microbes, which was noticeably lower than those of lignin-Au^0 (94–113 μg/mL), and lignin-Ag^0 (6.19–7.09 μg/mL) nanoparticles [273]. The enhanced antimicrobial activity of lignin bimetallic complexes was attributed to the high efficiency in generation of reactive oxygen species, which led to membrane damage and genomic DNA damage of microbial cells [273].

The antimicrobial activity of lignin bimetallic can be further improved by the cooperation of photosensitizers. Chandna et al. [274] used rose bengal (RB) as photosensitizer to synthesize lignin-Au0-Ag0-RB nanocomposite. The RB molecules were loaded onto the lignin bimetallic composites by esterification with lignin using carbodiimide and *N*-hydroxysuccinimide as activator [274]. After the incorporation of RB photosensitizer, the IC_{50} value of lignin bimetallic nanoparticles against the fungal *Candida tropicalis* decreased from 0.25 μg/mL to 0.1 μg/mL under green laser exposure [274]. The improved antimicrobial activity of lignin, bimetallic, and photosensitizer was attributed to the enhanced ability in reactive oxygen species generation by photosensitizer under light exposure.

Base metals. While precious metals, such as silver, are well known for its excellent antimicrobial activity, copper is recognized as a base metal antimicrobial of non-toxicity. It is because copper is an essential element with important function in the human metabolism. Haider et al. [276] used lignin as a reducing agent to deposit CuO nanoparticles on cellulose nanofibers. The researchers firstly coated the cellulose nanofibers with an alkali lignin aqueous solution and then immersed the coated nanofibers into a copper acetate solution to obtain CuO/lignin/cellulose composite nanofiber [276]. The CuO/lignin/cellulose composite inhibited the growth of both gram-negative and gram-positive bacteria by diffusion assay [276]. Also, the composite was characterized as non-toxic according to the ISO 10993-5 method [276]. Thus, it was be believed to be a potential dressing material [276].

Lignin can be used as both reducing agent and support to synthesize copper-based nanoparticles. Li et al. [277] synthesized broccoli-like Cu_2O nanoparticles by heating an alkaline solution of lignin and Cu^{2+} ions. The Cu_2O nanoparticles showed 100% bactericidal against *E. coli* and *S. aureus* within 30 min with 4.0 mg/mL dosage [277]. Xie et al. [279] synthesized lignin/copper sulfide nanocomposites using lignosulfonate as dual growth template and stabilizing agent. The researchers heated the aqueous solution of lignosulfonate, $CuSO_4$, and Na_2S at 80 °C, and dialyzed it for 2 days to obtain the nanocomposite [279]. The lignin/CuS nanocomposite reduced 5.9-$\log_{10}$ and 5.4-$\log_{10}$ CFU/mL of *E. coli* and *S. aureus*, repectivley, under near infrared light irradiation within 5 min with peroxidase-like mechanism [279]. These reports show that the synthesis of lignin/copper composite antimicrobial is quite straightforward: Simply mixing lignin with copper source and heating the mixture. Such feasible process would facilitate the application of lignin in the synthesis of copper antimicrobials.

Besides, copper has been long using as pesticide in agriculture. However, the prolonged and frequent use of copper-based antimicrobial would lead to copper accumulation in soil and groundwater [275]. Therefore, the European Community has lowered the annual maximum copper usage from 6 to 4 kg/ha [278]. To reduce the use of copper element in agriculture protection, the groups of Rogolino and Pelagatti performed a series of research on lignin/Cu pesticides [172,275,278]. The researchers synthesized hybrid lignin/$Cu_4(OH)_6SO_4$, and lignin/Cu_2O organic-inorganic materials by mixing ball mill and wet chemistry. All the *in vitro* antibacterial and antifungal assay, tests on crops in a greenhouse, and trials on crops in the field showed synergic activity of lignin and copper against tomato plants pathogen microbes. The synergic antimicrobial effect significantly reduced the copper dosage applied on plant pathogen therapy. Furthermore, an increase up to 25% in harvestable fruits was recorded when applying lignin/Cu_2O (2.5% Cu) as pesticide by field studies [278]. These results suggest that lignin/Cu hybrid material can lower the copper dosage in plant disease treatment while still achieving at high efficiency.

Zinc, in the form of ZnO, is another base metal that holds excellent antimicrobial activity and non-cytotoxicity to mammalian cells. The United States Food and Drug Administration listed it as a generally recognized safe material [287]. A major issue associated with ZnO nanoparticles is its aggregation and insolubility in water. Water soluble polymer are usually applied to modify the surface of ZnO nanoparticles which tunes the polarity of the nanoparticles and allows them well dispersible into water. Jose et al. [281] coated the ZnO nanoparticles with lignosulfonate by adding an ZnO in ethanol solution into lignosulfonate aqueous solution. The lignin encapsulated ZnO nanoparticles showed enhanced stability and antimicrobial performances by a variety of characterization technologies [281].

In other study by Pang et al. [282], lignin stabilized ZnO nanoparticles were synthesized by in situ formation of the ZnO nanoparticles in an aqueous lignosulfonate solution. Lignosulfonate was demonstrated to negatively charge the surface of ZnO nanoparticles which stabilized the nanoparticles in water by electrostatic repulsion [282]. The lignin stabilized ZnO nanoparticles showed strong antimicrobial activity with MIC values against *E. coli*, and *B. subtilis* of 125 μg/mL, and 62.5 μg/mL, respectively [282]. The action of mechanism of lignin/ZnO antimicrobial was attributed to the generation of reactive oxygen species, such as HO·, H_2O_2, which caused oxidative damage of biomolecules of microbial cells [282].

Because of the presence of multiple functional groups, lignin can be also used as environmental benign catalyzing, capping, and stabilizing source to synthesize ZnO nanoparticles in one pot [280]. Kaur et al. [280] synthesized lignin-ZnO nanocomposite by adding a lignin solution into zinc acetate aqueous solution at 50 °C, and stirring for another 24 h. The lignin-ZnO nanoparticles delayed both gram-negative and gram-positive bacteria growth for 12 h at a concentration of 50 μg/mL [280]. The same method could be applied to synthesize lignin-TiO_2 nanocomposite with improved antimicrobial performances. The lignin-TiO_2 nanocomposite inhibited 100% growth of *E. coli* at 400 μg/mL while commercial TiO_2 nanoparticles inhibited less than 35% growth of the bacterial at 400 μg/mL [283]. The improvement in generation of reactive oxygen species again was proven to contribute to the high antimicrobial activity of lignin-TiO_2 nanocomposite [283].

Summarily, lignin is conventionally used in the preparation of hybrid organic-metallic nanocomposite. Generally, lignin can be used as reducing, capping, and stabilizing agent to synthesize lignin-metal nanocomposite in one pot. In case of lignin and precious metal nanocomposites, lignin is used primarily to reduce the cytotoxicity of precious metals because precious metals are highly efficient as antimicrobial but associated with high cytotoxicity. In case of base metals, synergic antimicrobial activity is generally found between lignin and the base metals. Therefore, lignin can be used to reduce the dosage of base metal as antimicrobials. Large amount of research efforts have been done on the synthesis of lignin and metal nanocomposites. Most of these hybrid nanocomposites were involved in biological applications with antimicrobial activity. However, the safety issues of usage of lignin-metal nanocomposites as biological materials remain poorly known. Further studies on the environmental and biological metabolism will ascertain the safe application of those lignin and metal composition in a wide range of field.

7.5. Lignin derived molecular antimicrobial

As is frequently claimed, lignin related monophenols and bisphenols are good antimicrobials. For example, the MIC value of pinoresinol, a dimer of G units of lignin, is 31.25 μg/mL, and 15.60 μg/mL against *E. coli*, and *S. aureus*, respectively [144]. With state-of-the-art technologies of lignin depolymerization, a huge amount of lignin related phenolic compounds can be released. These released phenolic molecules provide a new library for drug identification. The downstream conversion of depolymerized lignin has open new window for the refineries of platform biochemicals (Fig. 10) toward molecular antimicrobials [288,289].

In the study by Piotrowski et al. [291], the antifungal activity of a collection of diferulates from lignocellulosic hydrolysates was assayed against the yeast *S. cerevisiae* to identify novel antifungal agents. Poacic acid was found to be an excellent antifungal agent with an IC_{50} of 324 μM (111 μg/mL) which was comparable with those of commercial fungicides, such as picoxystrobin (IC_{50} of 308 μM), and polyoxin D (IC_{50} of 340 μM) [291]. This study reveals that the lignin oil from depolymerization could be a potential pool for novel antimicrobial discovery.

In addition, through chemical modification technologies, bisphenols with weak antimicrobial activity may be converted into efficient ones. For example, Ochoa et al. [292] generated a library of honokiol analogs with more than sixty compounds by alternating the functional groups of the bisphenol. Their results showed that 5,5′-(ethane-1,2-diyl) bis(2-(*tert*-butyl)phenol) was highly effective against a dental pathogenic microbial *Streptococcus mutans* with an MIC value of 2 μM (0.65 μg/mL), while the honokiol had a noticeably high MIC value of 250 μM (66.6 μg/mL) [292]. Though honokiol is not a product from lignin depolymerization, the research by Ochoa et al. [292] shows a practical strategy to convert dimeric compounds of depolymerized lignin into efficient antimicrobials.

However, such biphenolic antimicrobial molecules are the minorities in the depolymerized lignin, which makes it difficult to be isolated with high purity and less profitable in view of lignin valorization. Therefore, it would be of great interesting to convert the major phenolic monomers from lignin depolymerization into novel efficient antimicrobials. In the study by Elangovan et al. [293], tetrahydro-2-benzazepines were synthesized in three highly selective steps from lignin derived monophenols taking advantage of the inherent phenylpropanoid moieties of monomers. The biological activity of the benzazepine derivatives was tested against *S. aureus, E. coli*, and a human hepatoma cell line [293]. The benzazepine compound with best antimicrobial performance showed an MIC value of 40 μM against *S. aureus*, which was comparable to the conventional antibiotic erythromycin [293].

Undoubtably, the inherent functionality and antimicrobial property of lignin-based chemical make it suitable for synthesis of biologically active molecules. A major challenge could be the desire of final products with pharmaceutical potential [290]. Given that nature synthesized

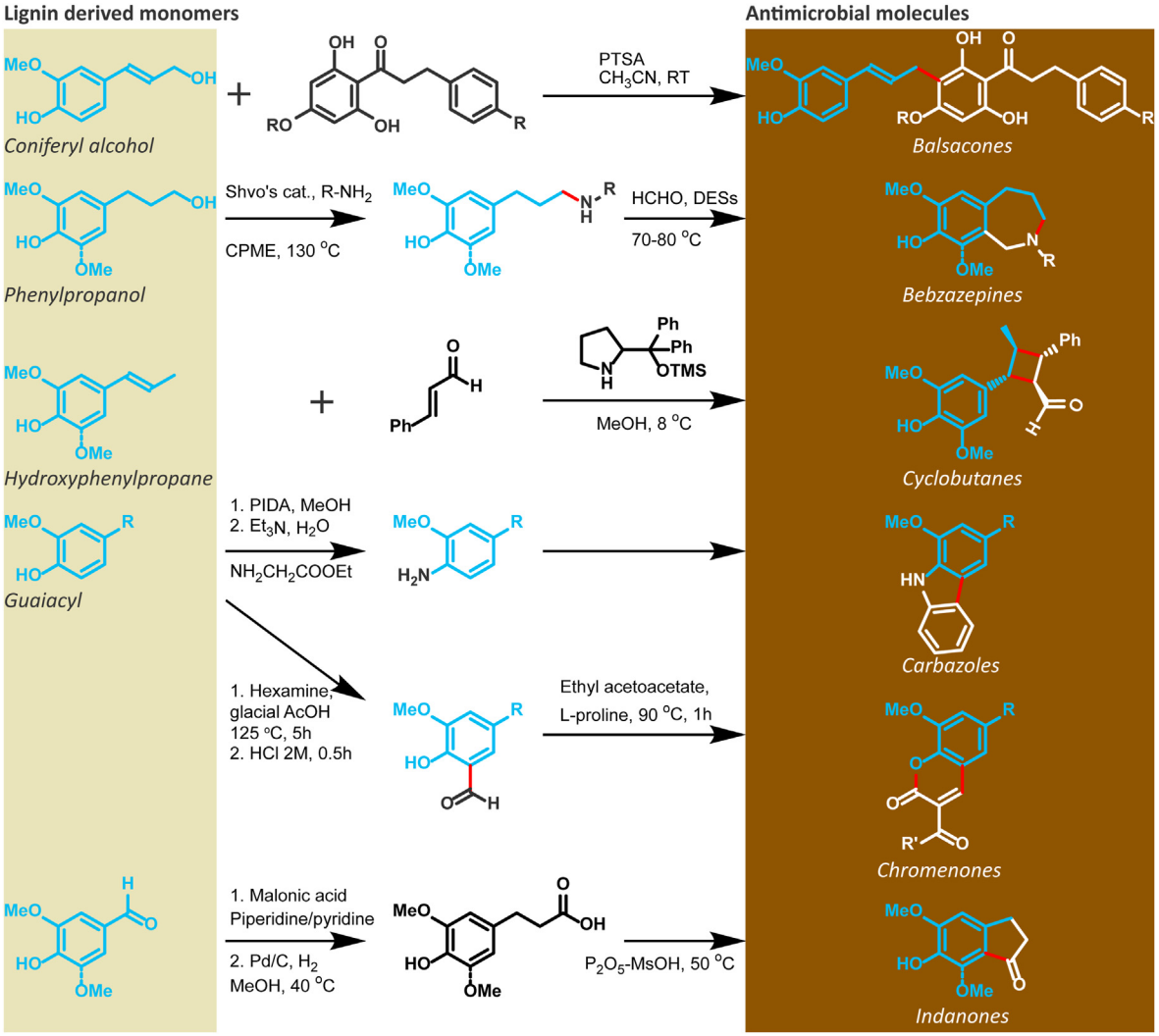

Fig. 10. Antimicrobial molecules synthesized from lignin related monomers [290].

lignin from cinnamic acid and use cinnamic acid divergently, a strategy is to synthesize analog of those natural occurred antimicrobials [294]. Readers interest in this aspect can find examples from some other excellent reviews [290,294,295].

8. Application of lignin as antimicrobial

It is well known that microorganisms are responsible for many diseases and causes of deterioration of industrial products. Antimicrobials help tuning the growth of microorganisms, which will provide a safe world for human being against pathogenic microbes and prevent our possession from rotted away by microbes. Lignin is attracting increasing interest for antimicrobial applications, because lignin is a cheap and renewable bioresource with low carbon footprint. Also, lignin has multiple mechanism of action of antimicrobial, which makes lignin a potential material combating the rise of drug resistant microbes. Lignin antimicrobial can find diverse applications in a wide range of different fields, from life science to industrial products, and to biomanufacturing. We will discuss how lignin are used in those different fields and the possible mechanism associated with when sticking to the antimicrobial properties of lignin.

8.1. Lignin in plant disease treatment

It is well known that many plant diseases are caused by pathogenic fungi, for example, grapevine disease esca is caused by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. fungi [253], while foot rot disease on tomato is caused by *Rhizoctonia solani* [278]. Lignin is one of the major plant cell wall components contributing to approximately 17 wt% of grape stalk [296]. Therefore, it is reasonable that lignin itself is not effectively biocide to plant disease fungi but even being decomposed by the plant disease fungi. Interestingly, scientists take advantages of the degradability of lignin to encapsulate biocides for plant disease treatment (Fig. 11).

The Wurm group used lignin as nanocarrier to encapsulate different fungicides for grapevine trunk disease esca treatment (Fig. 11) [253,254,262]. These fungicides included pyraclostrobin, azoxystrobin, tebuconazole, boscalid, and an antagonistic fungus. The fungicides loaded lignin nanocarrier were nonphytotoxic when injected into grapevine plants. The nanocarrier could remain "sleeping" in the plant body for at least five years without impact on the growth the host plant. The nanocarrier wake up upon esca-associated fungi invasion which secret ligninolytic enzymes to degrade lignin nanocarrier and release the encapsulated fungicide. Field studies showed that commercial F500 formulation of BASF (6 mg/mL pyraclostrobin) reduced esca severity at the first year after fungicide treatment, but the esca symptom level increased in the second and third years. In contrast, the esca symptom decreased over three years' time after lignin-fungicide nanocarrier treatment (0.7 mg/mL pyraclostrobin), and no symptoms were visible in the third year. This result suggests lignin-fungicide nanocarrier can be used to replace conventional formulations for plant disease treatment but without the loss of efficiency of plant disease therapy.

Following Wurm's studies, Luo et al. [297] used pyraclostrobin loaded lignin nanocapsules to treat tomato Fusarium crown and root rot caused by *Fusarium. oxysporum f. sp. radicis-lycopersici*. Greenhouse pot experiments suggested high efficiency on tomato crown and root rot treatment

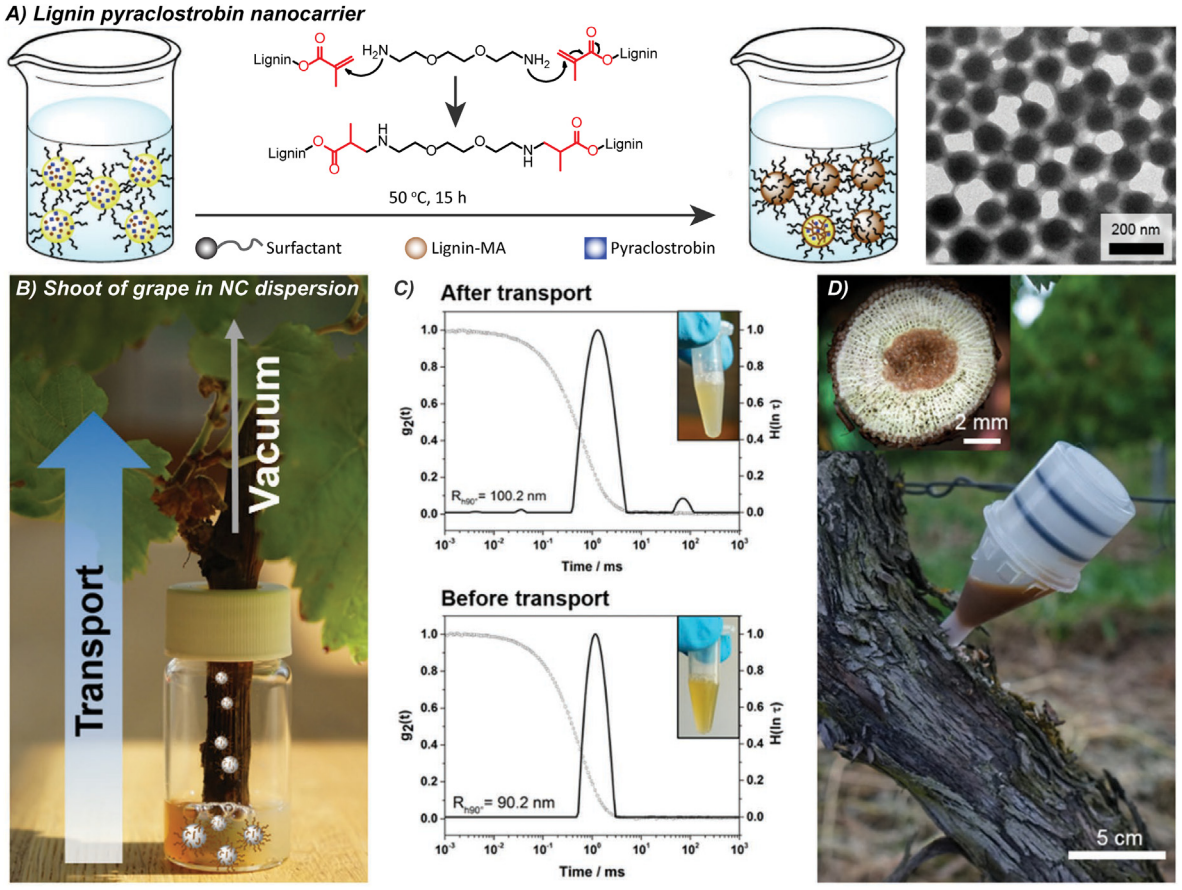

Fig. 11. Lignin pyraclostrobin nanocarriers developed for curative treatment of grapevine trunk disease esca. A) Synthesis of methacrylated-lignin and formation of lignin nanocarrier by crosslinking in miniemulsion; B) Shoot of a young grapevine plant through which a nanocarrier dispersion was sucked by vacuum simulating the natural transpiration; C) Photos and respective particle size distributions of the lignin nanocarrier dispersion before and after transport through a grapevine shoot. D) Injector filled with 5 mL of 1 wt% lignin nanocarrier dispersion and cross-section of a grapevine plant, showing the size of the vascular bundles [253].

by lignin nanocapsules and low drug residue in the soil. The negative charged lignin shell and reduced size of the nanocapsule were believed to enhance the soil mobility of pyraclostrobin, which significantly improved the efficiency of tomato disease treatment by the lignin-drug nanocapsules.

In another study by Vettraino et al. [251], lignin was used to encapsulated essential oils to reduce mortality of black pine caused by *Phytophthora cactorum*. The study showed that lignin encapsulated essential oils showed no phytotoxicity to pine seedlings, but more effective than pure essential oils in controlling *Phytophthora cactorum* [251]. The mechanism was that encapsulation slowed down evaporation of volatile essential oil, and continuously released the essential oil to inhibit the growth of *Phytophthora cactorum* [251].

Those above-mentioned case studies suggest that lignin can be used as nonphytotoxic enzymatic trigger for control release of biocide. It takes advantage of lignin as a plant cell wall component. Therefore, the trigger is compatible to the plant and able to 'sleep' long enough until the attack of ligninolytic enzymes secreted fungi. This concept provides both curative and preventive treatment of plant against pathogenic fungal infection.

On the other hand, lignin may show synergic antimicrobial activity with some compounds which provides a strategy to reduce hazardous antimicrobial use. Copper has been long used to protect crops against diseases. However, because of concerns about long term sustainability associated with the accumulation of copper into soil, the European Community has lowered the annual maximum copper limit from 6 kg/ha to 4 kg/ha [275]. To meet such a challenge, the Rogolino group perform series of studies on lignin and copper hybrid antimicrobial (lignin@Cu) for tomato and strawberry disease control [172,275,278]. In vitro antimicrobial studies showed that the lignin@Cu had noticeably lower MIC values against different agronomical pathogenic bacteria and fungi than the commercial Bordeaux mixture [172]. Green house studies on tomato revealed that the lignin@Cu achieved at better tomato disease control performance at a final copper concentration of 30 g/ha than that of the Bordeaux mixture of copper content of 600 g/ha [275]. Field studies performed with a copper concentration of 50 g/ha revealed that lignin@Cu performed better than either coprantol or copper sulfate in terms of incidence (percentage of attacked plants) and severity (average attacked area per plant) [278]. These studies revealed that the synergic antimicrobial activity of lignin and copper can be used to reduce copper content for efficient pathogen control [172].

In addition to the synthesis of lignin and fungicide nanocarriers, lignin can also be tuned for plant pathogens control by depolymerization. Hossain et al. [220] assayed the toxicity of pyrolytic oil of lignin against six plant pathogenic microbes. Their results showed that the lignin oil inhibited all the tested microbial growth at a concentration of 0.3 mg/mL [220]. The study showed the potential use of depolymerized lignin as biocide to control pathogen of important crops.

8.2. Lignin in animal farming

It is estimated that 80% of antibiotics are used for animal farming in the north America [298]. The potential threatens of antimicrobial resistance to global health is pushing farms to reduce antibiotics use. However, antibiotics provide clear benefits for food animal health and welfare [299]. Lignin provides a potential alternative to antibiotics because of its multiple antimicrobial mechanism, which do likely not cause antimicrobial resistance.

Lignin may be used for three different purposes in animal farming, namely feed preservation, growth promoter, and curative treatment. Although, all these purposes are achieved by adding lignin to the feed. In a study by Reyes et al. [300], lignosulfonates were found to inhibit the growth of three different fungi and one yeast isolated from spoiled forage. Accordingly, the researchers added lignosulfonate as preservative into baled alfalfa hay. Microbial counts studies showed that lignin was able to decrease fungi and yeast populations of the baled hay upon storage [300]. As a result, lignin was as good as commercial propionic acid in preventing the deterioration of hay's proteins and carbohydrates, and preserving its nutritional value [300].

When fed with lignin, animals' fermentation pattern is altered because lignin inhibits growth of certain bacteria [301]. Riche et al. [302] fed chicks with purified kraft lignin at a 4–10% level. Lignin feeding was found to promote the growth of chicks with low cecal volatile fatty acid concentrations comparing to diets without lignin [302]. The reason was explained by the fact that lignin inhibited certain gut microbes and thus tuned the metabolism products of the chicks [302]. When feeding cattle with high lignin diets, Khatoon et al. [303] found that the abundance of ruminal bacterial genera was dosage dependent on lignin content.

Baurhoo et al. [304] studied the effect of lignin on intestinal microbial population in the ceca and litter of broiler chickens to evaluate lignin as potential alternative to antibiotic growth promoter in broilers. The researchers fed chicken with alcell lignin, antibiotic (virginiamycin), and antibiotic-free diet and assayed the microbes of *lactobacilli*, *bifidobacteria*, *salmonella*, and *E. coli* at day 28 and 42 [304]. Their results showed that lignin performed better than the antibiotic, virginamycin, in increasing population of beneficial bacteria (*lactobacilli,* and *bifidobacteria*) in the ceca while reducing population of pathogenic bacteria (*E. coli*) in the litter [304]. Colombo et al. [305] observed similar results when the researchers fed Atlantic salmon with hydrolysis lignin diets. Low abundance of *Proteobacteria* pathogens and increasing abundance of *Lactobacillaceae* probiotic in the digesta of salmon were evidenced [305]. These studies suggest that lignin may be used to replace antibiotic as growth promoter in meat production.

Besides, lignin may be also used as curative treatment against animal and fish diseases caused by microbial infection. Oh-Hara et al. [306] reported lignin could be used for *E. coli* or *P. aeruginosa* infected mice treatment. Nelson et al. [307] reported that addition of lignin to mice diet reduced the amount of bacterial translocation after burn injury. Srisapoome et al. [188] showed that lignin could effectively protect shrimp from yellow head virus infection. Baurhoo et al. [308] used *E. coli* serotypes from poultry carcasses to challenge 29 days old chickens of dietarily treatment with lignin. Their results revealed that lignin performed better than virginiamycin in inhibiting the growth of the pathogenic strains of *E. coli* in the cecal of chicken [308]. These studies reveal the potential application of lignin in replacement of conventional antimicrobial in animal farming curative treatment.

8.3. Lignin in medical applications

The use of lignin for medical purpose is an expanding field. There have been many reviews discussing the medical potency of lignin [10,14,244,290,309,310]. The driving force is that lignin is a nontoxic, biocompatible, and bioactive material. As antimicrobial polymer, lignin may find application in antimicrobial therapy, drug delivery, wound healing, and tissue engineering (Fig. 12).

The reason of using lignin for antimicrobial therapy is direct and obvious. Early at 1979, Zemek et al. [138] had been looking into the potency of lignin as antibiotic alternative. However, crude lignin did not really success in therapeutic application because of its weak antimicrobial activity. Therefore, modified lignin is usually used for therapeutic purpose. Chee et al. [168] synthesized cationic lignin-based hyperbranched polymers for treatment of corneal infections with *P. aeruginosa* (Fig. 12A). *In vivo* study with a rabbit model of *Pseudomonas* keratitis showed that the modified lignin treatment decreased corneal edema by complete clearance of the bacterial bioburden [168]. However, few are known about the biosafety of these efficient modified lignin antimicrobial, while cationic antimicrobial polymers are nonselective toxicity toward mammalian cells [311].

Lignin has found extensive use in the field of drug delivery because of its amphiphilic structure and ability to form stable nanoparticles in water. A wide range of drugs have been loaded into lignin nanocarriers

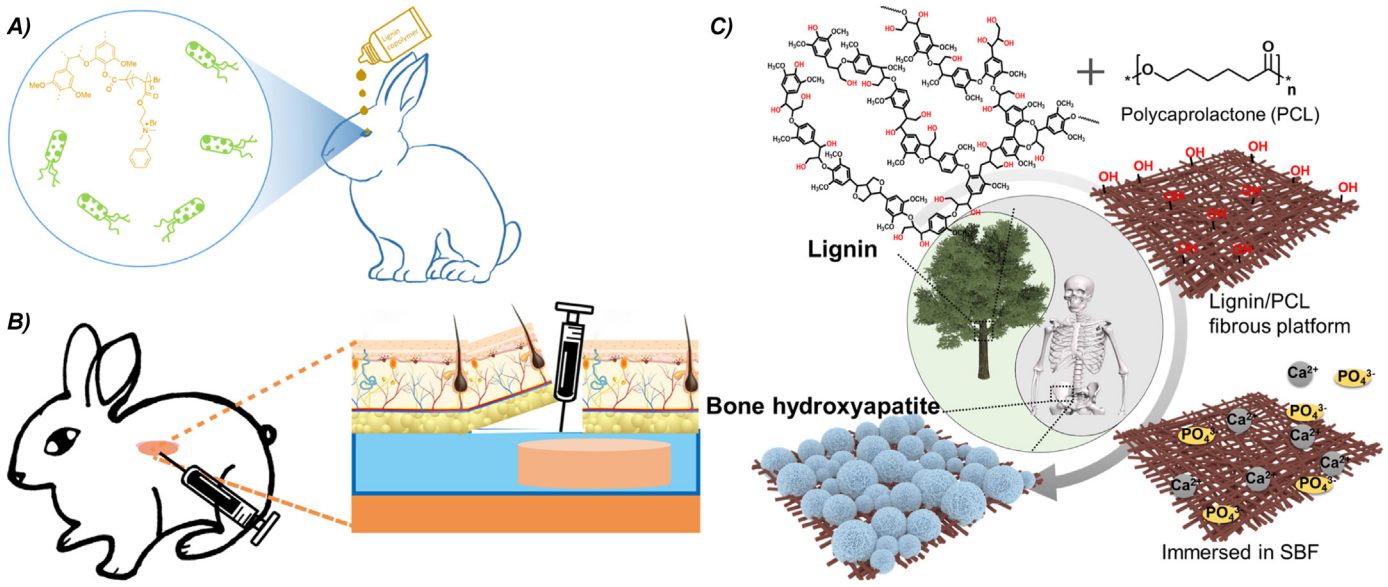

Fig. 12. Application of lignin as antimicrobial in medicine. A) Cationic lignin hyperbranched polymer that circumvents drug resistance in *Pseudomonas* keratitis. Reprinted (adapted) with permission from Ref. [168]. Copyright © 2021, American Chemical Society. B) Lignin based adhesive hydrogels for wounds healing [89]. C) Schematic description for the biomineralization of bone HAp induced by lignin. Reprinted (adapted) with permission from Ref. [312]. Copyright © 2019, American Chemical Society.

and released in a well-controlled way [313]. However, as is discussed above, current studies are focused on understanding the performance of lignin as the carrier. To our knowledge, no research addresses the advantage of lignin as antimicrobial carrier over conventional ones. On the other hand, antibacterial polymeric nanocarriers are potential to achieve complementary and synergistic effects with antibiotic therapy [314,315]. It is worthwhile for further research exploring the benefit associated with the antimicrobial properties of lignin as drug delivery vehicle.

Wound infection by microbes is a major cause of delayed healing [316]. The biocompatible character of lignin makes it an interesting antimicrobial material in promoting wound healing treatment. Spasojevic et al. [317] synthesized an antimicrobial hydrogel for wound treatment by incorporating lignin model polymer into alginate. The hydrogel showed strong antimicrobial activity against wild bacterial strains isolated from patients with chronic wounds [317]. Tests with sterile wound on mice showed that the lignin alginate hydrogel did not induce any damaging effect on the wound [317]. It is suggested that lignin is an excellent antimicrobial for wound healing purpose but without any toxic effect on human epithelial cells.

The application of lignin in wound treatment would create a sterile environment and thus promote the healing process. Eivazzadeh-Keihan et al. [318] synthesized a cross-linked lignin, agarose, silk fibroin, and $ZnCr_2O_4$ nanocomposite scaffold for wound healing purpose. *In vivo* assay with Balb/c mice showed that microbial infection of the wound was well eliminated, and the wound was healing faster with lignin derived nanocomposite treatment than those without lignin [318]. Li et al. [319] synthesized lignin based polyurethane, and silver composite transparent foam for wound healing. Evaluations in mice indicated that the lignin based foam performed better than lignin free polyurethane foam, and commercial Tegaderm film in accelerating the wound tissue and skin regeneration processes [319].

Also, the phenolic character of lignin makes it both a good reducing agent and excellent material to synthesize long-lasting adhesive. Taking advantages of this feature of lignin, Gan et al. [89] developed a catechol chemistry based hydrogel with both long term adhesiveness and antimicrobial activity for wound healing (Fig. 12B). *In vivo* wound healing test with mouse model showed that the lignin derived hydrogel performed better than any other tested hydrogel without lignin in repairing the wound and regenerating the skin tissue [89]. The reason was explained by the good cell affinity, tissue adhesiveness, and antimicrobial properties of lignin [89]. It is suggested that lignin could be a multi-functional antimicrobial material for wound treatment.

Microbial infection could be fatal if the infection is deeply localized. The use of inherently antimicrobial material in tissue engineering to combat infection is of significant importance because of their long-term antimicrobial activity [320,321]. As a multi-functional and natural antimicrobial material, lignin had found important use for microbial infection controlling of tissue engineering (Fig. 12C). Bilal et al. [322] synthesized guided tissue regeneration membrane (GTR) from lignin, and zinc oxide nanocomposite. The lignin derived GTR showed excellent antibacterial properties by diffusion assay and no cytotoxicity by MTT assay [322]. There are some reviews discussing the use of lignin in tissue engineering [24,323]. However, the advantages of lignin as inherent antimicrobial for tissue engineering use remain unclear.

8.4. Lignin in preservation

In addition to the impact on the health of life, microbial infection may also rot away our possessions. To keep the products “fresh”, preservatives are conventionally used in food, cosmetics, wood, and metallic materials to keep them away from microbial deterioration. Traditionally used preservatives in these fields are usually related to toxic, and hazardous biocides. As natural antimicrobial of plant cell wall, lignin is finding its way as an environmental benign, carbon neutral, and nontoxic alternative of preservative.

Preservatives are usually used to prevent food from microbial spoilage. Because of concerns with health and environmental pollution, plant extractives are attracting increasing interest for food preservatives. As a polyphenolic component of plant cell wall, lignin is believed to be an underutilized and valuable material for food industry [324]. It is unambiguous that lignin and its related compounds can effectively inhibit the growth food related microbes. Zhou et al. [144] reported that the MIC values of pinoresinol against five food related microbes were 3–32 μg/mL. Guo et al. [157] reported that commercial lignin could reduce *Listeria monocytogenes* from 9.4 log CFU/mL to 0.7 log CFU/mL. However, to our knowledge, there are no *in vivo* studies that adding lignin into food for human being, though lignin has been used to feed animals [301]. The

reason may be explained by the conclusions by Tao et al. [324], that is: 1st. The safety of isolated lignin as food additive is unknown, especially those components generated by the isolation processes; 2nd. The mechanism of *in vivo* bioactivities of lignin is unknown; 3rd. The interaction of lignin and food constituents is unknown.

Cosmetics comprise of large amount of nutrients which favors microbial multiplication. Antimicrobials have to be added to cosmetics in concerns of both microbial safety and product preservation. As natural nontoxic antimicrobial, lignin is believed to potential ingredients for use in cosmetic industry [325]. To evaluate the potential use of lignin and chitosan nanocomposite in cosmetic, Kim et al. [237] assayed the toxicity of the nanocomposite against the bacterial of *E. coli*, *S. aureus*, and *Bacillus subtilis*, and human skin fibroblasts cell line (BJ-5ta). Their results showed that lignin derived nanocomposite was effective antimicrobial and safe to human skin [237]. Kaur et al. [280] synthesized lignin and zinc oxide nanoparticle and added it into commercial Nivea body cream. The nanocomposites showed good antimicrobial properties against gram-negative and gram-positive (*B. megaterium*) bacteria with strong UV-blocking property. However, a major challenge of using lignin as cosmetic antimicrobial ingredients is the dark color of lignin.

Wood decay by microbes alters its permeability, chemistry, and physical properties, like mechanical strength. The use of preservatives can noticeably extend wood's service life, and thus reduce wood use. Alkaline copper quaternary (ACQ), and creosote, a brown oil from coal tar, are the most representative commercial wood preservatives. Because of environmental concerns of these compounds, lignin has been extensively studied as alternatives (Table 6).

In the study by Durmaz et al. [328], kraft black liquor was used as preservative to enhance the wood durability against microbial attack. The researchers impregnated pine sapwood with black liquor at concentrations of 2.5–7.5% under a vacuum of 650 mm/Hg for 30 min followed by atmospheric pressure for 15 min [328]. The treatment with black liquor reduced mass loss of the pine wood from 26.3% to 0.15% caused by brown-rot fungi *C. puteana* and *P. placenta* according to the "European standard 113 - Wood preservatives" test [328]. Different isolated lignins, including alkali lignin, lignosulfonate, kraft lignin, organosolv lignins were also used for wood preservation. Comparable results were reported that lignin could noticeably reduce the mass loss of wood upon microbial decay tests [326,327,329]. On one hand, the reasons why lignin could serve as preservatives because it inhibited the growth of a wide range of microbes. On another hand, Chirkova et al. [326] concluded that lignin filled partially the channels of wood, and thus hampered the diffusion of fungal enzymes to the cell wall of wood.

In addition to lignin macromolecules, depolymerized lignin oils are also used to treat wood. Along with the coal tar creosote, wood tar oil has been long used for wood preservatives [334] and abandoned because of the rise of oil refinery industry. Mohan et al. [330] showed that lignin should be the major contributor to the preservation property of whole biomass oil, because lignin oil showed greater fungal inhibition than the whole biomass oil when being used for wood treatment [330]. In addition, Kim et al. reported [221] that the active components of whole biomass oil as wood preservatives were mainly phenolic compounds derived from lignin. Dos Santos et al. [217] synthesized lignin oil by base catalyzed depolymerization of lignin at 300 °C for 40 min. The researchers treated pine sapwood with 1% lignin oil in aqueous acetone (5%) solution according to ASTM D 1413-99 standard methods [217]. After exposure to fungi attack for 48 weeks, the wood with lignin oil treatment showed a reduced mass loss of 45.9% when compared with untreated samples [217]. It is suggested that the use of depolymerized lignin oil as wood preservatives is very promising.

To achieved at improved performance, lignin is also used along with other compounds for wood preservation. Popa et al. [331] showed that lignin nanoparticle and copper ion was better than copper ion on preserving wood. Their results revealed that the mass loss of wood treated with lignin/copper complex after soil burial test for six month was 5.3%, which was lower than those of treated with copper ion (7.2%), and untreated sample (80%) [331]. In the study by Fernández-Costas et al. [332], the use of kraft lignin associated with laccase was found to limit the leaching of copper ion from the treated wood, and thus provided long term protection. In the study by Dumitrescu et al. [333], lignosulfonate, zinc oxide, and acrylic copolymer composite showed better wood preservation activity than those composites without lignin addition.

Table 6
Lignin used for wood preservation.

Ingredients	Impregnated Solutions	Impregnation method	Preservation assays	results	REF
alkali lignin	Aqueous solutions	Under vacuum	Brown and white rots challenges for 6 weeks	Decreases wood macropores by third	[326]
Lignosulfonate	Aqueous solutions 0.5–1.0 wt%	Alternating vacuum-pressure treatment	European standard EN 113 (2000)	Mass loss <6 wt%	[327]
Kraft lignin	Aqueous solutions 0.5–1.0 wt%	Alternating vacuum-pressure treatment	European standard EN 113 (2000)	Mass loss <6 wt%	[327]
Hydrolysis lignin	Aqueous solutions 0.5–1.0 wt%	Alternating vacuum-pressure treatment	European standard EN 113 (2000)	Mass loss <9 wt%	[327]
Alkali lignin	Aqueous solutions 0.5–1.0 wt%	Alternating vacuum-pressure treatment	European standard EN 113 (2000)	Mass loss <5 wt%	[327]
Kraft black liquor	Concentrations 2.5–7.5 wt%	Under vacuum of 650 mm/Hg for 30 min	European standard EN 113	Mass loss <3%	[328]
Organosolv lignin	1 wt% in water/ketone	Under vacuum	ASTM D2017–81	Weight loss <1%	[329]
Hydrothermal lignin oil	1 wt% in water/ketone	Under vacuum	ASTM D2017–81	Weight loss <1%	[259]
Base catalyzed depolymerized lignin oil	1% bio-oil, 5% acetone, 94% water	ASTM D 1413-99	ASTM D 2017-94	Reduced weight loss by 39–46%	[217]
Pyrolysis lignin oil	5%–25% in MeOH	Under vacuum	Soil block testing AWPA Standard E22-06	Compression strength loss of 5%.	[330] [221]
Lignin/Cu complex	5 wt% in 0.1 N ammonia solution	Immersion for 5 min	Soil burial test for six months	Weight loss <1%	[331]
Kraft lignin/Cu laccase composite	20% lignin, 25 mM Cu, in water	Vacuum impregnation and heat treatment	European Standard EN 113	Mass loss 3%	[332]
Acrylic polymer, lignosulfonate, ZnO composite	Emulsion	Immersion for 30 min	Exposing 28 days to microbes	Trace microbial growth visually	[333]
Lignin propiconazole nanocapsules	Lignin: 0.53 mg/mL Propiconazole: 0.04 mg/mL	Dip diffusion and vacuum impregnation	12 Weeks brown rot soil-block test	Weight loss (19.9%) comparable to CCA (16.4%)	[256]

In addition, lignin may have synergetic antimicrobial activity with other wood preservatives, which should benefit the reduction of preservative use. In the study by Andeme Ela et al. [256], lignin-propiconazole double shell nanocapsules were synthesized and used as wood preservative. Though the applied kraft lignin did not inhibit any tested fungal growth, the radical quenching capability of lignin enhanced the antifungal activity of the encapsulated propiconazole [256]. Compared to free propiconazole (39% mass loss of the treated wood), lignin-propiconazole nanocapsules could noticeably reduce mass loss of the treated wood (20%) subjected to brown rot soil-block test according to the American Wood Protection Association standard [256].

Lignin in different forms has been used as wood preservatives, and promising results were reported. Although, the performance of lignin as wood preservatives is seldom compared to those of commercial ones, like alkaline copper quaternary (ACQ), and creosote. It is therefore far away to conclude that lignin is a potential alternative to conventional wood preservatives. Further efforts are expected on the comparison of performances of lignin with conventional wood preservatives.

8.5. Lignin in antimicrobial functional products

We discussed the curative and preventive use of lignin in different field in the previous sections. This section will be focused on using lignin as antimicrobial additives to produce different antimicrobial functional products, including fillers, fabrics, coatings, and packages (Fig. 13). Instead of protection of the products from microbial spoil, the implementation of antimicrobial function to these products is to protect the goods they contact with.

Antimicrobial bottles. Additives, including filler, antimicrobial et al. are usually incorporated into polymeric products to improve their performances. Lignin has found wide application as additives of polymeric products because of its biodegradability. In the study by Klapiszewski et al. [335], kraft lignin, and zinc oxide hybrid material was synthesized by planetary ball mill treatment of the kraft lignin, and zinc oxide mixture. The researchers added subsequently the as-prepared composite of 5 wt% as filler into high-density polyethylene to prepare antimicrobial containers by a blow molding process [335]. However, the major challenge of using lignin as antimicrobial filler of plastic bottles is the compatibilization between the polar lignin and non-polar polyolefins [335]. This issue is out of the scope of this manuscript, and we avoid discussing it in-depth here. Readers who interest in this issue may refer to some other references focused on lignin and polymer composites [23, 338–342].

Antimicrobial fabrics. Adding antimicrobial property to fabrics will certainly increase the value of its products which might be widely applied in various fields from medicine to daily life [159,343]. Lignin can be incorporated into fabrics as an additive to the spinning solution. In the study by Aadil et al. [344], a solution of poly(vinyl alcohol), acetone organosolv lignin from *Acacia* wood, and silver nanoparticles was used to fabricate ultrafine nanofiber by electrospinning technique. The nanofiber was effective against both gram-negative *E. coli*, and gram-positive *B. circulans* with inhibition zone of 11 mm, and 13 mm by diffusion assay, respectively, which suggested its potential use as antimicrobial fabrics [344].

In addition, lignin may be incorporated into fabrics as antibacterial textile finishing. Sunthornvarabhas et al. [159,345] used both lignin in ethanol/dimethyl sulfoxide solution and lignin nanoparticle aqueous solution to treat glass fiber sheets by immersing the fabrics into lignin solutions. The lignin treated fabrics showed antimicrobial property comparable to those treated with silver nanoparticles, which inhibited the growth of 99.9% of the tested bacterial [159,345]. One of the disadvantages of lignin is that it is not dissolvable in water whilst aqueous solutions are the most environmental benign process for textile finishing. In a study by Chen et al. [229], lignin was chemically modified with amino acids by Mannich reaction to synthesize water soluble polyampholyte from lignin. The modified lignin was loaded onto non-woven fabrics by soaking the fabric in an aqueous solution of modified lignin (2.5 wt%) [229]. Compared to the fabric without lignin treatment which

Fig. 13. A) Antimicrobial polyethylene container of capacity 500 cm^3 obtained from composite filled with 5% by weight of 1:5 wt/wt ZnO/lignin [335]. B) Antibacterial mask with amino acid-functionalized lignin in the inner filtering layer [229]. C) Lignin nanospray was coating onto the surface of a glass slice [336]. D) Digital images of gelatin/lignin antimicrobial package film [337].

allowed penetration of 58% of the tested microbes, the fabric treated with lignin could effectively inhibited the adhesion of bacterial [229]. These studies suggest the potency of lignin as alternative to conventional metal based antimicrobial compounds used in fabrics [343].

Antimicrobial coating. Antimicrobial coatings are generally used to protect material from bacterial contamination on surfaces. The synthesis of lignin derived antimicrobial coatings can be achieved via several different ways. In the study by Paul et al. [336], nano spray coating was developed using lignin as the only ingredient. The researchers coated glass slides with lignin nanoparticle solution by directly spraying the solution onto the glass slides [336]. The lignin nanoparticles were found homogeneously distributing over the glass slides, which inhibited microbial colony formation on the surface of glass slides [336]. The study suggested that lignin could be used to fabricate a sustainable antimicrobial coating directly by peeling into nanoparticles.

In another approach, lignin can be used as polymeric block to synthesize antimicrobial coatings. Klein et al. [346] synthesized antimicrobial polyurethane coatings from unmodified and demethylated lignins. To synthesize the polyurethane coating, the researchers mixed lignin with polyethylene glycol to obtain polyol blend, then polymerized the polyol blend with 4,4-diphenylmethandiisocyanate [346]. The researchers coated the lignin derived polyurethane onto the surfaces of different materials, including untreated glass, plastic sheets of polypropylene, transparent polystyrene films and stainless steel, which showed significant microbial reduction against *S. aureus* with coating of polyurethane from demethylated lignins [346]. This study showed a potential strategy to synthesize antimicrobial coatings using lignin as polymeric block.

Lignin can be also used together with metal oxide to synthesize organic-inorganic hybrid antimicrobial coatings. In the study by Pang et al. [282], lignosulfonate stabilized zinc oxide nanocomposite was synthesized by in situ precipitation of Zn^{2+} ions with sodium hydroxide in the presence of lignosulfonate. Subsequently, the researchers coated alternately the nanocomposite associated with poly(-diallyldimethylammonium chloride) onto Whatman filter paper by a layer-by-layer method [282]. The paper coated with lignosulfonate and zinc oxide nanocomposite showed "good" antimicrobial activities against *E. coli* and *B. subtilis*, while the control one showed no inhibition against the two bacterial according to diffusion assay [282].

In addition, lignin can also be added into conventional coatings as antimicrobial ingredient. Kaur et al. [283] synthesized antimicrobial lignin and titanium dioxide nanocomposites by in situ formation titanium dioxide in a lignin solution followed by homogenization treatment. The nanocomposite was then added into a coating formulated with 10% lignin (w/v), 5% polyvinyl alcohol (w/v), and 2.5% succinic anhydride in water [283]. Glass slide and cloth coated with the coating formula comprising 5 wt% lignin and titanium dioxide addition showed good antimicrobial activity according to colony testing [283].

Antimicrobial packaging. Antimicrobial packages can efficiently prevent microbe contaminations, and therefore fulfill consumer demands for fresh, safe, and health products [347]. Lignin can be blended with different polymers to prepare antimicrobial packaging films. Mehta and Kumar synthesized choline citrate stabilized homogeneous gelatin and lignin films using a hot water solution of the polymers [337]. The composite films inhibited the growth of gram-positive bacteria *B. subtilis* according to ASTM E 2149 tests. Monteiro et al. [348] synthesized polyvinyl alcohol and lignin composite films by casting method using a dioxane/water solution. The lignin and polyvinyl alcohol inhibited the growth of *E. coli* by diffusion assay [348]. It was therefore believed that lignin could replace the traditional antimicrobials, such as potassium sorbate, in food industry for packaging purposes [348]. Sirvio et al. [349] extracted lignin contained nanofiber from wood by mechanical disintegration of cationic wood sawdust. Lignin contained nanofiber films were fabricated using a solvent-casting method, which showed antimicrobial and anti-adhesive properties against *E. coli* and *S. aureus* by diffusion assay [349]. In the study by Ogan et al. [350], cross-linked poly(acrylic acid), lignin, and caprolactone was synthesized, which showed strong antimicrobial activity against *S. aureus* and *E. coli* by diffusion assay. These studies suggest that lignin could be an environmental benign antimicrobial raw material for the synthesis of antimicrobial packages.

8.6. Lignin in biomanufacturing

Biomanufacturing selectively utilize beneficial biological systems to produce desirous products, such as bioethanol production [351]. However, bacteria contaminations during fermentation could result in low yield of the desirous products [224]. In the study by Kalinoski et al. [224], depolymerized lignin oil by peracetic acid oxidation showed up to 90% inhibition against lactic acid bacteria at 4 mg/mL, but without any inhibition effect against industrial ethanol production yeast strain. Adding lignin oil to the ethanol fermentation with lactic acid bacteria contamination showed an 8% increase in ethanol yield [224]. This study suggests that lignin oil may be used as antibiotic alternative in biomanufacturing to increase the products yield.

9. Conclusions

To show light on the synthesis of tailored antimicrobial alternatives from lignin, we discussed the chemistry of lignin, antimicrobial activity of isolated lignin, mechanism of antimicrobial action of lignin, approaches to improve antimicrobial performance of lignin, and potential applications of lignin as antimicrobial compound. Lignin chemistry parameters, including monomers, linkages, molecular weight, and functional groups, can significantly affect its antimicrobial performances. Different theories are applied to explain the mechanism of antimicrobial action of lignin, which can be classed into two catalogs, including the interaction with cell membrane, and binding with cytoplasmic components. Accordingly, several methods are applied to improve the performance of lignin as antimicrobial by tailoring lignin structure, including fractionations, depolymerization, chemical modification, blending with other antimicrobials, and upgrading of depolymerized lignin. Overall, lignin is a promising antimicrobial material that has found wide applications in diverse fields, from agriculture, to health, and to industrial products. On the other hand, novel lignins, including lignin derived from new identified monolignols, and lignin isolated by new technologies of biomass fractionation, continue emerging. Future study on these novel lignins must be encouraged and promising. Regardless of the significant development of lignin antimicrobial, the inherent antimicrobial activity of most reported lignin is weak compared with traditional antibiotics. Further improvement of the antimicrobial activity of lignin, and knowledge of the mechanism associated with remain the key challenges of research of lignin antimicrobial. To this end, the introduce of additional chemical structures, such as cationic functional groups by chemical modification, to lignin would be practical approaches to further improve the antimicrobial activity of lignin for future studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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