Antibacterial Compounds in Predominant Trees in Finland: Review

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Abstract

The extracts of Scots pine, Norway spruce, silver and white birches stem, bark, roots, leaves and needles contain several useful bioactive compounds that exhibit antibacterial activity against pathogens. Both phenolic extracts and essential oils are bacteriostatic against several bacteria. The main individual antibacterial phenolic compounds in Scots pine are pinosylvin that effectively inhibit growth of pathogens such as Bacillus cereus, Staphylococcus aureus and Listeria monocytogenes. From other phenolic compounds lignans appeared to be the least bacteriostatic and flavonoids tend to occur as glycosylated forms which have lower antibacterial activity than their aglycones. Gram-positive bacteria are generally more susceptible to plants bioactive compounds than gram-negative bacteria.

Keywords: Antibacterial compounds; Norway spruce; Extract; Hydrolysis; Fermentation

Introduction

Plants synthesise low molecular mass compounds, phytoalexins, which protect them against attacks by fungi, bacteria and insects [1,2]. Several studies of these plants used as traditional folk medicine have recently been published [3-10]. Interest in natural bioactive compounds has arisen for their multiple biological effects, including antioxidant, antifungal and antibacterial activity. The potential use of these compounds in food preservation and pharmaceutical applications as oxidants and for cancer chemoprevention has been investigated [1,11,12]. Their potential use against pathogenic microorganisms and infections that are currently difficult to treat because of the resistance that microorganisms have built against antibiotics would be one interesting application. Essential oils and phenolic extracts have been tested against multi-drug-resistant human pathogens and intestinal bacteria, like meticillin-resistant Staphylococcus aureus (MRSA) [8,13-19].

Essential oils are very complex natural mixtures which can contain about 20-60 components at quite different concentrations [20]. They are characterised by two or three major components at fairly high concentrations (20-70%) compared to others components present in trace amounts. The main group is composed of terpenes and terpenoids.

The main groups of bioactive phenolic compounds in plants are simple phenols and phenolic acids, stilbenoids, flavonoids and lignans, which are derivatives of phenylpropanoid metabolism via the shikimate and acetate pathways [11,12,21,22]. These secondary metabolites are often bound to a mono- or oligosaccharide or to uronic acid [2]. The saccharide or uronic acid part is called glycone and the other part the aglycone. Flavonoids, phenolic acids, stilbenoids, tannins and lignans are especially common in leaves, flowering tissues and woody parts such as stems and barks. In bark and knots they are especially important defence against microbial attack after injury. Hence, the bark and knot extracts have been observed to be more active against bacteria than the wood extracts [23]. The effectiveness of the defence varies among plant species. The long-lived and slow growing plants have been observed to be more active than the fast growing ones [24].

The aim of this literature review is to clarify the antibacterial compounds present in the predominant tree species in Finland, Scots pine (Pinus sylvestris), Norway spruce (Picea abies), silver birch (Betula pendula) and white birch (Betula pubescens). The extraction of these valuable compounds from forest biomass is of special interest as they are available in different wood harvesting and industrial residues, such as bark, knots, stump and roots.

Phenolic Compounds

The bioactive compounds are present in wood and, thus, they can be solubilised by different solvents [12]. In published studies phenolic compounds have been most often obtained through ethanol, methanol or acetone extraction as alone or after hexane extraction (Table 1). The most frequently used method to determine the total phenols in the extracts is colorimetric measurement with the Folin-Ciocalteu reagent. However, this reagent may react with any reducing substances other than phenols and therefore measures the total reducing capacity of a sample. By this method total phenol content is expressed in terms of gallic acid (GAE) or tannic acid (TAE) equivalents. The individual components have been identified by using gas (liquid) chromatography (GC, GLC) and mass spectrometry (MS) or high-performance liquid chromatography (HPLC).

The evaluation methods for antibacterial activity can be divided into diffusion, dilution and optical density methods. The most commonly used screen to evaluate antimicrobial activity is the agar diffusion technique. In this method, the diameter of inhibition zone is measured at the end of incubation time. The usefulness of diffusion method is limited to the generation of preliminary data only [25]. With respect to that, the activity values are not comparable, since the studies are made with different procedures and chemicals. Thus, extracts, extraction methods, assay of antimicrobial activity and strains of test organisms vary in the publications.

The minimal inhibiting (MIC) and bactericidal (MBC) concentrations are defined as the lowest concentrations of tested compounds present in the medium that inhibits the growth of the tested bacteria.

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compounds which completely inhibited bacterial growth and which results in more than 99.9% killing of the bacteria being tested, respectively. The MIC and MBC have been determined by the liquid dilution method.

**Scots pine**

Antioxidant, antifungal and antibacterial [26-34] activity of phenolic extracts of Scots pine growing in Finland have been investigated during the last decades. In most of these studies activity of knotwood extracts were detected and heartwood extracts were detected only against brown-rot fungus. The total phenolic concentration has been observed to vary a lot between the different parts of the tree: 76.0, 57.0, 17.5 and 6.7-13.6 mg TAE g⁻¹ for dried bark, needles and cork, respectively [35-38]. For the heartwood the total phenolic concentration of 4.55-17.5 and 1.1 mg GAE g⁻¹ for dried bark, needles and cork, respectively, have been reported.

| Tree species | Part            | Drying method, Solvent extraction | Compounds                        | Identification                      | Ref.            |
|--------------|-----------------|-----------------------------------|----------------------------------|-------------------------------------|-----------------|
| Scots pine   | heartwood       | Oven-dried 60 °C, 48h, 80% acetone| Total phenolics 6.7-13.6 mg TAE⁺¹ | Folin-Ciocalteau                    | [26]            |
|              | heartwood       | Oven-dried 60 °C 48h, acetone     | Total pinosylvins 4.7-7.5 mg⁻¹    | GLC-MS                              | [26]            |
|              | heartwood       | Oven-dried 60 °C 48h, 80% acetone | Total phenolics 4.55-4.66 mg TAE cm⁻³ | Folin-Ciocalteu                     | [27]            |
|              | heartwood       | Oven-dried 60 °C 48h, acetone     | Pinosylvin, PMME and PDME 2.07-3.16 mg cm⁻³ | GLC-MS                              | [27]            |
|              | knotwood        | Freeze-dried, 1. hexane, 2. acetone water | pinosylvin, PMME, DHPMME chromatographic purification, crystallization | GC after                          | [28]            |
|              | knotwood        | Freeze-dried, 1. Hexene, 2. Acetone | Nortrachelogenin fractionated by flash chromatography | GC-MS                              | [29,30]        |
|              | knotwood        | N.a., 1. Hexane, 2. Acetone water (95:5) | Lingnans, stilbenes and flavonoids | GC-FID                             | [30]            |
|              | knotwood        | N.a., 1. hexane, 2. acetone       | Pinosylvin, PSME Fractionation by flash chromatography | GC-MS                              | [30]            |
|              | knotwood, bark  | Freeze-dried, 1. Hexane, 2. Acetone | Pinosylvin Fractionation using flash chromatography | GC-FID HPSEC                      | [31]            |
|              | knotwood, bark  | Resin, Methyl-tert-buty ether     | Pinoserinol Purification with flash chromatography | GC-FID HPSEC                     | [31]            |
|              | phloem, bark    | Freeze/vacuum dried, 80% methanol | 22 phenolic compounds            | HPLC-DAD HPLC-ESI-MS                | [33]            |
|              | phloem, bark    | Air-dried, 1. 2.                | 70% acetone Chloroform            | GC-FID                             | [30]            |
|              | needle, cork,   | Air dried, 80% methanol          | Phenolics                         | Folin-Ciocalteau                   | [34]            |
|              | bark            | Distillation                      | Essential oil (mainly terpenes and terpenoids) | GC                                  | [35]            |
| Norway        | knotwood        | 1. Hexane, 2. Acetone water (95:5) | Lingnans, stilbenes and flavonoids | GC-FID                             | [30]            |
| spruce        | knotwood        | Freeze-dried, 1. Hexane, 2. Acetone water (95:5) | Hydroxymatairesinol | GC-MS                              | [30]            |
|              | phloem          | Freeze-dried, 80% ethanol         | Isorhapontin, astringin, piceid   | GC-MS                              | [37]            |
|              | phloem          | Freeze-dried, 80% methanol        | Isorhapontin, astringin, piceid, taxifolin guloside, (+)-catechin | HPLC                              | [38]            |
| Silver birch  | bark            | 1. Hexane, 2. Acetone water (95:5) | Lingnans, stilbenes and flavonoids | GC-FID                             | [30]            |
|              | phloem, bark    | Air dried, 80% methanol           | Phenolics                         | Folin-Ciocalteau                   | [34]            |

Table 1: Extraction and identification methods of antibacterial substances.

AE = accelerated solvent extractor, DHPMME = dihydroxyinosylvin monomethyl ether, FID = flame ionization detector, PDME = pinosylvin dimethyl ether, PMME = pinosylvin monomethyl ether, TAE = tannic acid equivalent.
been observed to be stilbenes, lignans (31%) nortrachelogenin being the most abundant lignan (30%) and oligolignans (6%) [31]. In the heartwood of the brown-rot fungus resistant and susceptible trees, the average total stilbenes concentration 6.4-7.5 mg g⁻¹ and 4.7-5.0 mg g⁻¹ of dry weight, respectively, have been measured [26].

Stilbenes (Figure 1) are 1,2-diarylethenes, the A ring usually having two hydroxyl groups in the m-position, while B ring is substituted by hydroxy and methoxy groups in the o-, m- and/or p-positions [11]. Stilbenes are synthesised mainly by forest trees [12], in monomeric form and as dimeric, trimeric and polymeric stilbenes, the so-called viniferins. They are commonly found in the roots, barks, rhizomes and leaves [11]. The most abundant stilbenes in Scots pine extracts are pinosylvins: 38% in knotwoods [31] and 6-7% in heartwoods [27], whereas in the bark extracts they have not been found [40]. Pinosylvin and pinosylvin monomethyl ether (PMME) are the main pinosylvins, pinosylvin dimethyl ether (PDME) being less abundant [26,28,31,41]. Dihydropinosylvin monomethyl ether (DHPMME) has been isolated from Pinus strobus knotwood [30]. The pinosylvin-3-O-methyltransferase enzyme catalyses the conversion of pinosylin to the monomethyl ether that plays a role in the resistance of the plant to stress including ozone and infection [41]. Hence, a high concentration of PMME relative to pinosylvin may be an indication of high stress levels of the trees.

The highest antimicrobial activities of the pure compounds present in Scots pine have been observed with pinosylvin and PMME, followed by DHPMME and flavanone pinocembrin [30] (Table 3). Very strong inhibition effects (62-100%) have been observed against human pathogens B. cereus, S. aureus and L. monocytogenes. Pinosylvin, DHPMME, PMME and flavonoid pinocembrin (from P. cembra) have shown a very similar activity against bacteria as the Pinus extracts where they have been isolated [30]. Both Välismaa et al. [30] and Lindberg et al. [28] have observed that the antibacterial activity correlate with the stilbene content of the extracts and, hence, stilbenes have been concluded to be the main antibacterial compounds of hydrophilic extracts of Scots pine.

The precise mechanism of antibacterial action of stilbenes is unclear. One possibility is that they destroy the membrane structure resulting in burst of the cell [42]. Välismaa et al. [30] suggested that two hydroxyl groups in meta position in one of the aromatic rings and the double bond in the carbon chain between the rings may play an important role. From phenolic acids chlorogenic acid has shown stronger activity against E. coli than ferulic acid [43].

Flavonoids consist of a central three-ring structure (Table 4). Their activity is proposed to be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [1]. Flavonoids and oligomers of flavonoids and proanthocyanidins, frequently occur as glycosides [2]. Different flavonol glycosides are typical in pine needles and the sugar residues are found to be

| Tree species       | Bacteria                  | Activity     | Method                   | Ref.   |
|--------------------|---------------------------|--------------|--------------------------|--------|
| Scots pine, knotwood | Escherichia coli          | 5 ± 13%      | Well microplates          | [30]   |
|                    | Salmonella infantis       | 0 ± 4%       | and turbidity reader     |        |
|                    | Pseudomonas fluorescens   | 13 ± 2%      |                         |        |
|                    | Bacillus cereus           | 79 ± 3%      |                         |        |
|                    | Staphylococcus aureus     | 30 ± 23%     |                         |        |
|                    | Listeria monocytogenes    | 47 ± 20%     |                         |        |
|                    | Lactobacillus plantarum   | 0 ± 3%       |                         |        |
| Scots pine, knotwood extract | Burkholderia multivorans | strong, > 20 mm | Agar diffusion         | [28]   |
|                    | Bacillus coagulans        | strong, > 20 mm |                         |        |
|                    | Alcaligenes xylosoxydans  | moderate, 16-19 mm |                         |        |
| Scots pine, phloem  | Escherichia coli          | slight, 1-3 mm | Cylinder diffusion       | [32]   |
|                    | Staphylococcus aureus     | clear, 4-10 mm |                         |        |
| Norway spruce, knotwood | Burkholderia multivorans | 0            | Agar diffusion           | [28]   |
|                    | Bacillus coagulans        | 0 small, 11-15 mm |                         |        |
| Norway spruce, knotwood and bark | Escherichia coli | 0            | Well microplates          | [30]   |
|                    | Salmonella infantis       | 0 knotwood 38%, bark 0% | and turbidity reader |        |
|                    | Pseudomonas fluorescens   | knotwood 15%, bark 0% |                         |        |
|                    | Bacillus cereus           | 0 0 0 0      |                         |        |
|                    | Staphylococcus aureus     | 0            |                         |        |
|                    | Listeria monocytogenes    | 0            |                         |        |
|                    | Lactobacillus plantarum   | 0            |                         |        |
| Norway spruce, needles | Escherichia coli          | slight, 1-3 mm | Cylinder diffusion       | [32]   |
|                    | Staphylococcus aureus     | clear, 4-10 mm |                         |        |
| Silver birch, knotwood | Burkholderia multivorans | 0            | Cylinder diffusion       | [32]   |
|                    | Bacillus coagulans        | 0 small, 11-15 mm |                         |        |
| Silver birch, knotwood and bark | Escherichia coli | 0            | Well microplates          | [30]   |
|                    | Salmonella infantis       | 0 knotwood 20%, bark 22% | and turbidity reader |        |
|                    | Pseudomonas fluorescens   | knotwood 14%, bark 0% |                         |        |
|                    | Bacillus cereus           | 0 0 0 0      |                         |        |
|                    | Staphylococcus aureus     | 0            |                         |        |
|                    | Listeria monocytogenes    | 0            |                         |        |
|                    | Lactobacillus plantarum   | 0            |                         |        |
| White birch, leaf  | Escherichia coli          | slight, 1-3 mm | Cylinder diffusion       | [32]   |
|                    | Staphylococcus aureus     | clear, 4-10 mm |                         |        |

MBC = minimal bactericidal concentration, MIC = minimal inhibiting concentration

Table 2: Antibacterial activity of wood extracts and essential oils against selected bacteria.
 bonded mainly at the 3-position [28,44-48] (Appendix 1). However, the glycoside contents on other parts than needles are not available. Dihydro-flavonol type taxifolin and flavanone type pinocembrin were the main flavonoids in knotwood [29]. Pinocembrin has been observed to inhibit growth of several bacteria, the strongest activity being against B. cereus [30] (Table 3).

Lignans isolated from knotwoods of conifers are strong antioxidants [29], but their antibacterial activity is observed to be low. Purified lignans (Figure 2), maatiresinol, hydroxymatairesinol, lariciresinol and secoisolaricinol, have not shown activity against any of the tested bacteria and isolariciresinol and nortrachelogenin have shown slight activity only against B. cereus [30] (Table 3).

**Norway spruce**

The average amount of extractable phenolic compound in Norway spruce knotwood is around 15% (w/w), but as high values as nearly 30% (w/w) have been detected [49]. The amount of phenolic compounds in the stem wood has been observed to be much lower, usually 0.15-0.3%. Malá et al. [50] observed that in Norway spruce the soluble glycoside-bound forms of phenolic acids accounted for ~85% of the total content, followed by the methanol-insoluble cell wall-bound phenolic esters (7%-8%). The amount of methanol soluble esters and free phenolic acids were low, accounting for ~2 and 4.5% of total phenolic contents, respectively [50]. Free, ester-bound (released after alkaline hydrolysis) and glycoside-bound (released after acid hydrolysis) phenolic acids were obtained from a methanol extract [50]. Two cinnamic acid derivatives, p-coumaric and ferulic acids and five benzoic acid derivatives (anisic, p-hydroxybenzoic, vanillic and syringic acids) were found in the Norway spruce cells. p-Hydroxybenzoic acid glucoside and native ferulic acid have been reported in the extracts of roots [51].

Several stilbenes and stilbenes glucosides have been detected in different parts of Norway spruce. Astrinigin and isorhapontin are the main constitutive stilbenes glucosides in Norway spruce [36,37,50]. Zeneli et al. [36] detected the contents of astrinigin and isorhapontin of 20.2 and 71.8% in sapwood phenolics and 38.8 and 46.5% in bark phenolics, respectively, in trees growing in Norway. Wood phenolics contained also 5.1% piceid and bark phenolics 7.7% piceid and 0.4% piceatannol. Viiri et al. [37] have detected stilbene glucoside concentration of ~7-8 µg mg⁻¹ in fresh phloem tissue. Over half of it was isorhapontin and rest astrinigin and piceid, while resveratrol was the most abundant aglycone (~0.5 µg mg⁻¹). These stilbenes aglycones and glucosides have been detected also in bark extracts [31]. In healthy phloem stilbenes typically occur as glucosides. Piceoside, piceatannol and its glucoside, isorhapontin have been detected in roots [51]. On the contrary, Willför et al. [49] have not found stilbenes in the hydrophilic knotwood extractives of Norway spruce. They reported that more than a half of the knotwood extracts are lignans, the rest being mainly oligolignans. The most abundant lignan was hydroxymatairesinol [29,31]. Its two isomers constitute over 70% of the lignans [29].

Shan et al. [45] evaluated antibacterial activity of resveratrol and its glucoside piceid against five bacteria (Table 3). In general, efficacy of aglycone and glucoside appeared to be almost the same. The MIC values of both compounds were 313-625 mg L⁻¹ and in the case of L. monocytogenes MIC was also bacteriostatic concentration.

Needles have considerably high content of phenolic substances: 155.3 mg GAE g⁻¹ dry weight of the original sample [34]. Five types of flavonoids (flavones, flavonols, flavanones, dihydro-flavonols

**Figure 1:** Chemical structures of stilbenes and stilbenes glucosides found in conifers.
| Substance class | Substance | Bacteria | Inhibitory effect, % | Method | Ref. |
|-----------------|-----------|----------|----------------------|--------|-----|
| **Hydroxycinnamic acid** | Ferulic acid (commercial) | *Escherichia coli* | Slight | Agar well diffusion | [43] |
| | | *Salmonella enterica* | Slight | | |
| | | *Enterococcus faecalis* | No inhibition | | |
| | Escherichia coli Bacillus licheniformis | *Micrococcus luteus* | MIC 375 mg L⁻¹ | Not announced | [44] |
| Chlorogenic acid | Escherichia coli | *Salmonella enterica* | Clear | Agar well diffusion | [43] |
| | | *Enterococcus faecalis* | No inhibition | | |
| | Pinosylvin (Scots pine knotwood) | *Escherichia coli* | 54 ± 8 | Well microplates and turbidity reader | [30] |
| | | *Salmonella infantis* | 42 ± 20 | | |
| | | *Pseudomonas fluorescens* | 50 ± 15 | | |
| | | *Bacillus cereus* | 101 ± 6 | | |
| | | *Staphylococcus aureus* | 76 ± 2 | | |
| | | *Listeria monocytogenes* | 62 ± 15 | | |
| | | *Lactobacillus plantarum* | 0 ± 0 | | |
| | Stilbene | Pinosylvin monomethyl ether (Scots pine knotwood) | *Burkholderia multivorans* | Disc diffusion and liquid culture | [28] |
| | | *Bacillus coagulans* | 29-34 mm | | |
| | | *Acaligenes xylosoxydans* | 21 mm | | |
| | | Stilbene glucoside | Dihydropinosylvin monomethyl ether (Scots pine knotwood) | *Burkholderia multivorans* | Disc diffusion and liquid culture | [28] |
| | | *Bacillus coagulans* | 15-17 mm | | |
| | | *Acaligenes xylosoxydans* | 18 mm | | |
| | | Resveratrol (C. baiesii) | MRSA | Liquid microdilution | [15] |
| | Resveratrol (commercial) | *Escherichia coli* | MIC 313 mg L⁻¹ | Agar well diffusion | [45] |
| | | *Salmonella anatum* | MIC 313 mg L⁻¹ | | |
| | | *Bacillus cereus* | MIC 313 mg L⁻¹ | | |
| | | *Listeria monocytogenes* | MIC 625 mg L⁻¹ | | |
| | | *Staphylococcus aureus* | MIC 313 mg L⁻¹ | | |
| | | Stilbene glucoside | Piceid (commercial) | *Escherichia coli* | MIC 625 mg L⁻¹ | | |
| | | *Salmonella anatum* | MIC 313 mg L⁻¹ | | |
| | | *Bacillus cereus* | MIC >2500 mg L⁻¹ | | |
| | | *Listeria monocytogenes* | MIC 625 mg L⁻¹ | | |
| | | *Staphylococcus aureus* | MIC 313 mg L⁻¹ | | |
| | | Flavone | Apigenin | *Escherichia coli* | No inhibition | Agar well diffusion | [43] |
| | | | *Salmonella enterica* | No inhibition | | |
| | | | *Enterococcus faecalis* | No inhibition | | |
| | Flavanone | Pinocebrin (Pinus cembra knotwood) | *Escherichia coli* | 15 ± 2 | Well microplates and turbidity reader | [30] |
| | | | *Salmonella infantis* | 14 ± 8 | | |
| | | | *Pseudomonas fluorescens* | 7 ± 3 | | |
| | | | *Bacillus cereus* | 74 ± 6 | | |
| | | | *Staphylococcus aureus* | 30 ± 9 | | |
| | | | *Listeria monocytogenes* | 55 ± 16 | | |
| | | | *Lactobacillus plantarum* | 4 ± 5 | | |
and flavans [22]) occur in Norway spruce (Appendix 1). Several glycosides of quercetin, isorhamnetin, kaempferol, myricetin, 3-caffeoylquinic acid and syringetin have been identified in needles of Norway spruce [52]. Glucose at the 3- or 7-position is the most common glycone part of the glycosides. However, majority of the antibacterial activity data concerns aglycones and only limited data on glycosides are available.

Table 3: Pure antibacterial substances and their activity against selected bacteria. 

| Flavonoid | MIC | Method |
|-----------|-----|--------|
| Quercetin (commercial) | 500 µg | Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis | Slight-moderate inhibition |
| | | | Hole-plate diffusion |
| Quercetin | Staphylococcus aureus | MIC 20-400 mg L⁻¹ | Hole-in-plate |
| Isoquercetin (quercetin-3-glycoside) (commercial) | | Escherichia coli, Salmonella enterica, Enterococcus faecalis | No activity |
| | | Staphylococcus aureus, Staphylococcus epidermidis | No activity |
| | | Bacillus subtilis, Staphylococcus aureus | No activity |
| Kaempferol (commercial) | | Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis | No activity |
| | | | Hole-plate diffusion |
| Myricetin | Escherichia coli, Salmonella enterica, Enterococcus faecalis | MIC 25-400 mg L⁻¹ | Agar dilution |
| Flavanone | Naringenin (commercial) | Escherichia coli, Pseudomonas aeruginosa, Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis | Moderate-clear inhibition |
| | | | Hole-plate diffusion |
| Flavan | Catechin | Escherichia coli, Salmonella enterica, Enterococcus faecalis | No activity |
| Tannins | Gallic acid | Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus | No inhibition |
| Lignans | Matairesinol (synthesized), Hydroxymatairesinol (Norway spruce), Lariciresinol (balsam fir), Secoisolariciresinol (Brazilian pine) | Escherichia coli, Salmonella infantis, Pseudomonas fluorescens, Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Lactobacillus plantarum | MIC 125 mg L⁻¹ |
| | | | MIC 62 mg L⁻¹ |
| | | | MIC 31 mg L⁻¹ |
| | | | MIC 62 mg L⁻¹ |
| Isolariciresinol, Nortrachelogenin (Scots pine knotwood) | Escherichia coli, Salmonella infantis, Pseudomonas fluorescens, Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Lactobacillus plantarum | 0 | Well microplates and turbidity reader |

MIC = minimal inhibiting concentration, MRSA = methicillin-resistant Staphylococcus aureus
The leaf extracts of white birch have been observed to inhibit clearly the growth of gram-positive bacteria *S. aureus* [54]. Omar et al. [23] found that the bark extract of *Betula papyrifera* was active against gram-positive bacteria *S. aureus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Mycobacterium phlei*, whilst the wood extract showed activity only against *S. aureus*. None of the extracts inhibited growth of gram-negative pathogens *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Klebsiella pneumonia*.

The growth of *S. aureus* was inhibited very effectively by quercetin, kaempferol and naringenin [32,54], whereas *+/- -catechin and (+)-catechin were inactive against it. In addition, Tsuchiya et al. [19] observed the antibacterial activity of kaempferol and naringenin against methicillin-resistant *S. aureus* (MRSA).

### Essential Oils

The primary constituents of the essential oils of conifers are terpenes [55] and when they contain additional elements, usually oxygen, they are termed terpenoids [1,20]. Monoterpenes are the most representative molecules constituting 90% of the essential oils [20]. Terpenes are derived through isoprenoid pathway in plants [21] and

| Structure | Compound | R1 | R2 | R3 | R4 | Trees  |
|-----------|----------|----|----|----|----|--------|
| ![Flavanones](image1.png) | Pinocembrin | H | H | P | | P |
| | Naringenin | H | OH | B | | B |
| | Eriodictyol | OH | OH | S | | S |
| ![Flavones](image2.png) | Apigenin | H | H | H | S, B | B |
| | Luteolin | OH | H | H | B | B |
| ![Flavonols](image3.png) | Kaempferol | H | H | OH | P,S,B | P,S,B |
| | Quercetin | OH | H | OH | P,S,B | P,S,B |
| | Isorhamnetin | OMe | H | OH | P,S | P,S |
| | Myricetin | OH | OH | OH | S | S |
| | Laricitrin | OMe | OH | OH | OH | S |
| | Syringetin | OMe | OMe | OH | OH | S |
| | Chrysirin | H | H | H | H | H |
| | Acacetin | H | OMe | H | H | B |
| ![Dihydro-flavanol](image4.png) | Aromadendrin | H | H | OH | S | S |
| | Taxifolin | H | OH | OH | P,S | P,S |
| | Ampelopsin | OH | OH | OH | S | S |
| ![Flavans](image5.png) | Catechin | OH | H | P,S,B | | P,S,B |
| | Epicatechin | H | OH | S | | S |
| | 3’-Me-Catechin | OMe | H | S | | S |
| | Galloacetin | OH | OH | S | | S |
| ![Anthocyanins](image6.png) | Pelargonidin | H | H | S | | S |
| | Cyanidin | OH | H | S | | S |
| | Peonidin | OMe | H | S | | S |
| | Delphinidin | OH | OH | S | | S |

Table 4: Flavonoids found in extracts of Scots pine (S), Norway spruce (S) and silver birch (B).
The leaf extracts of white birch have been observed to inhibit clearly the growth of gram-positive bacteria S. aureus [54]. Omar et al. [23] found that the bark extract of Betula papyrifera was active against gram-positive bacteria S. aureus, Bacillus subtilis, Enterococcus faecalis and Mycobacterium phlei, whilst the wood extract showed activity only against S. aureus. None of the extracts inhibited growth of gram-negative pathogens E. coli, Pseudomonas aeruginosa, Salmonella typhimurium and Klebsiella pneumonia.

The growth of S. aureus was inhibited very effectively by quercetin, kaempferol and naringenin [32,54], whereas +/- -catechin and (+)-catechin were inactive against it. In addition, Tsuchiya et al. [19] observed the antibacterial activity of kaempferol and naringenin against methicillin-resistant S. aureus (MRSA).

**Essential Oils**

The primary constituents of the essential oils of conifers are terpenes [55] and when they contain additional elements, usually oxygen, they are termed terpenoids [1,20]. Monoterpenes are the most representative molecules constituting 90% of the essential oils [20]. Terpenes are derived through isoprenoid pathway in plants [21] and they are based on an isoprene structure (Figure 3). Different conifer species often contain the same terpenes but in different portions [55] (Appendix 2). They can be obtained by expression, fermentation, extraction or by steam distillation that is the most commonly used method for commercial production of essential oils [56]. However, greater antibacterial activity has been observed with essential oils extracted by hexane than the corresponding steam distilled essential oils.

Essential oils of pine needles and spruce have been reported to be inactive against gram-negative bacteria but to have significant activities against gram-positive bacteria S. aureus, E. faecalis and B. subtilis. L. monocytogenes and Listeria ivanovii [35,57] (Table 5). On the contrary, Hammer et al. [25] have noticed stronger activity against gram-negative E. coli than S. aureus.

Few studies have been published at the antimicrobial activity of terpenes present in conifer extracts. β-Pinene (from nutmeg) has been found to be particularly effective against E. coli O157:H7. Mourey and Canillac [55] studied activity of commercial terpenes, α-pinene, β-pinene, 1,8-cineole, R-limonene, S-limonene and borneol, against L. monocytogenes serovars, which is one of the most dangerous food pathogens. The terpenes studied had a significant anti-Listeria activity (Table 5). α-Pinene was the most active compound with an average MIC of 0.019-0.025% against L. monocytogenes, while 1,8-cineole was the least inhibitory and had the lowest activity against bacteria being 0.375-0.417%, although this concentration was directly bactericidal.
Furthermore, 1,8-cineole has exhibited low antibacterial activity against MRSA and vancomycin-resistant enterococci (VRE) E. faecalis [18].

Non-oxygenated monoterpene hydrocarbons, α-pinene, p-cymene and γ-terpinene have shown the least antibacterial activity among essential oil components [58-60]. Furthermore, these compounds may produce antagonistic effects and therefore, lower the antimicrobial activity of essential oil. Terpinen-4-ol from tea tree oil has been active on its own against P. aeruginosa and S. aureus, but reduced efficacy has been observed in combination with either γ-terpinene or p-cymene due to lowered aqueous solubility [61]. Also minor components in essential oil may play a role in antibacterial activity of the main component as interactions between components may lead to additive, synergistic or antagonistic effects [20,56].

**Mechanisms of Activity**

In general, the extracts were more active against gram-positive bacteria than gram-negative bacteria [23,28,58-66]. The main difference between gram-positive and gram-negative bacteria is the structure of their cell walls. Therefore it seems that the main target of the antibacterial activity is to destroy the cell walls of the bacteria. The gram-negative cell envelope is made up of lipopolysaccharide that renders the surface highly hydrophilic whereas the lipophilic structure of the cell membrane of gram-positive bacteria may facilitate penetration by hydrophobic compounds [13,57,66]. Thus, flavonoids and stilbenes with lower hydroxylation should be more active against bacteria than those with the several hydroxyl groups. However, there is no clear comparability between the degree of hydroxylation and toxicity to bacteria. Either the mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds [1,67,68].

**Conclusions**

The extracts of Scots pine, Norway spruce, silver and white birches stem, bark, roots, leaves and needles contain several useful bioactive compounds that exhibit antibacterial activity against pathogens. Both phenolic extracts and essential oils are bacteriostatic against several bacteria. The main individual antibacterial phenolic compounds in Scots pine are pinosylvins that effectively inhibit growth of pathogens such as B. cereus, S. aureus and L. monocytogenes. From other phenolic compounds lignans appeared to be the least bacteriostatic and flavonoids tend to occur as glycosylated forms which have lower antibacterial activity than their aglycones. Gram-positive bacteria are generally more susceptible to plants bioactive compounds than gram-negative bacteria.
| Substance                | Bacteria                        | Inhibitory effect, % | Ref.                                      |
|-------------------------|---------------------------------|---------------------|-------------------------------------------|
| Scots pine, needles     | Escherichia coli                | MIC > 100 mg L⁻¹    | Agar diffusion and liquid dilution methods |
|                         | Pseudomonas aeruginosa          | MIC > 100 mg L⁻¹    |                                           |
|                         | Proteus mirabilis               | MIC > 100 mg L⁻¹    |                                           |
|                         | Klebsiella pneumoniae           | MIC > 100 mg L⁻¹    |                                           |
|                         | Staphylococcus aureus           | MIC 25 mg L⁻¹,      |                                           |
|                         |                                | MBC >50 mg L⁻¹      |                                           |
|                         | Staphylococcus aureus           | MIC > 100 mg L⁻¹    |                                           |
|                         | Staphylococcus aureus           | MIC 12.5 mg L⁻¹,    |                                           |
|                         |                                | MBC > 50 mg L⁻¹     |                                           |
|                         | Enterococcus faecalis           | MIC 3.1 mg L⁻¹      |                                           |
|                         | Bacillus subtilis               | MIC 50 mg L⁻¹       |                                           |
|                         | Pseudomonas aeruginosa          | MIC 2.0 % (v/v)     |                                           |
|                         | Klebsiella pneumoniae           | MIC > 2.0 % (v/v)   |                                           |
|                         | Klebsiella oxytoca              | MIC > 2.0 % (v/v)   |                                           |
|                         | Enterobacter cloacae            | MIC > 2.0 % (v/v)   |                                           |
|                         | Staphylococcus aureus           | MIC > 2.0 % (v/v)   |                                           |
|                         | Staphylococcus aureus           | MIC > 2.0 % (v/v)   |                                             |
|                         | Listeria monocytogenes          | MIC 0.022-0.061%,   |                                           |
|                         | Serratia marcescens             | MIC 0.25%           |                                           |
|                         |                                | MIC 0.015-0.087%,   |                                           |
|                         |                                | MBC 0.20%           |                                           |
|                         |                                | MIC 0.025%,         |                                           |
|                         |                                | MBC 0.27 %          |                                           |
|                         | Pseudomonas aeruginosa          | MIC > 100 mg L⁻¹    |                                           |
|                         | Klebsiella pneumoniae           | MIC 2.0 % (v/v)     |                                           |
|                         | Klebsiella oxytoca              | MIC > 2.0 % (v/v)   |                                           |
|                         | Enterobacter cloacae            | MIC > 2.0 % (v/v)   |                                           |
|                         | Staphylococcus aureus           | MIC > 2.0 % (v/v)   |                                           |
|                         | Staphylococcus aureus           | MIC > 2.0 % (v/v)   |                                           |
|                         | Listeria monocytogenes          | MIC 0.019-0.025%,   |                                           |
|                         |                                | MBC 0.192-0.354%    |                                           |
| Norway spruce, essential oil commercial | Listeria ivanovii            | MIC 0.022-0.061%,   |                                           |
|                         |                                | MBC 0.25%           |                                           |
|                         |                                | MIC 0.015-0.087%,   |                                           |
|                         |                                | MBC 0.20%           |                                           |
|                         |                                | MIC 0.025%,         |                                           |
|                         |                                | MBC 0.27 %          |                                           |
| g-Pinene                | Escherichia coli                | MBC > 900 mg L⁻¹    | Broth dilution, visible growth            |
|                         | Pseudomonas aeruginosa          | MBC > 900 mg L⁻¹    |                                           |
|                         | Salmonella typhimurium           | MBC > 900 mg L⁻¹    |                                           |
|                         | Yersinia enterocolitica         | MBC > 900 mg L⁻¹    |                                           |
|                         | Staphylococcus aureus           | MBC > 900 mg L⁻¹    |                                           |
|                         | Staphylococcus epidermis        | MBC > 900 mg L⁻¹    |                                           |
|                         | Enterococcus faecalis           | MBC > 900 mg L⁻¹    |                                           |
|                         | Bacillus cereus                 | MBC > 900 mg L⁻¹    |                                           |
|                         | Listeria monocytogenes          | MIC 0.019-0.025%,   | Broth dilution, visible growth            |
|                         |                                | MBC 0.192-0.354%    |                                           |
|                         | Several bacteria                | No activity         | Vapour diffusion test                      |
|                         |                                |                    | Agar test                                 |
| β-Pinene                | Listeria monocytogenes          | MIC 0.041-0.060%,   | Broth dilution, visible growth            |
|                         |                                | MBC 0.55-1.167%     |                                           |
| R Limonene              | Listeria monocytogenes          | MIC 0.047-0.052%,   | Broth dilution, visible growth            |
|                         |                                | MBC 0.208-0.45%     |                                           |
| S Limonene              | Listeria monocytogenes          | MIC 0.028-0.062%,   | Broth dilution, visible growth            |
|                         |                                | MBC 0.15-0.45%      |                                           |
| Borneol                 | Listeria monocytogenes          | MIC 0.039-0.094%,   | Broth dilution, visible growth            |
|                         |                                | MBC 0.039-0.156%    |                                           |
| 1,8-Cineole             | Listeria monocytogenes          | MIC 0.375-0.417%,   | Broth dilution, visible growth            |
|                         |                                | MBC 0.375-0.417%    |                                           |
| Escherichia coli        | Pseudomonas aeruginosa          | MIC > 8 mg mL⁻¹     | Broth microdilution                        |
|                         | Klebsiella pneumoniae           | MIC > 8 mg mL⁻¹     |                                           |
|                         | Acinetobacter baumannii         | MIC > 8 mg mL⁻¹     |                                           |
|                         | Bacillus subtilis               | MIC 8 mg mL⁻¹       |                                           |
|                         | Staphylococcus saprophyticus    | MIC 32 mg mL⁻¹      |                                           |
|                         | Staphylococcus aureus, MRSA     | MIC > 8 mg mL⁻¹     |                                           |
|                         | Staphylococcus epidermis        | MIC 8 mg mL⁻¹       |                                           |
|                         | Staphylococcus agalactiae       | MIC > 8 mg mL⁻¹     |                                           |
|                         | Staphylococcus pyogenes         | MIC 16 mg mL⁻¹      |                                           |
|                         | Enterococcus faecalis, VRE      | MIC > 8 mg mL⁻¹     |                                           |

**Substances and Bacteria:**
- Scots pine, needles
- Norway spruce, essential oil commercial
- g-Pinene
- β-Pinene
- R Limonene
- S Limonene
- Borneol
- 1,8-Cineole

**Inhibitory Effects:**
- MIC (Minimal Inhibitory Concentration)
- MBC (Minimal Bactericidal Concentration)

**Methods:**
- Agar diffusion and liquid dilution methods
- Broth dilution
- Vapour diffusion test
- Agar test
### Table 5: Essential oils of conifers and pure monoterpenes and -terpenoids with their activity against selected bacteria.

| Species                        | MIC or MBC | Method     | Ref  |
|--------------------------------|------------|------------|------|
| Bacillus acereus               | Not active | Vapour diffusion | [59] |
| Escherichia coli               | MIC 1% v/v |            |      |
| Pseudomonas aeruginosa         | MIC >8% v/v|            |      |
| Staphylococcus aureus          | MIC 0.5% v/v|            |      |
| Escherichia coli               | MIC >8% v/v|            |      |
| Pseudomonas aeruginosa         | MIC >8% v/v|            |      |
| Staphylococcus aureus          |            | Vapour diffusion | [61] |
| p-Cymene                       |            | Broth microdilution | [58] |
| Escherichia coli               | MBC > 900 mg L⁻¹ | Broth |      |
| Pseudomonas aeruginosa         | MBC > 900 mg L⁻¹ | Broth |      |
| Salmonella typhimurum          | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Yersinia enterocolitica        | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Staphylococcus aureus          | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Staphylococcus aureus          | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Enterococcus faecalis          | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Listeria monocytogenes         | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Bacillus cereus                | MBC > 900 mg L⁻¹ | Microdilution | [58] |
| Staphylococcus, Micrococcus,   | MBC > 1 mg mL⁻¹ | Agar | [60] |
| Bacillus, Enteropacter sp.     |            |            |      |
| γ-Terpinene                    |            | Broth microdilution | [58] |
| Escherichia coli               | MBC > 900 mg L⁻¹ | Broth |      |
| Pseudomonas aeruginosa         | MBC > 900 mg L⁻¹ | Broth |      |
| Staphylococcus aureus          | MBC > 900 mg L⁻¹ | Broth |      |
| Staphylococcus, Micrococcus,   | MBC > 1 mg mL⁻¹ | Agar | [60] |
| Bacillus, Enteropacter sp.     |            |            |      |
| α-Terpineol                    |            | Broth dilution, visible growth | [58] |
| Escherichia coli               | MBC 450 mg L⁻¹ | Broth |      |
| Escherichia coli O157:H7       | MBC > 900 mg L⁻¹ | Broth |      |
| Pseudomonas aeruginosa         | MBC > 900 mg L⁻¹ | Broth |      |
| Salmonella typhimurum          | MBC > 900 mg L⁻¹ | Broth |      |
| Yersinia enterocolitica        | MBC > 900 mg L⁻¹ | Broth |      |
| Staphylococcus aureus          | MBC 900 mg L⁻¹ | Broth |      |
| Staphylococcus, Micrococcus,   | MBC > 1 mg mL⁻¹ | Agar | [60] |
| Bacillus, Enteropacter sp.     |            |            |      |
| Staphylococcus, Micrococcus,   | MBC > 1 mg mL⁻¹ | Agar | [60] |
| Bacillus, Enteropacter sp.     |            |            |      |

Na. = not announced

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