The Extractive Content of Scottish Roundwood

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1 Introduction

Although the composition varies from species to species, in general wood is made up of cell wall material comprising cellulose (40% - 50%), hemicelluloses (20-30%) and lignin (20-30%) which altogether account for about 90-95% of the mass of oven dry wood (Higuchi, 1997, Pereira, 2003). Other components, which make up approx. 0% to 10% of the dry mass of wood, are known as extractives. These are not part of the structure of the wood (Wiedenhoeft, 2013, Pereira, 2003) and are found in the cell lumen, resin cells (Higuchi, 1997) and also stored within the heartwood (Hillis, 1987). Extractives can also be found in sapwood where they are involved in the synthesis of cell walls (Pereira, 2003). As well as having many compounds that are not found in the wood, bark can also often have a higher extractive content compared to wood, though this difference can depend on the method used to extract the compounds (Rowell, 2013).

As described by Higuchi (1997), chemical extractives can be separated into four main categories:

Aliphatic compounds: hydrocarbons, alcohols, fats, waxes and others
Fats are found mostly in the parenchyma cells with more in sapwood than heartwood

Sugars: D-glucose, D-fructose, sucrose, raffinose, stachyose – found in sapwood
L-arabinose, D-xylose, L-rhamnose, D-glucose and D-mannose – found in heartwood

Aromatic compounds: phenols, stilbenes, flavonoids, tannins, quinones, tropolones, lignans – found in bark and heartwood

Terpenes: monoterpenes (e.g. α-pinene, camphene, cineole), sesquiterpenes, diterpenes (e.g. resin acids), triterpenes, polyterpenes (e.g. rubber)

Extractives are chemicals that can be removed using a number of different solvents and some have been used by humans for centuries. They are often classed by the solvent used to extract them and usually fall under two main categories - those that are water soluble i.e. hydrophilic and those that are lipophilic. Lipophilic extractives are extracted by non-polar solvents such as pentane, hexane, toluene and chloroform and include resin acids, fatty acids, sterols, stilbenes and terpenes. Hydrophilic extractives are extracted using solvents such as acetone, ethanol and water and include lignans, oligolignans, flavonoids, stilbenes and sugars. In most cases the sample is ground into small pieces or powder and then extracted with the relevant solvent or sequentially with lipophilic and hydrophilic solvents.

Within a tree stem, extractives are often accumulated in parenchyma cells at the heartwood sapwood boundary or in the older sapwood (Wiedenhoeft, 2013) as well as in other parts of the tree such as knots, bark and roots. However, not only does the nature of the extractives differ between species, the amount and distribution within a tree also differs depending on

the species and the extractive. This review study aims to pull together what is known about extractives in a number of important commercial species which are grown in Scotland.

A study commissioned in 2003 by The Forestry Commission resulted in an online website and database being produced (Watkins and Chaudhry, 2004) giving information on the chemical compounds found in the main commercial tree species found in the UK including alder, ash, aspen, beech, birch, cherry, Corsican pine, Douglas fir, larch, oak, poplar, Scots pine, Sitka spruce and willow. This study collated data from numerous databases, scientific research papers and conference proceedings as well as books and reports and is available at https://secure.fera.defra.gov.uk/treechemicals/review/index.cfm.

The species looked at in this study are all conifer species and were picked on the basis of volume grown in Scotland. The species are Sitka spruce, Norway spruce, Scots pine, larch and lodgepole pine.

2 Extractive by Tree Species and Tree Part

2.1 Sitka Spruce (*Picea sitchensis*)

Originating from the west coast of North America Sitka spruce has become one of the most commercially important tree species in the UK and the predominant plantation species of conifer in Scotland covering approximately 523,000 ha (Forestry Commission, 2011)

An extensive PhD project published in 2010 at The University of Glasgow investigated the extractive content of Sitka spruce grown in the UK (Caron, 2010). Some findings of this were also published in (Caron et al., 2013). As well as the nature of the extractives Caron (2010) also looked at the distribution of the extractives at different heights within the different parts of the tree (bark, roots, knots, heartwood and sapwood). The same study also investigated the variation in extractive content in trees grown in different sites throughout Scotland to determine if site factors or geographical location had any influence on the extractive content. The main findings of Caron (2010) indicate that Sitka spruce is generally lower in extractives than other species such as Norway spruce which corresponds to the findings in other studies (Pietarinen et al., 2006, Willfor et al., 2004a).

Using acetone as the solvent Caron (2010) found that heartwood extractive content is higher than in sapwood (heartwood extractive content ranged from 1.4% to 1.7% and sapwood extractive content ranged from 0.8% to 1.1% of dry mass) with this difference being consistent throughout the height of the tree. While there was no significant difference in the overall extractive content due to height there may be a suggestion of an influence of rootwood as the amount of extractives found were slightly higher in the samples from the base of the tree. As well as this, more aromatic compounds were found in the heartwood

and these had a tendency to increase with height in tree. Knot extractives were also found to be mainly composed of aromatic compounds and no esters were found in the knotwood though some (campesterol ester and sitosterol ester) were found in the heartwood, sapwood and rootwood. Bark was found to have the highest quantity of extractives followed by rootwood and knots (Table 2-1). Although rootwood had a similar composition of resin acids to the other parts of the tree, it was the only sample where the essential fatty acid α-linolenic acid was found (Table 2-2).

Table 2-1: The extractive content of different locations in Sitka spruce as a percent of dry mass (Caron, 2010)

Location	Extractives as a % of Dry Wood
Sapwood	1.3
Heartwood	1.5
Knotwood	9.0
Rootwood	11.5
Bark	28.3

Overall, although the extractive content was found to be low it was also found to be stable with little variability in amount of extractive found in trees at different sites and no statistically significant difference due to silviculture or geographical location (Caron, 2010). However, the ratio in the concentrations of chemical compounds can vary by provenance (i.e. the original geographical source) and even within provenance (Forrest, 1975a).

At least 34 different extractives were identified in Sitka spruce by Caron (2010), a list of which is shown, along with the parts of the tree in which they were found, in Table 2-2.

Table 2-2: Compounds identified by Caron (2010) in different parts of Sitka spruce. X indicates that the extractive was found.

Extractive	Knotwood	Rootwood	Heartwood	Sapwood	Bark
α-linolenic acid 1		X			
palmitic acid			X	X	
levopimaric acid	X		X		
pimaric acid	X	X	X	X	X
isopimaric acid	X	X	X	X	X
dehydroabietic acid	X	X	X	X	X
abietic acid	X	X	X	X	X
eicosanoic acid		X			
palustric acid			X	X	
stigmast-5-en-3 oleate					X
α-tocopherol (Vit E)					X
campesterol		X	X	X	X
isolariciresinol	X		X		
seicosalariciresinol	X	X	X	X	
divanillytetrahydrofuran		X			
matairesinol	X	X	X	X	
lariciresinol	X		X	X	
nortrachelogenin	X				
β-sitosterol	X	X	X	X	X
pinoresinol	X		X	X	
7-hydroxymatairesinol	X		X	X	
stigmast-3,5-dien-7-one			X	X	
stigmastone-3,6-dione		X	X	X	
7-oxositosterol			X	X	
cis eicosanyl ferulate			X	X	
trans eicosanyl ferulate			X	X	X
cis docosanyl ferulate			X	X	X
trans docosanyl ferulate			X	X	X
cis tetracosanyl ferulate			X	X	X
trans tetracosanyl ferulate			X	X	X
β-sitosterol acetate			X	X	
campesterol ester		X	X	X	X
sitosterol ester		X	X	X	X
triglycerides			X	X	X

2.1.1 Bark

Bark of Sitka spruce has been found to contain antifungal compounds, mainly in the form stilbene glucosides which can act as inhibitors to growth of certain decay-causing fungi (Solhaug, 1985, Solhaug, 1990). These stilbenes are hydrophilic extractives of which astringin is the most predominant, with isorhapontin and piceid also present in more minor amounts (Aritomi and Donnelly, 1976). In healthy bark of young trees, levels of astringin and

rhaponticin have been found of around 10–15 mg g^{-1} tissue based on fresh weight with highest amounts being found in the root bark (Woodward and Pearce, 1988). Similarly, astringin and isorhapontin are found in parenchyma cells in the bark tissue of more mature trees, again with more being found in the bark of the roots than that of the stem, while levels in needles, sapwood and feeder roots are relatively low (Toscano Underwood and Pearce, 1991a). Astringen and isorhapontin are also found in the bark of branches of Sitka spruce with total and relative amounts found depending on the type of branch and height in the tree (Forrest, 1975b). Provenance or site does not seem to have an influence over the amount of stilbenes found with a large variation between different trees which may be due to variation in individual genotypes (Toscano Underwood and Pearce, 1991b). The structure of both astringen and isorhapontin can be seen in Figure 3-2.

2.1.2 Knots

Knots are the remaining part of a branch which, as the tree grows, becomes embedded within the tree stem. Sitka spruce knotwood contains approximately 5% by weight of dry wood of hydrophilic extractives (Table 2-3) which is relatively low compared to other species such as Scots pine where between approximately 9% (Pietarinen et al., 2006) and up to 32% (Willfor et al., 2003b) have been found and up to 30% in Norway spruce (Willfor et al., 2003a).

Table 2-3: Gravimetric amount (% dry wood) of extractives in Norway and Sitka spruce stem sapwood and heartwood as well as dead knots and live knots. From (Willfor et al., 2004a)

Species	Sapwood	Heartwood	Live Knot	Dead Knot
P.sitchensis	0.33-0.43	0.62-1.5	4.7-5.0	4.9-5.0
P.abies	0.25-0.52	0.40-2.6	17-21	14-17

The hydrophilic extractives of Sitka spruce knotwood mostly contain lignans with liovil and secoisolariciresinol being predominant (Willfor et al., 2004a), the structure of which can be seen in Figure 3-1. Hydroxymatairesinol, which is the main lignan found in Norway spruce knotwood is only found in small amounts in Sitka (Willfor et al., 2004a). The smaller amount of lignans found in the knotwood of Sitka also means that the extract has a weaker antioxidant potency compared to other spruce species (Pietarinen et al., 2006). However there has also been 5 different flavonoids found in Sitka spruce wood which are not found in other spruce species such as Norway spruce (Willfor et al., 2004a). These are shown in Table 2-4 along with ligans and lignols which were also identified in Sitka spruce.

Table 2-4: Breakdown of the hydrophilic compounds found by (Willfor et al., 2004a) in Sitka spruce stemwood and knotwood. Note that stemwood concentrations are shown in µg and knot concentrations in mg.

Compounds	µg g^{-1}		mg g^{-1}	
	Sapwood	**Heartwood**	**Live Knot**	**Dead Knot**
Lignans				
7-Hydroxymatairesinol	trace	60-290	0.95–1.80	1.00-1.10
7-allo-Hydroxymatairesinol	trace	20-170	0.70–1.30	0.79-1.20
Secoisolariciresinol	3-6	30-60	0.42–1.00	0.46-1.60
Liovil	4-5	510-2100	2.60–4.10	2.80-5.20
Lariciresinol	11-28	140-360	0.10–0.30	0.17-0.34
Lignan A		30-150	0.55–0.89	0.65-0.73
Cyclolariciresinol	4-5	50-150	0.10–0.60	0.21-0.30
Pinoresinol	trace -3	20	0.05–0.09	0.05-0.10
Total amount of lignans	22-47	860-3300	6.20–9.40	6.50-10.0
Lignols				
Dilignols	5-6	36-55	0.07–0.12	0.12-0.13
Flavonoids				
Dihydrokaempferol	4-7	110-180		0.09-0.10
Pentahydroxy-flavanone	trace	50-340	0.11–0.22	0.17-0.32
Catechin	trace	0-30	0.00–0.08	
Pinocembrin		16-28	trace	0.03
Pinobanksin		trace		
Total amount of flavonoids	4-7	210-550	0.19–0.33	0.29-0.45

2.1.3 Needles

The outer part of conifer needles are surrounded by a layer of cells called the epidermis. The cuticle is the outer layer of the epidermis and with the formation of epicuticular waxy deposits acts as a protective barrier between the plant tissue and the atmosphere. This waxy layer contains a number of different compounds which not only varies between species but also can vary between different clones of the same species (Percy and Baker, 1990) and also changes due to the age of the needles (Gunthardt-Goerg, 1986). In Norway spruce age is known to not only affect the total amount of wax, but also cause an increase in alkane and decrease in alcohol content of the wax (Prugel et al., 1994). In Sitka spruce waxes (extracted using chloroform) make up approximately 1.7 to 1.8% of oven dry needle weight (Percy and Baker, 1990) and have been found at 10 to 12 mg g^{-1} of dry needles (Pruegel and Lognay, 1996). The wax extract is made up mainly of secondary alcohols, diols (mostly nonacosan-10-ol and various isomers of nonacosane-diol) and fatty acids (mostly hexadecanoic) along with other secondary (e.g. C_{31}) and primary (e.g. C_{30}) alcohols though the composition can vary greatly between trees (Percy and Baker, 1990). Pruegel and Lognay (1996) reported that Sitka spruce needle wax contained approximately 14% secondary alcohols (made up of C_{25}, C_{27}, C_{29},C_{30}, C_{31} and C_{33}), 7.5% diols (C_{27},C_{28} and C_{29}), less than 0.7% alkanes (C_{15}-C_{35}) and alkenes (C_{17}-C_{31}), 0.3% primary alcohols (even no's of C_{12}-

C_{30}) and about 1.3% fatty acids of various lengths. The same study also found lesser amounts of long chain aldehydes and ketones. This differs from Norway spruce where no aldehydes were detected (Pruegel and Lognay, 1996).

Conifer needles also contain ducts which produce and store resins comprising of secondary metabolites such as terpenoids. Concentrations of these resins have been found in Sitka spruce at 1.69% of the dry weight of the needle which is higher than other species studied such as Douglas-fir (0.77%) and Western hemlock (1.13%) and are thought to be produced as a defence against herbivores (Kelsey et al., 2009). The ratios of compounds which make up these terpenoids resins are different for each species. In Sitka the monoterpene myrcene is the most abundant (33% of needle resin) and together with limonene/β-phellandrene and piperitone make up almost 50% of the total resin (Kelsey et al., 2009). Ludley et al. (2008) found limonene to be the most abundant monoterpene in samples of freshly fallen needles, however the total monoterpene concentration of approximately 100 μg g^{-1} (dry weight) was several times lower than that found in both Norway spruce and Scots pine. This is also generally lower than what is found in green needles collected fresh from the tree where highly variable concentrations of between 32 μg g^{-1} and 4252 μg g^{-1} were found depending on the tree (Ludley et al., 2009). In these fresh green needles, myrcene rather than limonene was the dominant monoterpene found (Ludley et al., 2009).

2.2 Norway Spruce (*Picea abies*)

Native to northern and eastern Europe as well as the mountainous areas of central and southern Europe, there are approximately 61,600 ha of Norway spruce in Great Britain of which approx. 26,000 ha is in Scotland (Forestry Commission, 2011) where it is often processed by the timber trade in combination with Sitka spruce as British spruce (EN14081, 2005).

2.2.1 Stem and Knots

Although similar low total concentrations of extractives are found in the heartwood and sapwood of Norway and Sitka spruce (see Table 2-3 above), the knotwood in Norway spruce generally has higher total concentrations of hydrophilic extractives of approximately 30% of total weight (Willfor et al., 2003a, Pietarinen et al., 2006). The composition of the extractives also differs between the two species and unlike Sitka spruce the knotwood of Norway spruce does not contain flavonoids (Willfor et al., 2004a). A list of the hydrophilic compounds along with the quantities found in Norway spruce by Willfor et al. (2004a) are shown in Table 2-5.

Table 2-5: Breakdown of the hydrophilic compounds found by (Willfor et al., 2004a) in Norway spruce stemwood and knotwood. Note that stemwood concentrations are shown in µg and knot concentrations in mg.

Compounds	**µg g^{-1}**		**mg g^{-1}**	
	Sapwood	**Heartwood**	**Live Knot**	**Dead Knot**
7-Hydroxymatairesinol	20–190	39–6500	46–51	23–50
7-allo-Hydroxymatairesinol	trace–37	12–1700	18–37	13–19
Secoisolariciresinol	5–20	3–370	3.8–5.6	1.4–6.8
Liovil	14–58	22–1900	2.8–6.1	2.5–4.2
a-Conidendrin	trace	trace–460	3.4–4.8	2.5–4.4
Lariciresinol	5–21	17–370	1.3–2.5	1.0–1.5
Matairesinol	trace–12	10–520	3.2–5.5	1.7–2.8
a-Conidendric acid	trace–5	16–290	1.0–1.8	0.81–1.3
Lignan A		6–18	0.30–0.76	0.60–1.6
Cyclolariciresinol	5–8	25–90	trace–0.42	trace–0.32
Pinoresinol	18–22	10–20	trace	trace
Total amount of lignans	73–390	160–12000	85–110	54–83

2.2.2 Knots

Lignans are the main component of Norway spruce knotwood extractives (Willfor et al., 2004b, Kebbi-Benkeder et al., 2015) and although the extractive content varies between trees and even between knots in the same tree lignan quantities have been found between 6% and 24% by weight of the total knot material (Willfor et al., 2003a, Hovelstad et al.,

2006). A direct comparison of knotwood and stemwood found knotwood to be as high as 15% compared to 0.05% for the adjacent stemwood (Willfor et al., 2005). The predominant lignan found in Norway spruce knotwood is 7-hydroxymatairesinol which accounts for between 65% and 85% of the total lignans found (Kebbi-Benkeder et al., 2015, Willfor et al., 2003a, Hovelstad et al., 2006) as well as small amounts (2%-6%) of oligolignans (i.e. three to four phenylpropane units) are also found (Willfor et al., 2003a). The concentration of lignans found within a knot can vary radially within the tree stem with higher amounts found in the part of the knot towards the bark where concentrations up to 152 mg g^{-1} have been found (Willfor et al., 2005). Within the knot itself, higher lignan concentrations are generally found towards the centre of the knot (i.e. at the knot pith) decreasing towards the outer knot (Willfor et al., 2005) and higher in the upper parts of the knot where there is less compression wood (Willfor et al., 2003a). Resin acids have also been found in Norway spruce knots but these are only in minor amounts of less than 0.04% (Hovelstad et al., 2006).

2.2.3 Stem

In the stems of Norway spruce higher amounts of resin acids have been found in the heartwood and sapwood than in the knotwood though some dead knots can also concentrations up to 2% (Willfor et al., 2003a). The content of these lipophilic extractives varies between trees and within different parts of the same tree (Ekman et al., 1979, Willfor et al., 2003a), however, the main components found in varying quantities are dehydroabietic acid, isopimaric acid, palustric acid and levopimaric acid (Willfor et al., 2003a). Other lipophilic extractives found in small amounts are the free sterols sitosterol and campesterol, as well small amounts of esterified and free fatty acids such as linoleic, pinoleic, palmitic and oleic acids (Willfor et al., 2003a). Diterpenoid concentrations of 5.3 mg g^{-1} (resin acids, cis-abienol and thunbergol) have also been found in the sapwood of Norway spruce (Willfor et al., 2005) and these along with fatty acids and resin acids can be higher in the sapwood than heartwood (Ekman et al., 1979). The amount of lignans found in the stemwood is relatively small compared to knots (Willfor et al., 2005) with negligible amounts being found in the sapwood and in heartwood varied from trace amounts up to nearly 0.5% of the weight of dry wood (Ekman, 1979). The only lignan found in heartwood by Kebbi-Benkeder et al. (2015) was α-conidendrin which accounted for 13% of the total acetone extractives detected along with approximately 12% fatty acids (palmitic acid, oleic acid, stearic acid and linoleic acid). Figure 3-3 in Section 3 shows the structures of the main resin acids found in wood and Table 3-1 shows the formula of the main fatty acids.

2.2.4 Branches

Ekman (1979) found higher lignan content (approx. 4%-6%) in the heartwood of branches than in the stem of the same tree (0.1%) and within the branch there is a general pattern of decreasing lignin content from the branch pith outwards (Willfor et al., 2005). However

although later studies found relatively high concentrations of lignans (up to 145 mg g^{-1}) in the first 10cm of the branch, levels decrease rapidly along the branch and mostly disappear by 20 cm out from the tree (Willfor et al., 2003a, Willfor et al., 2005).

2.2.5 Bark

In Norway spruce the total extractive content of bark is relatively high and has been found at 21.6% of the total weight of dry bark of which approximately 78% consists of polar extractives (soluble in water and ethanol) with the remaining being non-polar (Miranda et al., 2012). The main constituent of acetone extractives from Norway spruce bark are stilbene glucosides (mainly astringen, isorhapontin and piceid) which can be found in concentrations ranging from 12 to 48 mg g^{-1} in 37 year old trees and 5 to 27 mg g^{-1} in 18 year old trees (Jyske et al., 2014). From these yields, it is been estimated that in total the older trees could produce approximately 1.6 kg of stilbene glucosides per cubic metre of timber (Jyske et al., 2014). The same study found that on average the bark from the 37 year old trees produced 618g of astringen, 324g if isorhapontin and 90 g of piceid with higher amounts coming from the base of the stem than the top (Jyske et al., 2014). Another component of Norway spruce bark are tannins which can be extracted using hot water. These can be found at as much as 10.7% of the bark during the summer, though the levels in winter could be slightly lower at 8.3% (Kemppainen et al., 2014). However, Bianchi et al. (2014) found lower amounts of tannins (only 3.3% of the dry bark weight) in Norway spruce (compared to 10.1% for silver fir). This difference may be due to the different method of analysing samples as the findings by Kemppainen et al. (2014) are based on bark which has had any excess wood removed. Bianchi et al. (2014) also suggested that stilbenes such as astringen are included in these tannin extracts. Procyanidins, which are chemicals forming part of the tannins have been found comprising 3.6% of Norway spruce bark (Matthews et al., 1997).

Table 2-6: Content of the petroleum ether (non-polar) extractives found in Norway spruce bark by Anas et al. (1983)

Extractive		Inner Bark mg/g	Outer Bark mg/g
Fatty Acids		**7.57**	**6.36**
	Triglyceride	4.86	2.60
	Mono & diglyceride	0.84	1.74
	Steryl Ester	1.44	0.60
	Free	0.43	1.42
Resin Acids		**6.26**	**1.94**
Sterols & Triterpine alcohols		**2.94**	**2.98**
	Free	0.72	1.91
	Esterified	2.22	1.07
Diterpene Alcohols		**1.24**	**0.30**
Diterpene Aldehydes		**0.33**	**0.10**
Fatty Aclcohols		**0.13**	**1.24**
	Ferulate-	0.08	0.94
	Wax-	0.03	0.07
	Free	0.02	0.23
Glyceryl Residues		**0.32**	**0.31**
Total		**18.79**	**13.23**

Non-polar compounds are also found in Norway spruce bark and using methylene chloride as the extractive solvent, Norin and Winell (1972a) found these lipophilic extractives consisted of a mixture of fatty acids, waxes (which contained esters of fatty acids ranging from C_{12} to C_{24}, with oleic acid and linoleic acid being predominant), resin acids, an equal distribution of odd and even number chains of alkanes (C_{15}-C_{38}), sterols (β-sitosterol and campesterol), fatty alcohols ranging from C_{18} to C_{26}, and a range of monoterpenes, sesquiterpenes, diterpenes and triterpines. Investigating the difference in non-polar extracts between inner and outer bark, Anas et al. (1983) found non-polar extractives were approx. 1.8 to 2.9% of dry weight of the inner bark and 2.0-4.8% of the outer bark. Fatty acids and resin acids, which were similar to that found by Norin and Winell (1972a), constituted about 1.5% of the total dry weight of the whole bark and sterols were between 0.2 and 0.5% (Anas et al., 1983). The content of the non-polar extractives found by Anas et al. (1983) are summarized in Table 2-6.

2.2.6 Stump and Roots

Like the bark of the main stem the acetone soluble extractives found in the root bark of Norway spruce are astringin and isorhapontin as well as smaller amounts of piceid (Mulat et al., 2014). However, unlike the stem bark, isorhapontin has been found to be the major component of the root bark with concentrations varying from 0.18% to 6.4% of the total dry weight, compared to 0.04% to 2.5% for astringin, both of which depend on location in the root system (Latva-Maenpaa et al., 2013). This compares to concentrations of isorhapontin

in Norway spruce root bark of 1.5%, 0.4% in the stem and 0.3% in the fine roots found by (Beyer et al., 1993). Fine roots of Norway spruce have been found to have between 15% and 29% extractives (Richter et al., 2007). Table 2-7 shows the results of the study in which Latva-Maenpaa et al. (2013) examined the stump and roots of Norway spruce. They found that the bark had much higher extractive content than the wood; the root zone nearest the stem (A) had the highest amount of extractives in the bark and the zone closest to the root tip (C) had the highest amount of extractives in the wood. While isorhapontin was the major stilbenoid found in the bark in all three of the root zones, astringin was the major compound found in the stump bark. Within the wood they mainly found saccharides such as fructose, glucose and sucrose as well as fatty acids and resin acids such as pimaric acid, abietic acid and dehydroabietic acid (Latva-Maenpaa et al., 2013). The same study also found that extractive concentrations, and especially isorhapontin, were higher in samples taken from trees grown in mineral soils as opposed to those grown on peaty soils (Latva-Maenpaa et al., 2013).

Table 2-7: Total amount of acetone extractives by % of dry weight for the root and stump of Norway spruce trees. The root nearest the stump is Zone A and the part of the root furthest from the stump is Zone C. Zone B is between the two. Taken from (Latva-Maenpaa et al., 2013)

Zone	Stump	Root Zone A	Root Zone B	Root Zone C
Wood	1.93%	2.09%	1.67%	3.46%
Bark	13.38%	18.18%	13.44%	16.40%

As well as finding the stilbenes astringen, isorhapontin and piceid in the root bark of Norway spruce, Pan and Lundgren (1996) also found a number of other phenolic substances including nine monoaryl compounds and ten lignans as well as five catechins and proanthocyanidins which are the some of the main compounds found in tannins (Matthews et al., 1997). Procyanidins have also been found in the mycorrhizas of Norway spruce along with astringen, isorhapontin, catechin, vanillin and ferulic acid (Weiss et al., 1999).

The root neck of Norway spruce stumps may also be a valuable source of lignans. Latva-Maenpaa et al. (2014) investigated the acetone-water extractives and found relatively high concentrations (approx. 10% of total dry weight) of hydroxymatairesinol in the heartwood of the lowest part of the root neck suggesting that this may be an alternative source of these bioactive compounds. They also found a difference in concentrations of stilbene glucosides between the inner bark and the outer bark of this part of the stump with high levels of approx. 20% found in the inner compared to 0.2% in the outer bark (Latva-Maenpaa et al., 2014).

2.2.7 Needles

Monoterpenes are found in freshly fallen Norway spruce needles, the most predominant being β-pinene and α-pinene which contribute 56% and 19% of the total monoterpene content respectively (Ludley et al., 2008). Total monoterpene concentrations of approximately 1531 μg g^{-1} have been found in the freshly fallen needles, which is slightly higher than Scots pine and several times higher than Sitka spruce (Ludley et al., 2008). Higher monoterpene concentrations are found in green needles taken directly from the tree (approximately 3628 μg g^{-1}) and concentrations have been shown to decrease rapidly in fallen needles (Ludley et al., 2009). A number of flavonoids are also found in Norway spruce needles (Slimestad et al., 1999). Waxes constitute 10 to 15 mg g^{-1} of Norway spruce needles of which approximately 10% is long chain secondary alcohols, ranging from C_{22} to C_{33}, with C_{29} (10-nonacosanol) being the most predominant. Approximately 3% of the wax is diols, ranging from C_{27} to C_{29}, and approximately 4% fatty acids (such as hydroxydodecanoic acid), with smaller amounts of alkanes (C_{16}-C_{35}), alkenes (C_{18}-C_{32}), primary alcohols (even no's of C_{12}-C_{28}), as well as ketones, methyl esters and ethyl esters also found (Pruegel and Lognay, 1996).

2.3 Scots Pine (*Pinus sylvestris*)

Native to Europe and Asia where it is found from Scandinavia across to Siberia and as far south as Spain, Scots pine is one of only a few conifers which are native to Great Britain where it formed part of the Caledonian Forest. It is also the only native conifer which is commercially grown for timber in Britain where it covers an area of approximately 241,300 ha, approx. 171,000 ha of which is in Scotland (Forestry Commission, 2011).

2.3.1 Stem

The heartwood of the stem of Scots pine can be a valuable source of extractives with total extractive content of approximately 21% having been found (Kebbi-Benkeder et al., 2015).Common extractives found within the stem of Scots pine, using acetone/water as a solvent, are the stilbene pinosylvin and pinosylvin monomethyl ether. These are mostly found in the heartwood with less in the inner heartwood than the outer heartwood and highest concentrations found at the heartwood/sapwood boundary (Bergstrom et al., 1999, Ekeberg et al., 2006, Philip et al., 1995). Total extractive content of 2.9% (Sable et al., 2012) and pinosylvin stilbenes concentration of 0.2% to 2% of the total wood weight have been found in the stemwood when sapwood and heartwood are measured together (Hovelstad et al., 2006) however neither Bergstrom et al. (1999) or Ekeberg et al. (2006) found any in the sapwood. Pinosylvin concentrations as high as 9.2 mg g^{-1} can be found within the heartwood/sapwood boundary zone decreasing to approximately 4 mg g^{-1} towards the inner heartwood (Bergstrom, 2003). There is no correlation between maximum pinosylvin content and factors such as tree age, tree height, crown width, stem diameter or climate however there is a large variation between trees in the maximum amount of pinosylvin (Bergstrom et al., 1999). Ekeberg et al. (2006) also investigated the effect of sample size (solid wood (5x10x30 mm), small particles (2x1x1 mm) and fine powder) on the extractives within Scots pine stems and found that the sample sizes gave different yields for different extractives. For example, the yield of pinosylvin and pinosylvin monomethyl ether were higher for small particles and powder than they were for solid wood. Conversely, the yield of resin acids and fatty acids was higher for solid wood and small particles than for powder (Ekeberg et al., 2006).

Resin acids and fatty acids (such as abietic acid, linoleic acid and oleic acid) are also found in the stemwood with concentrations in the inner part of the heartwood higher than in the outer portion (Ekeberg et al., 2006) and account for 1% - 4% of the total weight of stemwood (Hovelstad et al., 2006). Bergstrom (2003) found resin acid concentrations up to approximately 10 mg g^{-1} along with fatty acid concentrations up to a maximum of 7.9 mg g^{-1} in the inner heartwood. Arshadi et al. (2013) identified 21 fatty acids and 10 resin acids (Table 2-8) which together accounted for approximately 0.24% to 4.1% of dry material with heartwood containing between 2.5 and 5 times more of the extractive than sapwood. In the stemwood sitosterol, stigmastanol and campesterol are the predominant free sterols

(Saranpaa and Nyberg, 1987) and the amount of free sterols tends to be higher in the heartwood than sapwood. Steryl esters concentrations of approximately 0.83 mg g^{-1} can be found in the stemwood (Saranpaa and Piispanen, 1994) and while levels are similar across heartwood and sapwood, levels of lipids tend to be higher in the sapwood (Hoell and Lipp, 1987). The triacylglycerol faction of fatty acids are found mainly in the sapwood and although quantities vary greatly from tree to tree average concentrations of 25 mg g^{-1} (Saranpaa and Nyberg, 1987) and 26 mg g^{-1} (Piispanen and Saranpaa, 2002) have be found in the outer sapwood.

Table 2-8: Breakdown of the fatty acid and resin acid extractives found in the stem wood of Scots pine by Arshadi et al. (2013)

	Extractive	Max conc. (mg/g)
Fatty acids	Octadecanoic acid	1.43
	Oleic acid	1.26
	Docosanoic acid	0.91
	9,12-Octadecadienoic acid	0.89
	Linolenic acid, anteiso	0.64
	Hexadecanoic acid	0.54
	trans-9-Octadecenoic acid, anteiso	0.5
	Heptadecanoic acid, anteiso	0.42
	Heptadecanoic acid, anteiso	0.34
	Linolenic acid	0.32
	(E)-9-Octadecenoic acid	0.21
	Heptadecanoic acid	0.16
	Eicosanoic acid	0.15
	11-cis-Octadecenoic acid	0.08
	Tricosanoic acid	0.08
	Tetradecanoic acid	0.07
	Nonanoic acid	0.06
	Pentadecanoic acid	0.06
	Docosanoic acid, anteiso	0.06
	Octanoic acid	0.04
	dodecanoic acid	0.02
Resin Acids	Dehydroabietic acid	13.67
	Abietic acid	7.59
	7-Oxodehydroabietic acid	3.25
	Isopimaric acid, anteiso	3.03
	Pimaric acid, anteiso	2.87
	Isopimaric acid	2.75
	Pimaric acid	2.63
	Dehydroabietic acid, anteiso	2.06
	Isopimaric acid, anteiso	0.79
	Pimaric acid, anteiso	0.59

2.3.2 Stump and Roots

The stump and roots of a mature conifer tree can be 22-25% of the stem biomass (Hakkila, 2004) and in Scots pine this is made up of 19.3% fine roots, 46.8% coarse roots along with 16.2% sapwood and 17.7% heartwood in the stump (Eriksson et al., 2012). Coarse roots in Scots pine have a higher extractive content than the thin roots (approximately 8% by weight compared to approximately 2% respectively) and the heartwood of the stump contains approximately 19% extractives by weight compared to approx. 3.7% for the stump sapwood (Eriksson et al., 2012).

2.3.3 Knots

Knots of Scots pine are another source of extractives the composition of which differs from that seen in Norway spruce. Extractives of 8.8% by weight have been found in knotwood which consisted mostly of pinosilvans (22% pinosylvin, 16% pinosylvin monomethyl ether), which are a type of stilbene, and lignans, of which nortrachelogenin (30%) was predominant (Pietarinen et al., 2006). In fact, in a study encompassing 5 other softwood species (silver fir, Norway spruce, Douglas fir and larch) Scots pine was the only species in which the knotwood contained stilbenes (Kebbi-Benkeder et al., 2015). This study found total knotwood extractives to be almost 40% by weight of wood when extracting sequentially with dichloromethane, acetone and toluene/ethanol, with the resulting extractives being 42% pinosylvin monomethyl ether, 34% pinosylvin and 19% nortrachelogenin (Kebbi-Benkeder et al., 2015). The structure of these compounds can be seen in Figure 3-1 and Figure 3-1.

Investigating the phenolic and lipophilic extractives in Scots pine Willfor et al. (2003b) sequentially extracted the knotwood with hexane to remove the lipophilic compounds, then acetone/water to remove the hydrophilic extractives. With regards to hydrophilic extractives, the knotwood contain approximately 1-8% by weight of phenolic stilbenes (Willfor et al., 2003b, Hovelstad et al., 2006) and about 0.4-3% lignans, which again mostly consists of pinosylvin, pinosylvin monomethyl ether and nortrachelogenin (Willfor et al., 2003b). Small amounts of other lignans such matairesinol, secoisolariciresinol and liovil have also been detected as well minor amounts of the flavonoid pinocembrin (Willfor et al., 2003b) and oligolignans (Willfor et al., 2004b). Stilbene and lignan concentrations mostly stay the same or decrease slightly in the knots in a radial direction from the pith to the outer branch, however there is a rapid decrease in the outer branch with lignans almost disappearing within the first 20cm (Willfor et al., 2003b). Ekman et al. (2002) found Scots pine knot heartwood to contain 20 to 40 mg of nortrachelogenin per g wood and this was the same for dead and live knots.

Scots pine knots contain high amounts of lipophilic extractives (between 4.5% to 32% by weight of wood) mainly in the form of resin acids of which abietic acid is the most abundant (Willfor et al., 2003b, Hovelstad et al., 2006). The resin acids also decreased slightly or

stayed the same in a radial direction from the pith to the outer branch and again there was a rapid decrease once in the outer branch (Willfor et al., 2003b). Hovelstad et al. (2006) found resin acid content of Scots pine knots to be approximately 5-10% by weight compared to 0.04% for Norway spruce. While abietic acid is the predominant resin acid the knots also contain smaller amounts of related compounds neoabietic acid, dehydroabietic acid, levopimaric acid and palustric acid (Hovelstad et al., 2006). The high resin acid content can make recovery of the lignans from Sots pine knots more difficult (Holmbom et al., 2003).

2.3.4 Bark

There have been a number of studies investigating the extractive properties of the bark of Scots pine. Non-polar extractives, extracted using petroleum ether constitute approximately 2% – 7% of the weight of dry Scots pine bark (Anas et al., 1983) which is similar to that found by Norin and Winell (1972b) and also similar to Norway spruce. 50 to 80% of the non-polar extractive is made up of fatty acids (mostly oleic, linoleic and pinolenic acids), resin acids (mostly abeitic-type acids) and sterols with the rest consisting of diterpene alcohols and aldehydes, fatty alcohols and other unidentified compounds (Anas et al., 1983). Table 2-9 gives an indication of the nature and quantities of the non-polar extractives, found by Anas et al. (1983) in the inner and outer bark of a Scots pine tree.

Table 2-9: Content of the petroleum ether (non-polar) extractives found in Scots pine bark by Anas et al. (1983)

Extractive		Inner Bark mg/g	Outer Bark mg/g
Fatty Acids		**37.83**	**9.04**
	Triglyceride	33.40	1.71
	Mono & diglyceride	2.26	5.46
	Steryl Ester	1.54	0.19
	Free	0.63	1.68
Resin Acids		**7.16**	**2.39**
Sterols & Triterpine alcohols		**4.50**	**2.98**
	Free	2.56	2.73
	Esterified	1.94	0.25
Diterpene Aldehydes		**0.21**	**0.11**
Fatty Aclcohols		**1.33**	**1.25**
	Ferulate-	1.26	1.01
	Wax-	0.03	0.09
	Free	0.04	0.15
Glyceryl Residues		**1.76**	**0.68**
Total		**52.78**	**16.45**

Successively using dichloromethane, methanol, ethanol and water as solvents Miranda et al. (2012) found total extractives in Scots pine bark of 18.8%. The most predominant fraction were those that were soluble in water (9.2%) and ethanol (5%) with hydrophilic extractives

accounting for approximately 75% of the total extractives (Miranda et al., 2012). Similarly Valentin et al. (2010) found that the water extractives accounted for 13.7% of the total mass of dry bark and acetone extractives accounted for 5.6%. The composition of the acetone extract can be seen in Table 2-10.

Table 2-10: Composition of the extractives found in the acetone fraction from Scots pine bark by Valentin et al. (2010)

Extract	mg/100g	Extract	mg/100g
Phenolic acids, aldehydes, dicarboxylic acids		**Resin Acids**	
Malic acid	1	Pimaric acid	64
Vanillin	5	Sandarakopimaric acid	11
3,4-Dihydroxybenzaldehyde	7	Isopimaric acid	38
Vanillic acid	2	Palustric acid	30
3,4-Dihydroxybenzoic acid	30	Levopimaric acid	8
Glucose	29	Dehydroabietic acid	222
Cellobiose	2	Abietic acid	61
Unsaturated Fatty Acids		Neoabietic acid	14
C 16:1 (palmitoleic acid)	1	**Oxidized resin acids**	
C 18:3 (linolenic acid)	10	7-Hydroxy-dehydroabietic acid	24
C 18:2 (linoleic acid)	46	Hydroxydehydroabietic acid	41
C 18:1 (n-9; oleic acid)	54	Others	22
C 18:1 (n-11)	3	**Sterols**	
C 20:3	9	Campesterol	15
Saturated fatty acids		Sitosterol	176
C 18:0 (stearic acid)	6	Stigmasta-5,24(28)dien-3-ol	25
C 20:0 (arachidic acid)	11	Stigmast-4-en-3-one	56
C 22:0 (behenic acid)	41	7-Hydroxysitosterol	7
C 24:0 (lignoceric acid)	24	Pimaral	5
		Monomethyl pinosylvin	13
		Catechin	7
		Acid 22:0-monoglyceride	12

The main phenolic compounds that have been found in Scots pine bark extract include, monoaryl compounds, stilbene-glucosides, lignans, flavonoids, catechins and proanthocyanidins (Pan and Lundgren, 1996). This includes compounds such as β-hydroxypropiovanillone, vanillin, dihydroconiferyl alchohol, ferulic acid, pinoresinol, matairesinol, taxifolin and taxifolin 3'-O-β-D glucoside (Karonen et al., 2004). Lignans and sesquineolignans have also been identified in Scots pine bark.

2.3.5 Needles

Monoterpenes are found in needles of Scots pine and concentrations of approximately 4056 $\mu g\ g^{-1}$ of dry weight have been found in green needles however this can decrease rapidly when they fall from the tree (Ludley et al., 2009). α-pinene and 3-carene have been found to

be the predominant monoterpenes where they contribute 50% and 32% respectively of the total amount of monoterpenes found in the green Scots pine needles (Ludley et al., 2009) and 46%/37% respectively of the content of freshly fallen needles (Ludley et al., 2008). Scots pine needles also contain free sterols, steryl esters and lipid phosphorus with new needles containing higher amounts of free sterols but low levels of steryl esters (Fischer and Holl, 1991). This same study found that new needles contained the highest amount of lipid phosphorus, sitosterol made up 85-95% of the steryl ester fraction throughout the year and isofucosterol made up 25% of the total free sterols in newly emerged needles. The major fatty acids in the steryl ester fraction were palmitic, oleic and linoleic acid and others detected in minor amounts. One, two and three year old needles were similar in their composition of both free sterols and steryl esters. Other studies showed that the total amount and composition of the fatty acids in the sapwood and heartwood did not change over the year but here in the needles big changes were seen (Fischer and Holl, 1991).

2.3.6 Cones

The cones of five pine species were investigated in a study in Turkey, where Scots pine cones were found to have the lowest amount of lipophilic extractives (9.0 mg g^{-1}) of which resin acids were the main constituent with dehydroabietic acid being predominant (Kilic et al., 2011a). Concentrations of phenolic compounds in the cones were low and consisted of 3,4-dihydroxybenzoic acid and catechin both of which were measured at concentrations of 0.03 mg g^{-1} (Kilic et al., 2011b).

2.4 Lodgepole Pine (*Pinus contorta*)

Originates from, and covers a large area of north western USA, western Canada and Alaska. It was introduced to Europe in the 1950s and was more widely planted in both Europe and the UK in the 1960s, 70s and 80s (Elfving et al., 2001, Mochan, 2005). The 2011 Forestry Commission National Forest Inventory Report estimates that there is approximately 106,400 hectares of lodgepole pine in Great Britain of which 94,000 hectares is in Scotland (Forestry Commission, 2011) where it is the third most planted conifer species after Sitka spruce and Scots pine.

Hergert (1956) describes two varieties of lodgepole pine; one from the coastal region (*P.contorta* var. *latifolia Engelm*.) and the other from the interior (var. *Contorta*). Lodgepole pine has also been described as three varieties depending on provenance, where var. *latifolia* is a northern inland form and var. *murrayana* is a southern inland form and var. *contorta* is a coastal form (Elfving et al., 2001). A breakdown of how much of each of these (described as Alaskan, Inland and South Coastal) is grown in Scotland is discussed by Mochan (2005) and shows that there is differing quantities of each provenance grown throughout Scotland. Elfving et al. (2001) suggest that only the *latifolia* variety has done well in Sweden.

2.4.1 Stems

The stems of Lodgepole pine (var. *latifolia*) comprise of on average of 2.87% extractives, and although the extractive content of the stems of Lodgepole pine does not vary dramatically between varieties or over its full North American natural range, a positive correlation with latitude has been found (Kim et al., 1989). In the stem of the tree, sapwood of both *latifolia* and *murrayana* varieties contain less extractives than the heartwood (Table 2-11). However there may be a negative correlation between amount of extractives found and height in the tree (4.60% at the base vs 2.66% at 30% of height for *latifolia* and 3.06% vs 2.09% for *murrayana* at the base and 60% of stem height respectively) i.e. the base of the stem has a greater extractive content than higher up the stem (Campbell et al., 1990).

Table 2-11: Average extractive content in the stem of two Lodgepole pine varieties. From (Campbell et al., 1990)

	latifolia		*murrayana*	
	Sapwood	Heartwood	Sapwood	Heartwood
Extractives (%)	2.03	3.30	1.88	2.81

Arshadi et al. (2013) compared the stem wood extractives of mature Scots pine and lodgepole pine grown at the same location in northern Sweden. Scots pine had slightly higher fatty acid and resin acid content than lodgepole pine which had concentrations of between 2.3 mg g^{-1} and 26.0 mg g^{-1} which equated to 0.23% to 2.6% of dry material. This is

similar to that found by Sable et al. (2012) who found acetone extractives of lodgepole pine grown in Latvia to be approximately 2.6%. They also found that there was a difference between heartwood and sapwood concentrations with heartwood resin acid concentrations being 3.4 times higher and the concentration of fatty acids being 1.2 times higher (Arshadi et al., 2013). As well as this the same study found no significant differences between the same species at different sites.

2.4.2 Stem, Bark, Branch, Needles, Cones

Using hexane as the solvent, Backlund et al. (2014) investigated the lipophilic extractive content of different parts (stemwood, bark, branch wood, needles and cones) of lodgepole pine with a view to determining how the whole tree could be used by biorefineries. Although they found some variation between different trees the highest proportion of extractives were found in the bark followed by the branches (although branches included wood and branch bark). The average yield for each of the fractions is shown in Table 2-12.

Table 2-12: Hexane extracted yields found by Backlund et al. (2014) in different fractions of lodgepole pine

Fraction	Average Yield (% of Dry Weight)
Bark	16.4
Branches	7.4
Needles	6.3
Stem at base	1.6
Stem at top	0.8
Cones	1.7

Backlund et al. (2014) was able to quantify the extractives in each fraction (Table 2-13); In the stemwood and bark diterpenoids and ketones predominated, and the outer bark was also high in wax esters; Branches mostly contained diterpenes, ketones, fatty acids and wax esters; needles mostly contained wax esters and fatty alcohols and were high in aromatic compounds. From these yields Backlund et al. (2014) estimated that one hectare of lodgepole pine forest in the lower parts of northern Sweden could produce 950kg of extractives from the stem, 990kg from the bark, 660kg from branches and 370kg from needles working out at 2 to 3 tonnes of extractives per hectare.

Table 2-13: Breakdown of the hexane extractives found by Backlund et al. (2014) in the different fractions of lodgepole pine

Compound	Stem Base	Stem Top	Branches	Bark	Needles	Cones
	Average (μg g^{-1})	Average (μg g^{-1})	Average (μg g^{-1})	Average (μg g^{-1})	Average (μg g^{-1})	Average (μg g^{-1})
1-methoxy-4-[1-(4-methoxyphenyl)vinyl]benzene	-	-	-	-	38	62
13β-Methyl-13-vinyl-podocarp-7-en-3β-ol	-	11	-	-	3	16
Abietic acid		13	16	14	14	50
Andrographolide	239	53	189	91	-	-
Biformene	181	61	41	48	40	134
Cryptopinone	80	190	224	241	41	31
Dehydroabietal	27	72	172	81	-	20
Dehydroabietic acid	-	40	12	7	28	32
Epimanool	28	42	27	22	112	-
Germacrene D	20	29	-	19	2	17
Heptadecanoic acid	2	12	15	4	29	20
Hexadecanoic acid	67	40	29	27	-	-
Hexanal	-	6	-	-	-	-
Hexanoic acid	1	2	-	-	5	21
Linoleic acid	17	35	22	3	31	44
Linolenic acid	44	50	109	87	15	83
Methyl abietate	11	32	20	5	161	77
n-Heptacosanol	38	73	48	48	74	-
n-Nonacosanol	6	20	-	8	-	-
Nonanoic acid	-	-	-	3	21	232
Octadecanoic acid	113	125	152	149	-	-
Octanoic acid	0	7	-	-	25	13
Oleic acid	5	26	24	16	127	848
Pimaral/Androst-5-en-7-one/Androsta-3,5-dien-7-one	1105	1111	590	625	19	-
Pimaric acid	-	11	6	-	-	10
Podocarp-7-en-3-one/Pimara-7,15-dien-3-one	10	59	78	-	-	-
Rimuenel	56	31	31	21	37	99
Stigmastan-3,5-diene	1	-	-	-	46	-
Stigma-3,5-dien-7-one	-	-	73	18	-	-
Tetradecanoic acid	67	179	66	53	130	36
Wax esters	78	150	245	157	477	-

2.4.3 Bark

The predominant flavonoid in lodgepole pine bark is myrecitin (which accounts for approx. 90% of flavonoids), along with small amounts of quercetin, dihydromyricetin, aromadendrin and pinobanksin and although there is very little difference in the composition of the bark

extractives between different provenances, the coastal variety (var. *Contorta*) has been found to contain higher total amounts of flavonoids (Hergert, 1956). Using benzene as the solvent Rowe and Scroggin (1964) found the bark of lodgepole pine to be 28.7% extractives which when broken down consisted of free acids (42.6%), higher terpenoids (38%), combined acids, such as linoleic and oleic acid (9.4%), β-sitosterol (3%), wax alcohols (1.8%), campesterol (0.3%), steam volatile terpenoids, such as epimanool (2%) and hydrocarbons (0.02%).

2.4.4 Knots

Knotwood in lodgepole contains about 3% hydrophilic extractives which is lower than that found in other species of conifer such as Norway spruce and Scots pine (Willfor et al., 2003c). The composition (Table 2-14) is also slightly different as no oligolignans were found in lodgepole pine knots which are found in these other two species (Willfor et al., 2003c).

Table 2-14: Main components of hydrophilic knotwood extracts found by Willfor et al. (2003c) in Lodgepole pine knots

Extract	% of gravimetric extract
Lignans:	**10**
Nortrachelogenin	5
Liovil	3
Oligomers	3
Flavanoids:	**20**
Pinocembrin	15
Pinobanksin	7
Stilbenes:	**15**
Pinosylvin Monomethyl Ether	9
Pinosylvin	6

2.4.5 Needles

Gerson and Kelsey (1998), investigated piperidine alkaloids in lodgepole pine needles and found concentrations to be less than 6 μg g^{-1}. The most abundant alkaloid was euphococcinine, which is different for other pine species, for example, pinidine was only detected in small amounts in lodgepole pine but was the most abundant in ponderosa pine.

2.5 Larches (Hybrid: *Larix x marchlinsii* (syn. *L. × eurolepis*)), (European: *Larix decidua*), (Japanese: *Larix kaemferi*)

There are three main varieties of larch grown in the UK which together cover an area of approx. 68,400 ha in Scotland, and 133,000 ha in Great Britain (Forestry Commission, 2011). European larch originates from the European Alps and parts of Eastern Europe, Japanese larch originates in Japan and Hybrid larch is a cross between the two. All three of these varieties are treated the same by the timber industry in Scotland where they are processed together. In recent years larch has been greatly affected by the pathogen *Phytophthora ramorum* which has caused widespread mortality and has resulted in a lot of larch coming on to the market (Price and MacDonald, 2013).

2.5.1 Stem

One of the main features of larch extractives is the high concentrations of arabinogalactan found in the heartwood which can be found in quantities of 5 to 30% of wood depending on the larch variety (Cote et al., 1966). Unlike other hemicelluloses which are found in the cell wall, at least 90% of arabinogalactans are found in the lumens which is why it is classed as an extractive rather than a structural cell wall constituent although it may still have an effect on wood mechanical properties (Grabner et al., 2005b). The polysaccharide arabinogalactan is a water soluble hemicellulose which is found in small quantities in other softwood species, however in larch it can be up to 35% (Pereira, 2003). Grabner et al. (2005a) found total extractives in the heartwood ranged from 4% to 28.5% compared to 0.6% to 4.4% for sapwood of which most were hot water extractives (i.e. arabinogalactans) with amounts ranging from 3.3% to 25.1%. In the heartwood extractive filled tracheids were found in radially in rows in the earlywood as well as randomly throughout the yearly ring and this could suggest it may be having reinforcement properties for the wood. Extracting the heartwood had a big effect on mechanical properties while in the sapwood it was only a minor effect (Grabner et al., 2005a). Similarly removal of the extractives corresponded to a loss in density especially in the heartwood (Grabner et al., 2005b).

Although situated in eastern Canada, Keith and Chauret (1988) investigated European larch trees of approx. 25 and 30 years old where they examined water soluble and alcohol-benzene soluble extractives at two heights in the radial profile of the trees. Although there may be variation between trees, the concentration of water soluble extractives, mainly arabinogalactan, was similar over both heights measured and generally increased radially from the pith to the heartwood/sapwood boundary then decrease in the sapwood (Keith and Chauret, 1988, Gierlinger and Wimmer, 2004) with a similar pattern (though lower amounts) seen for alcohol benzene extractives (Keith and Chauret, 1988). This same study found total extractives reached almost 20% in the outer region of the heartwood with on average approximately 14.5% being water soluble and 3.5 – 3.8% being alcohol benzene extractives (Keith and Chauret, 1988).

Phenolic extractives have also been investigated in larch with the flavonoid taxifolin the most abundant (1.3-41.7 mg g^{-1} dry weight) compared to smaller amounts of dihydrokaempferol (0.5 -13.7 mg g^{-1}) (Paques et al., 2013). Taxifolin accounts for approx. 36% of total phenolic extractives in *Larix decidua* (Kebbi-Benkeder et al., 2015) and is also the predominant flavonoid in other larch varieties such as Siberian larch (*Larix sibirica* Ledeb.) (Neverova et al., 2013). However, there are differences in the relative amount of different phenolics found in the heartwood of the different varieties grown in the UK; Japanese larch had the highest total amount of phenols and highest amount of taxifolin of which European larch had the lowest. However Japanese larch had the least dihydrokaempferol compared to European larch with Hybrid larch having the most. Hybrid larch was high in both flavonoids (Paques et al., 2013).

2.5.2 Knots

Larch knots are a rich source of extractives with contents of almost 40% being found (Kebbi-Benkeder et al., 2015) in *Larix decidua* when extracted sequentially with solvents of increasing polarity (methylene chloride, acetone, toluene/ethanol and water). The main extractive component of this larch knotwood was the flavonoid taxifolin accounting for approximately 45% of the total extractives found (Kebbi-Benkeder et al., 2015). Lignans also constitute a large proportion of the extractives found with secoisolariciresinol being the most predominant (22.4% of extractives), and lesser amounts of nortrachelogenin (3.8%) which is the main lignan in Scots pine knots (Kebbi-Benkeder et al., 2015). Willfor et al. (2003c) also investigated the extractives in larch (*Larix decidua*) knotwood firstly by using hexane to remove the lipophilic extractives followed by acetone/water to remove the hydrophilic. Lignans were the main component of this extract of which secoisolariciresinol was predominant (Table 2-15) as well as finding taxifolin and other oligolignans.

Table 2-15: Main components of hydrophilic knotwood extracts found by Willfor et al. (2003c) in larch knots

Extract	% of gravimetric extract
Lignans:	**40**
Secoisolariciresinol	24
Lariciresinol	7
Isolariciresinol	6
Oligolignans	18
Flavonoids:	**17**
Taxifolin	14
Dihydrokaempferol	3

2.6 Summary of Extractive Content of the Different Species

There have been a number of studies on extractive content carried out into the different species. The table below gives a brief summary of the amounts found in the different parts of the tree by various studies.

Table 2-16: Relative amounts of extractives found by different studies into different parts of the trees.

Species	Sapwood	Heartwood	Knot	Bark	Roots	Branch	Needles	Solvent	Reference
Sitka	1.3%	1.5%	9.0%	28.3%	11.5%			Acetone/ Water	Caron 2010
Sitka			5.0%					Acetone/ Water	Pietarinen 2006
Sitka				10-15 mg/g					Woodward 1988
Sitka	0.33-0.43%	0.62-1.5%	4.7-5.0%					Acetone/ Water	Willfor 2004a
Norway			12.0%					Acetone/ Water	Pietarinen 2006
Norway	0.05%		15.0%					Acetone/ Water	Willfor 2005
Norway			6-24%					Acetone/ Water	Willfor 2003a
Norway	0.2%	1.0%						Acetone/ Water	Willfor 2004b
Norway	0.25-0.52%	0.4-2.6%	14-21%					Acetone/ Water	Willfor 2004a
Norway					2-3%	4-6%			Ekman 1979
Norway					15-29%			Methanol/ water	Richter 2007
Norway		4.0%	25.0%					Sequential	Kebbi-Benkeder 2016
Norway				21.6%				Ethanol/ water	Miranda 2012
Norway				1.8-4.8%				Petroleum ether	Anas 1983
Scots pine			8.8%					Acetone/ Water	Pietarinen 2006
Scots pine		21.0%	40.0%					Sequential	Kebbi-Benkeder 2015
Scots pine	1-3.5%		4.5-32%					Hexane	Willfor 2003b
Scots pine				18.8%				Ethanol/ water	Miranda 2012
Scots pine				2.1-7.2%				Petroleum ether	Anas 1983
Scots pine	0.24-4.1%							Petroleum ether/ acetone	Arshadi 2013
Scots pine	2.9%							Acetone	Sable 2012
Lodgepole	0.5-3.5%			13-20%		4.0%	10.0%	Hexane	Backlund 2014
Lodgepole (*latifola*)	2.0%	3.3%						Ethanol/ Toluene	Campbell 1990
Lodgepole (*murrayana*)	1.9%	2.8%						Ethanol/ Toluene	Campbell 1990
Lodgepole	0.23-2.6%							Non-polar	Arshadi 2013

Table 2-16 continued

Species	Sapwood	Heartwood	Knot	Bark	Roots	Branch	Needles	Solvent	Reference
Lodgepole	2.6%							Acetone	Sable 2012
Lodgepole			3%					Acetone/ Water	Willfor 2003c
larch (*decidua*)		15.0%	40.0%					Sequential	Kebbi-Benkeder 2015
larch	3.1-27.0%							Water	Grabner 2005b
larch	0.9-4.3%							Acetone	Grabner 2005b
larch (*decidua*)	3.5%	14.5%						Hot water	Keith 1988
larch (*decidua*)	3.8%	14.5%						Alcohol-benzene	Keith 1988
larch	0.6-4.4%	4-28.5%						Hot water	Grabner 2005a

3 Description of Extractives

Extractive compounds may be produced by trees for a number of reasons, for example as a response to stress at the base of the branch (Piispanen et al., 2008, Willfor et al., 2003c) or as a barrier against pathogen attack either chemically, by toxicity, or physically (Kirker et al., 2013, Kebbi-Benkeder et al., 2015).

A number of different hydrophilic phenolic and polyphenolic compounds are found in the tree species discussed in this report with each species having a different combination of the compounds. This section gives a very brief description of the main extractives found in the different species along with the chemical structure for comparison.

Lignans (Figure 3-1) are phenolic compounds found in large amounts in the knotwood, and in smaller amounts in other parts of the tree species studied. In Norway spruce the predominant lignan found in large amounts in the knotwood is hydroxymatairesinol; In Sitka spruce liovil and secoisolariciresinol predominate; Nortrachelogenin is the main lignan found in larch, Scots pine and lodgepole pine. Lignans have been found to have medicinal and pharmacological use for example hydroxymatairesinol has been found to have potential as antioxidants, anti-tumour and have potential for prevention of cancer (Saarinen et al., 2000), as well as having nutritional properties (Peterson et al., 2010), and it can also be used as a precursor to synthesise other lignans (Holmbom et al., 2003).

Stilbenoids (Figure 3-2 numbers 13 to 18) are stilbene derivatives which are also found in differing quantities in different parts of the trees investigated. Astringin and isorhapontin are the main stilbenoids found, along with piceid in Sitka spruce and Norway spruce bark; Scots pine stemwood and knots and also lodgepole pine knots contains pinosylvin and pinosylvin monomethyl ether. Stilbenes are known to have antioxidant properties, for example astringin, which is also found in red wine (Merillon et al., 1997) and also can be converted to other compounds e.g. piceid can be converted to resveratrol which has uses in food, cosmetics and medicine (Wang et al., 2007).

Flavonoids (Figure 3-2 numbers 19 to 31) are another phenolic substance found in trees which are known for their medicinal (Havsteen, 2002), antimicrobial (Rauha et al., 2000) as well as a number of other uses, such as nutritional supplements and adhesives as discussed in a review by Yazaki (2015). In Sitka spruce heartwood and knots the predominant flavonoid is dihydrokaempferol which is also found in larch stem wood and knots along with taxifolin. Scots pine and lodgepole pine knots have been found to contain pinocembrin and pinobanksin and lodgepole pine bark contains myrecetin.

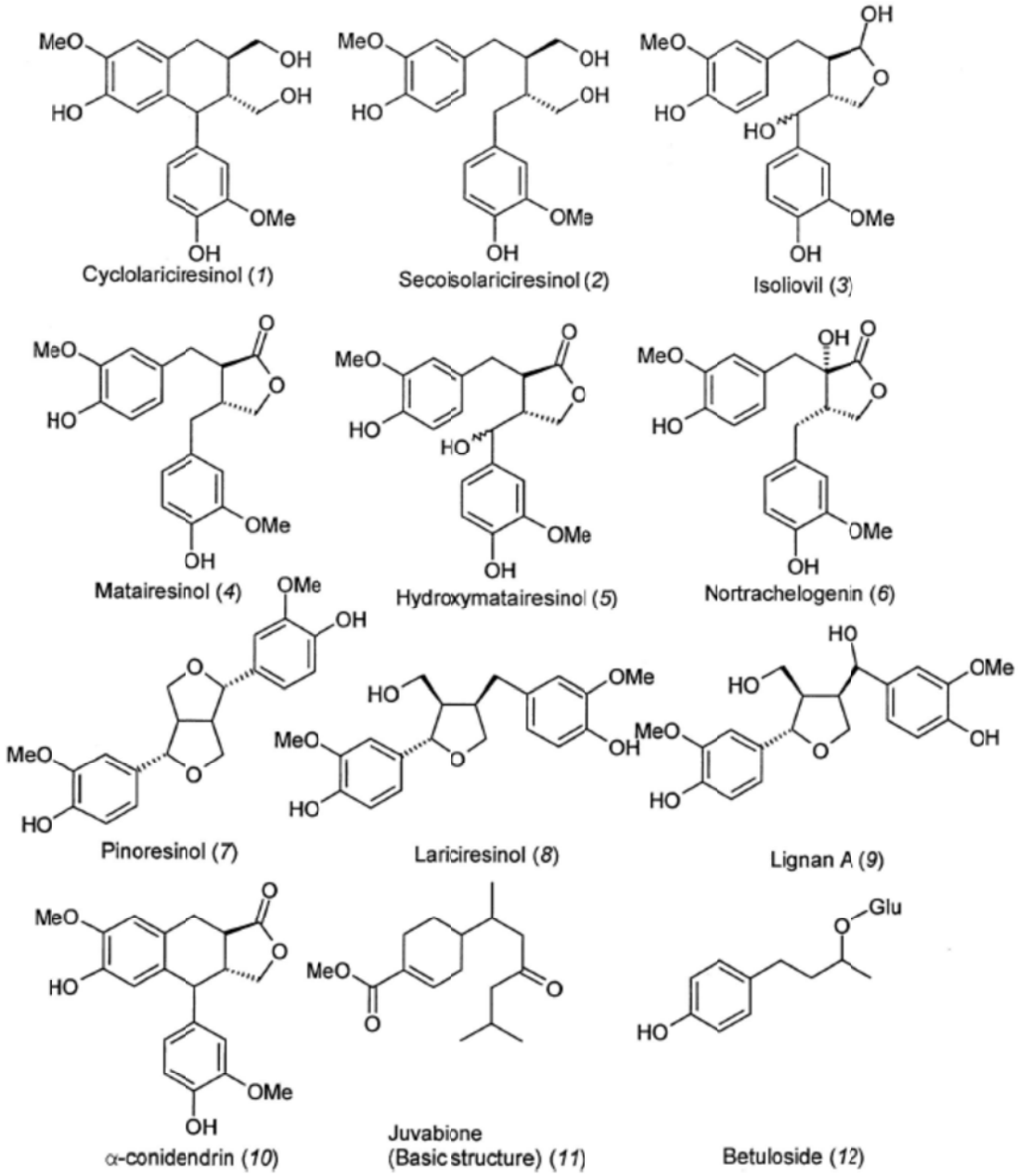

Figure 3-1: Structure of the main lignans found in tree extracts (Taken from Pietarinen et al. (2006))

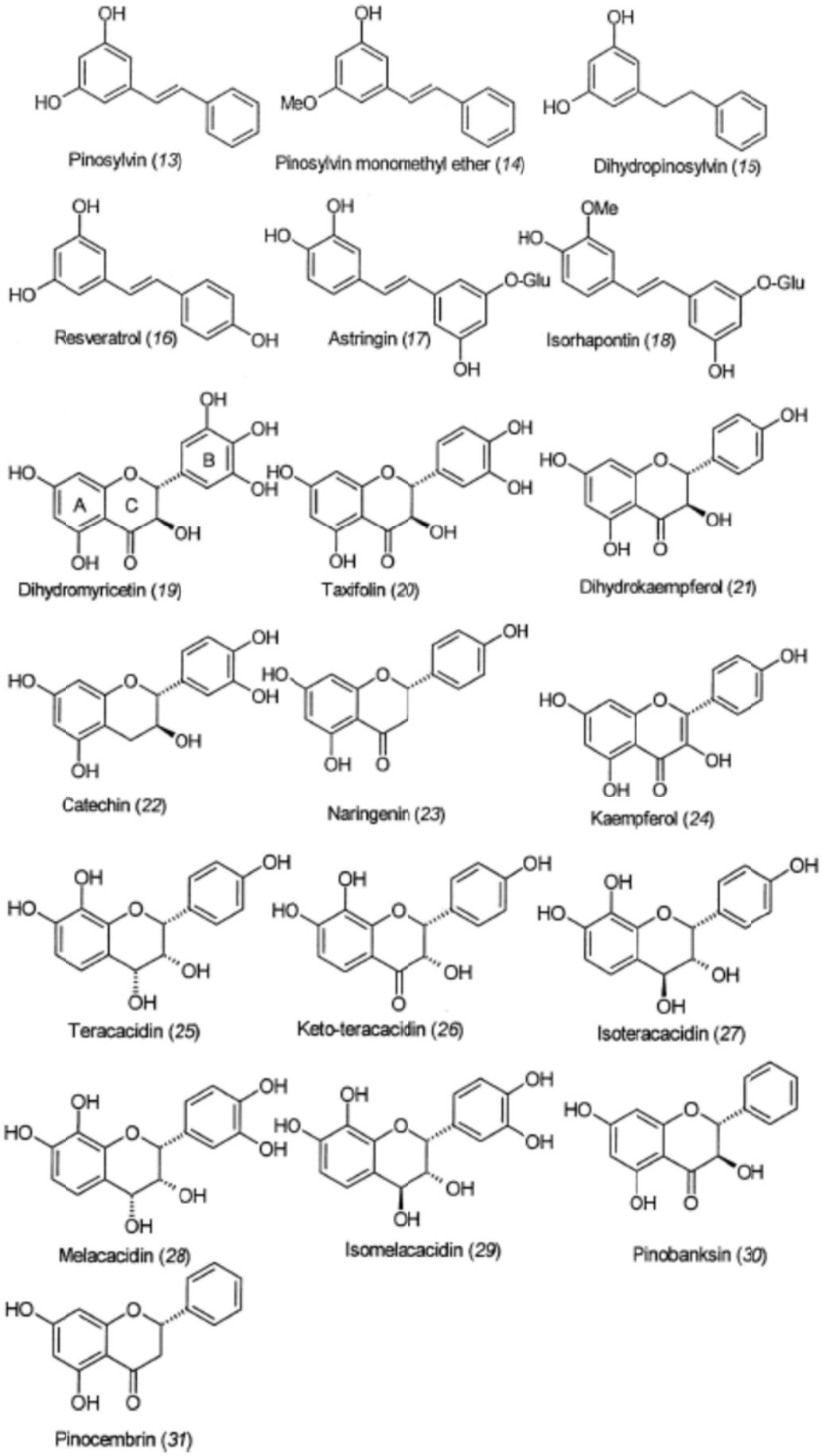

Figure 3-2: Structure of the stilbenes (13 to 18) and flavonoids (19 to 31) of tree extractives (Taken from Pietarinen et al. (2006))

Arabinogalactan is a water soluble branched polysaccharide based on arabinose and galactose monosaccharides found in high concentrations in the heartwood of larch. It is used in food processing as well as in pharmaceuticals and cosmetics and could be a valuable extractive due to its clinical and medicinal uses (Riede et al., 2013, Kelly, 1999).

Other compounds found in the tree species include terpenes, such as myrcene (7-Methyl-3-methylene-1,6-octadiene) which is a monoterpene found in Sitka spruce needles and potentially could be used as a renewable biological feedstock replacing hydrocarbons currently obtained from oil (Behr and Johnen, 2009).

Resin acids are found in various parts of the trees with quantity found depending where in the tree and which species of tree. These are carboxylic acids and most have a similar structure based around the same skeleton (Figure 3-3).

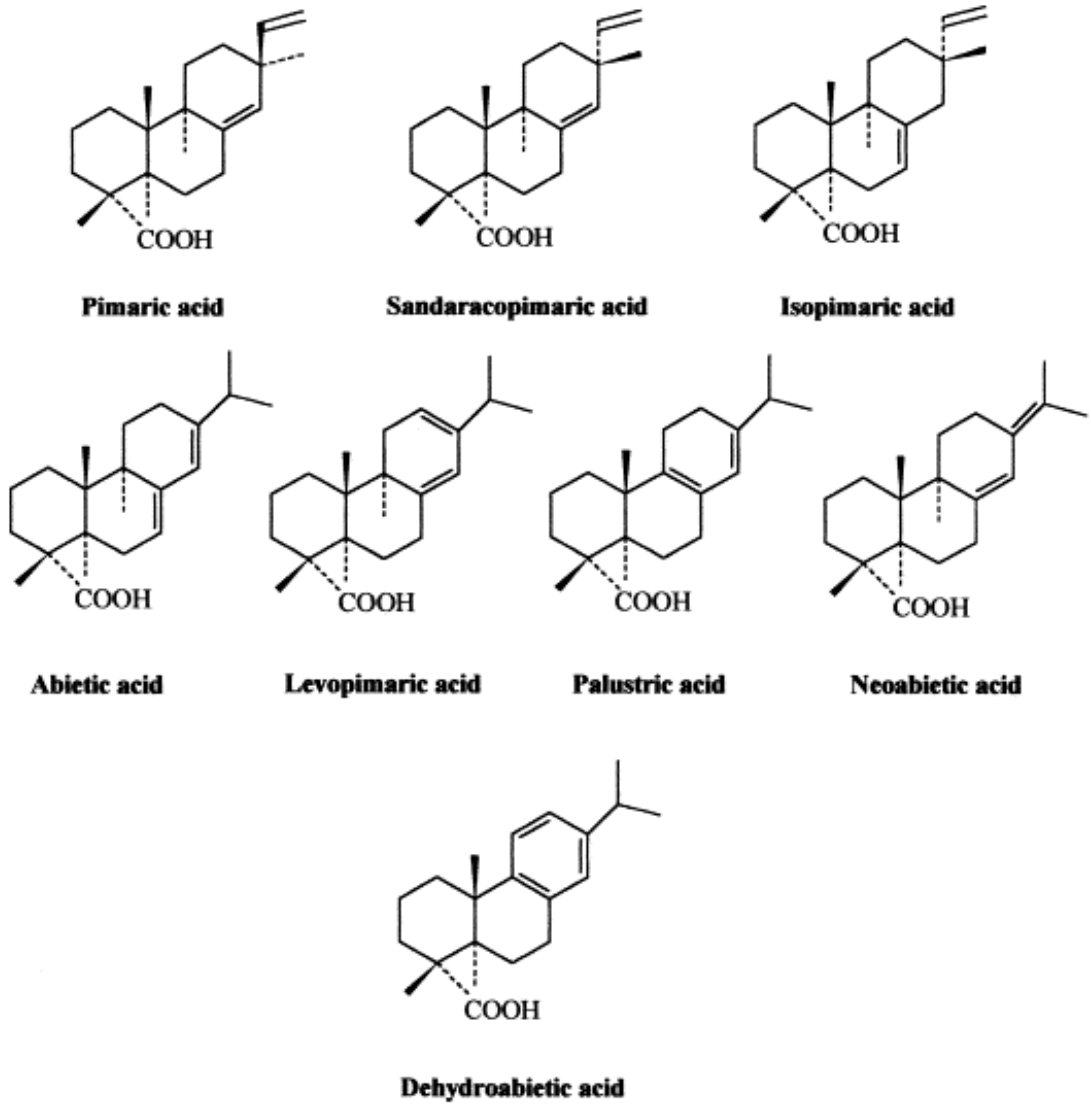

Figure 3-3: The chemical structure of the main resin acids found in the extractives of the tree species investigated. Taken from Peng and Roberts (2000)

Fatty acids, which are also found in varying quantities in different parts of the tree, are long chain carboxylic acids and are found both unsaturated and saturated. The chemical formula for these are shown in

Table 3-1: The chemical formula for the main fatty acids found in the tree species investigated

Unsaturated	**Formula**
Oleic	$C_{18}H_{34}O_2$
Pinoleic	$C_{18}H_{30}O_2$
Linoleic	$C_{18}H_{32}O_2$
Linolenic	$C_{18}H_{32}O_2$
Saturated	
Palmitic	$C_{16}H_{32}O_2$
Stearic	$C_{18}H_{36}O_2$

4 Discussion and Conclusion

This study has found that trees can be a large and valuable source of extractives. All of the species looked at contain different amounts of extractives, the content of the extractives is different for each species and where they are found within the tree are different between species.

Sitka spruce heartwood and sapwood is generally low in extractives which may be one of the reasons that Sitka spruce has been a species used in paper mills. However the stem wood still contains some extractives such as lignans and flavonoids, and the bark (which is where the highest amount of extractives is found in Sitka) contains other extractives such as astringin, isorhapontin and piceid. As well as this, monoterpenes such as myrcene and limonene are found in the needles. Although use of the extractives may give an opportunity to develop what has traditionally been waste into a usable product Sitka bark is now also used as bedding in horticulture and landscaping so any use may impact upon an already established bark market.

The extractives in the stemwood of Sitka spruce is similar in composition to the extractives of Norway spruce stemwood, however the knotwood composition is different between the two. Norway spruce has high concentrations of lignans, especially hydroxymatairesinol and while Sitka spruce also contains lignans these are predominantly liovil. Both Smeds et al. (2012) and Willfor et al. (2003a) quote Hakkila (1998) as saying that approximately 1% of the volume of a Norway spruce stems consists of knotwood and so there is a potential to produce extractives on an industrial scale. Knots also have little value and in fact could have a detrimental effect when manufacturing pulp at paper mills and it could be preferable if they could be separated before pulping (Holmbom et al., 2003). Knots are also seen as a waste product in many industrial processes such as structural timber and finger jointing, for example in cladding, where the knots are cut out of the material and disposed of.

Work has been done developing methods of separating the knotwood from the stem wood such as the ChipSep process patented by Eckerman and Holmbom (2004) where wood chips are ground, dried then separated in water where knotwood material sediments and stemwood material floats. Using this method Holmbom et al. (2003) estimates that 8 tons of knot could be produced from a mill using 1000 tons of Norway spruce a day which in turn could produce approximately 360 kg/day or 130 tons per year of hydroxymatairesinol. Liovil could also be extracted from Sitka on a smaller scale (Willfor et al., 2004a), however although Scots pine knots can contain a lot of lignans, mostly in the form of nortracheloginin, the main extractives are resin acids which make it more difficult to extract the lignans (Holmbom et al., 2003).

The stump and roots are usually left behind in the forest as waste when the timber is removed and this could also be a potential source of extractive compounds. However there

has not been a lot of work done into the extractive content of these especially with regards to Sitka spruce. Caron et al. (2013) found that rootwood of Sitka contained approximately 11.5% of extractives which is about ten times higher than that found in the stemwood however this may be due to the rootbark and rootwood being analysed together. In Norway spruce where the root bark and root wood have been measured separately the bark of the larger roots contained up to almost 20% extractives while the wood contained approximately 2% (Latva-Maenpaa et al., 2013) which is slightly higher than that found in the stemwood (Willfor et al., 2004a).

Scots pine and Lodgepole pine have both a similar amount and content of extractives. The main component of the pine knots are resin acids and other terpenoids. Scots pine stems also have slightly higher resin acid and fatty acid content than lodgepole pine but this is balanced out by the fact that lodgepole pine is faster growing : heartwood proportion is greater for wood the same age and stem volume is approx. 30% more (Backlund et al., 2014). Arshadi et al. (2013) also found that there was a difference between concentrations in heartwood and sapwood for both species, with Scots pine heartwood being between 5 and 2.5 times higher than sapwood. For lodgepole pine the heartwood was 3.4 to 1.2 times higher. They suggest that the amount of heartwood is therefore the most important thing when quantifying the amount of extractives in pine and that in an industrial sense it may be better to separate the two to get the best out of each part. They estimate that 100kg of dry material from breast height of scots pine will provide 0.1 kg of fatty acids and 1kg of resins, and for lodgepole pine the figures would be 0.1kg and 0.7kg respectively. However this difference would be negated by lodgepole pine being faster growing (36% higher yield for trees grown on the same site (Elfving et al., 2001)) and therefore having more biomass (Arshadi et al., 2013). Although within the stems of Scots pine most of the extractive content is found in the heartwood there is a great deal of variation in the size of the heartwood even between trees of similar diameter on the same site (Bjorklund, 1999). This not only makes it difficult to quantify the amount of extractives that could be removed but it also makes it virtually impossible to visually identify trees could or stands of trees which could potentially have higher yields.

The main extractive in larch is arabinogalactan. Although this is a hemicellulose it is seen as an extractive as it is found deposited in the tracheid lumen rather than as a structural component of the cell wall. This is found in large amounts, is relatively easy to extract using hot water and has been found to have medicinal, pharmacological and nutritional uses amongst others. Larch is potentially a very large and valuable source of this extractive. However, while there may currently be a lot of larch potentially available this may not be the case in the future as no new larch is being planted due to the outbreak of *Phytophthora ramorum*. This disease also means that the only market for larch bark at the moment is for burning as biomass and this is only under special licence (Hogan, 2013), though no studies on extractive content of larch bark could be found.

5 Future Research

Stump and root system of Sitka would probably be the biggest source of woody material but little research has been done on what is where in them. In Finnish spruce forests it is estimated that the mass of the stump/root system can be 23-25% of the stem biomass (Hakkila, 2004). Currently the stump and roots are not harvested and along with the crown (including stem, branches and needles) are left at the site as residues. This could be a huge resource though work would have to be done to quantify the amount of raw material and investigate fully the amount and type of extractives found.

Branches are also a potential source of extractives which has had little research. Like the stump, branches are also mostly left in the forest after felling and could be a useful resource along with the needles. This could also be another product for the forest industry that has often seen this as waste.

One other area in which there was very little research found was on the extractives in the bark of any larch varieties. However there has recently been problems with *Phytophthora ramorum* infecting and killing large amounts of larch, which although this means that there is potentially a lot of larch on the market would make it difficult to study due to the restrictions on the movement. It also means that larch is not being re-planted so may not be a viable resource in the future.

This report has shown that even within a species there is a lot of variation in the quantity of extractives found. However there have been few studies which have investigated the site to site variability to quantify how much the extractive content varies within a species and to investigate the factors which cause the variation.

Other questions that have arisen from this report include:

What is the potential value and uses of the extractives and what are the potential markets?

What is the availability of the tree parts where useful extractives are present and what would be the cost of extracting these products?

What is the preferred extraction system to obtain extractives, are these systems scalable?

Is extraction financially viable?

As a final suggestion for future research, it was thought that some sort of online database which mapped the extractives through the different parts of the tree for each species, which could be searched though the type of extractive or by tree part. This could be added to as new research was completed or if new species were added to the list and would help not only future research but also be of use to anyone within the biorefinery industry who wanted to utilise trees as a source of extractives.

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7 Appendix

The following diagrams which were developed by IBioIC, show a graphical representation of the information supplied within this report to help easily identify the chemicals available in each part of the tree and where possible the quantity available.

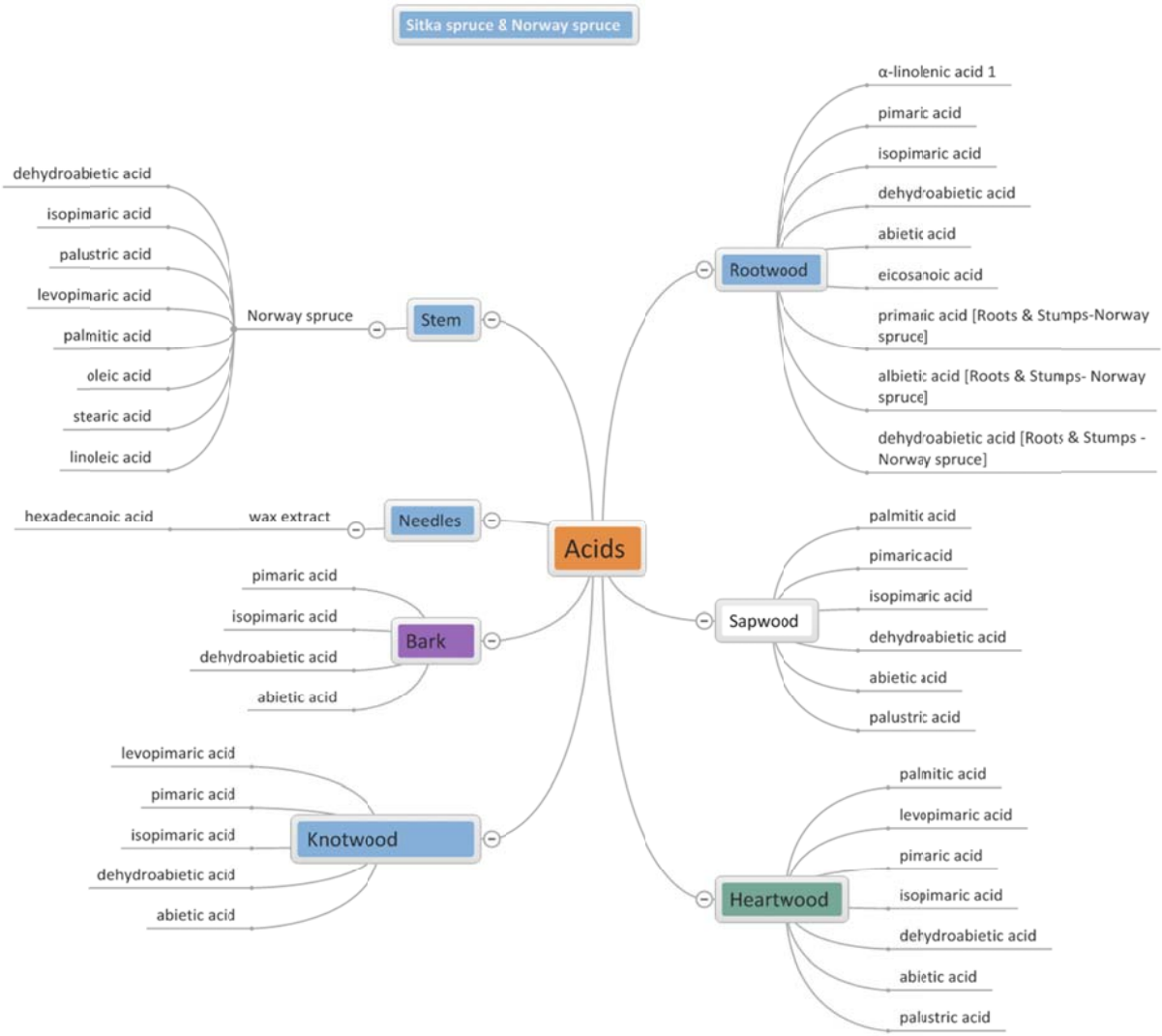

Figure 7-1: Resin Acids in Sitka spruce and Norway spruce

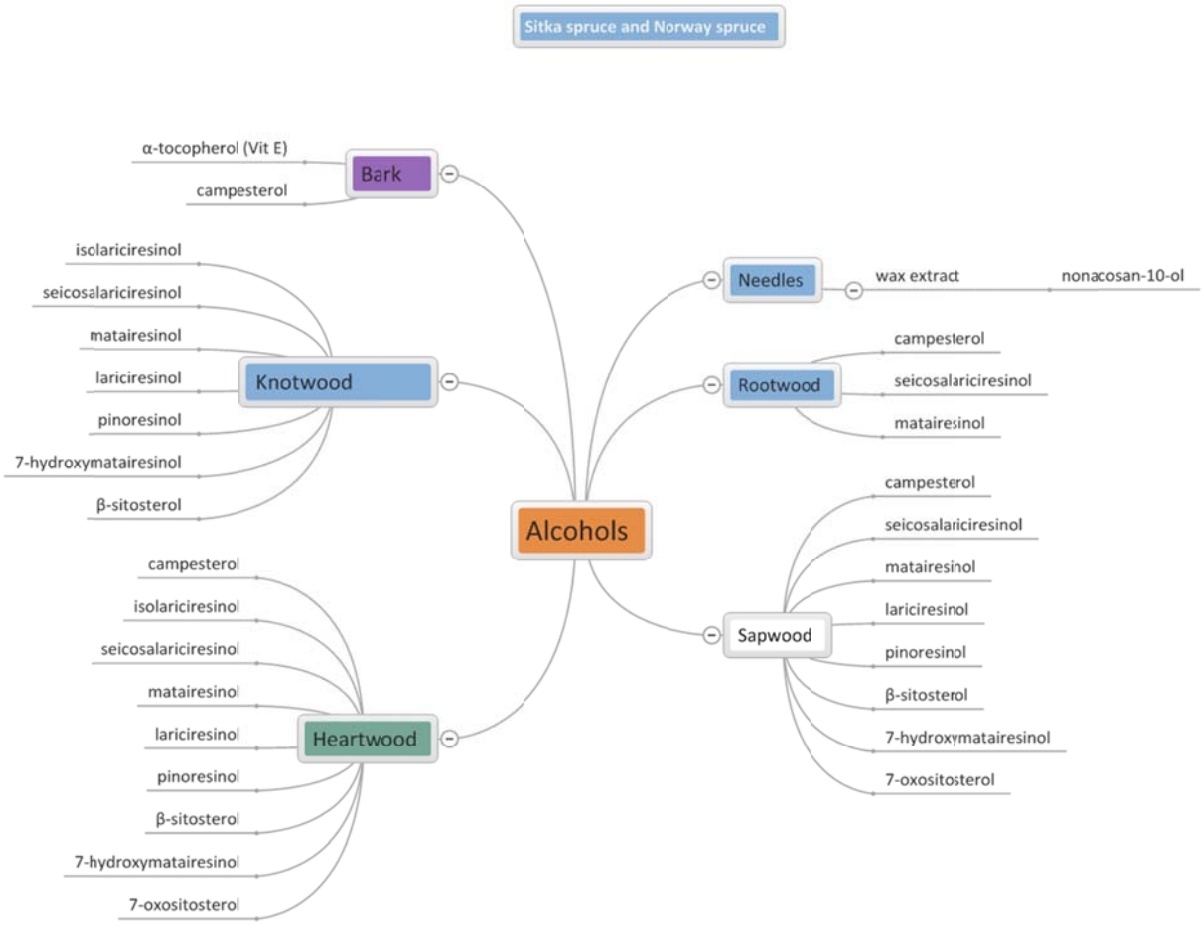

Figure 7-2: Alcohols in Sitka spruce and Norway spruce

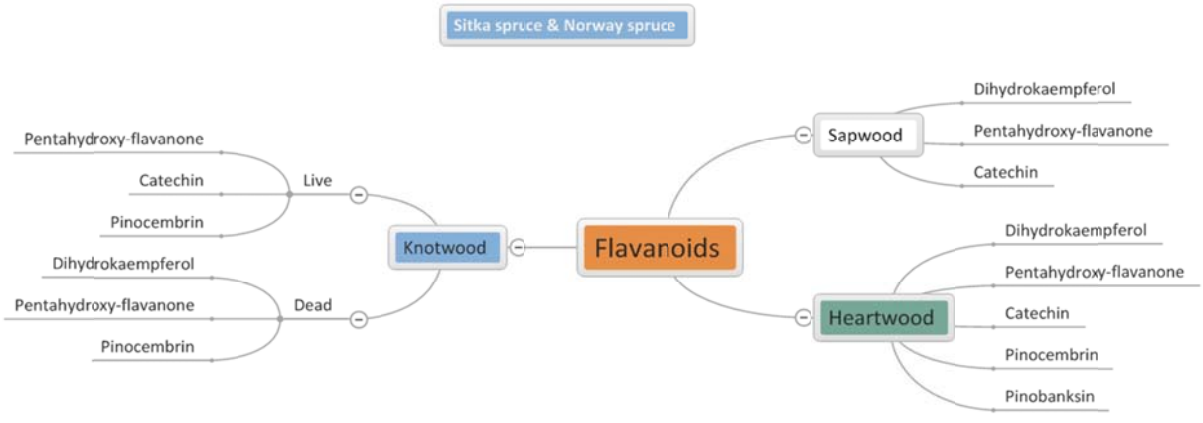

Figure 7-3: Flavonoids in Sitka spruce and Norway spruce

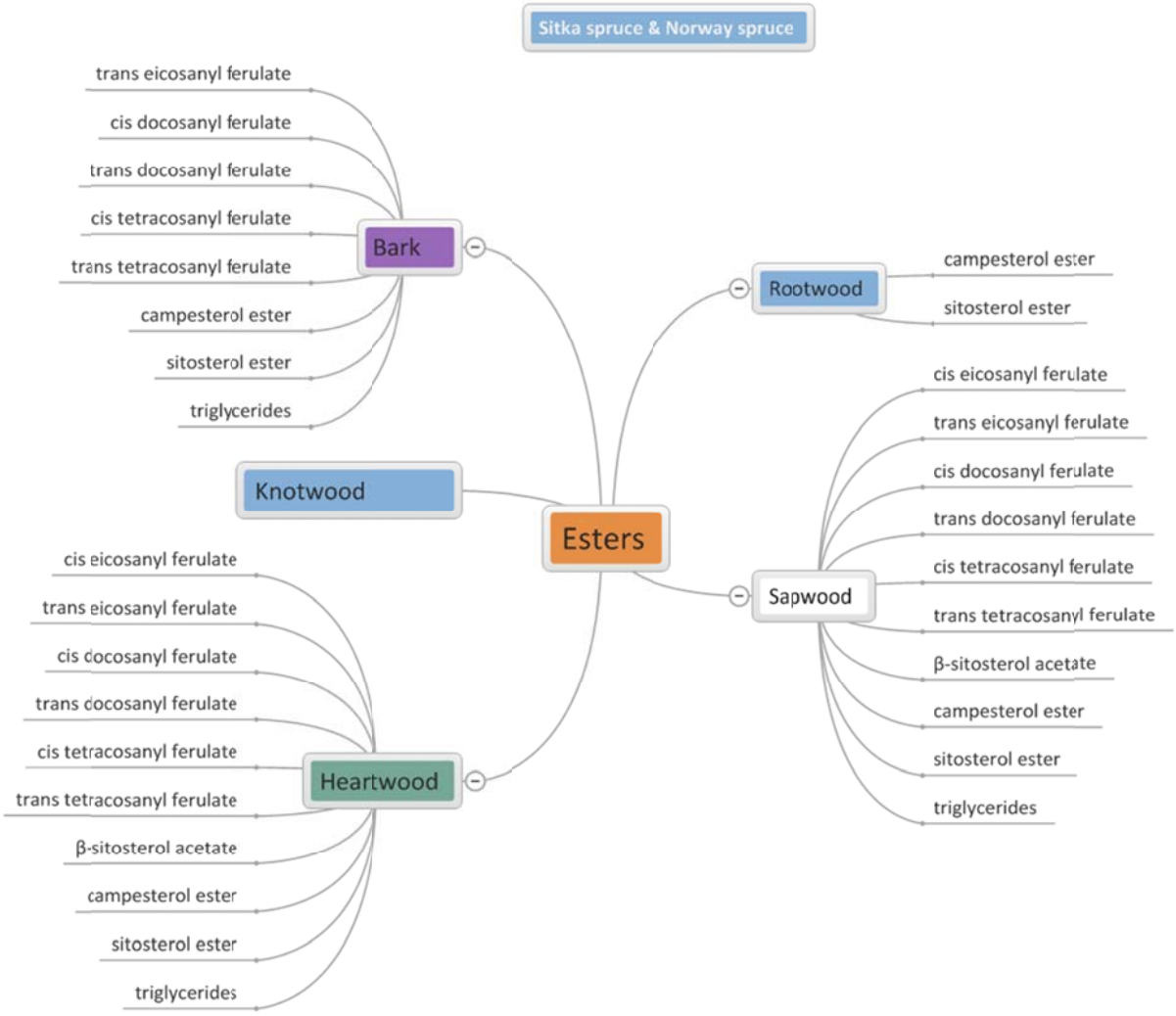

Figure 7-4: Esters in Sitka spruce and Norway spruce

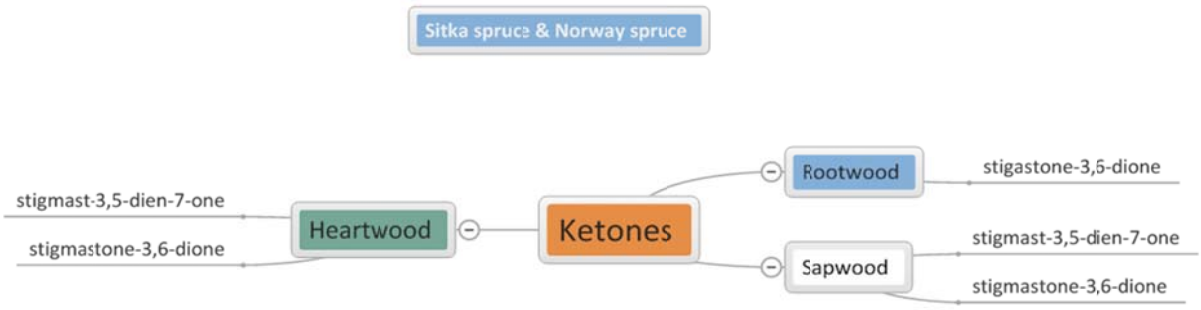

Figure 7-5: Ketones in Sitka spruce and Norway spruce

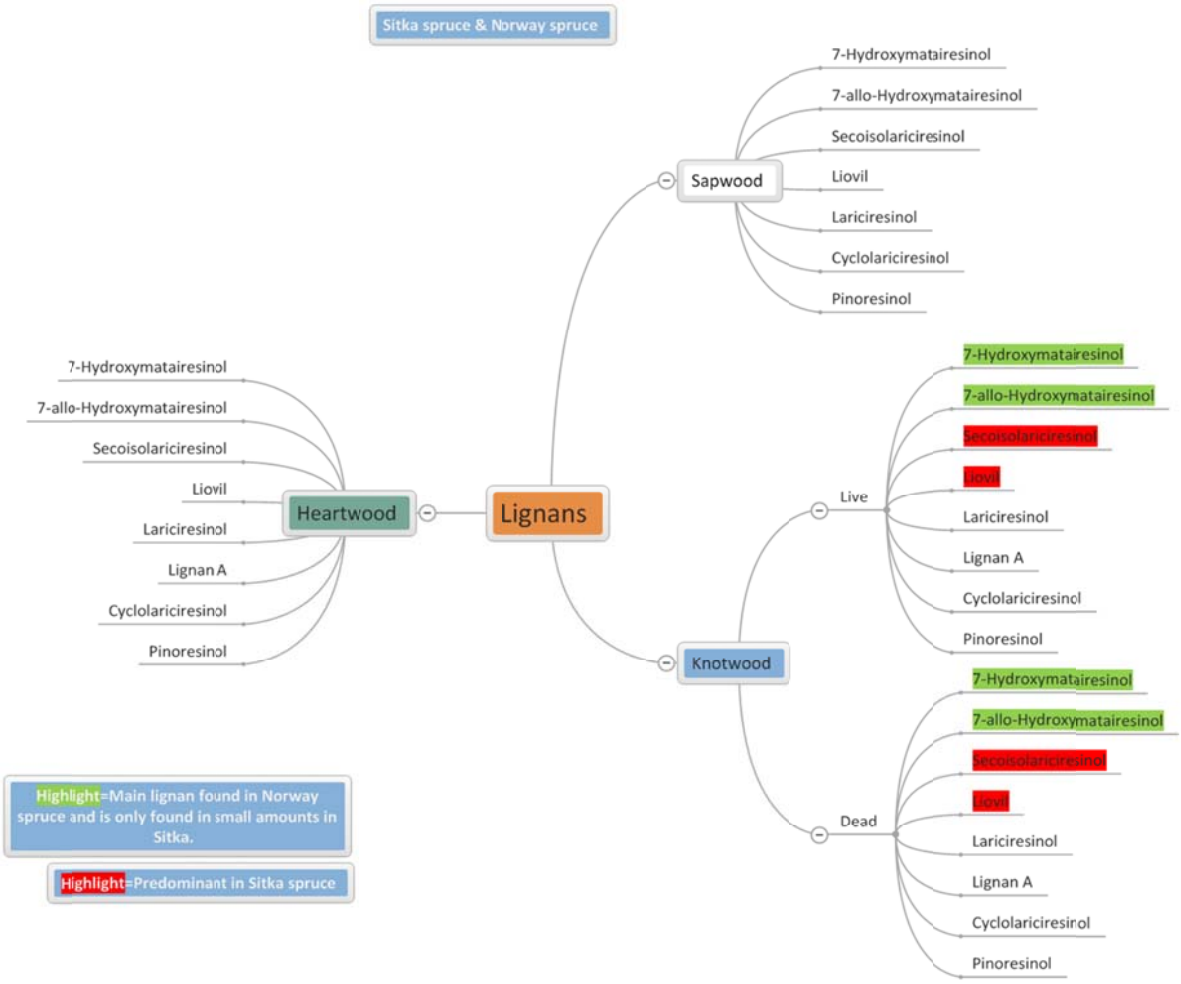

Figure 7-6: Lignans in Sitka spruce and Norway spruce

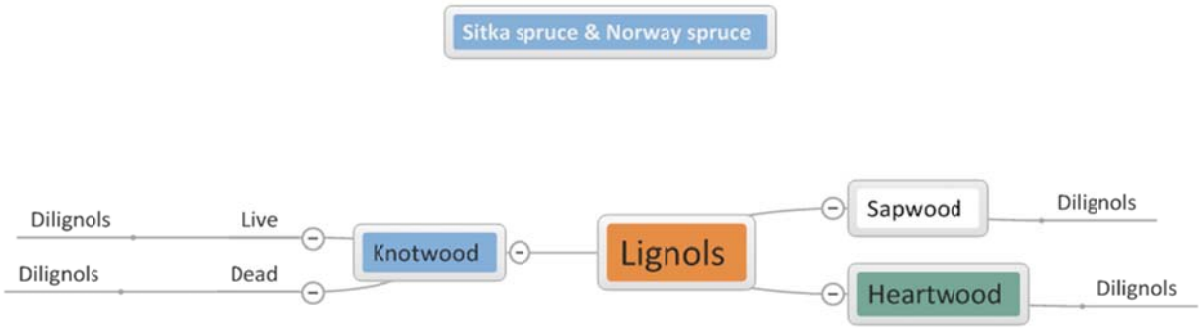

Figure 7-7: Lignols in Sitka spruce and Norway spruce

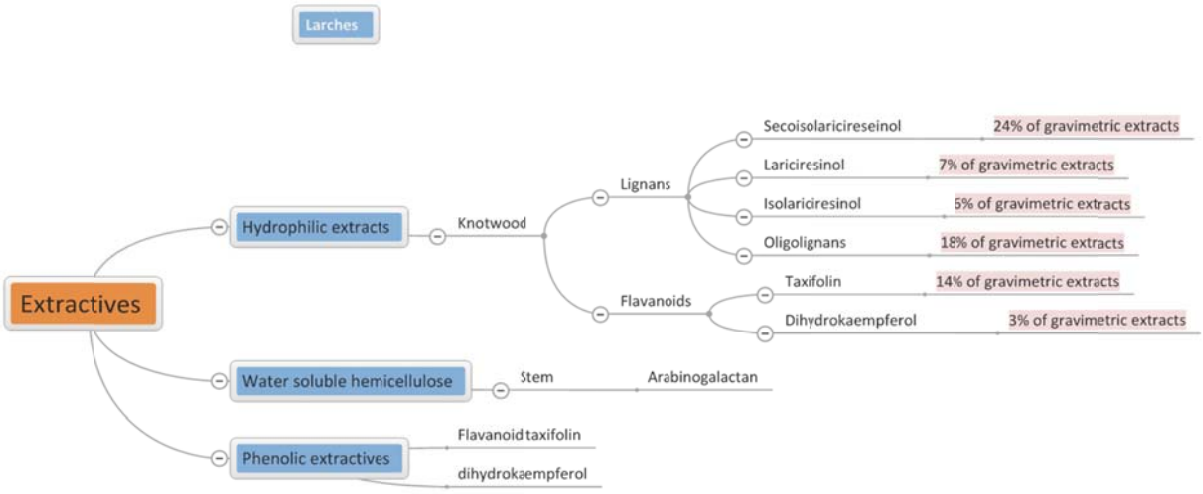

Figure 7-8: Extractives in larches

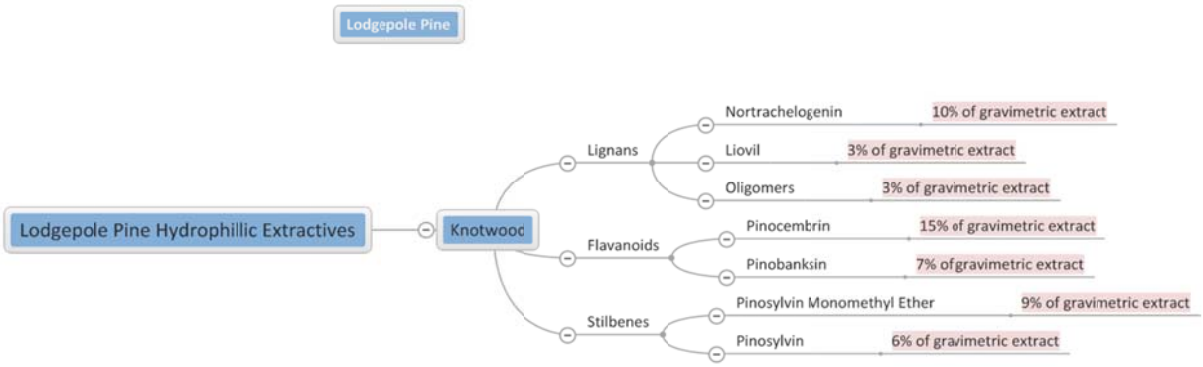

Figure 7-9: Hydrophilic extractives in lodgepole pine

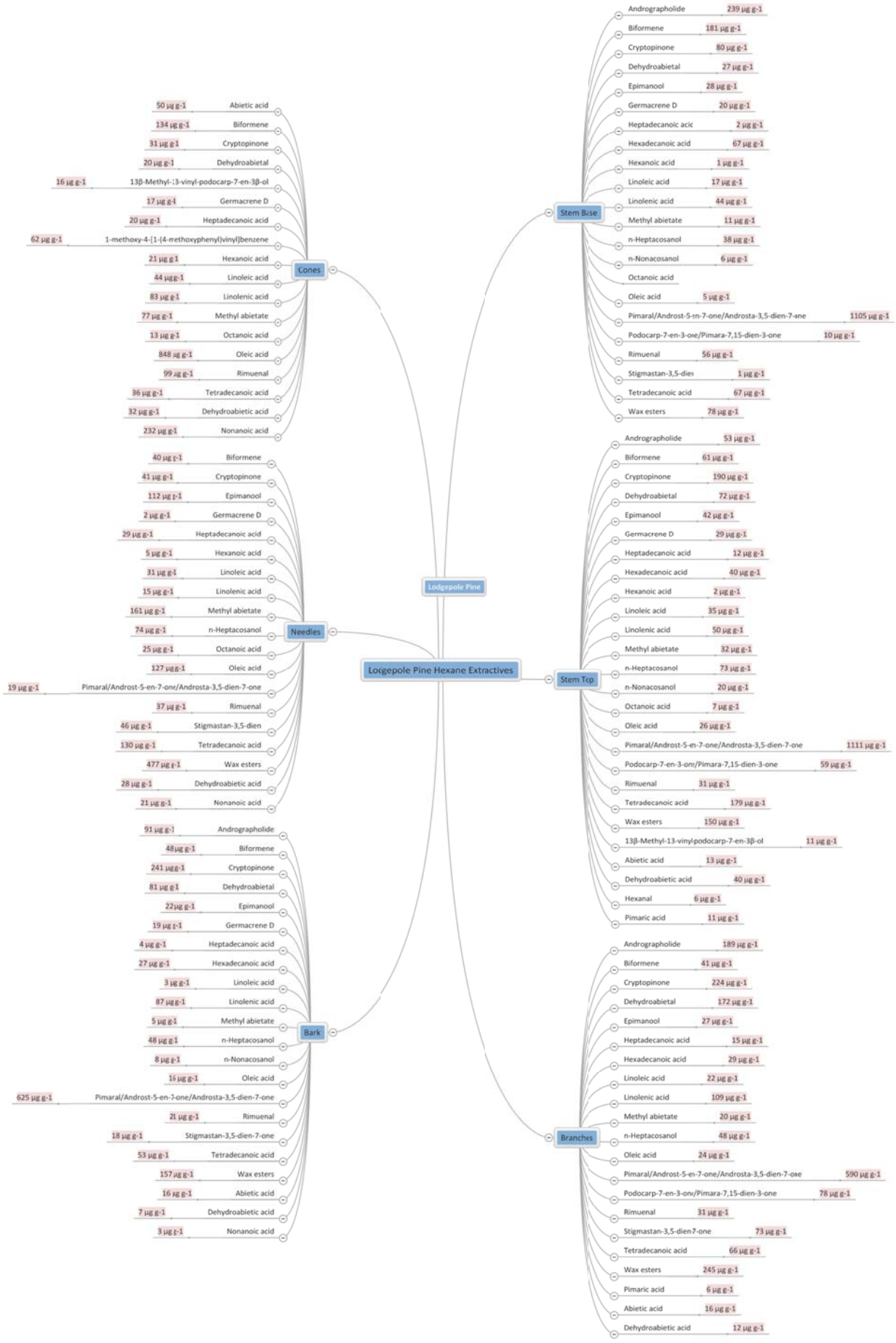

Figure 7-10: Hexane extractives in lodgepole pine

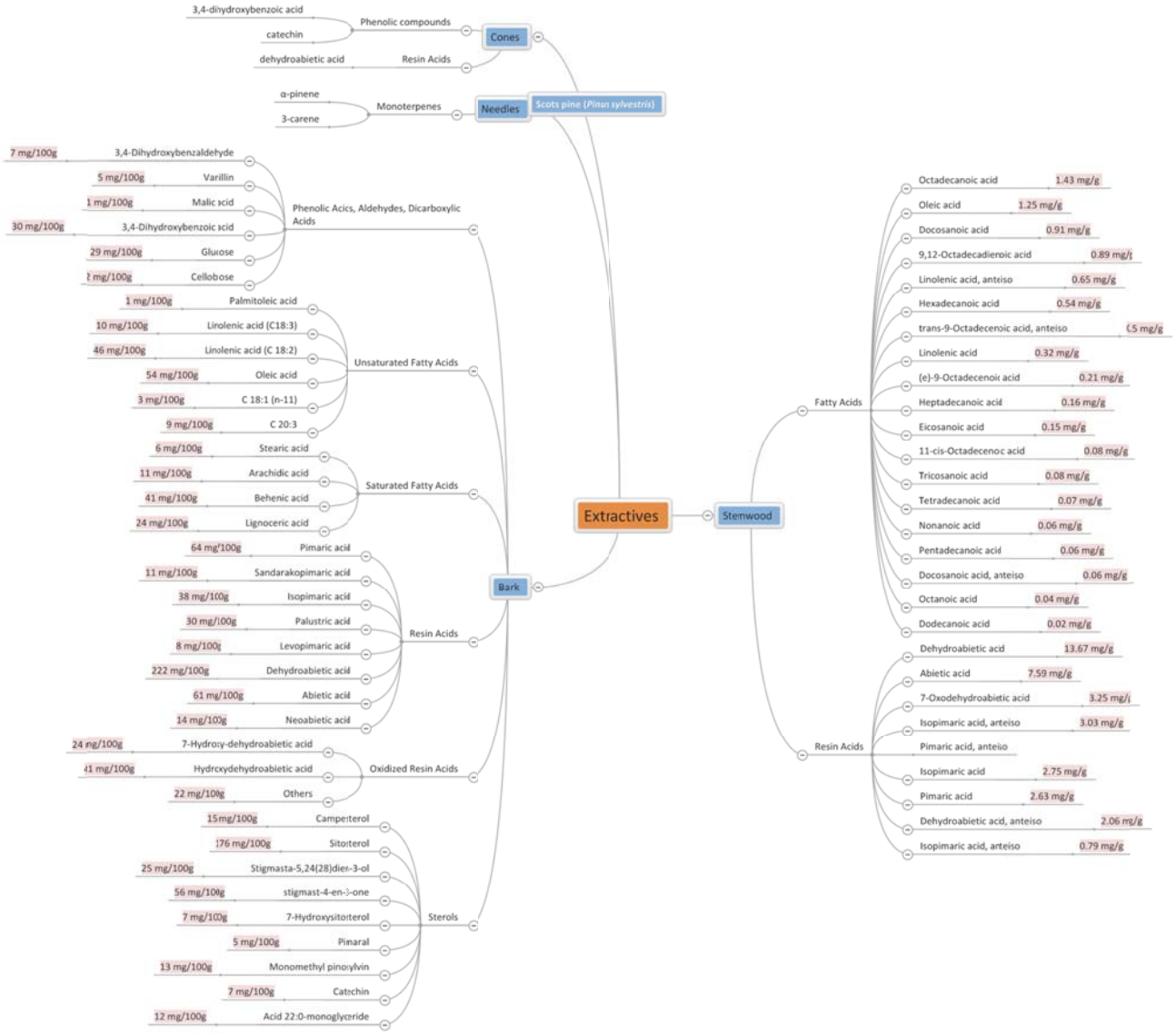

Figure 7-11: Extractives in Scots pine