Saimaa University of Applied Sciences
Unit of Technology, Imatra
Degree Programme in Paper Technology

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Extraction of Scots Pine with Non-polar solvents

Bachelor Thesis 2011

ABSTRACT

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Saimaa University of Applied Sciences, Imatra
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The purpose of this paper was to provide a general view of chemical distribution in the wood and the method for isolated extractives from woods; And Finnish forests' situation were also studied, covering themes included from forest resources distribution to extraction process and its products.

A research of the isolation of extractives for Scots pine with non-polar solvents, such as hexane, toluene and diethyl ether, was described. The experiment was carried out in Saimaa University of Applied Sciences, Imatra, Finland. Chemical compounds were extracted through isolation of pine stem chip, pine stump bark and pine sawdust . Identification of the extracted compounds was carried out by gas chromatography .

The aim of the experiment was to investigate the different effect of solvents and raw materials, extraction time, extraction equipment, temperature on the extractives from pine wood. The various species of extractive were mainly resulted from different solvents and various raw materials. On the other hand, the extraction time and extraction temperature shows minor influence on extraction process.

Keyword: Finnish Forest, Isolation, Wood Structure, Extractives, Non-polar solvent, GC analysis

CONTENTS

1 INTRODUCTION

The extractives are usually comprised 1%-5% of the wood and it is under genetic control with various species of tree. The extractives normally have relative small molecules ($<C_{40}$). In the literature study, a brief introduction was given for wood as raw material and wood-based chemistry. Furthermore, the certain type of extractives, i.e. terpenoids, was reviewed. Terpenoids, as one type of lipid extractive components, mainly existed in the softwood species. The chemical structures are different with diverse types of terpenoids. Moreover, various extractives were learned according to different tree species, e.g. pine, spruce, birch and aspen. The various applications of extractives are also introduced.

Some types of extractives can be dissolved in non-polar solvent. An attempt was made to isolate the extractives from wood, where pine root bark, pine stem chips and pine sawdust were used as raw materials. Non-polar solvents, e.g. toluene, hexane, and diethyl ether, were used in the extraction. The apparatus used in the experiments were steel-based rotation reactor and flask reactor with condenser. The different operating conditions were studied in the extraction of wood. Afterwards, the extraction solution from wood was analyzed with gas chromatography method in order to know the basic profile of extractives in wood and also the effect of operating condition on these profiles. In the experimental results the chemical compounds, which were extracted from pine, were discussed based on the raw materials, temperature and solvent.

2 WOOD RESOURCES IN FINLAND

Finland has widely distributed forest resources and the Finns have a developed and successful dependency on that to earn benefits. In Finland, the total land area is about 30.42 million m^3 including 22.82 million m^3 forest areas. Almost 300 million m^3 tree seeds are planted in the Finnish forests in each year. Annually the forest growth is 99.5 million metric tons and the production of logging wood is around 41.4 million metric tons per year. For the use of wood, 6 million metric tons of wood is taken as firewood. 31 million metric tons of round wood is contributed to pulp and paper industry and 21 million metric tons round is used in the timber product industry. The annual flow sheet for Finnish woods is displayed in the Figure 2.1. (Finnish Forest Research Institute 2010.)

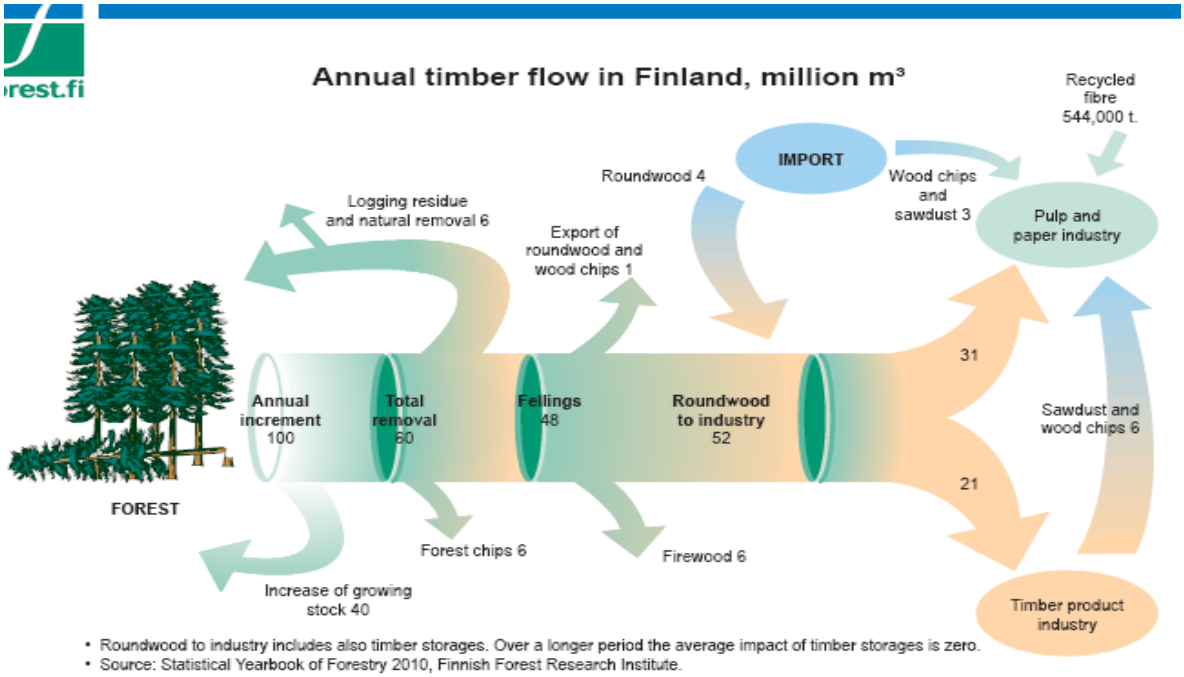


Figure 2.1 Delineate of commercial usage for timber in Finland per year million per m^3 (Finnish Forest Research Institute 2010.)

Diverse woods distributed in the various areas according to the temperature

and the soil condition in Finland. Harvested pine tree shared more than 50% of total land, while spruce tree occupied about 25% of land in Finland. There is just 10% land in Finland occupied by birch and other broadleaves trees. Total amounts of woods in various species and occupied percentages of the assorted species woods in Finland can be seen in Figure 2.2. (Finnish Forest Research Institute 2010.)

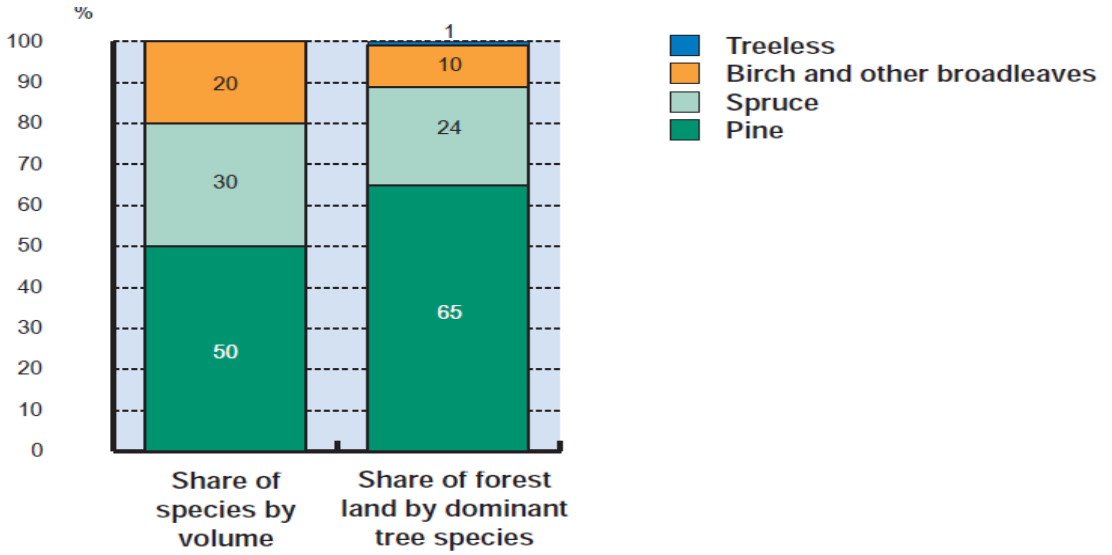


Figure 2.2 Total amounts of woods in various species and occupied percentages of the assorted species woods in Finland. (Finnish Forest Research Institute 2010.)

3 STRUCTURES OF WOOD

Structure of tree

In general, different tree species have similar exterior appearance structure but various chemical compounds inside. A full-grown tree makes up with 4 parts, i.e. foliage, branch, trunk, roots. By synthesis of carbon dioxide, water and trace elements, the complex, self-organized structure of tree is formed. The different parts of tree have different effective functions. The leaves of one tree has the

function of converting sun's energy into wood through breathing in carbon dioxide and exhaling oxygen through the pores on the leaves, and the pores also take in water vapor and allow excess moisture to evaporate. The leaves are held by branches which also send chemical energy back to the trunk for root growth in return. Nutrition for the tree absorbing by the roots of a tree is transported to branches by trunk. The structure for a tree is shown in the Figure 3.1. (Mish1828, 452.)

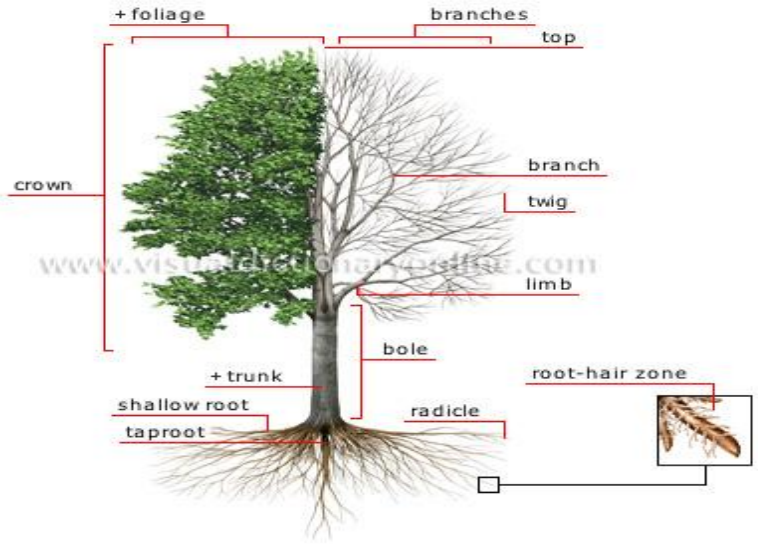


Figure 3.1 The segmentation of tree structures (Mish 1828,452.)

Sructure of truck

As for the structure of trunk, there are seven parts in the trunk of one tree. The sections of tree are shown in the Figure 3.2 (Mish 1828). From outside to inside of tree, the first part is the bark and the function of the surface of the trunk bark is to protect the tree itself, and to prevent disease invasion of the softer inner part. The water loss through evaporation can also be limited by outer bark. The second sector of trunk is the phloem which is the fibrous tissue transporting sugar from the leaves to the roots. The third part of a trunk is cambium, which produces phloem and internal sapwood enabling trees to grow in its diameters. The fourth part is the sapwood, light-colored zone around the heart wood.

Sapwood is composed of water, living parenchyma tissue, active xylem tissue (fat and starch). The nutrient of water and minerals are transported by sapwood from roots to the leaves. The fifth sector is annual ring; the age of a tree can be accounted by the number of rings. Sixth part is the heartwood with dark-colored zone around the center of stem. The pitch located in the center of the trunk contains nutrients essential for growth of tree. (Sjöström 1993, 90; Wiedenhoeft 2005, 3)

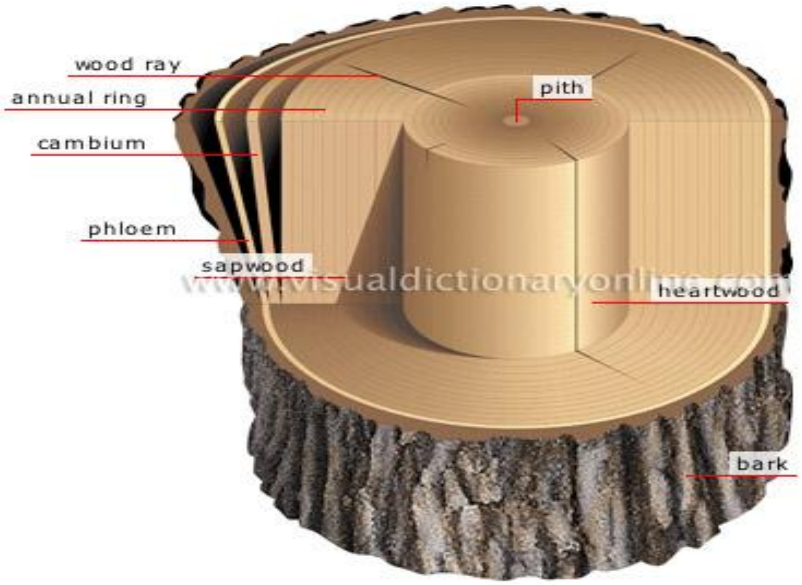


Figure 3.2 Cross section of the truck (Boudet 1998.)

Structure of wood cells

There are two main primary components in a single plant cell which are the protoplast and the cell wall. Protoplast consists of all the living contents bound by the cell membrane. The main function of plant cell wall is to provide mechanical support to the plant. The cell wall is an important structure in wood consisting of three main regions which are the middle lamella, the primary wall and the secondary wall. The different layers of the cell wall are shown in the Figure 3.3. It starts with the middle lamella (ML). Then the next layer is the primary wall (P). Interior to the primary wall is the secondary wall in its three

layers: S1, S2, and S3. The bordered pits are illustrated in the lower portion in both sectional and face view. The four domains of the pit are shown in terms of the pit aperture (pa), the pit chamber (pc), the pit membrane (pm) and the border (b). (Wiedenhoeft and Miller 2005, 3-2)

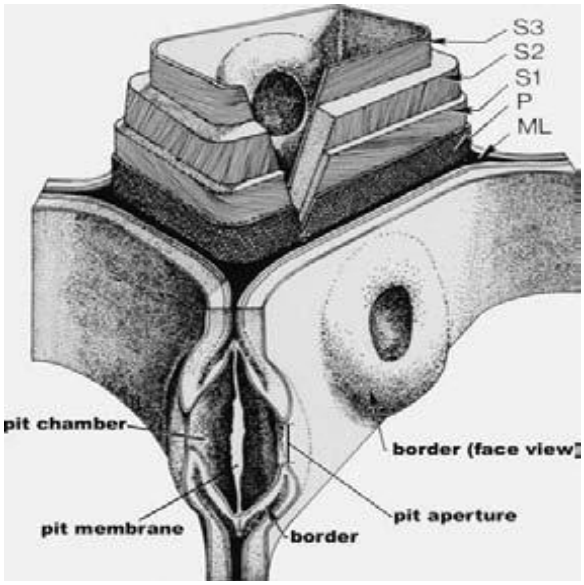


Figure 3.3 Specific drawing of cell wall included the structural details of a bordered pit. (Wiedenhoeft 2005,3-2)

Hardwood and Softwood

Tree is defined as perennial plant which is classified into two categories which are hardwoods and softwoods. Hardwood is mostly broadleaf and high density, such as aspen and birch. Softwood is mostly needle-leaved evergreen trees and low density, such as pine, spruce. Except the difference in the aspect of the types of trees from which they derive, they are also different in the aspect of their component cells. The hardwoods have a vessel element as shown in the Figure 3.4 whereas the softwoods lack these elements. The function of vessel elements is water transportation in the hardwood tree; water transport is only through tracheids within the tree in softwood. There are two types of cells: longitudinal wood fibers and transverse ray cells described in Figure 3.5 . (Wiedenhoeft 2005, 3-2)

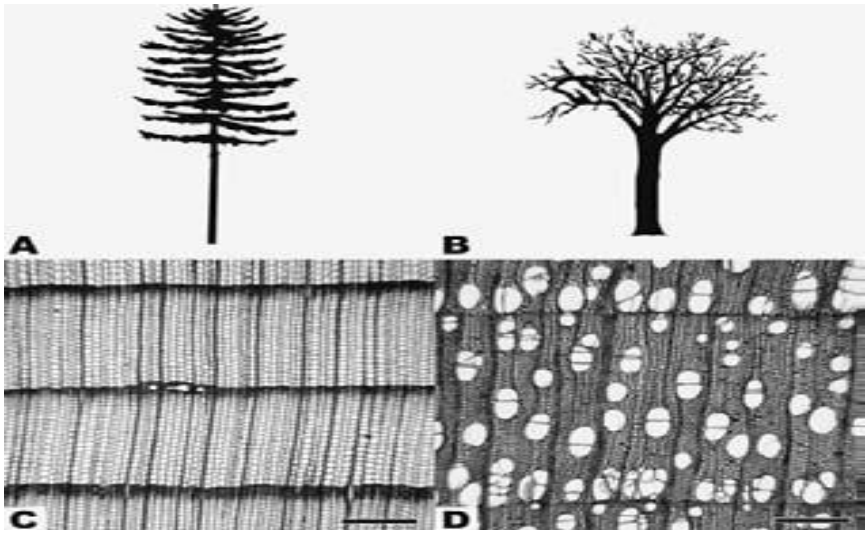
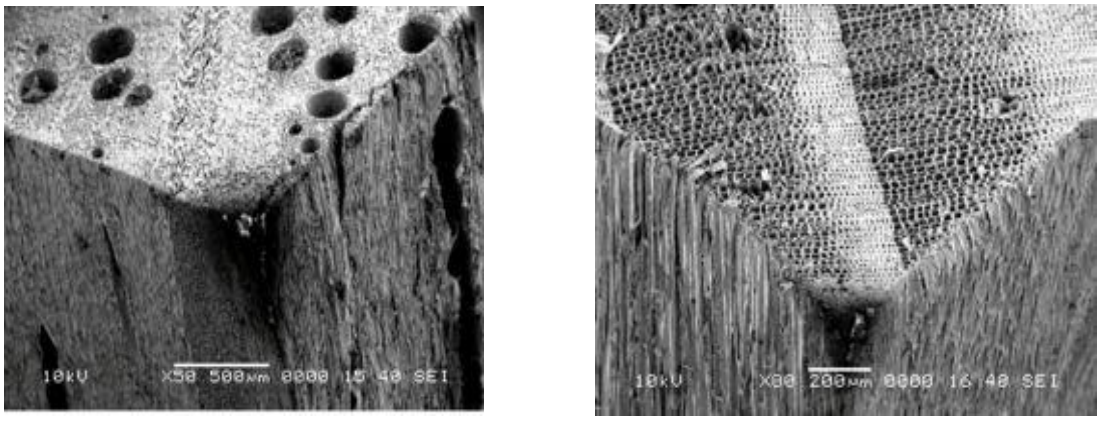


Figure 3.4 General forms of softwood and hardwood tree and the transverse section of typical softwood and hardwood.(Wiedenhoeft 2005,3-2)



Hardwood with pores Softwood without pores

Figure 3.5 Scientific Stock Photography images show comparison between softwood and hardwood under observation of microscope. (Wiedenhoeft 2005,3-2)

4 CHEMISTRY OF WOOD

4.1 Compounds in wood

There are four types of chemical compounds in the wood which are cellulose,

hemicelluloses, lignin and extractives. Lignin is a crucial component located between cells or within the cells and filled spaces between cellulose and hemi-cellulose. It is generated in primary walls as well as secondary walls. The main function of lignin is to strengthen the wood in the tree. Cellulose as one of the most abundant biopolymers generating in wood served as the main reinforcing phase in plant structures and hemicelluloses is contained 20-30% in tree. A section of the cell wall indicated the location of cellulose, lignin and hemi-cellulose in a plant cell is shown in the Figure 4.1 (Boudet 1998). Softwood contains more extractives than hardwood. There are 3± 2% of extractives are presented in hardwood and 5± 3% extractives in softwood. (Sjöström 1995, 92) The contents of wood components are shown in Table 4.1 and Table 4.2. (Laine 2005)

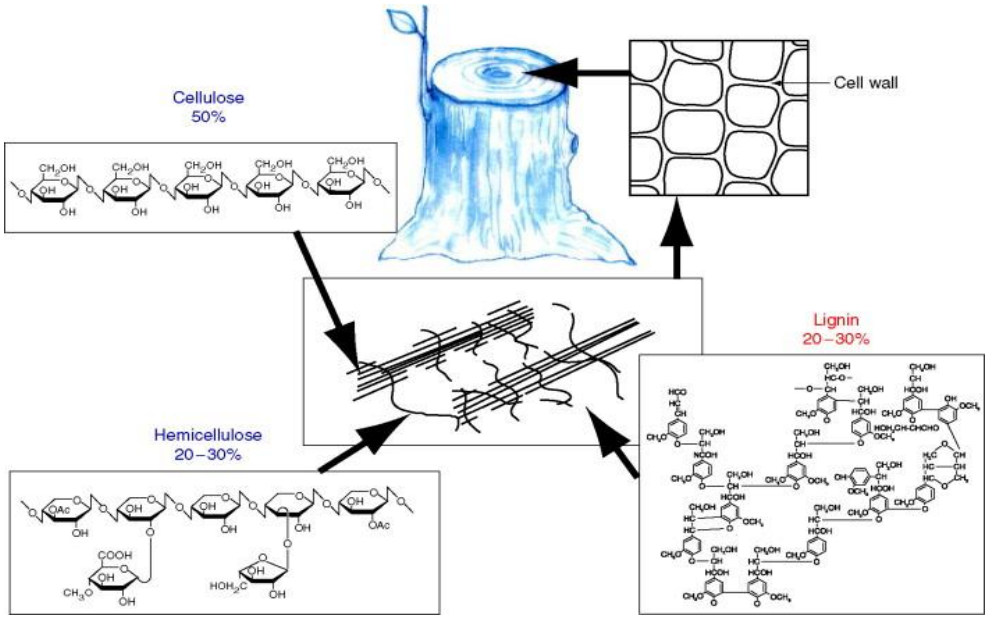


Figure 4.1 Distribution of cellulose, lignin, and hemi-cellulose micro-fibrils in the plant cell wall and their chemical structures. (Laine 2005)

Table 4.1 The wood components in hardwood and softwood (Laine 2005)

Wood Components	Hardwood (%)	Softwood (%)
Cellulose	40 - 50	40 - 50
Hemicellulose	25 - 35	25 - 30
Lignin	20 - 25	25 - 35

Table 4.2 The distribution of four components for different species of tree (Laine 2005)

Constituents	Pine	Spruce	Eucalyptus	Silver Birch
Cellulose (%)	40.0	39.5	45.0	41.0
Hemi-cellulose (%)	28.5	30.6	19.2	32.4
Lignin (%)	27.7	27.5	31.3	22.0
Total Extractive (%)	3.5	2.1	2.8	3.0

Cellulose

Cellulose, as the main stress-bearing compound of the plant cell wall, consists of 10nm wide crystallite of long and straight intently packed polymeric chains of exclusively b-(1-4)-linked D- glucopyranose units. The acetal linkage is beta that makes it different from starch. It is an odourless compound insoluble in water and most organic solvents. Cellulose can be resolved in concentrated acids under high temperature back to glucose form. There are various uses of cellulose concluding wood for building, paper products, cellulose acetate for films. The chemical structure of cellulose has been shown in the Figure 4.2 (Wertz and Mercier 2010.)

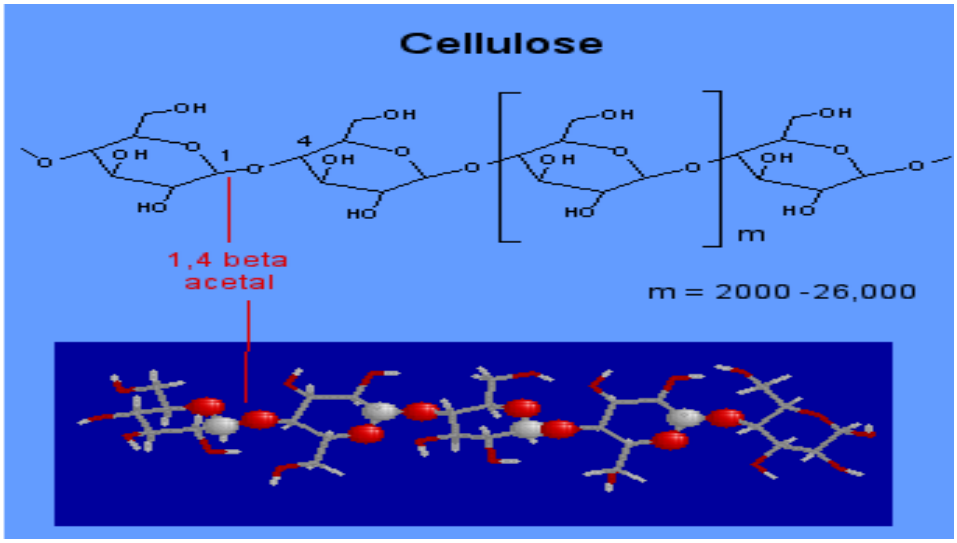


Figure 4.2 Chemical structure of cellulose and its molecular model. (Wertz and Mercier 2010.)

Hemicellulose

Hemicellulose contains 20% biomass presented with cellulose in most plant cell walls. It is derived from various sugars. Compared with cellulose, hemi-cellullose structure are branched and cellulose are unbranched. The main chemical compounds of hemi-celluloses in softwood are galactoglucomannan, glucomannan and arabinoglucuronoxylan, arabinogalactan, xyloglucan, and other glucans. (Wertz and Mercier 2010.)

Lignin

Lignin is a complex polymer composed of phenyl propane units such as P-hydroxyphenyl, guaiacyl, and syringyl units as shown in the Figure 4.3 distributed in the cell wall between cellulose, hemicelluloses components which can be linked by means of numerous types of chemical bonds (ether, ester,

carbon-carbon). The monomers of lignin are rearranged into polymers built up by three different units called: H units, G units, and S units. The relative proportion of these units greatly varies relating with the plant species and the developmental stage. The lignins from gymnosperms which do not contain S units are more resistant to the chemical degradation than lignins which contain both G and S units. The function of lignin is to transport liquid in the living plant and it enables trees to grow taller and compete for sunshine.(Stenius and Pakarinen 2000.)

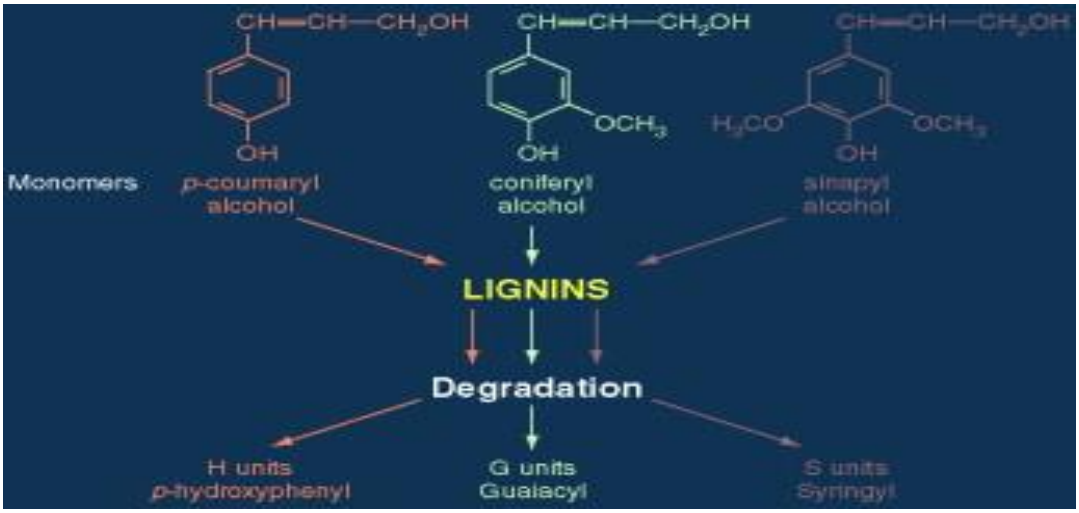


Figure 4.3 Monomeric constituents of lignins and degradation products (Stenius and Pakarinen 2000)

4.2 Extractives

Extractives contain various kinds of wood components which are soluble in neutral organic solvents or water. They occupied minor fraction of total wood chemical compounds. The extractives from wood compose every kind of raw materials for making chemicals and they have a significant impact on the pulping and paper making processes. Terpenoids and steroids, phenoilc constituents, fats and waxes, inorganic compounds can be found from the

extractives extracted. The extractives from wood provide different functions applied into different areas. (Bruce and Allen 2000.)

The fats are glycerol esters existing as triglycerides in the wood. The fatty acids, belong to linolic acid, are released from triglycerides during storage. More than 30 fatty acids including saturated and unsaturated, have been found in the woods. Phenolic compounds are derived from phenylpropanoid structure. The important Phenolic groups can be defined as Stilbenes, Lignans, Hydrolyzable tannins, Flavonoids and Condensed tannins. Especially heartwood and bark contain a large variety of complex aromatic extractives. Most of them are phenolic compounds.They protect the tree against microbiological attack because they have fungicidal properties.

Inorganic components exist in the wood as ash and are derived from various kinds of metal salts like carbonates, silicates oxalates and phosphates. The heavy metals, iron and managanese, existing in xylan and pectin can be extracted from wood through aqueous acids or other complex agents. However, because of metal salts in inaccessible areas of wood structure and just a small amount of metal salts being soluble, it is not easy to extract inorganic compounds from wood. (Sjöström 1993.)

4.3 TERPENOIDS

Terpenoids as one of the main types of secondary plant metabolization products can be classified in line with the number of isoprene units: Hemiterpenoids, Monoterpenoids; Sesquiterpenoids; Sesterterpenoids; Tetrepenoids. It is existed mainly in the soft wood.The description of various terpene types can be seen in the Table 4.3. (Buckingham and McDonald 1995.)

Table 4.3 Number of isoprene units, carbon atoms and some examples of different terpene derived group (Buckingham and McDonald 1995.)

Terpene type	Number of isoprene units	Carbon atoms	Examples
Monoterpene	2	10	Menthol, camphor, Limonene,
Sesquiterpene	3	15	Farnesol, carophyllene
Diterpene	4	20	Phytol, Vitamin A
Triterpene	6	30	Squalene, lanosterol
Tetraterpene	8	40	β-carotene (provitamin A)

Monoterpenoids

The compounds are violate oil, less dense than water, have boiling point range from 150℃ to 185℃ depending on the effecting atmosphere. Distillation plant matter with steam and digester relief condensate after softwood kraft pulping are two main ways to isolate monoterpenoids existing in softwood oleoresin as hydrocarbon or their derivatives from wood before fragmental purification by distillations. Monoterpenoids can be used as turpentine, flavor and fragrance chemicals. (Buckingham and McDonald 1995)

Different chemical structure with various function groups are described. Acyclic and monocylic types: β-myrcene (1), (-)-limonene (2) and (-)-β-penllanrene (3). Bicyclic types: α-pinene (4) and β-pinene (5), 3-carene (6), Camphene (7), borneol (R=H) and bornyl acetate(R= acetate group) (8), and β- thujaplicin (9). are shown in the Figure 4.4. (Buckingham and McDonald 1995.)

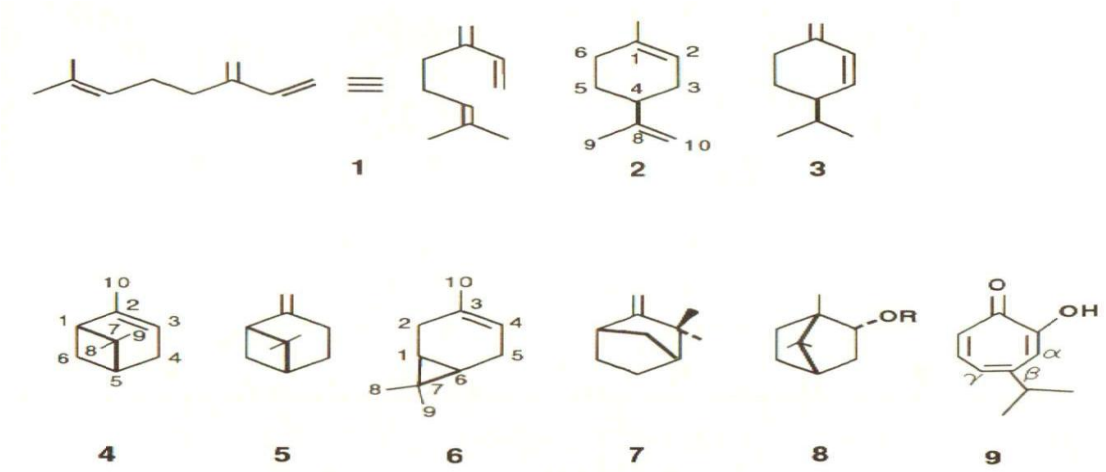


Figure 4.4 Common monoterpenoids chemical structure (Buckingham and McDonald 1995.)

General monoterpenoids donate in essential oils and in commercial turpentines. The ingredient of turpentine is generally consist of the bicyclic monoterpenoids foremost existing as the form α-pinene (4) and β-pinene (5), which can both occur in (+) and (-) form. Plenty of compound (-)-α-pinene are present in slash pine and Pinus species include predominantly 3-carene (6), Camphene (7), a minor hydrocarbon, and borneol (8) transpired as bornyl acetate in the pine wood. And the β- thujaplicin (9) as an type of C_{10}- tropolones can be found in Western red cedar heartwood. (Buckingham and McDonald 1995.)

Monoterpenoids' extraction technique is studied in two methods. The first one is extracted monoterpenoids in hexane solvent by soaking crushed juniper needles. The second technique is through steam distilling samples. The extracts contents are analyzed by using gas chromatographs. The first method yielded a lower total concentration and a decreased compositional diversity of monoterpenoids compared to the second method. The highest concentration and composition of monoterpenoids can be achieved by an 8-hours steam distillation. (Owen and Straka 1998.)

Sesquiterpenoids

Sesquiterpenoids are generated by oxidation or rearrangement of sesquiterpene which has chemical structure consisting of three isoprene units and has the formular $C_{15}H_{24.}$ More than 2500 sesquiterpenoids have been designated describing various skeletal types of compounds from acyclic to tetracyclic systems. Usually it occurs in small amounts and can be less used in commercially. Common sesquiterpenoids can be found in essential oils and in commercial turpentines. Some chemical structure of sesquiterpenoids eg. α-Muurolene (1),δ-cadinene (2) and α-cadinol(3) reveal a type named cadalanes. α-Cedrene (4), longifolene(5), and juniperol(6),. Nootkatin (7) and Chanootin (8) are shown in Fig. 4.5. (Buckingham and McDonald 1995.)

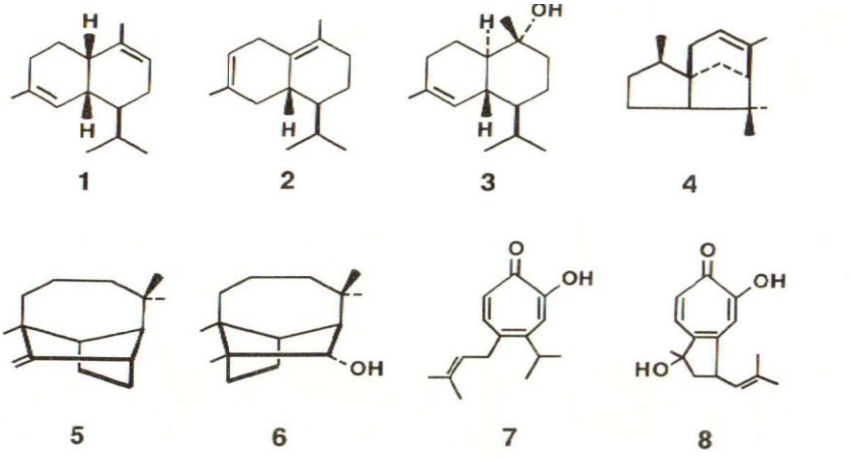


Figure 4.5 Common sesquiterpenoids (Buckingham and McDonald 1995.)

One type of sesquiterpenoids, γ-lactone sesquiterpenoids, named ugandenial A is isolated at the room temperature during a chemical investigation of Warburgia ugandensis bark. The solvent used in the experiment is ethyl acetate. (Xu and Litaudon 2009.)

Diterpenoids

Diterpenoids are one of the main components which can be found in oleoresin and present either as hydrocarbons or as derivatives with hydroxyl, carbonyl, or carbonyl groups. It can be used as rosin or sizing agent. The chemical structure can be grouped as acyclic, bicyclic, tricyclic, tetracyclic and macrocyclic systems. Some of diterpenoids being found in the wood eg. Geranyl-linalool (1), β-epimanool (2), cis-abienol (3), Manooyloxide (4), Pimaral (5), pimarol (6) and macrocyclic diterpenoid(7)are described in Figure 4.6. (Sjöström 1993 97.)

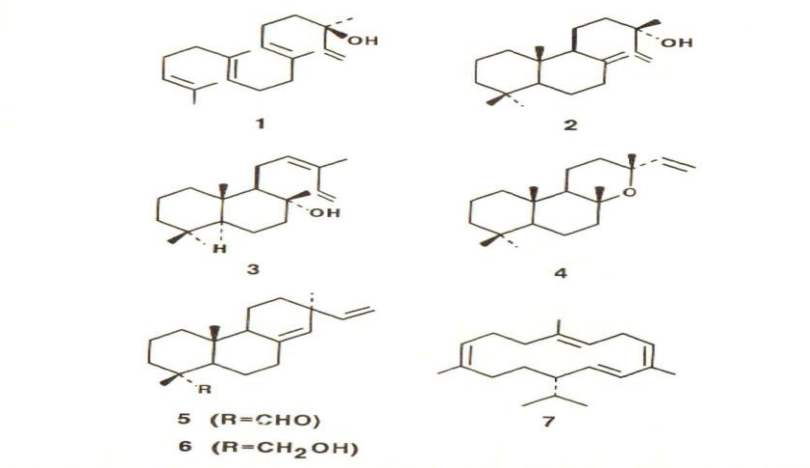


Figure 4.6 Chemical structure of diterpenoids (Sjöström 1993 ,97.)

Diterpenoids can be extracted from Taxodium distichum cones. The solvent used in the extraction process is the n-C_6H_{14}. Twelve diterpenoids including ten abietane-type components were isolated from the n-C_6H_{14} extract: 6,7-dehydroferruginol (1), ferruginol (2), 6,7-dehydroroyleanone (3), sandaracopimaric acid (4), taxodione (5), taxodal (6), taxodone (7), sugiol (8), xanthoperol (9), salvinolone (10), 5,6-dehydrosugiol (11), and 14-deoxycoleon U (12). (Kusumoto 2008.)

Resin acids are main composition in pine wood oleoresin and rosins. Tricyclic terpenoids group into pimarane and abietane, are the most common resin acids in softwood. Various chemical structures of resin acids eg. Pimaric acid (1),

sandaracopimaric acid (2), isopimaric acid (3), Abietic acid(4), levopimaric acid (5), palustric acid (6), neoabietic acid (7) and dehydroabietic acid (8) are shown in the Figure 4.7. (Sjöström 1993,98.)

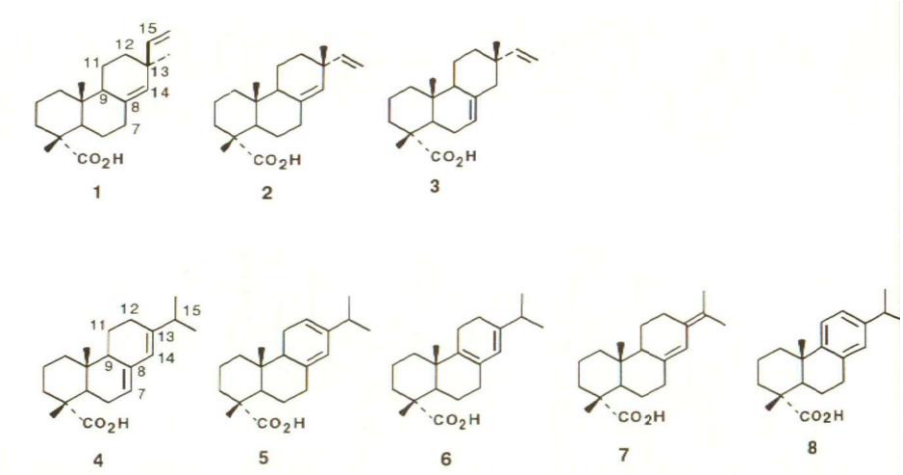


Figure 4.7 Compound structures of resin acids in oleoresin and commercial rosin. (Sjöström 1993,98.)

Pimaric acid (1) and its isomers sandaracopimaric acid (2) and isopimaric acid, comprised vinyl and methyl groups at the C-13 position, are three main components of pimarane type acids. Abietic acid(4), levopimaric acid(5),palustric acid(6), neoabietic acid(7) and dehydroabietic acid (8) include isopropyl or an isopropenyl group at the C-13 position, and are the main abietane type acids. Because of the chemical structure of hydrophobic skeleton in combination with a hydrophilic carboxyl group, the resin acid soaps are solubilizing agents. Joint with the fatty acid soaps, the neutral lipophilic substance compounds from wood in alkaline pulping and subsequent washing can be removed. (Sjöström 1993,92.)

Triterpene (steroids)

There are about 1700 triterpenes constructing mainly from squalene and squalene. D-vitamins and bile acids can be found from the triterpenes which are derived from the tetracyclic. The triterpenoids are relatively rare in nature as the form of tetracyclic and pentacyclic existed in nature. Terpenoids and steroids have similar characteristic in the aspects of structure and biogenetic. Self-defence and propagating are the two main functions of terpenoids and steroids for the plant. Due to the extraordinaraly components of hydrocarbon skeleton, sterols are powerfully hydrophobic except the hydroxyl group. Sitosterol(1), campesterol(2), Sitostanol(3), Citrostadienol(4), Cycloartenol (5), 24-methylnene-cycloartanol (6), Betulinol (7), Serratenediol (8) are shown in the Figure 4.8. (Sjöström 1993,92.)

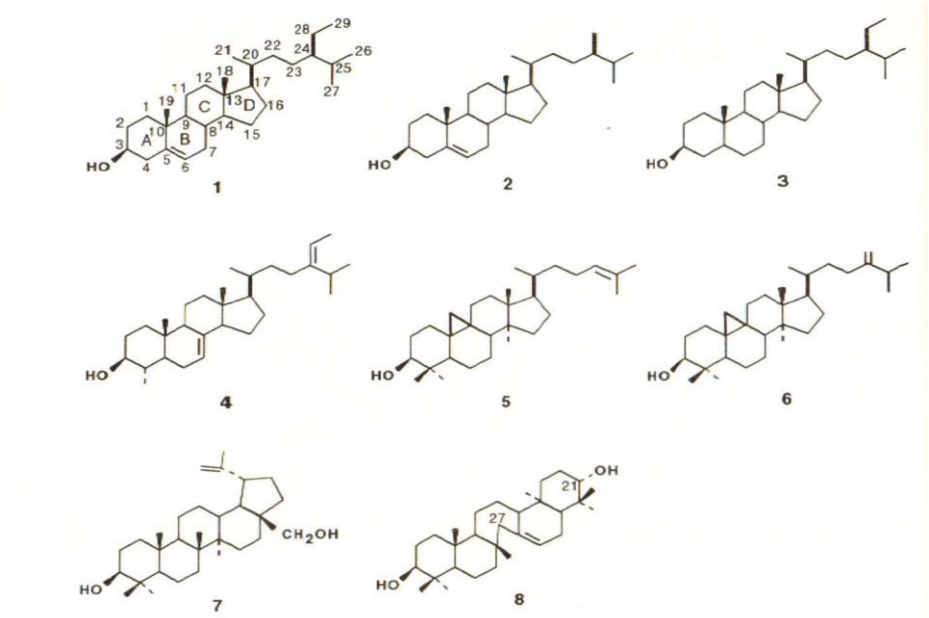


Figure 4.8. Chemical compounds of Steroids and triterpenoids in wood. (Sjöström 1993, 92.)

The most common steroids found in the wood is sitosterol(1). Smaller amounts of campesterol(2), which has similar monoenoic steroids structure, are distributed in the wood than sitosterol. Sitostanol(3) is the saturated parallel pattern of sitosterol. Citrostadienol(4) is a dienoic sterol with a 4α-methyl group. Cycloartenol (5) and 24-methylnene-cycloartanol (6) are intermediate in the biosynthesis of 24-alxylsteroids. Betulinol (7) is a pentacyclic triterpenoid taking place in the outer bark of birch. Serratenediol (8), has a seven-membered C-ring belonging to pentacyclic triterpenoids' subgroup located in the bark of pines. (Sjöström 1993, 92.)

Polyterpenoids

Betulaprenols, a type of polyprenols, existed as fatty acid esters in silver birch building up of 6-9 isoprene units with cis and trans constructions of the double bonds. The huge amount number of isoprene units decided that the polymerization is high in their relative products such as rubber, balata. They are distinguished in the aspects of their structures, such as nature rubber with all-cis configuration in contrast with balata which has the all-trans configuration as presented in the Figure 4.9. Rubber(1) with all-cis, balata(2) with all-trans, and betulaprenols(3) with mixed cis/trans double-bond structure.(Sjöström 1995,98.)

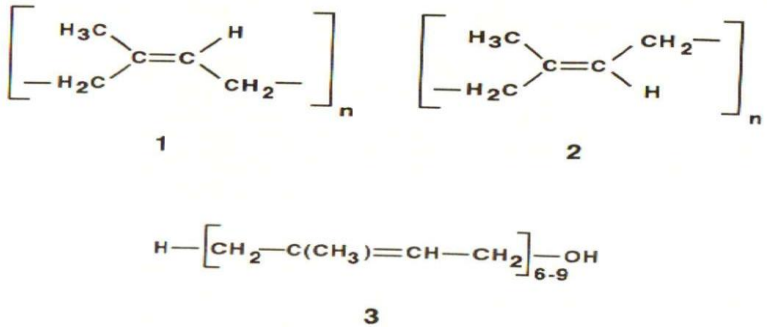


Figure 4.9 Chemical described structures of polyterpenoids. (Sjöström 1995,98.)

5 LOCATION OF EXTRACTIVES

The chemical compounds build up in structural components distributed in different location. The major compounds and their location can be found in the Figure 5.1. (Sixta 2011.) Various amounts and the species extractives located in different positions from the cambium to pith of one tree. For instance, the heartwood contains much more extractives than the sapwood.
(Sjöström 1995, 90.)

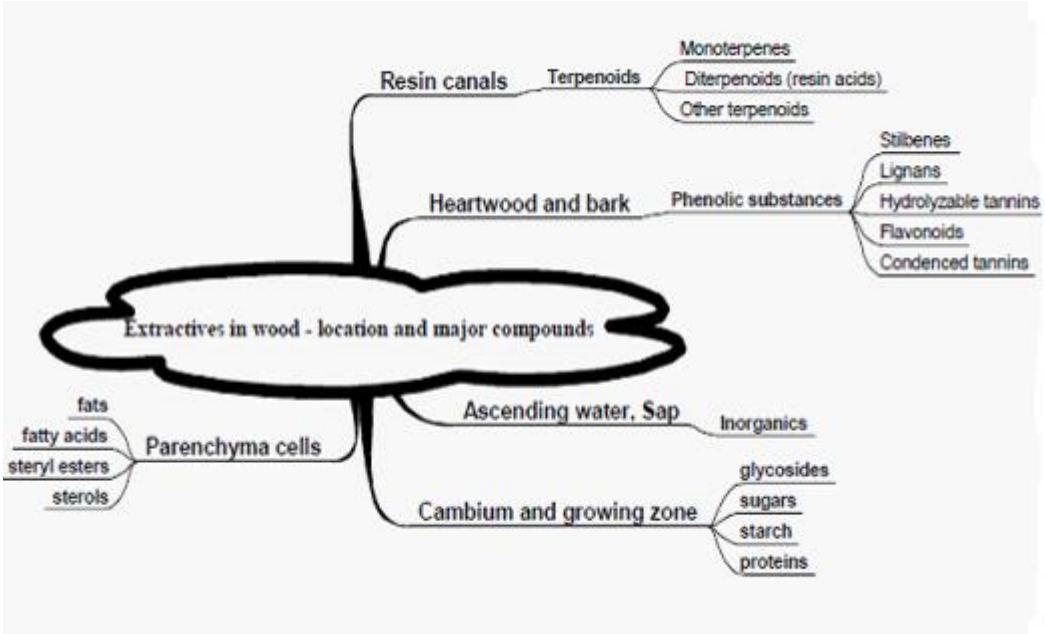


Figure 5.1 Distribution of extractives in wood (Sixta 2011.)

First, the terpenoids include monoterpense, diterpenoids (resin acids) and other terpenoids that can be found in the resin canals of trees. Secondly, Phenolic substances which include Stilbenes, Lignans, Hydrolyzable tannins, Flavonoidsa and Condenced tannis are located in the heartwood and bark. The third part of tree is sap of the tree. Fourth, inorganics are transported with the ascending water to the branches. Glycosides, sugars, starch and proteins can be found in the cambium and growing zone of tree. Fifth, there are also some other chemical compounds including fats, fatty acids, steryl esters, and sterols located in the parenchyma cells. (Sjöström 1995, 94.)

The heartwood sample contains the largest relative amount of sterols. The largest relative amounts of the resin acid compounds are identified in knotwood, sapwood and heartwood. Lignans and phenolic compounds are found in greatest relative concentration in heartwood. Sapwood and heartwood contain more esters, relative to the total extractives identified, than root and bark samples. (Taylor , Gartner, and Morrell 2006.)

For many bark species, extractives are existed in form of mixture chemical compounds and it is not easy to isolate one single pure chemical during extraction process. Advanced experiments have been made to isolate chemicals from bark in all areas of extraction process, including raw materials, production test technology, processing control and its application. Therefore material preparation methods vary from material to solvent depending on its aim. (Harkin and Rowe 1971.)

Terpenoids which include monoterpense, diterpenoids (resin acids) and other terpenoids can be found in the resin canals of trees. The observation of wood divided to three different kinds of resin canals is displayed by microscope in the Figure 5.2 A) Horizontal resin canal in a ray (tangential section) originating from the inner annual rings. The canal is surrounded by epithelial cells which secrete resin into the canal cavities. B) Horizontal resin canal in a ray originating from the outer annual rings. The canal is filled with epithelial cells because of their swelling during sample preparation. C) Vertical resin canal. (Sjöström 1995, 95.)

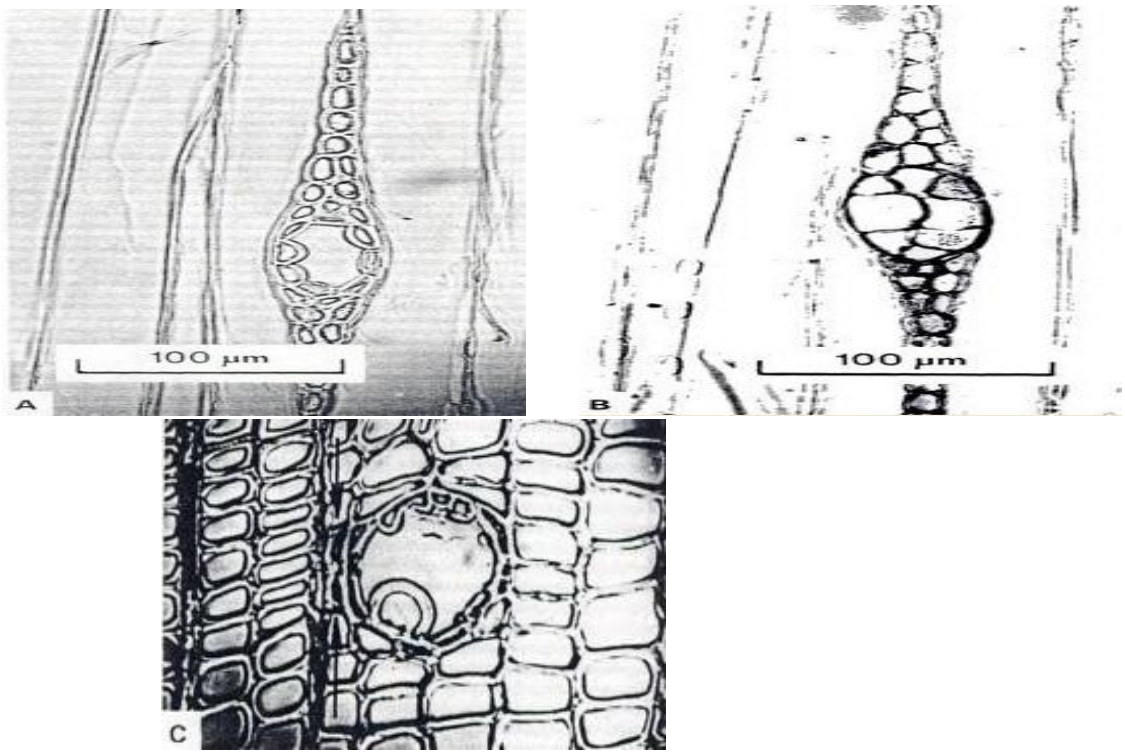


Figure 5.2 Location of terpenoids in three types of canal of one tree (Sjöström 1995, 95.)

Heartwood represents the inner layers of wood in the growing tree. The process of heartwood formation in conifers includes two major biological phenomena in the tissue: death of the parenchyma and extractive formation. Sapwood is defined as the part of the tree containing living cells. The role of the sapwood is to transport water from the root to the crown, and act as a water and energy reserve material for the tree. (Taylor, Gartner & Morrell 2006.) Heartwood contains more extractives than sapwood. The total extractive content will decrease with the increasing height of the trunk. The extractives are formed close to the heartwood/sapwood boundary by using local available compounds (such as carbohydrates) and material translocated from the phloem and sapwood. The rootwood sample had the second largest extractives content, with about one third of the bark extractive content, but ten times more than the heartwood and sapwood extractive content. (Taylor , Gartner, and Morrell 2006.)

The bark constitutes a barrier between the tree and the exterior environment. As extractives are active in defence against exterior attack, a higher concentration of extractives within the bark was expected. (Harkin and Rowe 1971.) The cork cells in the outer bark contain polyestolides or suberins. The suberin content in the outer layer of the cork oak bark (cork) is especially high and amounts to 20-40% in the periderm of birch bark. Polyestolides are complicated polymers composed of w-hydroxy monobasic acids which are linked together by ester bonds. (Sjöström 1995, 95.) Bark contains 2-5% inorganic solids of the dry bark weight. The metals are present as various salts including oxalates, phosphates, silicates, etc. Some of them are bound to the carboxylic acid groups of the bark substance. Calcium and potassium are the predominating metals. Most of the calcium occurs as calcium oxalate crystals deposited in the axial parenchyma cells. Bark also contains trace elements, such as boron, copper, and manganese. (Harkin and Rowe 1971.)

6 EXTRACTIVES FROM THE DIFFERENT WOODS

Various raw materials of tree are located in different regions according to the atmosphere of the environment, such as climate, the condition of soil. Aspen, birch, pine and spruce are introduced as main species widely planted in Finland. The different properties and function applications and extractives in these four woods are introduced.

Birch

Essential oil existed in birch tree is mainly methyl salicylate that can be extracted by distillation or macerated in water used for disinfection, in antibacterial or insecticidal agents. And three main extractives can be found

which are Betulin, Betulinic acid and Fatty acids bound with natural polymer suberin in the birch trees. The first one is betulin which belongs to an abundant naturally occurring triterpene protecting birch wood from the harmful actions of water and light. Betulin exists in large amounts of outer bark of silver birch, betulinic acid can also be isolated from silver birch bark and its purity is not less than 98%. American paper birch contains nearly 15-17% of betulin compared with European birch, Betula verucosa, which contains nearly 25% of betulin which is a hydrophobic compound. Furthermore, Betulinic acid, a natural molecular derivative from betulin, was found to be very active against melanoma cancer, one of the most dangerous forms of skin cancer. (Zhao , Yan and Cao 2007, 959–962.)

The heartwood of birch wood contains similar components, namely betulin, lupeol, β -sitosterol, and a very small amount of procyanidin **.** Sweet birch bark contains lupeol and betulin as major constituents with lupenone, oleanolic acid, β -sitosterol, methyl salicylate, and leucocyanidin; the heartwood contains lupeol, betulin, methyl acetylbetulinate,methyl salicylate, β-sitosterol, sitosterol-β -*D*-glucoside, and a small amount of a procyanidin. Yellow birch bark contains lupenone as the major constituent with betulin, lupeol and procyanidin. The heartwood contains betulin, lupeol, lupenone, methyl acetylbetulinate,β -sitosterol, sitosterol-β -*D*-glucoside and trace of procyanidin**.** Birch sap has been found to contain an arabinogalactan, *D*-glucose, *D*-fructose, sucrose, gentiobiose, melibiose, mannotriose, verbascotetraose, and two trisaccharides as well as a complex polysaccharide. Catechin and glycosides of pyroca-techol, coniferyl alcohol, and pyrogallol were found in the heartwood of birch. (Zhao, Yan and Cao 2006.)

Aspen

The extractive from aspen as medicine treatments can be used to reduce fevers and relieving eczema. Extractives of aspen bark as a water-soluble powder can also be used as traditional natural preservative for food. The aspen bark is rich in salycin, an aspirin-like substance, considered to be analgesic, anti-inflammatory, calming, and healing. The extractives of aspen are traditionally used as analgesics, the mechanism is invading parasites. Crude protein content of aspen bark is normally less than 3% on a dry basis. The chemical composition of aspen sawdust is similar to that of whole aspen tree. Protein content is less than 2%. (Leon 2009.)

Spruce

Linolenic, pinolenic, palmitic and oleic acid are main types of esterified and free fatty acids identified in Norway spruce.The esterified fatty acids are composed of di- and triglycerides, steryl esters and diterpenyl alcohol esters. The stemwood contained more esterified fatty acids than knotwood. Fats and fatty acids are accumulated in woody tissues in order to provide food reserves. (Obst 1997.) Different parts of spruce consist of extractives with various contents. The bark contained the largest total amount of extractives. Knotwood and the rootwood samples contained about one-third of the amount of extractives detected in the bark. Heartwood and sapwood were the parts of the tree where the total extractive content was lowest. The percentage of extractives in the dry mass determined for each sample is illustrated in Figure 6.1. (Krasutsky 2002.)

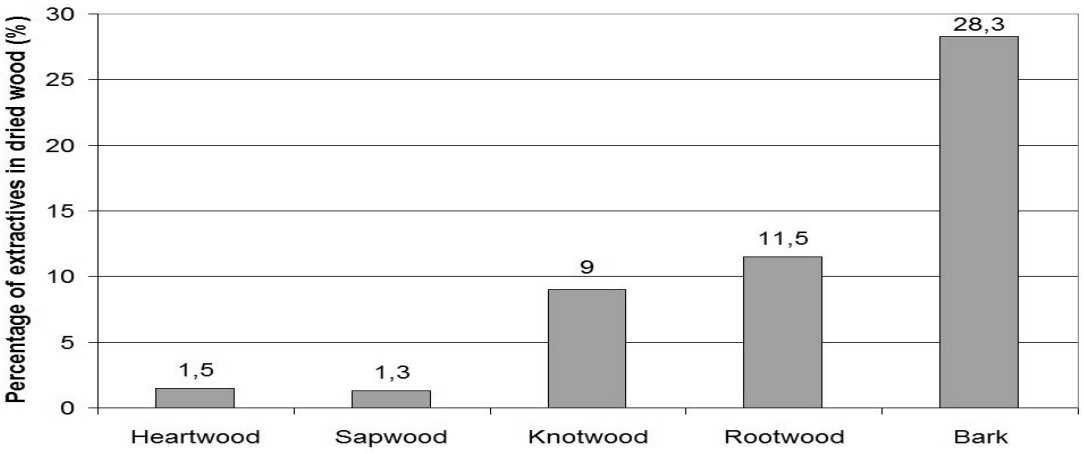


Figure 6.1 Extractive content in different tissues of Sitka spruce (% of dried mass) (Krasutsky 2002.)

At least 34 compounds from different classes were identified in the Sitka spruce. No fatty acids were identified in the other samples. The molecules identified were grouped by classes of extractive which are resin acids, lignans and phenolic compounds, steroid ketones, esters. The composition of the lipophilic extractives before and after storage of spruce logs is shown in the Figure 6.2: (Sjöström 1995.)

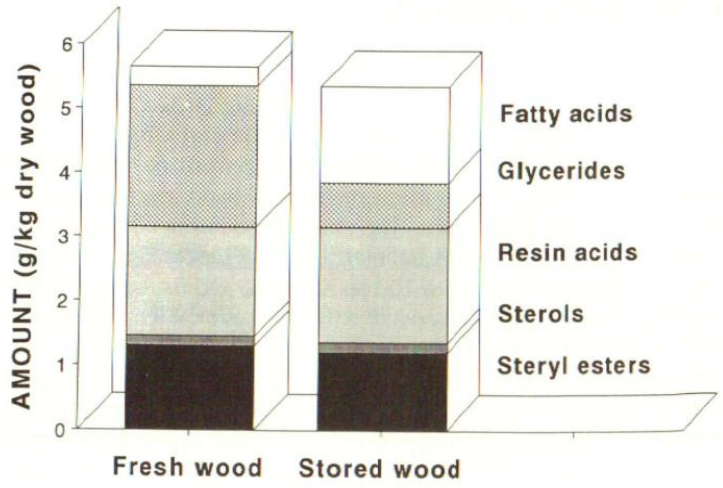


Figure 6.2 The composition of the lipophilic extractives (Sjöström 1995.)

Pine

Pine tree as the most commercially valuable used tree species . The color of bark is orangey-brown to grey. Pine woods have poor insect after logging, therefore the pine woods are better kept in reserve indoors. Pine bark extract contains chemicals called proanthocyanidins used for its antioxidant properties. Slash pines are essential material for extraction of monoterpenoids derivative. Turpentine can be used as a solvent to produce varnishes or used as clean scent because of its antiseptic properties and crude oils which are a flammable liquid used in manufacturing industry. Lastics and pharmaceuticals are produced from slash pine. (Verheijen, Jeffery and Bastos 2010.)

The ecotoxicity tests evaluate the effect of the chemical compounds existing in the extractives from the pine wood on the soil and aquatic ecosystems, The extractives of heat- treated and untreated pine wood are tested by thermophilic eubacterium used to determine the toxic affection of lipophilic compounds and the main extractives are found in the water and ethanol extracts. (Esteves, Videira and Pereira 2009.)

7 ISOLATION OF EXTRACTIVES FROM THE WOODS

Extractives are natural products and they can be removed with solvents such as ether, methane,water. It is critical to make sure the raw material is totally immersed into solvent. It is also important for the proper preparation of materials prior to extraction. The complexity of the processes, the scale of operation and equipment affect the species and content of extractives. The best extraction required an optimum size of raw material to be maintained. (Helm 2000.)

Birch bark rich in betulin and betulinic acid is used in pharmaceutical industry.

The extraction method and identification process are studied dried bark pieces were refluxed with dichloromethane, ethyl acetate, acetone, chloroform, methanol and 95% ethanol respectively. Different solvent extracts were collected and filtered and then dissolved with methanol. After filtering the solution with membrane, HPLC was used to identify the compounds. (Kemna 2007.)

For the extraction of silver birch bark, the ratio of the bark to the extraction solvent was 1:10. The content of the compounds was strongly dependent on the solvents with various polarities. Because of the co-solubility affection, 95% ethanol was a good extraction solvent allowing extraction of triterpenoid with the highest content. (Gore 1997.) Some other studies also show that 95% ethanol was found as a suitable extraction solvent that allows extraction of triterpenoids with a highest content. During the extraction process, fatty acid is the first compound being extracted from birch (betula penduala) bark. (Gore 1997.)

The salicylates extracted from aspen can be applied in cosmetic and personal care products. The extract has broad spectrum of antibacterial activity. Therefore, aspen bark extract is an excellent alternative to traditional preservatives and it inhibits the growth of mold and it can be used as a defensive mechanism against invading parasites. For the extraction process, a small amount of aspen bark, which is prepared from powder, was treated with a small amount of water at or below 60°C and the obtained salicylates can be used for treating lower back pain and it can also impart a smooth feel to skin. (Leon 2009.)

The extractives of pine wood were studied with the mass loss of the wood. Chemical composition changed with the heat treatment by the degradation of extractable compounds. At the same time, the fats and fatty acids are removed

by resin acids, small amounts of lignin also decomposed for a milt heat treatment leading to the formation of soluble phenolic compounds, but the reaction rate is much slower than hemicellulose. Cellulose as a resistant compound will be decomposed under much higher temperature with higher mass losses. For sugar, Arabinose and galactose are degraded followed by xylose and mannose. New compounds such as anhydrosugars and phenolic compounds are risen up with the original extractives disappeared. The whole process is environmental friendly since the compounds, which identified in the water and ethanol extracts, are not harmful at the existing concentrations. (Sijtsma and Tan 1996.)

For the pine wood with and without heat treatment, the testing result show that water soluble compound are no toxic for bacteria and some of the water soluble derivate compounds like sugars even offer nutrients for the growing of bacteria. Of the ethanol extracts of untreated and treated wood, the bacteria growth was affected due to the increasing of concentration. (Esteves 2009.)

8 EXPERIMENTAL STUDIES

8.1 Raw materials and methods

The raw materials for the experiments were pine chips, pine sawdust, pine stump chips and pine stump bark available from Saimaa University of Applied Sciences. The pine wood were stored in refrigerator or outside to keep fresh, as shown in Figure 8.1.

A B C

Figure 8.1 wood chips (A), saw dust (B) and root bark (C)

Raw materials from different parts of pine tree were pre-treated in various ways by using different tools during the practical work in laboratory of Saimaa University of Applied Sciences located in Imatra. The pine chips had to be chosen in proper size for the extraction, and therefore a screen was used to separate wood chips to be different sizes. The pine wood chips, sawdust and stump and stump bark were treated with a debarking tool and knife to small particle sizes. The preparation equipment for screening wood chips is shown in the Figure 8.2 The smallest fractions of chips were taken for experiments, the sizes of chips were in the range from 5 X 10 X 3 mm to 15 X 20 X 3 mm (length X width X thickness).

Figure 8.2 Screening equipment for raw material

8.2 Solvent selection

The extraction of wood carried out with an organic solvent and most resinous substances occurring in wood dissolved in those organic solvents. Various solvents applied for extraction to obtain the extractives from wood. Solvent can be broadly grouped into two categories which are polar and non-polar. Hexane, toluene and diethyl ether, which belong to non-polar solvents, are introduced in this experiment.

Hexane is considered to be the best solvent since the beneficial commercial price, edibility of the various products obtained from extraction, stable physical properties and its low boiling point (67°C / 152°F). Because it is a non-polar molecular, it is insoluble into the water and it can be miscible with alcohol and ether. The major use of n-hexane is to extract vegetable oils, cleaning agents and used as thermometer liquid. N-hexane is a volatile liquid easily evaporated into the air. It must be stored in a proper position that designed for inflammable liquid. (Wade 2003.)

Toluene is a colorless, flammable, water insoluble liquid produced from processes of making gasoline by a catalytic reformer. It is an important organic solvent for industry feedstock and it can also be used as an inhalant drug. Toluene does not vaporize until 70 degrees Celsius. (Wade 2003.)

Diethyl ether is a colourless organic compound. It has a low boiling point therefore it can be ignited at a relative low temperature and it may react with the oxygen in the air which leads to potential of fire. It is normally used for organic liquid and organic liquid extraction due to limited solubility in the water. (Normann and Morland 1987.)

Table 8.1 Boiling points and physical properties of hexane, toluene and diethyl ether displayed. (Wade 2003.)

Solvent	Chemical formula	Boiling point	Density
Diethyl ether	CH_3CH_2-O-CH_2-CH_3	35 °C	0.713 g/ml
Hexane	CH_3-CH_2-CH_2-CH_2-CH_2-CH_3	69 °C	0.655 g/ml
Toluene	C_6H_5-CH_3	111 °C	0.867 g/ml

8.3 Extraction process

Extraction of wood involved extracting with non-polar solvent, which had been processed by two instruments in Saimaa University of Applied Science in Imatra. The first equipment was a boiling flask. The flask had been heated and attached to a refluxing device, as shown in Figure 8.3.

The second equipment was a steel based rotating reactor in ethylene glycol bath, as shown in the Figure 8.4. Eight 200 ml containers were used in the extraction. It is worth to notice that the cover of container was closed tightly to prevent the pollution during extraction. The equipment needs 30 minutes for cooling down before the samples can be taken off. The solvent bath temperature was kept depending on different experimental setting, and the heating temperature was controlled through control panel.

Figure 8.3 Steel based rotating reactor in ethylene glycol bath

Figure 8.4 Control panel is clarified on the left hand and glycol bath is clarified on the right hand side. (Gladyshko 2011,24.)

Extraction with Flask Reactor

The fresh wood chips or bark were pre-treated to the appropriate size and the mass ratio of wood and hexane was 1:5. The operating conditions are shown in Table 8.2.

Table 8.2 Extraction of wood with hexane

Raw material	Solvent	Temperature	Time
Pine root bark, 41.57g	206.5 ml	69℃	4h
Pine saw dust, 40.32g	203.6 ml	69℃	4h
Pine stem chips, 42.71g	205.5 ml	69℃	4h

Extraction with Steel-Based Reactor

The pine root chips and bark, pine stem chips and pine saw dust were treated with hexane, toluene, and diethyl ether. The mass ratio of wood and hexane was about 1:4, the mass ratio of wood and toluene was about 1:6. The mass ratio of wood and diethyl ether was about 1:5.

Table 8.3 Extraction of wood with hexane

Raw material	Solvent	Temperature	Time
Pine root bark, 41.57g	206.5 ml	55℃	4h
Pine saw dust, 40.32g	203.6 ml	55℃	4h
Pine stem chips, 42.71g	205.5 ml	55℃	4h

Table 8.4 Extraction of wood with hexane

Raw material	Solvent	Temperature	Time
Pine saw dust, 25.65g	178.9 ml	55℃	2h
Pine stem chips, 25.76g	182.6 ml	55℃	2h
Pine root bark, 27.20g	180.5 ml	55℃	2h

Table 8.5 Extraction of wood with toluene

Raw material	Solvent	Temperature	Time
Pine saw dust, 25.65g	176.9ml	55℃	2h
Pine stem chips, 26.10g	180.7ml	55℃	2h
Pine root bark, 25.63g	180.5ml	55℃	2h
Pine saw dust, 25.50g	180.9ml	90℃	2h
Pine stem chips, 23.60g	180.5ml	90℃	2h
Pine root bark, 25.20g	180.5ml	90℃	2h

Table 8.6 Extraction of wood with Diethyl ether

Raw material	Solvent	Temperature	Time
Pine saw dust, 25.50g	180.9ml	26.8℃	2h
Pine stem chips, 23.60g	180.5ml	26.8℃	2h
Pine root bark, 25.20g	180.5ml	26.8℃	2h

8.4 GAS CHOROGRAPHY ANALYSIS

Pretreatment of Extraction Solution

Before GC analysis, the extraction solution was treated with gravity filtration and suspended waste solids were removed from liquid in this process. The filtration equipment and filtrated liquid are shown in Figure 9.1. The filtrated solution was then going to GC analysis.

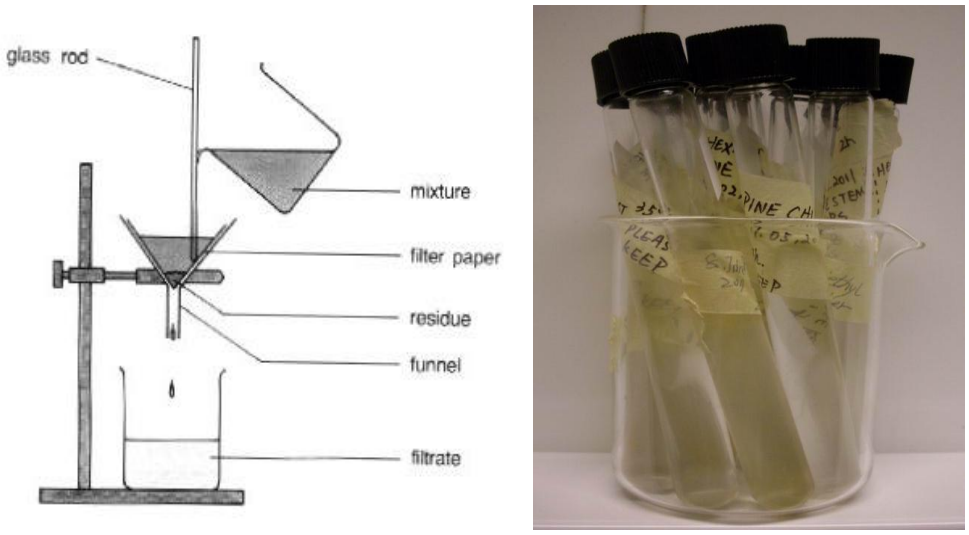


Figure 9.1 Process for filtration (Christian 2003.)

GC analysis

Gas chorography, composed of two parts which are the mobile phase (gas) and the stationary phase (a liquid or a solid compound), is used for obtaining specific information about various substances. The compounds are separated

based on various vapor pressures and dissolution to the stationary phase in a column according to the boiling point order. Not all chemical compounds can be analyzed by gas chorography analysis method. The analyzed compound has to be vaporized without decomposing and the suitable chemical compounds have to be pretreated in order to transform the components into a sufficiently volatile modification. In contrast with high molar mass compounds (molar mass over 500 g/mol) and ions, small molecular and lower molar mass organic compounds can be analyzed by gas chorography method. The structure of the equipment of analyzing gas chorography is described in the Figure 9.2 .(Christian 2003.)

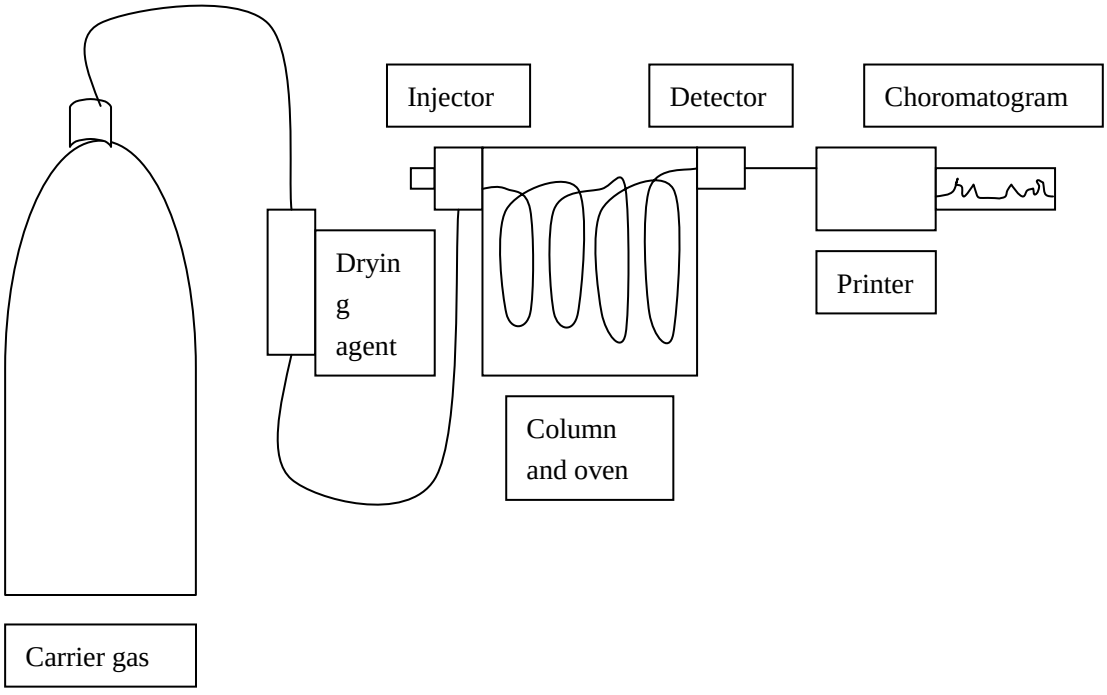


Figure 9.2 Equipment components' description of gas chorography analysis (Christian 2003.)

The carrier gas goes through three parts of the injector which are column, septum and by pass flow. The sample is injected through a gas tight thick rubber disk, vaporizing in the injector and flowing with carrier gas to the column

and the detector. Inert gas such as helium, nitrogen or hydrogen is used as carrier gases to samples and stationary gases. The drying agent is used to purify the carrier gas (at least 99,995%), and moisture is removed with the absorbing agents. Furthermore, a small amount of the sample (1:20-1:100) is injected to the column in a split injection. The temperature of the injector is near the highest boiling point of the compound, so that all compounds vaporize in the injector. (Christian 2003.)

Column has an internal diameter of 0.2-0.7mm and a length of 20-50 cm. The stationary phase is a thin layer in the inner surface of the column and the thickness is 0.1-1um. The longer the column the better result will be got in the resolution and the longer is the analyzing time. The smaller the diameter of the column is, the better result and the shorter analyzing time will be got in the separation. Out surface of the column is made of polyimide which makes the column durable mechanically. There is a layer of silica (pure silicon oxide) inside the polyimide. The stationary phase (inert liquids) stands high temperature without decomposing or vaporizing. Then the detector detects compounds flowing from the column, the signal detected is proportional to the amount of the compound and it is calibrated with reference compounds to find out the amount of compounds. At last, different compounds can be identified by their different retention times compared with reference compound. The amount of compounds can be found by the area of peaks. (Christian 2003.)

In this work, a small amount of the sample was injected to the column with a split injection. Column had an internal diameter of 0.25 mm and a length of 50cm. The temperature of the injector was 300℃. The temperature of oven was raised 10℃/min from temperature of 50 ℃to around 300 ℃.The temperature of detector was 200℃. Gas N_2 was chosen as carrier gas and it went through three parts of the injector which were column, septum and by pass flow.

8.5 Experimental Results

Concentration of Extracts from extraction

The extraction solution was dried in an evaporator at around 35 ˚C overnight, the concentration of extractives was determined gravimetrically. The table 8.7 shows the extractive contents of solution obtained at the different operating conditions.

Table 8.7 Concentration of extractives obtained by drying in oven

Raw material	Solvent	Concentration
Pine saw dust	Hexane	0.2%
Pine stem chips	Hexane	0.3%
Pine root bark	Hexane	0.5%
Pine saw dust	Toluene	0.2%
Pine stem chips	Toluene	0.2%
Pine root bark	Toluene	0.6%
Pine saw dust	Diethyl ether	0.4%
Pine stem chips	Diethyl ether	0.6%
Pine root bark	Diethyl ether	0.9%

The Table 8.7 show the extractive did not reach concentrations above 1% in the pine wood extraction solution. It is difficult to distinguish the difference between extractives under various operating conditions. The testing content of extractive was pretty low in pine bark, pine stem chips and pine sawdust according to this analysis method. It is worth to mention that some extractives might evaporate during the drying process in the oven.

GC analysis results

The extractives from stump bark, stem chip and sawdust of pine wood had been performed by GC analysis method. The GC analysis time for the extractive solution is 30 mins. The difference between the effects of solvents on extractive is significant as shown in the Figure 10.1, Figure 10.2 and Figure 10.3.

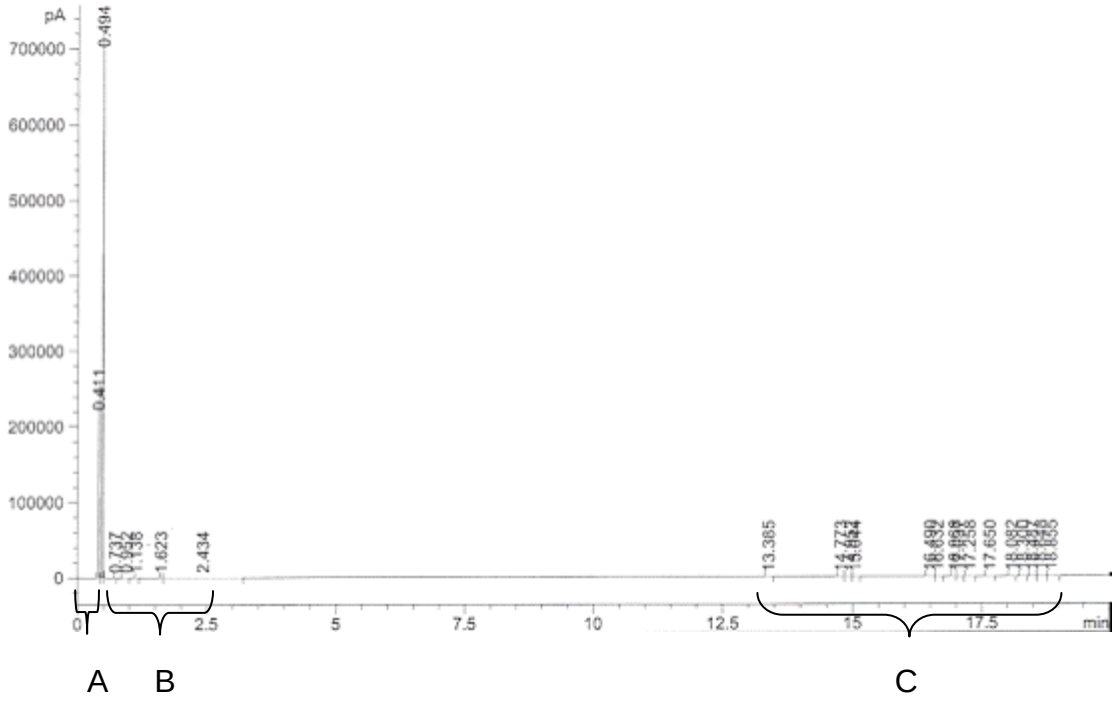


Figure 10.1 Gas chromatography analysis result of extractive from stem chip by hexane extraction through steel based reactor (2 hours)

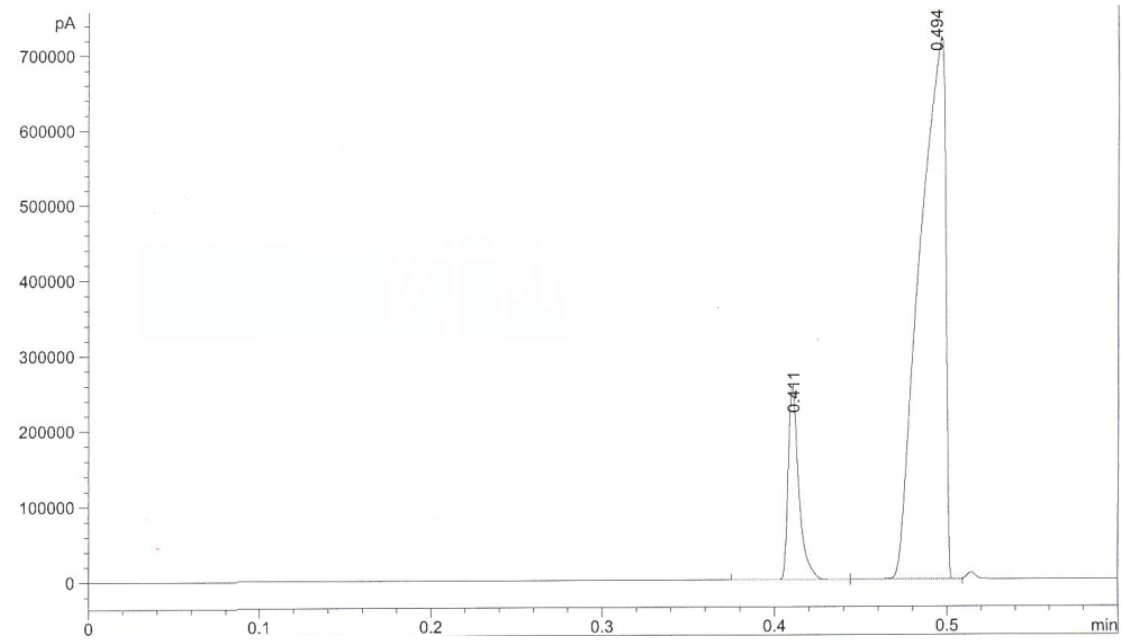


Figure 10.1 (A)

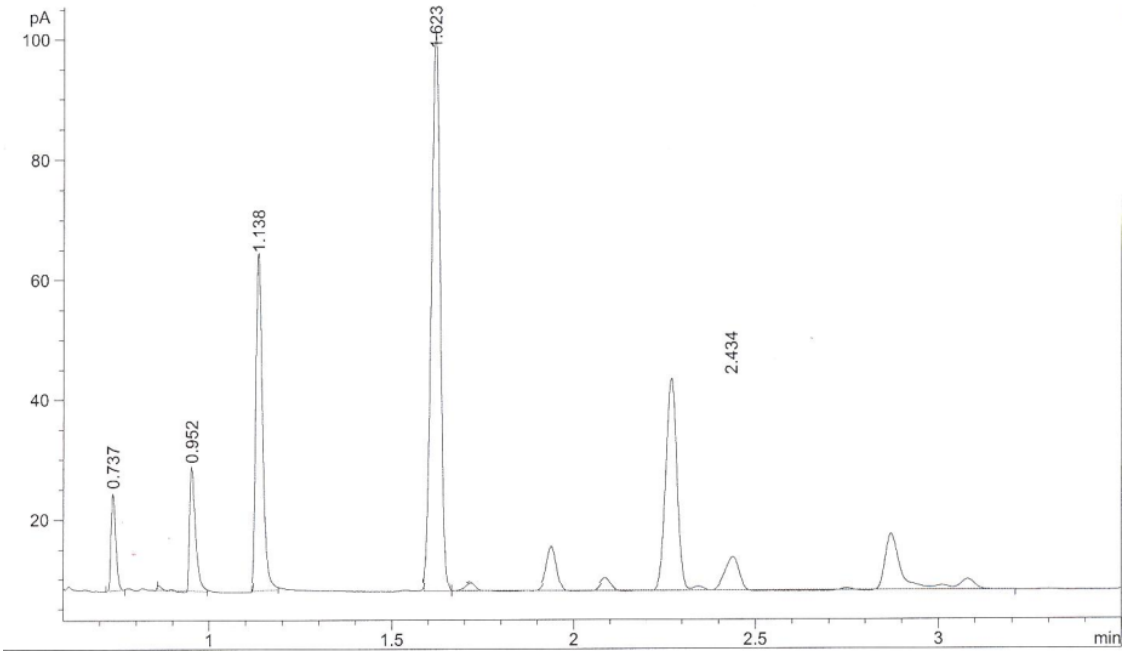


Figure 10.1 (B)

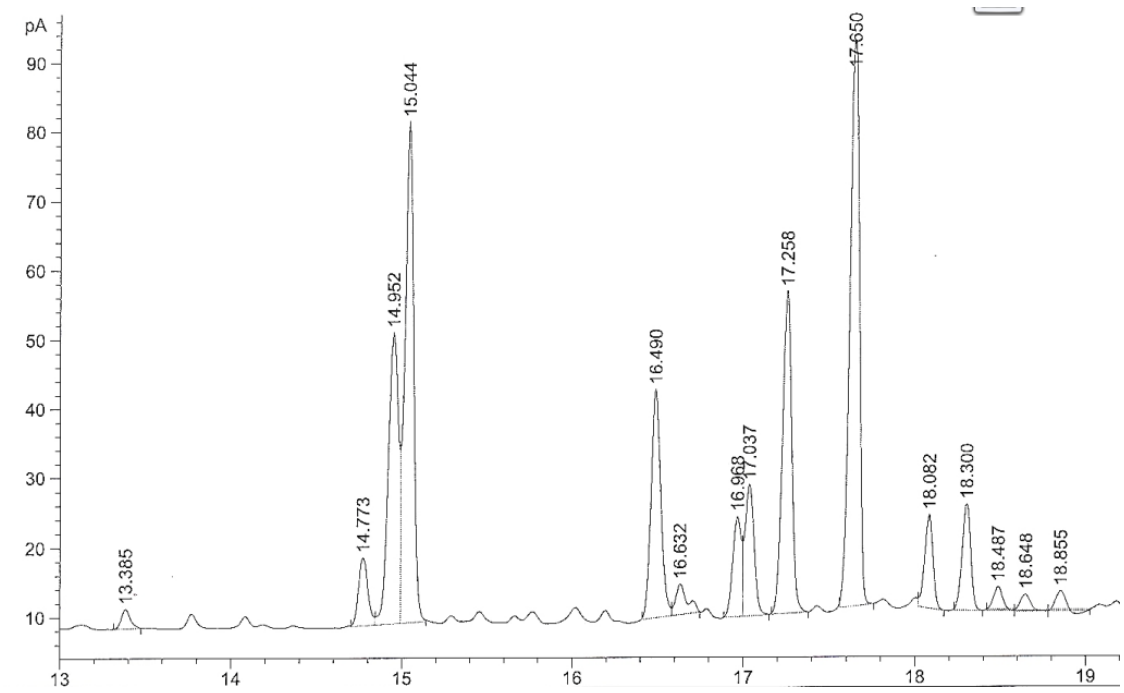


Figure 10.1 (C)

Fig. 10.1 shows that the certain chemicals were tested at the beginning analysis time, and also at the middle of analysis time. More chemical compounds were identified at the middle of analysis time. The detail pictures of the different retention time A, B, C could be seen more clearly peak profile. The extractives solution for this analysis was obtained with pine stem chip extracted with hexane.

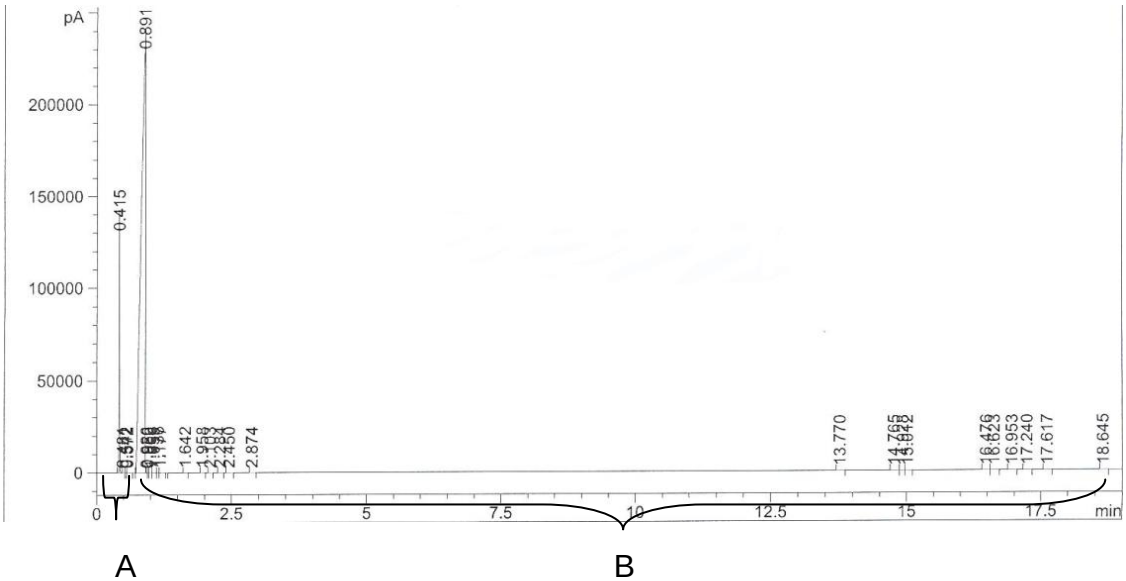


Figure 10.2 Gas chromatography analysis result of extractive from stem chip by toluene extraction through steel based reactor (2 hours)

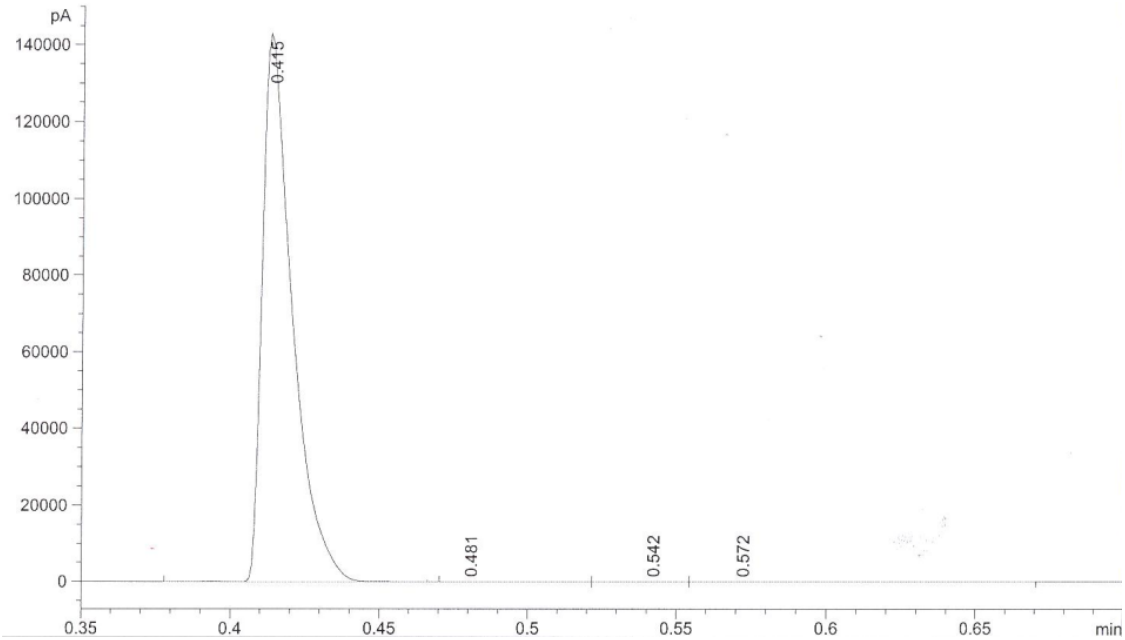


Figure 10.2 (A)

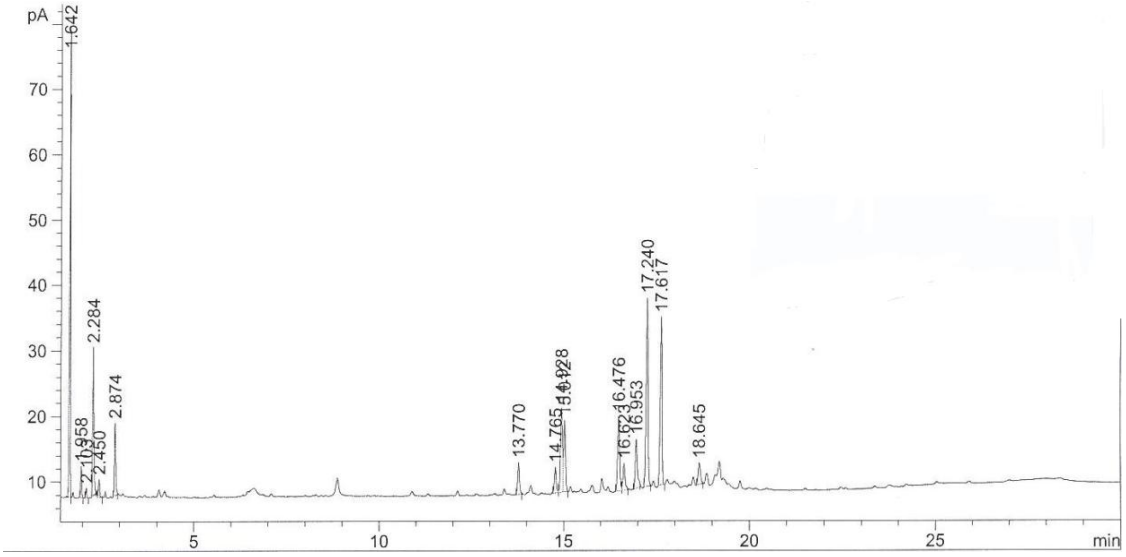
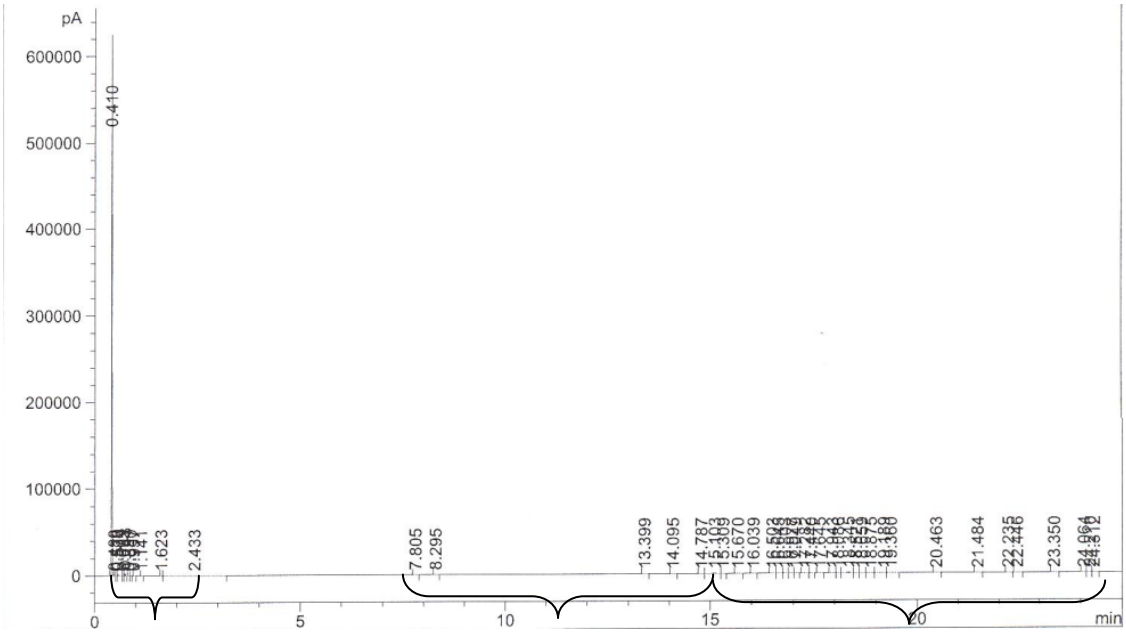


Figure 10.2 (B)

Fig. 10.2 shows that chemical compounds were identified at the beginning Figure 10.2 (A) and at the middle of retention time Figure 10.2 (B). The clear peaks profile of different analysis times were listed in the detail pictures A, B. The extractives solution for this analysis was achieved with pine stem chip extracted with toluene.



A B C

Figure 10.3 Gas chromatography analysis result of extractive from stem chip with diethyl ether extraction through steel based reactor (2 hours)

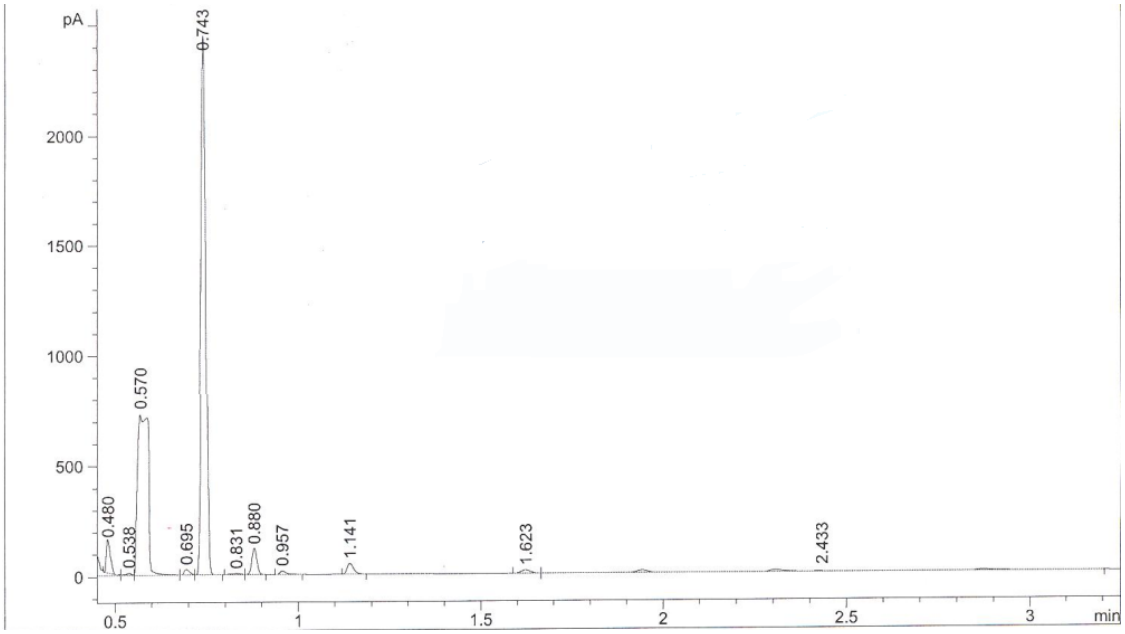


Figure 10.3 (A)

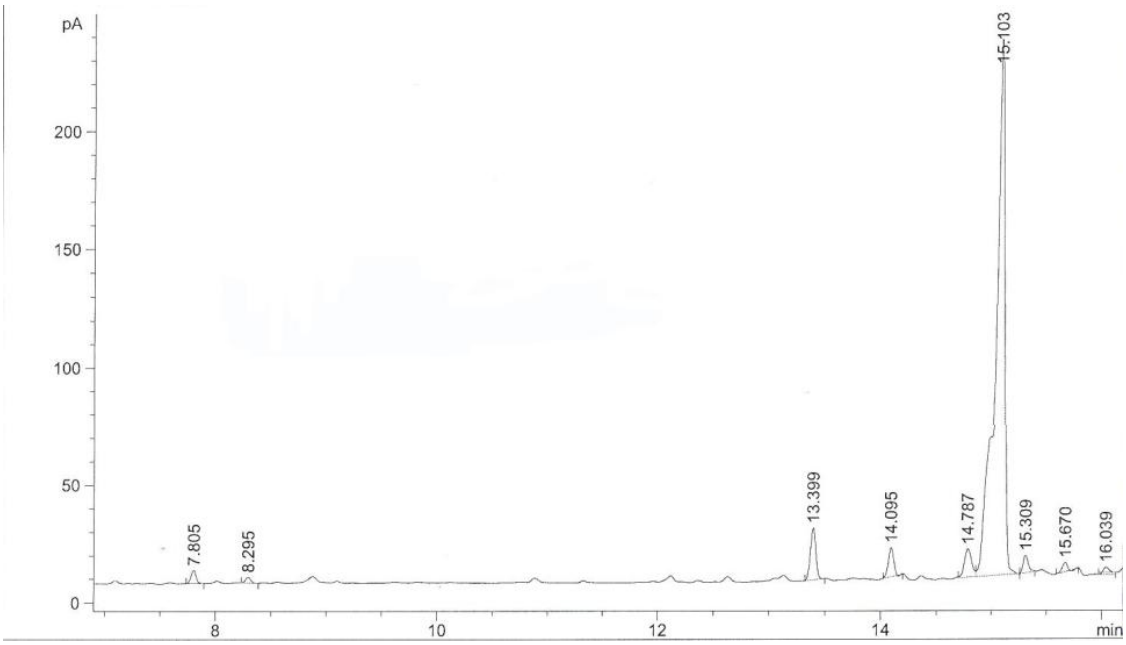


Figure 10.3 (B)

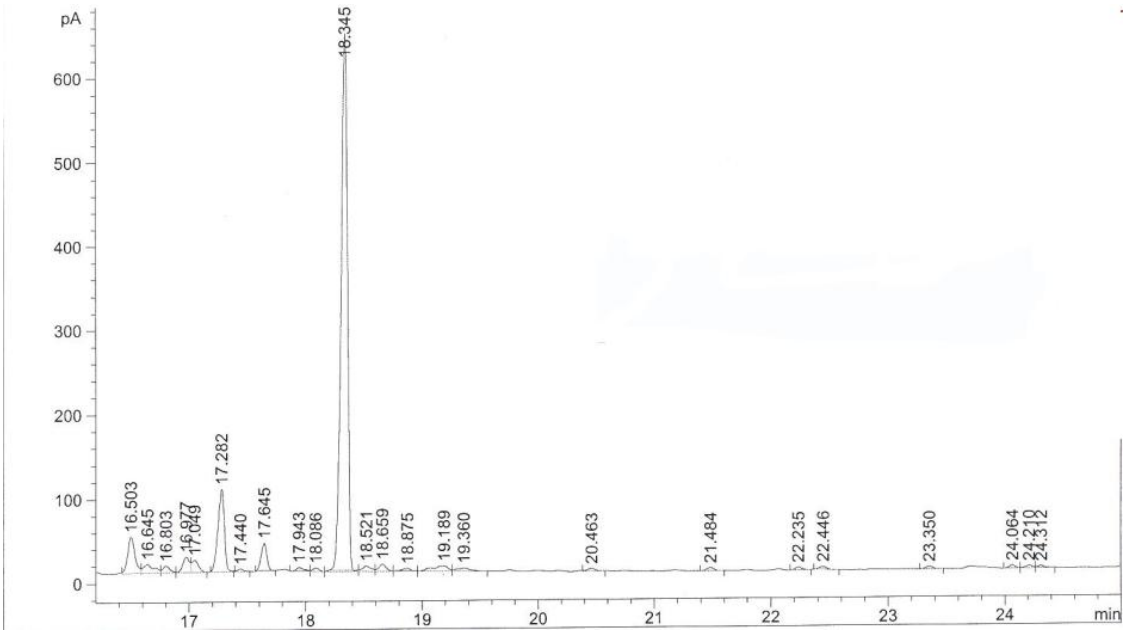


Figure 10.3 (C)

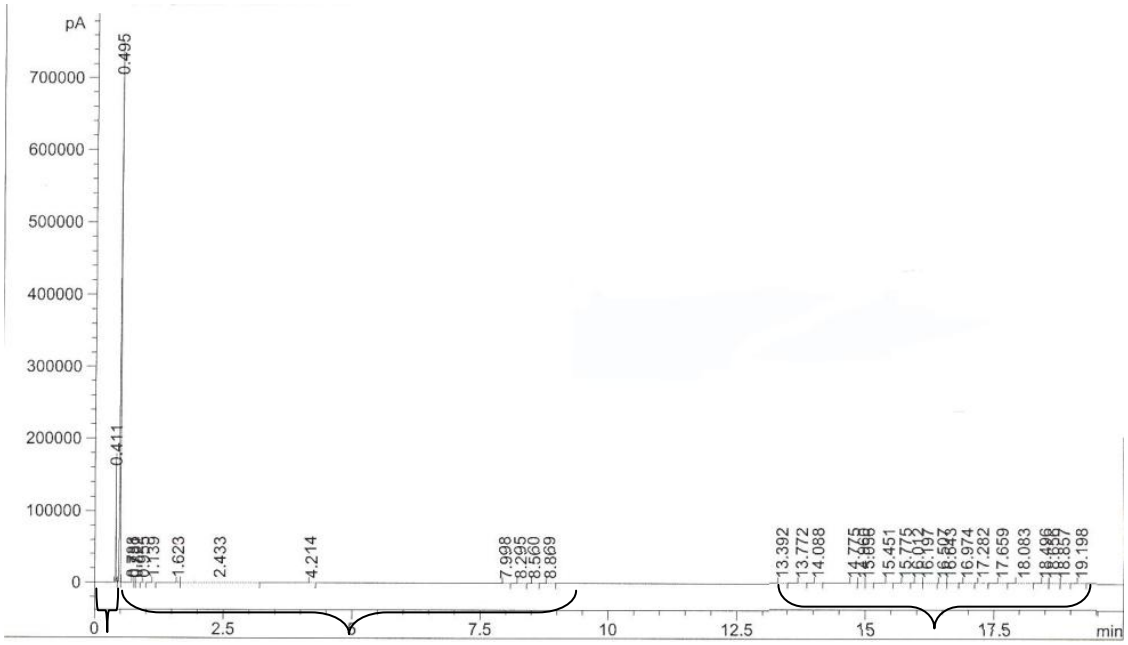
The clearly peaks profile of different analysis times were listed in the detail pictures A, B, C. For the pine stem chips, by using diethyl ether, more abundant extractive components might be obtained comparing with hexane and toluene.

The boiling points of different chemical compounds are listed in the Table 8.8. According to the boiling points, the operating column temperature and injector temperature were setting as 300°C during GC analysis. The different extractives have the different boiling points, and thus the chemicals with boiling point below 300˚C might be detected in GC analysis profile.

Table 8.8 Boiling points of chemical compounds (Ophardt 2003.)

Terpene type	Boiling point
Phenolic compounds	285°C- 320°C
Monoterpene	150°C-185°C
Sesquiterpene	223°C- 235°C
Diterpene	347°C-400°C
Triterpene (steroids)	71°C-210°C

For the different raw materials, e.g. pine saw dust, pine stem chip and root bark, the analysis results showed that root bark contained more abundant chemical compounds. The results can be compared from Figure 10.1 and Figure 10.4. However, the difference between the extractives profiles for pine stem chip and pine saw dust were relative small as shown in Appendix 1.



A B C

Figure 10.4 Gas chromatography analysis result of extractive from pine bark with hexane (2 hours 55 ˚C)

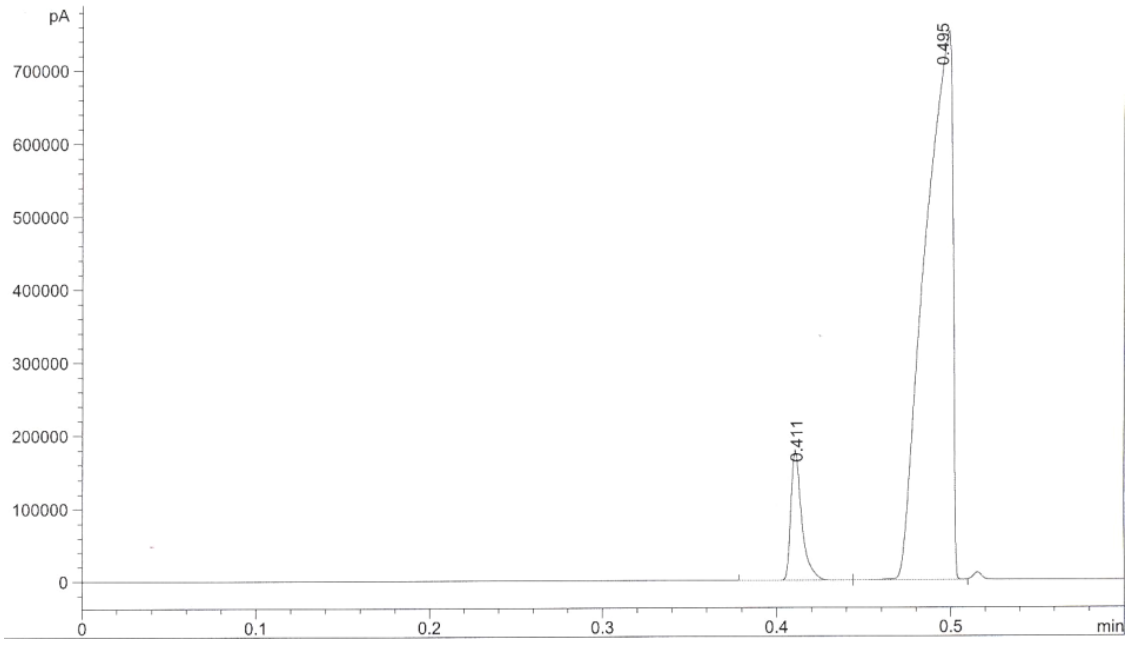


Figure 10.4 (A)

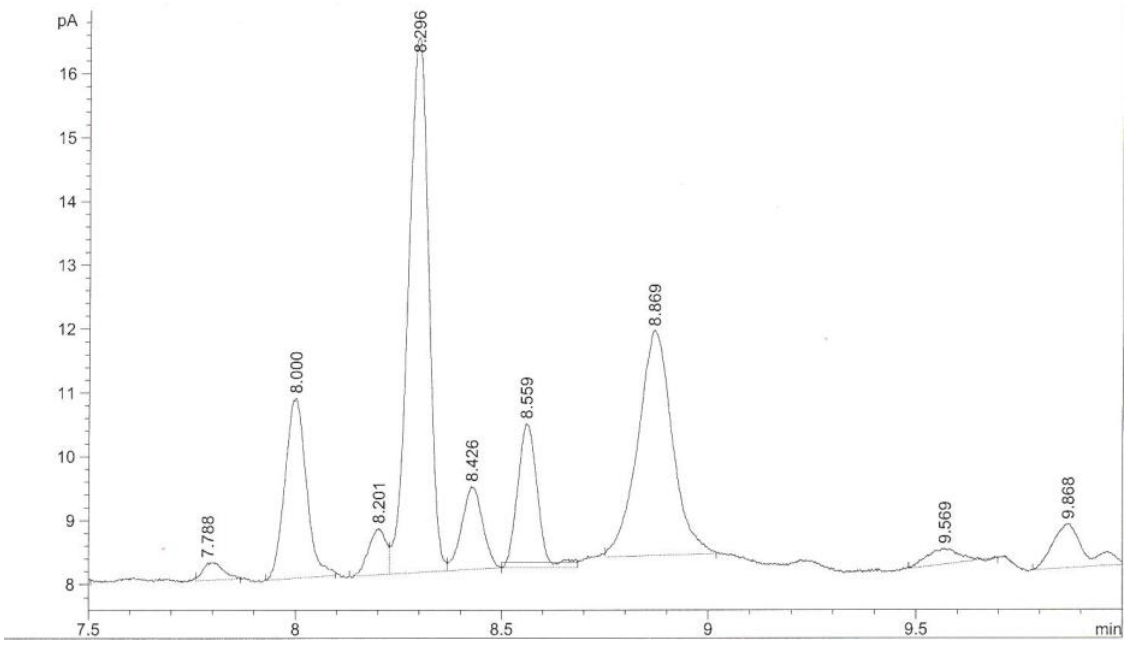


Figure 10.4 (B)

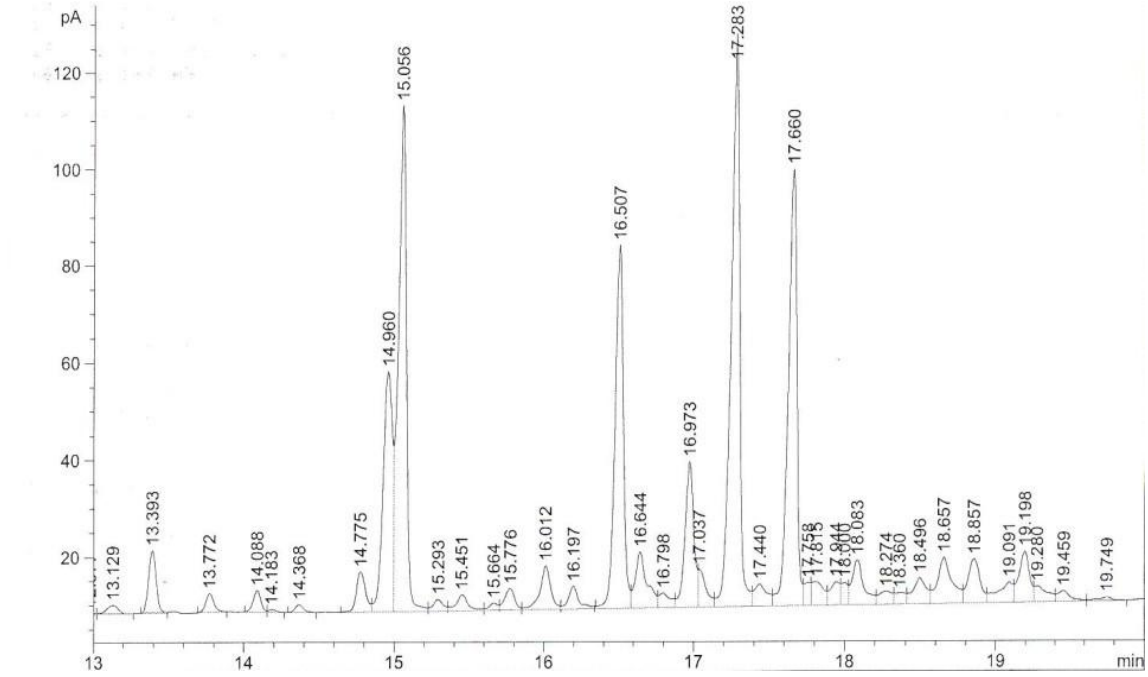


Figure 10.4 (C)

Fig. 10.4 shows that the certain chemicals were identified before 20 minutes. More chemical compounds were identified after 13 minutes. The detail pictures of the different retention time A, B, C could be seen more clearly peak profile. The extractives solution for this analysis was acquired with pine bark chip extracted with hexane

The GC analysis results show that the effect of operating temperature on extraction solution is also small. For example, the different raw materials of wood were extracted with toluene at the temperature of 55˚C and 90˚C, the GC analysis results show that the profiles are almost the same, as seen in Appendix 2. On the other hand, the different extraction equipments, e.g. flask reactor and steel-based reactor, also have no obvious effect on the extraction process, as seen in Appendix 3.

From GC analysis profile, the amount of extractives in the extraction solution could also be estimated, as seen in Table 8.9. It can be seen that the various solvent types used were crucial for extraction process. Solution treated with hexane and toluene solvents treatment could result in more concentration than that with diethyl ether.

Table 8.9 Concentration of extractives obtained by GC analysis

Raw material	Solvent	Concentration
Pine saw dust	Hexane	8.44%
Pine stem chips	Hexane	8.28%
Pine root bark	Hexane	8.76%
Pine saw dust	Toluene	6.57%
Pine stem chips	Toluene	6.95%
Pine root bark	Toluene	6.89%
Pine saw dust	Diethyl ether	1.81%
Pine stem chips	Diethyl ether	1.88%
Pine root bark	Diethyl ether	1.67%

To identify the single chemical component from extraction solution, the more detail analysis work should be done considering the property of the chemicals and also need to take the suitable standard solution as reference. For example, extractives compounds can be identified by comparing their analyzed spectra results with the library published spectra or with the spectra obtained from

standard compounds. This part of experimental work was not included in this experiment work.

9. SUMMARY

The purpose of this study is to know the chemistry of wood, especially, the extractives of wood by literature study. On the other hand, experimental work was carried out to investigate the isolation of extractives from woods. The three types of raw material i.e. pine saw dust, pine bark and pine stem chips and the three non-polar organic solvents, i.e. Hexane, Toluene and Diethyl ether, were used in the experimental work. Also, the different operating conditions, e.g. extraction time, extraction temperature and extraction equipment, were tested. The obtained extraction solution was analyzed by gas chromatography method.

The experimental results revealed that different non-polar solvents have significant effect on extraction results. Also, for the fixed solvents, the various raw materials also resulted in the different extraction solution due to the different raw material might contain different types of extractives. However, the temperature, time and equipment have no obvious effect on the extraction results from GC analysis profile, but might have the influence on the total amount of extracts.

The GC analysis identified different chemical compounds based on the different boiling points and structure of chemicals, the adsorption ability of chemical compounds also affect the retention time. The injector temperature was setting as 300°C according to the boiling points of chemicals. For the extractives with higher boiling points, certain chemicals need to be added. Therefore, the lower boiling points of extractive chemicals e.g. Phenolic compounds can be achieved .

The work provides the basic information to obtain the extractives from wood with organic solvents, and therefore, the suitable experimental conditions can be selected further when the work focus on the certain types of extractives.

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Appendix 1

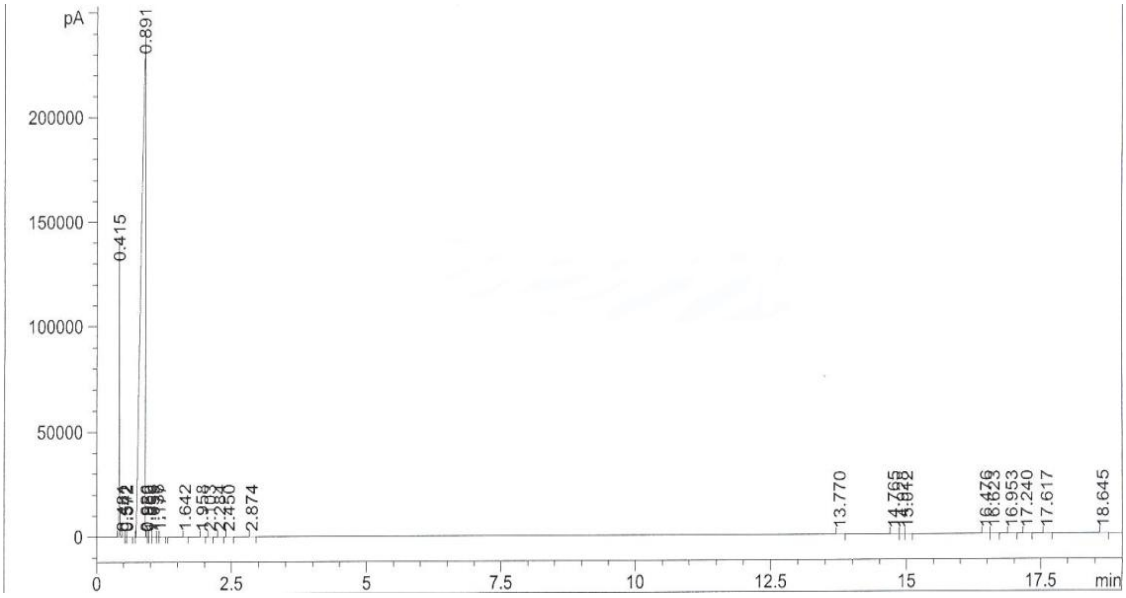

Figure 10.5 Gas chromatography analysis result of extractive from pine stem chip by toluene extraction through steel based equipment (2 hours)

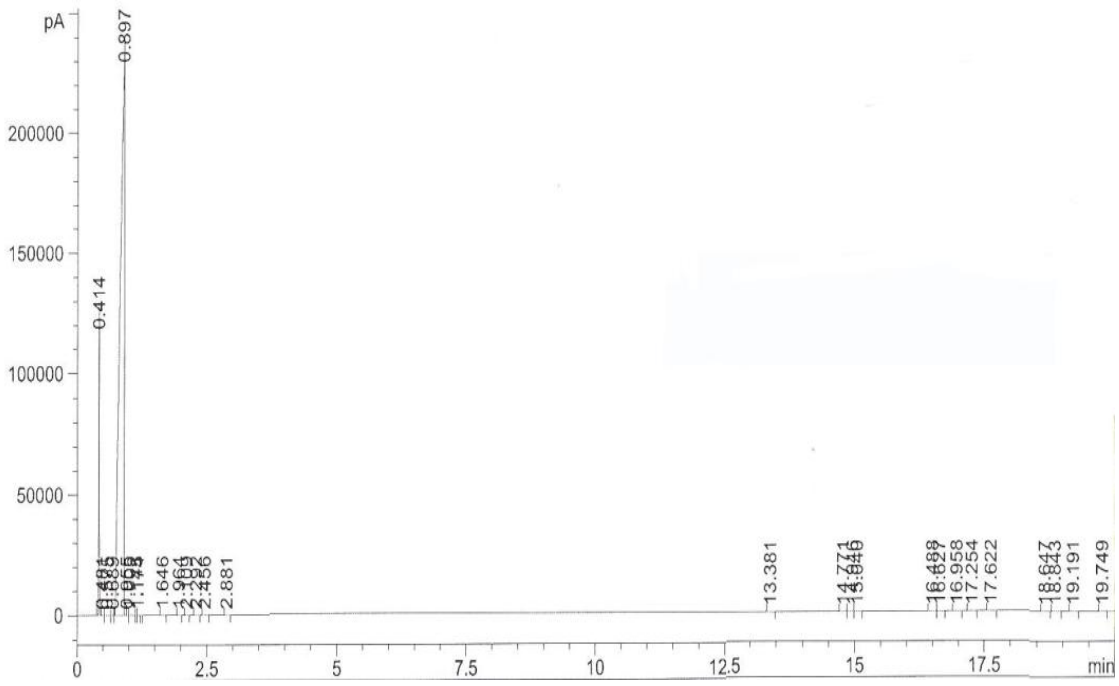

Figure 10.6 Gas chromatography analysis result of extractive from pine sawdust by toluene extraction through steel based equipment (2 hours)

Appendix 2

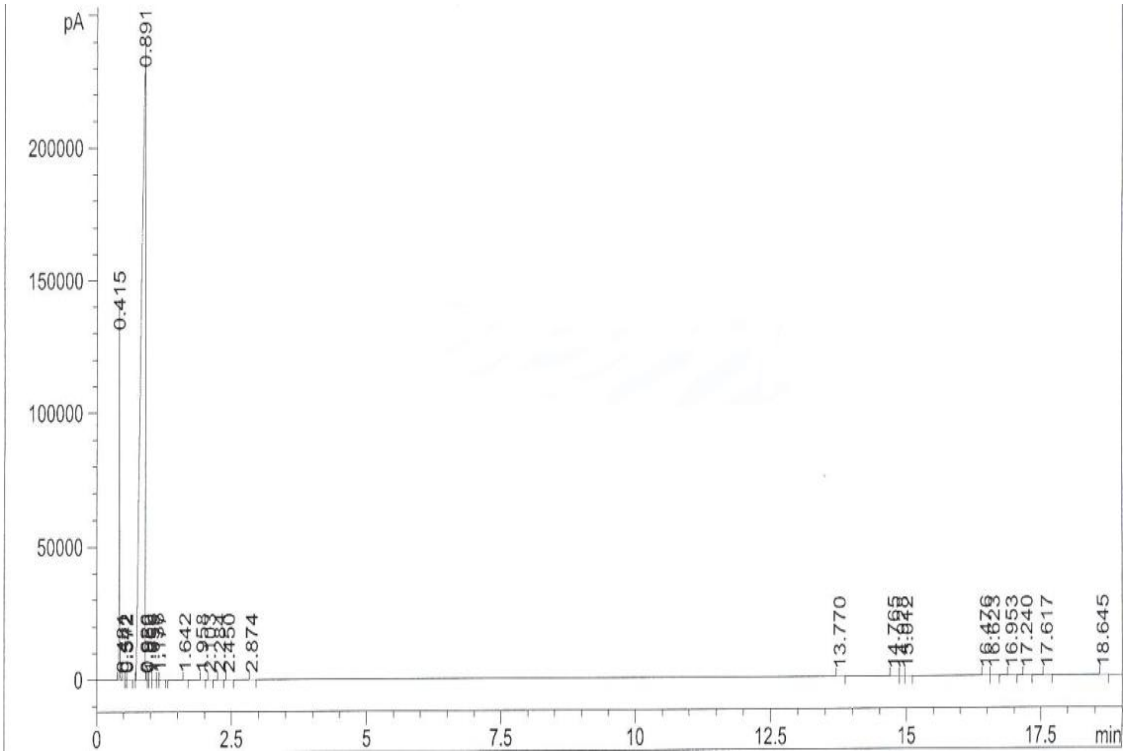

Figure 10.7 Gas chromatography analysis result of extractive from stem chip by

toluene extraction through steel based equipment (55 °C)

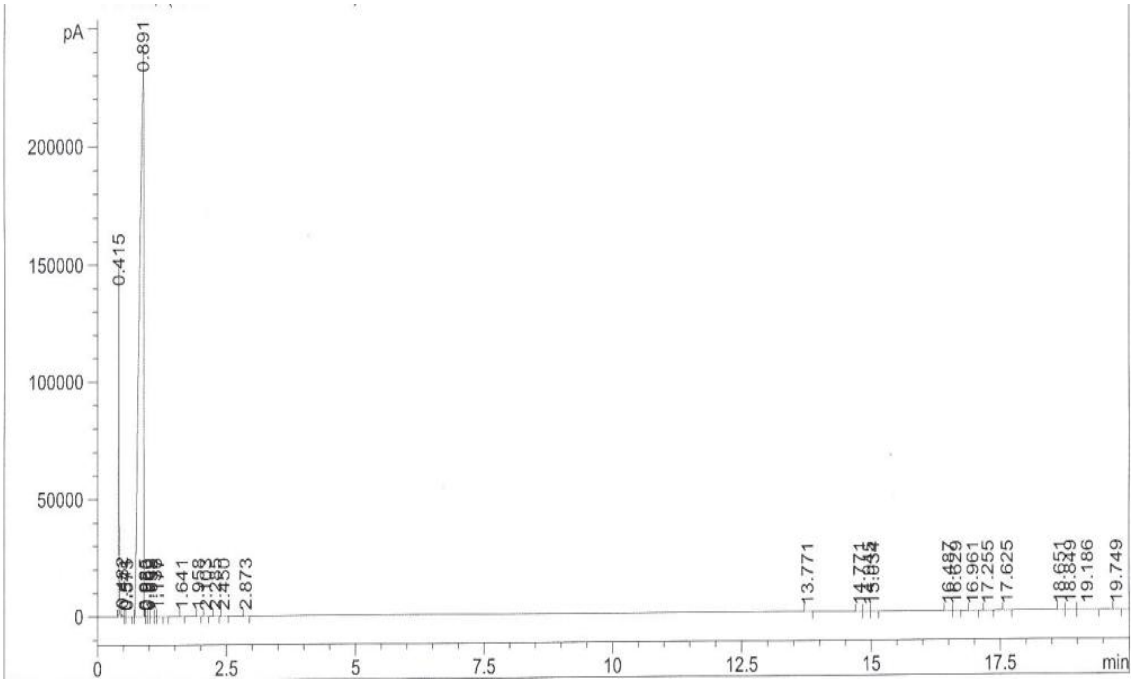

Figure 10.8 Gas chromatography analysis result of extractive from stem chip by toluene extraction through steel based equipment (90 °C)

Appendix 3

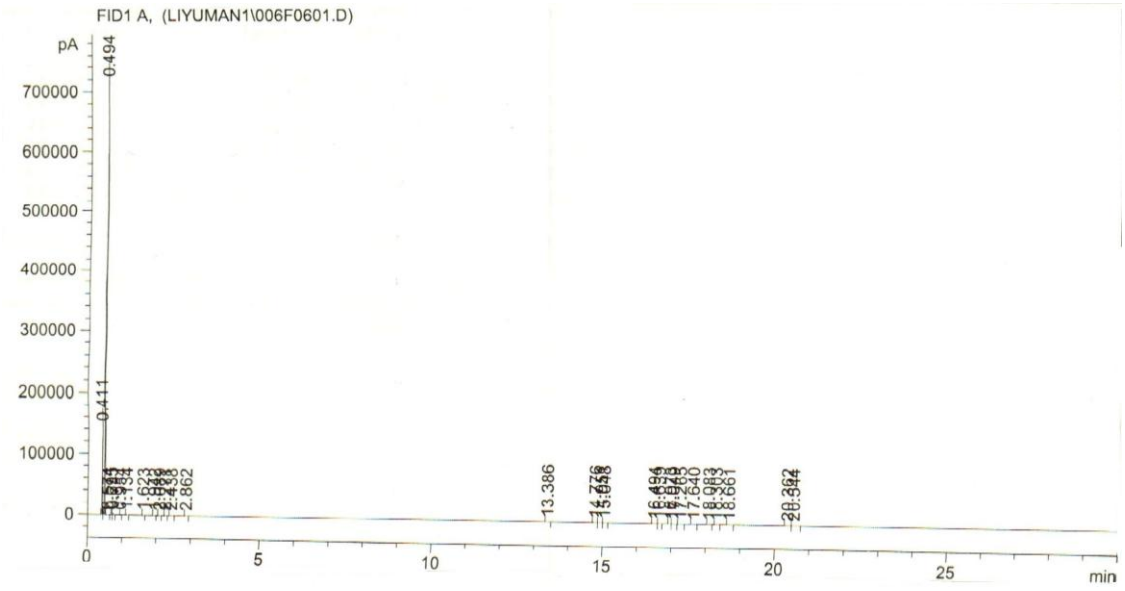

Figure 10.9 Gas chromatography analysis result of extractive from stem chip

by hexane extraction through boiling glass equipment

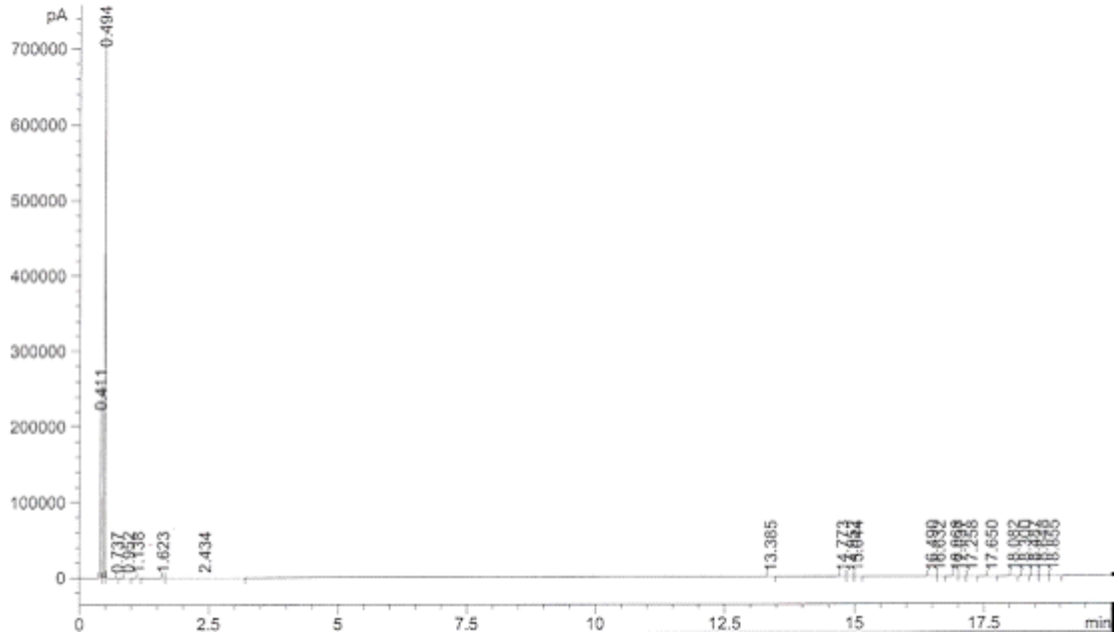

Figure 10.10 Gas chromatography analysis result of extractive from stem chip by hexane extraction through steel based equipment

FIGURES

TABLES