Saimaa University of Applied Sciences
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EXTRACTION OF SCOTS PINE BY POLAR SOLVENT

Bachelor's Thesis 2011

ABSTRACT

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Extraction of Scots Wood by Polar Solvent 62 pages, 4 appendices
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The aim of this thesis was to study, isolate, and analyze the extractives from pine chips, saw dust, root chips, and root barks by polar solvent such as water, acetone, and ethanol. The methods used in the extraction were boiling flask or rotation reactor. The different extraction time of 2 or 4 hour was also investigated. The obtained extraction solution were analyzed by gas chromatography.

In order to understand the experimental work theoretically, the wood chemistry, especially, phenolic extractives of wood was studied from literature. Phenolic extractives are a very large group of chemicals, which consists of many different families of aromatic secondary metabolites in plant. The application of phenolic extractives from wood was widely investigated by the different research groups.

The result showed that extracted at same condition with different raw material, the content of extractives from pine root bark is more than other raw materials. And the longer the extract time the more chemicals can be analysis by the GC analysis from the extracted samples. However, there has no big difference on the content of the extractives where the treatment organic solvent was different.

Key words: Phenolic Extractives, Gas Chromatograph, Scots Pine, Polar solvent

CONTENTS

1 INTRODUCITION

Wood is the most abundant and versatile natural materials, which has the wide application to support our life. However, the chemical components in the wood is quite complex and even has not been well understood nowadays. Recent year, wood-based chemicals were widely studied with the aim to obtain the valuable product by biotechnology. The wood is essentially composed of four major components, which are cellulose, hemicelluloses, lignins and extractives. Study should be focus on these components in order to use wood benefited.

The aim of literature study in this thesis is to learn the different types of phenolic extractives and their applications. Phenolic extractives are a very large group of chemicals, which consists of many different families of aromatic secondary metabolites in plant, and therefore, the different types of phenolic extractives like stilbenes, lignans, tannins, flavonoids are mainly introduced.

In the experiment part, different equipment, polar solvent, temperature, and time are used to isolate the extractives from different part of the pine wood, such as pine chips, saw dust, root chips, and root barks. The solvent selected for the experiment are acetone, water, and ethanol. The liquor samples from the treatment are analyzed in the gas chromatography. The concentration of the extractives and identified profile from GC are compared according to the different conditions.

2 WOOD CHEMISTRY

Wood logs are the principal strengthening and nutrient-conducting tissue of trees, and wood is one of the most abundant and versatile natural materials. Wood species commonly can be divided into softwood and hardwood. To hardwood is belonging to angiosperm trees and to softwood is belonging to conifer trees. In both groups there is an enormous variation in actual wood hardness. The range in density in hardwoods completely includes that of softwoods. (Stenius 2000, p.1.) Wood is anisotropic material in nature. Its appearance and physical properties vary according to its sectioned plane. The macrostructure of wood is shown in figure 2.1.

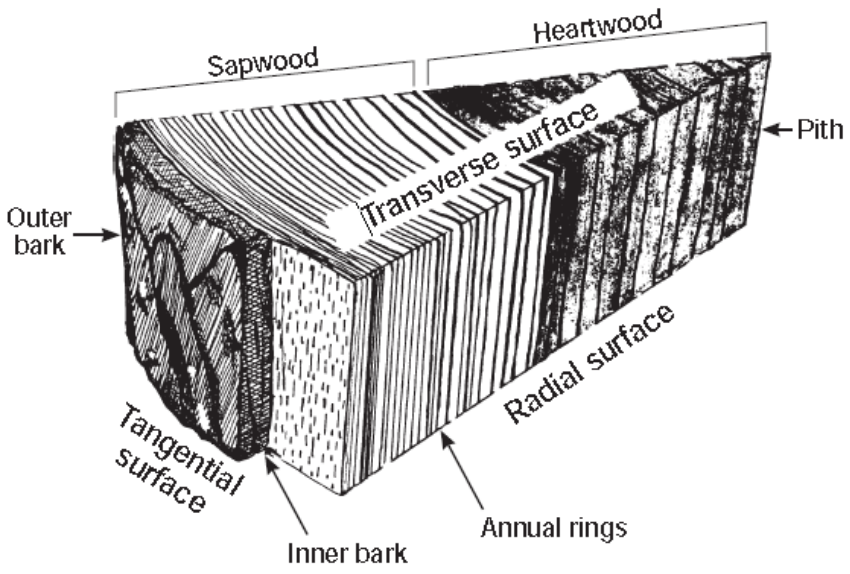


Figure 2.1 The transverse tangential and radial surface of wood (Kellomäki 1998, p. 139)

As can be seen from the figure 2.1, the wood can basically be separated into three parts – pith, wood and bark. The cambium zone which is not visible with the naked eye is between the inner bark and the wood. Pith is a very small part

of the wood and it is located in the middle of the transverse surface of wood. Wood is marked by the presence of concentric layers known as growth rings. (Haygreen & Bowyer 1996.)

Heartwood is wood that as a result of a naturally occurring chemical transformation has become more resistant to decay. Heartwood formation occurs spontaneously. Once heartwood formation is complete, the heartwood is dead. Some uncertainty still exists as to whether heartwood is truly dead, as it can still chemically react to decay organisms, but only once. (Shigo 1986, p. 54.)

Sapwood is the younger, outermost wood; in the growing tree it is living wood, and its principal functions are to conduct water from the roots to the leaves and to store up and give back according to the season the reserves prepared in the leaves. However, by the time they become competent to conduct water, all xylem tracheids and vessels have lost their cytoplasm and the cells are therefore functionally dead. All wood in a tree is first formed as sapwood.

Bark is the outermost layers of stems and roots of woody plants. Plants with bark include trees, woody vines and shrubs. Bark refers to all the tissues outside of the vascular cambium and is a nontechnical term. It overlays the wood and consists of the inner bark and the outer bark. The inner bark, which in older stems is living tissue, includes the innermost area of the periderm. The outer bark in older stems includes the dead tissue on the surface of the stems, along with parts of the innermost periderm and all the tissues on the outer side of the periderm. (Helena, 1981, p. 641)

Wood is one of the most abundant resources in the bio-based industry. However, it is also one of the most complex materials. Wood is composed of polymers,

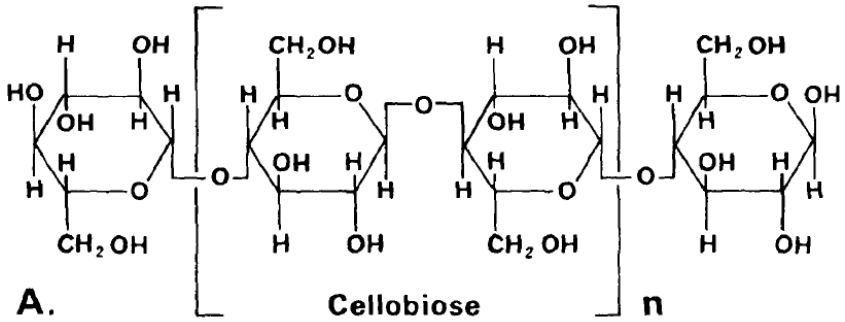
which are large organic molecules of lignin and carbohydrates that are physically and chemically bound together. Wood is essentially composed of four major components, which are cellulose, hemicelluloses, lignin, and extractives. Table 2.1 shows the major chemical compositions of some wood species. Each of these components contributes to the properties of the wood. However, the amount of extractives compounds which are found in wood is small. The extractives contribute to some properties of the wood such as colour, odour, durability, permeability and basic density. **(**Cassidy & Ashton 2007, p.169.)

Table 2.1 Chemical Composition of Some Wood Species (San Diego 1993)

Constituent	Pine	Spruce	Eucalyptus	Birch
Cellulose (%)	40	39.5	45.0	41.0
Hemicelluloses (%)	28.5	30.6	19.5	32.4
-Glucomannan (%)	16.0	17.2	3.1	2.3
-Glucuronoxylan	8.9	10.4	14.1	27.5
-Other polysanccharides (%)	3.6	3.0	2.3	2.6
Lignin (%)	27.7	27.5	31.1	22.0
Total extractives (%)	3.5	2.1	2.8	3.0

Celluloses

The cellulose is composed of carbon, hydrogen, and oxygen with the form of sugars, proteins and starches. It is the most affluent organic compound in the world, comprising 40%–50% of woody plant composition by weight. (Smith et al, 1994) Association with hemicelluloses and lignin, cellulose occurs mainly in the secondary cell wall. It is a polysaccharide chemical which has high resistance. (Browning 1967.)



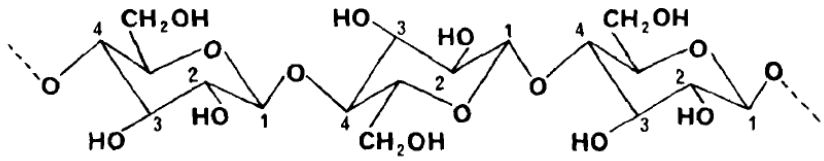


Figure 2.2 The chemical structure of a cellulose molecule as a linear polysaccharide of glucose units (Fogelholm 2000)

The figure 2.2 shows that the cellulose is a linear polymer composed of glucose units. Figure 2.2 A shows the simplified diagram, where the degree of polymerization in wood is about 10,000n. Figure 2.2 B shows the chair conformation of the glucose units and the b-1,4 linkage that cellulose polymer adopts in space. Cellulose molecules can subsist in either amorphous or crystalline form. (Fogelholm 2000.)

Hemicelluloses

Different from cellulose, hemicelluloses are comprised of carbohydrates with the polymer form of pentose, xylose, and hexose sugars. The hemicelluloses consist of 25%-35% dry weight of the wood. The proportion of hemicelluloses in softwood is samaller than that in hardwood. Hemicelluloses are relatively easily hydrolyzed by acids to their monomeric components consisting of D-glucose,

D-mannose, D-xylose, L-arabinose, and small amounts of L-rhamnose. (Sjöström 1993, p.60.)

The chemical and thermal stability of hemicelluloses is generally lower than that of cellulose, presumably due to their lack of crystallinity and lower degree of polymerization. In addition, hemicelluloses generally differ from cellulose with respect to their solubility in alkali. This characteristic property is most commonly utilized when fractionating different polysaccharides in lignin-free samples. It should be pointed out that some hemicelluloses, such as fragments of hardwood xylan and the arabinogalactan, especially from larch species are partly or even totally water-soluble. Therefore, in these special cases, the distinction between water-soluble hemicelluloses, sugars (mainly mono- and disaccharides), and extractive-derived compounds is sometimes difficult. (Stenius 2000, p. 35.)

Lignin

Lignin is most commonly derived from wood which is a complex chemical compound. Lignin is the main part of the secondary cell walls of wood. And lignin has been described as a random, three-dimensional network polymer comprised of variously linked phenylpropane units. Lignin is also the abundant biological material on the planet, exceeded only by cellulose and hemicelluloses. 20%-30% of the wood structure is made up of lignin depending on the species of the wood. It is the cementing agent which binds the individual wood fibers together to form a substance of strength and hardness. (Sjöström 1993, p.293.)

Generally, lignins are roughly divided into three major groups: softwood, hardwood, and grass lignins. And lignin is a quite insoluble compound. The basic structure of lignin is consist of phenylpropane with a phenol ring which is

substituted by zero, one or two methoxyl groups. Phenol ring substituted with one methoxyl group produces guaiacyl unit, and phenol ring substituted with two methoxyl groups generate syringyl unit. A guaiacyl lignin exists in softwoods and a guaiacyl-syringyl lignin is in hardwoods. (Sarkanen & Ludwing 1971, p.916.)

Table 2.2 Average Klason lignin content in softwood and hardwood of different countries. (Pettersen 1984, p.614.)

Country	Softwoods	Hardwoods
	Average content of Klason lignin, %	
USA	28.8±2.6	23.0±3.0
Former USSR	29.0±1.6	21.9±3.2
Japan	29.6±2.6	22.1±3.0

The record in table 2.2 shows the average klason lignin content for a large number of softwood and hardwood in different countries. It generally shows that lignin content in softwoods is higher than that in hardwoods (Pettersen 1984, p.614).

2.2 Wood Extractives

Extractives are kinds of organic compounds including fats, waxes, alkaloids, proteins, simple and complex phenolics, simple sugars, pectins, mucilages, gums, resins, terpenes, starches, glycosides, saponins, and essential oils. Although the extractives are not a structural part of the woody tissue, they contribute to many properties of wood, for example colour, basic density and durability. Different extractives can be found in different types of wood. (Semester 2000)

Wood extractives can chemically be classified into several different groups of compounds. In table 2.3 shown the main classification of extractives in woods. The main constituents can be divided into aliphatic and alicyclic compounds, phenolic compounds, and some other compounds. (Stenius 2000, p.44.)

Table 2.3 Classification of organic Extractives in woods (Per Stenius 2000)

Lipophilic compounds	Phenolic compounds	Other compounds
Terpenes and terpenoids	Simple phenols	Sugars
(including resin acids and	Stilbenes	Cyclitols
steroids)	Lignans	Tropolones
Esters of fatty acids	Isoflavones	Amino acids
(fats and waxes)	Condensed tannins	Alkaloids
Fatty acids and alcohols	Flavonoids	Coumarins
Alkanes	Hydrolyzable tannins	Quinones

The content of extractives and the quality of them depend on the type of tree, surrounded environment, and growth conditions. For example, the typical content of extractives in Scots pine is in the range of 2.5 to 4.5%, in Norway spruce is 1.0 to 2.0%, and in silver birch is 1.0 to 3.5%. Although the amount changes in different species, the content of extractives in hardwood is usually more than that in softwood. (Kellomäki 2009, p.150.)

The compounds are present in trees that can be removed by hot and cold water, petroleum ether, ethyl ether, ethylene dichloride, dichloromethane, ethanol, or acetone. All the extractives cnnnot be extracted by one single solvent. It is necessary to use an order of two or more solvents for reasonably complete removal of extractives. (Browning 1967, pp.75- 77.) For some technical aspects the extractives are important. The extractives compose valuable raw material for

making organic chemicals. And for pulping and papermaking process some kind of extractives will play a very important role. (Stenius 2000, p 44.)

2.2.1 Lipophilic Extractives

Lipophilic extractives in wood are complex mixtures which include many different compounds. For example, fatty acids, resin acids, waxes, alcohols, terpenes, sterols, sterol esters and glycerides. The lipophilic extractives, in particular, can be responsible for the formation of sticky deposits on machinery or give rise to dark spots in bleached pulp and paper, commonly known as pitch. (Sjöström 1993.)

Terpenes are the major compounds of lipophilic extractives. Terpenes and their derivatives consist of a large group of compounds with widespread appearance in the plant kingdom. Their basic structural unit of terpenes is isoprene. And they can be divided into subgroups on the basis of the number of isoprene units linked in a terpene. The classification of the main terpene structural types in wood are monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), polyterpenes ($C_{40}H_{64}$). Among these subgroups, the monoterpenes and sesquitepenes are of major industrial importance. (Stenius 2000, p 45.)

Alkanes, fatty alcohols, fatty acid, fats, and waxes are also existed in lipophilic extractives of woods. However, wood only has small amount of alkanes, free alcohols, and free fatty. The major part of the fatty acids in wood are esterifies with glycerol or with higher fatty alcohols. (Stenius 2000, p.49.)

2.2.2 Phenolic Extractives

Phenolic extractives are a very large group of chemicals, which consists of many different families of aromatic secondary metabolites in plant. Phenolic extractives exist in wood reaching from simple phenols to complex polyhenols and their related compounds. It is characteristic for polyphenols that they often are colored compounds. Phenolic ectractives are plentiful in the heartwood of many species, but only small amount of it can be found in the sapwood based on the literature study until now. (Stenius 2000, p 50.)

The phenolic extractives can be classified into non-soluble compounds such as condensed tannins, lignans, cell-walls bound hydroxycinammic acids, and soluble compounds such as phenolic acids, phenylpropanoids, flavonoids and quinines. (Williams 2000.) They are broadly distributed in the plant kingdom with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colors. (Dai & Mumper 2010.)

The phenolic extractives play a major role in the interaction of plant with their environment. They have fungicidal properties; these properties are used to protected trees against microbiological attack. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects. (Knogge & Weissenböck 1986)

3 TYPES OF PHENOLIC EXTRACTIVES

Phenolic extractives include phenolic acids, tannins, flavonoids, stilbenes, and lignans. The biosynthesis of extractives is controlled genetically and hence each wood species tend to produce specific substances. Hardwood contains a large number of phenolic substances, because of the secondary metabolites changes. It can be seen from the chemotaxonomical point that chemical structure of various flavonoids, lignans, stilbenes, and tropolpnes are of great interest. For example, according to the composition of the substances, species within families of Taxodiaceae, Cupressaceae, and Pinaceae and within genera of Pinus, Acasia, and Eucalyputs can be classified. (Sjostrom 1993, p.105.) The major different types of phenolic compounds in wood are introduced in the following text.

3.1 Stilbenes

For a long time, stilbene has been very important in heart wood resistance to fungal decay. Stilbenes are diphenylethylenes which can be found in many kinds of plant families, but commonly they occur in wood, bark and leaves of the forest trees. Almost all stilbenes which can be found in wood have a resorcinol-A ring. (Hart 1966.) Stilbenes derivatives of 1,2-diphenylethlene, process a conjugated double bond system. There are two isomeric forms of 1,2-diphenylethylene: trans-stilbene and cis-stilbene, And the chemical structure of these two stilbenes are shown in figure 3.1.

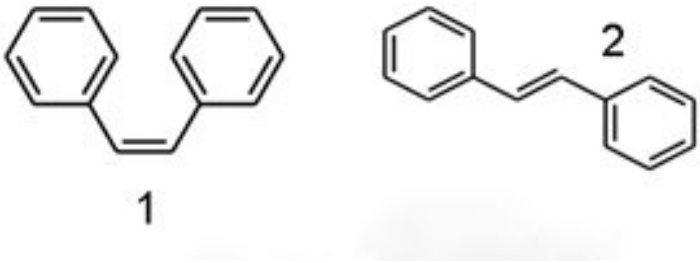
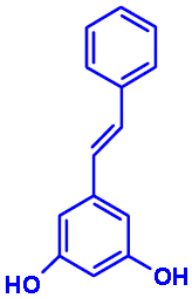


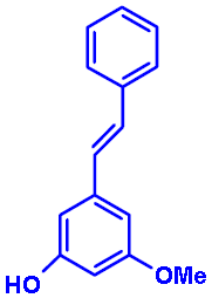
Figure 3.1 The chemical structure of stilbenes: cis-stilbene (1), and Tran-stilbene (2). (Hart 1966.)

The typical subgroup of stilbenes is pinosylvin, it can be found in pine at present. Pinosylvin is a typical member of stilbenes. This compound is present in pine wood, especially in heart wood and knotwood. In Table 3.1 shows the main member of stilbenes in Scots pine wood.

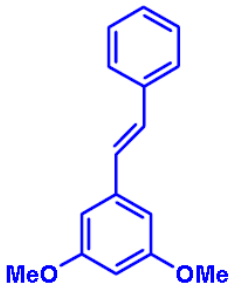
Table 3.1 Stilbenes in Scots Pine Wood (Willför et al.)



Pinosylvin (PS)



Pinosylvin monomethyl ether (PSMME)



Pinosylvin dimethyl ether (PSDME)

	PS (mg/g)	PSMME (mg/g)	PSDME (mg/g)	Other stilbenes (mg/g)
Sapwood	0.11-0.14	< 0.05	< 0.05	< 0.05
Heartwood	3.7-5.5	5.1-6.3	< 0.14	0.15-0.27
Knotwood*	4.5-19	13-49	0.09-0.89	0.14-2.6

* Knot with a dead branch

It has been found that pinosylvin is more effective in inhibiting fungal growth and wood decay. In Pinus, the sapwood of non-stressed trees is normally free of stilbenes, conversely the biotic stress contains the synthesis of pinosylvin (PS), pinosylvin monmethyl ether (PSM), and their glucosides. Pinosylvin and pinosylvin monmethyl ether are the major secondary phenolic compound in Scots pine heartwood. (Johnk et al. 1987.)

3.2 Lignans

Lignans are a group of compounds which can be found in plants. Mostly lignans are present found in nature in the free form. They constitute a complex family of skeletons and functionalizations. In the plant kingdom the lignans are widely distributed, and they can be found in species belonging to more than seventy families being represented in pterdophytes, gomnosperms and angiosperms. (Slanina & Glatz 2004.) One of the richest sources of lignans in the plant kingdom is the Norway spruce. High concentration of lignans can be found in the heartwood of branches and knots of the Norway spruce. Norway spruce knots comprise a portion of side branches embedded in the wood of stems or larger branches. Spruce knots consist of 6% to 16% of lignans. (Karst 1988.) Lignans are very important to protect the plant from different biological pathogens and pests.

Lignans are a group of secondary plant metabolites and produced by oxidative dimerization of two phenylpropanoid units. The major member of lignans group which exist in wood are pinoresinol, conidendrin, plicatic acid, and hydroxymatairesinol. The chemical structures of these compounds is shown in figure 3.2. (Sjostrom 1993.) At present, lignans which are related to conidendrin as shown in figure 3.2(2) exist in hemlock and spruce species. However, plicatic

acid can be found in western red cedar. Hydroxymatairesinol, which exists in two stereoisomeric forms that are abundant in Norway spruce. (Sjostrom 1993.)

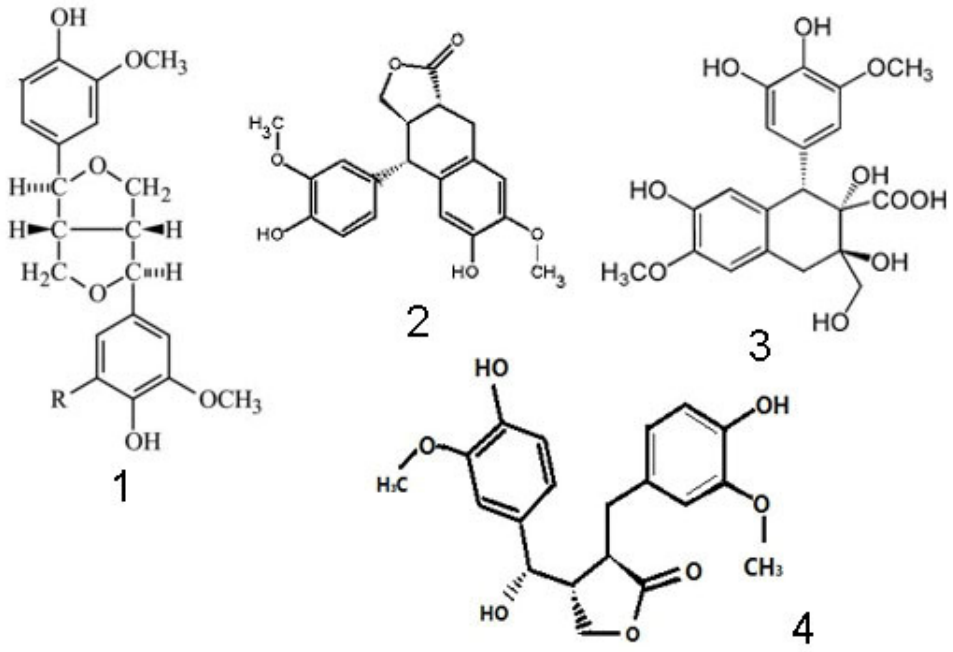


Figure 3.2 The chemical structure of lignans: pinoresinol (1), a-conidendrin (2), plicatic acid (3), and hydroxymatairesinol (4). (Sjostrom 1993)

Pinoresinol is a kind of lignans which is widely distributed in plants. It is exists as a minor component in the defensive secretion. In structure, pinoresinol is the simplest lignan that can be found in Styrax spruce. The structure of pioresinol is shown in figure 3.2(1). The pinoresinol is being a dimer of coniferyl alcohol, and it is frequently present in woody or fibrous plants. (Schroeder 2006.)

a - conidendrin, like other lignans, is synthesized in many kinds of plant, and it is a major phenolic extractive that exists in western hemlock and spruce wood. It has been assumed to originate in the same simple C_6C_3-precursors as lignin. The structure of conidendrin is shown in figure 3.2(2). In spruce wood, it contains 0.048% (w/w) a-conidendrin. (Freudenberg & Knof 1957)

Plicatic acid is a carboxylic acid from the resin acid group. It is naturally found in thuja and cypress resin. It is the main irritant and contact allergen present in thuja wood. The concentration of plicatic acid is very high in Thuja plicata, but it also can be found in Thuja occidentalis and Cryptomeia japonica. Plicatic acid can be dissolved in a relatively inert polar solvent. (Altman 1989.)

Hydroxymatairesinol is another group of lignans. A large quantity of hydroxymatariesinol extractives can be found in the knots of spruce trees. Spruce knots contain 6%-16% lignans extractives, and 65%-80% is hydroxymatairesinol extractives. The structure of hydroxymatariesinol is shown in figure 3.2(4). There are two types of hydroxymatairesinol, -7-allo-hydroxymatairesinol (HMR 1) and -7-hydroxymatairesinol (HMR 2). HMR may be the most abundant lignan in nature.The potentiality for the extraction of plant lignan 7-hydroxymatairesinol (HMR) in large scale from spruce tree has enabled the characterization of the metabolism and biological actions of HMR in rats. (Johannes H, 2007)

3.3 Tannins

Tannins can be classified into two broad groups, hydrolysable tannins and condensed tannins. Tannins are polyphenols and natural extractives that exist in many parts of different plants belonging to multiple species. Tannins are found as shapeless yellowish or light brown and they can be found in almost all plants and in all climates all over the world. Fungi and algae do not contain much tannins. Usually tannins exist in large quantities in the bark of trees and they are frequently used to protect materials as either a primer or as an inhibitor. (Zelinka 2011.)

Hydrolysable tannins are a group of substances which upon hydrolysis yield gallic and ellagic acid and sugars as main products. The structure of gallic and ellagic acid is shown in figure 3.3. Tannins are a basic ingredient in the chemical staining of wood. The tannins are already present in woods like oak, walnut, and mahogany. Hydrolysable tannins are not very common in wood. (Sjöström, 1993.)

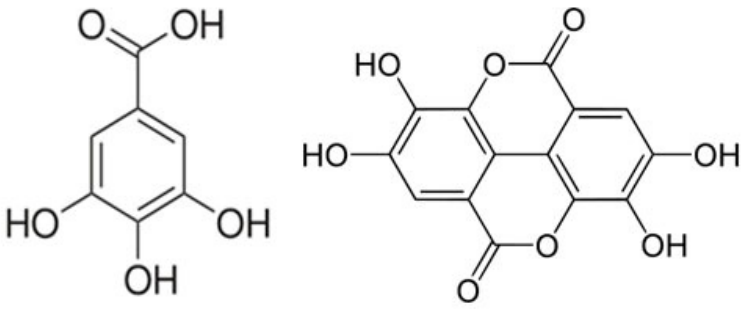


Figure 3.3 the chemical structure of Tannins: gallic acid (1) and ellagic acid (2). (Sjöström 1993)

Gallic acid is a trihydroxybenzoic acid, it is a type of phenolic acid and it is also known as 3,4,5-trihydroxybenzoic acid. It can exist in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants. The chemical structure of the gallic acid is shown in figure 3.3(1). Gallic acid acts as an antioxidant and helps to protect our cells against oxidative damage. Commonly gallic acid is used in the pharmaceutical industry. It is used as a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay. (Leyser & Pizzi 1990.)

Ellagic acid is a natural phenol antioxidant and it is very rich in the infructescence of Platycarya strobilacea. Plants produce ellagic acid to protect themselves from microbial infection and pests. Among the tannin phenolics

ellagic acid is acceptable as a single representative indicator of hardwood dust. The amount of ellagic ellagic acid in the total phenolic existing in Oak wood chips is about 10%. (Bianco et at. 1999.)

Condensed tannins are also known as non-hydrolysable tannins. Condensed tannins are not easy to split and hence, it is also very difficult to analyze. Condensed tannins are polymers of flavans and never contain sugar residues. Unlike hydrolysable tannins, condensed tannins do not possess any trace of hepatotoxicity. Quebracho, chestnut wood and wattle bark are the major source for the condensed tannins of the catechin type, although these polymers exist in many other barks belonging to species such as Betula and Eucalyptus. (Sjöström 1993.)

3.4 Flavonoids

Flavonoids are widely distributed throughout plant and they play an important role to protect the plant from microbe and insect attacks. Another important group of phenolic extraceivs found in wood is the flavonoids. Flavonoids are $C_6C_3C_6$ three ring structures. The common members of Flavonoids are chrysin, taxifolin, catechin and genistein. The structures of these compounds are shown in figure 3.4. Chrysin present in pines wood and taxifolin were first found in Douglas fir heart wood, and there are other species of wood containing taxifolin such as Larix species. Catechin also is a very important flavanol precursor. Genistein belongs to the subgroup of isoflavonoids. (Sjöström 1993.)

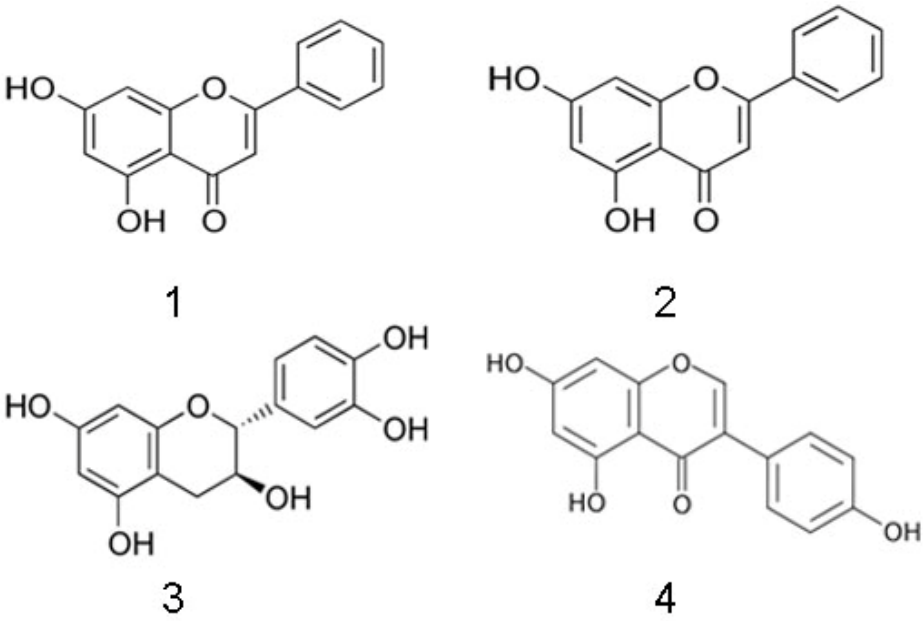


Figure 3.4 The chemical structure of flavonoids: chrysin (1), taxifolin (2), catechin (3), and genistein (4). (Sjöström 1993.)

Chrysin is the chemical name for a type of isoflavone molecule. Naturally it can be found in many types of plants, such as the Pelargonium species, which are germanium-like plants; passion flower species, which include tropical passion fruit; and the Pinaceae species, including pine trees. Chrysin belongs to a biologically active class known as bioflavonoids. Chrysin plays a potential role in drug metabolism and the chemoprevention of carcinogenesis. (Richard 2009.)

Taxifolin is a flavanonol which is a subgroup of flavonoid. It can be found in the Larix sibirica in Russia. A taxifolin molecule has two chirality centers and can exist in the form of four stereoisomers. (Kim 2007.) Taxifolin has shown a big effect to control the ovarian cancer cell growth in a dose-dependent manner. There is also a strong correlation between the antiproliferative effects of dihydroquercetin derivatives on murine skin fibroblasts and human breast cancer cells. (Yi 2008, p. 60.)

Catechin is a natural phenol antioxidant plant secondary metabolite. The term catechins is also commonly used to refer to the related family of flavonoids and the subgroup flavan-3-ols. Catechin exists in the form of glycoside. Catechin can be found in the traditional Chinese medicine plant Uncaria rhynchophylla. (Takano et al 2008, p. 329-331.)

Genistein is a species of isoflavones that is the subgroup of flavonoids. It exists in a number of plants, such as lupin, fava beans, soybeans, kudzu, and psoralea, being the primary food source. Genistein can also be found in the medical plants. Genistein has beenfound to be strong toxic. With this property, genistein has responsible on both anticarcinogenic and carcinogenic potential of the substance. (Weller M, 2008)

4 GAS CHROMATOGRAPHY

Gas chromatography (GC), is a common type of chromatography used in analytic chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture. In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture. (Gary M. Lampman, 2006) The gas chromatography can be used for organic compounds and the compounds have to vaporize enough and it must not decompose in temperature used in the instrument. The compounds where molar mass is over 500 g/mol or ions cannot be analyzed with gas chromatograph. (Hyver & Sandra 1989.) The gas chromatograph can be divided in to seven parts which can be seen in figure 4.1.

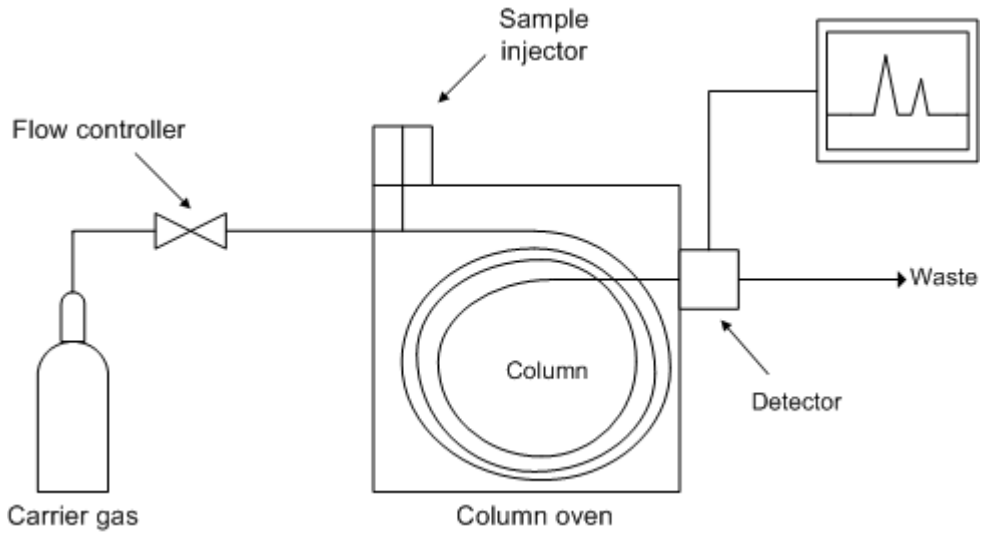


Figure 4.1 Diagram of a gas chromatograph (Hyver & Sandra 1989)

The mobile phase is called carrier gas. The sample vaporizes in the injector and flows with carrier gas to the column and to the detector. The column is in an oven and the temperature of the column can be controlled. The temperatures of injector and column have effect on vaporizations of compounds and they are very important parameters. After the column there is a detector to detect the compounds. (Hyver & Sandra 1989.)

4.1 Carrier Gas

In gas chromatography, an inert gas such as helium, hydrogen or an unreactive gas such as nitrogen is used as a carrier gas. The carrier gas has to be pure at least 99.995%. Hydrogen has the best properties chromatograph. Helium is the most typically used as carrier gas. Nitrogen requires the longest time to analyze to get same separation as helium or hydrogen. (Eiceman 2001.)

4.2 Injection Techniques

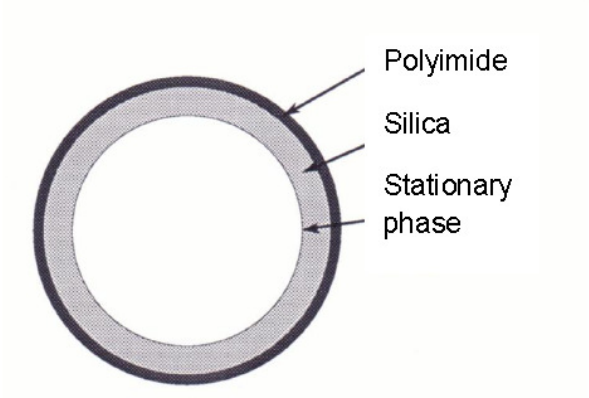
In gas chromatography the sample is injected through a gas tight thick rubber disk which is called septum. The most typical injection techniques are split injection, direct injection and on column injection. Only a small part of sample goes to a column in a split injection. The temperature of the injector is near the highest boiling point of compounds. All compounds vaporize in the injector. The temperature of the injector is usually higher than the temperature of the oven. (Harris 1999.)

Split sampling is the most popular method of injection because sample sizes of 0.1-2 µL can be injected without impeding peak height or sensitivity. The advantage to split sampling is that narrow-bore columns can be used to achieve superior resolution without overloading the column. The split injection method should always be monitored by running standard solutions of known concentration and checking for reproducibility. The split injection is usually done automatically. The whole sample flows to the column in the direct and the on column injection. So these techniques are suitable for small concentrations. (Harris 1999.)

Splitless sampling is mostly used for trace analyses of compounds where a large amount of sample (2-5 µL) must be injected onto the column for analyte sensitivity and detection. During splitless sampling, the sample extract is injected into the heated inlet and forms a vapor cloud consisting of sample, solvent and gas. Some of the vapor cloud exits the purge vent upon entering the liner as it expands. (Restek 1992, pp. 8-12.)

4.3 Columns

In gas chromatography typically capillary columns are used. The cross section of the capillary column is shown in figure 4.2. The column has an internal diameter of 0.2-0.7 mm and a length of 20-30 m. The resolution is proportional to the length of column. The stationary phase is a thin layer in the inner surface of the column. The thickness of the layer is 0.1-1 µm. The out surface of the column is made of polyimide. Polyimide makes the column durable mechanically. Inside the polyimide there is a layer of silica which is very pure silicon oxide SiO_2. (Skoog & Leary 1992.)



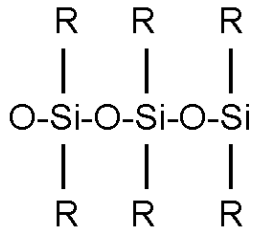


Figure 4.2 The cross section of the capillary column and the structure of polysiloxane (Skoog & Leary 1992.)

The stationary phases are inert liquids which withstand high temperatures without decomposing or vaporizing. The stationary phase must not move to the carrier gas. Liquid stationary phases are polymers, typically polysiloxanes. There are long sili-con – oxygen chains in polysiloxane but the properties of the column material depend on substitution groups in polysiloxane. (Harris 1999.) The R in the polysiloxane structure in figure 4.2 is typically methyl group, phenyl group, cyanopropyl group or trifluoropropyl group. The typical stationary phase is the polydimethylsiloxane phase. The longer the column, the better results in the

resolution and the longer analyzing time. The smaller the diameter of the column the better separation and the longer analyzing time. (Martínez-Crespiera 2011.)

4.4 Detectors

A number of detectors are used in gas chromatography. They are flame ionization detector (FID), electron capture detector (ECD), nitrogen phosphorus detector (NPD), flame photometric detector (FPD), photo ionization detector (PID), thermal energy detector (TCD), and mass spectrometer. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. TCDs are essentially universal and can be used to detect any component other than the carrier gas, FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. TCD can be operated in-series before FID, thus providing complementary detection of the same analytes. Other detectors are sensitive only to specific types of substances, or work well only in narrower ranges of concentrations. (David 1974.)

4.5 Interpreting the Chromatogram

Every compound has a different retention time, and compounds are identified by their retention times. The reference compounds are needed. The reference compounds are measured in the same conditions as the sample and the retention times are compared. The column material, the temperature of the injector and the oven has effect on retention times. The amount of compounds is found by area of peaks. And there has to be a reference compound amount with

linear relation of peak areas. (Harris 1999.)

5 EXPERIMENT PART

5.1 Equipment

The extraction of wood using polar solvent was done in the laboratory of Saimaa University of Applied Sciences, Imatra, Finland. There are two different equipments were used in this experiment. One is the rotating reactor in the ethylene glycol bath and another one is flask reactor with reflux condenser. The pictures of the equipments are shown in the figure 5.1 and 5.2.

Figure 5.1 The control panel and working part of rotating reactor in the ethylene glycol bath in the ethylene glycol bath

There are four buttons on the top of control panel. These four buttons are power, rotation, and heating controlling. There are eight small reactors with the volume of about 200 ml that are put inside the ethylene glycol bath. When the cover of

the bath is closed the eight reactors are submerged into the ethylene glycol. And then rotated continually. The small reactor is shown in the left-top side on figure 5.1.The reaction temperature can be adjusted on the control panel. The temperature of the bath can also be seen from the control panel.

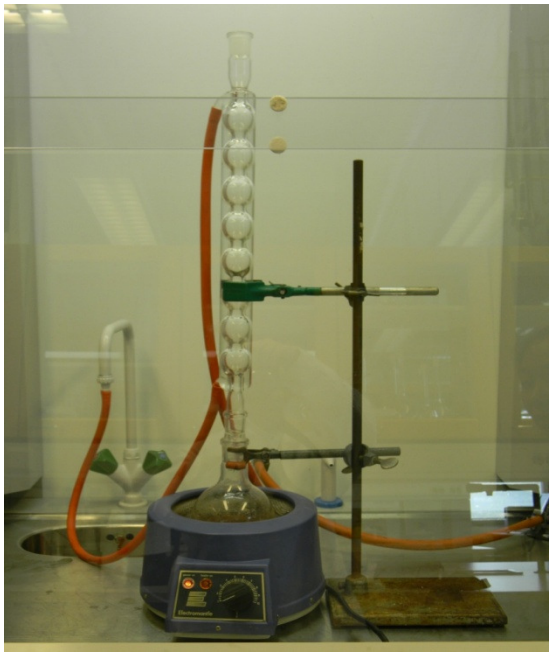


Figure 5.2 The flask reactor with reflux condenser used for the experiment

This equipment is built with a round bottomed flask with reflux, and the heater. The cold water was going through the reflux condenser from bottom to top. Condensation is the change of the physical state of matter from gaseous phase into liquid phase, when the extraction was carried out at boiling conditions.

5.2 Solvent and Raw Materials Used to in the Experiment

The solvent that we used in this experiment is polar solvent. Polar solvent is a kind of compound that is composed of polar molecules which have a slight electrical charge due to its molecule shape. Ionic compounds or covalent that ionize can be dissolved by polar solvent. Three polar solvents water, acetone,

and ethanol which were used to isolate the extractives from the wood. The table 5.1 shows some of their properties.

Table 5.1. Some properties of the solvent

Solvent	Chemical formula	Boiling point	Density
Water	H_2O	100℃	1 g/ml
Acetone	CH_3-C(=O)-CH_3	56℃	0.786 g/ml
Ethanol	CH_3-CH_2-OH	79℃	0.798 g/ml

As a solvent, water plays a very important role. The solvent properties of water are vital in biology, because many biochemical reactions take place only within aqueous solutions. In addition, water is used to transport biological molecules. Acetone is a good solvent for most polystyrene, polycarbonate and some types of polypropylene. Wood phenolic extractives are composed of simple and complex polyphenols, so most of the phenolic compounds can be separated from the wood by acetone compounds. Ethanol is a very polar molecule due to its hydroxyl group. As the second most important solvent after water, ethanol is a versatile solvent. It can miscible with water and many organic solvents. Ethanol can dissolve both polar and non-polar substances.

The raw materials that were used for this experiment were chips, saw dust, stump bark and stump chips of pine. These raw materials were fresh which were provided by UPM Company in Lappeenranta, Finland.

The wood stumps were debarked with tools and was cut in the suitable size before use. The chips were screened prior to the extraction. The raw materials were stored in the refrigerating chamber in the laboratory with the temperature of

about 5 ℃. The screener which is used in the laboratory is a gyratory screen that is shown in 5.3.

Figure 5.3 Gyratory screen In the paper laboratory

The screener is separated into several levels. The sizes of the hole in the screen are in the range from 32 mm to 6.5 mm from top to the bottom. The 12.7 mm and 6.5 mm fractions of chips were taken for the experiment.

5.3 Experiment Procedures

The raw materials were taken from the refrigerating chamber and loaded in to the small reactors. The solid in the small reactors were kept half full. Then the solvents were added to the small reactors. The cover of the reactors should be enough. After that the reactors were fixed on the glycol bath equipment, then the cover of the bath should be closed. Before the power was put on the temperature should be set first. The temperature on the control panel thet we choose for the experiment was below the boiling point of the solvent. The raw materials were extracted with the solvent in a certain time. After extraction, the liquid in the reactors was collected and filtrated for further analysis. The operating conditions for extraction are shown in tables 5.2 and 5.3.

Table 5.2 The conditions of extraction with glycol bath

Raw Materials	Solvent	Time (h)	Temperature (℃)
Pine Stem Chips Pine Stump Chips Pine Saw Dust Pine Stump Bark	Water	4	45.2 - 47.3
		2	
	Acetone	4	
		2	
	Ethanol	2	68.2 - 70.3

The table 5.1 shows that the different raw materials were extracted with water, acetone, and ethanol for two of four hours. The operation temperature of the acetone and water solvent were at 45.2 - 47.3 ℃, while the ethanol solvent was at 68.2 - 70.3 ℃.

The raw materials were added in to a 500 ml round bottomed flask. Then the solvent was added to the flask. The raw materials were submerged in the solvent. The equipment was heated up to the boiling point of acetone is 56 □. The extraction worked for a certain time. The table 5.3 shows the extraction conditions with acetone by flask reactor with reflux.

Table 5.3 The conditions of extraction with flask reactor with reflux

Raw Materials	Solvent	Time (h)	Temperature(℃)
Pine Stem Chips Pine Stump Chips Pine Saw Dust Pine Stump Bark	Acetone	2 and 4	56

After the extraction experiment, the filter papers were used to filtrate the extraction liquor samples. The filtrated liquor was stored in the cool storage room. A small amount of filtrated liquor was taken to a 50 ml beaker. The weight of the beaker and the beaker with liquor were measured. Then the beakers were put into an oven to evaporate the solvent. The temperature of the oven was at about 40 ℃. After drying for a certain time, the weight of the residue was measured out, and the concentration of the extracted solution could be calculated.

5.4 Extraction Liquor Analyzed by Gas chromatograph

The analysis of the filtrated liquor samples after extraction was carried out in the analytical chemistry laboratory by gas chromatography. The gas chromatography in the laboratory is shown in figure 5.4.

Figure 5.4 Gas Chromatography in the laboratory in Saimaa University of Applied Sciences, Imatra, Finland

In GC analysis the carrier gas is N_2. The column type of the GC is HP-1 GC columns. The length of the column is about 50 meters, and it is made of 100% dimethylpolysilloxane, the upper limit temperature of the oven is 350℃. The detector used is flame ionization detector (FID).

Firstly, the solid free liquor samples were put into small bottles. Eight samples can be analyzed automatical in one sequence. Then the analysis method was set. The wash liquid of the experiment is acetone and the volume used in washing is 2 µl. In the method, injector volume, oven temperature, inlet temperature, and the type of the injector are selected. After that the sequence were run.

The operating method in GC was test in the analysis experiment, for example the volume of the injector is 0.2 and 2µl, the inlet temperature is 200 and 300℃, the type of injector is split and splitless, the oven temperature is constant

temperature and temperature program. The temperature of the column was selected as shown in figure 5.5.

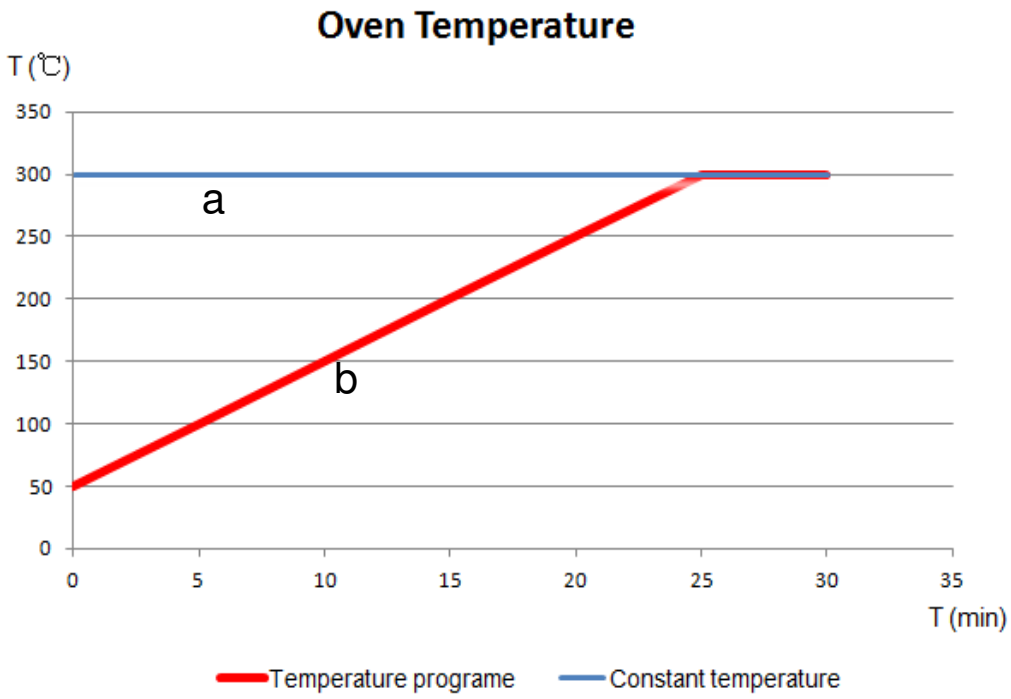


Figure 5.5 The two different temperature condition of the column oven

The figure shows the two different temperature conditions which were used in this analysis work. Line (a) shows a constant temperature at 300℃ during the whole measurement time of 30 minutes. Line (b) is a temperature program with with the initial temperature of 50℃ and final temperature of 300℃. The temperature increased 10℃ per minute, and it was held for 5 minutes, after it reached to 300℃.

6 RESULTS AND DISCUSSION

Different methods were used to detect the concentration and the content of the extractives in the different part of wood. Their results are discussed in detail in the following chapters.

6.1 Comparison of the Extracts after Treatment

Some of the extracted liquid samples are shown in figure 6.1. From left to right it shows the pine root bark extract with water and acetone, and pine stem chips extract with water and acetone. The equipment used in these extractions are rotation reactor in the ethylene glycol bath.

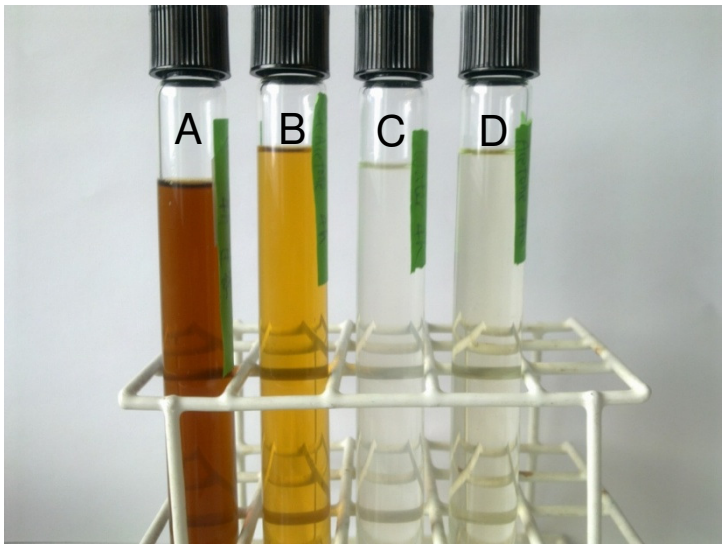


Figure 6.1 The filtrated liquor of some extracts A: Pine stump bark with water B: Pine stump bark with acetone C: Pine stem with water D: Pine stem with acetone

Visual comparison from figure 6.1 shows that the liquid sample obtained from the pine stump bark as the raw material has the deepest color. So it maybe contains more extractives than other samples. The phenolic extractives

contribute the color of the wood. The phenolic extractives such as tannins and phenol resins are in dark brown colors. (Dai & Mumper 2010.)

The liquor samples extracted from different treatments were put in to the oven to evaporate the liquid. The temperature of the oven was about 40 ℃. After one night, the residual extracts were in different forms, such as solid, or the gelatinous from. Then their weights were measured, and then the concentration was calculated. The results are shown in figures 6.2 and 6.3. The figures show the concentration of extraction liquor samples that were treated with different material, solvents, extraction time, and temperature.

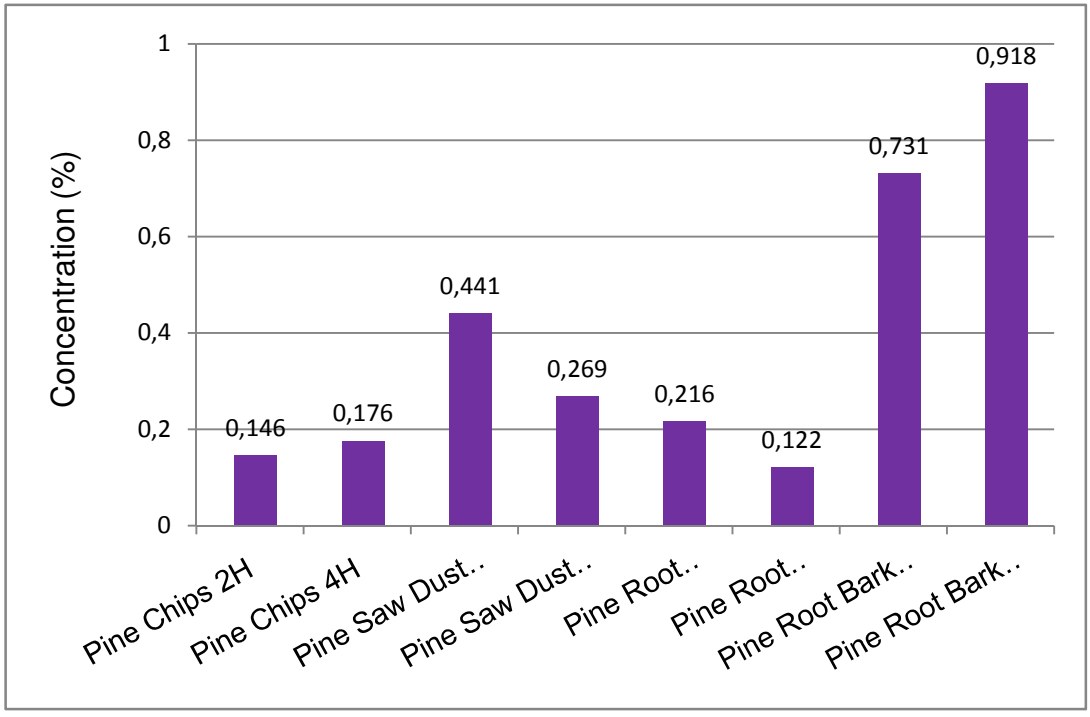


Figure 6.2 The concentration of extract solvent obtained with water solvent by the ethylene glycol bath at 45.2-47.3 ℃.

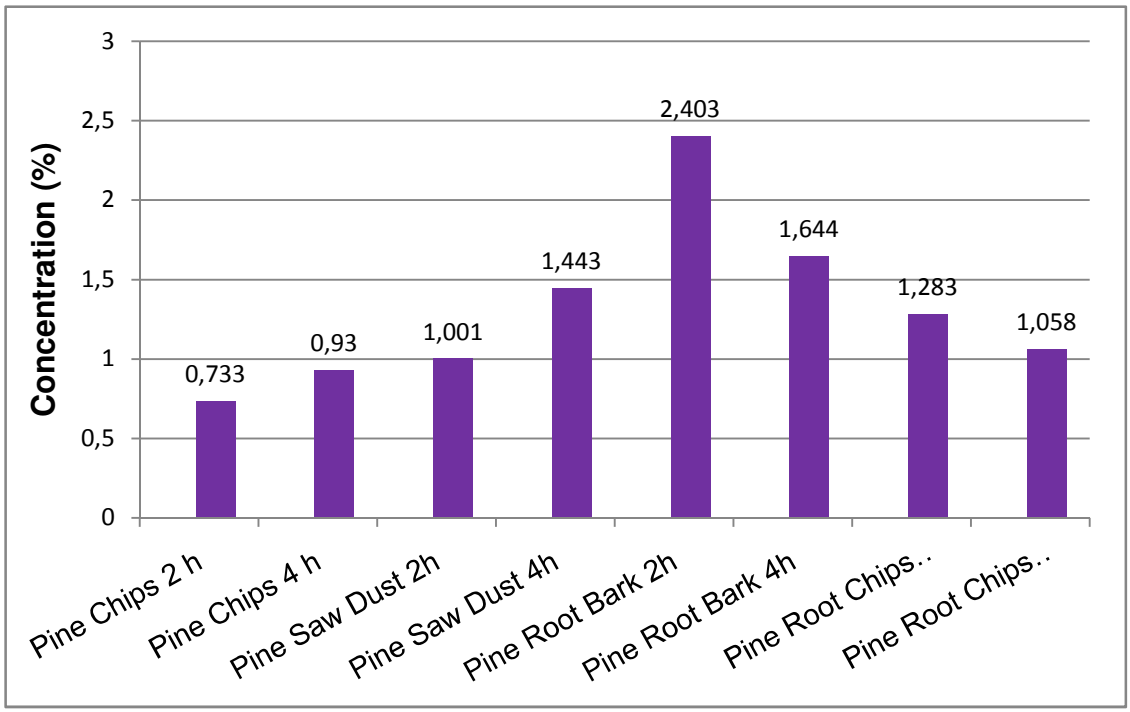


Figure 6.3 The concentration of extract solvent obtained with acetone solvent by the ethylene glycol bath at 45.2-47.3 ℃.

Figures 6.2 and 6.3 show the concentration of the liquor samples of the raw material extracted with same equipment, temperature, and different extraction solvent. Comparing the two figures, the concentration of the solvent which was extracted by acetone is much higher than that extracted by water solvent. For example, the concentration of solids in pine chips treated with water solvent is 0.164% while with acetone solvent the concentration is 0.773%. The extraction with both solvent indicated that the concentration of pine root barks liquor samples are highest comparing with other raw material liquor samples.

6.2 Comparison of Different GC Analysis Methods

The liquor samples are very complex compounds. In order to get more accurate results, the proper GC analysis method should be used. Some different GC

analysis methods of the GC have been test in order to find the best method, as shown in table 6.1. The liquor samples used for these GC analysis were pine chips extracted with acetone solvent by the ethylene glycol bath for 2 hours at 45.2-47.3 ℃.

Table 6.1 GC analysis method test

Method	Inlet T (℃)	Column T (℃)	Inject volume (µl)	Inject type
1	200	Temperature Program	2	Split
2	300	Constant Temperature	2	Split
3	300	Temperature Program	0.2	Split
4	300	Temperature Program	2	Splitless
5	300	Temperature Program	2	Split

From methods 2, 3, and 5, the different oven temperature and inject volume are used in these three GC analysis methods. The results are shown in figures 6.4, 6.5, and 6.6.

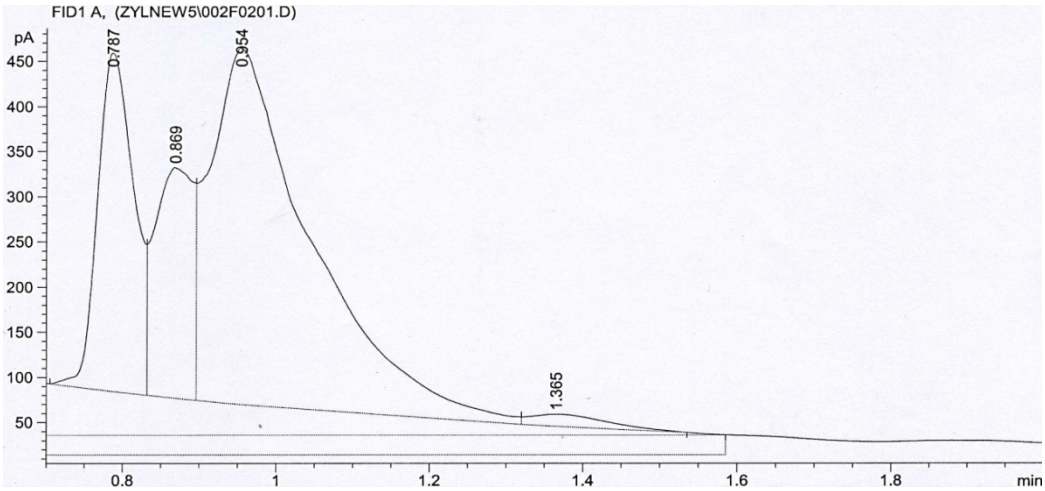


Figure 6.4 GS analyzed result from method 2

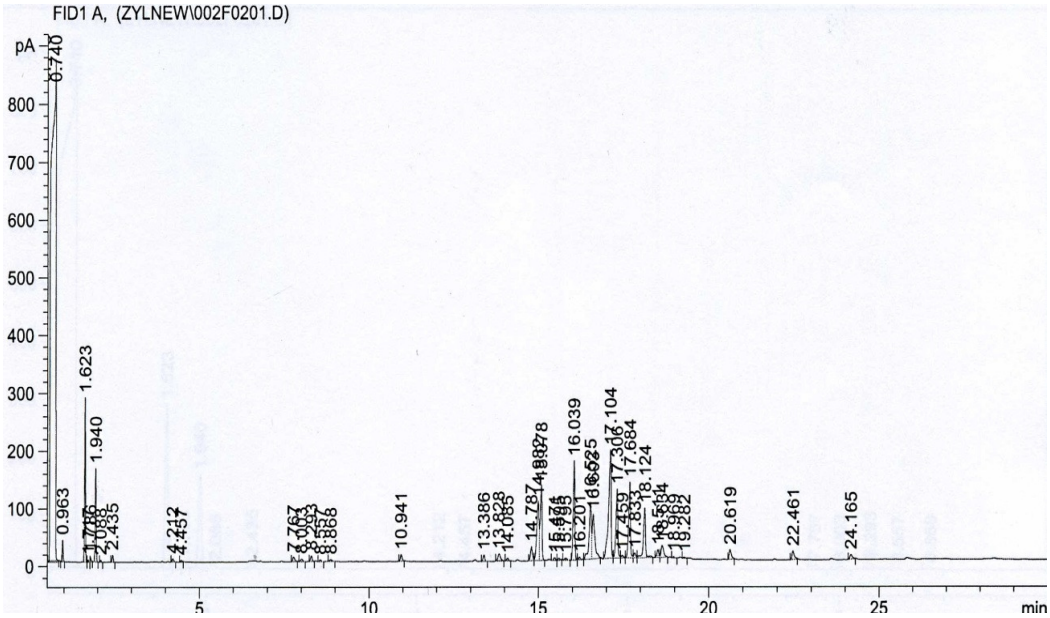


Figure 6.5 GS analyzed result from method 5

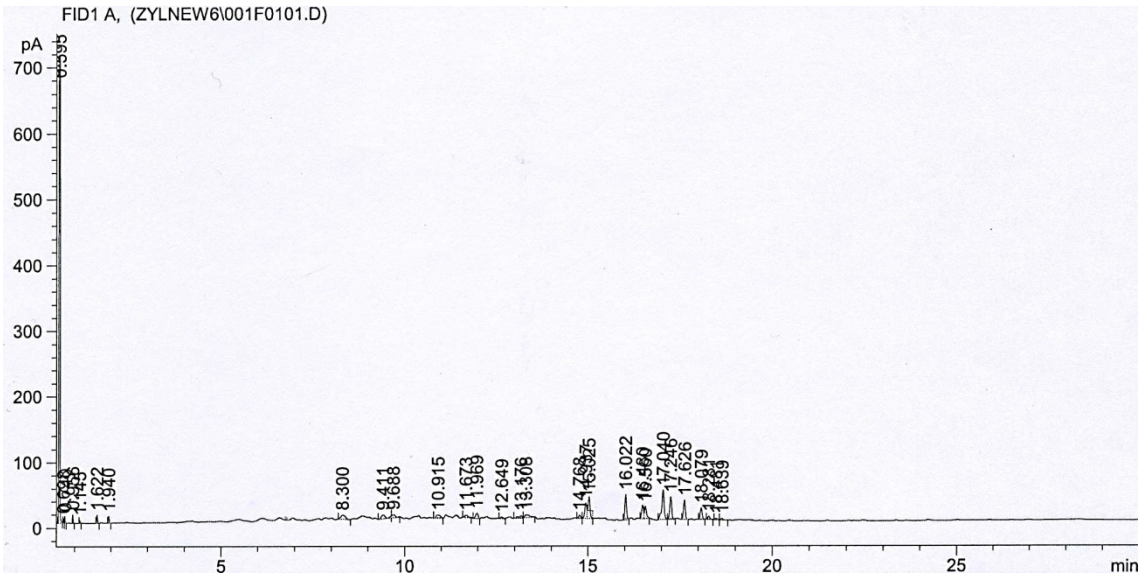


Figure 6.6 GS analyzed result from method 3

As can be seen from figures 6.4 and 6.5, comparing the two figures, the result are obviously shown that the peaks that appeared in constant temperature are much lower than that comes out from the temperature program. Comparing with figures 6.5 and 6.6, the smaller the volume of the injector, the fewer the peaks comes from the GC analysis results. Comparing method 1, 4, and 5, there is no big difference in the result of GC analysis, the result of GC analysis method 1 and 4 can be seen in figure 1 and 2 in appendix 1.

6.3 Comparison of GC Analysis Results

From the comparison of the different GC analysis methods, the method 5 (temperature program of column, 300 ℃ inlet temperature, split inject type, and 0.2µl inject volume) has been chosen to analyze following extraction samples. The GC analysis results are shown and discussed in the following text.

Raw material comparison

In figure 6.7, it shows the result of GC analyze of pine chips that extracted for 2 hours with acetone by the ethylene glycol bath equipment. However, in figure 6.8 only raw material has been changed which is pine barks. The A, B, and C parts of them are compared in the following text and figures.

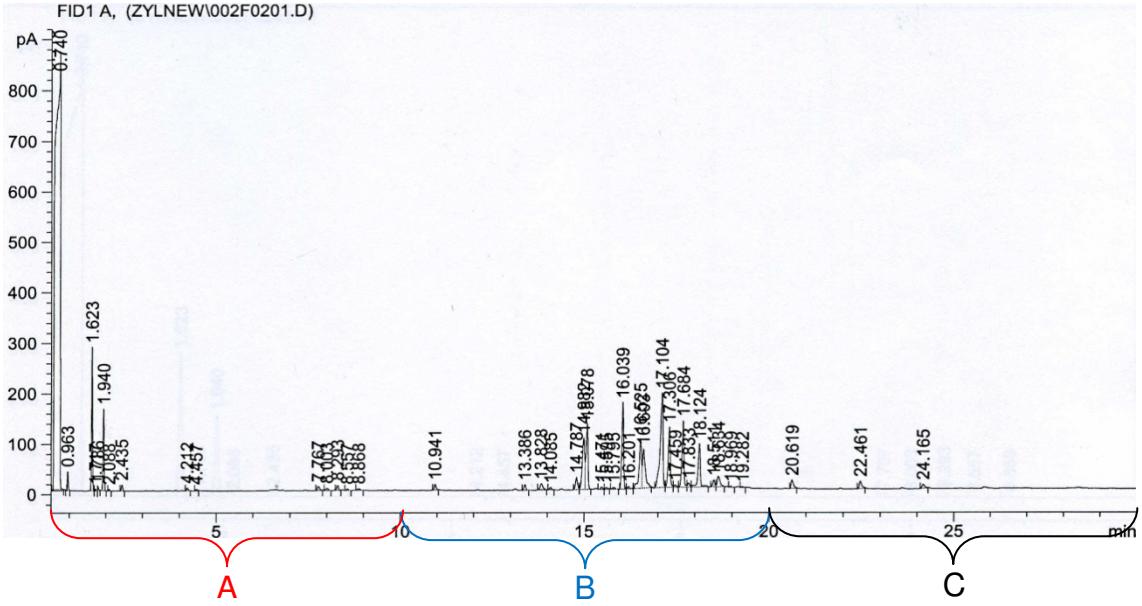
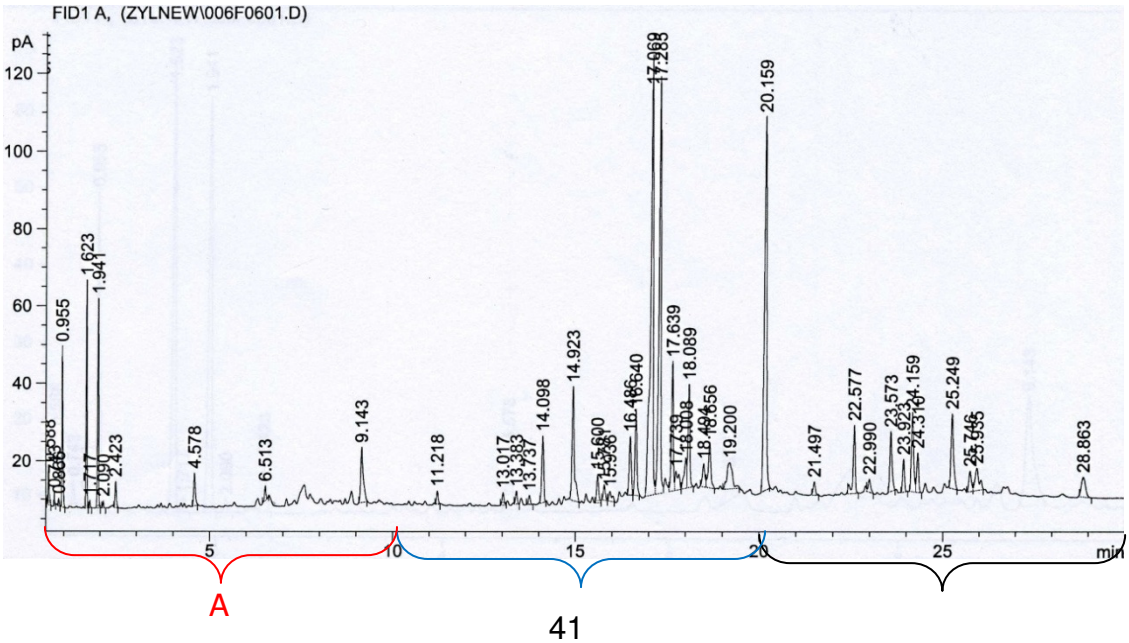


Figure 6.7 GS analyzed result of extraction of pine stem chips



B C

Figure 6.8 GC analyze result of extraction of the pine stump barks

From figures 6.7 A and 6.8 A, it shows the result of part A that the retention time between 0 to 10 minutes. Compare these two figures; there is no big difference in these two figures. However the peaks in the retention time 7-10 minutes in figure 6.7 A is a little more than that in figure 6.8 A. It means there are more chemicals in that liquor samples. And the height of peaks in figure 6.7 A are much higher than that in figure 6.8 A.

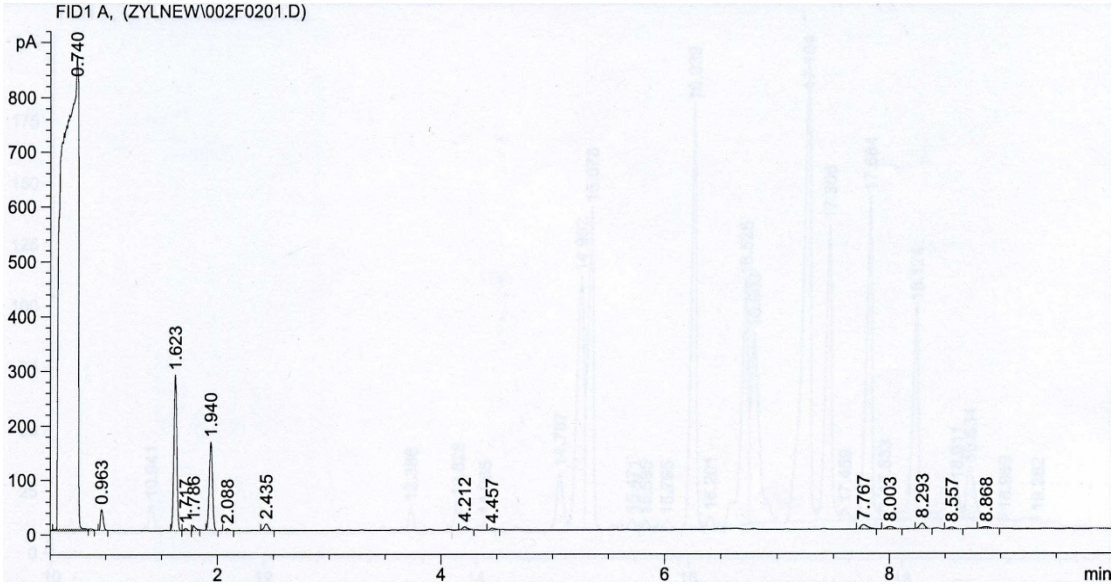


Figure 6.7 A

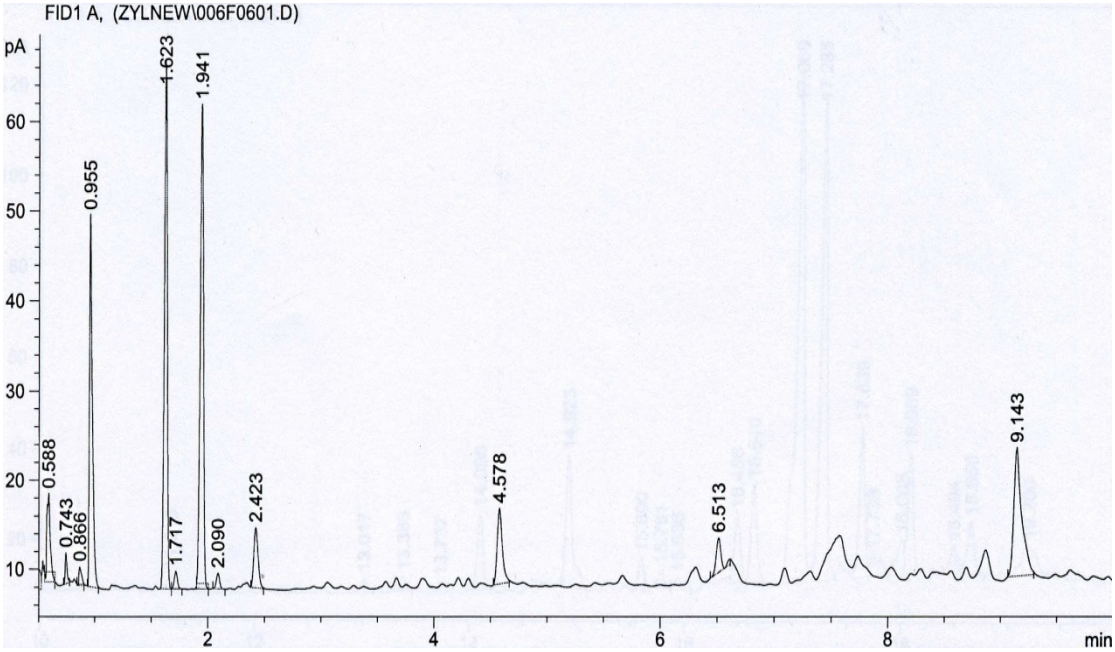


Figure 6.8 A

In figures 6.7 B and 6.8 B, it is the comparison of part B that the retention time between 10 to 20 minutes. In these two figures, the peaks are both very dense in the retention time. And there is no big difference on the height of the peaks in these two figures.

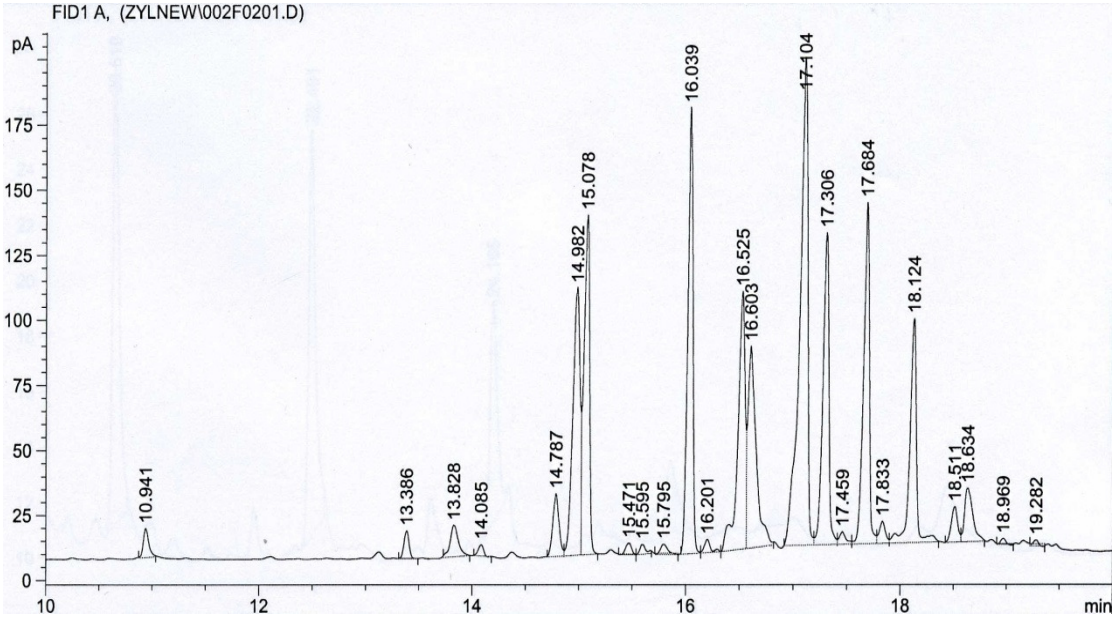


Figure 6.7 B

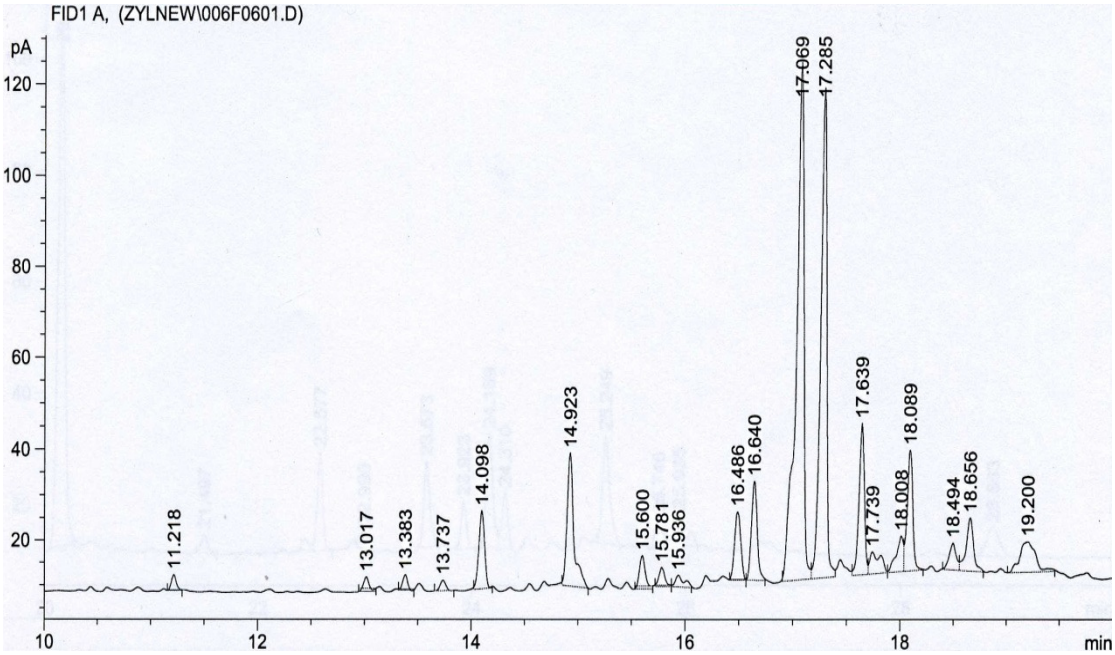


Figure 6.8 B

As can be seen from figures 6.7 C and 6.8 C, it shows the part C that the retention time between 20 to 30 minutes. Compare these two figures, the peaks of GC analysis results in figure 6.8 C is much more than that in figure 6.7 C at the retention time. Therefore there is much chemical content in that liquor samples.

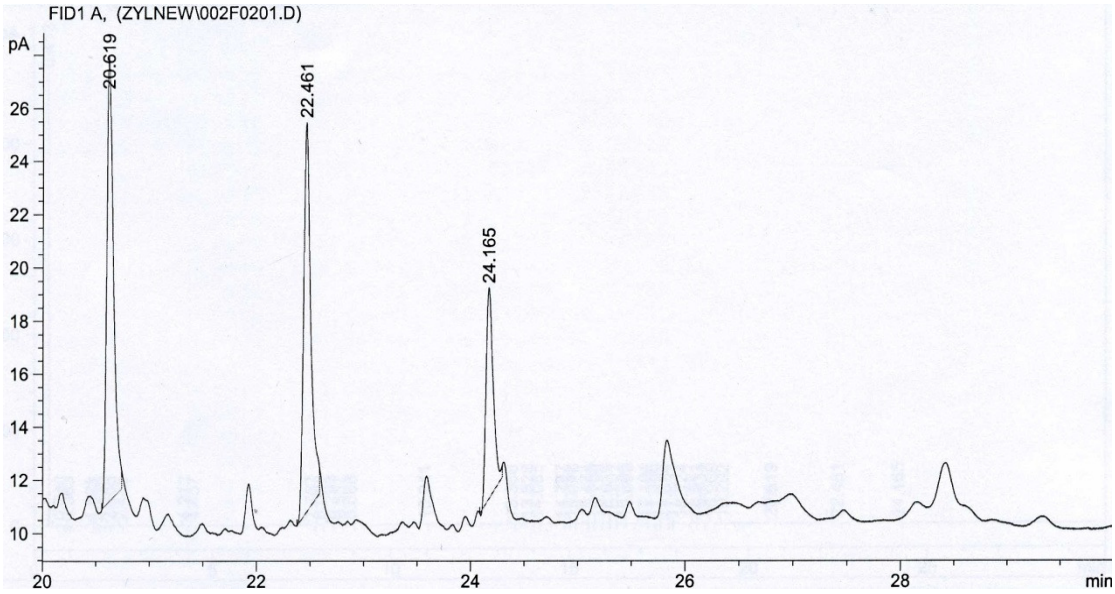


Figure 6.7 C

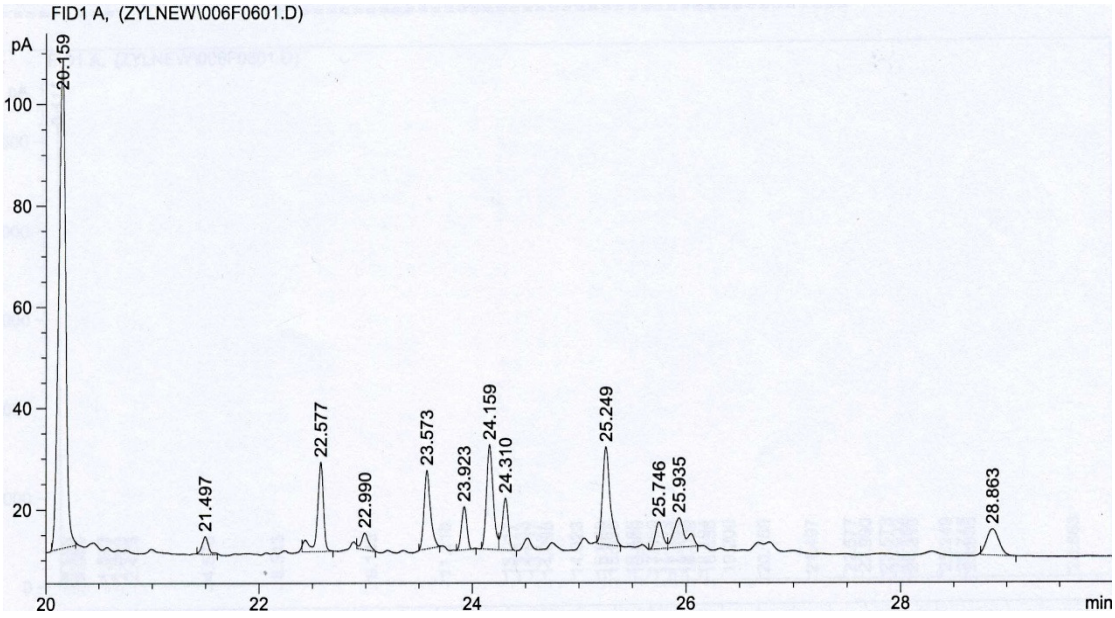


Figure 6.8 C

The GC analysis method and extraction condition are same for the pine stump chips, and pine saw dust. The GC analysis results of them are shown in figure 1 and 2 in Appendix2, the GC analysis result for the four raw materials show the chemical content at the retention time from 10-20 minutes are much more than that at other retention time. In addition, comparing these GC analysis results, the

pine stump barks have much more peaks than other three results. It means there are much more extractives in that liquor sample at the certain GC analysis method.

Extraction time and equipment comparison

Figures 6.9 and 6.10, it shows the results of GC analysis method of the pine chips liquor samples that are extracted in the same boiling temperature, and acetone solvent, with 2 or 4 hours by flask reactor with reflux condenser. The A, B, C part of figure 6.9 and 6.10 are compared in the following text and figures.

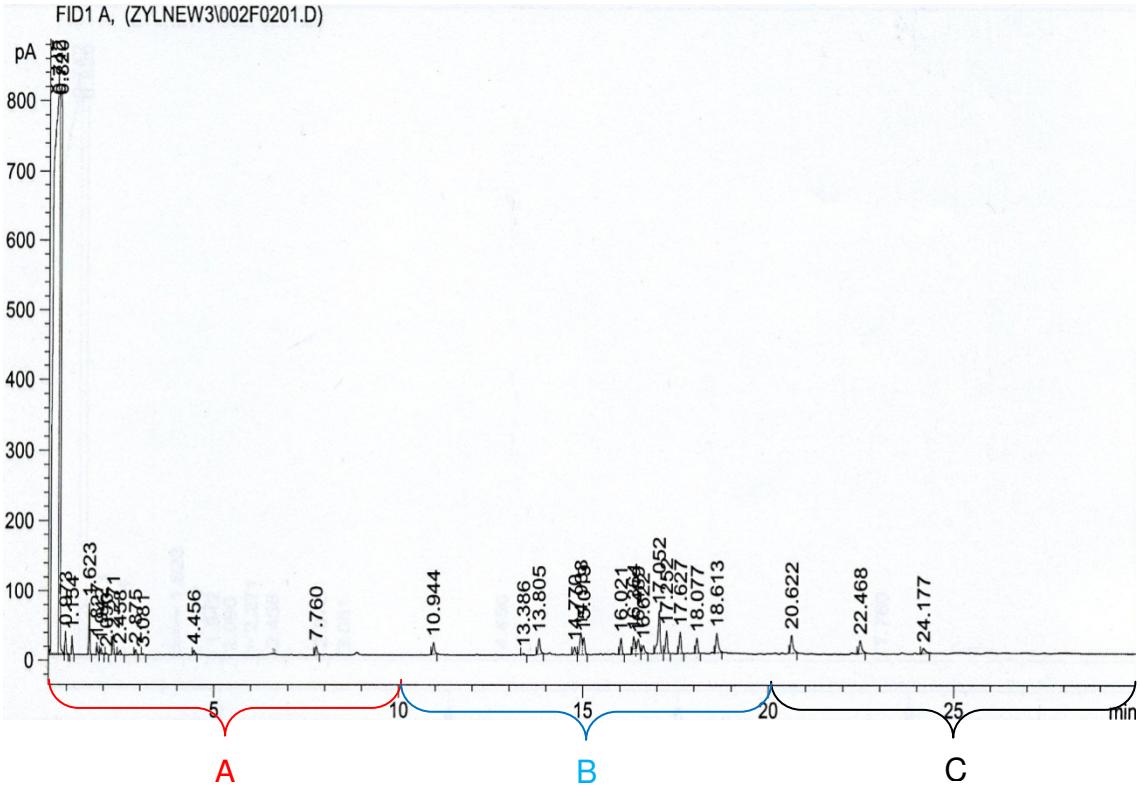


Figure 6.9 GS analysis result of extraction with 2 hours

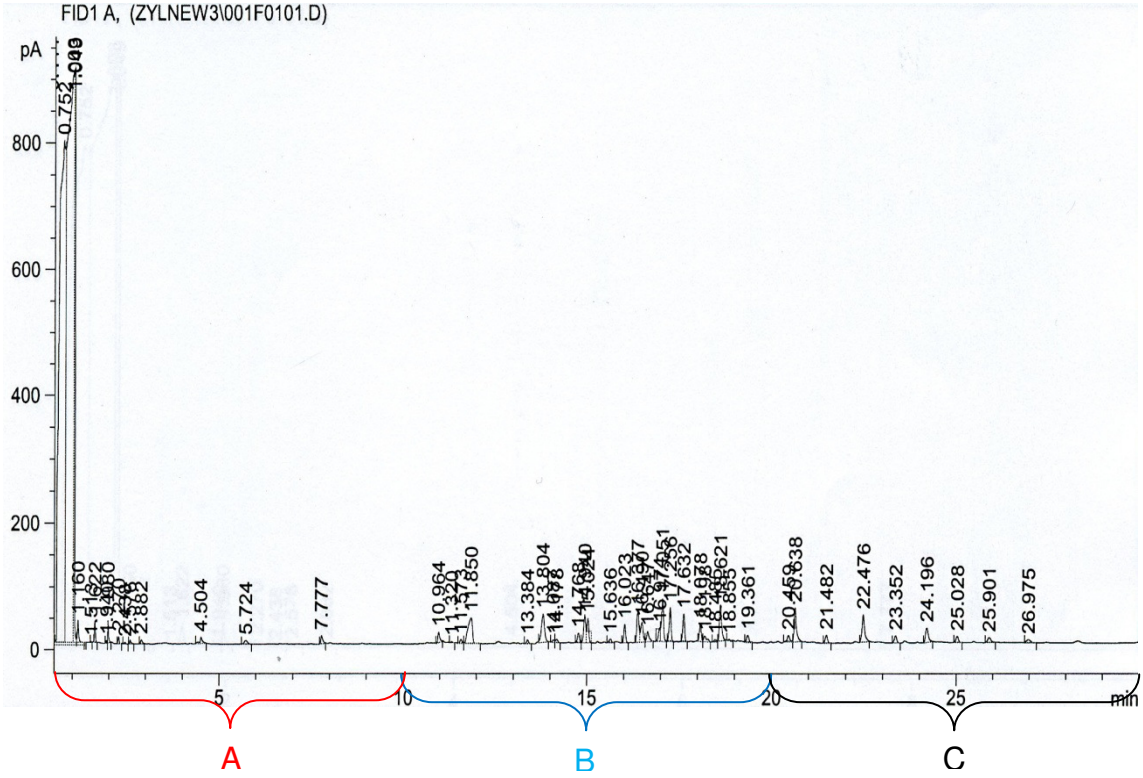


Figure 6.10 GS analysis result of extraction with 4 hours

The part A and part B of figure 6.9 and 6.10 that the retention time between 0 to 20 minutes is shown in figure 1 and 2 in appendix 3. The amount of the peaks in that retention time shows almost same. Therefore, at a certain GC analysis method, there is no big different of the quantity of the chemicals which can be analysis in that retention time. However, as can be seen from figure 6.9 C and 6.10 C, GC analysis result of the samples which extract 4 hours has more peaks on the retention time from 20 to 30 minutes.

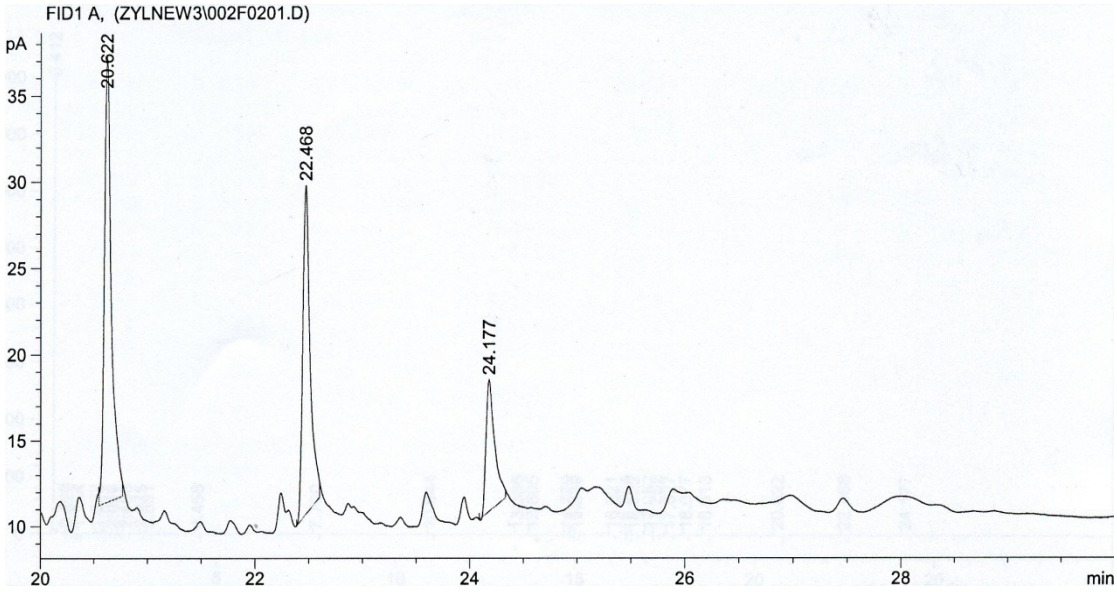


Figure 6.12 C

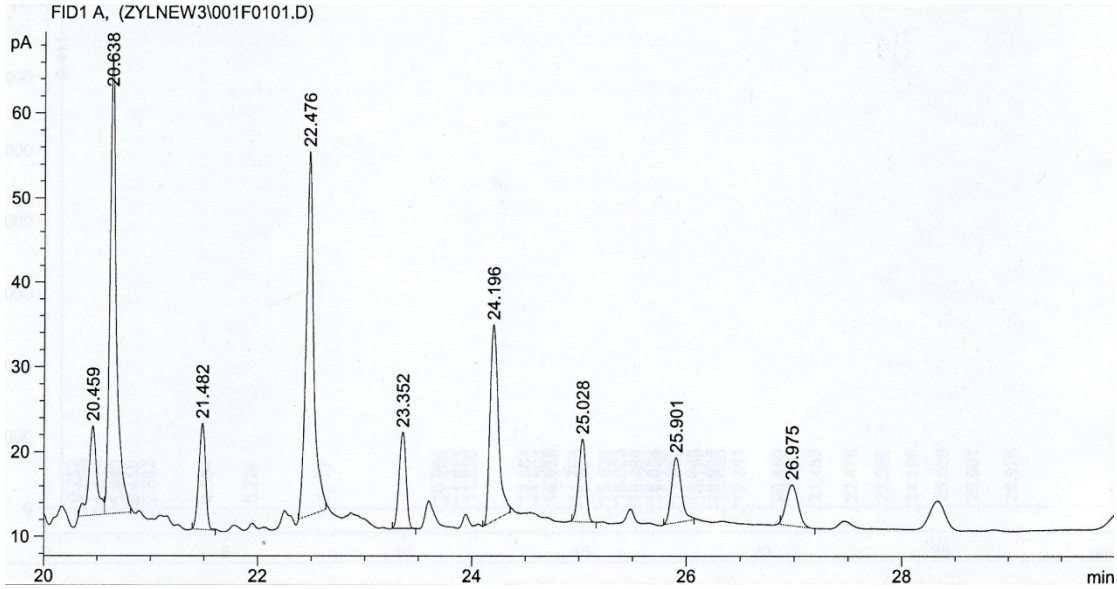
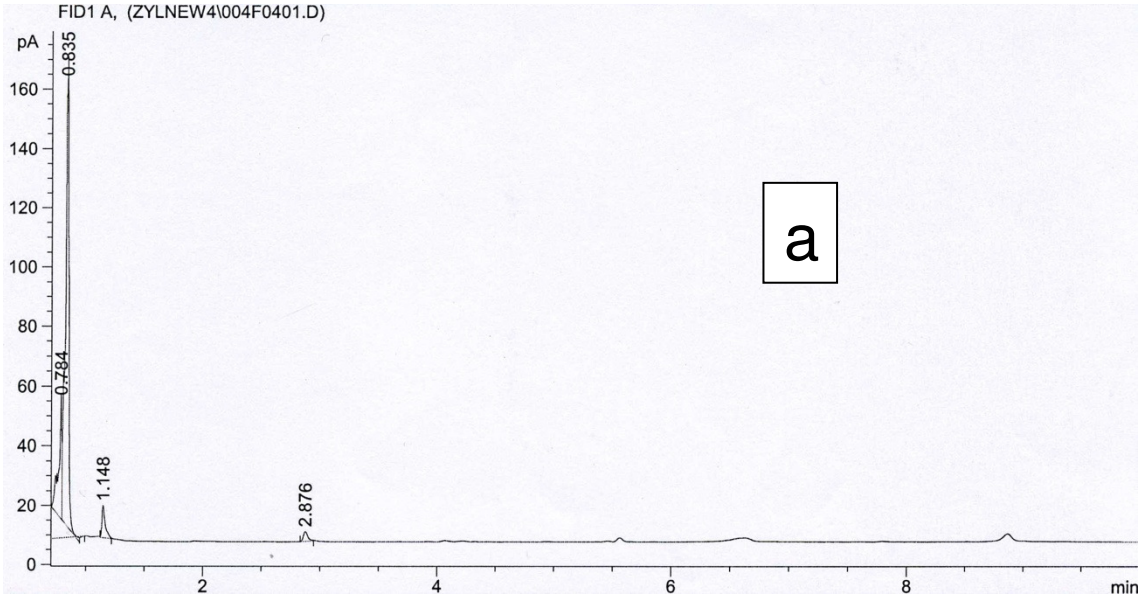


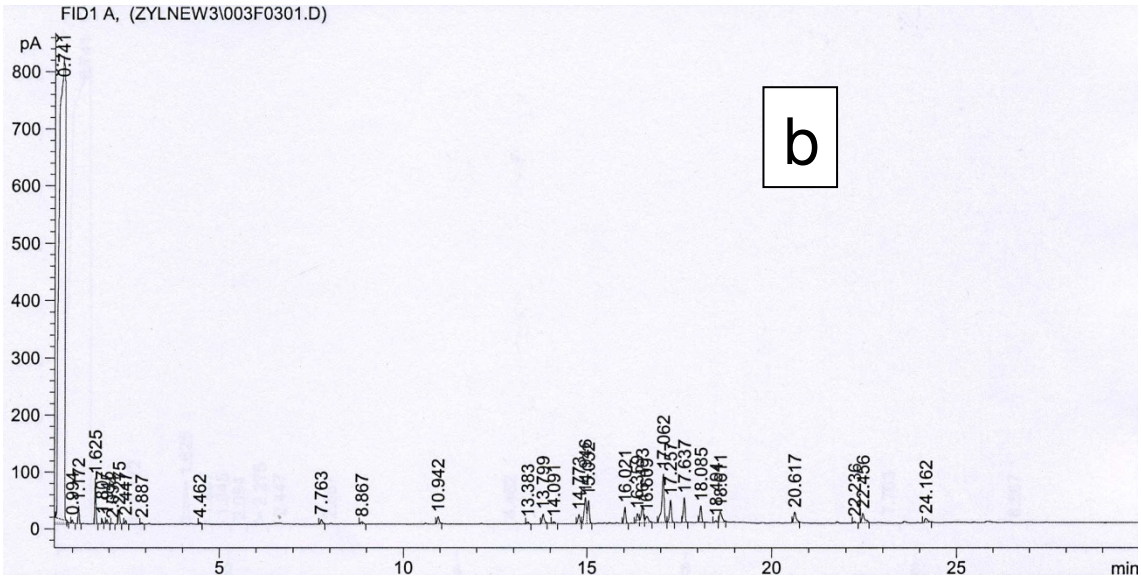
Figure 6.13 C

In addition, the GC analysis results show other extraction liquors obtained from pine root bark and pine saw dust also shows that the longer the extraction time the more chemical was identified from the GC analysis. Comparing the different equipment used for extraction at the same conditions, the GC analysis results show the peaks at the same retention time are almost the same. The results are shown in Appendix 4.

Solvent comparison

The GC analysis results of the pine stem chips liquor samples that extracted with water, ethanol, and acetone for 2 hours by the ethylene glycol bath are shown in figure 6.15.





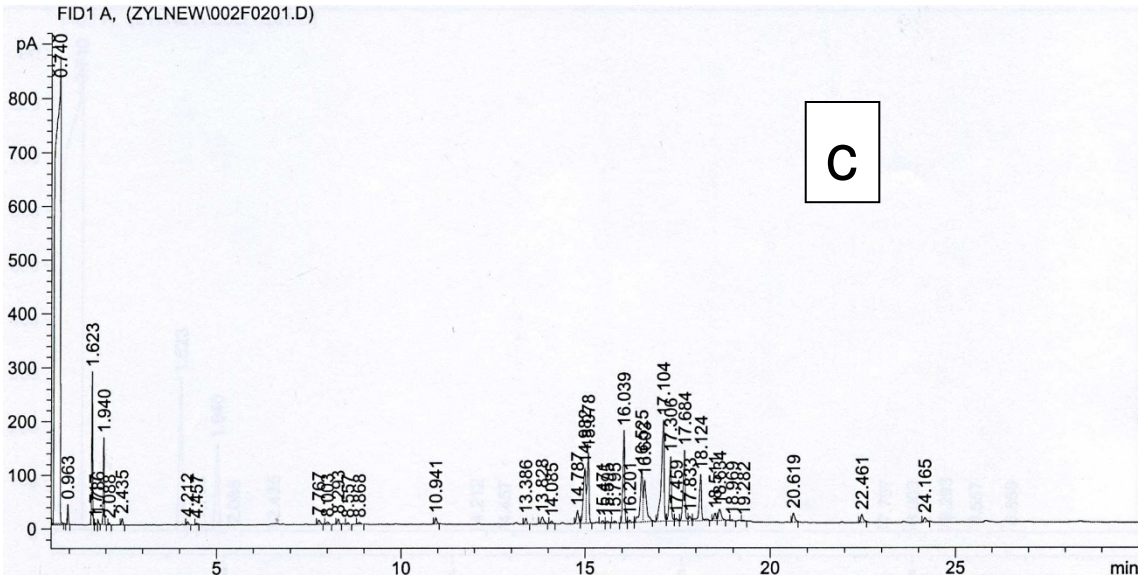


Figure 6.11 GC analysis method of extraction with water (a), ethanol (b), and acetone (c)

As can be seen from figure 6.11 (a), only few results come out of the GC analyze of the pine chips liquor obtained with water. These might be two reasons. One is the boiling point of the extractives that extracted by water might be very high, and the upper limit oven temperature of the GC that used in our experiment is 300 ℃. The other reason might be that the content of the extractives extracted by water is very few. However, comparing the GC analysis results of the same pine chips liquor sample that obtained ethanol and acetone solvent, no big difference can be seen. The peaks of the GC analysis results of them are almost the same on the same retention time.

The same extraction solutions are analyzed by GC analysis method and the oven drying method and the result are shown in figure 6.12. From figure 6.12, it shows the content of the extracts analysis by GC analysis method commonly has high values comparing that from oven drying method. However, bark and sawdust samples show the higher concentration by oven drying. For the drying

method, the extracts might be losing by evaporation during drying. However, by GC method, some content of extractives might not be detected out due to high boiling points of those compounds.

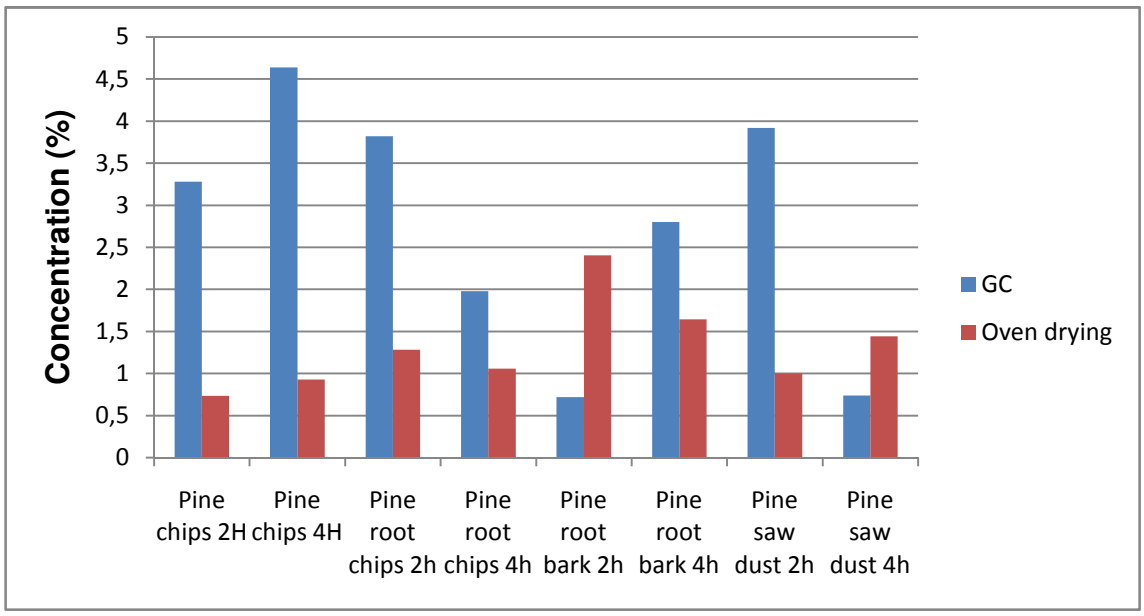


Figure 6.12 Concentration of the extracts from the different analysis method

7 SUMMARY

The aim of this experiment is to investigate the content of extraction from different parts of the pine wood such as pine chips, root barks, root chips, and saw dust extract by polar solvent. The obtained extractives were analyzed, such as by drying in the oven, or by GC analyzed method. The result shows that different GC method used for the samples affect the actual result of the content of the extraction. Using temperature program to control the oven temperature is important for analysis result.

In the comparison of the different analysis methods, it shows the content of the

extracts analysis by GC analysis method commonly has high values comparing that from oven drying method. The GC analysis results show that extracted at same condition with different raw material, the content of extractives from pine root bark is more than other raw materials. Moreover, the solvent used for the extraction have effect on the concentration of the extractives extract from the wood. At the certain GC analysis method in this work, the content of extractives in the liquor sample which extracted by acetone or ethanol solvent is richer than water used as the extraction solvent. From the result study, it also shows the longer extraction time is the more chemicals were identified from the GC analysis.

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TABLES

FIGURES

APPENDIX 1

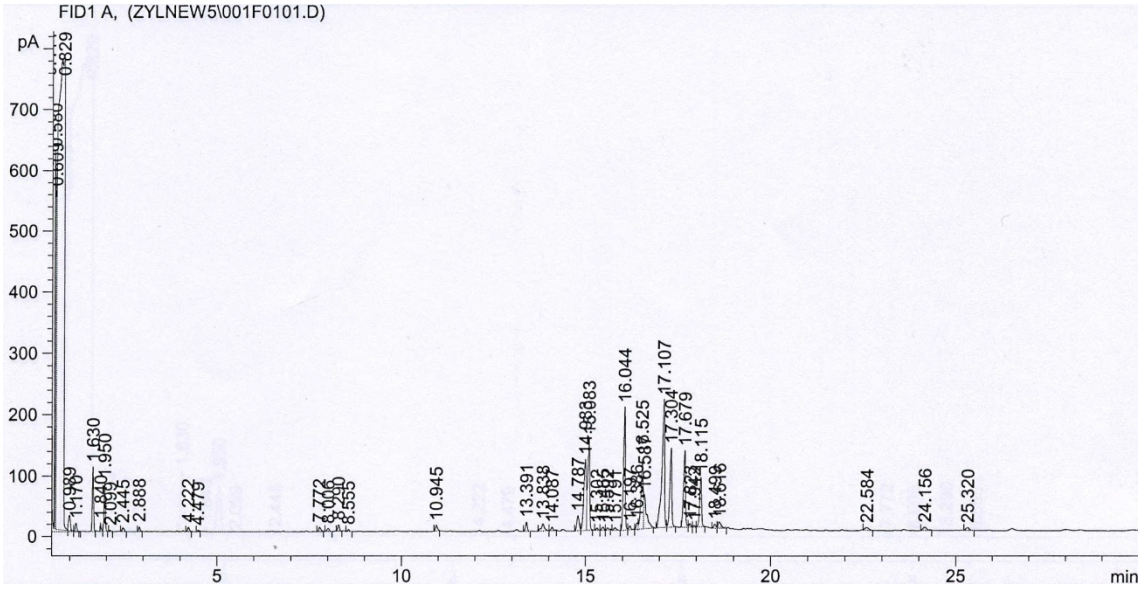


Figure 1 GS analyzed result from method 1

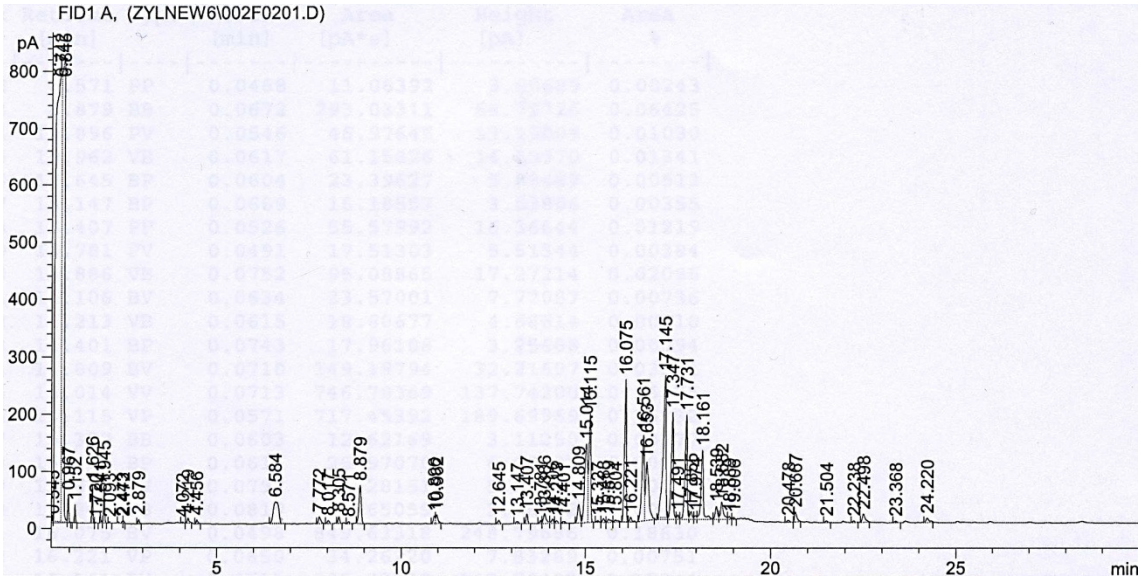


Figure 2 GS analyzed result from method 4

APPENDIX 2

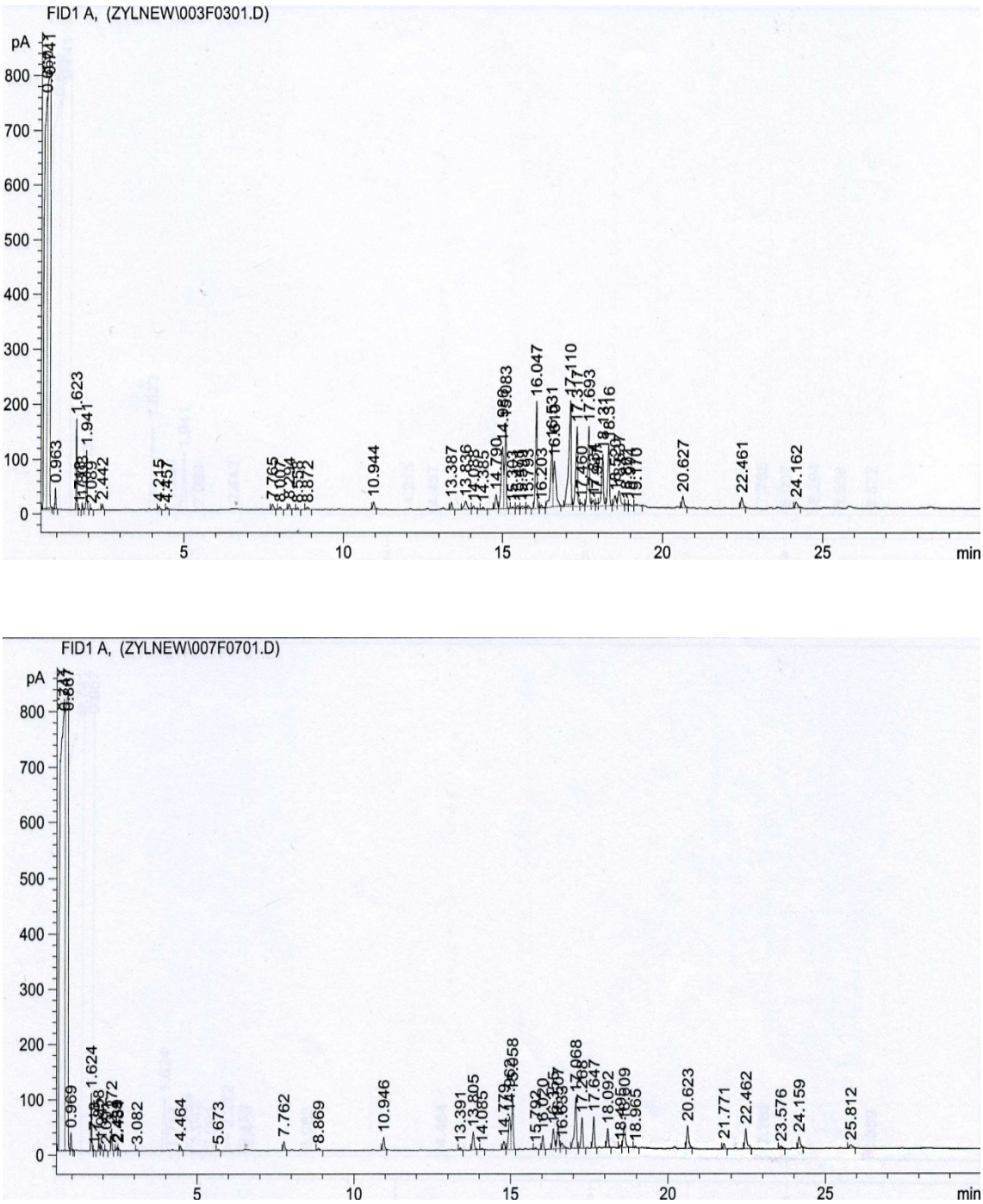
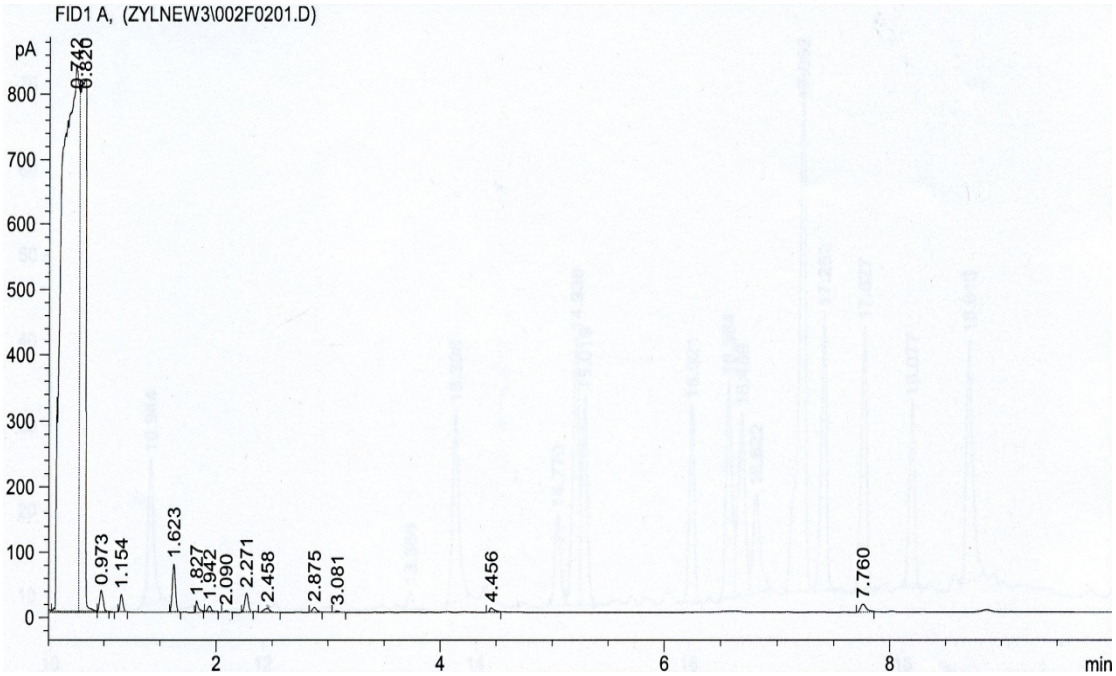


Figure 3 GC analyze result of pine root chips and saw dust extract with acetone solvent, 2 hours by the ethylene glycol bath

APPENDIX 3



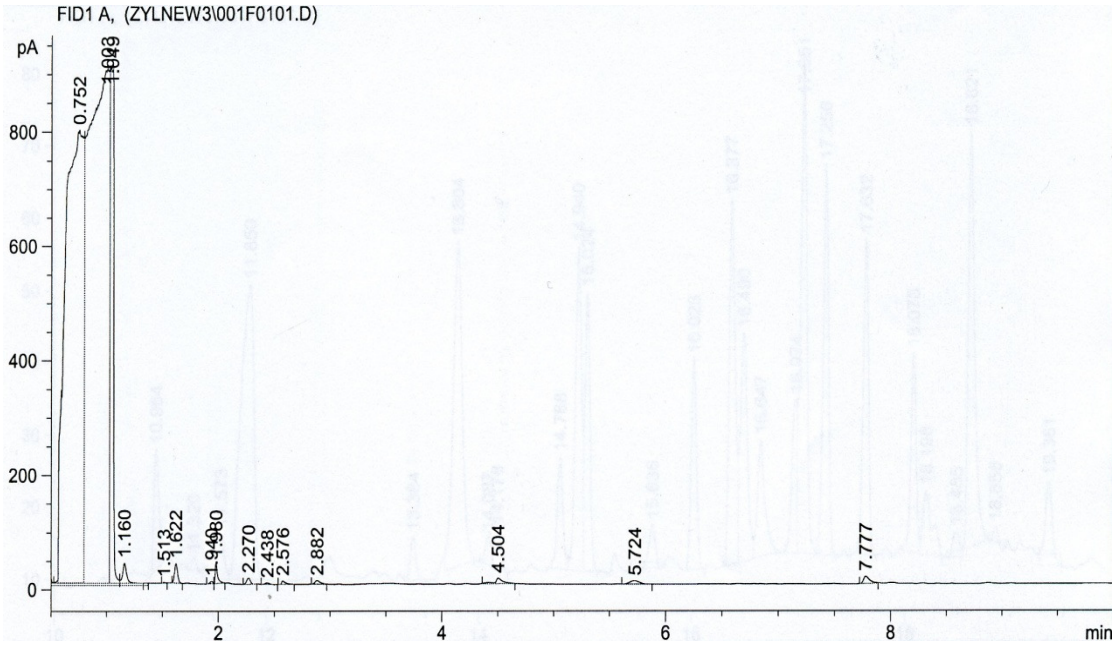


Figure 4 Part A of figure 6.12 and 6.13

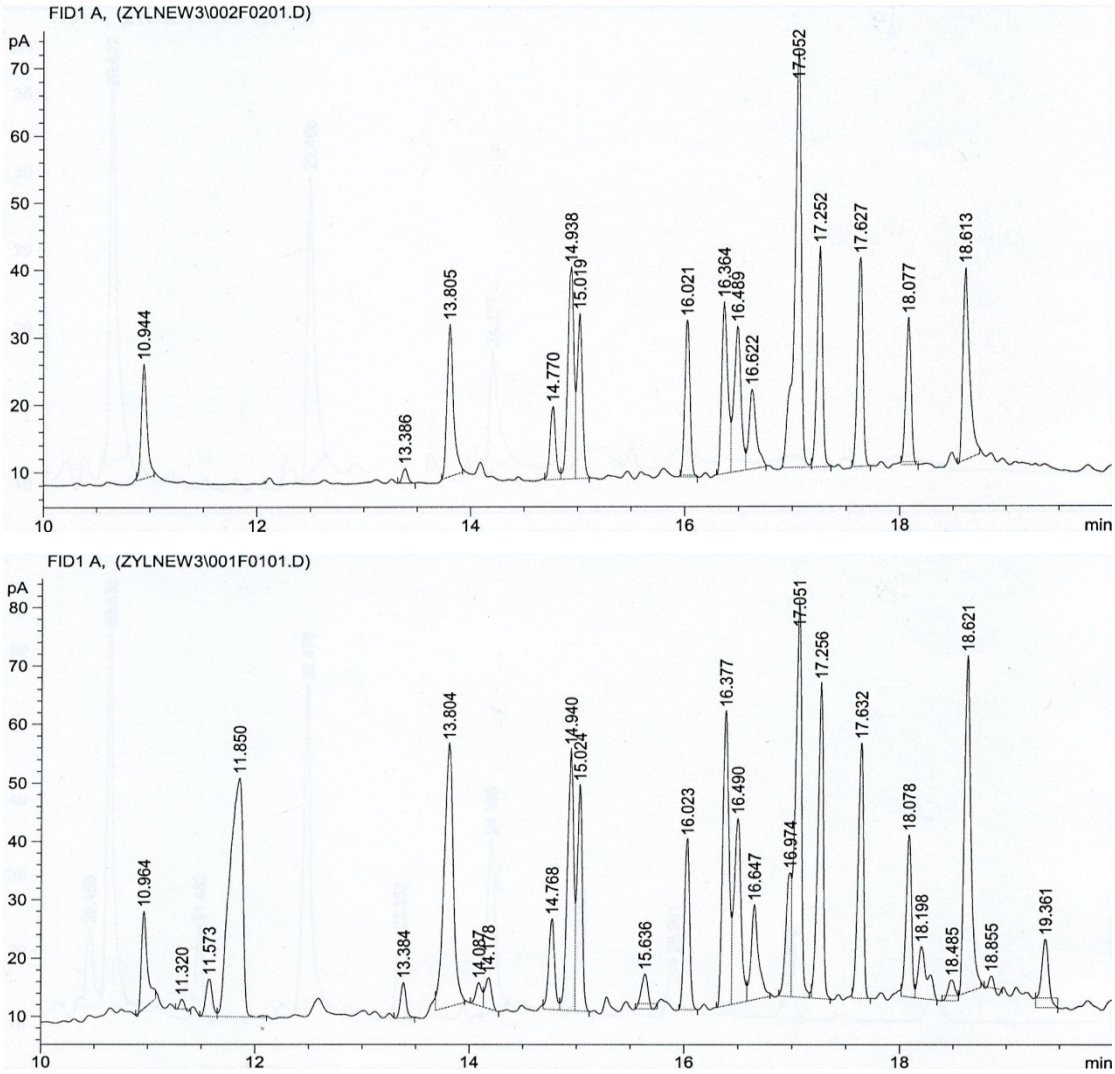


Figure 5 Part B of figure 6.12 and 6.13

APPENDIX 4

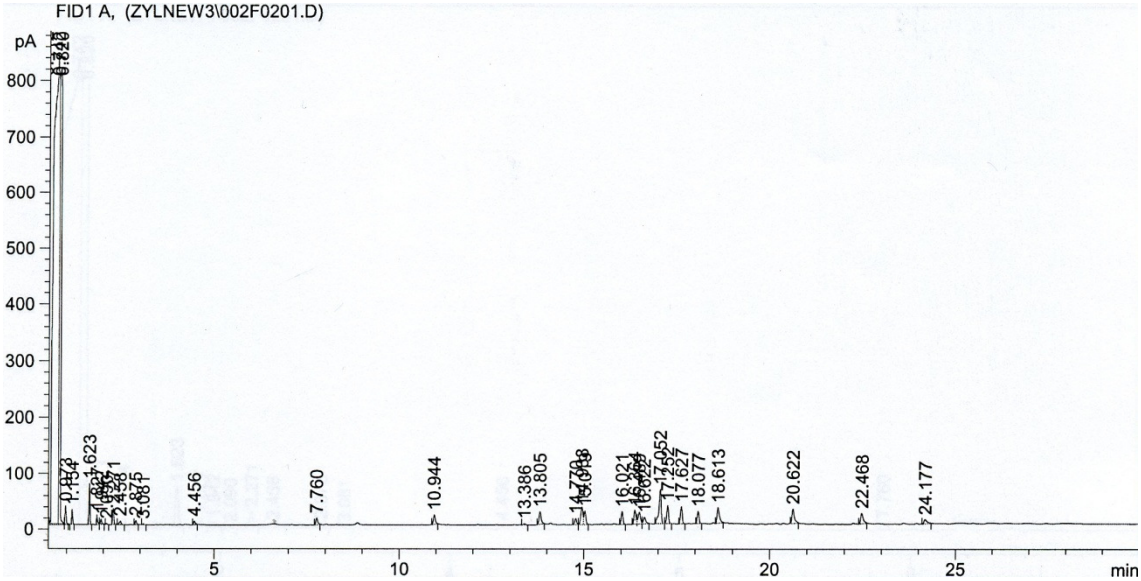


Figure 6 The GC analysis results of pine chips extracted 2 hours with acetone by flask reactor with reflux condenser

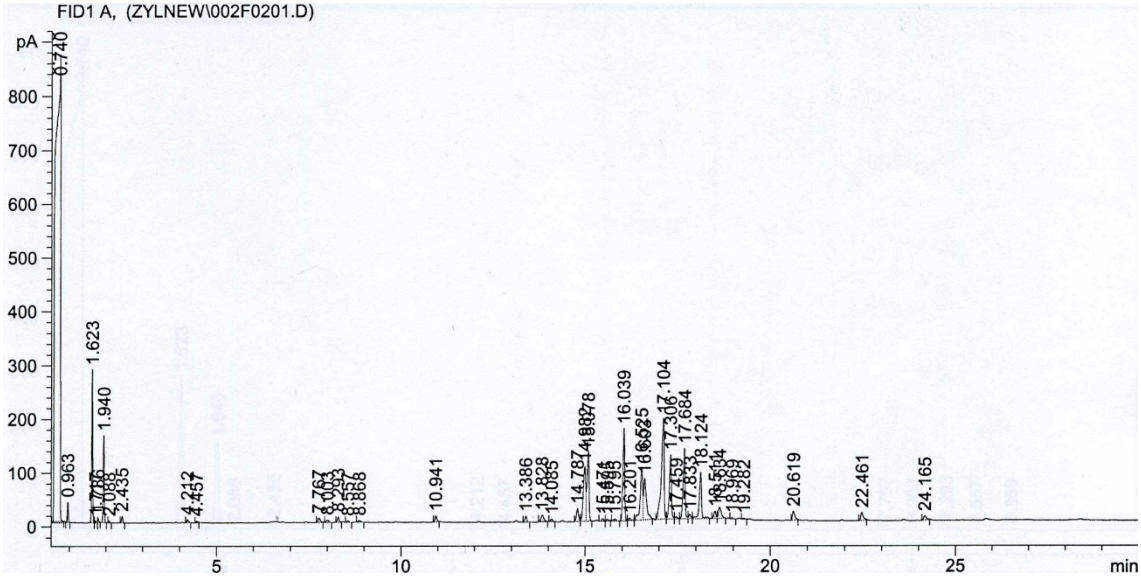


Figure 6 The GC analysis results of pine chips extracted 2 hours with acetone by the ethylene glycol bath