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# CHEMICAL ANALYSIS OF SPRUCE NEEDLES

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**ABSTRACT**


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The purpose of this thesis was to analyze the distinctive chemical compounds in spruce needles. This research was mainly focused on the extractives found in spruce with the help of its needles. The raw material used in this study was spruce needles. Different characterization of compounds had been studied throughout the literature. The chemical constituents found in spruce needles were cellulose, hemicellulose, lignin and extractives. Extractives like, fatty acids, resins, sterols, stilbenes, flavonoids and many different products were identified. These complex compounds were identified by separation method.

In laboratory, the extractives were isolated by using two different solvents, polar and non-polar. Different separation methods could be utilized but mainly acetone and hexane were two solvents used in this experiment to identify the extractives with spruce needles. Extracted solution was then ready and carried out for gas chromatography mass spectroscopy (GCMS) to analyze all the possible chemicals compounds. During the analyzation of compounds, various complex chemical was seen in the chromatograph with different spectra. These various compounds have wide range of applications in many fields.



**Key words**
Spruce needles, separation, extractives, polar and non-polar solvents and GCMS

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# 1 INTRODUCTION

Forest biomass is considered as a fundamental source of energy and raw materials for industrial production. Its chemical components produced as bio-materials, bio-chemicals, bio-fuels, and bio-refinery products have brought the attention to study individually for manufacturing chemicals, well-being and various products for customers. The main purpose of this research is to analyze the chemical compositions and primarily its extractives from spruce needles. The total amount of extractives in coniferous trees comprises up to 4 -5 % depending on the geographic location. The chemistry in spruce needles is complex and even though it has not been understood clearly yet. Nevertheless, those complex compounds contain in needles can obtain the valuable products from different method of technology. The extractives are usually complex chemical compounds having low molecular weights. The chemical compositions in needles are generally composed of four major components which are cellulose, hemicellulose, lignin and extractives. Some of the extractives are stilbenes, lignans, fatty acids, sterols, resins and waxes. Therefore, this study focuses on these various compounds in order to get many advantages from spruce needles for instance, extractives feeding to broiler chicken, medicines, essential oils in skin care, antioxidants, juice and beer, cosmetics and so on.

In the experiment part, the different types of solvents were dissolved, i.e. polar and non-polar solvents. The chemical compounds were isolated from spruce needles by using two different solvents. The polar solvent used during the experiment was acetone whereas non-polar solvent, hexane was introduced in the extraction method. The apparatus used in this experiment was Soxhlet extraction apparatus. The different operating conditions like, temperature, titration and time interval for extraction process were applied in the extraction process. The extracted solution from spruce needles were analyze from gas chromatography mass spectroscopy to identify the various complex chemical compounds and its wide applications in the market.

## 2 CHEMICAL COMPOSITIONS OF WOOD

Wood is composed of four different complex compounds which are cellulose, hemicellulose, lignin and extractives. The first and foremost component in the plant cell is cellulose which defines the wall structure. Cellulose is a high molecular polymer made up of glucose. It is a renewable source in nature and its raw material is also used for cotton fiber. Cotton fiber contain pure cellulose consisting 95% to 97% (Tan, Ong, Kah Hay & Chin Yiap, 2013). The diameter of this fiber is from 10 to 50 micrometer which explains the largest structural unit of cellulose (Carrasco, 2011). Lignin is another significant component in the cell wall. It has also large molecule polymer. The physical properties of lignin are, it gives hardness to the cell wall. About 15 – 35% of lignin is contained in wood (Ek, M., Gellerstedt, G., & Henriksson, G. 2009). The primary cell wall in trees are polysaccharides, proteins and many different enzymes and ions. The main polysaccharides are cellulose, hemicellulose and pectin. Cellulose contains 15-30% dry weight of the primary cell which relates to hemicelluloses and forms micro-fibrils (Ek et al. 2009). The various chemical compositions are explained as:

### 2.1 Cellulose

Cellulose is the richest renewable source and found in trees, bacteria, marine algae and biomass. Cellulose is the major component in the plant cells. It is an organic compound of a polysaccharide consisting of linear chain of thousands of β-1, 4- linked glucose units (Wertz, J., Mercier, J. P., & Bédué, O. 2010). Cellulose is combined with carbon (44.44%), hydrogen (6.17%) and oxygen (49.39%). Its molecular formula is $(C_6H_{10}O_5)n$:n which is called the degree of polymerization, signifies the number of glucose groups from hundreds to thousands. Cellulose is primarily used for making paperboard and paper. Smaller amounts of cellulose are converted into a wide variety of derivative products such as cellophane and rayon. Cellulose for industrial use is largely obtained from wood pulp and cotton. The cellulose contents in plant is usually from 35-50% of dry weight and more than 90% for cotton. The molecular structure of cellulose is in crystalline and non-crystalline phases. The crystalline phase forms many hydrogen bonds and non-crystalline phase is supposed to be amorphous. Cellulose is predominantly significant material used for different purposes in industries such as pulp, paper, and textile and so on (Chen H, 2014).

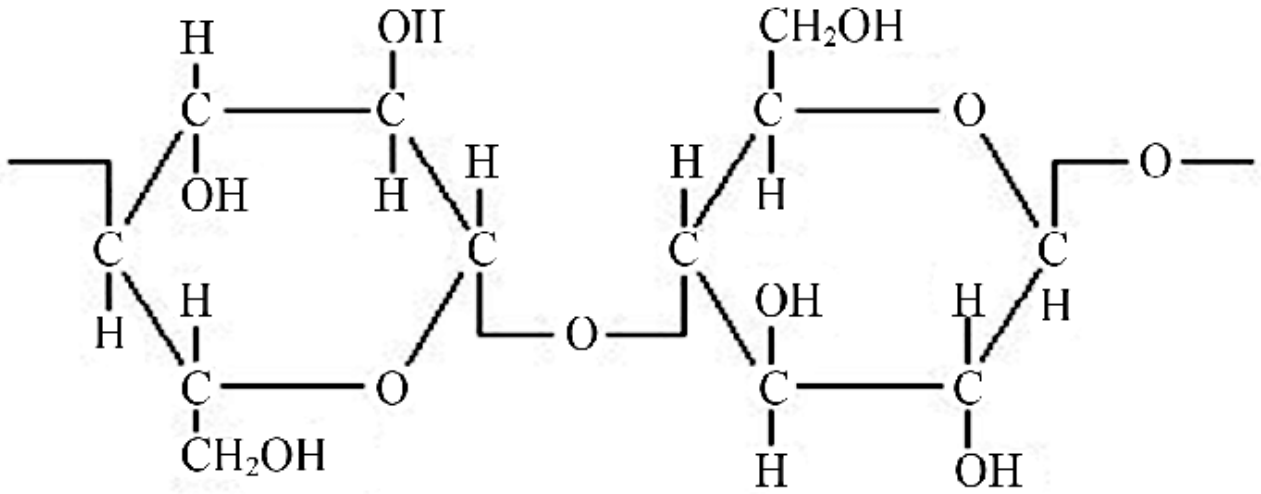


FIGURE 1: Cellulose (Richards, Baker & Iwuoha, 2012)

**2.2 Hemicellulose**

Hemicellulose is also the major element found in plant. The polysaccharides that can be easily separated from plant but could be incomplete products of cellulose molecules often referred as hemicellulose. Wood has generally 25 – 35 % hemicelluloses. Hemicellulose is derived from polysaccharides of plants consists different sugar monomers in branched chains which are D-xylose, D-mannose, D-glucose, or D-galactose and other glycosyls (Stokke, D. D., Wu, Q., & Han, G. 2013). Hemicellulose is purified by conducting different alkaline solubilities with cellulose (Chen H, 2014). The structure and content of hemicellulose is different from plants to plants. The chemical structure of hemicellulose is the composition of the branch chain of glucans. The main chain is the type of glycosyls. Basically, the glucan is the matrix of the cell in hemicellulose which are xylan, xyloglucan, glucomannan manna, and so on (Stokke et al. 2013).

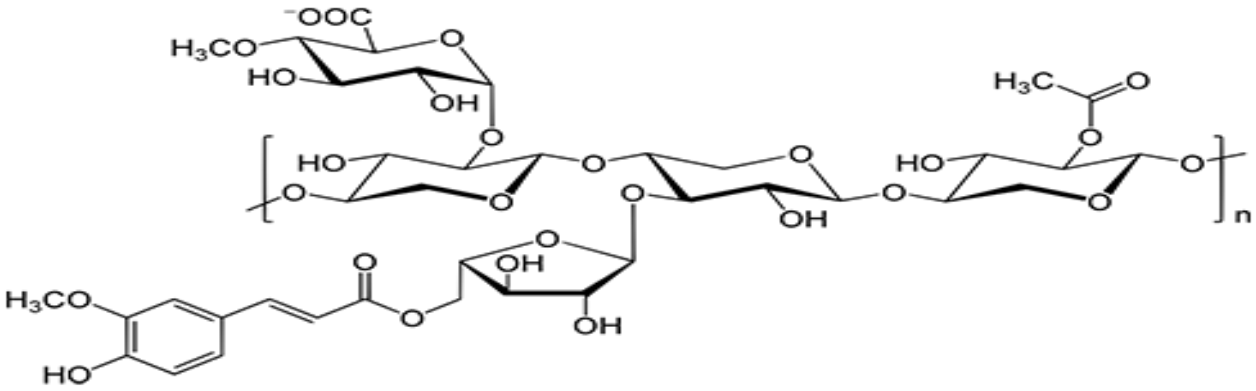


FIGURE 2: Structure of hemicellulose (Stokke et sl. 2013)

**2.3 Lignin**

Lignin is a complex organic polymer, which is placed just behind cellulose. It supports the tissues of vascular plants and some algae. Lignin's are classified as an important polymer because it helps formation of cell walls in wood and bark of a tree. Lignin content in wood is 20 – 40%. The composition of lignin differs from species to species. It is composed of complex phenylpropane units non-linearly linked with three monomers which are coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Calvo-Flores, F. G., Dobado, J. A., Isac-García, J., & Martín-Martínez, F. J. 2015). Lignin is not composed of carbohydrate monomers while the rest of polymers found in plant cell walls are composed of carbohydrate monomers for instance cellulose. The molecule of lignin comprises many reactive functional groups like phenol propane unit, hydroxyl groups, methoxyl groups, carbonyl groups etc. (Chen H, 2014)

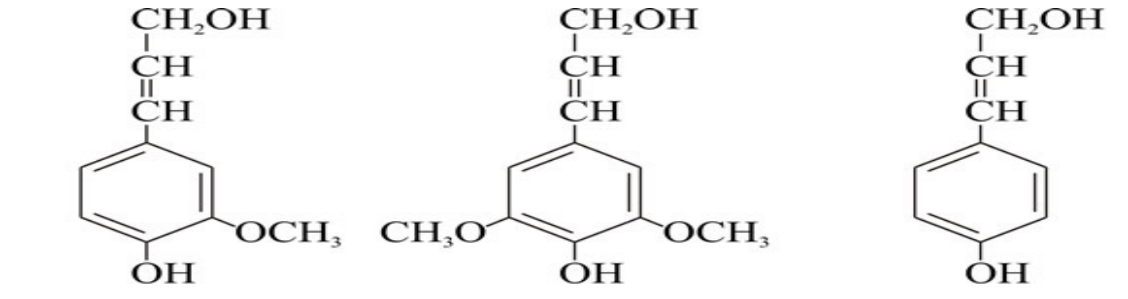


FIGURE 3: Structural unit of lignin (Calvo-Flores et al. 2013)

## 3 EXTRACTIVES

Wood extractives are compounds which has relatively low molecular mass that can be extracted from different part of trees. Extractives consist different kinds of wood components which can be soluble in organic solvents and water. The wood is the compositions of various raw materials with complex chemical compounds and its significant is applicable in different form for pulping, paper making and wide utilization in medicine, skin care, antioxidants and animal feeding. Terpenoids and steroids, fatty acids, waxes, resin and other inorganic compounds are the major chemical compounds found in the extractives. These extractives provide broad functions in different areas (Routa, J., Brännstorm, H., Anttila, P., Mäkinen, M., Jänis, J. & Asikainen, A. 2017).

The fats are mainly glycerol esters present in the woods and needles of spruce and pine trees. Several fatty acids comprising saturated and unsaturated can be found in the wood, barks and needles. The essential phenolic groups have also been derived from the different region of coniferous trees such as, stilbenes, lignans, tannins, and flavonoids. The largest group of aromatic compounds contain in phenolic groups for instance, toluene and benzoic acid. These compounds protect the trees causing from fungi and microbiological attack. Hence, the extractives from wood can be extracted from polar and non-polar solvents. Since, the standard method for extraction is done from water, alcohol, acetone, hexane, diethyl ether and etc. They have good solvent property which dissolves all the complex chemical compounds (Ek, M., Gellerstedt, G., & Henriksson, G. (Eds.). 2009).

Extractives of wood is chemically categorized in different groups as shown in TABLE 1 and they are lipophilic compounds, phenolic compounds and other compounds. Since the composition of extractives differs broadly from species to species, and the total amount of extractives in species rely on the growth conditions.

TABLE 1. Classification of organic extractives in wood (Alén, R. 2011).

| **Aliphatic and alicyclic compounds (Lipohilic)** | **Phenolic compounds** | **Other compounds** |
|---|---|---|
| Terpenes and terpenoids (resin acids and steroids) | Simple phenols Stilbenes Lignans Isoflavones Flavonoids Condensed tannins Hydrolyzable tannins | Sugars Cyclitols Tropolones Amino acids Alkaloids Coumarins Quinones |
| | Stilbenes | Cyclitols |
| Esters of fatty acids | Lignans | Troplones |
| Fatty acids | Isoflavones and flavonoids | Amino acids |
| Alkanes | Condensed tannins and Hydrolyzable tannins | Alkaloids |

The two-major type of extractives process; Phenolic and Lipophilic extractives which contain different complex organic compounds are explained below:

### 3.1 Phenolic extractives

Phenolic extractives are a complex compound which has different aromatic secondary metabolites in coniferous trees. It exists in wood starting from simple phenols to complex polyphenols and other compounds. The phenolic extractives have non-soluble compounds and soluble compounds. The non-soluble compounds contain tannins, lignans and soluble compounds include phenolic acids, flavonoids and quinines. Hence, the most predominantly composed phenolic compounds are phenolic acids, tannins, flavonoids, stilbenes and lignans (Ganthaler, A., Stöggl, W., Mayr, S., Kranner, I., Schüler, S., Wischnitzki, E., Maria Sehr, E., Fluch, S. & Trujillo-Moya, C. 2017). Each wood species produces specific substances. However, in hardwood, phenolic compounds are higher than softwood because of the secondary metabolite's changes (Routa et. al. 2017).

3.1.1 3.1.1 Stilbenes and lignans

Coniferous tress such as pine and spruce have comparatively high amounts typically contains 5 – 10 % of stilbene compounds. In Norway spruce trees, they are bounded in the bark and also in the needles of the tree. The chemical substances like isorhapontigenin, piceatannol, resveratrol and glucosides are found. Piceatannol is the major stilbenes. Stilbenes can be found in the heartwood of a tree. Stilbene is very significant in heartwood because it resists from fungal decay. Stilbenes is basically arising in many different species of the tree in the forest and commonly seen in the bark and leaves of the tree. Stilbenes in wood has resorcinol-A ring which is a derivative of 1,2-diphenylethlene in two-different double bond form, they are trans-stilbene and cis-stilbene. Stilbenes and its derivative are beneficial fighting against cancer, cardiovascular and neuro diseases (Alén, R. 2011).

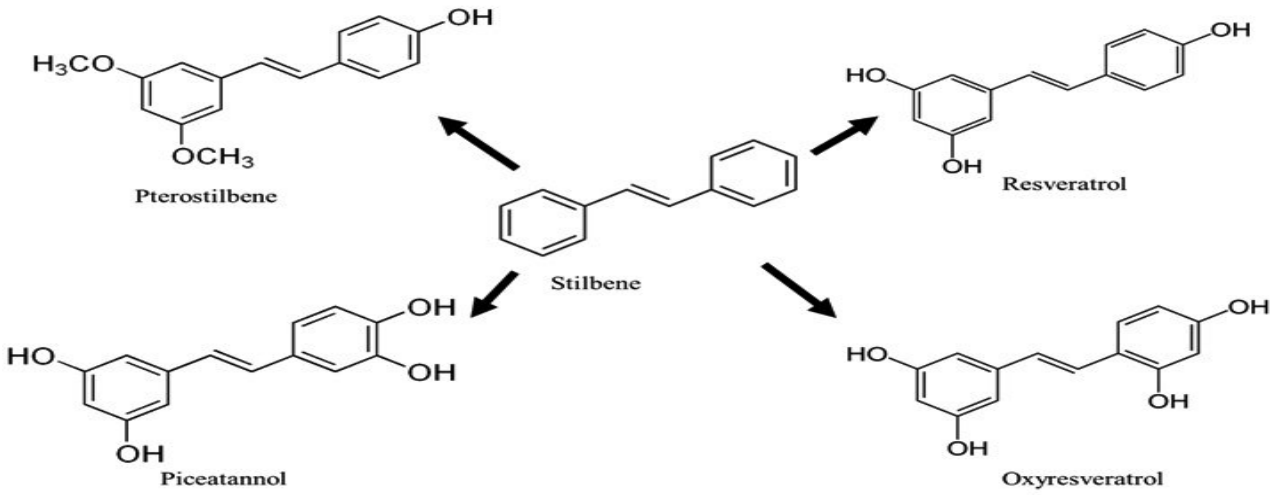



FIGURE 4: Compounds of stilbenes (Shelan, T., Ghazali, R., Fredalina Basri, D. & Nallance Lim, W. 2018)

Lignans are a group of complex compounds found in the tree which has low molecular phenolic compounds. It is the richest source in Norway spruce. The higher amount of concentration can be seen in the heartwood of branches and knots of Norway spruce. It contains 6% to 15% lignans (Holmbom, B.R., Eckerman, C., C Eklund, P., Hemming, J., Nisula, L., Reunanen, M., Sjöholm, R., Sundberg, A., Sundberg, K. & M Willför, S. 2003). The main function of lignan is to protect the substance from insects and microorganisms like bacteria, fungi and viruses. Hydroxymatairesinol (HMR) is one of the lignans found in spruce tree. In 1970's, HMR was analyzed in Åbo Akademi Univerisity to know the beneficial in human health. And it was known in 1998 that the amount of lignans is rich in knot of a spruce. This

HMR was first tested to those causing breast cancer in Turku, Hormos Medical and later became the interesting health product (Alén, R. 2011).

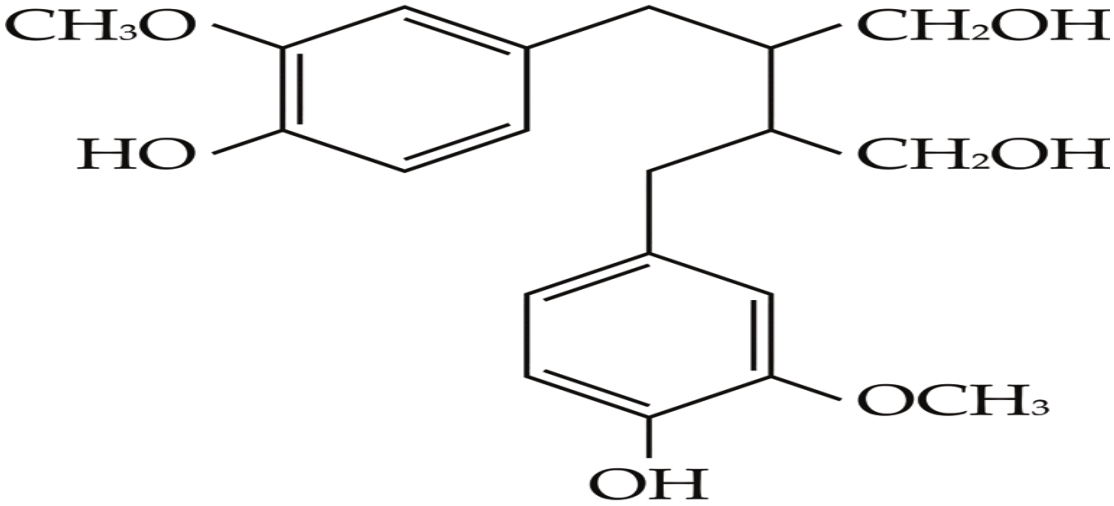



FIGURE 5: Structure of lignans (Mrduljas, N., Kresic, G. & Bilušić, T. 2017)

### 3.1.2 Tannins and flavonoids

Tannins are polyphenolic oligomers. It is water soluble compound with molecular masses between 500 to 3000. Tannins are primarily used for the preservation of leather and hence it is used in leather manufacturing industries. Tannins have divided into two different groups which are hydrolysable tannins and condensed tannins. Tannins are polyphenols exist in many different plants with different colors, such as yellowish or light brown. Tannins are usually in high amount in the bark of the tree. A group of substance is found in tannins which is called hydrolysable tannins. It yields gallic and ellagic acid and sugar when hydrolysis. Gallic acid is formed as 3,4,5-trihydroxybenzoic acid. It acts as an antioxidant (Alén, R. 2011).

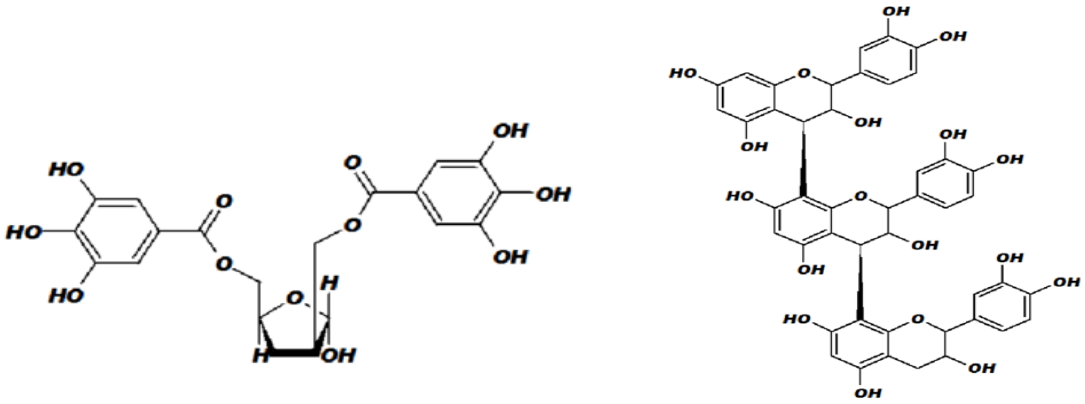


FIGURE 6: Structure of tannins (Andrade, J.N., Costa Neto, E.M. & Brandao, H.N. 2015)

Flavonoids are distributed throughout plant and they act an important role to protect the plant from fungal and insect attacks. It is another important group of phenolic extractives found in wood is the flavonoids. The structure of flavonoids is $C_6C_3C_6$. The common members of Flavonoids are chrysin, taxifolin, catechin and genistein (M. S. Nascimento, 2013). Various biotests is needed to extract the polyphenols like flavonoids. The polyphenol has many functions like antioxidants, bacteriocidic and fungicide. Flavonoids is organic biocides which is replacing the synthetic pesticides (Ganthaler, A., Stöggl, W., Kranner, I. & Mayr, S. 2017).

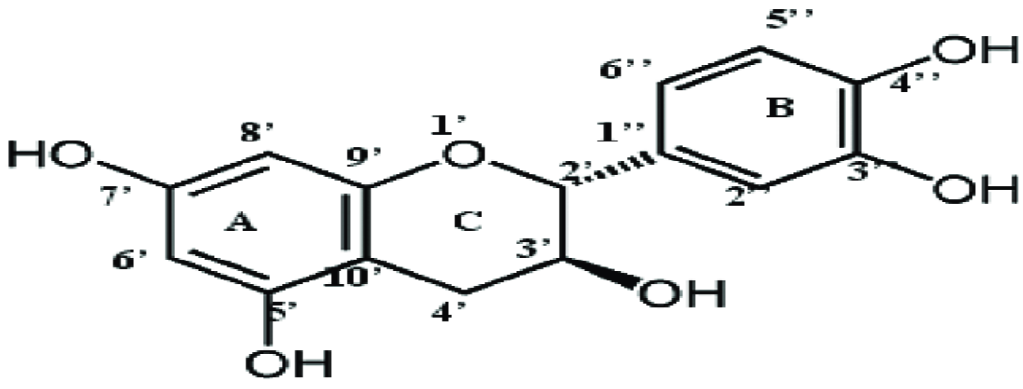


FIGURE 7: Flavonoids (Maher, P., Akaishi, T. & Abe, K. 2006)

### 3.2 Lipophilic extractives

Lipophilic extractives in wood are very complex compound attached with many other compounds. For instance, fatty acids, resin acids, waxes, terpenes, sterols, sterol esters and glycerides. The terpenes and its derivatives are a large group of compounds made for fragrant and flavored substances. Oleoresins and other secretions are found in terpenes. The chemical of terpenes is isoprene units. The number of isoprene units linked in a terpene has different classes: monoterpenes (2 units), sesquiterpenes (3 units), diterpenes (4 units), sesterterpenes (5 units) and triterpenes (6 units). In softwood, all type of terpenes is contained. Fats and waxes are extracted from wood using different organic solvent like acetone and diethyl ether. The composition of fats is about 0.3-0.4% and waxes are about 0.08-0.09% relied on dry wood. The concentration of fatty acids is higher in heartwood than in sapwood (Carmen S.R, 2006).

#### 3.2.1 Terpenoids

Terpenoids is also known as isoprenoids are a large group of compounds with the derivatives of its five-carbon isoprene units by accumulating thousands of the compounds. It mainly consists of oxygen and a hydrocarbon. The majority of these complexes are found in plants. There are various number of isoprene units which are monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids and triterpenoids (Heras, B., Rodriguez, B., Bosca, L. & Villar, A.M. 2003). The structure of terpenes is made from isoprene units in empirical form. These phenomena are followed by isoprene rule (Perveen, S. 2018). Number of isoprene units with carbon atoms are subdivided as follows:

TABLE 2: Classifications of terpenoids (Perveen, S. 2018).

| Classifications | Isoprene Units | Carbon Atoms |
|---|---|---|
| Monoterpenes | 2 | $C_{10}$ |
| Sesquiterpenes | 3 | $C_{15}$ |
| Diterpenes | 4 | $C_{20}$ |
| Sesterterpenes | 5 | $C_{25}$ |
| Triterpenes | 6 | $C_{30}$ |

### 3.2.1.1 Monoterpenes

Monoterpenes hydrocarbons consists almost 40% of the oleoresin. The molecular formula is $C_{10}H_{16}$. It is a volatile compound having high boiling point ranges from 150 °C to 185 °C. It is derived biosynthetically in order to use in pharmaceuticals, cosmetics and agriculture. Monoterpenes are acyclic and also could be in ring structure. Acyclic and monocyclic types are β-myrcene, d-limonene and β-penllanrene. Bicyclic types of monoterpenes are the compounds with two rings in it which are β-pinene and β-thujaplicin. The largest group of compounds consisting different types of cyclic monoterpenes determines in essential oils (Eastman, R.H. & Kluger, R.H, 2019).

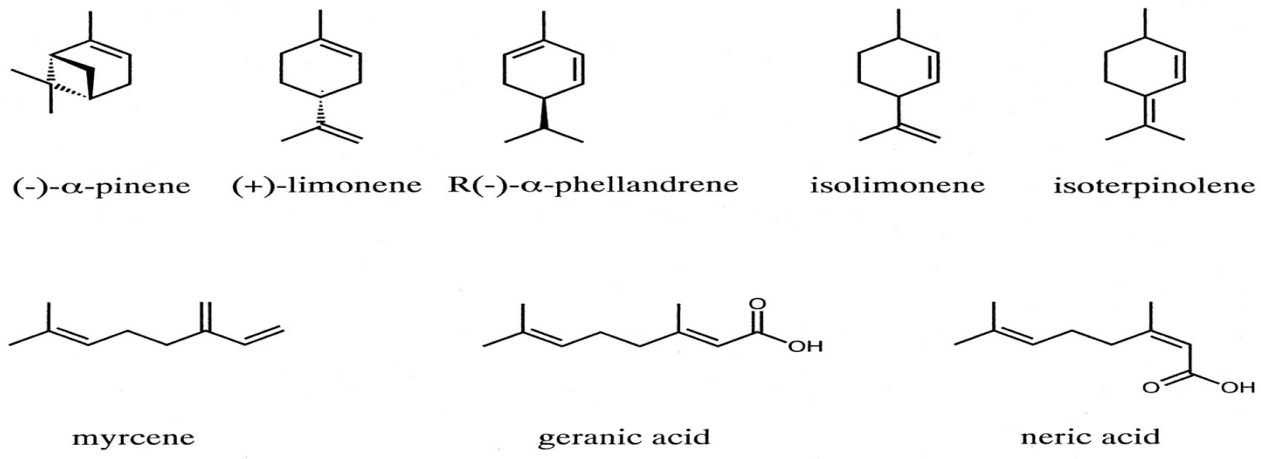



FIGURE 8: Monoterpenes chains (Heyen, U. & Harder, J. 2000)

### 3.2.1.2 Sesquiterpenes

Sesquiterpenes are a class of terpenes with molecular formula $C_{15}H_{24}$. It is basically produced from oxidation and rearrangement of sesquiterpenes. Large group of sesquiterpenes are arranged in acyclic to tetracyclic form. It is also commonly used in essential oils and many commercials turpentines. Some of the examples of sesquiterpenes are δ-cadinene, α-Muurolene and γ-lactone sesquiterpenoids (Prerveen et. al. 2018).

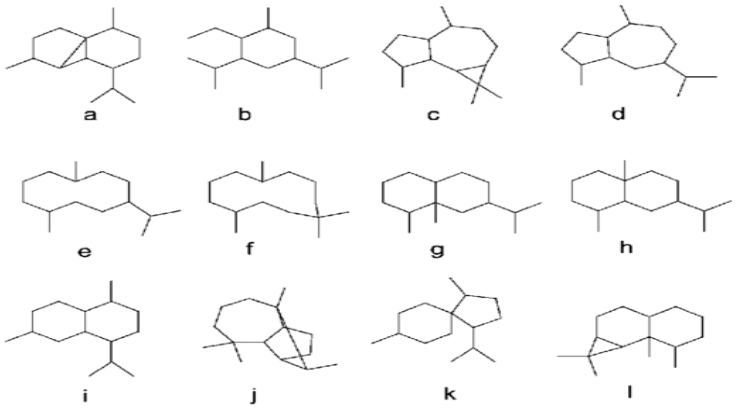



FIGURE 9: Structure of sesquiterpenes (Wang, S.W., Wu, C., Chu, F.H., Chien, S.C., Kuo, Y.H., Shyur, L.F. & Chang, S.T. 2005)

### 3.2.1.3 Diterpenes

Diterpenes is a complex chemical compound consisting of two isoprene units. Its molecular formula is $C_{20}H_{32}$. Diterpenes are the most crucial compounds of terpenes found in oleoresin and exists either as hydrocarbon or as derivatives with functional groups like hydroxyl, carboxyl and carbonyl groups. Its structure is categorized in different types as acyclic, bicyclic and tricyclic. Diterpenes are less volatile and can be found in lesser amount as compared to that of monoterpenes and sesquiterpenes. However, traditional extraction by distillation and separation method helps to identify the diterpenes in essential oils. Some of the diterpenes found in the wood is geranyl linalool, β-epimanoo, cis-abienol, monooxy-loxide and macrocyclic diterpenes (Perveen et. al. 2018).

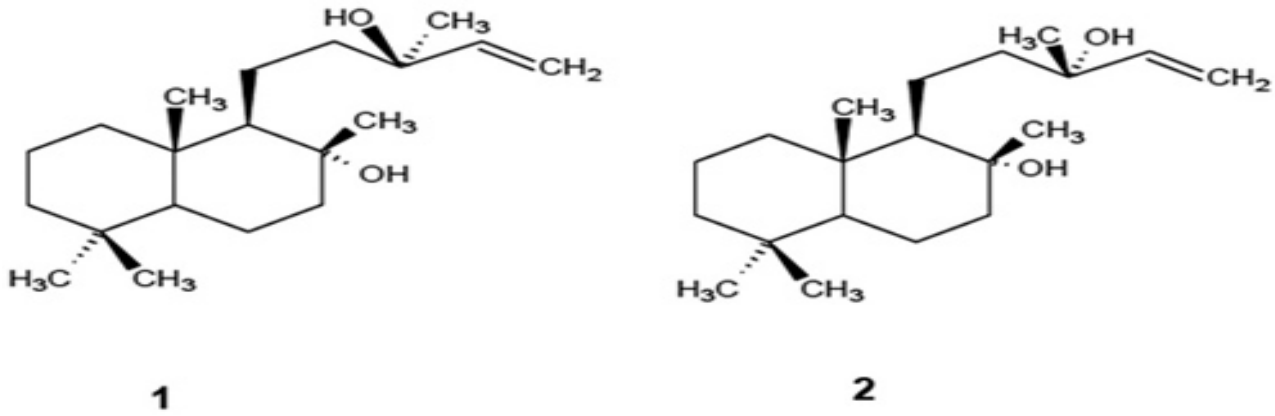



FIGURE 10: Diterperpenes (Huang, Z., Jiang, M.Y., Zhou, Z.Y. & Xu, D. 2014)

#### 3.2.1.4 Triterpenes

Triterpenes are hydrocarbons consisting more than thousands of different groups from squalene. It is formed mainly in pentacyclic structure which means it has 5 rings. Terpenoids and steroids have similar features with respect to their structure and genetic. Its major function in plant is propagation and protect the cell membrane. The large component of hydrocarbon present in triterpenes and steroids acts as hydrophobic except hydroxyl group. Sitosterol, phytosterol, cycloartenol, lanosterol and brassinosteroids, oleanolic acid, betulinic acids, corosolic acids are the common examples of steroids and triterpenoids found in conifer and other trees (Perveen et. al. 2018).

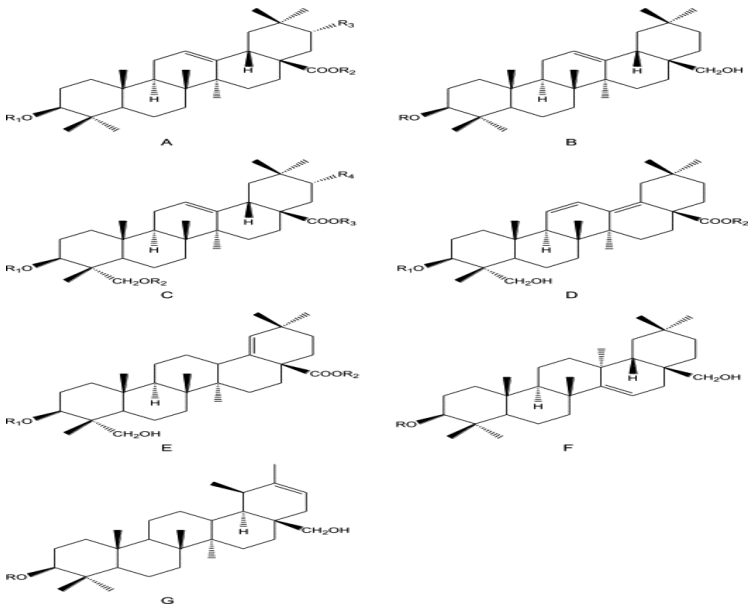



FIGURE 11: Structure of triterpenoids (Du, J.R. & Chen, C. 2014)

### 3.2.2 Fatty acids and sterols

Generally, most of the trees have fatty acids as major components in triglycerides and steryl esters. Coniferous trees like pines and spruces consist 0,5 – 1 % of total fatty acids. Palmitic acid, oleic acid and linolenic acids are the foremost fatty acids found in trees. Pine and spruce contain more pinolenic acid whereas the other trees do not have such component at all. More than 30 different types of fatty acids can be found in spruce combining the carbon chain from C-12 to C24 which also have about four double bonds in them. Some part of spruce has long chain saturated fatty acids ranging from C-26 to C-30. Tall oil fatty acids are mainly found in coniferous trees, which comprises resin acids, different fatty acids, fatty alcohols and some sterols. The fatty acids need distillation methods in Kraft pulping process to isolate form the complex chemical compound. These various derivatives of fatty acids are used in producing many products in manufacturing lubricants, paints, fuel additive, printing inks and cosmetics (Alén et. al. 2011).

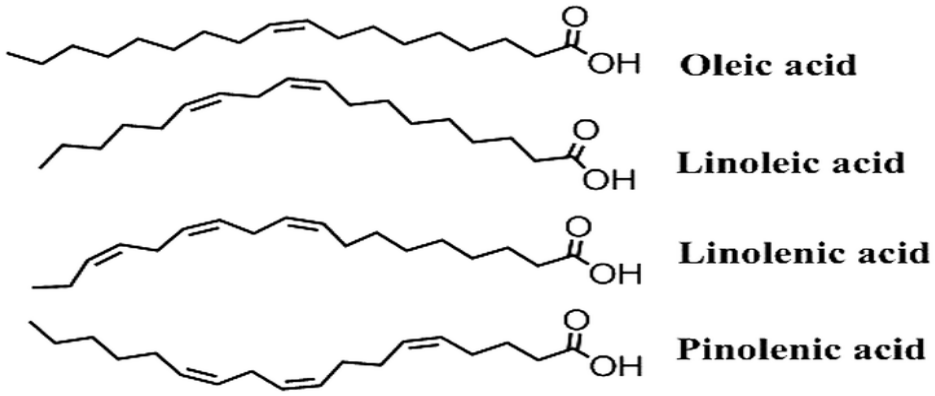



FIGURE 12: Examples of fatty acids (Alén et. al. 2011)

Likewise, sterols occur in coniferous trees and other plants in the form of fatty acid esters. Large number of sterols and its by-products such as sitostanol, campesterol and other sterols alongside methyl groups have been found in sufficient amount. These components relied on different geographical region and hence, only exist in temperate zone. The sterol esters are extracted in Kraft pulping process for the production of soap and lubricants (Alén et. al. 2011).

# 4 GAS CHROMATOGRAPHY

Gas chromatography is a technique of chromatography used in analytic chemistry for analysing and separating compounds. GC helps to analyse the purity of a specific substance and separate the distinguished components of a mixture. This method helps to identify the hundreds of comparatively low molecular weight compounds from the materials (Harris, D. C. 2007). To analysis the compounds, the molecules has to be volatile for instances, alcohols, aromatics, aliphatic and other molecules like esters, fatty acids, steroids, and resin acids (Falaki, F. 2019).

Gas chromatography mass spectroscopy starts with the gas chromatograph only when the samples are completely volatized. It generally composed of two different parts, the mobile phase and the stationary phase. The mobile phase is the liquid or gas that streams through a chromatography system and the substances which are separated is called stationary phase (Woodford, C. 2019). When the sample is brought into the GC inlet from where it is vaporized and later it flows through the column with the help of carrier gas. Usually, the mobile phase is so called a carrier gas. The carrier gas used in this technique is helium which is an inert gas helps to travel solutes through the column. As soon as the sample injected at the top of the instrument, the sample vaporizes into the gaseous phase and splits into the different individual components by means of capillary column filled in a stationary or so-called solid phase. Later, the components will be isolated and elute from the column at different interval of times. The time taken to remove the adsorbed substance is called retention time. Hence, the molecules go to the gas chromatography column which are supposed to ionized by the mass spectrometer in the aid of electron (Raja, P.M.V & Barron A.R. 2019). Then the ionized molecules are passes through the mass analyzer, that is quadrupole. Quadrupole is a device which distributes the electric charges. It will follow to the final process involving ion detection and analyses the compounds with several low and high peaks. A very complex compounds will be seen on the monitor with different peaks resulting mass spectrum.

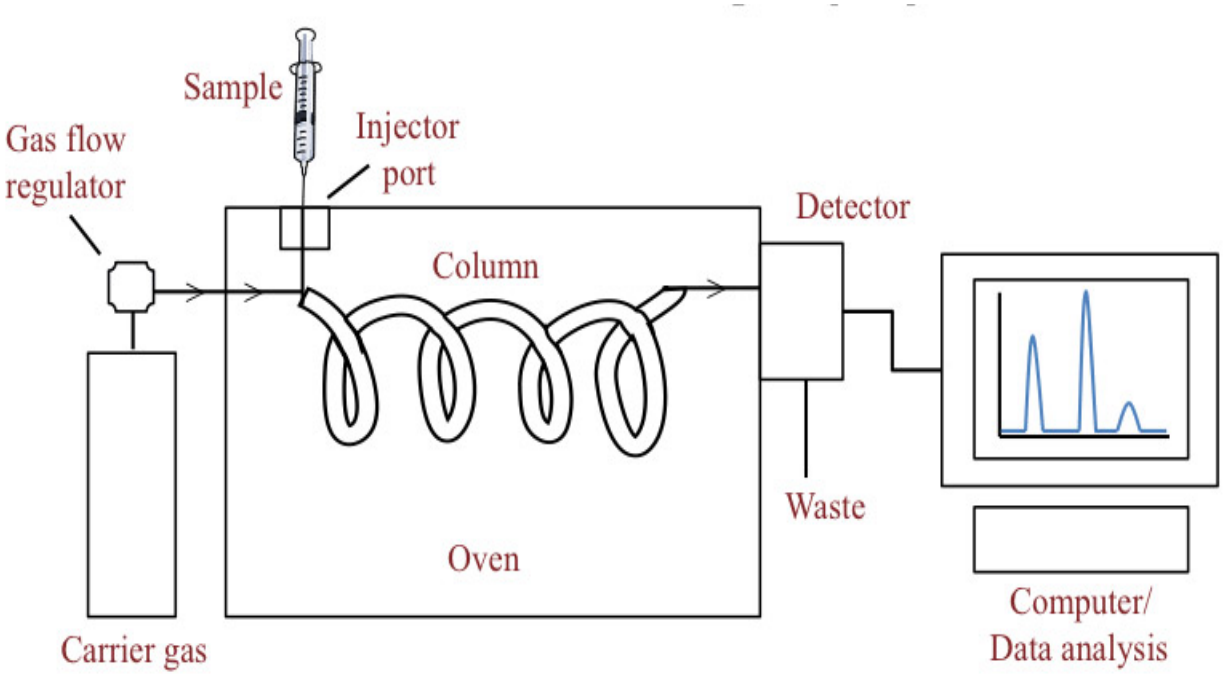



FIGURE 13: Gas Chromatography Mass Spectroscopy (Aryal, S. 2018.)

Gas chromatography is first introduced by injecting the sample into the septum which goes through the mobile phase. The mobile phase is usually constituted of a helium gas. It carries the injected sample until the stationary phase is reached inside the gas chromatography. The stationary phase normally comprises of a chemical which is in solid and liquid state and they are preferably a complex compound from the sample. At the stationary phase, the components get separated. The separation of components inside GC column happens because of gradually heating the sample inside the column. The compounds inside GC are with low and high boiling points by which the low boiling points compounds appeared to be eluted prior. Similarly, the pressure is adjusted in the mobile phase which is varied in order to achieve the separation of compounds. When the sample flows to the column, the capillary column begins to heat up at high temperature. A lot of chemical interactions can be seen between the sample and the stationary phase (Poole, C. (Ed.). 2012). During the interactions, the weaker components will separate and elute quicker than stronger components. The weaker and stronger components can be known from the retention time. The compounds retention time is a compound which is measured from the sample injected until its elution inside the GC column. As a fact, low molecular weight compounds appear sooner in a GC column than that of a higher molecular weight. This is ought to be the reason of boiling point differences (Woodford, C. 2019).

The eluted compounds will undergo electron ionization or chemical ionization. In an ionization, the electrons interact with solid or gas phase atoms or molecules to form ions. These ions will discharge in mass analysis in a mass spectrometer to measure the mass to charge ratio of ions. When the ratio of mass and charge is reported, the ion peaks will be displayed on the computer. The peak values shown on the computer are compared with the libraries of mass spectra by using analytical software programme (Poole, C. (Ed.). 2012). Eventually, it will start matching the spectra and the compounds will be identified and characterized. Hence, the mass spectroscopy analysis is done which finally helps to determine the molecular weight of compounds, molecular formula and functional groups respectively.

# 5 MARKET OVERVIEW OF SPRUCE NEEDLES

The extractives of spruce needles have significant purpose on utilization of various products by studying its individual chemical existence. The extractive compounds provide high functionalities for several manufacturing chemical companies and health products in the market. Since, the spruce needles have extensive range of applications in various forms from treating cancer to recreational purposes.

## 5.1 Spruce needles extractives in broiler chicken feeding

Spruce needles biomass is harvested from a forestry by-product to extract the naturally active substances. The main components in the naturally active substances of spruce needles are chlorophyll and its significant products which are carotenoids, vitamins, antioxidants, essential oils, minerals, fatty acids, polysterols, etc. All of these natural substances have a comprehensive beneficial and disease preventive impact on broiler chicken and human beings (Vitina, Krastina, V., Daugavietis, M., Miculis, J. & Cerina, S. 2011).

The extractions of spruce needles have many complex natural substances as mentioned above which is then transported in the poultry diet from the feed to meat. So many researchers had tested this phenomenon earlier in 1970's in Latvia and later they confirmed that it will be utilized in poultry farm as a feed. Since that time, the naturally active substances as an innovative product is transferred from feed to the value added and have been running successfully. The biomass of spruce needles is rich in active substances such as carotenoids, fatty acids etc. After being tested, it was then carried out to poultry farm to feed them. When tested, it showed up with many advantages like, it helped in increasing the weight of chicken, improvement of immunity, blood immune, sufficient amount of protein and minerals and decrease in cholesterol level. There was also clearly seen that the productivity of poultry was improved in its egg and meat quality. The amount of carotenoids is almost 0,45 to 0,57 mg $kg^{-1}$. Carotenoids are antioxidants which protects and fight against any diseases and enhance the immune system. When carotenoids converted into vitamin A, so as to help in growth of broiler chicken (Vitina et. al. 2011).

### 5.2 Spruce needles essential oils

Essential oils are organic aromatics which is extracted from mostly green plant materials such as, leaves, spruce and pine needles, flowers, peeling of fruits and many more. Essential oils cannot be made instead it has to be manufactured synthetically in a laboratory. There are many methods to extract this essential oil by introducing solvents, steam distillation, CO2 extraction, water extraction and so on. The extraction method is significantly relied in two factors which are temperature and pressure adjustment. Basically, the methods of extraction are experimented with respect to their plant materials. Hence, amongst all the extraction methods, solvent extraction is used for spruce needles in order to produce essential oils. For instance, spruce essential oil is traditionally used in therapeutic benefits by human beings. Essential oils have adequate amount of antioxidants which helps fighting the immune system (Elmore, L. 2019).

The solvent extraction methods use solvents like hexane and ethanol to separate essential oils from spruce needles. It extracts low amounts of essential oils but highly resinous with delicate aromatics compounds. However, this has very strong sweet smell and sometimes it is further used in making fragrances by using distillation process. Explaining of solvent extraction, the non-volatile substances like waxes, resins and other pigments are treated with the solvent which yields a waxy aromatic compound called a concrete. These concrete substances when contacted with an alcohol, the essential oils will be collected from the bottom of the chamber. Afterall, a wide range of applications of essential oils can be seen. Furthermore, it is used by the people who has respiration problem, conditioning for hair and beard, workout massage in muscles and joints (Elmore, L. 2019).

### 5.3 Medicine

Likewise, spruce needle is applicable in medicinal purpose. People used the needles for healing properties, causing from different infectious disease back 20$^{th}$ century and still in some part of European countries. The primary benefit of the spruce needles is in the tip of the growing needles which contains vitamin C. Basically, they harvest the needles in the spring season and grind them. Once its crushed into powder form, they drink up as an herbal tea. It helps from fatigue, nervousness and increase the blood circulation. Apart from those benefits, it is also signified as an antibiotic. The spruce herbal tea is taken who is also suffering from bronchitis, angina and flu (Sõrm, H. 2015). Additionally, spruce resin is valuable substances in treating chronic wounds. Chronic wound is extremely dangerous infectious disease which cannot be curable at all. By involving different academic researchers, Finland has studied on

different properties of Norway spruce resin in treating the chronic wound particularly, since 2002 until 2013. They have been focused on wound healing and its antimicrobial substances. The reason of investigating on this particular field is that the treatment of chronic wound is tremendously expensive, and many people have been caused by this infectious disease globally. Later, they got succeeded with a feasible treatment plans and also, very cost-effective (Jokinen, J.J. & Sipponen, A. 2013).

### 5.4 Cosmetics

Spruce tree is nowadays considered as a bio-economy resources because of its environment friendly production factor. The goods produce from spruce trees is referred as the best economy which is because of its biological renewable resources. The spruce resin is one of the substances that comes from the spruce trees. This substance is used in some of the cosmetic companies like aroma land in United States. healing from various skin problems. There are few start-up cosmetic companies in Finland which they produce different skin products from spruce resins.

Spruce resin has enormously fine oil and organic resin acids which contains hundreds of antioxidants and flavonoids. From these organic compounds, it can be clearly known that by using the products from resins helps from skin damage. Furthermore, it is widely used in the healing of many forms of skin problems, such as cracking of face and hands, lips, rashes, sunburn, dry skin, dry cuticles, as well as blisters (Solomon, D. 2017). In Finland, few years ago, the researchers had been working on spruce resins where few companies were able to launch new products in the market. Karelia Arctic Oy started a new company named Pihqa balm in 2016. They found out that spruce resins have many benefits concerning the healing properties of the skin. Pihqa is quite famous company which produces different skin products without containing any alcohol or solvents. It is said that no trees were destroyed while harvesting the resin. Hence, spruce needle plays significance application in skin regeneration (Solomon, D. 2017).

### 5.5 Spruce needle beverages

Spruce needle is harvested for manufacturing alcoholic and non-alcoholic beverages. As it has wide range of advantages in various forms of human's life. People in Estonia extract spruce needle juice because of its health benefits like cancer, swelling and other infectious diseases. During 18$^{th}$ and 19$^{th}$

century, North America, England and some parts of Europe had traditional brewery extracting from spruce needles. They drank as an herbal treatment as well as recreational purpose (Rail, E. 2019). Because of the valuable products of spruce needles, largescale markets have potential utilizations in many fields as mentioned earlier and also could replace synthetic chemicals. Extractives based chemicals and its raw materials could substitute fossil feedstock which helps to produce fuel additives, lubricants and etcetera.

# 6 EXPERIMENTAL PROCEDURES

Different methods of experiment have been done throughout the research by using different procedures which is clearly explained below. This experiment could not be possible without proper instructions on how to use various chemicals in right order and also proper guidance.

## 6.1 Pre-treatment of spruce needles

Pretreatment is generally defined as a treatment of something chemically or mechanically before its used. Pretreatment enables to separate biomass in such a way which makes it easier to go further steps in a laboratory. It is the first step to carry out the consequent processes and hence it is taken as a prior method in most of the cases. Relying on the desired products, different pretreatment methods are used. In this case, the method of pretreatment is mechanical, where the size of biomass is reduced mechanically. Size reduction is one of the predominantly means of mechanical pretreatment which is utilized frequently.

Firstly, spruce needles were harvested from the forest and brought into the laboratory. The needles were cut from the stem without mixing waste products. After cutting the fine needles, it was collected in a container and put it in a freezer for 3 to 4 days. Therefore, it was so called freeze-dried needles. Then it was further taken to grind the needles in order to reduce the size of the biomass. The initial size of the needles was about 3-4 mm which was then reduced to 0, 5 mm to 1 mm. The mechanical pretreatment has different size reduction methods such as hammer mills, stirred mills, centrifugal mills, ball mills, cutting mills and so on. The mills that I used in this process was cutting mills. The cutting mills has rotor inside the mill that rotates at high speed which is connected to the blades and cut into tiny particles like fiber. It was then again kept in a freezer so as to remain the moisture unchanged. Eventually, the grinded needles were processed in the laboratory aiming to find out the chemical compositions and extractives.

## 6.2 Extraction solutions of acetone and hexane

Now, to identify the chemical compounds, different solvents extraction was used. Acetone and hexane were the main solvents used to analysis the unknown compounds from spruce needles. When the Soxhlet

apparatus was ready, 150 ml of acetone was poured in a round bottom flask with grinded fresh spruce needles. In the same way, hexane extraction was set up in the same apparatus. The whole experiment was operated for 4 hours. The temperature was maintained in order to create any cause. Finally, the both extractions were ready and the solutions from acetone and hexane were taken for GC analysis.

After making the acetone and hexane solutions, it was then prepared for the GC analysis. Now, the 3 ml of acetone and hexane solutions were poured in the test tubes by using pipette. The pipette samples were dried in the hood by using a gentle stream of $N_2$ gas at room temperature. When the liquid in the test tubes was completely dried, small amount of compounds was settled down at the bottom. The dried samples at the bottom of the test tubes were considered as extractives groups with free individual compounds. The residue seen at the bottom was silylated by dissolving with other subsequent compounds like pyridine and BSTFA and TMCS. Furthermore, 0,7 ml of pyridine was added in both of the test tubes. Later, the mixture was transferred into a vial. To make it complete silylation, 0,7 ml of a mixture of BSTFA and TMCS compound was injected into a vial. The silylated samples were shaken occasionally for few minutes and it was then kept in an oven for 60 degree Celsius to dissolve the mixture entirely. Eventually, these samples were sent to the GCMS to find out the qualitative chemical compounds.

### 6.3 GCMS

When the samples were ready, it was then injected into the injector of GC. Once the samples injected, a small amount of the samples from the vial was begun to split to the column. An inert carrier gas which was helium was supplied from a gas cylinder to the GC in order to flow the sample through the column and into the detector. The sample from the vial was causally injected into the heated injection port which was slowly volatilized and carried away to the column with the help of carrier gas. As soon as the samples passes through the column, it was then started to isolate the compounds inside the column. The capillary column was 30m long with the diameter of 0,25mm. The samples when travelled through the capillary column, it was then coated with the stationary phase with 0,25 micron. The longer the column the better will be the separation. The compounds flowing through the column was detected by the detector. The compounds can be known by their distinct retention time and that of was compared with the reference compounds. Finally, the amount of compounds was seen on the computer by appearing distinguish spectra.

## 6.4 Calculations

From the experiment, different data have been gotten by using different methods which would help to determine the chemical compositions of spruce needles. The obtained data in each experiment will show how much percentage of individual compounds it has.

### 6.4.1 Moisture contents

Two samples were weighed and dried in an oven overnight.

| S. N | Weigh of porcelain crucible (without sample) | Weigh of sample before drying (Freeze-dried spruce needles) | Weigh of sample after drying (Porcelain crucible + sample) |
|---|---|---|---|
| 1. | 47, 7192 g | 2, 0325 g | 48, 9326 g |
| 2. | 46, 0076 g | 2, 0094 g | 47, 2009 g |

From the table, the moisture content is calculated as:

I. Initial sample = 2, 0325 g

Final sample = 49, 7517 g – 48, 9326 g

= 0, 8191 g

$$\text{Moisture content (\%)} = \frac{weight\ of\ dry\ sample}{Initial\ sample} * 100\%$$

$$= \frac{0{,}8191\ g}{2{,}0325\ g} * 100\%$$

= 40, 30 %

II. Initial sample = 2, 0094 g

Final sample = 48, 0170 g – 47, 2009 g

= 0, 8161 g

Moisture content (%) = $\frac{weight\ of\ sample}{Final\ sample} * 100\%$

$= \frac{0{,}8161\ g}{2{,}0094\ g} * 100\%$

= 40, 61 %

Hence, the average moisture contents is 40, 46%.

### 6.4.2 Ash contents

At the beginning, the weight of porcelain crucible was taken and also the weight of sample was measured. The standard weight of the sample was 1, 5 g. After measuring the weight of the samples, it was then placed in a muffle oven. To complete this experiment, it was followed by different steps such as the ramp from the room temperature was maintained at 105 degree Celsius and then held it for 12 minutes. The second step was the ramp was increased to the temperature of 250 degree Celsius at the rate of 10 degree Celsius per minute. Later it was hold for 30 minutes at the same temperature. Thirdly, the ramp was again increased to 575 degree Celsius for 3 hours. Finally, the sample was removed from the muffle oven dropping the temperature to 105 degree Celsius. I had to put my samples overnight in order to get the expected results allowing them to turn into ash completely. The crucible with the samples were cooled down in the desiccator and again measured the weight.

The ash content is calculated as follows:

| S. N | Weight of porce-lain crucibles | Weight of samples | Weight of samples after drying |
|---|---|---|---|
| 1. | 19, 5018 g | 1, 5083 g | 19, 5340g |
| 2. | 19, 8324 g | 1, 4999 g | 19, 8832 g |

By using the above values, the ash content is calculated.

I. Ash content (%) = $\frac{Weight\ of\ dry\ ash}{Weight\ of\ sample} * 100\ \%$

$= \frac{19{,}5340\ g - 19{,}5018 g}{1{,}5083 g} * 100\%$

= 2, 13 %

II. Ash content (%) = $\frac{Weight\ of\ dry\ ash}{Weight\ of\ sample} * 100\ \%$

$= \frac{19{,}8832\ g - 19{,}8324\ g}{1{,}4999 g} * 100\%$

= 3, 38 %

Hence, the average ash content is 2, 75 %.

### 6.4.3 Extractives

Extraction can be extracted by using Soxhlet extraction apparatus with the help of solvent, acetone. And, it takes 4 hours to complete the whole process. The first thing is to take the samples and weight of

thimbles before the experiment starts. It is done in duplicate in order to face the consequences. The weight of standard sample was 4 grams.

| S. N | Weigh of samples | Weigh of thimbles |
|---|---|---|
| 1. | 4, 0552 g | 3, 4855 g |
| 2. | 4, 0592 g | 2, 8893 g |

After 4 hours of extraction process, the solvent at the round-bottomed flask was taken to the rotavapor to evaporate. The extractives will be seen at the bottom which was then poured in a dish. The extractives were left in a hood to let it evaporate. Then it was further taken to an oven to dry it completely for one hour at 105 degree Celsius. Finally, it was cooled down in a desiccator.

| S. N | Weigh of dish | Weigh of dish + extractives (after drying) | Weigh of samples from thimble (after drying) |
|---|---|---|---|
| 1. | 2, 4700 g | 2, 9858 g | 5, 4496 g |
| 2. | 2, 4671 g | 2, 9877 g | 4, 8409 g |

The contents of extractive is calculated as:

I. $$\text{Extractives (\%)} = \frac{Weigh\ of\ dish\ and\ extractives - Weigh\ of\ dish}{Weight\ of\ oven\ dried\ sample} * 100\%$$

Here, the weight of an oven dried sample is 59, 70 % of 4, 0552 g.

The moisture content calculation says that 40, 30 % is moist and 59, 70 % is dried. That is why the oven dried sample is calculated with this outcome.

So, weigh of oven dried sample = $\frac{59,70}{100} * 4,0552\ g$

= 2, 4209 g

Now, oven dry sample will be = 4, 0552 g – 2, 4209 g

= 1, 6342 g

Hence,

Extractives (%) = $\frac{Weigh\ of\ dish\ and\ extractives - Weigh\ of\ dish}{Weight\ of\ oven\ dried\ sample} * 100\%$

= $\frac{2,9858\ g - 2,4700\ g}{1,6342\ g} * 100\%$

= 31, 56 %

II. Extractives (%) = $\frac{Weigh\ of\ dish\ and\ extractives - Weigh\ of\ dish}{Weight\ of\ oven\ dried\ sample} * 100\%$

let's find out the weight of an oven dried sample which is 59, 39% of 4. 0592 g.

= $\frac{59,39}{100} * 4,0592$ g

= 2, 4107 g

Now, oven dry sample will be = 4, 0592 g – 2, 4107 g

= 1, 6485 g

Hence,

$$\text{Extractives (\%)} = \frac{Weigh\ of\ dish\ and\ extractives - Weigh\ of\ dish}{Weight\ of\ oven\ dried\ sample} * 100\%$$

$$= \frac{2{,}9877\ g - 2{,}4671\ g}{1{,}6485\ g} * 100\%$$

$$= 31,\ 58\ \%$$

Therefore, the average moisture content is 31, 60 %.

It is supposed to determined that the moisture content from 1 g of extractives free samples which is from the thimble after the experiment.

Initial weigh of sample = 1, 0053 g
weigh of crucible = 80, 1707 g
Final weigh with crucible = 79, 2439 g

So, after drying the weigh becomes = 80, 1707 g – 79, 2429 g
= 0, 9278 g

$$\text{Moisture content (\%)} = \frac{weigh\ of\ dried\ sample}{weigh\ of\ initial\ sample} * 100\%$$

$$= \frac{0{,}9278\ g}{1{,}0053 g} * 100\%$$

$$= 92,\ 29\ \%$$

### 6.4.4 Lignin

The weight was taken from the extractive free samples. The samples were heated in a water bath by adding 4 ml of 72% of $H_2SO_4$ solution in a test tube. The water bath was maintained at 30-degree Celsius temperature. It took one hour to complete the experiment. The heated samples were stirred with a glass in an interval of few minutes so, it can be dissolved very well. After all, the samples were transferred to

the flask for autoclave. Then it was mixed with 112 ml of distilled water on it. The mixture was placed in an autoclave at 112 degree Celsius for one hour along with 1 bar. Then it was prepared for the filtration. About 100 ml of hot distilled water was added to the samples which was kept in an autoclave so as to remove all the acid insoluble lignin from the samples. These samples were taken for filtration and then kept in an oven for overnight at 105 ± 3 degree Celsius. Finally, the samples were dried and taken for the measurements.

| S. N | Weigh of samples (Extractives free samples) | Weigh of flask with filter paper (Without sample) | Weigh of flask with filter paper and samples (After drying) |
|---|---|---|---|
| 1. | 201, 6 mg (0,2016 g) | 30, 3584 g | 30, 3896 g |
| 2. | 208, 5 mg (0,2085 g) | 19, 6583 g | 19, 6896 g |

Now, the amount of acid-insoluble lignin (AIL) is determined,

I. $$\text{AIL (\%)} = \frac{Filtered\ solid\ residue\ with\ sample - Weigh\ of\ filter\ and\ flask}{Weigh\ of\ oven\ dried\ sample} * 100\%$$

$$= \frac{30{,}3896\ g - 30{,}3584\ g}{92{,}29\%\ of\ 0{,}2019\ g} * 100\%$$

Here, the weight of oven dried sample is calculated as 92, 29% of moisture content from extractive free samples multiplying with the initial sample of lignin.

$$= \frac{0.0312\ g}{0{,}1839\ g} * 100\%$$

$$= 16, 77\%$$

II. AIL (%) = $\frac{Filtered\ solid\ residue\ with\ sample - Weigh\ of\ filter\ and\ flask}{Weigh\ of\ oven\ dried\ sample} * 100\%$

$= \frac{19{,}6896\ g - 19{,}6583\ g}{92{,}29\%\ of\ 0{,}2085 g} * 100\%$

= 16, 27%

Hence, the average content of lignin is 16, 52 %.

### 6.4.5 Holocellulose

The weight of standard extractives-free sample was 1, 5 g. The sample was poured in the Erlenmeyer flask. About 48 ml of hot distilled water was added in it. Then again 0, 3 ml of acetic acid and 0, 75 g of sodium chlorite was added to the same flask. The mixture was heated in water bath at 70 degree Celsius and was shaken occasionally in order to mix it completely. Every 1 hour, 0, 3 ml of acetic acid and 0, 75 g of sodium chlorite were added in a flask for two times. During the reaction, the neck of flask was covered. This reaction was completed in 4 hours and then finally the flask was taken to cool down in a water bath.

After heating the mixture for 4 hours, the solid residue was filtered. Before the filtration process, the flask containing mixture was put in centrifuge to separate the particles which enabled to filter the solvent easily. While filtering the residue, it was supposed to wash it everything which was remained in a flask and also until the yellow color was removed. Eventually, the solid residue was washed with acetone and dried in an oven at 105 degree Celsius overnight.

| S. N | Weigh of extrac-tives-free samples | Weigh of a jar without samples (For Filtration) | Weigh of dried samples |
|---|---|---|---|
| 1. | 1, 5013 g | 45, 8213 g | 46, 5204 g |
| 2. | 1, 5007 g | 48, 4930 g | 49, 1944 g |

Now, the amount of holocellulose is determined as,

weight of holocellulose = 46, 5204 g – 45, 8213 g

= 0, 6991 g

Here,

I. Holocellulose (%) = $\frac{Weigh\ of\ holocelllose}{Weigh\ of\ dried\ oven\ sample} * 100\%$

$= \frac{0{,}6991\ g}{92{,}29\ \%\ of\ 1{,}5013\ g} * 100\%$

= 50, 45 %

Weight of holocellulose = 49, 5204 g – 48, 4930 g

= 0, 7014 g

II. Holocellulose (%) = $\frac{Weigh\ of\ holocellulose}{Weigh\ of\ oven\ dried\ sample} * 100\%$

$= \frac{0{,}7014\ g}{92{,}29\ \%\ of\ 1{,}5007\ g} * 100\%$

= 50, 64 %

Hence,

Average holocellulose = $\frac{50{,}45\ \%+50{,}64\ \%}{2}$

= 50, 54 %

### 6.4.6 Cellulose

Firstly, 0, 5 g of standard sample was taken from holocellulose. Then 25 ml of 17, 5 % NaOH was poured in a 250 ml of Erlenmeyer flask. The holocellulose was stirred completely to dispersed in a flask. The stirrer was then removed and washed with 5 ml of 17, 5 % NaOH to make it total reagent content in the flask to 30 ml. It was again stirred thoroughly with a glass rod to disperse the mixture in a flask. The mixture was kept in a water bath at 25 degree Celsius. After 30 minutes from the first addition of NaOH

reagent, it was again added with 30 ml of distilled water and stirred thoroughly with the glass rod. The flask was kept for another 30 minutes. And then the suspension was stirred very well and ready for the filtration. The solid residue was washed with distilled water and then 15 ml of 10 % acetic acid was added in it. Finally, it was washed with 400 ml of distilled water. Afterall, the solid residue was dried overnight at 105 degree Celsius and was measured the weight.

| S. N | Weight of a jar without sample | Weight of the samples | Weight of a jar with samples (After drying) |
|---|---|---|---|
| 1. | 11, 4252 g | 0, 5017g | 11, 6134 g |
| 2. | 11, 6241 g | 0, 5061 g | 11, 8965 g |

Now, the content of cellulose is determined,

I. Cellulose (%) = $\frac{Weight\ of\ cellulose}{Weight\ of\ holocellulose} * 100\ \%$

$= \frac{11{,}6134\ g - 11{,}4252\ g}{0{,}5017 g} * 100\%$

= 37, 51 %

II. Cellulose (%) = $\frac{Weight\ of\ cellulose}{Weight\ of\ holocellulose} * 100\ \%$

$= \frac{11{,}8965\ g - 11{,}6241\ g}{0{,}5061 g} * 100\%$

= 53, 82 %

Hence, the average cellulose is 45, 65 %.

### 6.4.7 Hemicellulose

The hemicellulose content was obtained by subtracting the α-cellulose content from the holocellulose content.

Here, weight of hemicellulose = Weight of holocellulose – weight of cellulose

= 0, 5061 g – 0, 2724 g

= 0, 2337 g

To get the amount in percentage = $\frac{0,2337g}{0,5061g} * 100\%$

= 46, 17 %

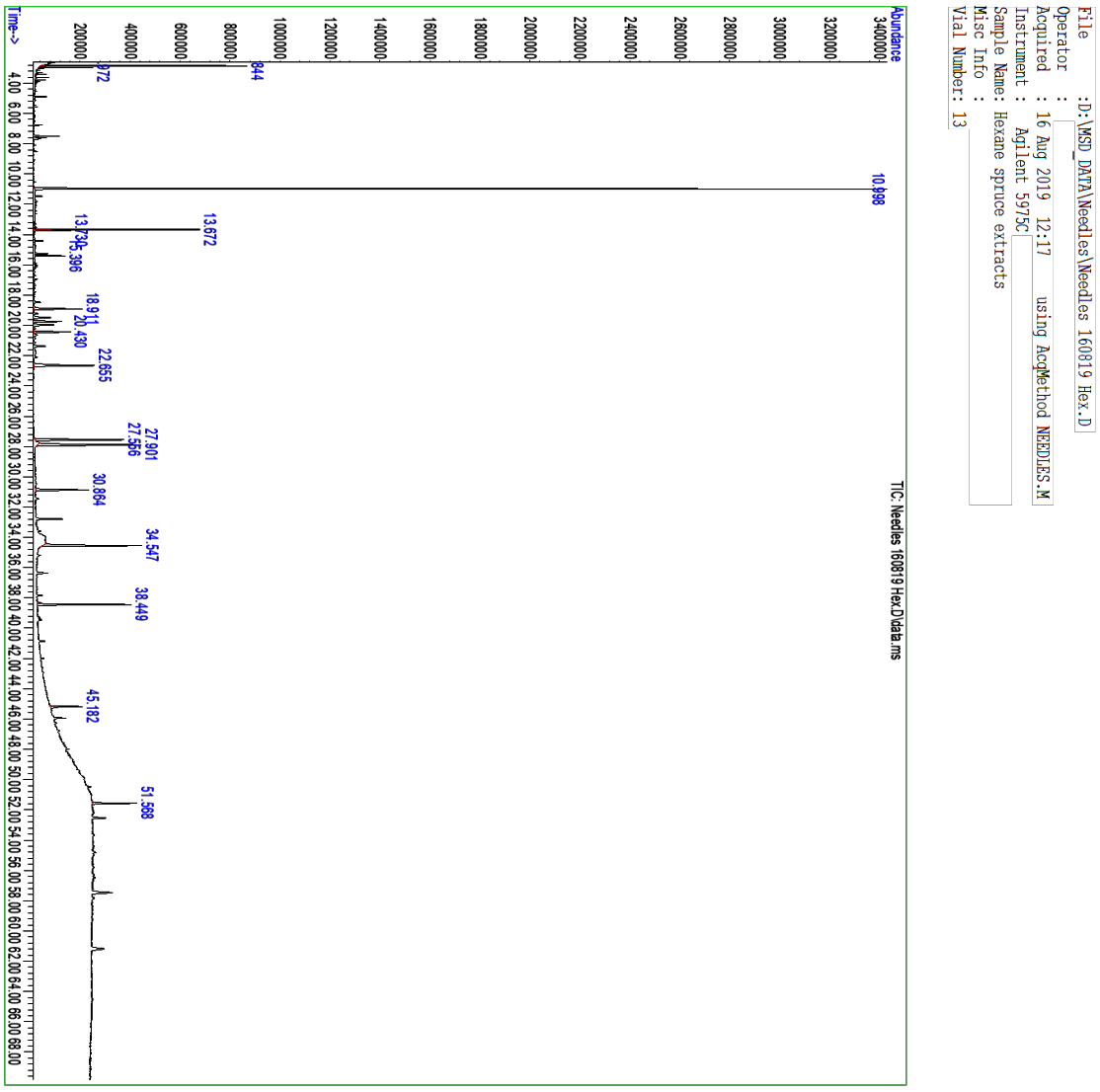
# 7 RESULTS

Spruce needles have chemical compositions of different components which can be found with varying chemical contents in them. In TABLE 3, it shows the compositions of spruce needles from acetone during the period of the experiment.

TABLE 3: Chemical compositions of spruce needles

| S. N | Chemical compositions | Chemical contents (%) |
|---|---|---|
| 1. | Ash Content | 2, 8 |
| 2. | Extractives | 31, 6 |
| 3. | Lignin | 16, 5 |
| 4. | Holocellulose | 50, 5 |
| 5. | α-Cellulose (Cellulose) | 45, 6 |
| 6. | Hemicellulose | 46, 2 |

Hexane is another solvent used to identify the extractives from spruce needles. Once the sample injected to the GCMS, it begins to show the different compounds fluctuating the spectra. It also functions as acetone. Higher boiling point compounds boils faster than lower boiling point. Based on the boiling points, the different chromatograph will appear on the monitor alongside high and low peaks. This all matters with the most crucial role plays in this analysis which is the retention time. The retention time measures the time taken for a compound to go through the column and later it shows on the chromatograph. The extractives from spruce needles in hexane solvent is shown in GRAPH 1.




GRAPH 1: Extractives of spruce needles from hexane

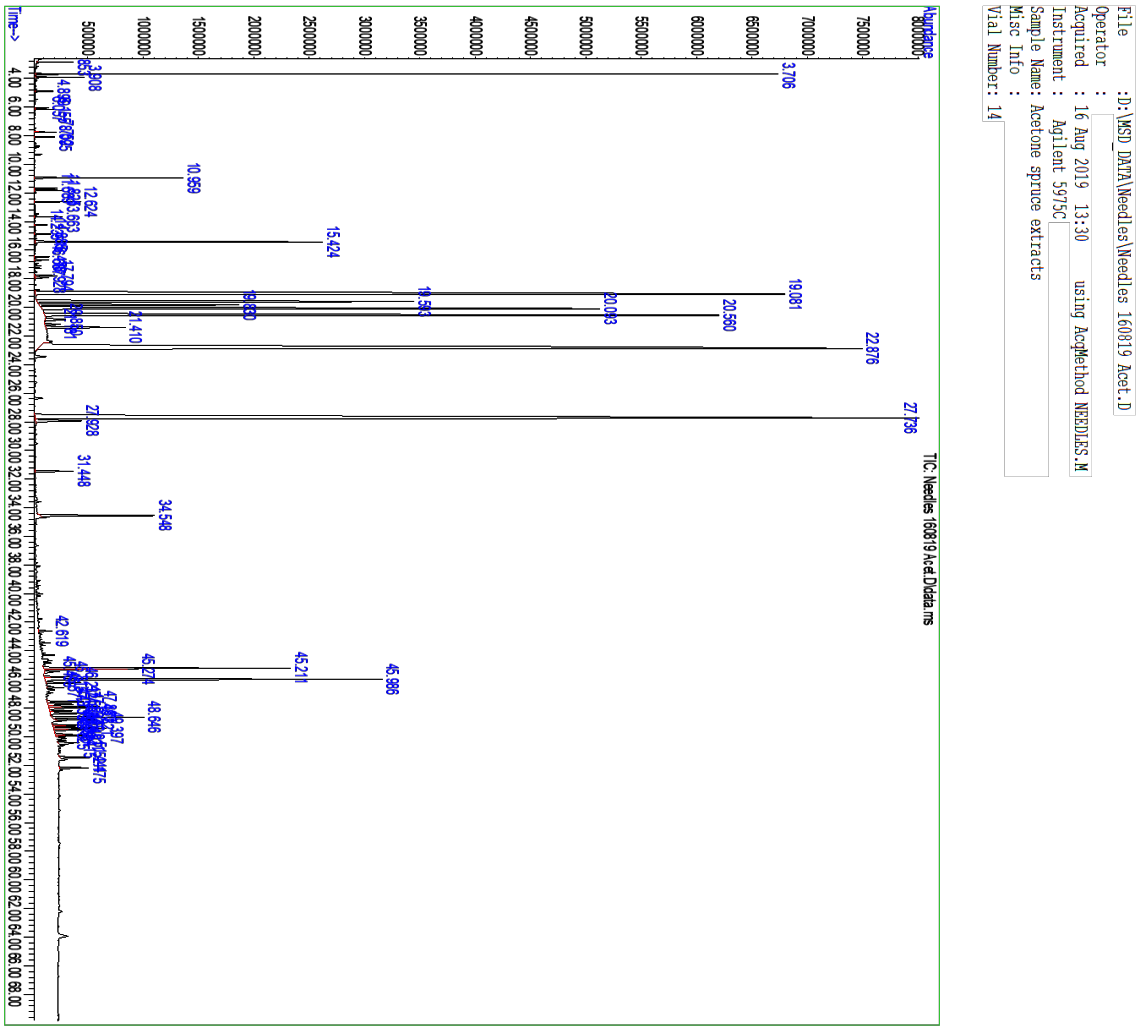
Various chemical compounds are known after experimenting in GCMS. Out of more than hundred compounds, some of them are listed below in TABLE 4.

TABLE 4: Compounds like fatty acids and resins from acetone extraction

| S. N | Retention Time (Minutes) | Compounds | Probability (%) |
|---|---|---|---|
| 1. | 10, 998 | Acetophenone | 32, 9 |
| 2. | 13, 672 | 4-(1-hydroxyethenyl) phenol | 71, 0 |
| 3. | 16, 887 | Vanillic acid | 77, 5 |
| 4. | 18, 412 | 3-Vanilpropanol | 81 |
| 5. | 27, 901 | Hexadecenoic acid | 96, 7 |
| 6. | 32, 71 | Phytol | 58 |
| 7. | 33, 249 | Lioleic acid | 51, 4 |
| 8. | 33, 371 | α-Linoleic acid | 59, 1 |
| 9. | 33, 524 | Oleic acid | 66, 7 |
| 10. | 37, 34 | Dehydroabietic acid | 45, 5 |
| 11. | 51, 568 | Tetradecanoic acid, hexadecyl ester | 22, 2 |

Now by using acetone solvent solution, different complex compounds are found in gas chromatography. Although, it is hard to know the compounds in real life, so GCMS helps to identify the unknown compounds which still they have significant applications in many forms. It can be seen in the chromatograph that; the graph fluctuates corresponding to the retention time. It is because the higher boiling points of compounds have low peak and lower boiling point of compounds have high boiling points. That's why, the peaks are arranged with directly proportional to the temperature of the compounds. Most importantly,

it doesn't mean that, all the higher boiling point compounds have to be boiled initially in the chromatography. Some compounds take less time to boil completely so as it could appear later in the chromatograph. The extractives of spruce needles from acetone is shown in GRAPH 2.




GRAPH 2: Extractives of spruce needles from acetone

There might be hundreds of different compounds in spruce needles. Here are some preliminary compounds analyzed in gas chromatography. In TABLE 5, it demonstrates the extractives of spruce needles.

TABLE 5: Compounds like glucose and phenolic acids by using acetone solvent

| S. N | Retention Time (Minutes) | Compounds | Probability (%) |
|---|---|---|---|
| 1. | 7, 737 | Glycerol | 87, 9 |
| 2. | 8, 081 | Butanedioic acid | 82, 3 |
| 3. | 8, 722 | Glyceric acid | 85, 1 |
| 4. | 10, 959 | Acetophenone | 33, 1 |
| 5. | 14, 206 | P-hydroxybenzoic acid | 56, 9 |
| 6. | 15, 424 | 2,5-Dihydroxyacetophenone | 47, 1 |
| 7. | 19, 081 | 1-Cyclohexane-1-carboxylic acid | 77, 9 |
| 8. | 34, 548 | Hexadecenoic acid | 33, 1 |

# 8 CONCLUSIONS AND DISCUSSIONS

The main objective of this research was to distinguish the chemical compositions, specifically, the extractives of spruce needles. Consequently, this research wouldn't be possible without investigating the multidisciplinary studies with my supervisors. The experiment was conducted in order to isolate the extractives from spruce needles. The raw needles were the main materials of my research to classify the chemistry behind it. The needles were first harvested and carried out in the laboratory for further process. Alongside, the extraction method was integrated with polar and non-polar solvents, i.e. acetone and hexane. During the experiment, different operating conditions were applied such as, the time interval of extraction, accurate temperature, dissolving the compounds and the proper equipment's. The extracted solution obtained from two different solvents were analysed in gas chromatography mass spectroscopy.

By using two different solvents polar and non-polar, have substantial consequence on obtaining the extraction results. Although, the raw materials used in this experiment was unchanged but still resulted different extractives and this was obviously the reason of different solvent properties. Nonetheless, the different operating conditions have nothing to do with the extraction results, but it might have certain impact on the total amount of extracts. Afterall, the gas chromatography analysis was identified showing distinctive chemical compounds in different boiling points, chemical structure and the adsorption ability of compounds corresponding to the retention time. The injector temperature was maintained at 280 °C depending on the boiling point of various chemical compounds. Therefore, the lower boiling has high peak and higher boiling point has low peak in the chromatograph. Eventually, the various complex chemical compounds were isolated by contacting polar and non-polar solvents in appropriate experimental conditions.

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