The Optimization Of Various Extraction Methods Of Dioscorine From *Dioscorea hispida* Dennst

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DECLARATION

I hereby declare that the work embodied in this report is the result of my own work except the excerpt and summary I recently described the source.

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The Optimization of Various Extraction Method of Dioscorine from *Dioscorea hispida* dennst tuber

ABSTRACT

Dioscorea hispida dennst (D.hispida) is a type of yam in the Dioscoreacea family that can be abundantly found in the tropical and subtropical country. It is commonly found in second growth forest and grows under shaded areas. The *D.hispida* tuber has become staple food in some part of place and brings benefit for many people as a therapeutic tools and insecticide. The tubers of D.hispida contain a neurotoxic of dioscorine which can cause food poisoning, vomiting, and paralysation of nervous system. Before consume it the detoxification must be performed to remove dioscorine. This study is to optimize the best extraction methods for isolating dioscorine from the *D.hispida* in powder form. The dioscorine was extracted by solid-liquid extraction methods and liquid-liquid extraction method. Different extraction methods were leaded to the different yield of extractive and functional group content of *D.hispida*. The results show that maceration assisted with ultrasonication probe type extraction method showed the highest amount yield of extractives is 6.74%, while Soxhlet extraction method showed 5.45% amount yield of extractives and ultrasonication bath was showed 5.36% and the least amount yield of extraction was chemical extraction method about 4.35%. The dioscorine functional group content from *D.hispida* was being identified by using Fourier Transform Infrared (FTIR) Spectroscopy and two methods extraction were showing complete compound of functional group of dioscorine. In this study dioscorine was utilize as an insecticide and evaluated using cognitive analysis.

Keywords: *Dioscorea hispida dennst*, dioscorine, extraction, FTIR, insecticide.

Pengoptimumkan Beberapa Cara Untuk Mengekstrak Dioscorine dari Umbi *Dioscorea hispida* Dennst

ABSTRAK

Dioscorea hispida dennst (D.hispida) adalah jenis keladi dalam keluarga Dioscoreacea yang boleh didapati dengan banyaknya di negara tropika dan subtropika. Ia biasanya ditemui di hutan pertumbuhan kedua dan tumbuh di bawah kawasan yang berlorek. Pokok *D.hispida* telah menjadi makanan ruji di beberapa tempat dan membawa manfaat kepada ramai orang sebagai alat terapeutik dan racun serangga.. Umbi *D.hispida* mengandungi neurotoksin dari dioscorine yang boleh menyebabkan keracunan makanan, muntah, dan paralysis sistem saraf. Sebelum mengkonsumi, detoksifikasi mesti dilakukan untuk menghilangkan dioscorine. Kajian ini adalah untuk mengoptimumkan kaedah pengekstrakan terbaik untuk mengasingkan dioscorine dari *D.hispida* dalam bentuk serbuk. Dioscorine diekstraksi oleh kaedah pengekstrakan pepejal-cecair dan kaedah pengekstrakan cecair-cecair. Kaedah-kaedah pengekstrakan yang berbeza telah menghasilkan hasil kumpulan ekstraktif dan fungsi *D.hispida* yang berlainan. Hasil kajian menunjukkan bahawa makerasi yang dibantu dengan kaedah pengekstrakan ultrasonication jenis peranti probe menunjukkan hasil ekstrak ultrasonik tertinggi ialah 6.74%, manakala kaedah pengekstrakan Soxhlet menunjukkan 5.45% jumlah hasil pengekstrakan dan mandian ultrasonik menunjukkan 5.36% ekstrak hasil dan jumlah pengekstrakan hasil paling sedikit adalah kaedah pengekstrakan kimia sekitar 4.35%. Kandungan kelompok fungsional Dioscorine dari *D.hispida* telah dikenal pasti dengan menggunakan Spektroskopi Fourier Transform Infrared (FTIR) dan hanya dua kaedah pengekstrakan yang menunjukkan kompound molecule dioscorine yang lengkap. Dalam kajian ini, dioscorine digunakan sebagai racun serangga dan dinilai menggunakan analisis kognitif.

Katakunci: *Dioscorea hispida dennst,* pengekstrakan, dioscorine, FTIR, racun serangga

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LIST OF ABBREVIATIONS

		Page
D.	*Dioscorea*	1
IITA	International Institute of Tropical Agriculture	4
FTIR	Fourier Transform Infrared	4
CD	Celiac Disease	9
$C_{13}H_{19}O_2N$	dioscorine	11
DPPH	1,1-diphenyl-2-picrylhydrazyl) and hydroxyl	11
TLC	Thin Layer Chromatography	12
w/w	Weight over weight	12
CN^-	Cyanide	13
HCN	Hydrogen cyanide	13
rpm	Rotation per minute	29
K_2CO_3	Potassium Carbonate	30
HCl	Hydrochloric acid	30
N_2SO_4	Sodium Sulphate	31
GC-MS	Gas Chromatography- Mass Spectrometry	49

LIST OF SYMBOL

		Page
%	percent	4
mg	milligram	13
β	Beta	12
℃	Degree celcius	12
kg	kilogram	13
kHz	Kilo Hertz	18
MHz	Megahertz	18
g	gram	26
ml	Milliliter	27
M	Molarity	30
W/cm^2	Watt per cubic centimeter	36
cm^{-1}	Reciprocal wavelength	39

CHAPTER 1

INTRODUCTION

1.1 Research Background

Dioscorea hispida dennst (*D. Hispida*) is unpopular species of yam and it is available in almost parts of the east coast of Malaysia. In Malaysia, *D.hispida* regularly it is known as Gadog, Gadong, Gadong lilin, Gadung Mabuk, Ubi Gadung, Ubi akas, Ubi arak, Taring pelanduk and Sulur Gadung (Mat, Tajuddin, NurAtiqah, O.Siti, & al, 2012). *D. hispida* is in one of the members of genus Dioscoreacea family plant it is a climbing tree with glabrous leaves characteristics which can reach up to 20 meters in height, and helix readily around (Hudzari, Ssomad, Syazili, Musa, & Asimi, 2012).

D.hispida is commonly found in second growth forest and it grows under shaded areas or near streams (Hahn, 1995; Salmah, 2013). *D.hispida* has been served as a primary foods, particularly by community in the tropical and subtropical countries (Liu, 2006; Hahn, 1995; Udensi & O.H, 2008). The tuber of *D.hispida* was consumed as a scarcity of food in some state in Malaysia and some other parts of the world. Stastically, there are 1137 species *Dioscorea,* however 537 species from it is harmful (Poornima &

Rai, 2009). This is the main reason why this *D.hispida* plant is critically ostracized as a primary food source,due to the presence of alkaloid dioscorine content in its tuber which can be fatal for the human consumption (Leete, 1989).

The alkaloid dioscorine capable of causing paralysant of the nervous system, diziness, nausea, vomiting and produce the narcotic effect (Sasiwatpaisit, Thitikornpong, Palanuvej, et al, 2014; Webster, Beck, Ternai, 1984; Rajyalakshmi, 1994). The proper preparation of fresh tuber is needed in order to avoid side effect after being consumed it. Traditionally, the detoxifixcation of dioscorine will take up until seven days of soaking *D. hispida* tubers in flowing water to ensure all the toxic content being remove and safe to consume by human (Razali, Muhammad, Mohd, & Wan Ismail, 2011; Udensi & O.H, 2008).

The toxics component of *D.hispida* plant exhibit insecticidal and antifeedant activities for some species of insect. Therefore, the study of that component potentially to utilized as insecticide and pesticide. The previous study, in Heal.R.E in 1950 reported that injection or extraction from this rhizome plant was effective against American cockroach but less effective against the German cockroach, and the milkweed bug. In the Philippines, the crude extract of the *D.hispida* tuber has been utilized by the farmers mixed with lime and soap to control some pest, after getting tested crude of extract of *D.hispida* could against larvae of the Lime butterfly and it was mean the crude showed deterrent activity and toxicity (Ecological Farming Program, 1990).

The process of isolating dioscorine from *D.hispida* tuber in powder forms by used two types of extraction methods namely liquid-liquid extraction and solid-liquid extraction. Through the time, there were so many extraction methods were used in

extracting phytochemical plant to isolating the desired product were found from different reseachers (Lujan & Luque De Castro, 2008; Mustafa & Turner, 2011). In this study, there were four extraction methods used for such as Soxhlet extraction, ultrasonication bath method, maceration assisted with ultasonication probe type method and chemical extraction method.

The Soxhlet extractor name were took from its founder who is Franz Ritter von Soxhlet the German agricultural chemist, he was first purposed in 1879 during upon of paper with the determination of milk fat (Onay, Sonmez, Oktem, & Yucel, 2016). Next, the scientific history and discovery of ultrasound are origin from study of sound by Sir Isaac Newton, he was proposed his theory in 1687 upon of sound waves (Mason, Paniwnyk, & Lorimer, 1996). The characteristics of sound such as frequency,intensity, viscosity, surface tension, temperature and pressure is vital matters on capability of ultrasound to cause cavitation. This procedure requires a liquid form, an energy generator and a transducer which transform the electric, magnetic or kinetic energy into acoustic energy (Pico, 2012).

The early implementation of liquid-liquid contacting were involving the removal of pigment from oil by using water as the solvent (Blass, Haeberl, & Liebel, 1996). Then, in the middle to late 19th century the modern practice of liquid-liquid extraction became an important laboratory technique. In 1872, Berthelot and Jungfleisch was introduced the division of ratio concept reporting on how the solute separate between two liquid phases at equilibrium (Frank, Dahuron, Holden, Prince, et al, 2008) .

Presently, the alkaloid disocorine in *D.hispida* is not explored more. Thus, in this study it was interested to investigate the dioscorine of *D.hispida* by using distinct type of extraction methods application on insecticide. The various extraction methods were adopted from various previous research journals and books. The Fourier Transform Infrared (FTIR) was used to analyse the discorine functional group compound of *D.hispida* tuber. The direct analysis also used to identify the effectiveness of application of insectide from dioscorine.

1.2 Problem Statement

Genus of Dioscorea, is a monocotyledon and categorizes to the Dioscoreacea family which is used as famine food in some parts of the country (Sharma & Bastakoti, 2009). According to the International Institute of Tropical Agriculture (IITA), there are many researchers interested upon significance and advantages of this species. This plant has been cultivated for own consuming as it is beneficial in nutritional and some for medicine. In certain parts of the country, these wild edible tubers contributed as major economic and cultural importance's which account 95% of the world population has been reported by Food and Agriculture Organization of the United Nations.

In previous study, they are more intending on the innovated of the removal alkaloid machine (Hudzari, Ssomad, Syazili, Musa, & Asimi, 2012), phytochemical screening (Sasiwatpaisit, Thitikornpong, Palanuvej, & et, 2014) , ethnobotany and distribution (Mat, Tajuddin, NurAtiqah, O.Siti, & al, 2012), physiochemical characterization of starch (Salmah, 2013) and antimicrobial activity (Udensi & O.H,

2008). Nevertheless, comparing the best method for removing dioscorine has been not explored wholly, there are only limited input could be found.

To fill this gap, the current research, is focusing on the best efficient methods for extracting the dioscorine toxic content in the *D.hispida* tuber. Various methods were adopted for extraction namely Soxhlet extraction, ultrasonication bath, maceration assisted with ultrasonication probe type and chemical extraction method. This is because there are not much information about extraction methods that could be apply for this dioscorine in laboratory for intention of pursue upon of capability and benefit it could bring for society. So, in this experiment it could give information which extraction methods are the most efficient to isolate the dioscorine from *D.hispida* tuber. Then, the crude that obtain from this extractor will further analyzing using FTIR to identify and to confirm the functional group of dioscorine compound exist in the yield of extraction. Then, the sample will undergo product process of insecticide which is in previous research this toxic could be a pesticide and insecticide that control the plant from pest like weds, fungi, or insect with the characteristic of anti insect and anti-mutagenic. (Banaag, Hiroshi, & Shono, 1997)

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1.3 Objectives

1. To determine the most effective extraction methods of dioscorine from *D.hispida* tuber.
2. To identify the functional group of dioscorine that content in *D.hispida* tuber using Fourier Transform Infrared (FTIR)

3. To utilize the extracted substance into insecticide by observation analysis.

1.4 Scope Of Study

The study will focus on the determination of the most efficient of extraction and isolation of the dioscorine content using various extraction methods. First procedure was the extraction by using four different methods of extraction namely Soxhlet extraction, ultrasonication bath extraction, maceration assisted ultrasonication probe type extraction and chemical extraction method. The fresh samples were collected from the nearer farmer in Tanah Merah, Kelantan area.

After the extraction process, the crude obtained was analyzing the functional group of sample. From here it could be determined or identify the chemical composition or chemical compound contained in it. To analyzing it will using Fourier Transform Infrared (FTIR) for conformation. After the identification of chemical contains, it was followed by measuring the most suitable elements and compound to produced an insecticide.

1.5 Significance Study

This study was significantly crucial to interpret the most effective extraction methods and identify the functional group of dioscorine contain in *D.hispida.* Information from this study be allow contribute to the development of scientific knowledge especially for *D. hispida* species. Besides extract and isolating of dioscorine

content in this tuber for the application in insecticide, the dependency on chemical insecticide could be reduce and will bring the beneficial on human and environmental in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 *Dioscorea hispida* Dennst (*D.hispida*)

Ubi Gadong or intoxicating yam with a scientific name is *Dioscorea hispida* Dennst (*D.hispida*). It is belongs to Dioascoreace Family (Ayensu, 1972). Morphological, of this plant is growing up by climb to the other plants and has a palmately compound leaves and its veins are a net-like pattern. The stem was covered with trichomes, and thorns with wide range diameter, thick walled, and stout. For the tuber, it has large, bristly globose in shape and hairy that specified as a root crop. It takes time almost a year for entirely growth period of stem and the plant replace tuber in every year (Tajuddin S. , Mat, Yunus, & Bahri, 2013).

D.hispida ordinarily grows under shaded areas or near streams (Salmah, 2013; Hahn, 1995). As reported by Terengganu Forestry Department, the distribution of *D.hispida* in forest area is limiting yet it still can be found in abundance near river. The growing of human habitation and land clearing give notable pressure on *D.hispida* (Salmah, 2013; Mat, Tajuddin, NurAtiqah, O.Siti, et al, 2012).

2.1(a) Uses of *Dioscorea hispida*

The tuber of *D.hispida* now days used as an exotic food along with scarcity of food of importance in Malaysia, especially in Terengganu and Kelantan state and some parts of the world. Some local communities are still planting this species as a potential food resource, the local Malay villagers in Besut, Marang, Setiu, Tanah merah area are using this tuber of *D.hispida* for baking the prominent traditional local food such as Kuih Putri Mandi, kuih onde-onde and pengat (Mat, Tajuddin, NurAtiqah, O.Siti, & al, 2012) Apart from being consumed, this tuber also contains medicinal properties and consider as excellent medicine to decrease the blood glucose.

This tuber crop has a potential as an alternative energy due to the essential source of carbohydrates contain. The *D.hispida* have nutritionally beneficially, as one of carbohydrate source, with no gluten, content make it as a potential substance in the reduction of the extent of Celiac Disease (CD) or others allergic reactions (Rekha and Padmaja, 2002). It is also has been related with a slow digestion in the lower parts of the human gastrointestinal tract, resulting in slow liberation and absorption of glucose because the resistant starch contained in this tuber. Digestive property of this tuber has suggested in order to decreasing the risk of obesity, diabetes, and other related disease by utilizing this gadung tubers species (Aprianita et al., 2009).

From the point of view in medicinal aspects, some studied assert that apart of Indian in Rajasthan area is using these Dioscorea species as a therapeutic plant to cure several of ailment and illness (Choudhary, Singh, & Pillai, 2008). According to Surnasih E.S et al. (2007), the corn infuses *D.hispida* dennst can decrease the blood glucose. In

Temuan tribe Malaysia they are using leaves from this intoxicating yam for curing sores of yaw (Hanum & Hamzah, 1999). However, it is a different way how the Jeypore people in India using this yam, they are using its toxic in *D.hispida* to forget their sadness it same effect as a beer could make them drunk (French, 2006).

Liu et al. (2011) had studied that tuber of Dioscorea species possesses a high amount of polyphenolics compound. Moreover, there are several researchers reported that this tuber has anti-oxidative, anti-fungal, antimutagenic, hypoglycaemic and immunomodulatory effects (Son, 2007). With these features, some of them used as essential ingredients of dietary supplements and add the ingredient in cosmetics also in pharmaceutical industries (Black, 2007). With these benefits, an effort on gadung processing into edible food materials was undertaken.

2.1(b) Alkaloid dioscorine

Alkaloid is the biggest group from second metabolites that possessing nitrogen atom. The biggest part of nitrogen atom share with a heterocyclic ring. Alkaloid generally is base, so that was first mentioned in 1819 by W. Meibner. An apothecary from Halle was identified that these compounds perform “like alkali” and he named as alkaloids (Clayden, Greeves, Warren, & Wothers, 2001). Even in low doses of alkaloid it is still greatly reactive substances with biological activity.

All alkaloid have a bitter in taste and come into white solid form, except if present of nicotine which has a brown liquid. Mostly, the alkaloid form as water-soluble

salts and they are well-defines crystalline substances which unite with acids and form salts. It is may occur in plant, in the free state and as N-oxides (Aniszewski, 2007).

The alkaloid generally can give impact to animal's nervous system, yielding the functionality of the organism practicable change. One of natural phenomena when the activity of alkaloid molecules on a psycho mental level, plants in the process of species self-protection and interactions between producers (plants) and consumer (herbivores). The good results of natural selection mechanism and results (Aniszewski, 2007).

The dioscorine is ($C_{13}H_{19}O_2N$) is water soluble alkaloid has that extracted from *D.hispida*. It has very toxic compound in the tuber and contains high angiotensin convert to enzyme-inhibitory. Significantly, toxic of dioscorine when it consumed could cause paralysant of the nervous system dizziness, nausea and vomiting (Sasiwatpaisit, Thitikornpong, Palanuvej, & et, 2014). Besides, the toxic compounds of *D.hispida* tuber have superiority to give positive impact as a therapeutic, antimicrobial effect and providing the impact for insecticidal and antifeedant activity.

The present of carboxylate ester makes the dioscorine belong to the carbonyl family, a non-aromatic ring with five carbon atoms, one double bonds ring and two oxygen atoms. Eventhough dioscorine is toxic, it is also could use in treating hypertension by inhibit the activity of angiostensin-activity enzyme. Recent research also found that dioscorine is able to control high blood pressure (Hsu et al., 2002) due to ability of scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) and hydroxyl free radicals(Hou et al., 2001) and it is also can enhance immune modulation (Fu et al., 2006)

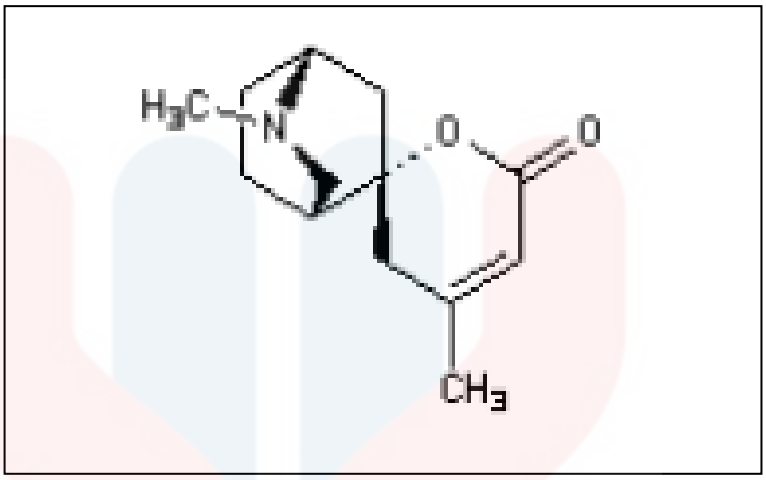

Figure 2.1: 2D of Dioscorine chemical compound structure

Source: Liu et al. (2009)

The dioscorine is light yellowish to greenish in colour with the boiling point 50-55℃. The dioscorine also could soluble in water, alcohol, acetone and petroleum ether but slightly insoluble in ether, and benzene (Merck, 1999). Tuber *D.hispida* was undergoing TLC-densitometry and TLC image analysis was reported by Sasiwatpaisit et al. (2014) resolved dioscorine values were 0.72% w/w and 0.66 %w/w respectively. Webster and his worker also employed TLC method however they are reported that content of dioscorine in *D.hispida* dried tuber was only 0.12% w/w.

The high content of toxic hydrogen dioscorine in both free and bound forms is main reasons related to the limitation of gadung tuber utilization to prefer as a food source for human. The bounds forms, known as cyanogenic glucosides, occur as linamarin [2-(β-D-Glucopyranosyloxy)-2-methyl-propanenitrile] and lotaustralin (Conne, 1969). If undergo to heat treatment, or hydrolytic enzyme is presence appropriately, these glucosides release hydrogen cyanide. When this reaction takes place

in the human system the cyanide toxicity results, the degree of toxicity which depends on the quantity of the cyanide released (Edijala e*t al.*, 1999). The liberation of hydrogen cyanide via hydrolysis, also it is take place during the preparation of the food based of these tubers (Akintowa *et al.*, 1994).

The outcome of dioscorine toxicity can causes several diseases and disorder, if untreated well it may result in death. Only free cyanides (CN^-) is toxic, and if hydrolysis process does not occurred the glycoside remains stable and the foods produces from food material are safe. Moreover, by daily consumption of ubi gadong products with unsafe cyanogens level as a staple food in long term impact could cause chronic dioscorine toxicity (Cooke and Maduagwu, 1978). As set by World Health Organization in 1988 the effective processing operation may reduce all cyanogens in cassava products to get below the safe level of 10 mg HCN equivalent per kg body weight (Mlingi, Bainbridge, Poulter, & Rosling, 1995).

2.1(c) Isolating of Dioscorine from *D.hispida* tuber

According to the previous study, *D.hispida* acknowledged as tubers that cannot be consumed raw without removing toxic first since it contains the toxic alkaloid. Amidst the yam species, *D.hispida* considered as one of the most underutilized species because of the presence of particular toxic, it is alkaloid dioscorine. Some studies also had denoted that *D.hispida* also contains some toxic compound which could impart serious health complications. In order to avoid any undesired cases of intoxication, local

people need to prepare the tubers before they consume it for make it edible. It will undergo the detoxifying process such as roasting, boil, and soak in flowing water for one week (Hudzari, Ssomad, Syazili, Musa, & Asimi, 2012).

In 2011 Hudzari et al. disclosure information upon the innovation about of new system of removal dioscorine equipment called as Spin and Wavy Type of water Circulation Operation. Purpose of this device is to isolate the alkaloid compound efficiently and cut off the time for preparing for *D. hispida* instead using the conventional method which soaking in flowing water in 7 days.

2.2 Extraction of Dioscorine using various methods from *D.hispida*

Extraction is a method of isolating the desire solute compound from its mixing form with using amount of solvent as a media. Process of extraction prior selected when extraction desire compound isolated from other components with the nearest boiling point, which is sensitive to heat and it is azeotrop mixtures.

Based on solute phase and solvent, extraction could be differentiating on extraction liquid-liquid, extraction solid-liquid, and extraction gas-liquid. Extraction of solid-liquid usually called as leaching. If undesired component is solute it needs to remove from its solid samples using variety of chemicals in liquid forms so that the leaching process called as chemical extraction. The process extraction solid-liquid basically use in food material industry, pharmaceutical and extraction essential oil. The

organic solvent are mostly used in this extraction method are hexane, alcohol, chloroform and acetone (Ibarz & Canovas, 2003).

Process extraction influence with some factors (Gartenbach, 2001). Types of solvent used will give the significant impact to the amount of solute that been extracted and also influence the speed of extraction. Generally, petroleum ether was used as a solvent to extract alkaloid in the plant materials due to its efficiency and cheapest solvent compared to other organic solvent. Besides, the temperature also plays an important role in the extraction process. The higher temperature will increase the solubility of the solute in the solvent. In fact that temperature of solvent and desired component has its restriction and limits of boiling point. In this study the solvent was used for all extraction methods is petroleum ether, this is due to the criteria of petroleum ether with the boiling point range is 40℃ to 60℃ while the melting point for dioscorine as a desired compound is 54℃. Thus, the selected petroleum ether as a solvent of isolating dioscorine is the most compatible among other solvents and will not degrading the disocorine compund .

The ratio of the solvent also one of the important factor that contribute to the efficiency of the extraction process, the greater ratio of solvents over the sample will increase the concentration solute dissolved due to increasing the gradient concentration in and on the surface of solid particles. Consequently, the extraction rate will increase. Particle size of sample also important in the extraction process, the *D.hispida* tuber was grind drying to become a powder form because the finer the sample the higher the rate extraction.

2.2(a) Extraction Using a Soxhlet extractor

In 1879 a Soxhlet extractor is one of a laboratory apparatus that was invented by Franz Von Soxhlet (Dingler's *et al*., 1879). This method was proposed to determine the milk fat. In order to extract of lipid from food, Soxhlet method was applied for semi continuous method. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent usually hexane or petroleum ether under reflux using a special glassware. Moreover, extractor is not only limiting to the extraction of lipids. Recently, the Soxhlet extraction is required where the targeted compound has limited solubility in a solvent and also the impurity is insoluble in certain solvent.

Soxhlet extraction method which has been utilize for a long time. It is a common technique for evaluating the performance as the reference of other solid-liquid extraction or leaching methods and suitable for extracting the desire compound from solid sample. The Soxhlet apparatus is divided into three parts where the top part is the solvent vapor reflux condenser, middle part is the container of thimble with the siphon device complementary with side tube and bottom part is the round bottom flask that held the solvents and extract as shown in figure 2.2

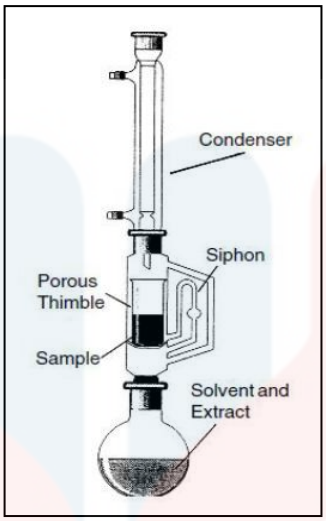

Figure 2.2: Schematic diagram of Soxhlet apparatus

Source: Wells, (2003)

Soxhlet system, the plant material was added in the thimble holder. In the solvent flask, solute is isolated from the solvent by distillation process. When the solvent of extraction reaches the overflow level, the siphon suck in the solution of the thimble holder and discharge it back into the volumetric flask and lifting extracted solutes into the bulk solvents. After extraction the solvent is vaporize and clear out by a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and discarded. It is the most conventional of all methods and consists of a simple distillation process repeated a number of times.

The advantage of this extraction method is that rather of using many portions of warm solvent being passed through the sample, it is just one batch of solvent is recycled. In fact the sample phase is always in contact with fresh solvent so intensify the displacement of target compound from the sample and the compound are not discomposed due to moderate extraction condition (Lee *et al*., 2000). Soxhlet extraction is straightforward and inexpensive (Luque de Castro *et.,al* 2004). In fact, it can maintain

a relatively high extraction temperature with heat from the distillation flask and no filtration of the extract is required.

The disadvantages of this procedure are indigent extraction of polar lipids. Agitation is impossible in the Soxhlet device in order to maximize the contact sample with solvents. Besides, for the extraction process were idle away times. The extraction normally occurs at the boiling point of the solvent for a long time and possibility of thermal decomposition of the target compounds must be acknowledged. It also disclose to the hazards of boiling solvents and concern large volumes of solvents.

2.2(b) Extraction using Ultrasonication Probe Type

In present day, there have been countless reports on the operation of power intensity or high intensity in the extraction of assorted phytochemicals, such as alkaloids, polysacharide, flavonoids, essential oil and proteins and from various parts of plants and plant seeds (Sargenti & Vichenewski, 2000). Ultrasound assisted extraction is a process that using acoustic energy and specific solvents to extract targeted compounds from various plant. Ultrasonic waves could occur at frequencies above 20 kHz which are a branch of sound waves and it exhibits all the characteristics properties of sound waves.

It is depends on the frequency, ultrasound is divided into three categories, namely power ultrasound (20–100 kHz), high frequency ultrasound (100 kHz–1 MHz), and diagnostic ultrasound (1–500 MHz). Ultrasound ranging from 20 to 100 kHz is used

in chemically important systems, in which chemical and physical changes are desired as it has the ability to cause cavitations of bubbles (Pilli et al. 2011; Rastogi 2011). It has been recommended that the development of solvent extraction from plant material by ultrasound is considering to the mechanical effects of acoustic cavitation, which intensify both solvent penetration into the plant material and the intracellular product release by breaking the cell walls (Mason, Paniwynk, & J.P., The uses of ultrasound in food technology, 1996).

Generally, accepted explanation for solvent extraction enhance by using ultrasound is the propagation of ultrasound pressure waves and resulting cavitation phenomena, the breaking cell walls and release the contents of the cell into extraction medium by collapse of cavitation bubbles and highly temperature.

The advantages of this technology include potential chemical-free and simultaneous oxidation, thermolysis, shear degradation, enhanced mass-transfer processes together. Overall, sonochemical oxidation uses ultrasound to produce cavitation phenomena, which is defined as the phenomena of the formation, growth and subsequent collapse of micro bubbles, releasing the targeted compound from plant easily.

The method requires an equipment of ultrasound, which is not used in the conventional method, demanding higher costs and must train by professionals. In addition, the mechanical effect of ultrasound is obtained only in low frequencies below 50 kWh; after all higher frequencies may not show good results in relation to structural rupture, impairing the bioactive compound release.

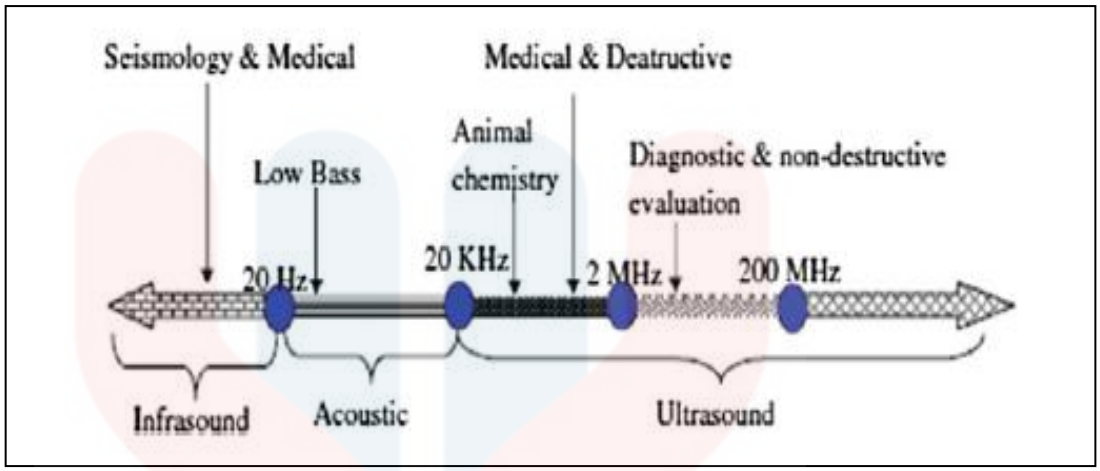

Figure 2.3: Diagram of ultrasound range.

Source: Pilli, Bhunia, Yan, LeBlanc, & Tyagi (2011)

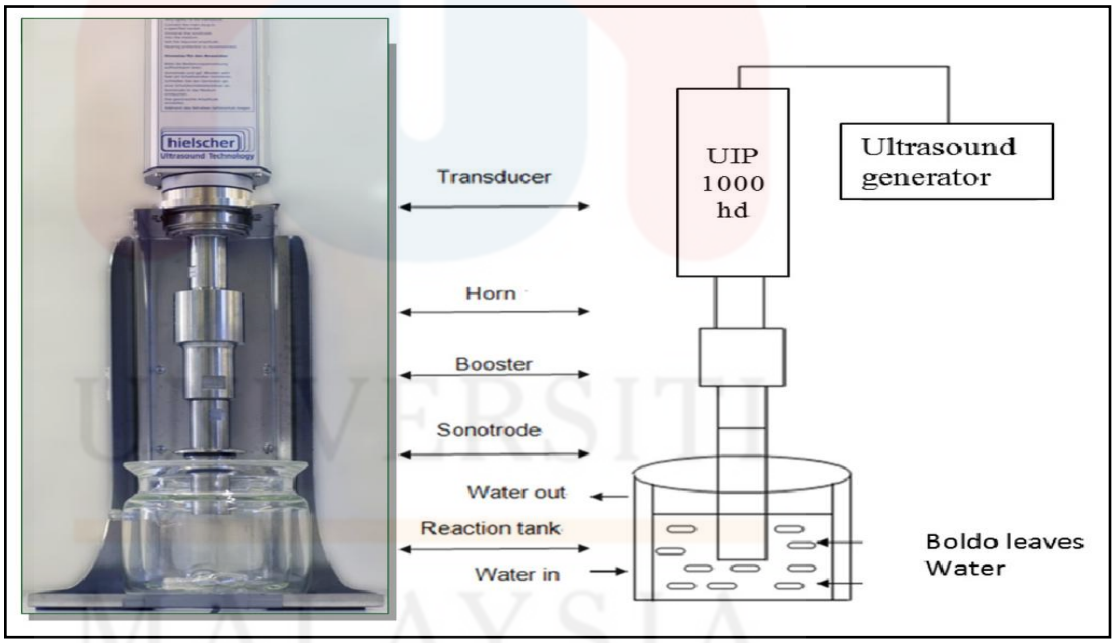

Figure 2.4: Ultrasonication probe type extractor

Source: Chemat. F. (2013)

2.2(c) Extraction using Maceration method

Maceration is involving soaking plant materials likes grainy or powdered with a solvent and allowed to stand at room temperature with persistent agitation for a period of minimum 3 days in a closed container to avoid vaporizarion of solvent. (Handa et al, 2008a).The expected of process to release the soluble phytochemicals for soften and break the plant's cell wall. In few days, the mixture was pressed and filtered by filtration. In this conventional method, heat is transferred through conduction from hot plate and the right choice of solvents will resolve the type of compound extracted from the samples (Azwanida, 2015). Maceration has been suggested by Vongsak et al as more applicable, convenient and less costly method. In this study the maceration is combining with ultrasound assisted extraction.

2.2(d) Extraction using Ultrasonication Bath

Bath sonication is type of indirect sonication. The indirection sonication definition is the ultrasonic waves travel across the bath liquid and before reach the suspension it will pass through the wall of the sample container. It is contrast with the direct sonication which is directly. The acoustic energy for indirect sonication, to uniform the distribution of ultasonic energy using the reactor, energy transfer from the transducer to reacting medium that via a water bath and the wall of reactor are important. The important thing is, the energy transfer need to be maximisd from the transducer to the reactor medium.

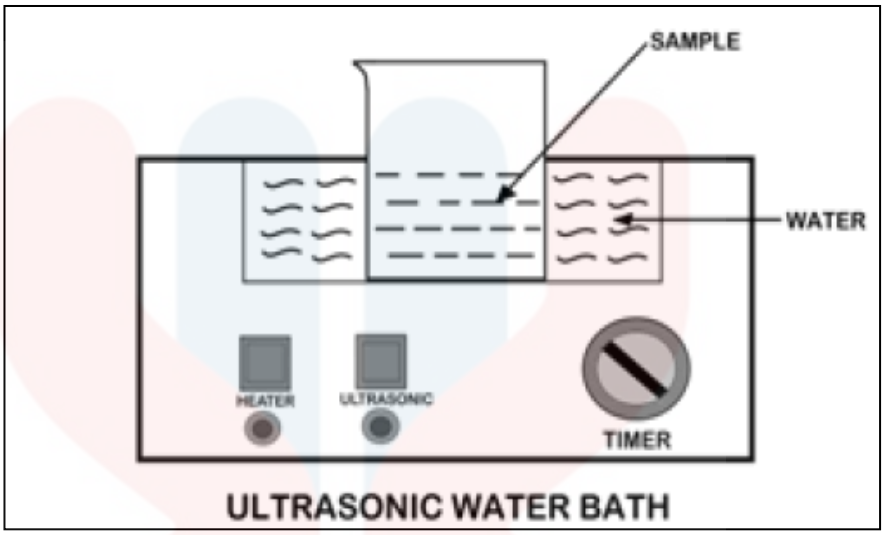

Figure 2.5: The schematic ultrasonication bath instrument.

Source: Akram, Chowdhury, & Chakrabarti,(2016)

2.2(e) Chemical extraction

Chemical extraction or sometime known as liquid-liquid extraction it is process of separation component which is form on the different distribution of the components intend to be segregate between two liquid phases. It is rely on the mass transfer of the component to be extract from a first liquid phase to a second one.

In separation process, immiscible or partially miscible is added to the second liquid solvent with the samples and the solutes of the mixture are dispense between two phases. The ratio of the concentration solute in the two distinct solvent when the system reaches equilibrium it is namely as distribution coefficient. The distribution coefficient is a quantitive measure of the constituent will distribute between two phases. Usually,

liquid-liquid extraction was used when the material is heat sensitive and non-volatile (Kubek, 1994).

2.3 Fourier Transform Infrared (FTIR)

FTIR has justified to be a great tool or machine for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt et al., 2007; Hazra et al., 2007). In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds.

The FTIR uses infrared radiation on the sample where the radiation will absorbed by the sample and some will pass through. The working principles of FTIR is shown when the result representing spectrum molecular absorption and transmission produce a unique molecular fingerprint based on peaks of absorprion that is correlate with frequencies of vibration in between atomic bonds that make it the materials. The amount of materials present by size of peaks showing. Because of uniqueness result features it is able to identify unknown materials, by determining the mixture components.

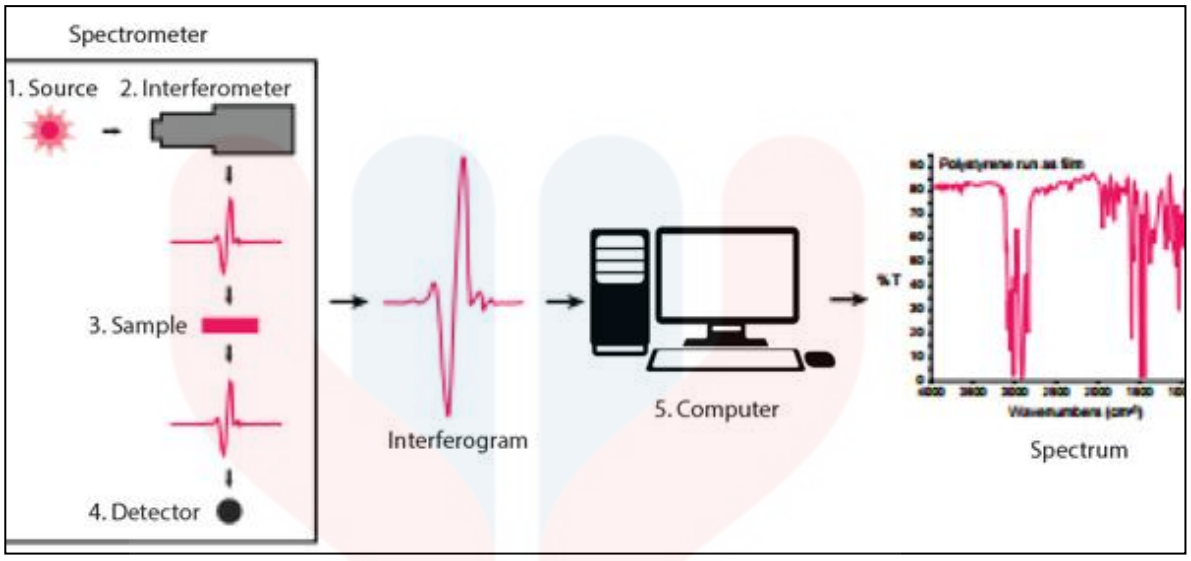

Figure 2.6: The working principle of FTIR

Source: Gurumuthy, Bhatia, & Ramesh, (2017)

2.4 Application of Dioscorine for Insecticide

Being satisfied had been viewed as an integral part of the success of the agricultural sector when synthetic chemicals in pest control for higher yield had used widely. In recent years there has been growing concern that pesticide constitutes a potential risk to the advantage of nature and natural resources including human (Pimentel D, 2006). In fact, less than 0.1% of the pesticide is effective when applied for pest control each target; the rest 99.9% is back to the environment by contaminating soil, water, and atmosphere environment where it is untoward affect public health (Pimentel D., 1995). Significant consequences of using heavy pesticide are human pesticide poisoning and illness (Jaga & C., 2006).

The increasing awareness towards the environmental effect, the significance to sustainable agriculture contributes to the concept of Integrated Pest Management (IPM) during 1970's for alternatives beyond IPM has been felt by the world community towards the end of 20th century (Jayaraj & Rabindra R.J., 1993). There has been a shift in the focus from pest control to pest management based on ecological approach (Thomas, 1999). The significant of biological control and bio-pesticides has been advocated (Gehardson, 2002) on an urgent basis.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample preparation

The material *D.hispida* tubers used was collect in Tanah Merah. The apparatus were used knife, slicer, aluminium foil, tray oven, blender, desiccators, weighing balance and zipper bag. In this study, only one sample species of *D.hipida* tuber were used. The fresh *D.hispida* tubers were peeled to remove the skin.

Then, the tuber was sliced in thin and in small size. It was dried in 60℃ oven and after overnight dried, it was grinded in a blender to reduce the sizes. Then, the samples were turn into fine powder form, it was secured in zipper bag and put inside the desiccators to prevent the moisture content would increase until further action were take. (Kalra, 1998)

3.2 Soxhlet extraction

The materials used were *D.hispida* tuber powder, petroleum ether. The apparatus were used Soxhlet set apparatus, thimble, cotton wool, weighing balance,

fume chamber and rotary evaporator. The powder sample of *D.hispida* is weighed 30g. Then the samples were put into the extraction thimble and on top of thimble was inserted cotton wool to avert the powder sample from departing the extraction thimble. The ratio of solvents and samples that were use in this extraction method was 1:10 which are sample is 1 then the solvent is 10. The amount of petroleum ether as a solvent was used 300 ml and approximately the extraction process took 8 hours inside the fume chamber. Then, using rotary evaporator the extractives were evaporated to transfer out the unwanted component and solvents and gaining the only extractives. Next, the extractives were weighed and calculated using equation 3.1.

$$\text{Extractives (\%)} = \frac{\text{Weight of extractives } (g)}{\text{Weight of dried sample}} \times 100\% \qquad (3.1)$$

Figure 3.1: The extraction process of dioscorine using soxhlet

3.3 Ultrasonication Bath

The materials used were *D.hispida* tuber powder, petroleum ether, tap water. The apparatus were used Erlenmeyer flask, water bath sonicator, fume chamber, rotary evaporator. Then, the *D.hispida* powder was added in Erlenmeyer flask with the petroleum ether as a solvent. The solvent and sample were used in ratio 1:10 which is 20g for *D.hispida* powder whereas 200 ml of petroleum ether, and was filled in flask. The tap water was filled inside the water bath tank until its indicator water level, the Erlenmeyer flask which is contain of sample and solvent were put onto the water bath tank where it is approximately 4cm above the transducer system. The operation of extraction was taken 30 minutes in fume chamber. Then, using rotary evaporator the extractive was evaporated to transfer out the unwanted component and solvents and gaining the only extractive. Next, the extractive was weighed and calculated using equation 3.1.

Figure 3.2: The extraction process of dioscorine using Ultrasonication bath

3.4 Maceration assisted with Ultrasonication Probe Type

The materials were used *D.hispida* and petroleum ether. The apparatus were used probe-type sonicator, orbital shaker, beaker, rotary evaporator and Erlenmeyer flask. The solvent and sample were used in ratio 1:10 which are 30g of *D.hispida* powder and 300 ml of petroleum ether. The powder sampled was weighed. Then, the powdery sample was added in Erlenmeyer flask with the petroleum ether as a solvent. The mixture was left 3 days in orbital shaker with room temperature and 150rpm. After 3 days, the mixture was undergoing extraction using probe-sonicator in condition room temperature, 20 kHz and 10 minutes times of extraction operation. Then using the rotary evaporator to remove the solvents the extractives were evaporated and obtained the desire extractive only. Then the extractive was put into the beaker and weighed it. The extractive content was calculated using equation 3.1.

Figure 3.3: The maceration process using orbital shaker

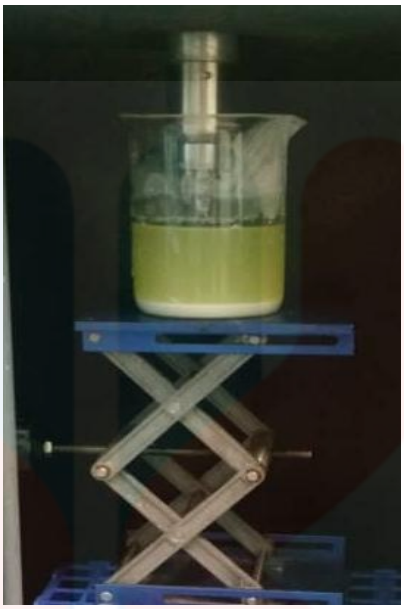

Figure 3.4: The extraction process of dioscorine using Ultrasonication Probe

3.5 Chemical extraction

The materials were used *D.hispida* powder, hydrochloric acid, potassium carbonate, sodium sulphate and petroleum ether. The apparatus were used separatory funnel, pH meter, measuring cylinder, conical flask and rotary evaporator. The powder of *D.hispida* weighed in 30g and was placed on Erlenmeyer flask. Then, the powder was mix with 200 ml of 0.5426M HCl. Then, the mixture was stirred in 2 days using hot plate and stirrer and pH was checked used pH meter in order to ensure the mixture was still in acidic condition. Next step is to make mixture to become alkaline 200 ml of K_2CO_3 was added. This is because to isolating the alkaline compound which is dioscorine compound because it is an alkaline substance. The sample was extracted using 300 ml of petroleum ether in separating funnel. The separating funnel was inverted

slowly inside the fume chamber until the separating layer was formed. Those steps were repeated 3 times. The upper was collected and combined with all three. Then, the N_2SO_4 was added and was dried. The dry extract was filtered and using rotary evaporator the extractives were evaporated to transfer out the unwanted component and solvents and gaining the only extractives. Next, the extractives were weighed and calculated using equation 3.1

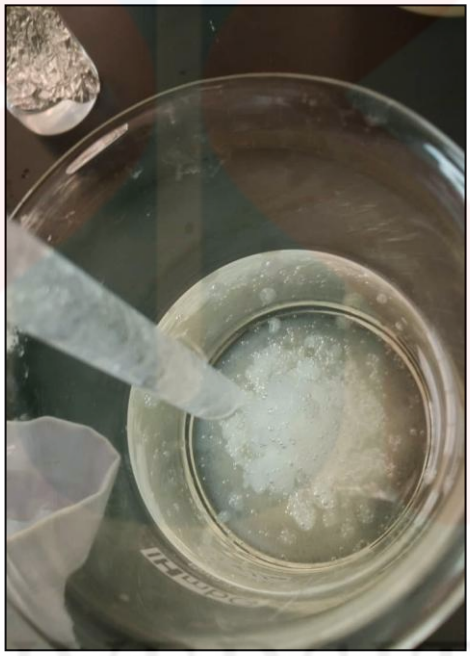

Figure 3.5: The extraction process of chemical extraction using separatory funnel

3.6 Extraction for insecticide

The materials were used *D.hispida* and petroleum ether. The apparatus were used probe-type sonicator, orbital shaker, beaker, rotary evaporator, spray bottle and Erlenmeyer flask. The powder sampled was weighed in 30g. Then, the powdery sample

was added in Erlenmeyer flask with the petroleum ether as a solvent. The solvents were used in ratio 1:10 which is 300 ml of petroleum ether was filled in flask. The mixture was left 3 days in orbital shaker with room temperature and 150rpm. After 3 days, the mixture was undergoing extraction using probe-sonicator in condition of room temperature, 20 kHz and 10 minutes times. Then the solution was transfer in spray bottle

3.7 FTIR Spectroscopy

In FTIR the extracted materials were analyzed it were extract from varies extraction methods. The graph data from FTIR was cluster for analysis. The FTIR brand used was Nicolet™ iN10 infrared microscope & iZ10 FT-IR Spectrometer.

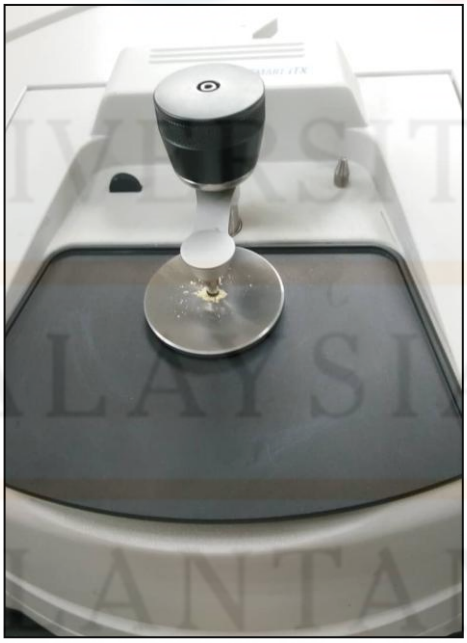

Figure 3.6: The extractivces were being analysed using FTIR

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Extractives

The extraneous component of extractives is consisting protein, resin, pectin, essential oil and glycosides (Pettersen, 1984). In this experiment, the sampled were used *D.hispida* tuber in powdery formed. The extractions were used four different methods to analyze its efficiency in extraction which were chemical extraction (liquid-liquid extraction), Soxhlet extraction, indirect sonication by ultrasonication bath and direct sonication by ultrasonication probe-typed. The solvents were used in this study was petroleum ether. The petroleum ether is a non- polar molecule, which is suitable for alkaloid which is has non-polar molecule which is fulfilled characteristic for good solvent and solute "like dissolve like" (Aguilera, 2003). Generally, the selection of solvent for extraction due to the number of characteristics, such as capacity, selectivity, chemical inertness, flammability, thermo-physical properties, toxicity, cost and availability. The best solvent should be select which has a higher affinity towards the

solute of desire and less soluble for the interest constituent. In the correct combination of solvent and solute, a better product yield and good quality could be achieved.

Table 4.1: The extractives values from various extraction methods of *D.hispida*

Methods	Solvents	Sample mass (g)	Extractives mass (g)	Extractives (%)
Soxhlet	petroleum ether,	30	1.63	5.45
Chemical	petroleum ether N_2SO_4, K_2CO_3, HCl	30	1.30	4.35
UB	petroleum ether	30	1.60	5.36
MUP	petroleum ether	30	2.02	6.74

UB=Ultrasonication bath; MUP= Maceration assisted with Ultrasonication Probe

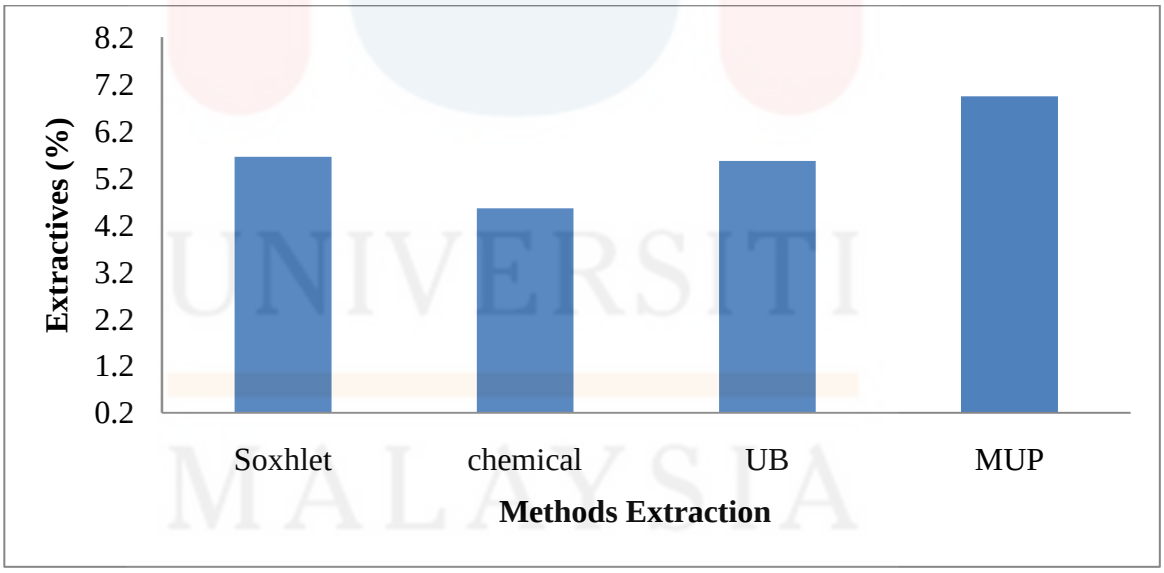

Figure 4.1: The extractives value from *D.hispida* tuber powder

According to table 4.1, the extraction using maceration assisted with ultrasonication probe type extraction was obtained 6.74% extractive, for Soxhlet extraction were obtained 5.45% and for ultrasonication bath it is 5.36% and for the last obtained extractives is the chemical extraction method which is 4.35%. From this result, the ultrasonication probe-type method was the most effective in extraction process by extracting the highest value of extractives. Second was Soxhlet extraction, third is ultrasonication bath and lastly chemical method extraction. It is shown in this results, it was vividly that method extraction types have influenced to the extraction process. The extraction method can have various factors such as thermo resistance of samples and solvent, the duration of process and cost.

The maceration assisted with ultrasonication probe type method extraction is able to proven to be the best method to extract the alkaloid dioscorine because as a result shown it is get the highest extractives because the potential of desired component free is high, simultaneously oxidation, thermolysis shear degradation, and could enhanced mass-transfer. The maceration first was play as an important role in this extraction method to soften and lysis the plant's cell wall to set free the dioscorine component. Then, the uses of ultrasound to produce the cavitation to releasing the targeted compound from *D. hispida* tuber powder form were easier way to isolating the desired extractives (Ta, Ningqun, Chee, & Xiao, 2013). It is due to the frequency of the ultrasonication.

Generally, the ultrasound are divided into three categories, for the 20-100 kHz it was known as power ultrasound, high frequency ultrasound (100 kHz- 1 MHz), and diagnostic ultrasound (1-500 MHz). Basically, ranging ultrasound of 20-100 kHz used in

physical and chemical alter and it has capability to done cavitations of gas bubbles with sound intensities from 10 to 100 W/cm^2 (McClements, 1995; Feng and Yang, 2005). In this study, 20 kHz was used to sonicate the samples and proof it is worked.

According to Mason, T.J. (1998) the ultrasonication bath usually has lower power due to avoiding cavitational damage to the walls tank and equipment so this is why the extractives percentage was not high as much as using probe sonication. The energy of ultrasound from the ultrasonic bath is not uniformly, only a little fraction of the whole liquid volume affected on cavitation which is in the immediate nearness from the ultrasound source (Majumdar, Kumar, & Pandit, 1998). The ultrasonic intensity in the baths sonication was depends mainly on design and placing of the transducers (Mason, 1990).

The dioscorine were extracted using chemical extraction from *D.hispida* tuber in powder form and the result of extractives is least good of extraction method. Probably, it is due to the desired compound are not dissolve much at the solvent eventhough the extraction was repeated three times. The *D.hispida* is bringing base features. Then, the base extraction is fit to carry on this studied in order to remove or isolate it from the solute. The 37% HCl were diluted to get 0.5 Mol of concentration using dilution calculation which is only 8.40 ml of HCl were added into 200 ml distilled water. The mixture was left in 2 days and stirred to give the acidic solvent were absorb into the samples powder more. After that, 200 mL K_2CO_3 were added to basify it back, until the mixture were reached pH 9 and it was measured by digital pH meter, this is because the dioscorine is alkaline so that it will attach to the alkaline solution. Then, the solution

mixture was placed into the separatory funnel and the 300 ml petroleum ether was added into it.

The mixture inside the separatory funnel was mixed vigorously in the fume hood. The two separating layers were formed, it is known as partitioning between two layers, the less dense layer will be on the top and the denser will be at bottom. According to Sigma-Aldrich the density of petroleum ether is 0.64 g/mL while the base solvent component had higher density. Then, the distinct layer substance formed, the petroleum ether at the top and the basic component at the bottom. The alkaloid was migrated to the organic solvent because it is soluble to the petroleum ether. Besides, a substance would dissolve in the solvent with the most identical polarity to its own. Since the partitioning of substance never perfect at the first trial perhaps some organic material remains in aqueous solution and some inorganic material in solvent solution. Then, the extractions were performed three times. All three extractions were combined. The N_2SO_4 was added as a dry extract to make it crystallization, and filter it before evaporated the solvents using rotary evaporator.

Lastly, the Soxhlet extraction was performed the second best of efficient to extract. This is because the dioscorine are very sensitive with heat it is could degrade due to the temperature Soxhlet set up was too high for it. The principle that learned the *D.hispida* powder form placed inside the thimble which is made from the thick filter paper, which is loaded into the main part chamber of the Soxhlet extractor. The soxhlet extractor is place onto a flask that was containing petroleum ether as extraction solvent. The solvent was heated to reflux by fixed temperature which is 50-70℃. The vaporization of solvent was travel up to a siphon arm, and loaded into the chamber

holder of the thimble of *D.hispida* powder. Some of the desired compound will dissolve in the warm solvent. The cycle was done repeatedly over 8 hours. To get more extractives the, cycle should done repeatedly over a day.

4.2 Analysis of dioscorine content using Fourier transformed infrared spectroscopy (FTIR)

There are some functional group should be present in the FTIR analysis to confirm the dioscorine was present in the extractive was namely as carboxylate ester, aliphatic amines, primary amines, alkanes, and alkenes.

The Figure 4.2 shows the FTIR peaks spectrum of extractive by using the ultrasonication bath extraction method extracted from *D.hispida* powder form tuber where it has a likely value compared with other method extraction.

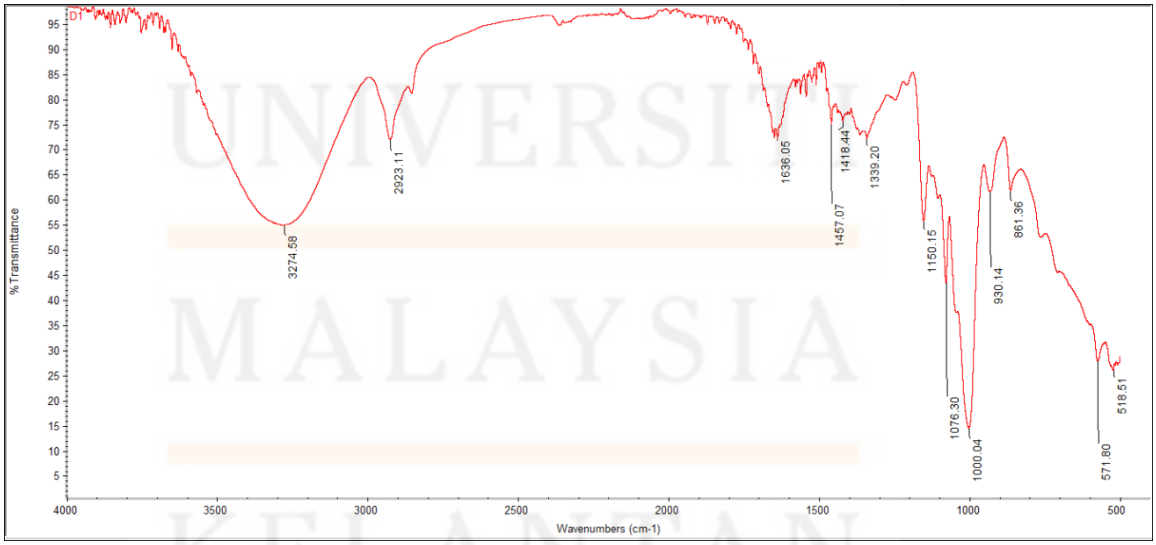

Figure 4.2: The FTIR graph of extractives of *D.hispida* used of ultrasonication bath extraction method

The FTIR peakment was achieve at the range of 4000-400 cm^{-1} along with a resolution of 4 cm^{-1}. It was use to analyze the spectra functional group of dioscorine. The first group is in the range 1020-1259 cm^{-1} corresponding to the aliphatic amines C−N stretching vibrations. In this analysis it was peaked at 1076.30 cm^{-1} that assigned to the C−N stretching vibrations, this band showed dioscorine compound was existed in this extractives. Next, the group band should have in this extractives is primary amines it is range between 3250-3400 cm^{-1} assigned as N−H stretching and it is successfully showed in this extractive where there is a peak at 3274.58 cm^{-1}. The cyclohexane group peak at 2923.11 cm^{-1} assigned to be absorption of C−H to become strong bond for this compund. The next group of bond is should presence in this extractive is between ranges 1130-1620 cm^{-1} it was assigned to C−C and the peak successfully showed in the graph was 1150.15 cm^{-1}. The peak 1636.05 cm^{-1} belong to the C=C also showed. Finally, the other peak can be seen at the 1000.40 cm^{-1} which is corresponding to the C−O ester stretch also successfully presen.

Table 4.2: Summary of FTIR peakment for ultrasonication bath extraction method

Wavelength (cm^{-1})	**Assignments**
1076.30	C−N stretch (aliphatic amines)
3274.58	N−H stretch (primary amines)
2923.11	C−H stretch
1000.40	C−O stretch (ester)
1636.05	C=C stretch
1150.15	C−C stretch

For the FTIR graph of extractives of maceration assisted with ultrasonication probe type extraction method was shown in figure 4.3. The graph shows all the peaks responsible to trace dioscorine presence during the extracted procedures.

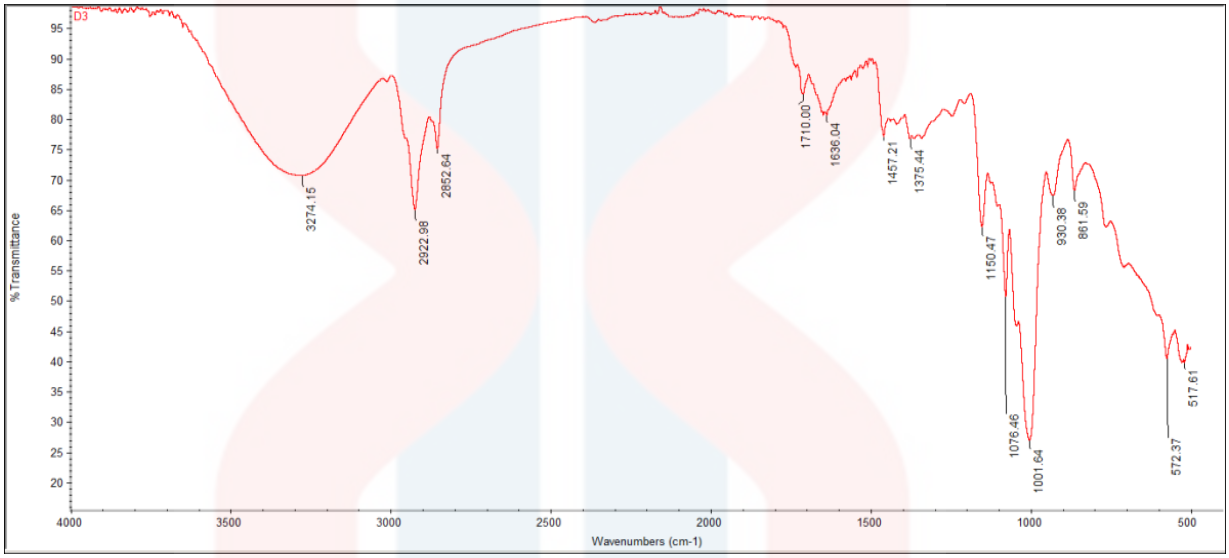

Figure 4.3: The FTIR graph of extractives of *D.hispida* tuber used maceration assisted with ultrasonication probe type extraction method

The peak for dioscorine at carboxylate ester group which is at 1710 cm^{-1} assigned to C=O stretch vibration and C−O at peak 1001.64 cm^{-1} stretching vibration. Next peak, 1076.46 cm^{-1} assigned to the aliphatic amines C−N stretching vibrations. While the band range 3250-3400 cm^{-1} is due to the primary amines stretching and in this extractive it was peak at 3274.15 cm^{-1} it was assigned to the N−H stretching vibrations. The cyclohexane group peak at 2922.98 cm^{-1} and 2852.64 cm^{-1} assigned to be absorption of C−H for become strong bond. The next group of bond is found at peak 1150.47 cm^{-1} it was assigned to C−C. Last peak that can be found in graph is assigned to C=C at 1636.04 cm^{-1}.

Table 4.3: Summary of FTIR peakment for maceration assisted with ultrasonication probe type extraction method

Wavelength (cm^{-1})	Assignments
1076.46	C−N stretch (aliphatic amines)
3274.15	N−H stretch (primary amines)
2922.98, 2852.64	C−H stretch
1001.64	C−O stretch (ester)
1636.04	C=C stretch
1150.47	C−C stretch
1710	C=O stretch (ester)

The Figure 4.4 shows the FTIR peaks spectrum of extractives used chemical extraction method was extracted from *D.hispida* powder form tuber where it has a unique value compared with other method extraction.

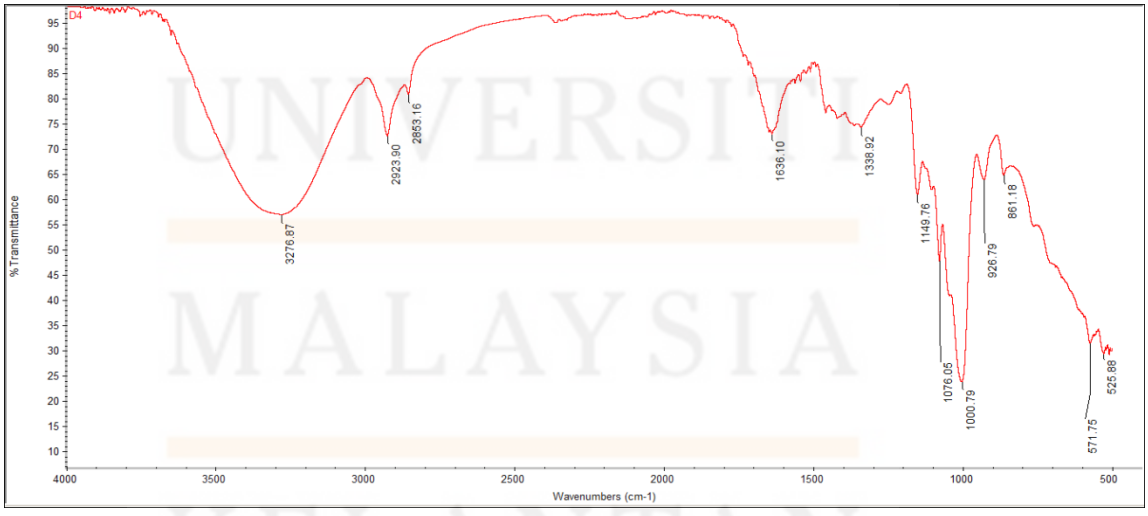

Figure 4.4: The FTIR graph of extractive of *D.hispida* tuber used chemical extraction method

The third FTIR graph was an extractive from chemical extraction method used as shown in Figure 4.5. The first showed in the graph at peak for C−O at peak 1000.79 cm^{-1} stretching vibration. Next peak, 1076.05 cm^{-1} assigned to the aliphatic amines C−N stretching vibrations. While for the primary amines stretching it was peak at 3276.87 cm^{-1} it was assigned to the N−H stretching vibrations. The cyclohexane group peak at 2853.16 cm^{-1} and 2923.90 cm^{-1} assigned to be absorption of C−H become strong bond. The next group of bond is found at peak 1149.76 cm^{-1} it was assigned to C−C. Last peak that can be found in graph is assigned to C=C at 1636.10 cm^{-1}.

Table 4.4: Summary of FTIR peakment for chemical extraction method

Wavelength (cm^{-1})	**Assignments**
1076.46	C−N stretch (aliphatic amines)
3274.15	N−H stretch (primary amines)
2922.98, 2852.64	C−H stretch
1001.64	C−O stretch (ester)
1636.04	C=C stretch
1150.47	C−C stretch

The Figure 4.5 shows the FTIR peaks spectrum of extractives used Soxhlet extraction method was extracted from *D.hispida* powder form tuber. The graph shows all the peaks responsible to trace dioscorine presence during the extracted procedures.

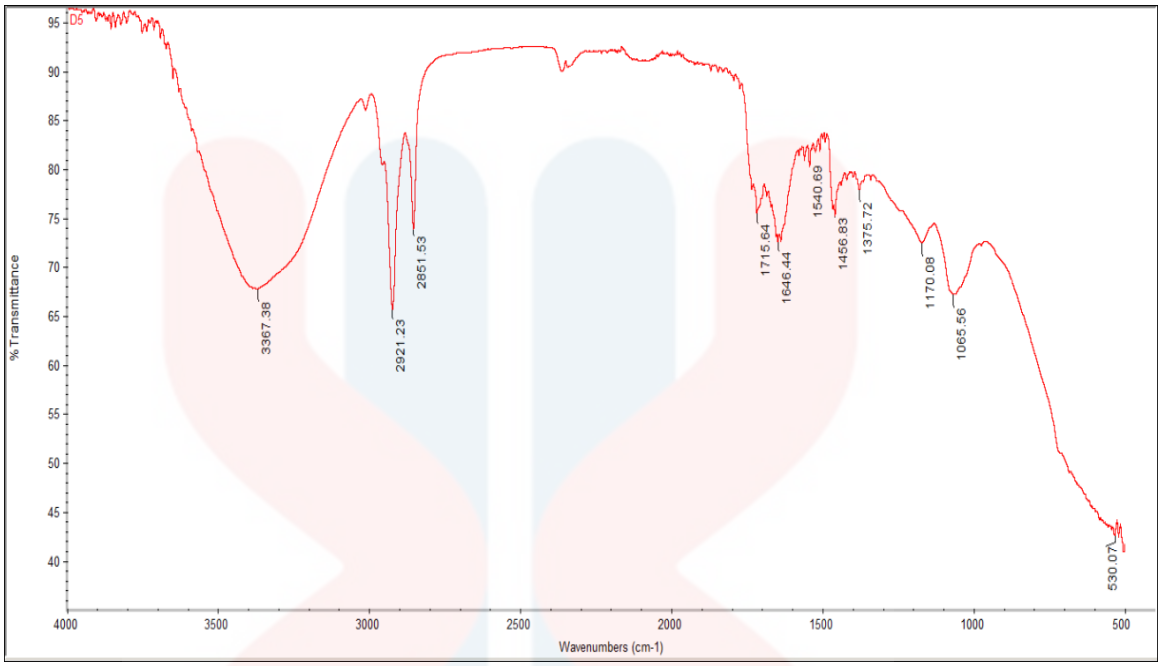

Figure 4.5: The FTIR graph of extractive of *D.hispida* tuber used Soxhlet extraction method

Next FTIR graph was an extractive from Soxhlet extraction method used as shown in Figure 4.5. The peak for dioscorine at carboxylate ester group which is at 1715.64 cm^{-1} assigned to C=O stretch vibration and C−O at peak 1065.56 cm^{-1} stretching vibration. Next peak is 1170.08 cm^{-1} assigned to the aliphatic amines C−N stretching vibrations. While the primary amines stretching showed at peak 3367.38 cm^{-1} it was assigned to the N−H stretching vibrations. Besides, the cyclohexane group peak at 2851.53 and 2921.23 cm^{-1} assigned to be absorption of C−H become strong. The next group of bond is found at peak 1375.72 cm^{-1} it was assigned to C−C. Last peak that can be found in graph is assigned to C=C at 1646.44 cm^{-1}.

Table 4.5: Summary of FTIR peakment for Soxhlet extraction method

Wavelength (cm^{-1})	Assignments
1170.08	C−N stretch (aliphatic amines)
3367.38	N−H stretch (primary amines)
2921.23, 2851.53	C−H stretch
1065.56	C−O stretch (ester)
1646.44	C=C stretch
1357.72	C−C stretch
1646.44	C=O stretch (ester)

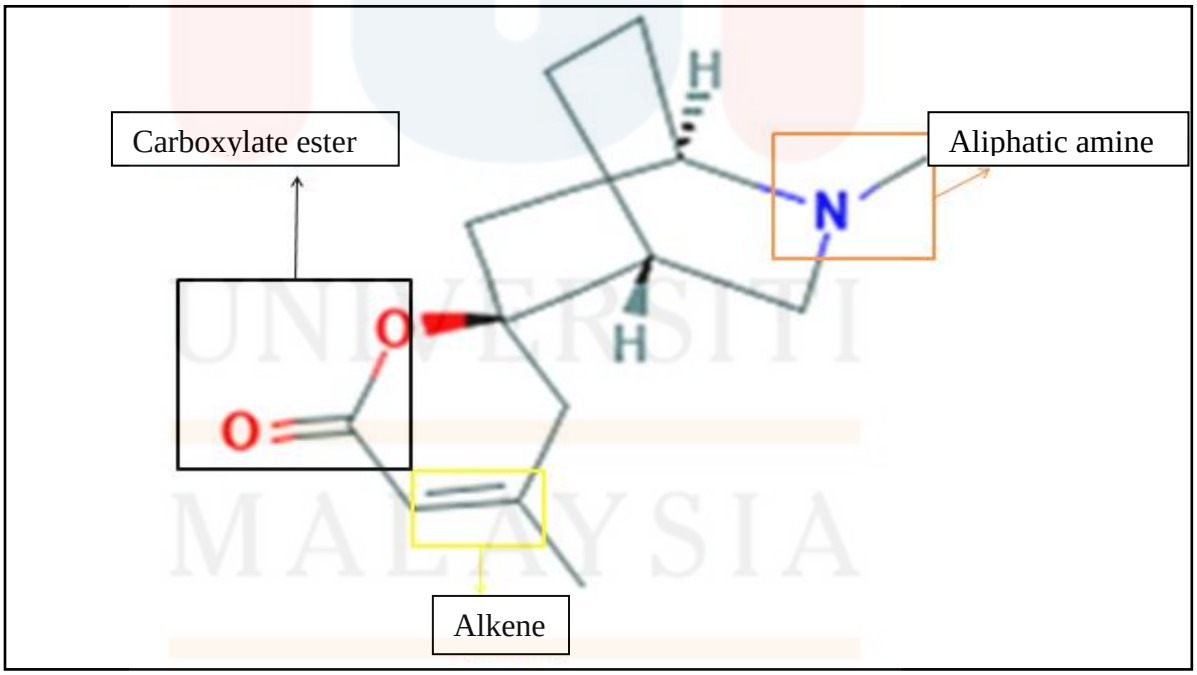

Figure 4.6: The dioscorine molecular structure and some of its functional group

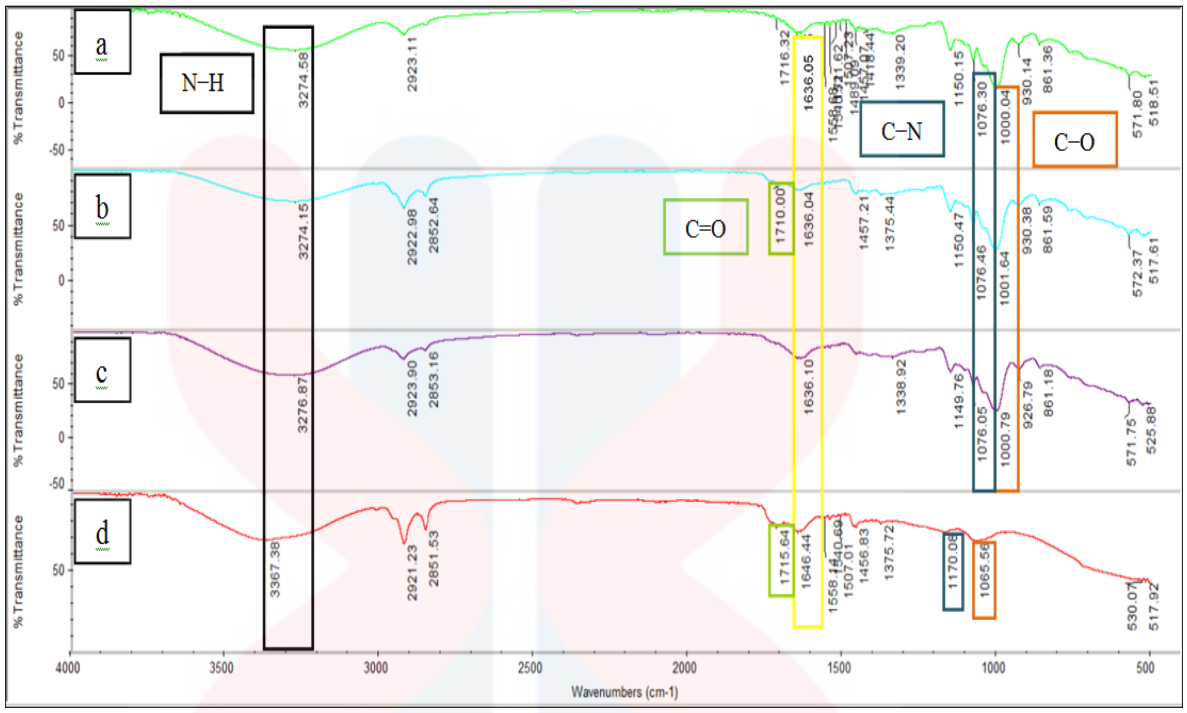

Figure 4.7: Combination of FTIR graph from all extraction methods a) ultrasonication bath extraction b) maceration assisted with ultrasonication probe type c) chemical extraction d) soxhlet extraction

Based on the figure 4.7 the results were shown there were one functional group was not present in two extraction methods. The spectrum of C=O stretch which is one of the carboxylate ester functional group component were not found in ultrasonication bath extraction method and chemical extraction method. Based on the extractives result these two methods were obtained the lowest yield extraction and it is could be relate why the peakment of spectrum of C=O stretch could not be found. The yields were too little to express the stretch of that particular compound. For Soxhlet extraction method and maceration assisted with ultrasonication probe type were success and completely obtained all the peakment spectrum of functional group for dioscorine. It can be conclude these two methods, Soxhlet extraction and maceration assisted with

ultrasonication probe type was the best efficient to extract the dioscorine from the *D.hipsida* tuber.

4.2 Application Dioscorine For Insecticide

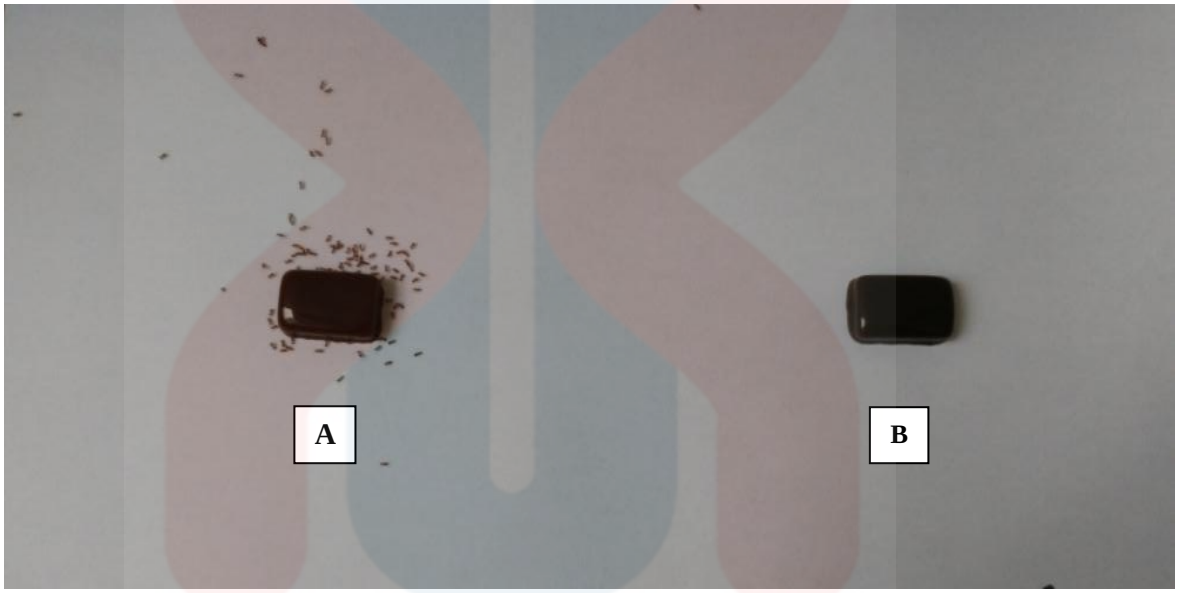

Figure 4.8: The observing result between two sweets A) with sprayed by crude petroleum ether B) without sprayed by crude petroleum ether

Based on the Figure 4.8 the results of significant feeding deterrent activity at different situation, the left one (cross) sweet without sprayed the crude petroleum ether extract of *D.hispida* tuber and the right one (tick) was sprayed by it. Obviously, result was showing that insect or ants were not come to the sweet that was sprayed by the crude. The extraction method used was maceration assisted with ultrasonication probe type because it was attained the highest yield of extractives and at the same time it was successfully present all the component of functional group should have in dioscorine.

The crude is contained the alkaloid dioscorine, which is also toxic in several organism such as cockroaches, milkweed bug, insect and causes dizziness, nausea, vomiting and sleepiness to human. This study was did not confirm the precise effect of the dioscorine on the insect because there is no death happen that have taken place when they consumed the dioscorine treated sweet. They are just avoiding from the sweets area that was sprayed by crude contain dioscorine,

With this antifeedent characteristic the Scoonhoven 1982 was suggested in order protecting plants from insect attacks of eating and sucking, this deterrent should be absorbed and transported inside the plant to ensure the parts of plant are protected. The dioscorine of alkaloids and assist with right method and solvent could give action and uses.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study to optimize of various extraction methods of *D.hispida* tuber which methods is the best efficient to extracting the dioscorine, the extraction methods namely as maceration assisted with ultrasonication probe type extraction, ultrasonication bath extraction, chemical extraction, and Soxhlet extraction. The constant variables in this study were using petroleum ether as solvent and the ratio of solvent and sample was 1:10. Next is to determine the functional group of dioscorine and at the same time to determine whether the extraction was successfully to isolate the dioscorine or not by using FTIR analysis. Lastly, the study and identify of application dioscorine in the environment as an insecticide was successfully or not.

The findings that were gathered, the highest yield of extractives by maceration assisted with ultrasonication probe type method followed by Soxhlet and ultrasonication bath and lastly is chemical extraction. For the FTIR the chemical extraction and ultrasonication bath the only are not showing at peak of C=O the bond between 1710-

1730 cm^{-1} which is the main part of dioscorine as known as Carboxylate ester group. The other two methods which are Soxhlet and maceration assisted with ultrasonication probe type extraction were managed to show every peak of dioscorine functional group so that the most efficient method is Soxhlet and maceration assisted with ultrasonication probe type Lastly, the insecticide study was show that insect do not come to the sweet that sprayed by crude extraction so it is consider as antifeedent toxic for insect. In conclusion, the objective which is to determine the efficient extraction method, the functional group of dioscorine by analysis using FTIR and make the dioscorine as insecticide have been successfully achieved for this study.

5.2 Recommendations

The researcher would like to recommends of several ideas for further study of *D.hispida*. First, the dioscorine can be extract by using different solvents such as ethanol which is near to the water characteristics to get more extractives, it is could be tested using two different methods such as in biological method using enzyme and the pharmaceutical characteristics. These different methods can be tested for its efficiency and accuracy in extracting dioscorine. Next researcher could use alkaloid test such as Dragendroff's test, Mayer test, and Gas Chromatography–Mass Spectrometry (GC-MS) to detect the component of dioscorine from extractives. The GC-MS in Universiti Malaysia Kelantan was not available of dioscorine standard so for next experiment hopefully dioscorine standard was update into the system. The extractives of a sample

can contain much valuable information and can be further analyzed to study its content. Advance research can consider the different solvent to extract dioscorine, different drying method, pretreatment and tuber condition of *D. hispida* tubers to identify the effects they could influence. The further work on *D. hispida* was hoping to get it commercialized on insecticide and used broadly in industry because of its high potential.

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APPENDICES

The calculation of extractives for each extraction methods:

$$\text{Extractives (\%)} = \frac{\text{Weight of extractives } (g)}{\text{Weight of dried sample}} \times 100\%$$

i) Extractives of Soxhlet extraction method:

$$\frac{1.63\ g}{30\ \text{g}} \times 100\% = 5.45\%$$

ii) Extractives of chemical extraction method:

$$\frac{1.30\ g}{30\ \text{g}} \times 100\% = 4.35\%$$

iii) Extractives of ultrasonication bath extraction method:

$$\frac{1.60\ g}{30\ \text{g}} \times 100\% = 5.36\%$$

iv) Extraction of maceration assisted with ultrasonication probe:

$$\frac{2.02\ g}{30\ \text{g}} \times 100\% = 6.74\%$$