ANNONA MURICATA (SOURSOP) : EXTRACTION OF AGEs FROM LEAVES

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Abstract— Since the dawn of medicine, natural products, especially those derived from plants, have been used to help mankind sustain their health. In the last century, phytochemicals in plants have been a pipeline for pharmaceutical discovery. The importance of plant active ingredients in agriculture and medicine has stimulated considerable scientific interest in the biological activities of these substances. Plants with a long history of use in ethno- medicine are a rich source of active phytoconstituents in a pharmaceutical landscape that offer medicinal or health through benefits against various diseases. Annona Muricata is also commonly referred to as Gunbanana Graviola or Soursop. Therefore, the aim of this work is to study the effect of solvent concentration towards the extraction of bioactive Acetogenin compounds in Annona Muricata . Extraction involved during experiment was liquid – solid extraction. The Annona Muricata leaves were picked and collected for about 500 gram were shade dried and powdered. The powder was macerated with 0 - 95% ethanol and water to obtain six different fraction F1-F6 which were bringing into analysis. HPLC and FTIR analysis showed that with different combination of water – ethanol concentration added into fractions affect the presence of acetogenin compounds and chemical groups. Four acetogenin were found in HPLC analysis. Besides, each fractionation showed different level of chemical groups presence in FTIR analysis.

Keywords : Acetogenin; Phytochemical; Annona Muricata Leaves

I. INTRODUCTION

Natural products, especially plant-derived ones, have been used since the dawn of medicine to help humanity sustain their health. Phytochemicals in plants have been a pharmaceutical discovery pipeline over the past century. The importance of plant active ingredients in agriculture and medicine has stimulated significant scientific interest in these substances biological activities (Moghadamtousi SZ, et. al, 2013). Despite these scientific studies, a limited number of plant species have undergone detailed scientific inspections and knowledge about their potential role in nature is relatively insufficient. Therefore, the achievement of a reasonable perception of natural products requires comprehensive research on the biological activities of these plants and their key phytochemicals. (Moghadamtousi SZ, et. al, 2014). The use of traditional complementary medicine, which includes herbal medicine in the treatment of multiple diseases, has rapidly increased in both advanced and emerging countries, due to affordability, accessibility and effectiveness. Documented and undocumented medical conditions related to herbal medicines make it relevant that pre-clinical toxicological studies on these natural products are carried out. Herbal medicinal plant remedies have traditionally been used in many parts of the world where access to formal healthcare is restricted. (Bailey and Day,1989). They may also have toxic side-effect (Keen et. al, 1994).

For thousands of years, plants have been the foundation of the world's traditional medical system and continue to provide humanity with new remedies. Medicinal plants and plants contain substances in ancient civilizations known for their healing properties. Those medicinal plants and herbs are used by people without access to conventional medicine to treat diseases. Annona Muricata's leaves have been confirmed to contain a few groups of substances simply called annonaceous acetogenins, including murihexocin and annocuricin. (Kim et al., 1998), annopentocin A, B and C,(2,4-cis)-annomuricin- D- one, murihexocin A and B,(2,4-trans)-annomuricin- D- one, 4-acetyl gigantetrocin and cis- gigantrionin(Zeng et al, 1996). The high effectiveness, quantification, wide chemical and biological diversity and effectiveness of these compounds against bacterial resistance could well make them the next class of useful natural antitumor and pesticidal agents(Alali et al., 1999) and other biochemical effects, the basic photochemical screen has revealed Annona Muricata to contain saponins, glycosides, tannins and flavonoids(Arthur et al., 2011). The essential oils with parasitocidal, anti-diarrheal, rheumatological and anti-neuralgic properties contain in leaves of Annona Muricata (Khan et al, 1997), help treat diabetes and gastric upset (Adewole and Ojewole, 2006), jaundice (Mshana et al, 2000) and used in treating kidney ailments (Duke, 1970).The leaves are also hepatoprotective against carbon tetrachloride and acetaminophen-induced liver damage and in streptozotocin treated diabetic rats (Adewole and Ojewole, 2008).

In a pharmaceutical landscape, plants with a long history of use in ethno-medicine are a rich source of active phyto-constituents offering medicinal or health benefits against various diseases. Also commonly known as Gunbanana Graviola or Soursop is Annona Muricata. Cherimoya(A.Cherimola) and sugarapple(A.Squamosa) are related species due to the sweet and sour taste of its great fruits; the paw paw(Asimina triloba) is also part of the family. The soursop is native to tropical Central and South America and the Caribbean, but is now frequently preserved in tropical areas worldwide, including southern Florida and South East Asia, from sea level to approximately 1150 meters above sea level. Soursop is one of the most commonly used medicinal plants in the Caribbean. Inside the

fruit there is a pulp that is eaten and used as ingredients for food and beverage. Soursop tea is often produced and used with other herbs for drinking. Soursop is an intolerant tree that is slender, small and cold, usually 4-6 meters high. The soursop is adapted to high humidity areas and relatively warm winters; temperatures below 5oC may cause damage to leaves and small branches, and temperatures below 3oC may be fatal (MS Sejal and Jayvadan, 2016).

II. METHODOLOGY

A. *Preparation of raw materials and chemicals*

The Annona Muricata also known as Soursop or Graviola were taken at Bukit Beruntung's farm, Selangor which was planted by Madam Rustazah Salwa. The Annona Muricata plant part which were leaves were picked and collected for about one kilogram and inserted into a plastic bag for transport to Laboratory of Food Technology at Faculty of Chemical Engineering UITM Shah Alam. The leaves were shade dried and powdered using mechanical blender. In order to extract the Acetogenins properties, the leaves had undergoes several steps. The dried leaves were crushed and sent to the grinder machine to become powder. The purpose of powder was to reduce the space and mobility. Pre-treatment steps were conducted first to give better raw samples preparation before main analysis. Ethanol and Acetone was used for the macerated as its ability for opening the micropores in leaves for determining in analysis.

B. *Process Flow*

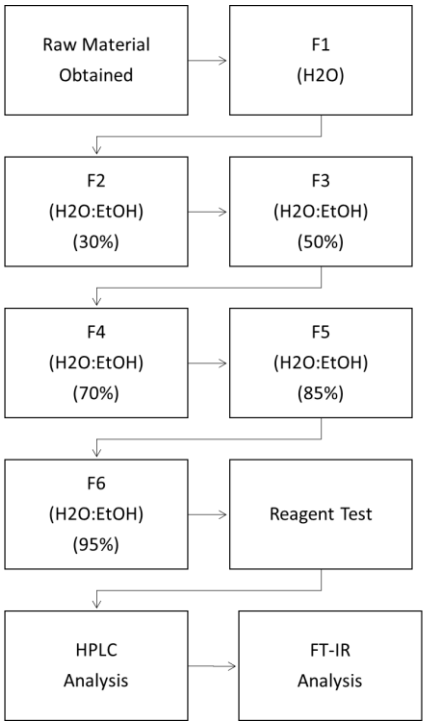

Figure 1: Process Flow Diagram

C. *Extraction of Annona Muricata*

50 g of Annona Muricata powder was macerated with 0 - 95% ethanol for one week. There were 6 fraction F1 – F6. The ethanol was evaporated using a rotary evaporator and the sludge was redissolved in acetone. The solution was filtered by using Buchner funnel with silica gel 60 on a filter paper. The fractions obtained by using the solvents water, water-ethanol different concentration (0 – 95%) to leach the solid crude extract. The ethanol concentration is prepared by mathematics formula M1V1 = M2V2.

The crude extract leaves were divided into 6 fraction with combination of Water – Ethanol. The first fraction were added with 20 ml of distilled water followed by Fraction 2 with 70:30 of water – ethanol. Besides that, 50 : 50 for Fraction 3, 30:70 water – ethanol for Fraction 4, 1.5:8.5 for Fraction 5 and 100% ethanol for Fraction 6.

Dry leaves (g)	Ethanol (95%)	Acetone + Sludge	F1 (+water)	F2 (H2O:EtOH) (7:3 v/v)	F3 (H2O:EtOH) (1:1 v/v)	F3 (H2O:EtOH) (3:7 v/v	F3 (H2O:EtOH) (1.5:8.5 v/v)	F3 (+EtOH)
10	60	30	20 ml					
10	60	30		14 : 6 ml				
10	60	30			10 : 10 ml			
10	60	30				6 : 14 ml		
10	60	30					3 : 17 ml	
10	60	30						20 ml

Table 1 : Fraction and concentration of ethanol

D. *Analysis of Functional Group*

The composition, and characteristics of Acetogenin group of Annona Muricata were analyzed to evaluate the content of the bioactive and properties with the aim of observed the components by the peak analysis. 6 fraction had prepared with different of water-ethanol fraction added. The sample Annona Muricata were set up into 6 different tube for analysis functional group according its chemical and acetogenin compound. FTIR and HPLC are suitable machine for determining these compounds. FTIR is a powerful functional group identification tool due to similar absorption frequencies in different molecules for these groups. Functional group identification is a cornerstone of IR spectroscopy and organic chemistry (Mike Bradley,2015). HPLC is a chromatographic method used to separate a compound mixture in analytical chemistry and biochemistry to identify, quantify or purify the mixture's individual components. Then, the samples were sent to Instrumentation Laboratory for functional group analysis. A small amount of liquid sample was injected into the FTIR and HPLC Spectroscopy in order to give a data on function groups and also to identify organic material in the sample. The data was collected and repeated several times using same samples to give constant value.

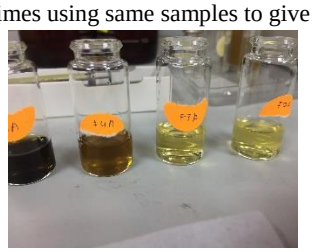

Figure 2 : Tube Samples

Figure 3: HPLC analysis

III. RESULTS AND DISCUSSION

The results are shown and discussed in this chapter. There were 6 fraction for this experiment. All fraction were equally put 10 g of dry powder leaves. 60 ml of ethanol with 95% concentration were added in all fractions. The ethanol macerated were left for one week

to let the chemical reaction reacted inside and vaporized the ethanol by using the rotary evaporator. Then the acetone were added combine with the sludge and filtered to get the crude extract leaves.

A. High – Performance Liquid Chromatography (HPLC)

According to Yang, (Yang et al,. 2009) the optimal extract sample was screened with slight modification through High Performance Liquid Chromatography (HPLC). It illustrated representative chromatograms of the acetogenin analytes for the optimally conditioned sample. The figure showed that in the HPLC screening process, the four acetogenins compound analytes were well separated and detected. Other sample compounds did not interfere with analyzing the four analytes of the acetogenin compounds. Each samples may have contain all four compounds and may have less than four.

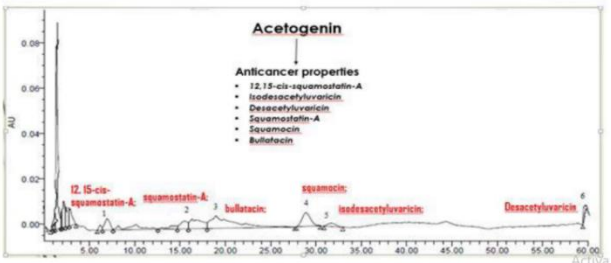

Figure 4 : HPLC test Acetogenin Determination

HPLC analysis were taken from "Preparation & evaluation of Annona Muricata extract against cancer cells with modified release". The results were confirmed and been compared to this studies to find Acetogenin Compound present in the samples

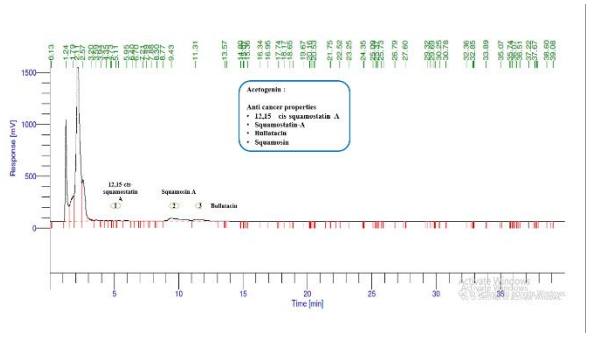

Figure 5 :HPLC F1 (Adding Distilled Water)

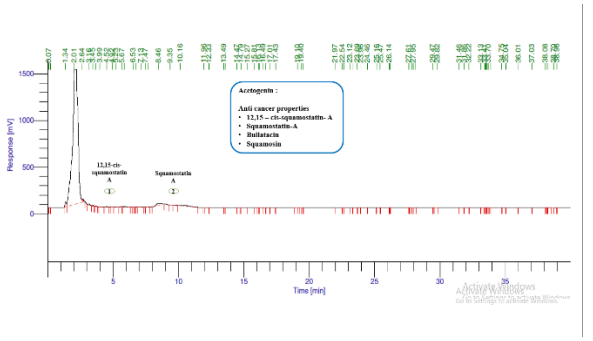

Figure 6 : HPLC F2 (7:3 water - ethanol)

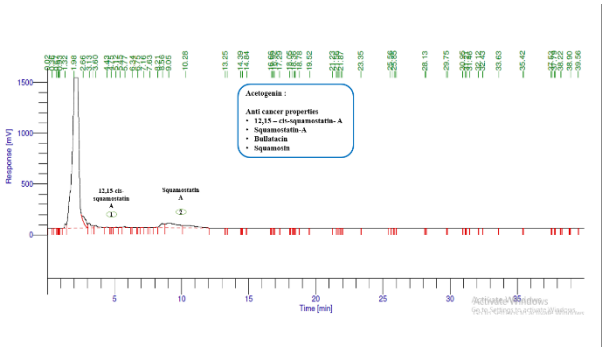

Figure 7 : HPLC F3 (1:1 water - ethanol)

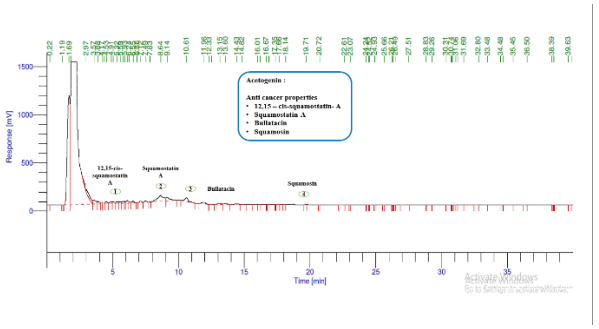

Figure 8 : HPLC F4 (3:7 water - ethanol)

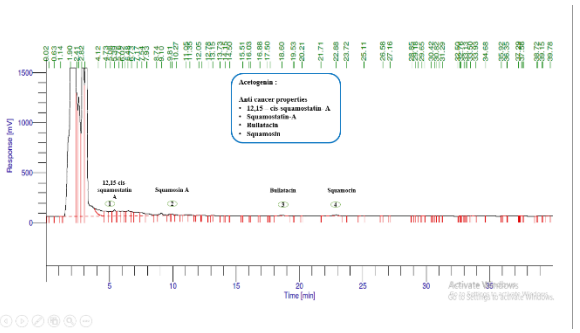

Figure 9 :HPLC F5 (1.5:8.5 water - ethanol)

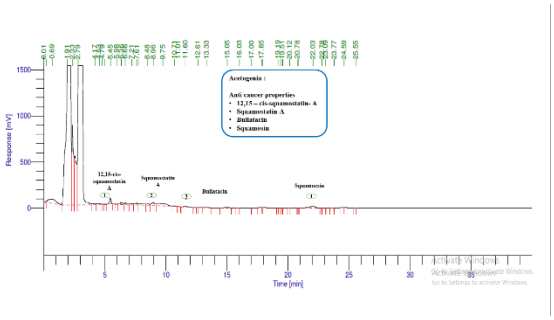

Figure 10 : HPLC F6 (Added ethanol)

F1 was analyzed with the total time 40 minutes. The graph peak was observed. For the first 3 minutes, unstable high peak level at more than 1500 mV was seen. This results was come from detecting the reaction component between water and crude extract. Later on, the observation of other samples also showed the early high peak performance. The next peak can be seen in minute 4 – 6. The component result was 12,15-cis-squamostatin A.

The observation were continued on F2 which only 2 -acetogenin

compound found (12,15-cis-squamostatin A and Squamostatin A). Both phytochemical were found at the first 10 minutes. The rest of 30 minutes, there were no signed of phytochemical present. These might because of the other acetogenin compound were reacted with water-ethanol. The ratio of 7:3 water-ethanol had changed the results (ZM. Gu et all, 1999). According to the journal, hydroxyl groups and acetogenin have a skeleton consisting many carbon atoms. These types atom have a weak UV absorption.

Figure 8 shows the sample F3 with adding 50% water and 50% ethanol ratio. The first three minutes resulted a high peak level containing Ethanol component in sample. The next 3 minutes, there were a few peak in the same level which presented by Acetogenin compound. After minute of 6, the graph showed static level then Squamostatin A appeared at the minute of 8 – 12. Only 2 acetogenin compound present in this sample.

In addition, F4-F6 were observed that all 4 acetogenin were present in the samples. But when comparing to the Journal studies, there were another 2 Acetogenin left that not present in the samples. Isodesacetyluvaricin and desacetyluvaricin were the compound that not be found in the sample. During lab experimental, it take longer for time taken for conducted due to the limited machineries in laboratory. Oxidation between sample and air inside the fraction might changes the reaction. These might change the components inside the fraction sample.

Acetogenin compound	Yang et al, 2009	F1	F2	F3	F4	F5	F6
	Time (min)	Time (min)	Time (min)	Time (min)	Time (min)	Time (min)	Time (min)
12,15-Cis-squamostatin A	9-10	4-7	3-6	3-5	3-6	4-7	4-6
Squamostatin A	15-18	9-11	8-11	9-12	7-10	9-11	8-11
Bullatacin	19-21	13	-	-	11-14	19-20	12-15
Squamocin	25-27	-	-	-	20	24-25	21-23

Table 2 : HPLC data of acetogenin compounds obtained in this study compared (Yang)

B. Fourier – Transform Infrared (FTIR)

FT-IR was used to identify the active component functional group based on the peak value in the infrared radiation region. All spectra were obtained on an FTIR spectrophotometer with the help of an OMNI-sampler attenuated total reflection (ATR) accessory. A small quantity of powdered leaves was placed directly on the infrared spectrometer's Germanium piece with constant pressure applied and infrared absorption data collected over the wave number ranging from 4000 cm–1 to 675 cm–1 and computerized for analysis). The melting points were measured on a micro-melting point device from Leica Gallen III Kofter. The optical rotations with Perkin Elmer 241 polarimeter were measured in CHCl3. The spectra of ultraviolet (UV) was recorded on the spectrophotometer of Shimadzu UV 1601PC.

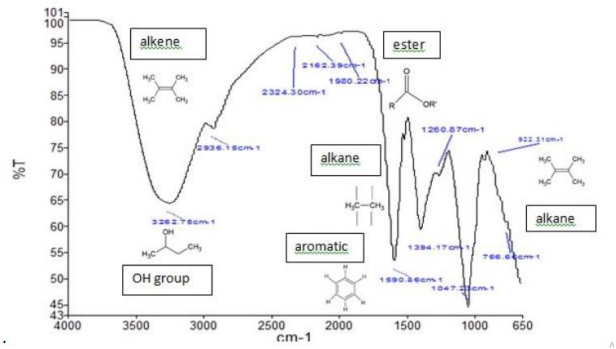

Figure 11 : FTIR test Chemical Group Determination

FTIR analysis were taken from "Preparation & evaluation of Annona Muricata extract against cancer cells with modified release". The results were confirmed and been compared to this studies to find chemical group present in the samples

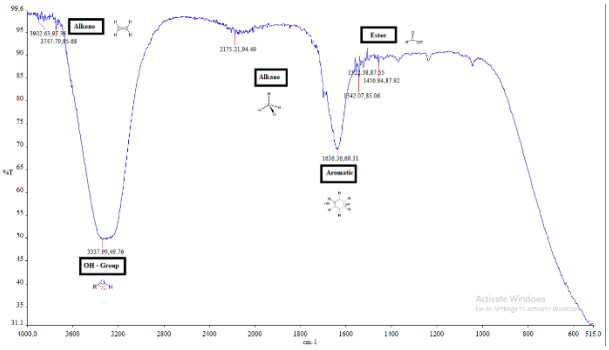

Figure 12: FTIR F1 (added 100% Distilled water)

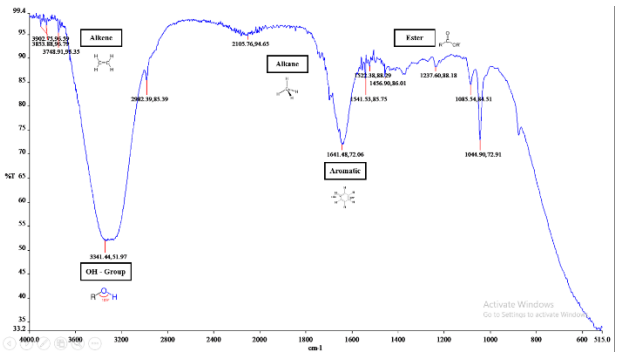

Figure 13: FTIR F2 (added 7:3 water-ethanol)

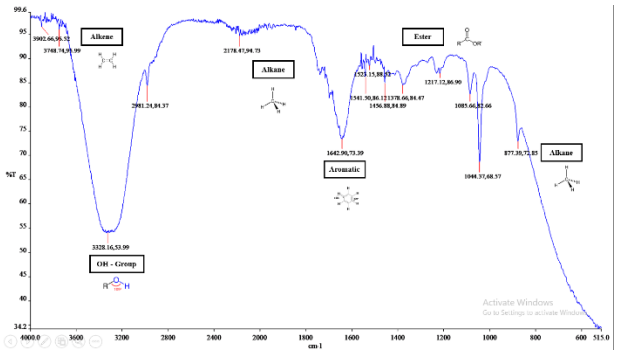

Figure 14: FTIR F3 (added 1:1 water-ethanol)

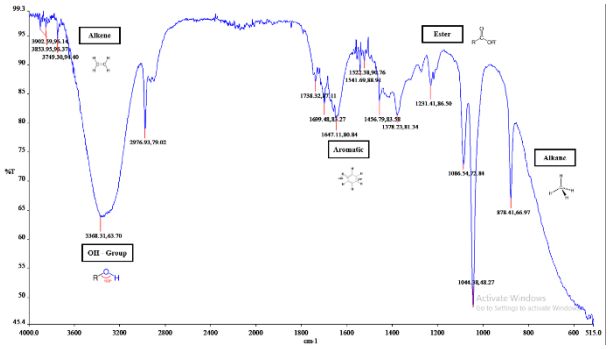

Figure 15: FTIR F4 (3:7 water - ethanol)

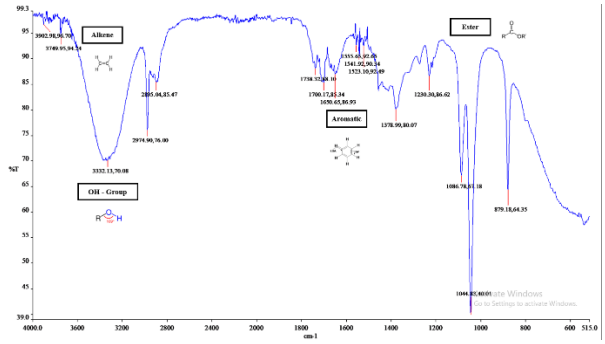

Figure 16: FTIR F5 (added 1.5:8.5 water-ethanol)

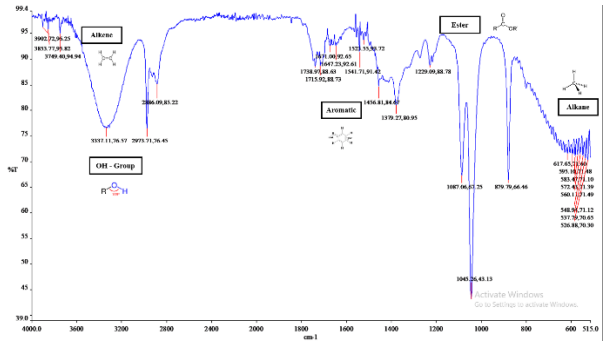

Figure 17: FTIR F6 (added 100% ethanol)

FTIR analysis were done for the first sample F1 after water has been added. During analysis, the machine were tested by put the water drop and scan by the crystal in the machine. The peak were obtained and compared to the previous studies. It was found that 5 group of chemical compound were determined by their presence from the peak. Alkene group was found at the peak number 3700-3900 cm-1. Besides that, for OH-Group, the ranging peak is from 3300-3400 cm-1 and from this sample, it was found at 3337.99cm-1 at 49.76 %T. The remaining group Alkane, Aromatic and Ester were found one after another and compared from previous study.

In addition, sample F2 were performed and being observed. According to (Mike Bradey, 2015) chart, there were different kinds of chemical group. It being classified accordingly to their wave. F2 sample show correct way of OH-group which in chart ranging number 3350 cm^{-1}. This was because of the presence of ethanol during sample preparation and also by adding into the fraction. Besides, Ester group in the range of 1200 – 1740 cm^{-1} also present in the result.

Sample F3 was performed by adding same amount of water-ethanol. From this observation, the peak at 2981 cm-1,84.37%T present in this sample because of ethanol concentration is higher. In addition, there was another group found which was Alkane at 877.39cm-1,72.85%T. Sample F3 showed more or less same as Journal studies by (Dilipkumar & Agliandeshwari, 2017). This studied showed differences at Ester group peak which much more complicated than Journal Studies.

There were some significant difference for samples F4 – F6 compared to F1 – F3. The graph shows very high peak level of Ester Group. Even still in the range of 1200 – 1740 cm^{-1}, the concentration in these samples were higher than others. The chemical reaction of OH-group might oxidised to ester group. (BCcampus)

C. Recommendation

Pure acetogenin compound determination need to be done qualitatively and quantitatively correct by many trial and error to obtain its presence in samples. A practical studies of separation and isolation is desirable. Due to the Annona Muricata is categorized in food group, kept it for longer period will affect itself as it can oxidise which can affect the chemical reaction inside the samples. For action to be taken, an immediately analysis samples need to be done after eluent added into samples.

IV. Conclusion

A practical fractionation technique involving an open column chromatography containing several eluents with different concentration to detect the presence of bioactive compounds has been developed. Water and ethanol were selected as eluents as to compared the detection of acetogenin and to see its reaction extraction. Six fractionation was prepared and four acetogenin were found in HPLC analysis. Besides, each fractionation showed different level of chemical groups presence in FTIR analysis.

Acknowledgment

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