Antibacterial Activity of Pineapple (*Ananas comosus*) Waste (Peel and Crown)

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the Report of this final year project entitled "Antibacterial Activity of Pineapple (*Ananas comosus*) Waste (Peel and Crown)" by Muhammad Asyraf Bin Mohd Nasrum, matric number F15A0087 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Husbandry Science) with Honours,

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LIST OF ABBREVIATIONS AND SYMBOLS

ºC	degree Celcius
nd	no detected
mm	Milimiter
mg	Milligram
cm	Centimetre
FAO	Food and Agriculture Organization
CAM	Crassulacean Acid Metabolism
G	Gram
MHA	Mueller-Hinton agar
TSA	Trypticase soy agar
TSB	Trypticase soy broth
SCP	Single cell protein
GRAS	Generally recognized as safe
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand

Antibacterial Activity of Pineapple (*Ananas comosus*) Waste (Peel and Crown).

ABSTRACT

The aim of this research study is to determine the antibacterial activity of pineapple (*Ananas comosus*) waste (peel and crown) against *Streptococcus sp.* There are four different extraction solvents that been used which are ethanol, methanol, hexane and distilled water. The antibacterial activity was carried out using agar well diffusion method. The result showed that the highest inhibition zone was observed for ethanol crude extract which was 1.20±0.10 mm compared to inhibition zone of methanol extract, 1.00±0.10 mm. For the hexane and distilled water, no detected inhibition was observed in the agar. This finding showed that the *A. comosus waste* (peel and crown) contain high level of antibacterial properties that can be used to prevent any bacteria which can attack the aquaculture species and human health. Further study need to be conducted to find the full structural component of antibacterial compound derived from the *A. comosus*. The result obtained can be used as a guideline for further research on this field in the future.

Keywords: *Ananas comosus* waste, Antibacterial activity, Extraction, Agar well diffusion, Bacteria.

Aktiviti Antibakteria Nanas (*Ananas comosus*) Sisa (Kulit dan Mahkota).

ABSTRAK

Tujuan kajian ini adalah untuk menentukan aktiviti antibakteria nanas (*Ananas comosus*) (kulit dan mahkota) terhadap *Streptococcus sp.* Terdapat empat pelarut ekstraksi yang digunakan iaitu etanol, metanol, heksana dan air suling. Aktiviti antibakteria telah dijalankan menggunakan kaedah penyebaran agar. Keputusan menunjukkan bahawa zon perencatan tertinggi diperhatikan untuk ekstrak etanol mentah yang adalah 1.20 ± 0.10 mm berbanding dengan zon perencatan ekstrak metanol, 1.00 ± 0.10 mm. Untuk heksana dan air suling, tiada perencatan yang dikesan diperhatikan dalam agar. Penemuan ini menunjukkan bahawa sisa *A. comosus* (kulit dan mahkota) mengandungi paras antibakteria yang tinggi yang boleh digunakan untuk mencegah sebarang bakteria yang boleh menyerang spesies akuakultur dan kesihatan manusia. Kajian lanjut perlu dijalankan untuk mencari komponen struktur penuh antibakteria yang berasal dari *A. comosus*. Hasil yang diperolehi boleh digunakan sebagai panduan untuk penyelidikan lanjut mengenai bidang ini pada masa akan datang.

Kata kunci: Sisa *Ananas comosus*, Aktiviti antibakteria, Pengekstrakan, Kaedah penyebaran agar, Bakteria.

CHAPTER 1

INTRODUCTION

1.1 Research background

Pineapple can be considered as one of the most important commercialized fruits globally. It can be protecting, cooked, making juice and consume freshly. Calcium, potassium, vitamin C, carbohydrate, crude fibre, water and mineral can be found in the pineapple. In pineapple industries, processing of pineapple cause a higher quantity of waste. The remaining pineapple consists of pineapple peel and crown. Pineapple peel has more nutritional ingredients that can be eaten because it has high of fiber and protein (Farid, Akhtar & Anwar, 2015).

Pineapple is a great source of vitamin C, and it can be a viable antioxidant and offer assistance to the absorption of iron (Farid et al., 2015). It's too can be utilized to battle bacterial and viral contaminations. Each 100 gram of pineapple waste, it contain 26.5 mg of ascorbic acid and each 100 gram of pineapple pulp it contain 21.5 mg of ascorbic corrosive. So, the vitamin C contains in pineapple pulp is lower than pineapple waste.

In this research, the pineapple (*Ananas comosus*) waste (peel and crown) was processed as extraction with different dilution by using soxhlet method. *Ananas Comosus* can create a bigger amount of waste during the processing in pineapple industries. Pineapple waste comprises of pineapple peels and pineapple crown. The point of this study is to discover the antibacterial activity of pineapple waste.

1.2 Problem Statement

In Malaysia, all of the waste products produce are useless and usually the consumers throw all the pineapple waste just like other waste product like banana waste and coconut waste. However this waste has the specific nutritional value that can replace the expensive substance that been used in agriculture business. Pineapple wastes contain antioxidant that useful for aquaculture. For this research, the pineapple (*Ananas comosus*) waste (peel and crown) been used to observe either it can be adding to the fish feed as disease resistance to the bacteria which is *Streptococcus sp.* and immune defense to prevent the fish from died. This can reduce the cost for the farmers to culture the fish.

1.3 Hypothesis

H_0: The Pineapples (*Ananas comosus*) Waste (peel and crown) extract not have antibacterial activity properties against *Streptococcus sp.*

H_1: The Pineapples (*Ananas comosus*) Waste (peel and crown) extract have the antibacterial activity properties against *Streptococcus sp.*

1.4 Objectives

1. To determine the antibacterial activity of Pineapples (*Ananas comosus*) waste extract using agar well diffusion method.

1.5 Scope of Study

For this research, the Pineapples (*Ananas comosus*) Waste (peel and crown) was obtained around the market in Jeli, Kelantan. The pineapple wastes were washed using tap water to make sure the waste cleaned from unnecessary things. The *A. comosus* wastes were dried in the oven for one night and then were processed into the powder form for making the extraction process run smoothly. Then, the *A. comosus* wastes undergo extraction using soxhlet method in different solvent (ethanol, methanol, hexane, distilled water) for the antioxidant testing. After that, the extraction undergoes

rotary process evaporation to get the crude extract. Lastly, the antibacterial activity in pineapple waste was observed.

1.6 Significance of Study

In this study, the antibacterial test in *Ananas comosus* wastes (peel and crown) being conducted to check the inhibition zone in agar plate. This information study either can be useful to prevent the fish from any disease from bacteria like *Streptococcus sp.* Besides that, through this study it also can provide information about pineapple wastes which can be used in the production of feed. Production of high quality feed with low cost will enhance the production of tilapia. On the other hand, the increase of tilapia demand also will help the farmer to expand their farming. Thus, contribute to the extension of tilapia farming for aquaculture industries in Malaysia.

1.7 Limitation of the Study

There limitation of the study such as less of information result the *Ananas comosus* waste (peel and crown) on the antibacterial activity. This study also affected by the handling technique during the experiment and surrounding environment at the place of the experiment. Other than that, limitation of using equipment's like soxhlet apparatus during researched due to the lack of budget.

CHAPTER 2

LITERATURE REVIEW

2.1 Pineapple (*Ananas Comosus*)

In the world, Pineapple (*Ananas comosus*) commonly known as one of critical natural products and as a queen of fruits based on its good in texture and flavour. *A. comosus* is the fruits that came from the family of *Bromeliaceae.* After the orange and apple juice, pineapple is the third most favored fruit juice (Cabrera, Menezes, Oliveira & Batista, 2000). The development of pineapple plant up to a tallness of 75 to 150 cm with spread of 90 to 120 cm. Based on the Tran (2006), *A. comosus* can creates to a plump fruit with crown on top. In 2007, the plantation of pineapple is almost 920 349 ha that be estimated of production more than 18 million tons (FAO, 2007). In commercially, the pineapple normally manufacture as canned fruits products and being devoured in around the world. Other than that, it is additionally can be processed as juices, concentrates and jams.

Pineapple (*A. comosus*) photosynthesis process is quite different from the other type of plants. It undergoes process called Crassulacean Acid Metabolism (CAM) which allows them to remain moisture during dry season. It works on the plant at night when the stomata opens and carbon dioxide is fixed to be stored and start to produce sugar and starch. Carbon dioxide that stored within the plants, serves as malic acid which allows them to close the stomata during the day for photosynthesis showed the quality of the CAM plants (Engebos, 2012).

According to Farid et al., (2015), mature pineapple fruit contains 14% of sugar, protein digesting enzyme, bromelin, excellent amount of citric acid, malic acid, vitamin A and B. Pineapple may be a great source of vitamin C, and it can be a viable antioxidant and offer assistance to the absorption of iron. It's also can be used to fight bacterial and viral infections. Every 100 g of pineapple waste it contain 26.5 mg of ascorbic acid and every 100 gram of pineapple pulp it contain 21.5 mg of ascorbic acid. Vitamin C contains in pineapple pulp is lower than pineapple waste (Farid et al., 2015).

In Malaysia, a variety of N36 pineapples are among the four different pineapples grown. This assortment is primarily utilized for canned product. The leaves of pineapple crown are the load of the canned pineapple industry with the reason that the requirements of the pineapple crown to reproduce are generally slightly different from the size of the resulting refusal. Furthermore, different options with their proficient use are important. On the other hand, the pineapple crown consist bromelain enzyme. Protease or proteolytic enzyme contained in the bromelain. It is an aqueous extract of pineapple contains a complex mixture of proteases and non-protease components. Bromelain is used in sustenance to process meat tenderization.

Based on Devakate, Patil, Waje & Thorat (2009), they utilized ammonium sulphate to quicken bromelain or utilized ion exchange chromatography to eradicate bromelain from fruit crude juice with dialysis layer to expel salt. At last, to deliver bromelain powder, the desalted bromelain solution is dried employing a shower dryer or cooler dryer. In this investigation, bromelain powder from the pineapple crown has been delivered through filtration, desalting handled by drying using cation trade chromatography, continuous diaphiltrators and cooler dryer, individually. At first, the physico-chemical properties of the pineapple crown extract was found. At that point, bromelain action in pineapple crown extract, filtered bromelain, desalted bromelain and bromelain powder were finished.

2.1.2 Taxonomy

Taxonomy is the science of naming, describing and classifying organisms. (Bartholomew, Paull & Rohrbach, 2003).

Domain : Eukarya

Kingdom : Plantae

Phylum : Anthophyta

Class : Lilopsida

Order : Bromeliales

Family : Bromeliaceae

Subfamily : Bromelioideae

Genus : *Ananas*

Species : *Ananas comosus*

2.1.3 Nutritional Value

A pineapple fruit contains contains calcium, potassium, vitamin C, carbohydrates, crude fibre, water and other types of minerals that good for the digestive system (Farid et al., 2015). Table 2.1 shows the nutrients contents in the pineapple (*Ananas comosus*).

Table 2.1: Nutrient content of Pineapple (*Ananas comosus*) (Farid et al., 2015)

Nutrients	**Amount**
Energy	52 calories
Dietary fibre	1.40 g
Carbohydrate	13.7 g
Protein	0.54 g
Iron	0.28 mg
Magnesium	12 mg
Calcium	16 mg
Potassium	150 mg
Phosphorus	11 mg
Zinc	0.10 mg
Vitamin A	130 IU
Vitamin B 1	0.079 mg
Vitamin B 2	0.031 mg
Vitamin B 3	0.489 mg
Vitamin B 6	0.110 mg
Vitamin C	24 mg

2.2 Potential of using Pineapple in aquaculture

Nile tilapia (*Oreochromis niloticus*) is a freshwater and source of financial everywhere throughout the world since it is well known for utilization and surrounding. According to FAO (2015), about 371 519 tons of tilapia production. Approximately 60% of the cost of aquatic animal production is influenced by the factor of making the feed which affecting the intensive culture system (Naylor, 2000). Cost increments and supply deficiencies of fishmeal which is the primary part of the fish feed extremely confine aquaculture development (Tacon and Metian, 2008). Many studies have been made to find out the accepted alternative protein sources to reduce the cost on fishmeal for fish feed that include animal proteins, plant proteins and single cell protein (Hardy, 2010). The capacity for nutrient utilization by fish, as well as growth rates can be lower because of anti-nutritional properties that contain in the plant protein sources (Francis, Makkar & Klaus, 2001).

In worldwide, Thailand became the biggest exporter of cannery pineapple product. Based on FAO (2008), approximately 2.5 million tons of pineapples were delivered. From that amount, around 520 000 tons and 150 000 tons of pineapples were sending out as canned pineapple and pineapple juice product. During pineapple processing, the crown and the stem are cut off before being peel and the core of the pineapple is expelled for advanced processing. According to Ketnawa, Chaiwut & Rawdkuen (2012), the waste portions of the pineapple like peel, core, stem and crown represent 29-40%, 9-10%, 2-5% and 2-4%. Apart from that, the pineapple waste increases rapidly because of increasing pineapple production.

Usually waste disposal can cause the microbial spoilage which causes the serious environment problems. To reduce the production of waste from processing, an alternative have been carried out to exploit the pineapple waste which would be useful. Bromelain with the other cysteine proteases are familiar enzyme that show within the different portion of the pineapple. Walsh (2002) said that, bromelain has been used commercially like within the food industry, certain beauty care products additionally in dietary supplements. It is recommended that the utilized of pineapple waste as a proteolytic enzymes in fish diets is one of the elective implies of waste management. In this way, the proteolytic enzyme action of pineapple waste extract can influence the development, feed utilization and nitrogen excretion of Nile tilapia.

In Mudfish, a part of the pineapple which is pineapple juice being used within the elimination of egg stickiness and progressed hatchability rate. Pineapple juice contain photolytic enzyme which can digest protein. It also contains ascorbic acid, calcium, potassium, magnesium, fiber and vitamin. Cultured of warm water fisheries in the aquaculture industry has become important because of the high demand of the fish lingerings. Breeding of the fish has turn from being science concept to art since the pre and post management of fires require an additional expertise to be done. In reality, the accessibility of fish seed is exceptionally imperative for coherence. In Nigeria, a household fish production has appeared that the artisanal fisheries sector contributes around 90% of the annual fish production while the industrial sector contributes about 5% and the aquaculture sector contribute about 3%.

Producing of quality fish seed can increased the fish production and the raise of the fish. The breeding of fish using different hormonal materials such as Ovaprim,

Ovulin and stimulants like pituitary various fish species can increase the fish production (Lawson & Ishola, 2010). Two males and three females broodstock of Mudfish (*Heterobranchus bidorsalis*) being used. Hormone ovaprim was injected into the female at rate of 0.5ml/kg. After 9 hours of latency period, the female eggs which already oozing out were transferred into dry bowl and being fertilized with milt gotten from the males. To eliminate egg stickiness of *Heterobranchus bidorsalis*, four treatments that include three different concentration of pineapple juice with 1%, 3% and 5% and the control without the juice were used. The mean of the hatchability rate were found to be 78%, 88%, 45% and 27% for the control. The utilization of natural product like pineapple can reduces the danger of fish kill when excess of applying and also increases the hatchability rate of the incubated egg.

Common carp (*Cyprinus carpio* L.) is one of the most cultured fish in world, with an annual production of nearly three million metric tons. Artificial propagation methods of common carp have been presented by Horvath, Tamas & Seagrave, (2002). Applying of artificial propagation method in hatcheries was not always successful because of poor facilities. One problem that confront in hatchery incubations of common carp is egg stickiness, which need to be reduced before eggs can be incubated successfully. Normally, eggs stickiness is reduced by using traditional methods like treating with a solution of sodium chloride and carbamide which 40 g of urea and 30 g of salt dissolved in 10 L clean water then followed by a tannin solution which 5 g tannin in 10 L of clean water. However, this method requires significant labor and experience and requires at least one hour.

Pineapple is commonly found in many tropical countries. Pineapple juice contains an abundance of proteolytic enzymes, principally bromalinases which can digest protein (Michael, 2001). Pineapple juice was prepared by squeezinf peeled fresh fruit. Solutions were made up at 1% (100 ml juice to 10 L clean water), 3% and 5%. A small volume of solution was first poured over the eggs and stirred continuously with a feather for about 1 min. Then while stirring continuously sufficient solution was added to just cover the eggs, for a further 1 min and the supernatant decanted. The procedure was repeated with fresh juice and the eggs washed with clean hatchery water and maintained in hatchery Weiss jars in running water. The traditional treatment methods which were salt, urea and tannin were carried out for 1 h.

Five hundred eggs which randomly selected from each treatment were incubated in separate Weiss jars and fertilization rates were calculated for all treatment after 12 h incubation period. All the treatments reduced the stickiness of the eggs but treatment with 1% pineapple juice showed the highest fertilization rate and hatching rate. Desticking of eggs using pineapple juice is quick and simple and required only 3 min. This technique not only increases fertilization and hatching rates, but has the advantage over conventional procedures by dramatically reducing egg handling process.

2.3 Used of Pineapple on others species

In the stem of Pineapple, it contain bromelain which the proteolytic enzyme that finding wide applications in pharmaceutical and food uses (Hebbar, Sumana & Raghavarao, 2008). According to Omojasola, Jilani & Ibiyemi, (2008), pineapple has

widely been used as a meat tenderizer. For example, bromelain had been used to tenderize beef (Ketnawa and Rawdkuen, 2011), mutton (Bille and Taapopi, 2008), chicken meat (Koide et al., 2010) and Pork (Leowsakulrat, Theerasamran, Chokpitinun and Pinitglang, 2011). Bromelain which is plant thiol affects the structure of actin and myosin filaments of myofibrillar proteins. Based on Gerelt, Ikeuchi and Suzuki, (2000), it is reported that proteolytic enzymes not only stimulate the fragmentation of myofibrils but it also can affect the structure of intramuscular connective tissue in the meat. In United States federal agencies, bromelain is recognized as safe (GRAS) to improve the tenderness of the meat (Sullivan and Calkins, 2010).

Amino acids are the protein which works as the major basic components of the body cells. Amino acids have two different types which are essential and non-essential amino acids. Non-essential or unnecessary amino acids are the amino acids that the body can make out of other chemicals found in the body. Meanwhile, necessary or on the other hand essential amino acids are the amino acids that can't be made and therefore, the only way to get them is through food. The failure to get enough essential amino acids can cause adverse health impacts. Bromelain been used in testing the Brahman meat. The tenderness of the bromelain-treated meat increase due to the proteolysis of muscle protein by bromelain. Additionally, the action of bromelain by denaturing protein and breaking down the collagen, muscle fibers and tissues that connect it also contributed to increase of beef tenderness.

Other finding which was made by Mensah & Twumasi (2016), on the single cell proteins (SCPs). Single cell proteins (SCPs) refer to dead, dry microbial cells or total proteins extracted from pure microbial cell culture produced using a number of different

microorganisms including bacterium, fungus, mould, yeast, and algae (Anapama & Ravindra, 2000). Single cell protein (SCP) is used as a protein supplement in animal feed and protein rich foods for humans. In this study, extracts of pineapple waste were used as substrate for SCP production using Saccharomyces cerevisiae sp. (Baker or brewing yeast). The study also focused on the effect of the pineapple substrate concentration on the yield of SCP. Pineapple waste were collected from food processing companies in Kumasi and processed into pineapple juice extracts. The residual soluble sugars in the extract were measured and the concentration adjusted (40–100% v/v) for various submerged yeast fermentations to produce the SCPs. Single cell proteins are utilized mainly as food supplement for human beings and animals. Several other applications of single cell proteins are available. It is used as a health food to control obesity, and provide instant energy to especially sports men. It is also used in therapeutic and natural medicines to reduce body weight, cholesterol, stress, lower blood sugar in diabetic patients, promote healthy eyes and skin, and increase lactation. It is further used in the technical field for paper and leather processing, and as foam stabilizer.

In recent years, the use of agricultural by-products for feeding ruminants has been the subject of several studies in Brazil and the rest of the world (Costa et al., 2007). Among the reasons for growth in the field of research is the high cost of food for animal production, which leads to low food costs that deliver satisfactory performance and reduce production costs. In addition, if it is not disposed of properly, the product can trigger environmental problems that potentially represent the loss of energy and high nutritional value feeds that can be used in the nutrition of various species such as sheep.

Pineapple (*Ananas comosus*) is a fruit that grows extensively in tropical and subtropical regions worldwide, its juice is one of the most important non-citrus juices and ranked fourth in the amount of fruit juices used in the United States (Corzo et al., 2012). Thus, agro-industrial by-products from pineapple made of bark, fruit crown, fruit, axial center and pulp have been praised as a promising alternative to ruminant nutrition especially in the form of silage as pineapple by-product silage (PS) has a greater energy value than maize silage and partly replaces the energy of concentration in ruminant food (Costa et al., 2007; Corzo et al. 2012). This study evaluated the effect of replacing elephant grass (EG) with moist pineapple by-products silage (PS) on the apparent digestibility, consumption of digestible nutrients and performance of 25 castrated male lams Santa Ines crossbreeds. There was no significant effect of PS replacement proportions on the intake of dry matter (DM), organic matter (OM), total carbohydrates (TC) and total digestible nutrients (TDN).

2.4 Pineapple Waste

Large quantities of waste material are generated annually from agricultural activities and processing of agricultural products. This includes wastes such as corn stover, sugarcane bagasse, rice and wheat straw. These wastes currently under investigation whether it's have potential feedstock for value-added products such as bioethanol production. However, a different category of waste from the food processing industry remains largely underutilised and should be investigated for further beneficiation. Examples of such wastes include citrus, apple and grape waste, often

referred to as pomace, as well as sugar beet pulp remaining after processing of sugar beet. These wastes contain high levels of polysaccharides which could potentially also be utilised for production of bioethanol.

Another way to dispose of food waste is through its use as animal feed, mostly for livestock feed. The waste may be dried and formed into pellets before being sold as animal feeds. However, most food waste has low protein content and therefore is not suitable for animal feed. High lignin content in some waste, such as olive and sugarcane waste, also limits the use of animal feed as it makes the waste hard to digest. Different wastes have different potentials for animal feed. For example, potatoes are very high in potassium and therefore can be used for livestock feed because they are not suitable for other animals. Where dry waste before use as animal feeds, additional costs may be incurred, which rarely recover from the cost of the sale. The use of apple pulp, especially, as animal feeds is limited due to rapid damage unless drying can occur immediately after processing.

Pineapple waste is a by-product that came from canning processing of pineapple that produce about 35% of fruit waste and lead to serious environmental pollution (Roha, Zainal, Noriham & Nadzirah, 2013). The wastes of pineapples are core, peel and the crown. Waste product that cannot be use being throw such as pineapples waste although these wastes contain high nutrient value like sucrose, glucose and fructose. The peel of pineapple contains high level of vitamin C or ascorbic acid. This vitamin C reacts as biological agent for hydrogen transport. According to Farid et al., (2015), vitamin C is known the first soluble antioxidant that increases the immune defense to the fish. Vitamin C also is required in formation of collagen and normal cartilage (Shiau

& Lin, 2006). For the crown of the pineapples, it acts as ethno-medicine related to wound healing, antimicrobial activity and toxicity of its enzymatic activities.

Fruit waste can cause serious environmental problems, as they gather in agro industrial areas without need any important and commercial value. An environmental problem can be caused by the disposal expensive waste due to high transportation costs and the limitations of disposal sites. Furthermore, the problem of disposal of the waste was raised by legal restrictions. The highest levels of BOD and COD in pineapple discharge add to the difficulty in the disposal. According to Alvarez and Liden, (2007), some researchers have studied on digestion pineapple waste together with other fruit and vegetable waste, dirt and abattoirs to reduce volatile solids by 50 to 65%. Recently, it is reported that composting of pineapple wastes using earthworm (Mainoo, Barrington, Whalen & Sampedro, 2009). About 99% of pineapple pulp wet and 87% of peel had loss in weight because of vermicomposting. The pH of the waste change from acidic condition to a neutral and to the alkaline during composting process. However, the effectiveness of the cost used is not being studied yet.

2.5 Bacteria in Tilapia

In aquaculture, it has an important role in the development and meets the increase demand for aquatic animal production (Haylor & Bland, 2001). The health of the fish can be related between fish, environment and pathogens. Tilapia is one of the disease resistances in aquaculture farming and have immune defense. Usually, they are not easily to get the disease but when it comes to infectious diseases in their rework

system, it's hard to be eradicated. Skin of the fish act as mirror since some pathogens attack the skin not only from the surface contamination of aquaria but also because of pathogenic microorganisms. According to Abd El-Latif & Adawy, (2004), some pathogens that isolated from the skin and the internal organs of tilapia were *Aeromonas sp., Pseudomonas sp*., and *Streptococcus sp. Streptococcus sp.* is the main bacterial species that give the major effect to the production of tilapia.

The first case of *Streptococcus sp*. infection involved in tilapia is determined by Wu, (1970). *Streptococcus iniae* is the cause of mortality in culture fish species and soft-tissue infections in human (Fuller et al., 2002). The sign of *Streptococcus sp.* infection on tilapia as darkening of the skin, unilateral or bilateral corneal turbidity that developed into abnormal swimming behavior whirling and simple activity. The infected fish will swarm close to the water surface, showed loss of balance and hyperventilated.

Tilapia (*Oreochromis niloticus*) is one of the most cultured freshwater fish in the world. Tilapia cultured has contributed to the world aquaculture since the ancient Egyptian days. Tilapia is well known of it resistant to unfavorable water quality than other freshwater fish but it is reported to be easily affected with *Streptococcus sp*. This disease which also known as 'pop eye' has been reported in many other species, contributing to an annual loss. For tilapia, the common signs that show it are infected by the disease are distended abdomen and erratic swimming. *Streptococcus sp.* common attack tilapia because it have become the perfect host for the bacteria. Other than that, water quality also plays an important role in tilapia farming which should always be maintained to prevent "stress" in fish that can lead to outbreaks of the disease (Amal & Zamri-Saad, 2011).

2.6 Antimicrobial activity

An antimicrobial is an agent that kills or prevents the growth of microorganisms. The microbial agent may be a chemical compound and physical agents. These agents interfere with the growth and reproduction of causative organisms like bacteria, fungi, parasites and virus. The increase in antibiotic resistant bacteria is high due to the extensive use of antibiotics in medicine, animal care and agriculture. This problem occurs because of the lack of new antibiotics to attack bacteria in various ways to kill the resistant genes. The research study been made to produce new antimicrobial agent which act as alternative therapies in difficult handling infections. Intestinal infections are causes by some bacteria like *Escherichia coli, Salmonella paratyphy B* and *Shigella sonnei*.

In worldwide, emergences of *E. coli*, *Staphylococcus aureus* have become a major therapeutic problem. All of the infectious disease can be kill using fruit juice that contain anti-infective agent. For pineapple juice, it contains enzyme bromelain which has several therapeutic properties including malignant cell growth, control of diarrhea and skin debridement. The bromelain is well absorbed with the therapeutic that effect the growth of the bacteria. Thus, for this research studies the pineapple waste (peel and crown) being used to know whether it can prevent and kill the *Streptococcus sp.* bacteria that occur in the Nile tilapia.

In human, periodontal diseases have been considered as one of the major health problems. Periodontal disease is a chronic condition which starts with gingival inflammation and progressively develops toward hard and soft tissue destruction and

tooth loss. There are various factors that inhibit the periodontal disease; the main factor is microbiological insult to the periodontal tissues. According to Praveen *et al*., (2014), microorganisms that cause the periodontal disease are *Aggregatibacter actinomycetemcomitans* (Aa), *Enterococcus fecalis*, *Porphyromonas gingivalis* (Pg) and *Streptococcus mutans*. Various treatments have been tried in the form of mechanical therapy and surgical therapy. Antimicrobial agents have been used as a monotherapy and as an adjunct with mechanical debridement. Different types of plant extract have been used as antibacterial agent. One of the agents is bromelain that produced from pineapple extract. The result inhibit that *S. mutans* showed sensitivity at the lowest concentration compared to *E fecalis*. Bromelain exerts an antibacterial effect against potent periodontal pathogens.

2.6.1 Agar Well Diffusion and Disc Diffusion Method

Agar well diffusion method is generally used to evaluate the microbial extracts or the antimicrobial activity of plants (Balouiri, Sadiki & Ibnsouda, 2015). Agar diffusion method is similarly to the disc-diffusion method where the surface of the agar plate is inoculated by spreading some volume of microbial inoculum over the entire of the agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip. And a volume of antimicrobial agent or extract solution at serial dilutions is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The inhibition zones will appear on the agar plate where the antimicrobial compound contact with the agar layer.

This method been used in the research study based on the antibacterial activity of pineapple waste.

The disk diffusion method allows for the simultaneous testing of a large number of antimicrobial in an easier way. For this method, the bacterial inoculum is adjusted to certain concentration and swab using sterile cotton-tipped to the entire surface of a Mueller-Hinton agar (MHA) plate (Jiang, 2011). The diluted antibiotic solution was placed on the surface of each MHA plate with 6mm in diameter hole. Then, the plates were incubated and the inhibition zone of the agar was measured using a ruler. The bigger the diameter of the inhibition resulting the more susceptible the microorganism to the antimicrobial.

2.7 Extraction

2.7.1 Aqueous extraction

According to Lee, Salleh & Mamat (2015), 10 grams of dried powder were extracted by using Soxhlet equipment with 150 mL deionized water. Ratio 1:15 of the dried powder and solvents were select for this Soxhlet extraction process. The extraction process was run for 24 hour and temperature was set at 150 ºC. Extract was dried in oven for 3 days in 40 ºC until the solvent is evaporated and only left dried extract. The dried extract was weighed and recorded.

Other finding was observed by Amarasinghe, Kumarasiri & Gangodavilage, (2009) on the extraction of rice bran oil using aqueous extraction. Rice bran oil (RBO) was extracted from raw rice bran using distilled water as the solvent. Rice bran and water were mixed in a container to the required bran to water ratio and the mixture was continuously stirred for a specific time at constant temperature and pH. The sample was then centrifuged to separate cream, supernatant and solid phase. The cream collected was stored overnight in the refrigerator. The cream was kept at room temperature before further processing. Then the content was boiled gently to break emulsion until oil appeared. Separated upper layer of oil and cream was washed with warm distilled water and then allowed to settle. Aqueous phase which were clear and oil free was discarded, and the oil was decanted. Then the oil was kept in hot air oven for 2 h at 105 ºC until constant weight was obtained. Aqueous extracted oil was named aqueous extraction rice bran oil (AERBO).

Based on the research from Parekh, Nair & Chanda, (2005) for the in-vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae), 10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged a 5000 g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours, was pooled together and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121 ºC temperature and at 15 lbs pressure and store a 4 ºC.

2.7.2 Ethanol extraction

Ten grams of dried powder were extracted by using Soxhlet equipment with 150 mL of ethanol (70%). The solvents used were of analytical grade. Ratio 1:15 of the dried powder and solvents were select for this Soxhlet extraction process. The extraction process was run for 24 hour and set at 80 ºC for ethanol extraction. Extract were dried in oven for 3 days in 40 ºC until the solvent is evaporated and only left dried extract. The dried extract was weighed and recorded (Lee, Salleh & Mamat, 2015).

According to Yu et al., (2003), grain samples were cleaned and milled on a Brabender Quadromat Jr. experimental mill for separating into bran and flour fraction. Two grams of bran were ground to 80 mesh and extracted for 15 h with 20 ml of absolute ethanol, 70% ethanol under nitrogen at ambient temperature, respectively. The grain also can be extracted with 125 ml absolute ethanol using a Soxhlet extractor. The ethanol extract obtained through the Soxhlet extraction was concentrated under a reduced pressure, and the final volume was adjusted to 25 ml. The antioxidant extracts were kept in the dark until further analysis.

In the research made by Parekh et al., (2005), for the solvent extraction, 10 g of air-dried powder was taken in 100 ml of organic solvent which were ethanol and methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4 ºC in airtight bottles.

CHAPTER 3

METHODOLOGY

3.1 Location of Study

For this researched study, it was conducted at Aquaculture Laboratory, Faculty of Agro Based Industry, University Malaysia Kelantan (UMK) Jeli Campus, Kelantan. The Pineapples (*Ananas comosus*) Waste (peel and crown) were obtained around the wet market in Jeli.

3.2 Materials

3.2.1 Raw Materials

There were many materials been used in these researched which were Pineapples (*Ananas comosus*) Waste (peel and crown), distilled water, hexane, methanol, ethanol and pathogen.

3.2.2 Equipment

The equipment that being used in the experiment were Soxhlet apparatus, Oven, Incubator, Rotary evaporator, Laminar air flow, Electric blender, Weighing scale, Tryptone soya broth (TSB), Trypticase soya agar (TSA), Petri dish, Beaker, Eppendorf tube, Glove, Glycerol and Schott bottle. All of the equipment was obtained from the FIAT's laboratory.

3.3 Methods of the Experiment

3.3.1 Preparation of *Ananas comosus* Waste (Peel and Crown)

For this experiment, pineapples (*Ananas comosus*) were bought from the wet market around Jeli, Kelantan. Pineapples were cleaned with tap water to get off the dirt. Skin of pineapples was peeled and the crown was separated from the flesh. In this experiment, *A. comosus* wastes which were peel and crown were used. The wastes were weighted using weighing scale and grinded using electric blender. The wastes then placed into oven at 60 ºC for one night. By using soxhlet apparatus, the wastes undergo the extraction process using different dilutions which were distilled water, hexane, ethanol and methanol. The liquid from the soxhlet process being placed into rotary evaporator until it become crude extract.

3.3.2 Anti-bacterial Activity

All the tools that were used in this procedure will undergo autoclave to avoid the contamination that cause by bacteria. The amount TSA and TSB which was 1 L each be prepared. Prepared ten tubes of serial dilution 10^{-1} until 10^{10} using micropipette 1000 µl and put into agar plate 1-10 respectively. By using micropipette of 1000 µl, the bacteria which were *Streptococcus sp.* been pour in agar plate of TSA and spread the bacteria at all space in agar plate gently with cotton bud. Then, the well diameter hole in agar was cut using gel puncher and added with 50µl crude extract of water, hexane, ethanol and methanol. The agar then incubated for 24 hours at 37 ºC. The inhibition zone and the diameter were measured and recorded.

3.3.3 Data collection and analysis

Data was subjected to the Statistical Package for Social Science (SPSS) version 2.4 and One-way analysis of variance (ANOVA) followed by the Durchan multiple comparison tests. The means at a significance level had been tested as ($P<0.05$).

CHAPTER 4

RESULT AND DISCUSSION

Antibacterial activity of pineapple (*Ananas comosus*) waste (peel and crown) was evaluated based on the diameter of clear inhibition zone surrounding the agar that was filled with pineapple extract with four different solvents which were ethanol, methanol, hexane and distilled water. The reading of inhibition zone was measured in milimiter (mm) unit by using a ruler.

Many of the laboratory procedures involve the use of dilutions. It is important to understand the concept of dilutions. These dilutions have to be considered as they make a quantitative difference in what is going on. A serial dilution is any dilution where the concentration decreases by the same quantity in each successive step. This dilution method was used in the study to determine the effect of samples concentration against the diameter of inhibition zone in the agar. Table 4.1 showed the inhibition zone (mm) of ethanol, methanol, hexane and distilled water against *Streptococcus sp.*

Table 4.1 inhibition zone (mm) of ethanol, methanol, hexane and distilled water against *Streptococcus sp.*

Treatment	Serial dilution (mm)
	10^{-2}
Methanol	1.00 ± 0.10^{b}
Ethanol	1.20 ± 0.10^{a}
Hexane	nd
Distilled water	nd
Control	nd

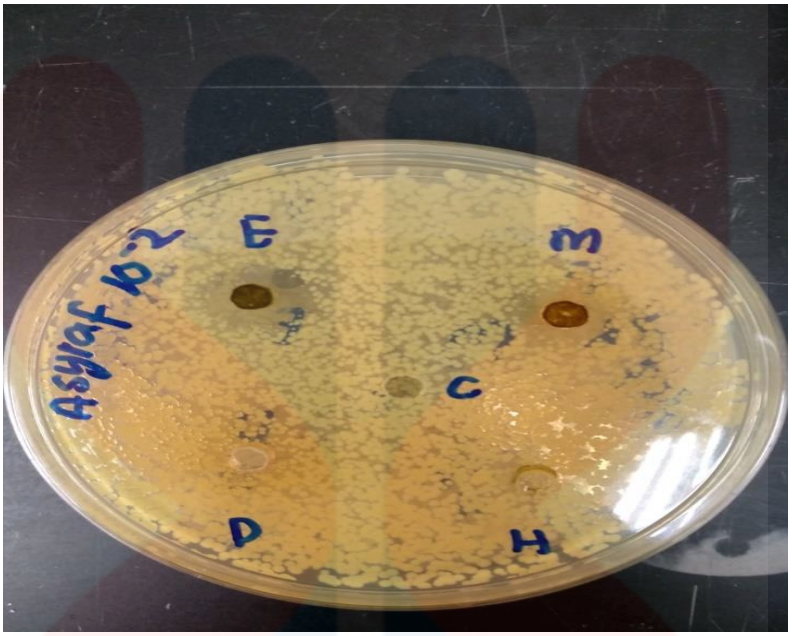
Values are the mean of triplicate reading (Mean±SD)

The result showed that the highest inhibition zone was observed for ethanol crude extract by using serial dilution 10^{-2} which showed the inhibition diameter 1.20 mm for the mean and 0.10 for the standard deviation. The diameter of inhibition zone of methanol crude extract for serial dilution 10^{-2} showed 1.00 mm for the mean and 0.10 for the standard deviation. On the other hand, the ethanol crude extract showed the largest diameter of inhibition zone in all replications compared to the methanol crude extract.

The lowest diameter of inhibition zone was observed in the serial dilution of 10^{-1} for each ethanol and methanol crude extract. The diameter of inhibition zone of ethanol crude extract for serial dilution 10^{-1} was much lower than 10^{-2} with the result showed the inhibition zone diameter 1.03 mm for the mean and 0.15 for the standard deviation. For the methanol crude extract by using serial dilution 10^{-1}, the value

observed was 0.90 mm for the mean and 0.10 for the standard deviation. The result also showed that there were no detected diameter for inhibition zone for hexane crude extract, distilled water crude extract and control. The hexane and distilled water showed no result because both are not strong to inhibit the bacteria growth. The control showed no inhibition zone because control agar was not filled with any extracts. According to Sugesh et al., (2013), antimicrobial activities depend on the nature of the solvent and compound extracted. For this study, distilled water showed no effect because it doesn't dissolve the intermediate polarity and non-polar components of dried extract. Apart from that, an alternative have been made by using different solvents like ethanol, methanol and hexane.

This finding is related with the previous study carried out by Sugesh et al., (2013) which conducted about antibacterial activities of marine gastropod which is *Hemifusus Pugilinus*. The body of *H. pugilinus* was extracted with three different solvents like ethanol, methanol and water. The result observed that the ethanol extracts was showed maximum antibacterial activities against *Staphylococcus aureus* with 3 mm diameter compared to the methanol extracts that is 2 mm. Water extract of *H. pugilinus* did not showed any antibacterial activity against the *S. aureus.* Figure 4.1 showed the inhibition zone (mm) of ethanol, methanol, hexane and distilled water against *Streptococcus sp.*



E= Ethanol, M= Methanol, D= Distilled water, H= Hexane, C= Control

Figure 4.1 inhibition zone (mm) of ethanol, methanol, hexane and distilled water against *Streptococcus sp.*

CHAPTER 5

CONCLUSION AND RECOMMENDATION

From the research study, it had been proven that the extracts of pineapple waste (peel and crown) could be used as the antibacterial agents for the tilapia to prevent the disease from the bacteria *Streptococcus sp.* in both ethanol and methanol. Ethanol was the best solvent to extract with the pineapple waste compared to methanol, hexane and distilled water. *Ananas comosus* waste showed the positive result against *Streptococcus sp.* because it contains an antioxidant agent which was vitamin C that could increase the immune defense to the tilapia. Thus, this plant had a huge potential to be the new source of aquaculture feed. This because it shows it's capability to inhibit the growth of bacteria *Streptococcus sp*.

Regardless of the result obtained, further research need to be conducted in order to classify the exact compound responsible for the bacterial inhibition. Future work is also needed to investigate if the compound has the ability to improve aquaculture species performance economically and whether it can pose health hazard to the human when they consumed the product. This can help the farmer to come with a better resolution in replacing the usage of antibiotic and vaccine with a more natural compound.

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APPENDIX

Descriptives

InhibitonZone

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Methanol	3	1.0000	.10000	.05774	.7516	1.2484	.90	1.10
Ethanol	3	1.2000	.10000	.05774	.9516	1.4484	1.10	1.30
Hexane	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Distilled Water	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Control	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	15	.4400	.56417	.14567	.1276	.7524	.00	1.30

F1: Descriptive of Inhibition Zone

ANOVA

InhibitonZone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.416	4	1.104	276.000	.000
Within Groups	.040	10	.004		
Total	4.456	14			

F2: One-way Anova

InhibitonZone

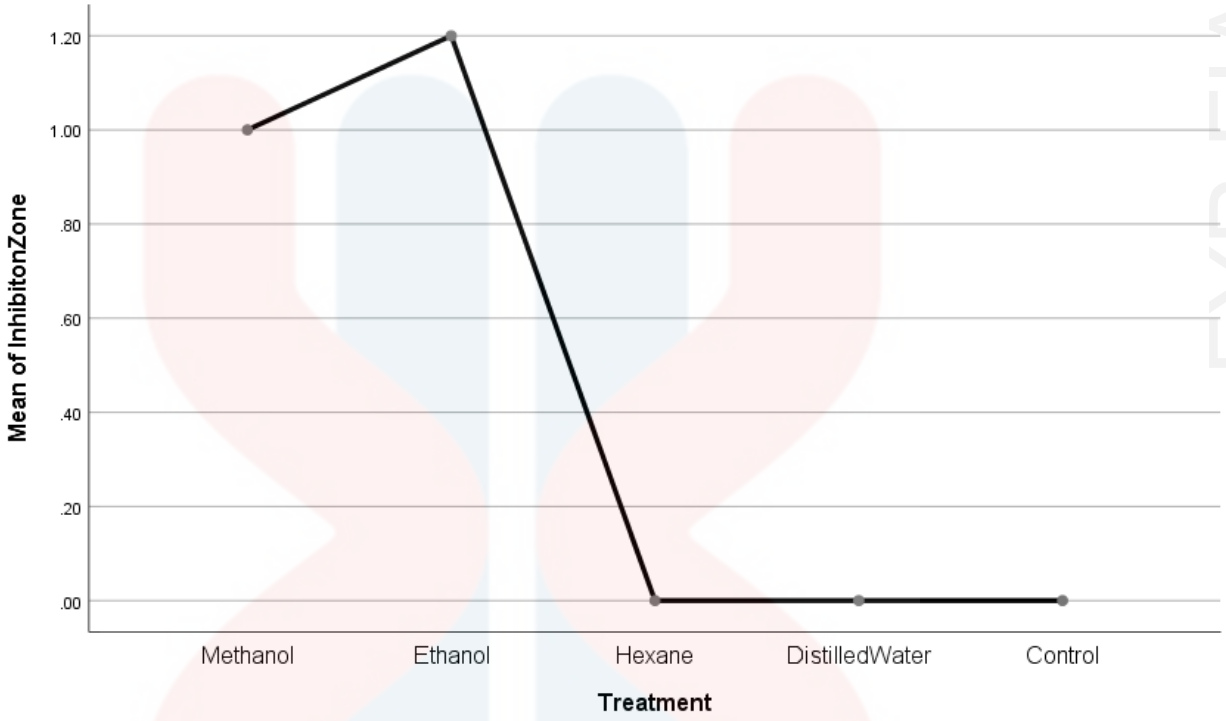
Duncan[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Hexane	3	.0000		
DistilledWater	3	.0000		
Control	3	.0000		
Methanol	3		1.0000	
Ethanol	3			1.2000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

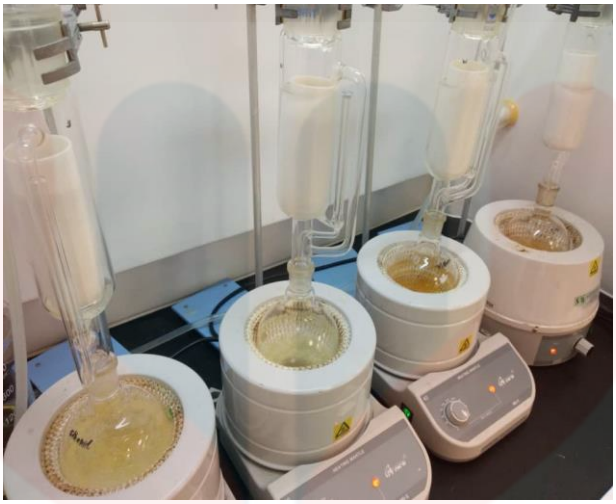
a. Uses Harmonic Mean Sample Size = 3.000.

F3: Post Hoc Test



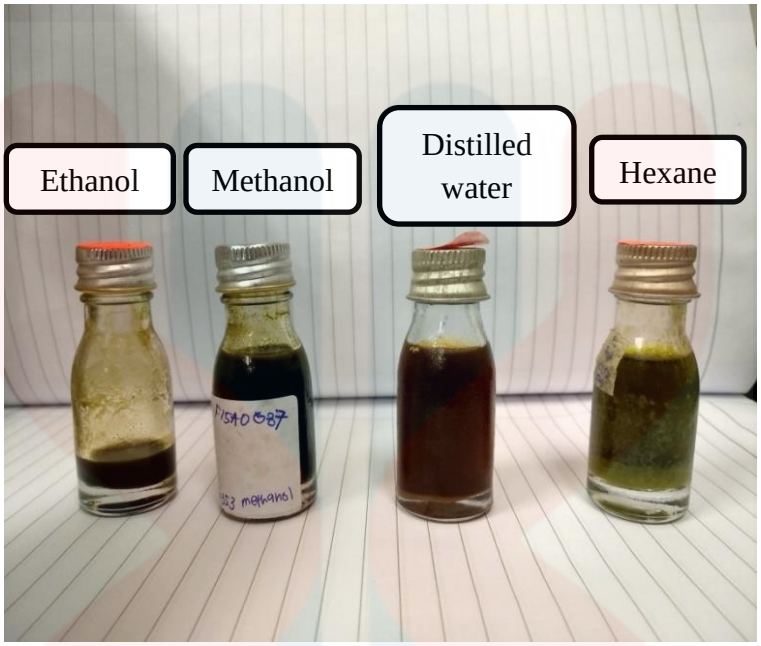
F4: Mean Plots of Inhibition Zone

F5: Dry sample of *Ananas comosus* waste (peel and crown)



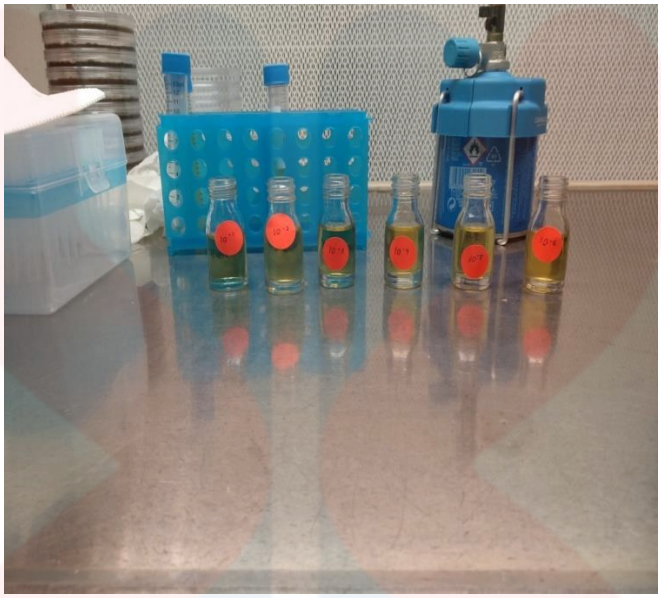
F6: Extraction process of sample using Soxhlet apparatus

F7: Removing solvent using Rotary evaporator to obtained crude extract,

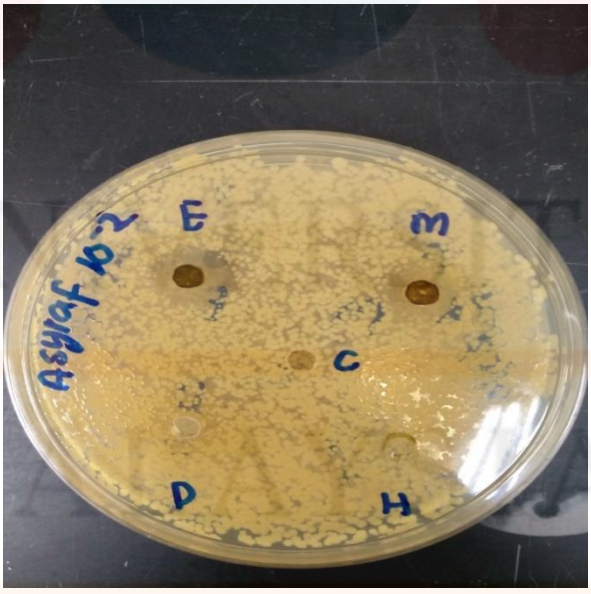


F8: Crude extract sample.

F9: Single colony of bacteria *Streptococcus sp.* in Trypticase soy broth (TSB).



F10: Single colony of bacteria *Streptococcus sp.* in Trypticase soy broth (TSB).



E= Ethanol, M= Methanol, D= Distilled water, H= Hexane, C= Control

F11: Inhibition zone of *Streptococcus sp.*