Antibacterial activity of Ulam Raja (*Cosmos caudatus)*

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A thesis submitted in fulfilment of the requirements for the Degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours

Faculty of Agro Based Industry

UNIVERSITI MALAYSIA KELANTAN

2019

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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Student

Name:

Date:

I certify that the report of this final year project entitled "Antibacterial Activity of Ulam Raja (*Cosmos caudatus*) by Nur Aliah Fatihah binti Rosdi, matric number F15A0127has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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ACKNOWLEDGEMENT

First of all, praise is due to almighty Allah S.W.T. with His compassion and for giving me the strength to allow me finishing this final year project as the requirement for the degree of Bachelor of Applied Science (Animal Husbandry).

I would like to acknowledge the authority of Faculty of Agro Based Industry, University Malaysia Kelantan for giving me an opportunity to do my research for the final year project. Also, I would like to express my gratitude to a number of people who had been supporting me in the achievement of this final year project.

Special appreciated to my supervisor, Dr. Suniza Anis binti Mohammad Sukri for her patience, knowledge, supervision and constant support. Her invaluable help of constructive comment, suggestion and ideal thought the experiment and thesis works have contributed to the success of this research.

My deepest gratitude goes to my beloved parents, Mr Rosdi bin Mat Nawi and Mrs Esmawati binti Mamat and my siblings foe their prayers, encourage and endless love. Also thanks to all my friends especially my cource mate friend who helping me in every ways

Last but not least, a special thanks to the laboratory assistance, Encik Shamsul, Encik Khalid and other lab assistance that help and assist me a lot in the laboratory. Your kindness means a lot to me.

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ABSTRACT

Cosmos caudatus or people called "Ulam Raja" is one of good herb plant with the present of antibacterial activity. The aim of study is to determine the potential of *C. caudatus* with different solvent extraction such as ethanol, methanol, hexane and distilled water against *Streptococcus sp*. The antibacterial activity were determine by using agar well diffusion method. The result showed, the largest inhibition zone was 1.11±0.02 mm in *C. caudatus* extract with ethanol and followed by methanol 0.93±0.12 mm and hexane 0.5±0.00 mm. There are no inhibition zone *in C. caudatus* plant species in distilled water and control treatment. The result showed that, there are present of antibacterial properties in *C. caudatus* extraction against *Streptococcus sp.* Further study need to be continue to know other component in *C. caudatus* that can be used in other research. Besides that, *C. caudatus* also can be used in other pathogen to analysis the reaction towards.

Keywords : *Cosmos caudatus*, Antibacterial activity, Bacteria, Extraction

Aktiviti Antibacteria Ulam Raja (*Cosmos caudats*)

ABSTRAK

Cosmos caudatus atau orang panggil "Ulam Raja" adalah salah satu tumbuhan herba yang baik dengan kehadiran aktiviti antibakteria. Tujuan kajian adalah menentukan potensi *C. caudatus* dengan pengekstrakan pelarut yang berbeza seperti etanol, metanol, heksana dan air suling terhadap *Streptococcus sp.* Aktiviti antibakteria telah ditentukan dengan menggunakan kaedah penyebaran agar. Keputusan menunjukkan, zon perencatan terbesar adalah 1.11 ± 0.02 mm dalam *C. caudatus* ekstrak dengan etanol dan diikuti oleh metanol 0.93 ± 0.12 mm dan heksana 0.5 ± 0.00 mm. Tiada zon perencatan dalam *C. caudatus* spesies tumbuhan dalam rawatan air sulingan dan kawalan. Keputusan menunjukkan bahawa terdapat ciri-ciri antibakteria dalam pengambilan C. caudatus terhadap *Streptococcus sp*. Kajian selanjut perlu diterus dalam mengetahui komponen lain dalam aktiviti antibakteria di *C. caudatus* yang boleh digunakan dalam penyelidikan lain. Selain itu, *C. caudatus* juga boleh digunakan dalam patogen lain untuk menganalisis reaksi terhadap.

Kata kunci: *Cosmos caudatus,* Aktiviti antibakteria, Bakteria, Pengekstrakan

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LIST OF ABBRECIATION AND SYMBOLS

sp.	Species
Mg	Magnesium
Fe	Iron
Ca	Calcium
ANOVA	Analysis of variance
TSA	Tryptose Soy Agar
TSB	Tryptic Soy brooth
SD	Standard deviation

µl	microliter
ºC	degree celsius
%	percentage
cm	centimetre
g	gram
m	meter
ml	milliliter
mm	millimeter
mg	milligram
µg	microgram
h	hour

CHAPTER 1

INTRODUCTION

1.0 Research Background

Cosmos caudatus or 'King's Salad'. It has antibacterial and anti-fungul effect, strong antioxidant that can reduce inflammation. It is also used as potential source of antioxidant for food and medicinal application. *Cosmos caudatus* also rich in source of bioactive which are have phenolics, flavonoids, carbohydrate, proteins, minerals and vitamin. Basically, *C. caudatus* has been used to boost blood circulation, to strength the bones, to reduce body heat, as an anti-aging agent ad to treat infection disease (Shi, Yusof, Joseph & Amin, 2015)

In plants biological, have their own source of medical agent. *Cosmos caudatus* is of medical agent that have potential to treat disease infectious with not have side effect to fish culture (Syahidah, Saad, Daud & Abdelhadi, 2014).

n anti-microbial there is agent which have natural antioxidant give health benefit to all (Anderson, Reggiani, Josefia & Maria, 2017). Mostly, plants are potential source of antioxidant. It is to prevent antibiotic resistance by using new compound with present of antimicrobial activity (Emad, 2011).

Plants have played one of significant main role in the inhibition of pathogens. And also it will improve of the quality and yield of food (Sheikh, Rahid, Meghavashi & Irsha, 2012). The uses of plant extraction with knowing antimicrobial properties can be great significant in human and animal disease treatment (Sheikh, Rahid, Meghavashi & Irsha, 2012). According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs (Hamidan, Syarul, Noraini & Noriha, 2014)

In general, *C. caudatus* contain are protein (2.9g), carbohydrate 0.6g), fat (0.4g), water (93.1), energy (18kcal), vitamin C (64.4mg), β-coretene (3568µg), vitamin B1 (0.13mg), vitamin B2 (0.24mg/100). In terms of mineral composition, it has potassium (426mg), calcium (270mg), phosphorus (37mg), magnesium (50mg), iron (4.6mg), zinc (0.9g), sodium (4.0mg) and copper (0.2g). Cheng (2015) and Zeinhom (2004) found that, fish fed diet containing 15% algae increased significant by the digestibility coefficient of dry matter (92.5%), crude protein (87.63%), ether extract (88.45%) and energy (81.41%) (Cheng, Mohd Yusof, Josep & Amin, 2015).

1.2 Problem statement

Nowadays, there are so many plant source in Malaysia that not discover their potential. They have really good in nutritional value and good quality. Besides that, there also have an issues about the usage of synthetic antibacterial agents. There are some of researcher say that *Cosmos caudatus* can be used in aquaculture as it also antibacterial. Since the aquaculture system was sensitive management, there was highly interest to replace, prevent and treat any disease. This study also, can reduce the cost of feed ingredients and maintenance the nutrition contents of feed. Furthermore, it also have potential to tolerance with disease resistance to the pathogen.

Besides that, common extraction method has their own disadvantage and low yields, formation by-product and formation of pure extract. Thus, this study was conducted in order to introduce various extraction modern techniques. One of them is soxhlet extraction method as a alternative applicable method to extract *C. caudatus.*

1.3 Hypothesis

H_0 = *Cosmos caudatus* did not have antibacterial properties against *Streptococcus sp.*

H_1 = *Cosmos caudatus* have antibacterial properties against *Streptococcus sp.*

1.4 Objective

1. To determine the antibacterial activity of *Cosmos caudatus* extract with different types of solvent against *Streptococcus sp.* using well diffusion method.

1.5 Scope of study

This research project focused on the testing antibacterial activities in *Cosmos caudatus* extraction, using agar well diffusion method against *Streptococcus sp*. The sample of *C. caudatus* collected around Jeli were washed with tap water and rinsed with distilled water to remove the impurities. The *C. caudatus* leaves were dried and was processed into powder to increase efficiency of extraction. Then, the samples were extract using soxhlet method with difference solvent such as methanol, ethanol, hexane, distilled water respectively. After that, rotary process evaporation was carried out to get the crude extract. Finally, the crude extract were proceed to antibacterial activity. Thus, the antibacterial activity in C. caudatus crude extract was observed.

1.6 Significance of study

This study may provide more information about *Cosmos caudatus* and provided new bioactive compound in aquaculture system. Furthermore, aquaculture system especially Tilapia is very 'disease- resistance'. Once the pathogen attach the fish culture, it is difficult to recover the system. In this study, it will help farmer to preventing or used it in the fish culture that use raw materials if have any problems.

1. 7 Limitation of study

The studies of *Cosmos caudatus* leaves is not exposed in aquaculture studies .Thus, this research must to find out that herbs plant suitable or not for aquaculture culture.

CHAPTER 2

LITERATURE REVIEW

2.1 *Cosmos caudatus*

Herbs are one of the most famous and useful resources in Malaysian socio-culture due to their nutritional value and also their medicinal benefits respectively. *Cosmos caudatus* belongs to family of Asteraceae that can grows up about 1-8 feet tall, contain shoot and some have different types of colour such as purple,pink and white grey in other country (Patil & Nikm, 2013).

Furthermore, it also contained high amount of antioxidant (Dian, Noriham, Nooraain & Azizah, 2014) In this content, *C. caudatus* knows as Ulam raja is one of the important herbs in Malaysia. It is also delight a good food serve for tourists mostly in hotels as well as their fresh leave and good aroma (Hafpah, Othman, Abubakar & Qamar, 2010)

and one side ingredients in ‘kerabu’ meal which is famous Malays traditional food especially in Kelantan (Perumal, 2014; Norhanom et al., 1990).

According to Rasaga et al. (1997) and Ikhlas et al. (2011) said that, antimutagen, antifungal and antioxidant activities is the most health benefit in plant. Other study by, Mustapha, Jai, Hamidon, Sharif & Yusof, (2017) antibacterial agent such as flavonoid, tannin and saponin which are active compound in the sample of *C. caudatus*. The extract concentration of *C. caudatus* can be used antibacterial agent influence by antibacterial agent potential (Theresia & Lauda, 2017). Thus, study by Rameli, Kader, Alia & Najiha (2018), based on their investigation, green aquaculture had leading because of their function of natural product as alternative antibiotic. Besides that, the bacteria, *C. caudatus* extract also give effective on controlling fungus for example, Phytophthira palmivora that in plant (Salehan, Meon & Ismail, 2013).

The effect from the association of antibiotic with plant extract that against resistance bacterial leads as a new choice for the treatment of infection disease as the present of respective antibiotic (Gislene, Nascimento, Juliana, Paulo & Guiliana, 2000). The herbs plant have been investigate in others treatment from many country because of their contribution to health care (Selvamohan, Rmadas, Shibila, 2012).

Cosmos Caudatus also can reduce body temperature, improving blood circulation, as ageing agent, have good strengthening bone marrow because of contain high calcium content, to treat infection associated with pathogenic microorganism and also to promote fresh breath are that have in *C. caudatus* (Farah, Nooraain, Noiham, Azizah and Nurul, 2013)

In water system, total phenolic content which are (1274 ± 98 GAE mg/100 g fresh weight) in *C. caudatus* is higher than others herbs such as *Curcuma domestica, Kaempferia galanga, Piper betle, Piper sarmentosum, Polygonum minus, Centella asiatica, Hydrocotyle bonariensis, and Barringtonia racemosa* (Shi, Yusof & Amin 2015). In aqueous extract, *C. caudatus* have higher contains of phenolic content (Yusuf, Syahida, Mansor & Maziah, 2010). Then, one of the source of natural antioxidant that active group of antioxidant agents was phenolic compound (Mediani, Fraidah, Alfi & Chin, 2013). Furthermore, the phenolic compound is important to maintain of fruits, vegetable and herbs because of having more antioxidant than vitamin C,E and β-carotene (Dong et al., 2016).

The biologically active compound were present in plant make the scientific more interesting working to evaluate plat possessing antibacterial activity for various disease coming (Clark, 1993).

Table 1.1 Biological active compound in *C.caudatus*

Compound	Total (mg/100g)
Ascorbic acid	108.83±0.50
Quercetin	51.28±4.06
Kaemferol	0.90±0.05
Chlorogenic acid	4.54±0.18
Caffeic acid	3.64±0.14
Ferulic acid	3.14±0.28
Anthocyanin	0.78±0.05
B-carotene	1.35±0.03

(Shin et al., 2015)

Nowadays, many countries realize and use the traditional medical plant as a primary source because of their healthcare and can give many benefits to people and also to the animals (Calixto, 2005; Maria, Nathalie, Pierre & Denis, 2017). The studies have been proved about Ethnobotanical, it very useful to check the present of bioactive plant. There are many researcher had study on the biological activities and chemical composition about ethnomedical plant (Banskota et al., 2003; Ayyanar et al., 2011; Maria et al., 2017). The interest about the medical plant, there are many encouragement for human and veterinary health as their side effect and higher in cost of prescription drugs (Hoareau and Da-Silva, 1999; Maria et al., 2017). Therefore the increasing prevalence of multi-drug resistance strain of microorganism that raise an urgent need to

search a new source antimicrobial agent because in recent year, the pathogenic bacteria and fungi has been reported (Nirmala & Pandian, 2015).

In addition, the extraction of *C. caudatus* also had been use in chicken meat to reduce microorganism contamination in food material (Yusof, Noor & Rukayadi, 2015). Even though there are many process or step have been done to overcome the preservation of food, still day by days have cannot reduce this problems (Yudof, Noor & Rukayadi, 2015). There are some compound that found out in the sample that react as antimicrobial agent such as terpenoids, alkaloids, flavonoids, tannins and phenols which have their own function and suitable as "second preservative" because of content natural antimicrobial compound (Naidu, Ababutain, Yusof, Noor & Rukayadi, 2015).

2.1.1 Important herbs plant in aquaculture system

The report from FOA, (2014) & Maria et al. (2017) showed that, aquaculture is the fastest growing in animal food-sector with increase 6.2% per year in 2000 to 2012. Though the excellent aquaculture system, there are some factors that will cause the spread of pathogens and spread disease outbreak and it also can mortality. The factors which related are culture intensification, higher turbidity in culture system and water pollution, higher density. The growth rate between 5 and 15% have been estimated by the world market for herbal plant and raw materials (Maggon, 2005; Thavasimithu, 2009).

According to Balasubramanian et al. (2007) and Cox et al. (2010), most scientific studied using herbs plant in aquaculture because of contain of biological activity rather that natural product determinantion. Herbs plant also can be administered to fish and shellfish by injection (intramuscular and interperitoneal), oral administration and through immersion (Ji, Lu, Kang, Wang & Chen, 2012). Furthermore, herbs plant also can help to better understand their mode of action because present of antimicrobial, antifungi, radioprotective cardioprotective, safe guarding against possible deficiencies and anti-inflammatory activities (Miriam, Pierre, Nathalie & Denis, 2017).

Mostly, all medical herbs have their own active ingredient which are responsible for various biological activities for viral disease in fish culture and give the positive impact in fish muscle. (Sivasankar, Anix & Kanaga, 2015). Some of herbal plant are found that have potential in anti-viral as well as antibacterial and anti-fungi properties and have importance properties in controlling the disease due have an antioxidant and antibacterial activity (Kurva & Gadadhar, 2013). Besides that, traditional herbal plants have potential immunostimulation because of poor immune response also can reduce ability of fish to fight pathogen.

Usually, it contains secondary metabolites which have therapeutic and prophylactic effects in fish disease in natural products like plant or herbs extract (Talpur & Ikwanuddin, 2013). Herbs plant also higher in hormone, antibiotic, vitamins and other can been tested as growth promoter, antibacterial activity and other purpose in mariculture (Thavasimithu, 2009). There are some local herbs such as *Acalypha wilkesiana, Leucaena leucocephala, Acalypha wilkesiana* and *Peperomia pellucida* have ability to inhibit the pathogenic bacteria in aquaculture (Direkbusarakom, 2004; Muiruzzaman & Chowdhury, 2004).

According to Maria et al. (2017) & Caruso et al. (2013) , in West Java (Indonesia), 46% of fish farmer can survived by using *Cosmos caudatus* from their own farmer and they use traditional way in human pharmacopoeia. It proved the medical plant so good in many ways such as in aquaculture in fresh plat, can increase water quality, reduce stress fish culture, have higher immunity resistance to attack the pathogen and against disease.

Is primary gives the benefit by using plant-derived herbs that are relatively safer that synthetic alternative and also more affordable treatment (Ahmed & Beg, 2001). It also will low in cost in treating drug-resistance infection due to high treatment of new alternative drug and also have long duration of treatment period compared to susceptible bacterial infection (Hassan, Najmeh & Leila, 2018).

Herbs plant that have specific compound showed very effective alternative of antibiotic which can increase consumption, induce maturation and have antibacterial capability in the culture of shrimps and other fin fish without any environment and hazard problems (Thavasimuthu, 2009). Herbs plant showed highest potential for used in aquaculture as substitute in the treatment of disease outbreak.

Plant have been reported to produce various effect such as present of antistress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and most have antipathogen properties showed in fish and shrimp due to have active compound (Chakraborty & Hancz, 2011; Citarasu, 2010).

Study by Miriam, Pierre, Nathalie & Denis, (2017) there are some plant species that have been used because highest potential for use in aquaculture such as garlic(*Allium sativum*), pomegranate (*Punica granatum*), bermuda grass (*Cynodon dactylon*), Indian ginseng (Whitania somnifera) and ginger (*Zingiber officinale*),

Most studies on the use of medical plant in aquaculture used were 37% of plant leaves, 22% used the whole plant as powder, plant essential oil or crude, 18% on root followed by 8% of seed, 6% of bark and fruits and 4% for flowers (Miriam et al., 2017)

2.1.2 Taxonomy

The taxonomy *Cosmos caudatus* were describe and justify according to Said, Manraj, Zuraini, Zainul & Nazrul (2017).

Phylum: Spermatophyta

Domain: Eukaryot

Kingdom: Plantae

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Asterales

Family: Asteraceae

Genus: Cosmos

Species: *Cosmos caudatus*

***Figure 2.1 Cosmos caudatus* leaves**

2.3 Used of *Cosmos caudatus* on others

According to Dian et al. (2014), consume of the young leave of *C. caudatus* will help to improve human health because *C. caudatus* is good in antioxidant. Furthermore, *C. caudatus* also the best test ingredient on human type-2 diabetes patient. It will low the blood insulin that allowing the cell to utilize circulating blood and inhibiting alpha-glucosidse. The enzyme will break down from larger carbohydrate into smaller glucose molecule then, the body will release glucose slowly into the bloodstream with more amounts. Through this, it will not have too much free glucose in the blood circulation. Study by Seyedreihani, Thuan, Abbas and Azhar (2017),

Ulam raja was used in mice as supplement to extraheoatic organ protection from xenobiotic and oxidative injury and it showed useful chemoprotective measure (Azman et al., 2014). *Cosmos caudatus* is one the medical plats have therapeutic value have been use for a long time ago as medical as alternative for human disease. (Demetrio,

Jeannie, Juliana, Esperanza & Windell, 2015). Hence, this bioactive compound may higher in life processes within of disease-cause one by itself or have combination with therapeutic agents (Demetrio et al., 2015).

Study by Tan & Charles (2011), *Cosmos caudatus* and *Persicaria hydopiper* may play an importance roles in preventing the formation of free radical and also can reduce the damage that cause the radical compared to *Centella asiatica* and *Artemisis argyi.*

Herbs plant also have been used in other tested as for biological, antimicrobial and hypoglycemic activity and also have been reported that the plant rich in volatile oils for mainly used in the treatment of diarrhea and ear infection in human being (Selvamohan, Ramadas & Shibila, 2012). The herbs plant investigated are know to have healing powers and also can be used for the treatment of various diseases among people (Omer, 2006).

2.4 Antioxidant

The natural antioxidant from plant have been expose increasing due to high potential of antioxidant capacity (Afeffa, 2013). There are strong antioxidant activities present in Ulam raja compared the others herbs (Shui, Leong and Wong, 2005). According to Seyedreihani et al. (2017), many types of antioxidant compound through beneficial of health in Ulam Raja properties. The first five ranking of Ulam raja in Malaysia in terms of antioxidant activity are “selom” (*Oenanthe javanica*), curry leaf

(*Murraya koenigii*), "pegaga" (*Centella asiatica*), Ulam raja, and the seeds of "petai" (*Parkia speciosa*). The present of superoxide dismutase and 2,2-diphenyl-1-pircryhdrazyl (DPPH) scavenging activity also showed in lemon grass, garlic, turmeric and 'Hempedu bumi' (Seyedreihaini et. al. 2017).

Study by Shui et al. (2005), the number of proanthocyanidins that exists some chemical in the sample was the major antioxidant in *C. caudatus.* Besides that, they can be considered to be active metabolites that involve in antioxidant activity because of the total amount of phenolic compound which directly defence against oxidative stress (Mediani, Abas, Ping, Khatib & Najis, 2012).

Moreover, in *C. caudatus*, it noticed that in this herb there are more than 20 antioxidant present which can be reduce oxidative stress among the aquaculture system and had higher potential in inhibiting pathogens (Shui et al. 2015). Based on experiment by Abas et al., it proved that the higher antioxidant and have free radical scavenging agents have in young leave *C. caudatus.*

The potential antioxidant is processing to help them removed the electrophiles and active oxygen species. They have to limit auto-oxidant by slow down nitrogation ad chelate metal ion (Median et al., 2013). Then, the process also can adjust some enzyme action by increasing the ability. In another study by Wong et al., (2006), 25 tropical plants are observed to see the anti-oxidant activities of aqueous extraction by using DPPH scavenging and ferric ion to decrease the anti-oxidant potential (FRAP) assays

In study on Wong, Leong & Koh (2006), C. caudatus is one of the famous use in Southeast Asian region to evaluate the antioxidant activities of aqueous extract of tropical plant followed by *Centellab asiatica, Piper betel, Sauropus androgyus, Coriandrumsativum, Eugenia polyantha, Polygonum hydropiper* and *O.basilicum.*

2.5 Antimicrobial

Antimicrobial is one of microbe that have genetic to against disease or can provide resistance to drug uses as a therapeutic agent. Besides that, antimicrobial activity also using for the treatment of disease inhibitory chemical employed that to kill the microorganism or it to prevent the growth. One of the way can uses to prevent antibiotic resistance is using new compound which synthetic antimicrobial agent absent (Hafipah et al., 2010; Shah 2005). Plant products yield extract with antimicrobial activities and it also give effective management of plant disease and microbial contaminantion (Sheikh et al., 2012). The growth of response of various microorganism can be dedicated by observing an antimicrobial activity (Akila, Prince, Bharathidasan & Krishnapriya, 2016). The increase in antibiotic resistance bacteria is high due to extensive uses of antibiotic in herbs plant for aquaculture. Thus, this is because lack of researcher for finding new antibiotic to attack pathogens in fish culture.

In herbal especially in *Cosmos caudatus*, there are many of antimicrobial agent. Herbal medicine is still the mainstay of about 75 - 80% world's population, mainly in developing countries for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects (Hafipah et.al., 2011; Tomoko et al., 2002). Medicine plant have rich source of novel therapeutic agents. It also have a biological active for treatment disease infections. The function antimicrobial in *C. caudatus* is against more pathogens to treat infectious disease. The experimental have been done to determine the antibacterial activities in the leaves *C. caudatus* by using agar disc diffusion method (Hafipah et al., 2010).

Antimicrobial in medicinal plant's extract and their derived silver nanoparticle also can against on honey nee pathogen. The medical plant and nano-silver have to use to against pathogenic microbes and used for healthcare because of they have low price and have rich source of antimicrobial (Shahid et. al., 2018). Antibiotics are sometimes associated with adverse effects on the host including hypersensitivity and immune-suppression thus necessitating the need for the development of novel antimicrobials especially from plant sources (Sieradski et al.,1999; Hafipah, Othman, Abubakar & Qamar, 2010)

The actual antimicrobial ingredient can be benefit to tolerance level in the human body as well as toxic effect on human and animal tissues (Pesaramelli, Vellanki, Keerthi & Chaitanya, 2012).

2.6 Antibacterial of herb plant

Plant are rich source of antibacterial agents ad botanical wealth because present of wide array of biotic molecule which are evolves the chemical defense against infection. In fact, herbs plant also produce a diverse range of bioactive molecule that make them rich in source of difference types of medicines produce (Chirag, Shailesh, Hitesh, Patel & Aviash, 2011).

The infection disease in public health is a useful treat because of antibacterial emergence of antimicrobial resistance and toxicity issues that use antibacterial agents (Malini, Abirami, Hemalatha & Annadurai, 2013). In Pakistan, present of their study,

antibacterial activity of three naturally plant and these plant extract can against pathogens especially in human bacterial (Bibi, Nisa, Chaudhary & Zia, 2011). The antibiotic is available on development of bacteria resistance in researcher of new antibacterial agents (Pamar & Rawat 2012)

It like co- friendly when use alternative method which is medicinal plant when manage in pathogenic microbes like bacteria, fungi and viruses and can be antibiotic to treat both human otherwise can also to promote growth in food animal like in aquaculture system and poultry (Uzma et al., 2013). This plant extract may give a new source of antibacterial, antifungal and antimicrobial agents (Jahan, Mehjabeen, Zia, Alam & Qureshi 2010). Study by Paul, Dubey, Maheshwari & Kang (2018), that reported remarkable antibacterial activity of *Trachyspermum ammin* against food borne bacterial was successful.

In central India, there are seventeen herbs plant were evaluate antibacterial activity that against pathogens that cause complicated urinary tract infection (Anjana, Chandraker & Padmini, 2009). Plant have been uses in medicine as present of antibacterial activity since ancient times that could provide solution for drug resistance species (Krishnananda & Ramakrishna, 2016).

The antibacterial therapy also can be threatened the spreading of multidrug-resistance that will cause increase in mortality, longer length of stay in hospital and higher cost of treatment and care (Bishnu et al., 2014).

2.7 Extraction of *Cosmos caudatus*

Extraction is the most important step in analysis because it necessary to extract the chemical compound for the separation and analysis characteristics in the sample (Sasidharan, Chen, Sravanan, Sundram & Yoga, 2011). According to the World Health Organization (WHO) researchers, it is first step to determine the biological active compound from the plant resource. The basic things before extraction such as washing drying the sample, grinding to get small shape to get easy kinetic of analytic extraction and also to get easy solvent absorb. Proper handling most important to make sure the potential active contain are not lost, destroy if the plant using extraction by traditional way (Fabricant & Farnworth, 2001).

There are available to extract the bioactive compound by using different type of solvent during extraction with the sample. (Sasidharan et al., 2011). Hence, the solvent were use of hydrophilic compound that have polar solvent such as methanol, ethanol and hexane (Sasidharan et al., 2011). Ethanol has a characteristic that the compound could be extracted efficiently. Soxhlet extraction (SE) was modern technique which have been utilize more than a century for isolation polyphenols (Kashif, Hafiz & Joong , 2017). Based on Cravotto, Binello & Orio (2011) there are some disadvantage using a domestic of conventional solvent extraction (CSE) which are uneconomical due to waste of time, energy and pollution solvent.

2.8 Agar Well Diffusion Method and Disc Diffusion Method

Basically, screening method is use for to identification of antibacterial from the natural source and to determine antibacterial in natural sample (Cleidson, Simone, Elza & Artur 2007). Generally, there are some method under screening process such as well diffusion method, disc diffusion, agar dilution and broth microdilution that can analyse the antimicrobial compound. But, the well-variant of the diffusion method is more sensitive among the others method. Furthermore, the diffusion method just alternative technique to check the present or absent of substance contain antimicrobial activity and also to determine the minimal inhibitory concentration in the sample (Cleidson et al., 2007). So, the latest technique is most suitable condition to determine the microbial growth.

Agar well diffusion method also one of common used to evaluate the antibacterial activity of plant extract because the method can provide the good result of antibacterial agents affect and have a better understanding of the impact to test the microorganism. (Mounyr, Moulay & Saad, 2015)

Disc diffusion is suitable and flexible method to analysis of a great number of antimoicrobial (Jiang, 2011). In this technique, some material had been used such as a pair of sterile forceps using the paper disks (BD Diagnostic System) was dilute together with diluted antibiotic solution and put on the surface each Tryptose Soy Agar (TSA). (Hmed, Sofy, EL-Monem, Sharaf & Khalid, 2017). Then, the plate were incubate and measured the inhibition zone by a ruler (Jiang, 2011). The result showed, if more susceptible in microorganism against to antimicrobial, the inhibition zone will become

bigger. If there is no inhibition zone, there is no susceptible of microorganism aganists antimicrobial. (Carson, Hammer & Riley, 2006)

2.9 Disease of tilapia

According to FOA (2014), tilapia is one of cultivated outside of their original habitat mainly in the Philipina, Indonesia, Malaysia, China, Chile, Brazil and Colombia that their production more than salmonid and crap production. The tilapia production were success due to their rapid growth, easy to propagate and tolerance in any environment condition (Huicab, Landeros, Cantaneda, Lango & Lopez (2016).

Tilapia is one of aquaculture disease-resistance. They difficult to get disease but once it have disease infectious in recirculation system, it difficult to eradicate. It is means in the presence of pathogen in system, tilapia are slow-to break with disease. The researcher shows that many bacteria that attack the tilapia culture. Common tilapia pathogens include *Streptococcus sp.*, *Gyrodactylus niloticus* and others (Klesius et al., 2008; Amal, Zamri, 2011).There are main bacteria species that effect the production of tilapia which is *Streptococcus sp. Streptococcus iniae* and *Streptococcus agalactiae* are the major bacterial species that affect the production of tilapias in the world (Evan et al., 2006). The disease have short period, if not more mortalities of fish and fish will dying each day. Some clinical sigh of streptococcus sp. have abnormal behavior for example, have different on their spinning, loss body control, have change in their eyes, gill and the sigh may cause increasing mortality time by times (Roy, Yanong, Ruth, 2016).

Other than that, the *Aeromonas hydrophila* is consider second infection as one of the pathogen that attack in tilapia culture (Hamid et al., 2016). Based on the study, clinical sigh showed with swimming is abnormal and loss in balancing. In Malaysia, Aeromonas hydrophilla call Motile *Aeromonas septicaemia*, MAS and also have more name because there are more numerous of bacteria toxic (Yardimci, Aydin, 2011).

There are some among the disease organism that cause mortality in tilapia especially in Nile tilapia culture such as *Flavobacterium columnare, Edwaedseiella tarda, Aeromonas spp., Vibrioa spp., Francisella spp., Sreptococcus agalactiae* and *S. iniae.* This cause the fish contain high bacterial diversity and tolerance by fish that protect them when adapting in nutritional changes and assimilation in the digestive tract (Huong, Thuy, Gallardo & Thanh, 2014).

CHAPTER 3

METHODOLOGY

3.0 MATERIALS AND METHODS

3.1 Location of study

Cosmos caudatus leaves were collected around Jeli and study were conducted at Aquaculture Laboratory, Faculty of Agro Based Industry, Universiti Malaysia Jeli Campus, Kelantan.

3.2 Source of ingredients

3.2.1 Material

Cosmos caudatus, distilled water, Hexane, Methanol, Ethanol and Pathogen (*Streptococcus sp*.).

3.2.2 Apparatus and equipment

Soxhlet apparatus (Laboof), Rotary evaporator, Oven, (Jeio Tech) Incubator, Laminar flow, blender, scale / balance (Sartorius), Trypticase soy agar (TSA), Tryptone soy broth (TSB), petri dish, beaker, eppendorf tube, Glycerol, schott bottles

3.3 Method

3.3.1 Preparation of *Cosmos caudatus*

Ulam raja or *Cosmos caudatus* were picked freshly from the tree at Jeli or surrounding nearby. *Cosmos caudatus* were clean and grinded the sample and dried in oven (Jeio Tech) 60°C for one night. Freshly dried powder from *C. caudatus* were extracted with distilled water (50g sample/ 300 ml of distilled water) in soxhlet apparatus (Lafoff) and the solvent had removed using rotary apparatus evaporator. The other extraction was performed with Hexane, Ethanol and Methanol (Sivakumar and Santhanam, 2011).

3.3.2 Preparation of 24 hours pure culture

One single colony of *Streptococcus sp*. was suspended in about 50µl in eppendorf tube. Each of these was streaked on to the appropriate culture slant and incubated at 37^0 for 24h. Autoclave the Trypticase soy agar (TSA), Trypticase soy broth (TSB), tips, tube, micropipette and other equipment. The well diffusion method was used to check the antibacterial activities. Prepared the approximately 10-10 of TSB with glycerol stock by using serial dilution. The TSA agar plate were related with pathogen strain under aceptic condition and filled 1000µl of test sample and incubate at 37ºC for 24h (Sivakumar & Santhanam, 2011)

3.3.3 Antibacterial activity using agar well diffusion

The bacteria inoculum 100µl was spread uniformly using cotton bud on TSA agar plate. The agar well was cutted using gel puncher and were pour with 50µl of crude sample. Those plates were incubated at 37ºC for 24h. After incubation, the diameter of growth inhibition zone were measured. The inhibition zone showed as a results and measuring in millimeter (mm).

3.3.4 Data analysis

Software was used to analyse the data using ANOVA one way. Post hoc test of Duncan test was analysis in order to identified the significance of difference ($P < 0.05$).

CHAPTER 4

RESULT AND DISCUSSION

Antibacterial activity of *Cosmos caudatus* was evaluated based on the diameter of clear zone in plate that was filled with different sample of extraction against *streptococcus sp*. The reading of the diameter on inhibition zone was measures in milimiter (mm) by using a ruler.

The result in Table 4.1 showed the largest of inhibition zone was ethanol crude extract by using serial dilution 10^{-3} in three replication. The diameter of inhibition zone was 1.11 mm for the mean and 0.02 for standard deviation. The diameter inhibition zone for methanol extract was 0.93 mm for mean and 0.12 for standard deviation. The lowest inhibition zone was hexane extract which is 0.5 mm for mean. There was no inhibition zone showed in distilled water and control. Hence, the result showed, ethanol extract was the highest in inhibition zone compare to methanol and ethanol

. The control treatment did not showed any inhibition because of not filled with any extract. The data was analyse by using one way ANOVA by Duncan.

Table 4.1: Diameter inhibition (mm) of crude extract against *Streptococcus sp.*

Pathogen	Diameter of inhibition zone (mm)				
	methanol	Ethanol	hexane	Distilled water	control
Streptococcus sp.	0.93 ± 0.12^{c}	1.11 ± 0.02^{d}	0.5±0.00	nd	nd

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

nd= no detected

Study by Setha, Laga, Mahendradatta & Firdaus (2014), the extract using difference solvent which are hexane, chloroform, ethyl acetate and methanol will effected antibacterial activity. Besides that, the study from Rubina, Priyanka & Ebenezer (2009) showed that, Aloe vera gel extract using ethanol crude have highest inhibition zone compared to methanol extract against *Bacillus cereus* which is 12.66-23.33 mm. In this study, it showed two different polarity that methanol have more polar properties compared to ethanol. The result show the bacteria have reaction toward less polar compound. Hence, *B. cereus* showed reaction in inhibition zone with less polar. Moreover, there no any inhibition zone showed for control and distilled water treatment.

The result were supported by Sugesh et al., (2013) not dissolve the polarity and non-polar with the crude extraction so, it not strong enough to inhibit the bacteria.

The finding is proved with another study by Demetrio, Esperanza, Juliana & Windell (2015), showed the experiment on antimicrobial activities of methanol, ethanol and supercritical CO_2 extract with Philippine *Piper betle* against *Staphylococcus sp.* The result showed of inhibition zone ranging on ethanol extract was 17-38 mm compare the methanol and supercritical CO_2 which are 15-34 mm and 15 MPa. Thus, the ethanol extract have more potential then methanol and supercritical CO_2.

In other country, the extraction *C. caudatus* with ethanol solvent can be used in rat as feed. The result showed that it is significant gaining of weight among the rat compared control treatment. This ethanol extract was non-toxic to animal can be proved (Farah et al., 2013).

The pathogen that used was *Streptococcus sp*. to test antibacterial activity which obtained from Biology laboratory. *Streptococcus sp*. has feature of Gram-positive. The selection of this bacterium because of it is one of common bacteria in the aquaculture species such as tilapia, catfish, crap and perch and also it suitable bacteria to test the antimicrobial test. Hence, it naturally present in environment.

In fact, Gram-positive bacteria more sensitive compared to Gram-negative bacteria to herbal extract (Koohsari, Ghaemi, Sadegh, Jahedi & Zahiri, 2015). This can be proved by study on Senouci, Hafida, Chahrazed & Djamel (2018), to determine the antibacterial activity of some medical plants such as *Berberis vulgaris'*s root against Gram positive and Gram negative bacteria. This researcher use *Staphylococcus aureus, Enterococcus faecalis* as gram positive and *Escherichia coli, Enterbacter cloacae, Klebsiella pneumonia* and *Pseudomonas aeruginosa* as gram negative. The result show

the diameter of inhibition zone for *S. aureus* and *E. faecalis* were 23 mm and 13 mm respectively and no inhibition zone showed towards other strain.

However, study by Wendakoon, Calderon & Gagnon (2012), showed that strong antibacterial activity strong against gram-positive bacteria which are *S. aereus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* extract with crude boldo, hops, licotice and yerba plant. The result showed no inhibition inhibit against gram-negative bacteria which are *E. coli*. *Pseudomonas aeruginosa* and *Salmonella enteridis*.

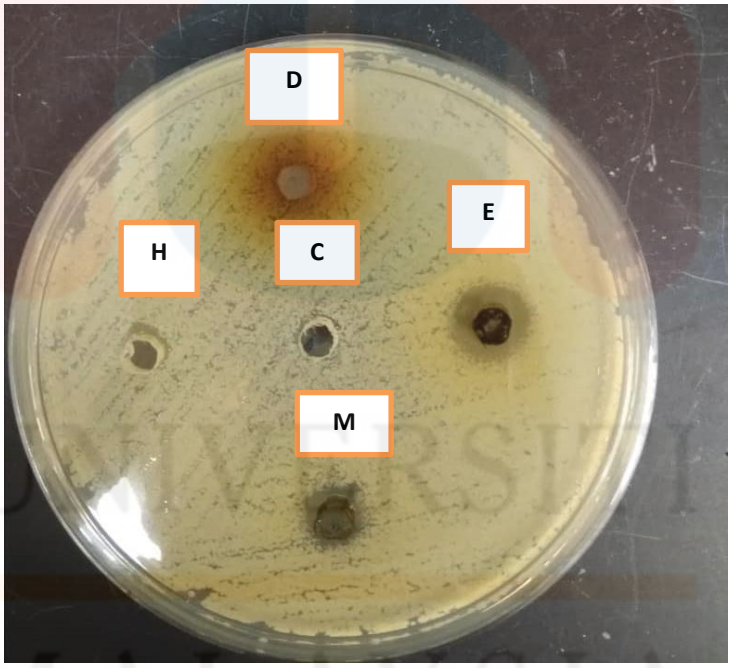

Figure 4.1 : Inhibition zone (mm) extract with different solvent against *Streptococcus sp.*

CHAPTER 5

CONCLUSION AND RECOMMENDATION

As conclusion, these plant can be use as bioactive compound as they have antimicrobial agents and it may serve for the development. *Cosmos caudatus* leaves was successfully isolated and screened for their antibacterial activities against *Streptococcus sp. Cosmos caudatus* were extract by using difference types of solvents (methanol, ethanol, hexane and distilled water). Ethanol crude was selected as the best crude extract and the best solvent compared to methanol, hexane, distilled water and control. Ethanol extract of *C. caudatus* shows a good antibacterial agents that has a potential source of medicine and making feed to animal especially in aquaculture system. Hence, this herbs plant have potential to be process in an aquaculture feed. This is because it show ability of inhibit the bacteria growth and it can help to reduce disease resistance in aquaculture species especially in tilapia, catfish, eel and perch.

Based on the result, further researcher need to continue in order to identify the extract compound for the bacterial inhibition. This also can improve the aquaculture system in economically and also human can consume the product. This can help the farmer to reduce the cost with using natural compound without using expensive vaccine or medical in system Moreover, *Cosmos caudatus* also can be use in other pathogen to analysis the reaction towards.

REFERENCE

Abdullah, A., Dhaliwal, K. K., Roslan, N. N. F., Lee, C. H., Kalaiselvam, M., Radman, H. M., Jaarin, K. (2015). The effects of *Cosmos caudatus* (Ulam Raja) on detoxifying enzymes in extrahepatic organs in mice. *Journal of Applied Pharmaceutical Science*, *5*(1), 082–088. https://doi.org/10.7324/JAPS.2015.50115

Ahmed Sheikh, H. M. (2010). Antimicrobial activity of certain bacteria and fungi isolated from soil mixed with human saliva against pathogenic microbes causing dermatological diseases. *Saudi Journal of Biological Sciences*, *17*(4), 331–339. https://doi.org/10.1016/j.sjbs.2010.06.003

Akila, P., Prince, L., Bharathidasan, R., & Krishnapriya, K. (2016). Antibacterial Activity of Herbal Plants. *Int.J.Curr.Microbiol.App.Sci*, *5*(4), 681–688. https://doi.org/10.20546/ijcmas.2016.504.078

Alavijeh, P. K., Alavijeh, P. K., Sharma, D., & Pharmacy, C. (2012). A study of antimicrobial activity of few medicinal herbs, *2*(4), 496–502.

Amal, M. N. A., & Zamri-Saad, M. (2011). Streptococcosis in Tilapia (*Oreochromis niloticus*): A review. *Pertanika Journal of Tropical Agricultural Science*, *34*(2), 195–206. https://doi.org/ISSN:1511-3701

Ameer, K., Shahbaz, H. M., & Kwon, J. H. (2017). Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(2), 295–315. https://doi.org/10.1111/1541-4337.12253

Andrews, J. M., Assous, M. V, Zedek, S., Ctr, M., Bart, H. Ö. R., Boland, T., Cdc. (2012). Antibacterial Screening Methods for Evaluation of natural product. *Food Chemistry*, *94*(2), 711–713. https://doi.org/10.1016/j.foodchem.2004.07.035

Astutiningrum, T., & Feroniasanti, Y. M. L. (2017). Kenikir leaves (*C. caudatus*) extract antibacterial test toward the growth of *Staphylococcus aureus* in-vitro. *AIP Conference Proceedings*, *1868*. https://doi.org/10.1063/1.4995202

Bereksi, M. S., Hassaïne, H., Bekhechi, C., & Abdelouahid, D. E. (2018). Evaluation of Antibacterial Activity of some Medicinal Plants Extracts Commonly Used in Algerian Traditional Medicine against some Pathogenic Bacteria. *Pharmacognosy Journal*, *10*(3), 507–512. https://doi.org/10.5530/pj.2018.3.83

Bonev, B., Hooper, J., & Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy*, *61*(6), 1295–1301. https://doi.org/10.1093/jac/dkn090

Bunawan, S. N., Bunawan, H., Baharum, N., Amin, N. M., & Noor, N. M. (2014). *Cosmos Caudatus* Kunth: A Traditional Medicinal Herb. *Global Journal of Pharmacology*, *8*(3), 420–426. https://doi.org/10.5829/idosi.gjp.2014.8.3.8424

Cheng, S. H., Barakatun-Nisak, M. Y., Anthony, J., & Ismail, A. (2015). Potential medicinal benefits of *Cosmos caudatus* (Ulam Raja): a scoping review. *Journal of Research in Medical Sciences*, *20*(10), 1000–1006. https://doi.org/10.4103/1735-1995.172796

Citarasu, T. (2010). Herbal biomedicines: A new opportunity for aquaculture industry. *Aquaculture International*, *18*(3), 403–414. https://doi.org/10.1007/s10499-009-9253-7

Cleidson Valgas;, Simone Machado de Souza, Elza F A Smânia; & Artur Smânia Jr. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*(2), 369–380. https://doi.org/10.1590/S1517-83822007000200034

Das, K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *of Medicinal Plants*, *4*(2), 104–111. https://doi.org/10.5897/JMPR09.030

Dhanani, T., Shah, S., Gajbhiye, N. A., & Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. *Arabian Journal of Chemistry*, *10*, S1193–S1199. https://doi.org/10.1016/j.arabjc.2013.02.015

Dian-Nashiela, F., Noriham, A., Nooraain, H., & Azizah, A. H. (2015). Antioxidant activity of herbal tea prepared from *Cosmos caudatus* leaves at Discovery Service for Universiti Kebangsaan Malaysia. *International Food Research Journal.*, *22*(3), 1189–1194. Retrieved from http://eds.b.ebscohost.com.www.ezplib.ukm.my/eds/detail/detail?vid=6&sid=b33afd3f-dccd-499c-9515-caef6fe05f9c%40sessionmgr4010&bdata=JnNpdGU9ZWRzLWxpdmU%3D#AN=102963639&db=fsr

Ernst, E. (2005). The efficacy of herbal medicine - An overview. *Fundamental and Clinical Pharmacology*, *19*(4), 405–409. https://doi.org/10.1111/j.1472-8206.2005.00335.x

Fagg, M. (2014). *Melaleuca alternifolia* Flower Photograph. *Atlas of Living Australia*, *19*(1), 50. https://doi.org/10.1128/CMR.19.1.50

Habibi, H., Ghahtan, N., & Karami, L. (2018). Analysis of Chemical Compounds and Antibacterial Effect of Five Medicinal Plant Essential Oils on Infectious Bacteria. *Trends in Pharmaceutical Sciences*, *4*(1), 51–58.

Hafipah, N., Rasdi, M., Samah, O. A., Sule, A., & Ahmed, Q. U. (2010). Antimicrobial studies of *Cosmos caudatus* Kunth. (Compositae). *Journal of Medicinal Plants Research*, *4*(8), 669–673. https://doi.org/10.5897/JMPR09.422

Hamid, N. H., Hassan, M. D., Sabri, M. Y. M., Hasliza, A. H., Hamdan, R. H., Afifah, M. N., Fuad, M. M. (2017). Studies on Pathogenicity Effect of *Aeromonas hydrophila* Infection in Juvenile Red Hybrid Tilapia *Oreochromis sp*. *Proceedings of International Seminar on Livestock Production and Veterinary Technology*, *0*(0), 532–539. https://doi.org/10.1016/j.jretconser.2018.06.007

Hao, H., Cheng, G., Iqbal, Z., Ai, X., Hussain, H. I., Huang, L., Yuan, Z. (2014). Benefits and risks of antimicrobial use in food-producing animals. *Frontiers in Microbiology*, *5*(JUN), 1–11. https://doi.org/10.3389/fmicb.2014.00288

Hmed, A. A., Sofy, A. R., Sharaf, A. E. M. A., & El-dougdoug, K. A. (2017). Effectiveness of Chitosan as Naturally-Derived Antimicrobial to Fulfill the Needs of Today ' s Consumers Looking for Food without Hazards of Chemical

Preservatives. *Journal of Microbiology Research*, *7*(3), 55–67. https://doi.org/10.5923/j.microbiology.20170703.02

Huda-Faujan, N., Noriham, a, Norrakiah, a S., & Babji, a S. (2009). Antioxidant activity of plants methanolic extracts containing phenolic compounds. *African Journal of Biotechnology*, *47*(3), 484–489. https://doi.org/10.1021/jf990146l

Indian, B., & Garden, B. (2015). Evaluation of Antioxidant Activities of Algae and Effect of Solvent, *6*(3), 1273–1278. https://doi.org/10.13040/IJPSR.0975-8232.6(3).1273-78

Jahangirian, H., Haron, J., Shah, M., & Al, E. (2013). Well Diffusion Method for Evaluation of Antibacterial Activity. *Digest Journal of Nanomaterials and Biostructures*, *8*(3), 1263–1270.

Javid, T., Adnan, M., Tariq, A., Akhtar, B., Ullah, R., & AbdElsalam, N. M. (2015). Antimicrobial activity of three medicinal plants (*Artemisia indica, Medicago falcata* and *Tecoma stans*). *African Journal of Traditional, Complementary and Alternative Medicines*, *12*(3), 91–96. https://doi.org/10.4314/ajtcam.v12i3.11

Jiang, L. (2011). Comparison Of Disk Diffusion , Agar Dilution , And Broth Microdilution For Antimicrobial Susceptibility Testing Of Five Chitosans by. *Fujian Agriculture and Forestry University, China*, *16*(5), 391–394. https://doi.org/10.1016/j.jscs.2010.11.002

Jiang, L., Wang, F., Han, F., Prinyawiwatkul, W., No, H. K., & Ge, B. (2013). Evaluation of diffusion and dilution methods to determine the antimicrobial activity of water-soluble chitosan derivatives. *Journal of Applied Microbiology*, *114*(4), 956–963. https://doi.org/10.1111/jam.12111

K, K. K., & Shabaraya, A. R. (2016). International Journal Of Pharmaceutical And Chemical Sciences Antibacterial Activity of Semisolid Dosage Forms Containing Extracts of Frontal Leaves of Tectona grandis (Family : Verbenaceae), *5*(4), 239–244.

Kabel, A. M. (2014). Free Radicals and Antioxidants : Role of Enzymes and Nutrition, *2*(3), 35–38. https://doi.org/10.12691/jnh-2-3-2

Khan, S. U., Anjum, S. I., Ansari, M. J., Khan, M. H. U., Kamal, S., Rahman, K., Khan, D. (2018). Antimicrobial potentials of medicinal plant's extract and their derived silver nanoparticles: A focus on honey bee pathogen. *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2018.02.010

Khan, U. A., Rahman, H., Niaz, Z., Qasim, M., Khan, J., Tayyaba, & Rehman, B. (2013). Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *European Journal of Microbiology and Immunology*, *3*(4), 272–274. https://doi.org/10.1556/EuJMI.3.2013.4.6

Koohsari, H., Ea, G., M, S. S., Jahedi, M., & Zahiri, M. (2015). The investigation of antibacterial activity of selected native plants from North of Iran. *Journal of Medicine and Life*, *8*(2), 38–42.

Koubaa, M., DorraDriss, Bouaziz, F., Ghorbel, R. E., & SemiaEllouzChaabouni. (2015). Antioxidant and antimicrobial activities of solvent extract obtained from rocket (Eruca sativa L.) flowers. *Free Radicals and Antioxidants*, *5*(1), 29–34.

https://doi.org/10.5530/fra.2015.1.5

Li, J., Xie, S., Ahmed, S., Wang, F., Gu, Y., Zhang, C., Cheng, G. (2017). Antimicrobial activity and resistance: Influencing factors. *Frontiers in Pharmacology*, *8*(JUN), 1–11. https://doi.org/10.3389/fphar.2017.00364

Mackeen, M. M., Ali, A. M., El-Sharkawy, S. H., Manap, M. Y., Salleh, K. M., Lajis, N. H., & Kawazu, K. (1997). Antimicrobial and Cytotoxic Properties of Some Malaysian Traditional Vegetables (Ulam). *Pharmaceutical Biology*, *35*(3), 174–178. https://doi.org/10.1076/phbi.35.3.174.13294

Magaldi, S., Mata-Essayag, S., Hartung De Capriles, C., Perez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, *8*(1), 39–45. https://doi.org/10.1016/j.ijid.2003.03.002

Mishra, M. P., Rath, S., Swain, S. S., Ghosh, G., Das, D., & Padhy, R. N. (2017). In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. *Journal of King Saud University - Science*, *29*(1), 84–95. https://doi.org/10.1016/j.jksus.2015.05.007

Modi, C., Mody, S., Patel, H., Dudhatra, G., Kumar, A., & Awale, M. (2012). Herbal Antibacterials: A Review. *Journal of Intercultural Ethnopharmacology*, *1*(1), 52. https://doi.org/10.5455/jice.20120207113423

Moshawih, S., Cheeme, M. S., Ahmad, Z., Zakaria, Z. A., & Hakim, M. N. (2017). A Comprehensive Review on *Cosmos caudatus* (Ulam Raja): Pharmacology, Ethnopharmacology, and Phytochemistry. *International Research Journal of Education and Sciences (IRJES) EISSN 2550-2158 Vol.*, *1*(1), 14–31. https://doi.org/10.1007/s00170-013-4743-1

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 253–258. https://doi.org/10.1016/j.sjbs.2017.02.004

Mushtaq, Z., Qayoom, U., Mir, I. N., & Mir, S. (2018). Tilapia lake virus : An emerging viral disease of tilapia industry, *6*(5), 141–144.

Mustapha, F., Jai, J., Hamidon, F., Md Sharif, Z., & Yusof, N. M. (2017). Antimicrobial agents from Malaysian plants and their potential use in food packaging material: Review. *Chemical Engineering Research Bulletin*, *19*(October), 57. https://doi.org/10.3329/cerb.v19i0.33797

Nascimento, G. G. F., Locatelli, J., Freitas, P. C., Silva, G. L., & Piracicaba, U. M. De. (2000). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-, 247–256. https://doi.org/10.4317/jced.52493

Nirmala, J., & Pandian, R. (2015). Studies on the antibacterial activity of Plant extract of *Kedrostis foetidissima* (Jacq.) cogn. *Int.J.Curr.Microbiol.App.Sci*, *4*(7), 779–786.

Nostro, A., Germanò, M. P., D'Angelo, V., Marino, A., & Cannatelli, M. A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology*, *30*(5), 379–384.

https://doi.org/10.1046/j.1472-765x.2000.00731.x

Perumal, V., Hamid, A. a, Ismail, A., Saari, K., Abas, F., Ismail, I. S., Khatib, A. (2014). Effect of *Cosmos Caudatus* Kunth Leaves on the Lipid Profile of a Hyperlipidemia-Induced Animal Model. *Journal of Food Chemistry and Nutrition*, *02*(01), 43–51.

Ramadhan, F., Mukarramah, L., Risma, F. A., Yulian, R., Annisyah, N. H., & Asyiah, I. N. (2018). Flavonoids From Endophytic Bacteria of *Cosmos caudatus* Kunth. Leaf As Anticancer and Antimicrobial. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(1), 200. https://doi.org/10.22159/ajpcr.2018.v11i1.21987

Rameli, N. M., Kader, M. A., Aznan, A. S., & Musa, N. (2018). Effect of *Cosmos caudatus* extract on antibacterial activity and lethality activity of brine shrimp. *AACL Bioflux*, *11*(3), 606–612.

Redfern, J., Kinninmonth, M., Burdass, D., & Verran, J. (2014). Tips & Tools Using Soxhlet Ethanol Extraction to Produce and Test Plant. *Journal of Microbiology and Biology Education*, *15*(1), 45–46.

Rubina Lawrence, Priyanka Tripathi, E. J. (2009). Isolation, Purification And Evaluation Of Antibacterial Agents From Aloe Vera Rubina Lawrence*, Priyanka Tripathi, Ebenezer Jeyakumar* Department of Microbiology & Microbial Technology, College of Biotechnology & Allied Sciences, Allahabad Agricultural In. *Brazilian Journal of Microbiology*, *40*(906–915), 906–915.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, Isolation and Characterization of Bioactive Compounds From Plants' Extracts. *Afr J Tradit Complement Altera Med*, *8*(1), 1–10. https://doi.org/10.4314/ajtcam.v8i1.60483

Setha, B., Laga, A., & Mahendradatta, M. (2014). Antibacterial Activity Of Leaves Extracts Of *Jatropha curcas, Linn* Against E*nterobacter aerogenes*. *International Journal of Scientific & Technology Research*, *3*(1), 3–5. Retrieved from www.ijstr.org

Seyedreihani, S. F., Tan, T. C., Alkarkhi, A. F. M., & Easa, A. M. (2017). Total phenolic content and antioxidant activity of Ulam raja (*Cosmos caudatus*) and quantification of its selected marker compounds: Effect of extraction. *International Journal of Food Properties*, *20*(2), 260–270. https://doi.org/10.1080/10942912.2016.1155055

Sheikh, M. (2012). Studies on Some Plant Extracts for Their Antimicrobial Potential against Certain Pathogenic Microorganisms. *American Journal of Plant Sciences*, *03*(02), 209–213. https://doi.org/10.4236/ajps.2012.32025

Sivasankar, P., & Santhiya A, A. V. Kanaga, V. (2015). A review on plants and herbal extracts against viral diseases in aquaculture. *Journal of Medicinal Plants Studies*, *3*(2), 75–79.

Suges, S., & Mayavu, P. (2013). Antimicrobial activities of two edible bivalves M. mretrix and M. csta. *Pakistan Journal of Biological Sciences*, *16*(1), 38–43. https://doi.org/10.3923/pjbs.2013.38.43

Sugesh, S., Mayavu, P., Ezhilarasan, P., Sivashankar, P., & Arivuselvan, N. (2009).

Screening of Antibacterial Activity of Marine Gastropod Hemifusus Pugilinus, *5*(0), 1–3.

Sumazian, Y., Syahida, A., Hakiman, M., & Maziah, M. (2010). Antioxidant activities, Flavonoids , Ascorbic acid and Phenolic contents of Malaysian vegetables. *Jouranal of Medicinal Plants Research*, *4*(10), 881–890. https://doi.org/10.5897/JMPR10.011

Syahidah, A., Saad, C. R., Daud, H. M., & Abdelhadi, Y. M. (2015). Status and potential of herbal applications in aquaculture: A review. *Iranian Journal of Fisheries Sciences*, *14*(1), 27–44. https://doi.org/10.1126/science.aad0095.Genomic

Thanh, H. N. T., Thuy, H. L., Gallardo, W. G., & Thanh, H. N. (2014). Bacterial population in intensive tilapia (*Oreochromi s niloticus*) culture pond sediment in Hai Duong province , Vietnam. *International Journal of Fisheries and Aquaculture*, *6*(12), 133–139. https://doi.org/10.5897/IJFA14.0425

Valle, D. L., Andrade, J. I., Puzon, J. J. M., Cabrera, E. C., & Rivera, W. L. (2015). Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pacific Journal of Tropical Biomedicine*, *5*(7), 532–540. https://doi.org/10.1016/j.apjtb.2015.04.005

Valle, D. L., Cabrera, E. C., Puzon, J. J. M., & Rivera, W. L. (2016). Antimicrobial activities of methanol, ethanol and supercritical CO2 extracts of philippine Piper betle L. on clinical isolates of Gram positive and Gram negative bacteria with transferable multiple drug resistance. *PLoS ONE*, *11*(1), 1–14. https://doi.org/10.1371/journal.pone.0146349

Value, S. J. R. I., Kumar, A., Jhadwal, N., Lal, M., & Singh, M. (2012). Available online http://www.ijddr.in Covered in Official Product of Elsevier , The Netherlands Antibacterial activity of some Medicinal Plants used against UTI causing Pathogens, *4*(2), 278–283.

Yanong, R. P. E., & Francis-floyd, R. (2002). Streptococcal Infections of Fish 1 Streptococcal Disease in Fish, 1–5. https://doi.org/10.1016/S1353-4858(01)00805-4

Yusoff, N. A. H., Noor, N. F., & Rukayadi, Y. (2015). Effects of *Cosmos caudatus* Kunth (Ulam Raja) Extract on Microflora in Ra Chicken Meat. *International Journal of Current Microbiology and Applied Sciences*, *4*(2), 426–435.

Yusoff, N. A. H., Sanuan, F. M., & Rukayadi, Y. (2015). *Cosmos caudatus* Kunth. extract reduced number of microflora in oyster mushroom (*Pleurotus ostreatus*). *International Food Research Journal*, *22*(5), 1837–1842.

Zamri-Saad, M., Amal, M. N. A., Siti-Zahrah, A., & Zulkafli, A. R. (2014). Control and prevention of streptococcosis in cultured tilapia in malaysia: A review. *Pertanika Journal of Tropical Agricultural Science*, *37*(4), 389–410.

ZG, H.-P., & C, L.-S. (2016). Current State of Bacteria Pathogenicity and their Relationship with Host and Environment in Tilapia *Oreochromis niloticus. Journal of Aquaculture Research & Development*, *07*(05). https://doi.org/10.4172/2155-9546.1000428

APPENDICES

Figure 5.1 : Dry sample of *Cosmos caudatus*

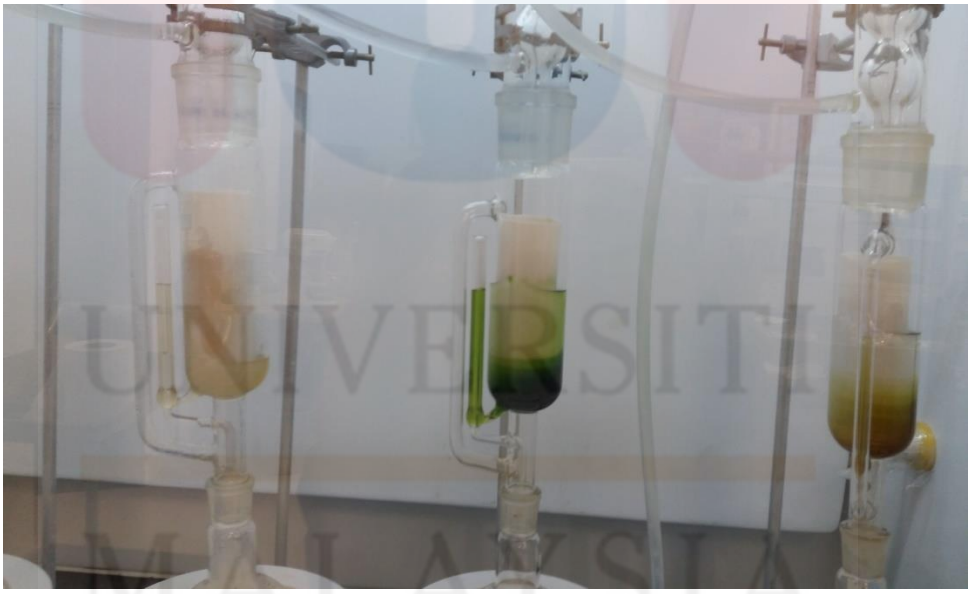

Figure 5.2 : Sample extrction process using Soxhlet apparatus

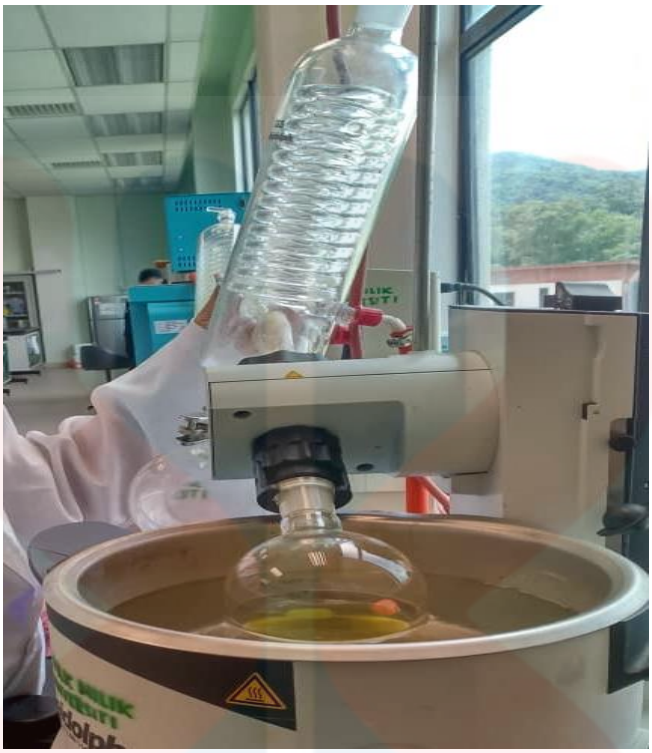

Figure 5.3 : Removing solvent using Rotary evaporator

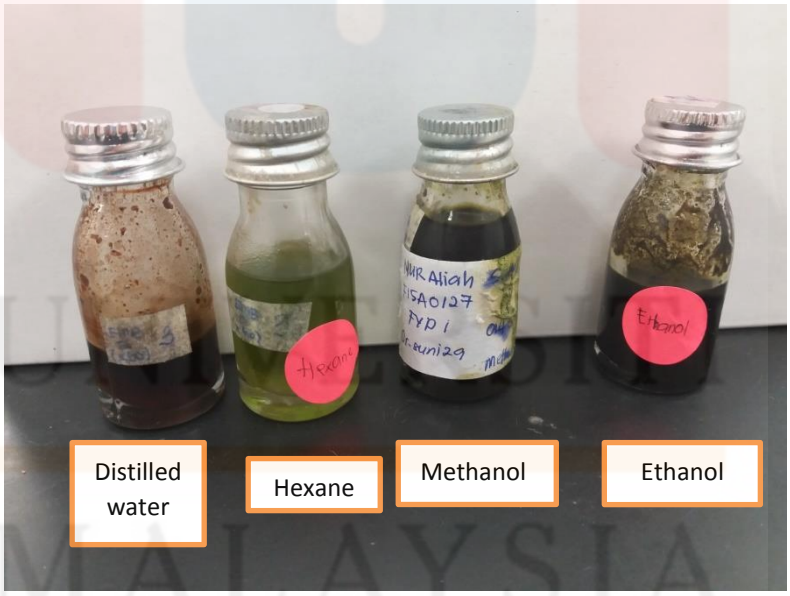

Figure 5.4 : Sample extraction.

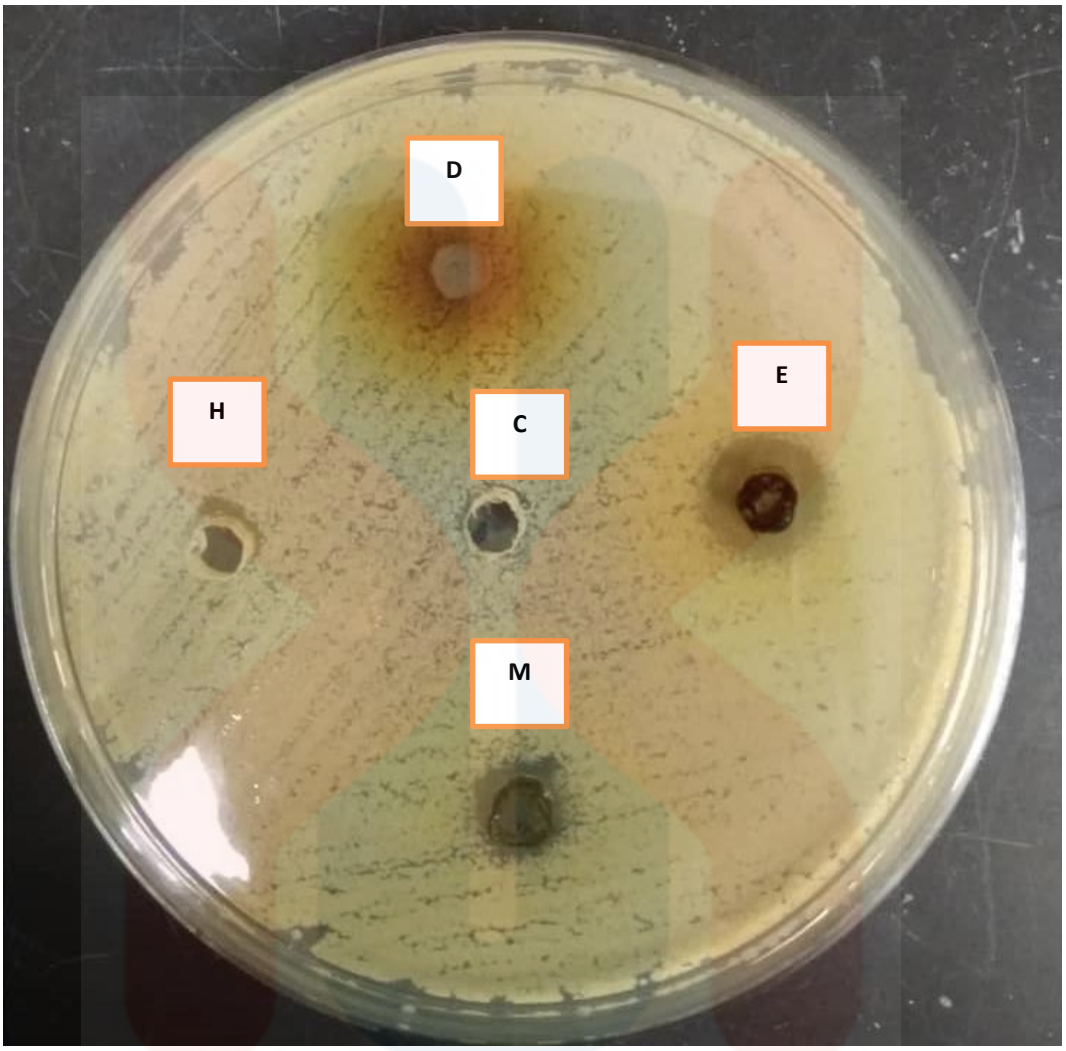

Figure 5.5 : inhibition zone

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

ANOVA

inhibitionZone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.203	4	.801	240.200	.000
Within Groups	.033	10	.003		
Total	3.236	14			

Post Hoc Tests

inhibitionZone

Duncan

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
distilled water	3	.0000			
control	3	.0000			
hexane	3		.5000		
methanol	3			.9333	
ethanol	3				1.1167
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Means Plots

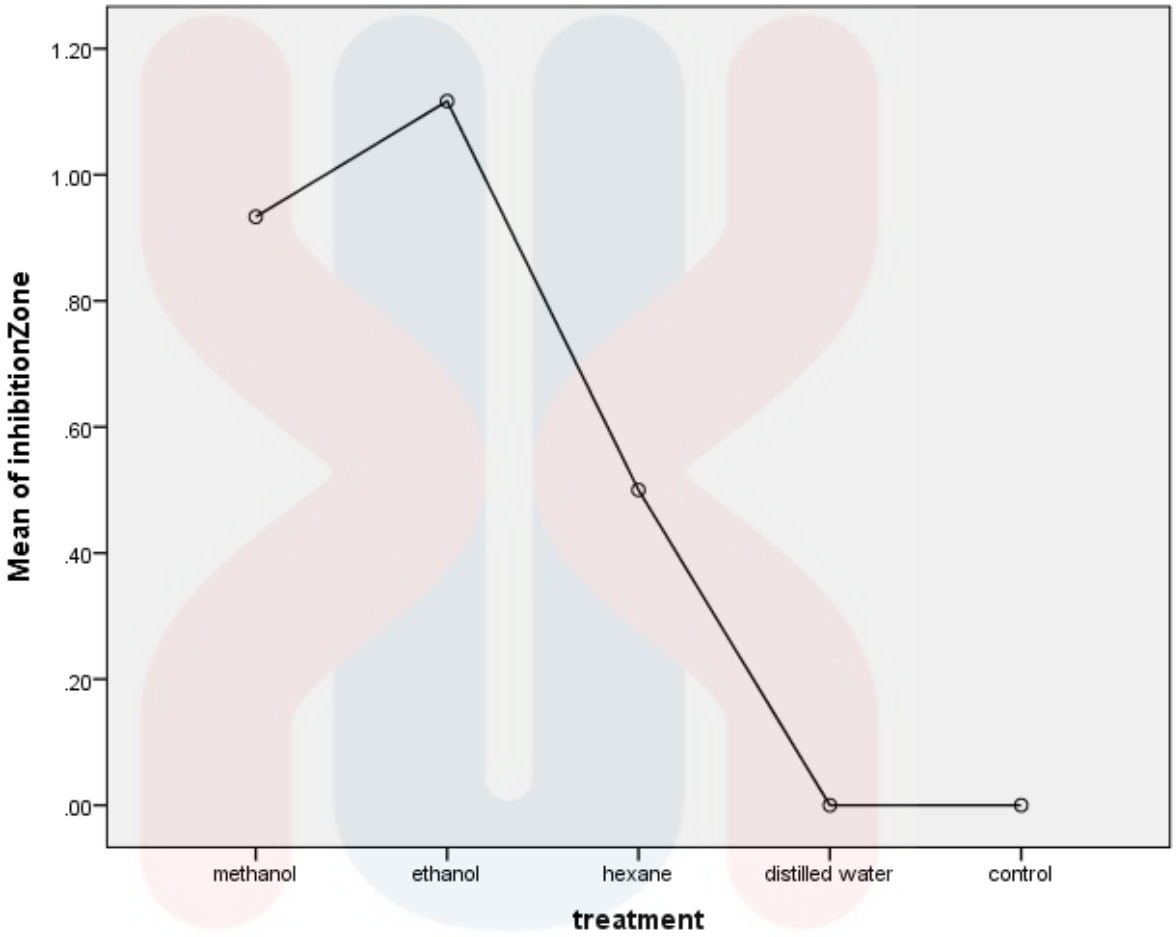

Figure A: Mean of inhibition zone