Antimicrobial Properties of *Euphorbia hirta* Crude Extract

by

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CERTIFICATE

This is to certify that the dissertation entitled

"Antimicrobial Properties of *Euphorbia hirta* Crude Extract"

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Abstract

Antimicrobial effects from the extracted whole plant of *Euphorbia hirta* were studied against several Gram positive and Gram negative bacteria. Water and petroleum ether fraction of the extract have been isolated by using the soxhet extraction method. The Minimum Inhibitory Concentration (MIC) tests were done to determine the minimum concentration of the crude extract that can inhibit bacterial growth. The minimum inhibitory concentration of both extract are 25.00 mg/ml (water extract) and 0.025 mg/ml (petroleum ether extract) respectively. Agar diffusion test was also done by impregnating different concentration of the water extract - 100 mg/ml, 50 mg/ml, 25 mg/ml and the petroleum ether extract - 0.1 mg/ml, 0.05 mg/ml, 0.025 mg/ml respectively onto the standard blank disc. Significant inhibition zone were observed with 0.1 mg/ml of petroleum ether fraction against methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* on a nutrient agar plates. However, no inhibition zones were observed against the Gram negative bacterial plates consisted of *Escherichia coli* (ATCC 25922) and *Salmonella sp.* for both extracts. This result however, primarily validates that the petroleum ether *Euphorbia hirta* crude extract has shown to be a promising effective antibacterial agents against certain Gram positive bacteria.

Abstrak

Kesan anti microorganisma dalam campuran ekstrak keseluruhan tumbuhan *Euphorbia hirta* dikaji ke atas beberapa bakteria gram positif dan gram negatif. Ekstrak air dan ekstrak petroleum eter telah diperolehi daripada tumbuhan *Euphorbia hirta* melalui kaedah pengekstrakkan Sohxlet. Prosedur ujian kepekatan minimum untuk menghalang pertumbuhan bacteria (MIC) dijalankan keatas semua bakteria ujian dalam menentukan kepekatan yang paling kecil untuk menghalang pertumbuhan bacteria. Ujian difusi agar dijalankan dengan memasukkan ekstrak ke dalam disk kosong dengan beberapa kepekatan tertentu. Kepekatan untuk ekstrak air ialah 100 mg/ml, 50 mg/ml, 25 mg/ml dan kepekatan ekstrak untuk petroleum eter ialah 0.1 mg/ml, 0.05 mg/ml, 0.025 mg/ml. Keputusan yang signifikan ditunjukkan pada plat yang mengandungi bakteria *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (ATCC 25923) dan *Staphylococcus epidermidis* apabila diuji dengan ekstrak petroleum eter 0.1 mg/ml. Tiada sebarang inhibitasi apabila kedua – dua jenis ekstrak diuji kepada bakteria gram negatif yang terdiri daripada *Escherichia coli* (ATCC 25922) dan *Salmonella sp.* Keputusan ini menunjukkan bahawa ekstrak daripada petroleum eter mengandungi bahan anti bakterial yang efektif terhadap sesetengah bakteria gram positif.

1.0 Introduction

The practice of medicine is as old as human existence itself. Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drug have been isolated from natural sources and many were based on their used in traditional medicine. These plant base traditional medicine systems continue to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Plant products also have an important role in the health care systems of the remaining 20 % who resides in develop country. At lease 119 chemical substances derived from 90 plant species has been isolated for important drugs and are currently in use. Of these 119 drugs, 74% were discovered as a result of research directed at the isolation of active compound from plants used in traditional medicine.

Indigenous people use a wide range of plants therapeutically to maintain their health. There are great discoveries based on traditional plant uses. These plant-base traditional medicines has been successfully used in many kinds of chronic or acute diseases such as cancer, musculoskeletal system disease, respiratory disease, urogenital disease, heart and circulation, gastrointestinal disease, and etc. Plant products have also been isolated and some were found to contain deterrent compound. These deterrent compounds are known as antibiotics,

antiseptics, pesticides and herbicides are well used for controlling the microbial infection and other communicable disease.

Malaysia is one of the twelve countries in the world with mega diversity bioresources. From 15,000 species of flowering plant known, about 1200 species were reported to have some kind of medicinal properties. But only 15% of these plants were phytochemically studied to some extent for their medicinal properties (NWFP-Digest, 2003). In Malaysia, plant products have been used traditionally or commercially for medical purpose. Mainly, people used it directly from the source or processed to obtain its crude extract. Most of the medicinal plant such as Tongkat Ali, Pokok Tutup Bumi, Pokok Misai Kucing, Kacip Fatimah are well known for their medicinal importance and its whole plant or its crude extract are easily available in market or herbs shopped sold by the traditional medical practitioner. *Euphorbia hirta* or its common name Pokok Patikan is commonly used as an asthma weed by boiling the whole plant with water and drinking its juice. We believe that this plant may have important medicinal properties like antimicrobial agent against several bacteria.

1.1 Ethnobotanical

Euphorbia hirta is included in the family of Euphorbiaceae. It is also synonyms with *Euphorbia pilulifera* or *Chamaesyce hirta*. Other common names are Asthma Weed, Cat's Hair, Fei Yang T's Ao, Daun Patikan, Gelang Susu, Gendong Anak. Dudhi, Euphorbe Indienne, Um Al Halbeeb and Kukon – Kukon. The most habitat

of *Euphorbia hirta* is in India and other tropical country. It grows well in the condition of tropical climate and under full sun.

1.2 Taxonomy

The name 'Hirta' means hairy. *Euphorbia hirta* annually can grow up to 0.3 m. The leaves green in color are opposite, toothed along the margin. The flowers are very small in size and present in the axils of the leaves. Seeds are very tiny, reddish with a wrinkled surface. It is frost tender. The plant is not very well tolerant of frost, though it can probably be grown successfully in tropical climates. The flowers are monoeccious. In the individual flowers either male or female, both seeds can be found on the same plant and it is also pollinated by insects. The plant prefers light, sandy and loamy soils and requires well drained soil. The plan can grows in any soil but also prefers acid, neutral and basic (alkaline) soils. Dried or moist soils are required for optimum growth. One of the unique characteristic is that it cannot grow in the shades.

Figure 1: *Euphorbia hirta*

1.3 Ethnopharmacological

Euphorbia hirta has been widely used and known for its etnopharmacological and medicinal importance. It is also known as asthma weed in Australia and has traditionally been used to treat bronchitic asthma and laryngeal spasm. In modern herbalism it is more used in the treatment of intestinal amoebic disentry (Stuart., 1979). It should not be used without expert guidance, since large doses can cause gastro-intestinal irritation, nausea and vomiting.

The plant is anodyne, antipruritic, carmaniative, depurative, diuretic, febrifuge, galactogogue, purgative and vermifuge (Duke., 1985). The aerial of the plant are

harvested when in flower during the summer and can be dried for later use (Bown., 1995). The stem of *Euphorbia hirta* is taken internally and it is famed as a treatment for asthma, bronchitis and various lung complications (Chopra., 1986). The action of the herbs is it relaxes the bronchiol but apparently depresses the heart and general respiration (Duke., 1985). For the anti-asthma purpose it is usually used in combination with other anti-asthma herbs such as *Grindelia camporum* and Lobelia inflate (Chevalier., 1996).

The whole plant is commonly decocted and used in the treatment of athlete's foot, desentery, enteritis and skin conditions (Duke., 1985). *Euphorbia hirta* has white latex inside its stem. The latex or sap has a characteristic of toothache remedies and is well known in Indonesia. The examples of phytosterol and potentially antimicrobial has been isolated from *Euphorbia hirta* are hydocyanic acid and callic acid (3, 4, 5-trihydobenzoic acid) (Walter., 1987). On the other hand, *Euphorbia hirta* also has been used as anti microbial in the treatment of syphilis infections (Grieve., 1984). The toxicology study has been done against *Euphorbia hirta*. The sap of the *Euphorbia hirta* contains latex which is toxic on ingestion and highly irritant externally. It also can cause a photosensitive skin reaction and severe inflammation, especially on contact with eyes or open cuts. The toxicity can remain high even in dried plant material (Huxley., 1992). Prolonged and regular contact with the sap is not advisable because of its carcinogenic in nature (Matthews., 1994). However, the phytochemical of *Euphorbia hirta* herb is still in research and exploration. The used of this plant for

many other medical purposes especially as an antimicrobial property needs further study to find the novel compound that can inhibit the growth of several pathogenesis microorganisms. The objective of this study is to determine whether *Euphorbia hirta* has anti-microbial properties. The study will also explore the used of *Euphorbia hirta* as an effective anti-microbial agent especially against the gram positive bacteria.

Several researches have been done with *Euphorbia hirta* to isolate its fraction and to search it use in different purpose. But there is not much study of the *Euphorbia hirta* for its anti-microbial effects. Besides, the use of the dried plant and the extraction using a soxhlet extraction with petroleum ether are rare. Then, the research will be followed with bioassay test. Several microorganisms consisting of Gram positive and Gram negative bacteria will be used to determine the antimicrobial effect of the plant. Minimum inhibition concentration (MIC) test will be done to determine the minimum concentration of the crude extract that can inhibit growth of microorganism. Then, MIC will be compared with the agar diffusion test using different concentration of crude extract and the selected commercials standard antibiotics.

2.0 Literatures Review

Many researches have been done to explore the usefulness of the *Euphorbia hirta* for medicinal purpose. Starting from extraction of the plant either the whole or parts of the plant to the bioassay test showed a significant of useful properties. Six compounds have been isolated from the leaves of *Euphorbia hirta* and identified as gallic acid, quercitrin, myricitriu, 3,4-di-O-galloylquinic acid, 2,4,6-tri-O-galloyl-D-glucose and 1,2,3,4, 6-penta-O-galloyl-beta-D-glucose on the basis of physicochemical and spectroscopic methods (Chen., 1991). The cytotoxic study has been studied done by Duez. *et al.*, (1991) using the *Amoeba proteus* model. Application of this model to *Euphorbia hirta* established that a 100°C aqueous extraction of fresh aerial parts allows efficient extraction of active constituents and that drying the plant material before extraction considerably reduces its activity.

Euphorbia hirta crude extract has been used for diuretics, sedative, anti-inflammatory, anti-diarrhea, anti-parasitic and antibacterial. The study of the effect of *Euphorbia hirta* crude extract against the diuretics effect has been done and shown that the crude extract of *Euphorbia hirta* can increase the urine output. The water and ethanol extracts (50 and 100 mg/kg) of the plant produced time-dependent increase in urine output. Electrolyte excretion was also significantly affected by the plant extracts. The water extract of *Euphorbia hirta*

increased the urine excretion of Na^+, K^+ and HCO_3^-. In contrast, the ethanol extract of *Euphorbia hirta* leaves increased the excretion of HCO_3^- , decreased the loss of K^+ and had little effect on renal removal of Na^+. This study suggested that the active component(s) in the water extract of *Euphorbia hirta* leaf had similar diuretic spectrum to that of acetazolamide (Johnson *et al.,* 1999).

Besides, *Euphorbia hirta* crude extract also has been studied for the sedative effects. Lanhers *et al.,* (1990) has proved that the lyophilised aqueous extract of *Euphorbia hirta* has a sedative properties and could be confirmed with high doses (100 mg of dried plant/kg, and more), by a decrease of behavioural parameters measured in non-familiar environment tests (activity test and staircase test), whereas anti conflict effects appeared at lower doses (12.5 and 25 mg of dried plant/kg), by an enhancement of behavioural parameters measured in the staircase test and in the light/dark choice situation test. In 1991, Lanhers *et al.* reported that lyophilised aqueous extract of *Euphorbia hirta* exerts central analgesic properties.

Other than that, in traditional practiced *Euphorbia hirta* is used as anti-diarrhoeic remedies. Tona *et al.,* (1999) has proved that the aqueous extracts from *Euphorbia hirta* whole plant can act as anti-diarrhoeic agents by a triple pronounced antibacterial, antiamoebic and antisplasmodic action. The lyophilized decoction demonstrated anti-diarrhoeic activity in experimental models in diarrhoea induced by castor oil, arachidonic acid, and prostaglandin E_2. In this

study, flavonoid, quercitrin, with antidiarrhoeic activity was isolated from this crude extract (Gelvez *et al.,* 1993).

Euphorbia hirta has an anti parasitic agent that can inhibit the parasite growth. Nqimbi *et al.,* (1999) found that the fraction of ethanol and chloroform extracts of *Euphorbia hirta* whole plant produce more than 60% inhibition of the parasites growth while performing in vitro test. It showed a significant chemosupression of parasitimia in infected mice. Tona *et al.,* (2000) reported that the active polyphenolic extracts from *Euphorbia hirta* whole plant inhibit *Entamoeba histolytica* growth with MIC < 10 micrograms/ml. The same extracts, at a concentration of 80 micrograms/ml in an organ bath, also exhibited more than 70% inhibition of acetylcholine and/or KCl solution-induced contractions on isolated guinea-pig ileum. The ethanol and CH_2Cl_2 extract of *Euphorbia hirta* whole plant, produced more than 60% inhibition of the parasite growth in vitro at a test concentration of 6 µg/ml. This crude extract also showed a significant chemosuppression of parasitaemia in mice infected with *P. berghei* at orally given doses of 100-400 mg/kg per day.

Euphorbia hirta also showed good bacteria inhibitory properties. The *Euphorbia hirta* methanol extract was found to be non-cytotoxic and effective anti microbial agents against *Shigella spp.* (Vijaya *et al.,* 1995). The study conducted by Emele *et Al.,* (1998) found an interesting finding. An addition of *Euphorbia hirta* leaves

extract to mycological media remarkably enhanced fungal growth on the media, and concomitantly suppressed bacterial growth to a similar extent as did with antibiotics. The results of this study suggest that *Euphorbia sapientum* glucose agar can safely be recommended as a cheap and efficient medium for routine isolation of fungi in both clinical and general mycological studies.

This research will focus to the effect of *Euphorbia hirta* crude extract which is obtained from aqueous and petroleum ether extract against several bacteria. The number of the studied of *Euphorbia hirta* crude extract can be a source of information in guiding this research. It is hoped that the findings in this research may achieve something new by exploring the nature s secrets.

3.0 Materials and Methods

3.1 Materials

3.1.1 Plant Source

The fresh plant of *Euphorbia hirta* was obtained from the surrounding vicinity of the Islamic Centre, Health Campus, USM. 2kg of the whole plant was collected and brought to the laboratory for processed. The plants were washed with distilled water then disinfected with 70% ethanol before rinse for the second time with distilled water to remove unnecessary materials. The plant was dried in an oven at 60°C until constant weight was achieved.

3.1.2 Microorganism

Five bacterial strains, *Staphylococcus aureus* (ATCC 25923), Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Escherichia coli* (ATCC 25922) and *Salmonella sp.* were obtained from the culture laboratory, School of Health Sciences, USM. The microorganisms were cultured and maintained by sub-culturing and agar plating in a nutrient agar twice a week to maintain its life and purity.

3.1.3 Chemicals

The analytical chemicals were used for this research. For the extraction solvent, petroleum ether (R&M chemicals) was used to isolate the fat compound in the plant. Dimethyl sulphoxide (BDH Analar, prod- 103234L) were used as universal solvent to dissolve the extracted compound in the water.

3.2 General Preparation

3.2.1 Preparation of 0.5% Dimethyl Sulphoxide (DMSO)

0.5 % Dimethyl sulphoxide (DMSO) was used to dissolve the aqueous and petroleum ether crude extract in water. 100 µl of Dimethyl sulphoxide was mixed with 19.9 ml of distilled water in making 20ml of the 0.5% Dimethyl sulphoxide (DMSO) volume per volume (v/v).

3.2.2 Preparation of 70% Ethanol

70 % ethanol was prepared by mixing 70 ml of absolute ethanol (Merck, Germany) to 30ml of distilled water. Mixed well and kept in a 100 ml Duran bottle. 70% ethanol is used for disinfectant.

3.2.3 Standard Antibiotics

The Oxoid™ antibiotic discs were used in the susceptibility test as a control references. The meticillin disc (5 µg) and ampicillin disc (10 µg) are used for all microorganisms to test its susceptibility.

3.3 Media Preparation

Nutrient agar are used for sub-culturing and susceptibility test. The nutrient broth was used to prepare the microorganism suspension for minimal inhibition test (MIC) and susceptibility test.

3.3.1 Nutrient Agar

The nutrient agar was prepared by suspending 25 g nutrient agar powder (OXOID™, Hampshire, England) in 900 ml of distilled water. The mixture was boiled to dissolve completely. Sterilization was achieved by autoclaving at 121°C for 15 minutes. The nutrient agar was poured in to the sterile culture plate (90 X 15 mm) and kept cooled until it completely solidified. To maintain it's moist, the nutrient agars were kept in the freezer at 4°C.

3.3.2 Nutrient Broth

The nutrient broth was prepared by suspending 8 g nutrient broth powder (OXOID™, Hampshire, England) in 100 ml of distilled water. The mixture was boiled to dissolve completely. Sterilization was achieved by autoclaving at 121°C for 15 minutes.

3.3.4 Instrument and Appliances

Several laboratory instrument and apparatus were used in this research such as soxhlet extractor set which consists of a condenser (Duran 50/42), timble container (Duran 50/42, 24/29), 500 ml round bottom flask (Duran 24/29), water inlet/outlet pipe and heating mantel (MS E103). The timble filter (Advantec 45 X 150 mm) is used to put the dried plant in the timble container. Retort stabilizer with double clamp was used to hang the soxhlet extractor.

For the petroleum ether extraction, the solvent soxhlet extractor (SER 148 solvent extraction) was used with a timble filter (Advantec 30 X 80 mm). Other equipments were used in this research are biosafety cabinet (LabConco, Kansas city, Missouri), oven (Memmert, western Germany), grinder (National), electronic weigher (Sartorius), electronic weigher (Dargon 204, Mettler Toledo groups), incubator (Binder), freeze dryer (iSHIN, Japan), autoclave machine (TOMY^{TM}), Appendorf pipette (France), laboratory glass ware (Pyrex) and etc.

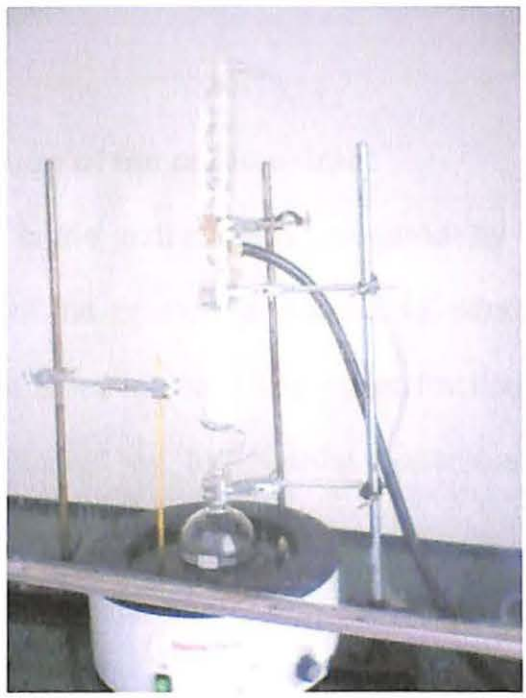

Figure 2: Sohxlet Extractor Apparatus

Figure 3: Sohxlet Extractor (SER 148 Solvent Extractors)

3.4 Method

3.4.1 Preparation of the crude extract

The *Euphorbia hirta* crude extract was prepared by the soxhlet extraction method. Two fraction of the *Euphorbia hirta* crude extract were obtained; water fraction and petroleum ether fraction. The water fraction of the *Euphorbia hirta* crude extract was obtained by the soxhlet apparatus. While the petroleum fraction of the *Euphorbia hirta* crude extract was obtained by the soxhlet extractor machine (SER 148 solvent extractors).

3.4.2 Preparation of the water fraction of *Euphorbia hirta*

The dried plant were cut into small pieces and blended to form fine grains. 60 g of the grains were put into the timble filter (Advantec 45 X 150 mm) and place into the soxhlet container. 500 ml of distilled water were filled into the flask. The soxhlet apparatus were set up and the condenser and heating mantel were turned on with temperature up to 100°C. The extraction process took 2 to 4 hours (10 to 15 cycles) to completely extract most of the compound in the plant. The extraction processed stops when the water in the flask turned to dark brown. The water in the flask contained with extraction compound and some fine grains were filtered by the filter paper (Whiteman No.1) to obtain a pure solution from the extraction. Using the water bath (Memmert, western Germany), the extract solution were vaporized to remove abscess water until the solution is concentrated. Freeze drier (iSHIN, Japan) was used to make the extraction

compound dried and turned to finest powder. The powder were kept in the universal bottle and sealed with parafilm tape (American National Can, TM) to avoid water moist and contamination.

3.4.3 Preparation of the peroleum ether fraction of *Euphorbia hirta*

The dried grains were weighted (3 - 5 g) and put in the timble filter (Advantec 30 X 80 mm). The soxhlet extractor machine (SER 148 solvent extractor) were turned on with running tap water and the heating temperature were set up to 120 – 130°C (refer to the machine manual). The timble filters contained with dried grains were placed on the machine and 80 ml of petroleum ether (R&M chemicals) were put in the solvent container then placed on the hot plate. The soxhlet extractor machine took 1 hours to completed its cycle consist with immersion, washed and recovery to separate most solvent from the extract grains. The solvent containers were cooled and the extracts were collected using the small spatula. The extract were kept in the sterile container and sealed with parafilm tape (American National Can, TM). The extract is place on the bench in room temperature.

3.4.4 The dilution of extract

The stock of *Euphorbia hirta* water extract solution were prepared by mixing 4 g of the fine crude extract powder with 2 ml of 0.5 % Dimethyl sulphoxide (DMSO) solution. For the petroleum ether extract of *Euphorbia hirta*, the stock solution

were prepared by mixing 4 g of the crude extract with 2 ml of absolute Dimethyl sulphoxide (DMSO) solution. The crude extracts were diluted with nutrient broth to get a different concentration. The serial dilution concentrations of the crude extract in water extraction were 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.12 mg/ml and 1.56 mg/ml. For the petroleum ether crude extract, 100µl of the stock were mixed with the 19.9 ml of distilled water to standardize the dimethyl sulphoxide (DMSO) concentration in the extract solution to 0.5 % (v/v). The serial dilution concentrations of the petroleum ether crude extract are 0.1 mg/ml, 0.05 mg/ml, 0.025 mg/ml, 0.012 mg/ml, 0.006 mg/ml, 0.003 mg/ml and 0.001 mg/ml after diluting with the nutrient broth. These serial dilutions were used to perform the Minimal Inhibitory Concentration (MIC) tests.

3.5 Bioassay Test

3.5.1 Minimum inhibitory concentration tests (MIC)

A working antimicrobial solution was prepared by diluting the crude extract solution in nutrient broth as above. The test was performed on sterile covered test tubes (13 by 100 mm). 1 ml of the stock crude extract solution is pipette in 1 ml of the sterile nutrient broth and labeled as tube 1 of the dilution series. To each remaining tube, 1 ml of nutrient broth was added. A sterile pipette was used to transfer 1 ml of the mixed working solution from tube 1 to the tube 2. The mixture was mixed, then by using a new sterile pipette 1 ml of the tube 2 solution is transferred to tube 3. The process was continued through the next-to-last tube, from which 1 ml is removed and discarded. No crude extract was received in the last tube and served as a growth control (sterility).

Four microorganisms were used in this bioassay. They were *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis*, *Escherichia coli* (ATCC 25922) and *Salmonella sp.* The inoculum was prepared to contain 10^5 to 10^6 CFU/ml by adjusting the turbidity of the broth culture to match the turbidity standard. 1 ml of the adjusted inoculum was added to each test tube. The test tubes were incubated in the incubator at 37°C for 16 to 20 hours.

The lowest concentrations of the crude extract that result in complete inhibition of visible growth represent the MIC. However, the crude extract itself was not

colorless; and differentiating the turbidity was difficult. The spectrophotometry turbidity tests were run to differentiate the different turbidities of the tubes. 1 ml of the tested sample was transferred to the microcuvette (Eppendorf microcuvette 10/2 mm) and the absorbance was measured by the Digital sprectrophotometry (Perkin Elmer).

3.5.2 Agar diffusion method

The testing method most frequently used is the standardized filter paper disk agar diffusion method or Kirby-bauer method. This method allows for rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that result from diffusion of the agent into the medium surrounding the disc. In this procedure, a number of small, sterile blank disc (OXOID™ Hampshire, England) of uniform sizes (6mm) that have been impregnated with 50µl of different concentration of the crude extract varies; 100 mg/ml, 50 mg/ml, 25 mg/ml for water extract and 0.1 mg/ml, 0/05 mg/ml, 0.0025 mg/ml for petroleum ether extract were placed on the surface of a nutrient agar plate previously inoculated with a standard amount of the organism to be tested. The plate was inoculated with uniform and close steaks to assure that the microbial growth will be confluent and evenly distributed across the entire plate surface. 4 to 5 colonies of each microorganism; *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis*, *Escherichia coli* (ATCC 25922) and *Salmonella sp* are touched with flamed and cooled inoculating loop. The colonies are emulsifying in 1 ml of the sterile nutrient broth

until the turbidity is approximately to standard. A sterile swab were used and dipped into the bacteria suspension and are inoculated on the surface of nutrient agar plate; first streak with the whole surface of the plate closely with the swab, then rotate the plate through a 45° angle and streak the whole surface again; finally rotate the plate another 90° and streak once more. The plates are allowed to dry.

The blank disc which was impregnated with different concentration of crude extract was placed on the inoculated nutrient agar. The disc was pressed gently to make a full contact with the agar with the tips of forceps. The procedure was repeated for other bacteria and different concentration of the crude extract and standard antibiotics; methicillin 5µg, ampicillin 10µg (OXOID™ Hampshire, England). The discs ware placed in even array on the plates, at well-spaced intervals from each other. When the discs were in firm contact with the agar, the antimicrobial agents diffuse into the surrounding medium and come in contact with the multiplying organisms. The plates were incubated at 37°C for 18 to 24 hours.

3.5.3 Determination of the Inhibition Zone

After incubation, the plates were examined for the present of zones of inhibition of bacterial growth (clearings) around the antimicrobial disks. If there is no inhibition, growth extended up to the rim of the discs on all sides and the organism reported as resistant (R) to the crude extract. The organism is

considered as susceptible with the crude extract when there is a zone of inhibition around the discs. The sizes of the inhibition zones were measured in millimeter (mm) and compared for size with values listed in a standard chart (Table 9). The standard measurement of the inhibition zones diameter were done by the AxioVs40LE (V4.2.0.0 Carl Zeiss GmBH) software.

4.0 Results and Discussions

4.1 Preparation of the crude extract of *Euphorbia hirta*

To obtain the fraction of the plant, soxhlet extraction method was used. Two fractions have been extracted; water extraction and petroleum ether extraction. Both extractions were used for bioassay test to determine its ability to inhibit bacteria growth. The total weight of plant that obtained from the ground is approximately 2 kg. After 1 week of drying in the oven the weight of the plant was reduced to 0.340 kg. The color of the plant has changed from dark green to light yellow. The whole plant was completely dried when its weight remains constant. Then the whole plant were crunched and kept in a clean container.

From the 0.340 kg of the plant grains, 126.58 g of the plant was used for the extraction in 800 ml of water using soxhlet extraction apparatus. After 6 hours of extraction, the water residues in the flask were kept in the reagent bottle. Approximately 500 ml of the water extract were concentrated to a volume of 47 ml. This concentrated extract became dark brown in color and were prepared for the freeze drying. The weight of the extract after water has been removed by freeze drying was 2.2150 g. This final weight of the extract was too little because the amount of compound which was soluble in the water and present in the plant was too small. The powders were kept in the sterile and tight containers to avoid from contamination and moist.

In the petroleum ether extraction, 59.52 g of the *Euphorbia hirta* grains was used for the extraction. After 3 hours of extraction, 1.5763 g of the extract was collected from the grains. The extract was in the paste form and the color was dark green. It was difficult to transfer the paste from the soxhlet collector container into a suitable container because, the extract is too sticky. When compare the both apparatus, the soxhlet extractor machine (SER 148 solvent extractor) is more efficient than the conversional soxhlet apparatus. It can dissolve the grains of plant in the solvent completely and at the final process it separated the extract and solvent distinctly.

Both extract were then dissolved in the 0.5% DMSO. The water extract was completely dissolved in the solvent, but the petroleum ether extract was not easily dissolved in 0.5% of DMSO. The petroleum ether extract was completely homogenized when it is dissolved in the absolute concentration of DMSO. The extract may contain a high and low density of oil and fat derived from the plant. This fat and oil have a low polarity due to its low affinity to dissolved in the water completely. In the bioassay test the concentration of the DMSO should not more than 0.5% in the solvent used. Further than that limit, it will react as a disinfectant to the microorganism. The stock solution of the petroleum ether was prepared by dissolving the extract in the absolute DMSO. Then only 0.5% (v/v) of the mixture were homogenized in the water before the serial dilution were prepared.